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KEYNOTE AND PLENARY LECTURES

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KEYNOTE AND PLENARY LECTURES

KL.3

Environmental influences on intestinal immune responses and epithelial cell homeostasis

Brigitta Stockinger[1], Kathleen Shah[1], Murali Maradana[1]

[1]*Francis Crick Institute*

The integrity of the intestinal barrier has implications for health even beyond the gut. Numerous genetic loci are involved in inflammatory disorders of the gut such as Crohn's disease or ulcerative colitis and the genetic susceptibility for disease is well documented. However, environmental factors, including smoking, diet, use of antibiotics also play a significant role is development of intestinal diseases. One of the mediators of environmental stimuli is the aryl hydrocarbon receptor, (AHR) a member of the basic helix loop helix PAS domain family of transcription factors, which function as environmental sensors of light, oxygen or in the case of AHR environmental pollutions such as TCDD. It has recently become clear that physiological AHR ligands such as dietary components and tryptophan metabolites, partly generated by bacteria from the microbiota, serve to drive beneficial functions of AHR in the immune system as well as in non-hematopoietic cells. Intestinal immune cell types express higher levels of AHR than found in peripheral lymphoid organs indicating readiness to receive environmental signals which are either required for their maintenance and/or their functional capacities. AHR deficiency in the immune compartment causes a generally increased inflammatory tone that predisposes such mice to gut inflammation. Apart from immune cells, AHR expression is pronounced in intestinal epithelial cells and necessary for the functioning of the epithelial barrier. This is particularly evident following intestinal injury by infection or mechanical stress, where AHR function is essential for timely repair of the barrier and differentiation of stem cells to epithelial subtypes. Thus, environmental triggers through AHR are maintaining a delicate balance of repair and differentiation to safeguard an effective intestinal barrier.

Keywords: AHR, intestinal barrier, intestinal immune cells, intestinal epithelial cells

References:

Stockinger, B., Shah, K., and Wincent, E. (2020) AHR in the intestinal microenvironment: safeguarding barrier function. Nat. Rev. Gastroenterol Hepatol https://doi.org/10.1038/s41575-021-00430-8

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PL.2

What we learned from COVID-19: cell by cell, cell type by cell type, patient by patient, cohort by cohort

Joachim L. Schultze[1-3]

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Single cell omics is changing how we define immune cells and their functions. Studies on COVID-19 have clearly illustrated the power of these high-resolution technologies to unravel pathophysiological mechanisms in otherwise unknown diseases. Using our own experience as an example, I will show, how these technologies lead to a better understanding of individual cells and cell types, what we learn about patient-specific disease severity and how we can apply these technologies in large cohorts and clinical studies. Based on this experience, I am outlining further developments to utilize immune parameters for decentralized machine learning when characterizing human immune responses to life-threatening diseases.

Keywords: Single cell omics, COVID-19, macrophages, monocytes, NK cells, T cells, plasmablasts, megakaryocytes

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MAIN SYMPOSIA AND SYMPOSIA

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MAIN SYMPOSIA AND SYMPOSIA

MS1.1 - T Cell Regulation

Molecular mechanisms of human T-cell differentiation

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T cells orchestrate immune system, and a proper balance of T-cell subsets is crucial for immune defense and fighting cancer. In autoimmune diseases, out-of-control T-cells drive pathologic inflammatory responses. We aim at mechanistic understanding of how human effector vs. regulatory T-cell differentiation is regulated. To complement extensive knowledge on the mouse system we have focused on studies on human T-cell differentiation and immune mediated diseases providing a basis for translational research. To achieve this, we have built and exploited an experimental set-up and data aiming at a truly holistic view of human T-cell differentiation. This has resulted in discovery of novel factors and pathways regulating early key decisions of T-cell specification. I will present and discuss our recent results on new factors involved in signaling, epigenetic and transcriptional regulation of human T-cell differentiation.

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Keywords: Human T-cell differentiation, Th17, Treg. transcription, cell signaling

References:

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MS1.2 - Regulation of Adaptive Immune Responses

Dendritic cell crosstalk in CD4 T cell help for the cytotoxic T cell response

Jannie Borst[1-2]

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[2]*Oncode Institute*

In this lecture, it will be highlighted that CD8 T cell differentiation into fully functional effector and effector/memory cytotoxic T lymphocytes (CTL), depends on help delivered by CD4 T cells during priming. Intravital imaging has highlighted that T cell priming is not a singular event, but that T cells collect information instructing their differentiation into effector and memory cells during successive interactions with different conventional dendritic cell (cDC) types in secondary lymphoid organs. Current data support a two-step priming model, in which CD4 and CD8 T cells are first activated separately on different types of migratory DCs, but subsequently can communicate in an antigen-specific manner with the same lymph node-resident cDC1. In this interaction, CD4 T cell help for the CTL response is delivered, in the form of specific cytokines and costimulatory signals. As a result of this input, the CD8 T cell can complete differentiation into effector/memory cell or short-lived effector cell. Whether the platform of CD4 T cell help delivery is created depends on the degree of inflammation, in particular type I interferon signaling. When help is not delivered, the CD8 T cell does not acquire full effector capability, as we have demonstrated by transcriptomic, *in vitro* and *in vivo* functional analyses in a mouse model of therapeutic anti-cancer vaccination. Analysis of available transcriptome data has pointed out that CTL raised in absence of help signals equate predysfunctional CTLs as identified in mouse and human chronic infection and cancer. These predysfunctional cells are the precursors of terminally exhausted cells and the target of "reinvigoration" as accomplished by PD-1 blockade, a key approach in cancer immunotherapy. A comprehensive view will be presented as to how insights into the cellular and molecular pathway of CD4 T cell help for the CTL response leads to clinically applicable strategies to prevent exhaustion of predysfunctional CTL and to make them differentiate into fully functional effectors.

Keywords: CTL, exhaustion, costimulation, PD-1, cancer immunotherapy

Acknowledgments: This work was supported by the Dutch Cancer Society and the Oncode Institute

A novel and human-specific role for the complosome in the regulation of chromatin landscapes

Claudia Kemper[1-2]

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[2]*University of Lubeck*

The complement system is traditionally seen as a liver-derived and serum operative ancient arm of innate immunity that detects and removes invading pathogens. However, recently, we identified a cell-intrinsic and intracellularly activated and functioning complement system – which we coined the complosome. The complosome is present in a broad range of immune and non-immune cells, where it is a central coordinator of nutrient influx, glycolysis and oxidative phosphorylation, and oxygen turn-over. We have now gathered evidence that the complosome contributes to the control of other central basic cell physiological pathways, aside from those of metabolic nature. Here, I will present data that show how the human-specific complement receptor and regulator CD46 controls, via its intracellular domain, DNA looping and chromatin organization in human $CD4^+$ T cells – and that this CD46-driven activity is needed for normal human Th1 induction. These new insights further underpin how intimately the non-canonical activties of ‹classic› innate immune sensors are connected with the basic molecular processes underlying normal cell activties. They also add to our better understanding of a species-specific function of the complement system and may help tackling CD46-driven human disease states in the future.

Keywords: Complement, complosome, CD46, T cell, transcription factors, DNA looping, chromatin structure, Th1 biology

References:

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MAIN SYMPOSIA AND SYMPOSIA

S1.1 - Latest Insight on B Cell Effector Functions

Sec22b is indispensable for plasma cell maintenance and antibody secretion

Marion Espéli[1], Amélie Bonaud[1], Laetita Poncet[2], Simon M Gilbert[3], Pablo Canales Herrerias[4], Julien Barbier[5], Danika Hill[6], Andres Aloatti[7], Daniel Gilet[5], Daniel Stockholm[8], Sebastian Amigorena[7], Ken GC Smith[3], Pierre Bruhns[4], Karl Balabanian[1], Michelle A Linterman[6], Andrew A Peden[9]

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Plasma cells (PC) are key actors of the immune response through secretion of large quantities of antibodies. To accommodate this important protein load, PC display unique cellular features including expansion of the endoplasmic reticulum (ER), adaptation of the unfolded protein response and of autophagy. Despite the essential role of PC in health and disease the cellular mechanisms controlling their development and their secretory function are still poorly understood. During the transition from a B cell to a PC, the newly generated antibodies transit from the expanded ER to the Golgi apparatus where the final maturation of the decorating oligosaccharides occurs. Finally, antibodies are transported from the Golgi to the plasma membrane via cellular cargo. Reports showed *in vitro* that some Soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) may allow antibody transport from the Golgi apparatus to the plasma membrane. However, the mechanisms driving antibody transport from the ER to the Golgi that constitutes the key bottleneck in this process are still unknown. Through a protein screening we identified SNARES highly expressed by PC and in particular Sec22b that is involved in ER-to-Golgi trafficking, autophagy regulation and plasma membrane expansion. Using a B cell specific deficient mouse model, we showed that in absence of Sec22b mature PC are almost completely absent and serum antibody titers are dramatically reduced. Accordingly, these mice fail to mount a protective immune response. Furthermore, our *ex vivo* results demonstrate that Sec22b is indispensable for efficient antibody secretion but also for PC maturation and maintenance through the control of ER expansion and mitochondria biogenesis. Altogether, our results demonstrate a unique and critical role for Sec22b in PC biology.

Keywords: Plasma cell, endoplasmic reticulum, SNARE

S1.2 - Myeloid Cells

The CD47-sirpalpha myeloid immune checkpoint in cancer

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Our group was among the pioneering ones to describe cloning of the inhibitory receptor SIRPalpha and the first to describe its myeloid-restricted expression. We have since been studying the physiological functions of CD47-SIRPalpha interactions and demonstrated that it acts as an immune checkpoint in the context of antibody therapy in cancer. The method of interfering with the CD47-SIRPalpha innate immune checkpoint in combination with tumor antigen-targeting monoclonal antibodies in cancer was patented (WO2009/131453), and this was exclusively licensed to Byondis BV who we are collaborating with to develop antibodies targeting SIRPalpha. During the seminar I will discuss various aspects of the CD47-SIRPalpha checkpoint, including its function and applicability, its mechanism-of-action, and clinical developments in the field. I will also present top-line preclinical data on BYON4228 (Byondis BV), a potentially best-in-class anti-SIRPalpha antibody.

Keywords: Immunotherapy, cancer, CD47, SIRPalpha, checkpoint, myeloid

S1.3 - NK Cell Biology from Basic Science to Clinical Therapy

Suppressing the suppressors - intracellular inhibitory checkpoint pathways as targets for nk-cell based therapies

Mira Barda-Saad[1]

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Natural killer (NK) cells provide a powerful weapon of immune defense against viral infections, tumor growth, and metastatic spread. NK cells demonstrate great potential for cancer immunotherapy; they can rapidly and directly kill cancer cells in the absence of antigen presentation and can initiate a robust immune response in the tumor microenvironment. Nevertheless, current NK cell-based immunotherapies have several drawbacks, such as the requirement for *ex-vivo* expansion of modified NK cells, and low transduction efficiency. NK cell infiltration into solid tumors is correlated with improved outcomes across a variety of different cancers. NK cell-based therapy is increasingly being harnessed for the treatment of advanced solid tumors. Nevertheless, no NK-based clinical trial has yet demonstrated a significant benefit in patients with advanced solid tumors. To overcome current obstacles in NK cell-based immunotherapies, we describe here a non-viral lipid nanoparticle-based delivery system that encapsulates siRNAs to gene silence key intracellular inhibitory NK cell molecules. Nanoparticles efficiently and specifically target NK cells in-vivo, silence inhibitory checkpoint signaling molecules, and unleash NK cell activity to eliminate tumors. Thus, the novel nanoparticle-based system developed here may serve as a powerful tool for future NK cell-based therapeutic approaches.

Keywords: NK signaling, inhibitory checkpoints, NK cells

S1.4 - Regulatory T Cells in Health and Disease

Metabolic control of Treg expansion in cancer

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Regulatory T cells (Tregs) are a subtype of CD4 T lymphocytes endowed with immunosuppressive functions. More and more data testify that Tregs are also capable to support tissue repair after injuries and to promote tissue homeostasis. The transcription factor that determines Treg program is Foxp3: its evolution in the animal kingdom suggests that the development of Tregs took place under the pressure of lactation, and the need to establish immunological tolerance to food and commensals. Tregs constitutively express a variety of costimulatory and cytokine receptors and are dynamically regulated by microenvironmental signals. Growing evidence demonstrates that these immune signals are integrated with metabolic signals and are regulated by both systemic and tissue metabolism. In adults, significant Treg expansion can be observed in the tumor microenvironment, which is characterized by nutrient restrictions, hypoxia and acidity. Our data indicate that Tregs proliferate at the tumor site thanks to a glycolytic-lipogenic circuit and to the activation of specific pathways, such as the biosynthesis of monounsaturated fatty acids and the uptake of iron bound to transferrin. The latter event also plays a crucial role in the proliferation of Tregs that physiologically occurs in neonatal life in mice and humans, according to our recent results. These observations suggest that some metabolic *modules*, involved in the physiological Treg expansion, can also appear in certain pathological microenvironments, contributing to the Treg proliferation that is detrimental in the tumor setting - a phenomenon recognizable as "antagonistic pleiotropy".

Keywords: Treg, metabolism, cancer

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MAIN SYMPOSIA AND SYMPOSIA

S1.5 - B Cell Differentiation and Function in Health and Disease

The survival and function of IL-10-producing regulatory b cells are negatively controlled by SLAMF5

Idit Shachar[1]

[1]*Weizmann Institute of Science*

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) leading to chronic neurological disabilities. It is characterized by extensive inflammation and demyelination, accompanied by axonal and neuronal damage. The disease is heterogeneous in its clinical manifestation and progression, as well as in its pathological mechanisms.

The SLAM (Signaling Lymphocytic Activating Molecules) family of immunomodulating receptors helps mediate the interaction of immune cells with their microenvironment. SLAMF5 (CD84), a member of the SLAM family, is a self-ligand receptor that forms homophilic dimers. In the current study, we investigated the role of SLAMF5 in EAE. We report here that SLAMF5 deficiency (SLAMF5$^{-/-}$) alleviated disease in MOG-induced EAE mice. The lack of SLAMF5 induces accumulation of Bregs both in the periphery and CNS and reduction of the microglia M1 population. *In vitro* blocking of SLAMF5 expressed on murine and human B cells resulted in a specific effect on Breg survival and induced expression of the transcription factor c-Maf, which was shown to upregulate IL-10 expression. Finally, treatment of EAE-induced mice with anti-SLAMF5 blocking antibody elevated Breg and M2 levels and mitigated disease progression, suggesting SLAMF5 as a specific negative regulator of Bregs and microglia funcion, and a potential target for MS treatment.

Keywords: CD84, SLAMF5, multiple sclerosis, Bregs, microglia, microenvironment

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MS2.1 - Immunomodulation and Aging

How can we predict vaccine responsiveness in older adults?

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This presentation will primarily focus on vaccines against T and B cell neoantigens (SARS-CoV-2), antigens requiring T cell memory (VZV), and both memory and neoantigen responses (influenza). First, let us examine the received wisdom that older adults respond less well to vaccination than young adults or children because of "immunosenescence". What are the recognized characteristics of immunosenescence in humans? Most data are from cross-sectional studies on differences between younger and older adults, now being backed up by increasing numbers of longitudinal studies investigating changes over time in an individual. One of the first of these was the OCTO/NONA series from Jönköping in Sweden following mostly women from 85 years of age, with biennial follow-up for 6 years. What emerges from all such studies is that older people possess fewer peripheral naïve T and B cells and more memory cells than younger adults, as well as more myeloid cells. The former is a reflection of adaptive immunity, not ageing per se; the latter of age-altered hematopoiesis. There are a few examples where some of these parameters have been shown to directly impact longevity; again, pioneering Swedish studies suggested that accumulations of dysfunctional late-differentiated CD8+ memory cells clustered together with a paucity of B cells to weakly predict 2-, 4- and 6-year all-cause mortality. Reduced numbers of naïve CD8+ T cells did not cluster with this risk phenotype. Which if any of these parameters predict vaccine responsiveness is unclear, but early studies suggested that the accumulation of late-differentiated CD8+ T cells did predict poor responses to seasonal influenza vaccination. However, In the case of influenza, there is strong evidence that vaccines can be equally effective in younger and older populations provided that the confounding factor of geriatric frailty is taken into account. The mechanisms by which overall organismal frailty affect immune function are manifold but not fully understood; they include soluble systemic factors, and intrinsic changes at the cellular and organ level. The initially unanticipated important message from the success in older adults of mRNA vaccines against SARS-CoV-2 is that even for neoantigens in a real-world setting, neither a paucity of immune cells or constriction of the antigen receptor repertoire, nor defects in antigen presentation or cellular function, nor the effects of "old blood" are sufficient to prevent an effective immune response provided that the vaccine formulation is appropriate. For vaccines that boost memory responses, such as herpes zoster, the key to overcoming the age-associated deficit in responsiveness lay in formulating an effective adjuvant and delivery mode.

Therefore, the answer to the question posed in the title of this talk is that, yes, we can predict and expect successful responses in older adults provided that we challenge the immune system in an appropriately effective manner.

Keywords: Immunosenescence, vaccination, influenza, SARS-CoV-2, herpes zoster, geriatric frailty, older adult

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Role of autophagy in immune aging

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Autophagy, an essential lysosomal degradation pathway, is a key regulator of many cell-intrinsic functions such as differentiation, cellular metabolism and inflammation. Adipocytes release free fatty acids (FFA) during times of high metabolic demand including inflammation. The importance of lipolysis for the release of FFA is well understood, in contrast our study explores the contribution of autophagy/lipophagy in this process. Inflammatory bowel disease, a highly metabolic disease, is characterized by creeping fat around the inflammatory site. Therefore, we dissected the impact of autophagy in adipocytes on intestinal inflammatory responses. Using a chemically induced colitis model, we found that autophagy is increased in adipose tissues during intestinal inflammation. We then created a mouse model in which we can induce adipocyte-specific deletion of the essential autophagy gene Atg7 (Atg7Ad). Our data showed increased intestinal inflammation and tissue destruction in Atg7Ad mice. In adipose tissues, Atg7Ad mice were unable to shift towards an anti-inflammatory macrophage polarization to resolve the colitis. Mechanistically, we found that release of FFA from adipose tissue explants from Atg7Ad mice was reduced upon TNF alpha stimulation, TNF alpha being the hallmark cytokine of this colitis model. We found that depleting FFA levels in macrophage culture medium suppresses specifically IL-10 production, while TNF levels remain unchanged. Moreover, IL-10 secretion is reduced *in vivo* and from adipose/ immune explants when autophagy is absent. This study highlights an underappreciated role of autophagy in adipose tissue in controlling anti-inflammatory processes during intestinal inflammation. Our current work aims to reveal whether this is relevant for human disease and whether this interaction could be exploited therapeutically.

Keywords: Autophagy, inflammation, adipocytes, colitis, lipolysis, lipophagy

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MAIN SYMPOSIA AND SYMPOSIA

MS2.2 - Cell Death in Immunology

Immunogenic cell death: endogenous and exogenous adjuvant signals

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In response to some selected chemotherapeutic and targeted agents, cancer cells undergo immunogenic cell death (ICD), thereby enhancing immune responses against stress-associated tumor antigens that account for therapeutic long-term effects beyond treatment discontinuation. ICD involves the exposure or secretion of adjuvant signals that are danger-associated molecular patterns (DAMPs) acting on pattern recognition receptor (PRRs). Mechanistically, ICD is characterized by autocrine stimulation of type-1 interferon (IFN) receptors, the pre-apoptotic exposure of calreticulin (CALR) on the cell surface, release of ATP during the blebbing phase of apoptosis, and post-apoptotic exodus of annexin A1 (ANXA1) and the chromatin-binding protein high mobility group B1 (HMGB1). Type-1 interferon secretion depends on the stimulation of toll-like receptor 3 (TLR3) and the cGAS/STING pathway, CALR exposure on an endoplasmic reticulum stress response, ATP release on pre-mortem autophagy, and annexin A1/HMGB1 exodus on secondary necrosis. ATP, ANXA1, CALR and HMGB1 interact with four receptor types present on the surface of dendritic cells, namely, purinergic P2Y2 or P2X7 receptors, formyl peptide receptor-1 (FPR1), CD91, and toll-like receptor 4 (TLR4), respectively. P2Y2, FPR1, CD91, P2RX7 and TLR4 promote chemotaxis of dendritic cells (DC), juxtaposition of DC and dying cells, engulfment of dead-cell antigen, production of interleukin-1β and cross-presentation of tumor antigens by DC, respectively. Local induction of the integrated stress response (phosphorylation of eukaryotic initiation factor 2a, eIF2a) in the tumor bed and systemic induction of autophagy increases anticancer immune responses. Altogether, these molecular events form a cascade that explains the mechanisms of ICD. In the advent of suboptimal ICD responses, the provision of exogenous DAMPs or PRR ligands can restore therapeutic responses.

Keywords: Cancer, immunogenic cell death, immunosurveillance

S2.1 - Microbiota and the Immune System

Secretory antibodies in the intestinal ecosystem

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Secretory IgA is the only component of the adaptive immune system that is present at high concentrations out in the gut lumen. Here it has to perform its protective functions in the context of a highly dense, diverse and actively growing, metabolizing and evolving microbial consortium and within an environment that is continuously flowing from mouth to anus. While IgA functions have been hard to explain in the context of a static picture of bacteria-antibody interactions, much becomes clear when we incorporate intestinal content flow, bacterial growth and bacterial evolution into our models. In this talk, I will explore techniques that can be used to probe and better understand antibody-microbe interactions in the gut, and the consequences of such models for our understanding of immune-microbiota interactions.

Keywords: IgA, microbiota, salmonella, fluid dynamics, affinity, within-host population dynamics

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S2.2 - Innate to Adaptive

Why we get sick: interactions between the immune and endocrine systems during viral infection

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Being sick makes us miserable. Following infection with a pathogen we lose appetite, get a temperature, and feel weak. We experience these feelings as pathology, but in fact they are a carefully orchestrated physiological response. Upon infection, the immune and endocrine system directly communicate to change systemic metabolism and induce a state that we experience as 'being sick'. The purpose of this state is to impair replication of the invading pathogen and at the same time generate an optimal environment for immune cell function. The underlying molecular mechanism of this process have long remained unknown, but recent advances have made clear how the immune system mediates changes in endocrine function upon infection and how these changes subsequently benefit the anti-viral response. In the context of pre-existing metabolic disease, this system derails and may promote development of pathologies such as diabetes mellitus type 2 (DM2). Importantly, patients with metabolic disease fail to induce the immune-mediated anti-viral changes in systemic metabolism, which predisposes them to severe disease outcome following infection with pathogens such as SARS-CoV-2. Indeed, DM2 is one of the biggest risk factors for morbidity and mortality in the context of COVID-19, as well as other infectious diseases. In this lecture, our recent discoveries on immune-endocrine interactions in the context of infection will be discussed.

Keywords: Viral infection, metabolic disease, COVID-19, CD8 T cells, diabetes mellitus type 2, virus, infection

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Antigen-specificity in human immune pathologies

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The majority of human immune pathologies are caused by inappropriate immune reactions against more or less defined antigens. CD4 T cells are the central orchestrators of adaptive immunity and provide important diagnostic and therapeutic potential. However, the analysis of antigen-specific CD4 T cell responses in human disease is still challenging, representing a major hurdle for patient and disease-specific "personalized" medicine approaches. We have established sensitive tools for the analysis of human antigen specific CD4 T cells, including regulatory T cells. I will give an overview on the characterization of T cells involved in antigen-specific tolerance, immune pathology and protective immunity in humans, which does in many aspects deviate from results obtained in experimental models. I will use coronavirus disease 2019 (COVID-19) as an example and try to illustrate how detailed quantitative and qualitative analysis of SARS-CoV-2-specific immunity in unexposed donors and COVID-19 patients or vaccinees may help to understand the clinical variability as well as differences in response to vaccination. In particular, the role of pre-existing T cell memory in unexposed donors has caused significant controversies. Our data suggest that pre- existing memory is a general phenomenon in the human immune system with strong impact on response to novel antigens in the ageing immune system. It correlates with a globally increased memory T cell repertoire upon ageing most likely due to frequent and diverse microbial encounter, rather than infection with a distinct related virus, such as common cold corona virus. Functional characterization suggests that pre-existing SARS-CoV-2 specific memory T cells are not protective but may in fact contribute to severe COVID19 disease and poor response to vaccines.

Taken together, our results highlight the need and potential of human antigen-specific T cell characterization for personalized "precision" medicine.

Keywords: Antigen-specific T cells, ARTE, regulatory T cells, tolerance, SARS CoV-2, pre-existing memory, human immunology

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S2.5 - Non-Coding RNA and Epigenetic Regulation in Immune Cells

Microrna-mediated regulation of primary human T lymphocytes

Silvia Monticelli[1], Michele Chirichella[1], Niccolò Bianchi[1], Stefan Emming[1]

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Through their ability to target a variety of mRNAs and regulate their translation and stability, microRNAs (miRNAs) modulate all aspects of the biology of T lymphocytes, including cell differentiation, activation and proliferation. The effect of any given miRNA is dependent on its expression level relative to that of its targets, and also on the specific context and cell-specific usage of target sites in the 3' untranslated region (3'UTR) of mRNAs, resembling the cell type-specific regulation of gene expression mediated by transcription factors. The quantitative analysis of miRNA expression in different T cell subsets and in response to T cell receptor (TCR) triggering may thus provide clues on the functional impact of individual miRNAs on T cell responses. I will discuss our recent findings about the key regulatory functions that miRNAs exert in the immune system, including the mRNA targets that are regulated by miRNAs specifically in T cells and how selected miRNAs are themselves transcriptionally regulated.

Keywords: miRNAs, T lymphocytes, gene regulation

MS3.1 - Mucosal Immune Response

Role of IL-17-secreting tissue-resident memory CD4 T cells in protection against bordetella pertussis nasal infection

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Tissue-resident memory (TRM) cells provide a first line of protection at mucosal surfaces and play an important role in long-term protective immunity induced by previous infection or vaccination against mucosal pathogens. We have examined the function of TRM cells in protection against the respiratory pathogen *Bordetella pertussis*, the bacterium that causes whooping cough (pertussis). Pertussis is a re-emerging infectious disease in part due to waning immunity after immunization with current acellular pertussis (aP) vaccines and their failure to prevent nasal infection with *B. pertussis*. We found that antigen specific TRM cells accumulate in the lungs and nasal tissue during infection with *B. pertussis* andthese cells expand rapidly after challenge of convalescent mice and conferred long-term protection against re-infection. In the nasal cavity, the majority of *B. pertussis*-specific TRM cells secreted IL-17 and conferred protection against infection by promoting recruitment of $SiglecF^+$ neutrophils to the nasal tissue. Ablation of IL-17 or depletion of CD4 T cells or neutrophils from the respiratory tissue abrogated protection against re-infection with *B. pertussis*. In addition, we found that respiratory TRM cells are maintained in the tissue by non- specific activation through cytokines produced by innate immune cells stimulated with unrelated pathogens or PAMPs. However, immunization with aP vaccines failed to generate TRM cells and this may in part be due to the suppressive effects of IL-10 that the vaccine induces. The addition of a novel adjuvant combination, LP-GMP, comprising a TLR2 lipopeptide from *B. pertussis* and a STING agonist, overcome the deficits of the alum-adjuvanted aP vaccine by promoting the induction of respiratory TRM cells. In particular, nasal immunization with aP vaccine formulated with LP-GMP was highly effective at inducing IL-17-secreting respiratory TRM cells and conferred long term protection against infection of the lungs and nasal mucosa with *B. pertussis*. Our findings underline the crucial role of TRM cells in protective immunity against respiratory pathogens and suggests that vaccines against mucosal pathogens, as well as inducing systemic humoral and cellular immune responses, should be designed to induce adaptive immunity and immunological memory at mucosal tissues.

Keywords: Tissue-resident memory T cell, respiratory tract, vaccine, IL-17, mucosal infection

Acknowledgments: This work was supported by Science Foundation Ireland

MS3.2 - Innate Immune Response

Regnase-1: an endoribonuclease involved in inflammation, immunity and metabolism

Shizuo Akira[1]

[1]*Osaka University*

Innate immune responses are initiated by pattern recognition receptors. Among them, Toll-like receptors (TLRs) play a major role in triggering innate immunity and subsequent adaptive immunity by recognizing different microbial components. Through the analyses of the genes induced in response to TLR stimulation, we have shown that Regnase-1 encoded by the Zc3h12a gene is an endoribonuclease involved in destabilization of a variety of mRNAs including IL-6, IL-12, and Regnase-1 itself mRNAs via the stem loop structure present in the 3'UTR of these mRNAs. Although originally identified as the LPS-inducible gene, Regnase-1 protein is present in unstimulated cells, and disappears in response to TLR ligands via an IKK-dependent proteasome degradation pathway or in response to T cell receptor stimulation through the cleavage by Malt-1. Thus, Regnase-1 acts as a brake in unstimulated cells as well as a negative feedback regulator after cellular activation. We also found that IL-17 signal also inhibits the function of Regnase-1. Regnase-1 is evolutionally highly conserved. C. elegans Regnase-1 homologue, REGE1 promotes fat accumulation by degrading the mRNA encoding a fat-loss promoting transcription factor. Drosophila Regnase-1 is required for metamorphosis from larva to adult fly. Mammalian Regnase-1 controls colon epithelial regeneration via regulation of mTOR and purine metabolism. Thus, Regnase-1 plays an important role in metabolism in addition to inflammation and immunity.

Keywords: Posttranscriptional regulation, mRNA stability, toll-like receptor, MyD88, RNA binding protein

S3.1 - Immunometabolism

Generating and maintaining tissue resident T cells

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Tissue resident memory (T_{RM}) CD8 T cells are one of the most abundant T cell populations and offer rapid protection against invading pathogens. Generated from circulating T cells, T_{RM} cells metabolically adapt to their tissue niche, such as the intestinal epithelial barrier. There, intraepithelial lymphocytes (IELs) contain high levels of cytotoxic molecules and express activation markers, indicating their heightened state of activation. We hypothesize that the tissue environment may affect IEL activity. We will present previous and novel data on generation and maintenance of intestinal resident T cells and their metabolic requirements.

Keywords: Metabolism, T cells, mucosal immunology

Metabolite-mediated regulation of immune-inflammation

Claudio Mauro[1]

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The Mauro's group investigates the interconnections between metabolic and inflammatory pathways and how systemic and local metabolic alterations in diseases with an inflammatory component led to aberrant immune cell responses, which favor both the establishment and the propagation of inflammation. In particular, they focus on unveiling how specific metabolites, including lactate and fatty acids, can surprisingly act as signaling molecules modulating many aspects of the immune-inflammatory response. The most recent developments of our research in this area will be presented.

Keywords: Metabolism, inflammation, immunometabolism, metabolite, lactate, lipids, chronic inflammation

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S3.2 - Aging of the Immune System

Impact of inflammatory status on resilience in the elderly

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Hip fracture (HF) is a common and devastating injury as well as a major health issue in old age. HF has a one-year mortality >30% in the elderly and is a frequent cause of institutionalization. The reasons for such poor outcomes in this trauma are multifactorial but we aim here at identifying immunological factors, which can influence and/or predict the outcome of hip trauma in elderly patients' post-surgery. We analyze immunological parameters evocating of the Immune Risk Phenotype in sequential pre- and post-surgical samples collected from HF patients over 75 years of age. The study revealed that HF is associated with an immune scar depicting a transient T-cell leucopenia and an acute hyper-inflammation. Importantly, we show that blood level of a molecule released by activated macrophages is predictive of one-year mortality in these patients. Its plasmatic level correlated negatively with the time of survival and functional autonomy after HF surgery. In conclusion, HF patients exhibit transient changes in innate and adaptive immunity. Meanwhile, profound acute inflammatory processes measurable pre-surgery occurs, which are predictive of long-term survival after HF surgery. We propose to use the identified predictive biomarker to improve medical interventions and follow-up of patients at risk of early death.

Keywords: Inflammation, aging, stress, hip fracture, cellular immunity, metabolite

Acknowledgments: Emergence Sorbonne University grant.

Role of modified proteodynamics in aging of the immune system and its consequences

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Proteodynamics is an umbrella term that we have coined in order to include all stages of cellular protein existence, from translation to various ways of posttranslational modifications, to functional life in the cell to final aggregation, misfolding and proteolytic elimination. The latter stages, including identification and elimination of aggregated or misfolded proteins are known as proteostasis and are described to be faulty in the cells from old organisms, leading to accumulation of dysfunctional proteins and cellular senescence or death. This loss of proteostasis is included in the nine hallmarks of aging. Also, the immune cells, forming both the innate and adaptive branch of the immune system, exhibit aging-related loss of proteostasis, adversely affecting their functionalities. This, however, is not all. It was demonstrated that the immune cells of older donors exhibit lower efficiency of mRNA translation and higher numbers of both transcription and translation errors. Also, various means of posttranslational modification of proteins are affected in the aging immune cells. These include the additive ones like glycosylation, prenylation, SUMOylation etc., and notably phosphorylation and dephosphorylation, as well as subtractive modifications, where part of the originally translated peptide must be cleaved by limited proteolysis in order to make it functional (active) or inactive. The latter are executed in the cytoplasm notably by the calpain-calpastatin system (CCS). We have found that ubiquitous members of the CCS, the Ca^{2+}-dependent cysteine proteases m- and m-calpain, as well as their endogenous inhibitor calpastatin are present and active in resting mononuclear peripheral blood immunocytes (all populations of T and B lymphocytes and monocyte/macrophages). Furthermore, we have demonstrated that inhibition of this resting CCS activity in the T cells prior to stimulation leads to decreased proliferation, lowered synthesis of multiple cytokines, and reduces the levels of phosphorylation of important signaling molecules, notably the NFkB, p56LCK, ZAP-70 and phospholipase PLCgamma. This makes the CCS one of the important regulators of the immune response. Not surprisingly, we have later demonstrated that the amounts and activities of member proteins of the CCS are significantly reduced in the peripheral mononuclear immune cells from people aged 65-85 years compared to those 20-30 years old, but the cells coming from healthy individuals aged 95-101 years retain the amounts and activities of both proteases. We can speculate that this phenomenon is a bit of the puzzle concerning relatively good activity of the immune system in the oldest old.

Keywords: Proteodynamics, proteostasis, aging, immunosenescence, inflammaging, T cells, proliferation, cytokine secretion, calpai, calpastatin, modulating proteolysis

S3.3 - Immunotherapy in Transplantation

The future of Treg therapy: from polyclonal to genetically modified Tregs

Giovanna Lombardi[1]

[1]*King's College London*

Human regulatory T cells (Tregs) are a subset of T cells expressing the surface molecules CD4 and CD25 together with FOXP3, their master transcription factor, and low levels of CD127. Tregs function to maintain self-tolerance and prevent inappropriate immune activation. They are currently under investigation as an adoptive cell-based therapy to prevent transplant rejection and to cure autoimmune diseases. Polyclonal Treg-based cell therapy approaches yielded early promising results for the prevention of graft-*versus*-host disease (GvHD), and maintenance of Cpeptide levels in Type 1 diabetes. We have completed two Phase I/II clinical trials, the ONE Study (NCT02129881) and ThRIL (NCT02166177), both assessing the safety and feasibility of adoptive transfer of polyclonal Tregs. We have shown that Treg therapy is safe, well tolerated, and with some signs of efficacy.

Importantly, we and others have demonstrated that donor-specific Tregs were superior compared to polyclonal Tregs in pre-clinical models of transplantation, whereby donor-specificity was achieved by culturing Tregs with allogeneic antigen presenting cells (APC) or by transduction of Tregs with T cell receptors (TCR) specific for alloantigens. A new way to confer antigen specificity is genetically engineering Tregs to express chimeric antigen receptors (CAR). CARs are synthetic fusion proteins that comprise of an extracellular antigen-targeting domain, hinge and transmembrane domains, one or more intracellular costimulatory domains and a TCR-derived intracellular signaling domain. We have demonstrated that CAR-Tregs are superior compared to polyclonal Tregs for preventing allograft rejection. All this work has led to the start of a new company (Quell Therapeutics) that is aiming to use genetically manipulated Tregs in the treatment of transplant patients and patients with autoimmune diseases.

More recently, we have been addressing a series of questions to inform the future of Treg therapy. One such question is where Tregs traffic to and localize, and this has been addressed using imaging modalities such as SPECT/CT. Another question is whether combining Tregs with other strategies, such as low dose IL-2, inhibition of innate immune responses or depletion of B cells, can further increase the likelihood of achieving transplantation tolerance by improving Treg survival, function, and stability.

Keywords: Regulatory T cells, transplantation tolerance, clinical trials

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MAIN SYMPOSIA AND SYMPOSIA

S3.4 - Cell Signaling

The role of SLy proteins in immune responses

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The SLy/SASH1-adaptor family consists of three highly homologous proteins SLy1/SASH3 (SH3 protein expressed in lymphocytes 1), SLy2/HACS1 (hematopoietic adaptor containing SH3 and SAM domains 1), and SASH1/SLy3 (SAM and SH3 domain containing 1). They all share conserved structural motifs, such as a bipartite nuclear localization signal, a SH3 and a SAM domain. Despite their pronounced structural homology and sequence similarity, the three SLy/SASH1-members fundamentally differ with regard to their expression and function in intracellular signaling, with all three proteins playing an important role in human health and disease. SLy1 plays a crucial role in immune cell development and function. Mice expressing a truncated form of SLy1 ($SLy1^{d/d}$) and SLy1-deficient ($SLy1^{Ko}$) mice show dramatically reduced sizes of various lymphoid organs such as thymus, spleen, lymph nodes and Peyer's patches. In addition, B and T cell responses, such as T-cell-dependent and -independent antibody and cytokine production, respectively, were impaired. During thymocyte development, SLy1 integrates Notch and preTCR signals. Our latest results demonstrate that SLy1 might be also involved in the IL-7 receptor signaling. In peripheral T cells, classical TCR signaling is not altered in SLy1-deficient T cells. Instead, SLy1 is part of a complex, mediating Foxo1-phosphorylation and inactivation by nuclear export as a response to TCR stimulation, most likely via a 14-3-3 interaction. In its absence, premature reactivation of Foxo1 and thus expression of cell cycle inhibitors p130 and p27 prevents the effector T cells of proper expansion in response to *Listeria monocytogenes* infection. In addition to B and T lymphocytes, the adaptor protein SLy1 is also expressed in Natural Killer (NK) cells. We found, that $SLy1^{Ko}$ mice had fewer numbers of NK cells, with reduced degranulation activity and IFN-$_\gamma$ production, consequently decreased *in vitro* clearance of NK cell targets, and higher susceptibility to Lung Lewis carcinoma. Mass spectroscopy revealed an increase in multiple free ribosomal proteins in $SLy1^{Ko}$ NK cells, including small ribosomal protein 3 (RPS3) and large ribosomal protein 5 (RPL5). This ribosomal stress leads to higher levels of unbound p53, indicating that the overabundance of RPL5 in $SLy1^{Ko}$ cells inhibits Mdm2-mediated clearance of p53. Hence, SLy1 crucially contributes to the maintenance of ribosomal stability in NK cells. SLy2 is relatively low expressed in naïve B cells, whereas its expression is highly upregulated in $B220^+$ murine splenocytes and $CD19^+$ peripheral human B cells upon *in vitro* treatment with stimulants such as IL-4 and CD40L via the PI3K-/ PKC-dependent signaling. Anti-IgM stimulation of B cells leads to endogenous association of SLy2 with several tyrosine-phosphorylated proteins, indicating a potential role of the adaptor downstream of the BCR. Specifically, SLy2 was shown to directly associate *via* the SH3-domain with the immunoreceptor tyrosine-based inhibition motif (ITIM) of the paired immunoglobulin-like receptor B (PIR-B), an inhibitor of the BCR-associated kinases Syk and Btk, which negatively affects B cell responses. $SLy2^{Ko}$ mice have normal numbers of B cellprogenitors in the BM as well as splenic mature and transitional B cell populations. Splenic B cells from $SLy2^{Ko}$ mice display enhanced proliferation towards B cell stimulants suchas IL-4, anti-IgM and CD40L. The natural B-1 cell compartment of $SLy2^{Ko}$ mice was altered with a significant increase in peritoneal B-1a cells and increased rates of BM-resident B-1bcells (Jaufmann et al., IID 2020). In line with that, we found significantly increased natural IgM levels in the serum of $SLy2^{Ko}$ mice. The antibody responses towards immunization with TI and TD antigens are enhanced in these mice. Elevated levels of *Pneumococcus*-specific IgM and IgG_2 antibodies point to a role of SLy2 for immune responses towards *Streptococcus pneumoniae*. However, increased levels of *S. pneumoniae*-specific antibodies in $SLy2^{Ko}$ mice were not sufficient to provide survival advantages in the course of acute pneumococcal pneumonia. The overexpression of SLy2 in lymphocytes impairs proper B-1 cell function by lowering the percentage of peritoneal B-1 cells and levels of global serum IgM. Upon immunization with pure pneumococcal vaccine, SLy2-Tg mice show significant deficits regarding their specific antibody responses. Moreover, the IL-5-dependent IgM production inpurified splenic B cells from SLy2-Tg mice is significantly decreased, accompanied by attenuated expression levels of IL5Rα on B-1a cells. IL-5/IL-5Rα-signaling is essential for survival, proliferation and differentiation of B-1 cells and is promoted by the transcription factor OCT2. Conclusively, SLy2 is involved in the regulation of both, innate and adaptive B cell responses towards B cell stimulatory signaling molecules and TI and TD antigens.

Keywords: SLy1/SASH1 adaptor proteins, signaling, adaptive immune responses, infection, cancer

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MS4.3 - New Technologies in Immunology Research

Decoding the developing human immune system

Muzlifah Haniffa[1]

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Muzlifah has used functional genomics, comparative biology and single cell RNA sequencing to study human mononuclear phagocytes. In this seminar she will demonstrate the applications of single cell genomics to decode the developing human immune system.

Keywords: Single cell RNA sequencing, single cell genomics, developing human immune system

Cell atlas technologies to decipher immunity

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Despite their crucial role in health and disease, our knowledge of immune cells within human tissues, in contrast to those circulating in the blood, remains limited. Here, we surveyed the immune compartment of lymphoid and non-lymphoid tissues of six deceased adult donors by single-cell RNA sequencing, including alpha beta T-cell receptor (αβ TCR), gamma delta (γδ) TCR and B-cell receptor (BCR) variable regions. To aid systematic cell type identification we developed CellTypist, a tool for automated and accurate cell type annotation. Using this approach combined with manual curation, we determined the tissue distribution of finely phenotyped immune cell types and cell states. This revealed tissue-specific features within cell subsets, such as a subtype of activated dendritic cells in the airways (expressing *CSF2RA, GPR157, CRLF2)*, *ITGAD*-expressing γδ T cells in spleen and liver, and *ITGAX+* splenic memory B cells. Single cell paired chain TCR analysis revealed cell type-specific biases in VDJ usage, and BCR analysis revealed characteristic patterns of somatic hypermutation and isotype usage in plasma and memory B cell subsets. In summary, our multi-tissue approach lays the foundation for identifying highly resolved immune cell types by leveraging a common reference dataset, tissue-integrated expression analysis and antigen receptor sequencing.

Keywords: Tissue immunity, immunogenomics, antigen receptor repertoire, scRNA-seq

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MAIN SYMPOSIA AND SYMPOSIA

Looking into the past and future of cells: single-cell analysis of epigenetic cell states in immunology and cancer

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Most diseases develop through the complex interplay of genetic and environmental influences, involving signaling pathways, metabolic changes, immune deregulation, and diverse cellular phenotypes. Our research is based on the hypothesis that the "epigenetic landscape" constitutes a highly informative intermediate layer of information processing that allows cells to maintain their regulatory state and cellular identity over time, while retaining the flexibility to respond swiftly to a broad range of perturbations. In our definition, the "epigenetic landscape" is not restricted to epigenetic marks such as DNA methylation and histone modifications. Rather, it reflects the full spectrum of transcription regulation by which cells translate various inputs into sustainable changes in their cell state. Notably, the epigenetic landscape not only reflects a cell's current state, but also its developmental history (e.g., cell-of-origin in cancer) and its potential for future adaptation (e.g., plasticity in response to an immunological challenge). I will present our work within and beyond the Human Cell Atlas, dissecting epigenetic cell states in immunology and cancer; and I will present methods for causal, mechanistic analysis at scale (CROP-seq and KPNNs) and forultra-high throughput transcriptome profiling in millions of single cells (scifi-RNA-seq).

Keywords: Medical Epigenomics, Bioinformatics & ML/AI, Single-cell Sequencing, Cancer Immunology, CRISPR Technology

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S4.1 - Visualizing Lymphocytes Signaling

Intracellular traffic of the linker for activation of t cells (LAT): role in T cell activation

Claire Hivroz[1], Andres Ernesto Zucchetti[1], Laurence Ardouin-Bataille[1], Juan-José Saez[1], Stéphanie Dogniaux[1]

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T-lymphocyte activation is induced by the cognate recognition by the T-cell receptor (TCR) of antigenic peptides presented by the MHC molecules (pMHC) express at the surface of antigen presenting cells (APC). This leads to the dynamic formation of a cognate contact between the T lymphocyte and the APC: the immune synapse (IS). Although the engagement of the TCR takes place at the plasma membrane, the TCR/CD3 complexes and the signaling molecules involved in transduction of the TCR signal are also present in intracellular organelles. These intracellular pools, both endocytic and exocytic, have a regulated intracellular traffic from and to the IS. Our team was among the first to analyze the traffic to the IS of a key molecule of the TCR-induced signaling: the linker for activation of T cells (LAT). LAT is a transmembrane protein that plays a key role in T lymphocyte signaling and function This transmembrane protein, phosphorylated on multiple tyrosines upon TCR activation, scaffolds numerous proteins involved in T lymphocyte activation, forming LAT signalosomes. LAT is present at the plasma membrane and more abundantly on intracellular membranes. The purification of LAT-containing vesicles performed by our team and their proteomic analysis revealed the presence of several molecules involved in membrane trafficking of cargo proteins. We will present the mechanisms involved in the intracellular traffic of LAT and their fine-tuning and discuss the potential functional role(s) of the different intracellular pools in T-cell activation.

Keywords: TCR signaling, immune synapse, LAT

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S4.2 - Cancer Immunotherapy

Local interventions with global impact: targeting tumour-draining lymph nodes

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Immune checkpoint blockade has changed the therapeutic landscape of oncology. Whereas CTLA4 blockade has generally been perceived to be effective in lymph nodes during the T cell priming phase, PD-1 blockade has been assumed to predominantly target interactions between PD-L1 expressing tumor cells and/or antigen-presenting cells and PD-1$^+$ effector T cells in the tumor microenvironment (TME). However, recent findings have shown tumor-draining lymph nodes (TDLN) to also be vital for the efficacy of PD-1 blockade. The importance of TDLN has been demonstrated by the observation that waves of tumor-specific, proliferating central memory and early effector T cells, newly primed or invigorated in TDLN, are vital for PD-1 blockade efficacy. Both tumor-derived migratory dendritic cell (DC) subsets and DC subsets residing in TDLN, and an interplay between them, have been implicated in the induction of these T cells, their homing imprinting, and their subsequent provision of loco-regional and distant tumor control. We propose that novel therapeutic approaches, involving local delivery of immune modulatory agents aimed at overcoming hampered DC and T cell activation in TDLN, will enable immune checkpoint blockade by promoting T cell recruitment to local as well as distant tumor sites. Comparative analyses of healthy, tumor positive, and tumor negative TDLN from melanoma, breast and cervical cancer patients revealed progressive suppression of the activation state of lymph node resident DC and allowed for further unravelling of immune suppressive mechanisms(involving amongst others Tregs and/or M2-like macrophages) that facilitate tumor invasion and spread. These observations prompted us to clinically explore targeted interventions to counteract suppression of resident DC and T cells in TDLN. Obtained clinical evidence now shows that local CpG-B mediated activation of resident DC or anti-CTLA4-mediated depletion of Tregs in melanoma TDLN can indeed overcome local immune suppression and boost systemic anti-tumor immunity. In the case of local CpG-B delivery this resulted in significantly prolonged recurrence-free survival of early-stage melanoma patients. Whereas local CTLA4 blockade in melanoma resulted in profoundly decreased activated Treg (aTreg) rates in both TDLN and peripheral blood, local PD-L1 blockade in cervical cancer resulted in increased Treg proliferation in TDLN and elevated systemic aTreg frequencies. Our findings make a strong case for the local immune modulation of TDLN to halt metastatic spread in early stages of cancer development and provide clues for more effective therapy combinations.

Keywords: Cancer, immune checkpoint blockade, CpG, tumor-draining lymph nodes, local immunotherapy

Reference:

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S4.3 - Emerging Tools in Immune Response

Integrated approaches to decipher the TCR signaling network

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The ligation of T-cell antigen receptor (TCR) triggers a multitude of intracellular molecular events that control T cell activation. By applying Mass spectrometry (MS)-based approaches to primary T cells, we identified and monitored the dynamics and stoichiometry of hundreds of molecular events associated with T cell activation. This approach allowed to refine the TCR signaling network and bring to light novel effectors that control the magnitude of T cell effector functions. By developing computational analyses, we unveiled signaling marks providing insights about the cellular programs engaged. These results lay the foundation for an integrated and comprehensive understanding of the molecular mechanisms that regulate T cell functions, a prerequisite for the design and production of drugs for therapeutic treatments.

Keywords: T cell signaling; TCR; mass spectrometry

Quantitative tools to investigate the interaction of colloidal nanoparticles with biology

Wolfgang J. Parak[1]

[1]*Universität Hamburg*

Colloidal nano- and microparticles can be used for investigating cellular signaling. By using analyte-sensitive fluorophores connected to colloids these analytes can be read-out in situ. By using plasmonic nanoparticles, encapsulated signaling molecules can be released.

Keyword: Effector molecules, phagocytosis, cellular interactions

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S4.4 - Perspectives in Single Cell Analysis

Deciphering the transcriptional and clonal heterogeneity of adaptive immune cells in inflammation

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The combination of traditional and cutting-edge single-cell technologies allow us to study the heterogeneity and clonality of adaptive immune cells at unprecedented resolution in the context of chronic inflammatory and infectious diseases as well as protective immune responses. These technologies provide us with the ability to understand the disease at the cellular level, to dissect the interaction of different immune cells with each other and, through their transcriptomes, to additionally get an idea about the microenvironment at the site of the immune response. As an example, I would like to share a study that we did in the context of severe COVID-19. Using flow cytometric techniques, we enriched specific immune cells from peripheral blood and inflamed tissues of severe COVID-19 patients and analyzed their transcriptomes and immune receptor repertoires at the single cell level. In this way, by zooming in on specific cell populations, we were able to identify the role of TGF-β in the pathogenesis of severe COVID-19. TGF-β induces a chronic inflammatory response that leads to sustained production of antibodies that are no longer directed against SARS-CoV2. Moreover, early after infection, elevated TGF-β levels cause a defect in the cytotoxic function of cells necessary for viral clearance. This functional defect can be reversed by neutralizing TGF-β in the serum of COVID-19 patients. In conclusion, single cell sequencing of flow cytometrically highly enriched immune cells represents a new strategy to uncover new pathomechansisms that could not only be used for stratification but also lead to the development of novel therapies for patients with inflammatory conditions.

Keywords: Single cell sequencing, immune receptor repertoire, COVID-19, T and B cell interaction, flow cytometry

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JOINT SYMPOSIA

JS.1 - Immunogenetics of Cancer

Immunogenetics of the MHC and NK cell receptors

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Natural Killer cells are able to respond rapidly to infection, cancer and other forms of cell stress by producing cytokines and killing infected target cells. Their deployment for cancer therapy is showing promise, at least for hematopoietic malignancies, but with limited success so far for solid tumors. NK cells have other roles in secreting growth factors, in the uterus for example. Their regulation is strictly controlled by arrays of both activating and inhibitory surface receptors. MHC class I molecules are central to the development and function of NK cells. Receptors of two main types, lectin-like (CD94:NKG2) and Ig-like (KIR), on the surface of NK cells, detect perturbation of MHC class I in response to pathological changes. MHC class I molecules are polymorphic and NK cells undergo a process of education to calibrate their response to the normal level of MHC in the individual's tissues. The necessity of responding to the threat of continually evolving pathogens, in relation to polymorphic MHC class I molecules, has spurred extreme complexity in the arrangement and variation in NK receptors. We have been studying variation in KIR receptors encoded in the leukocyte receptor complex (LRC) on chromosome 19q. By analyzing the genetics of KIR at the nucleotide level we are probing how variation in KIR is restrained by the need to balance resistance to infection with regulating the other activities of NK cells.

Keywords: NK receptors, KIR, CD94/NKG2, immunogenetics

JS.2 - Immunosensing and Antiviral Immunity

Metagenome data on intestinal phage-bacteria associations aids the development of phage therapy against pathobionts

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Our intestinal tract harbors a large number of bacteria in its lumen as commensal microorganisms. Traditionally, it has been thought that the beneficial indigenous bacteria in our intestines help our digestion and peristalsis, while putrefactive bacteria produce harmful substances and promote aging and cancer. However, recent studies have shown that dysbiosis, an abnormality in the composition of the gut microbiota, is found in a variety of diseases ranging from infectious diseases, inflammatory bowel disease, obesity, diabetes, and even mental disorders, and has a significant impact on our health. In fact, the number of cells and genes in the intestinal microbiota far exceeds that of the human host, and it has become impossible to ignore not only the influence of host genes but also the influence of the indigenous bacterial flora when considering the state of health and disease. The development of next-generation sequencers has been a major turning point in the study of intestinal microflora, and this has led to dramatic changes in the study of intestinal microflora. In the classical analysis, each intestinal bacterium was cultured one by one, and the sequence was confirmed. This required the establishment of a culture method for difficult anaerobic bacteria, which was extremely time-consuming and labor-intensive, and limited the analysis. However, with the development of next-generation sequencers, it has become possible to extract the entire genome from feces and perform comprehensive sequencing, thereby enabling the immediate detection of the bacteria present. These analyses have revealed not only abnormalities in the composition of intestinal bacteria in diseases, but also many reports of pathobionts, which are pathogenic factors directly involved in the development of obesity, diabetes, and cancer. As a new way to control diseases, methods to correct dysbiosis and to specifically control and eliminate pathobionts are required. In our laboratory, we are conducting metagenomic analysis by whole genome sequencing. We have constructed a pipeline for ultra-fast metagenomic analysis by using homology search software driven on a supercomputer, which enables high-speed analysis. In this talk, I will give an overview of the ultra-fast pipeline and introduce the analysis of intestinal bacteria and virus flora using the pipeline. In addition, we will report on the development of a therapeutic platform for the correction of dysbiosis and the specific elimination of pathobiont.

Keywords: Dysbiosis, metagenome analysis, bacteriophage

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Antiviral innate immunity targeting RNA

Osamu Takeuchi[1]

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Antiviral immunity is induced by the recognition of virus-specific molecular patterns by host pattern-recognition receptors (PRRs). Among them, RIG-I-like receptors and Toll-like receptors sense viral nucleic acids like double-stranded (ds)RNA and initiate signaling pathways leading to the production of type I interferons and proinflammatory cytokines. A set of interferon-inducible proteins function as the effector to eliminate viruses. N4BP1 is one of an interferon-inducible protein in immune cells. We identified N4BP1 as an endoribonuclease critical for the restriction of HIV-1 infection in T cells and macrophages. N4BP1 inhibits the growth of HIV-1 and other lentiviruses by degrading viral RNAs. Although the expression of N4BP1 is induced in response to HIV-1 infection, the activation of T cell receptor (TCR) signaling results in the cleavage of N4BP1 by a MALT1 protease at R509, which inactivates N4BP1. N4BP1 expressed in HIV-1 latently infected cells undergoes MALT1-mediated cleavage in the cause of reactivation of HIV-1. The HIV-1 latent cells expressing R509A mutant N4BP1 are resistant to reactivation induced by TCR stimulation, indicating that MALT1-mediated degradation of N4BP1 is involved in the reactivation of HIV-1 latent cells. Thus, N4BP1-mediated viral RNA regulation is essential for the control of immune reactions to pathogen infection at the levels of direct control of foreign RNAs. The RNA decay system also controls host inflammatory responses by degrading mRNAs involved in inflammatory responses. Indeed, N4BP1 is also involved in the control of host innate immune responses. Besides, immune-related mRNAs are also known to be degraded by an RNase Regnase-1 downstream of the PRR signaling. Regnase-1 recognizes stem-loop structures in 3′ untranslated regions of cytokine mRNAs for degradation and is critical for preventing aberrant tissue damage and autoimmunity. These studies demonstrate that RNA decay machineries are critical for the control of both antiviral immunity and the prevention of dysregulated immune responses.

Keywords: Anti-viral immunity, RNA decay, posttranscriptional regulation, cytokine, interferon

JS.4 - Single Cell Rheumatology

Fibroblast subsets in arthritis

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The synovium is a thin mesenchymal membrane encapsulating the joint space and is the major site of pathology in rheumatoid arthritis. Synovial fibroblasts comprise a key cell type in the hyperplastic pannus that invades and destroys cartilage and bone via their production of matrix degrading enzymes. However, they are also major contributors to inflammation by providing an amplificatory loop that drives the production of cytokines such as IL-6. Until now, functional subclasses of fibroblasts have proven difficult to define, characterize and study in health and disease. In contrast the identification of leucocyte subsets with non-overlapping effector functions provided a molecular framework for the development of targeted therapies that have demonstrated spectacular success in immune-mediated inflammatory diseases (IMIDs). Furthermore, it remains unknown whether fibroblast mediated inflammation and tissue damage always coupled, reflecting cellular plasticity residing within a single fibroblast population or instead, are uncoupled and mediated by different subsets of fibroblasts In this lecture I will explain the interrelationships between synovial fibroblast subsets in the lining and sub-lining layers of the synovium and observe how selective deletion of these subsets or changes in their biology alter the balance between persistent inflammation and tissue damage during the development of arthritis. Next, I will describethe functional relationships between alterations in fibroblast subsets and disease outcome during the development of human rheumatoid arthritis. Finally, I will speculate on how clinical trials targeting fibroblasts in patients with IMIDs might be delivered given these new findings.

Keywords: Fibroblasts, arthritis, synovial membrane

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JOINT SYMPOSIA

Coexistence of synovial T lymphocytes driving and regulating chronic inflammation in juvenile idiopathic arthritis

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T lymphocytes accumulate in inflamed joints of patients with juvenile idiopathic arthritis (JIA). In addition, a genetic linkage of JIA to MHC genes suggest that T lymphocytes might mediate the pathogenesis of this disease. But their role in established disease is less clear. We aimed to define the transcriptional and clonal identity of autoreactive memory T cells in patients with JIA. We isolated paired samples of antigen experienced conventional $CD4^+CD45RO^+CD25^{lo}$ T helper memory cells (T_{cons}), regulatory $CD4^+CD45RO^+CD127^{lo}CD25^{hi}$ T memory cells (T_{regs}) and cytotoxic $CD8^+CD45RO^+$ T memory cells (CTLs) by flow cytometry from the synovial fluid (SF) and the blood of seven patients with JIA. Subsequently, we performed single-cell sequencing combined with T cell receptor (TCR) sequencing on 74.891 cells to dissect their cell heterogeneity due to their transcriptional profiles and clonal repertoire. We then performed shared nearest neighbor-clustering using dimensional reduction analysis by t-distributed stochastic neighbor embedding (t-SNE). Our data reveal transcriptional heterogeneity among the different subsets of T memory cells both in peripheral blood as well as in cells derived from inflammatory tissues. TCR sequencing and gene expression of TCR signaling-induced genes enabled us to distinguish autoreactive from bystander memory T cells. Gene expression profiles of expanded recently activated clonotypes showed elevated expression of *PDCD1*(encoding for PD-1) compared to non-enriched bystander T helper memory cells from the inflamed tissue. A PD-1^+*TOX*$^+$*EOMES*$^+$population of $CD4^+$T lymphocytes expressed immune regulatory genes and genes attracting myeloid cells. A PD-1^+*TOX*$^+$*BHLHE40*$^+$population of $CD4^+$, and a mirror population of $CD8^+$ T lymphocytes expressed genes driving inflammation as well as genes supporting B lymphocyte activation. This dichotomy among *PDCD1*-expressing cells represents a general, lineage-transcending signature of memory T lymphocytes in chronic inflammation, since both $CD4^+$and $CD8^+$ T memory cells possess analogous populations. Finally, we identified autoreactive T lymphocyte clones and transcriptional signatures of recirculating SF-derived cells in the blood of JIA patients. Taken together, these results might offer a basis for developing diagnostic and therapeutic strategies for patients with JIA i), by developing biomarkers on the basis of recirculating autoreactive memory T cells and ii), by treating patients with agents to selectively deplete memory T cells driving pathology in chronic inflammation.

Keywords: chronic inflammation, juvenile idiopathic arthritis, T cells, single cell RNA sequencing, T cell receptor repertoire, inflamed tissues

Reference:

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JS.5 - The Immune Challenge of COVID-19 Infection for Allergic Patients

Biologicals for allergic diseases and asthma, COVID-19 infection and COVID-19 vaccines

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Immune modulation is a key therapeutic tool for allergic diseases and asthma. It can be achieved in an antigen-specific way via allergen immunotherapy or in endotype-driven approach using biologicals that target the major pathways of the type 2 (T2) immune response: IgE, IL-5 and IL-4/IL-13. COVID-19 vaccine provides an excellent opportunity to tackle the global pandemics and is currently being applied in an accelerated rhythm worldwide. It works as well through immune modulation. Thus, as there is an obvious interference between these treatment modalities recommendations on how they should be applied in sequence are expected. The European Academy of Allergy and Clinical Immunology evaluated the evidence and formulated recommendation on the administration of biologicals for allergic diseases and asthma during the pandemics and for the COVID-19 vaccine in patients with allergic diseases and asthma receiving biologicals. The panel also formulated recommendations for COVID-19 vaccine in association with biologicals targeting the type 1 or type 3 immune response. In formulating recommendations, the panel evaluated the mechanisms of COVID-19 infection, of COVID-19 vaccine, of biologicals and considered the data published for other anti-infectious vaccines administered concurrently with biologicals.

Keywords: Asthma; allergic diseases, biologicals; COVID-19; immune mechanisms

Allergen immunotherapy, COVID-19 infection and COVID-19 vaccines

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Immune modulation is a key therapeutic tool for allergic diseases and asthma. It can be achieved in an antigen-specific way via allergen immunotherapy (AIT) or in an endotype-driven approach using biologicals that target the major pathways of type 2 (T2) immune response: IgE, IL-5 and IL-4/IL-13. Allergen immunotherapy (AIT) is an intervention for allergic diseases and asthma-inducing tolerance to the allergen responsible for eliciting the symptoms. By continuous administration of a high number of relevant allergen(s), a tolerogenic immune response is generated. Main mechanisms involve early effector cell desensitization and progressive onset of a regulatory B and T cell response followed by significant decreases in allergen-specific Type 2 especially Th2 cells and Type 2 ILCs in circulation and the affected tissue. Although AIT induced changes are antigen-specific, recent data support a positive effect in the overall rebalance of Th2 skewed innate immune system. COVID-19 does not considerably increase in severity in allergic disease, with conditions such as rhinitis, urticaria, and atopic dermatitis or even asthma, if controlled under background treatment. The immunological mechanisms of the AIT and COVID-19 vaccine do not seem to interfere as both primarily target the immune system in a specific, non-overlapping manner. The effect of AIT on the effector cell desensitization, especially mast cell desensitization is rather limited, antigen/allergen-specific and occurs early during AIT. However, mast cells are not considered to be relevant for antiviral immune response. COVID-19 vaccine provides an excellent opportunity to tackle global pandemics and is currently being applied in an accelerated rhythm worldwide. It works as well through immune modulation. Thus, as there is an obvious interference between these treatment modalities recommendations on how they should be applied in sequence are expected. The EAACI recommendations are based on the mechanistic evaluation as well as clinical experience and evidence involving other anti-infective vaccines. The current assessment does not suggest any relevant interference compromising neither the safety nor the efficacy of AIT, biologicals or COVID-19 vaccines. Further evidence from disease registries and other real-world databases must be accumulated in order to refine current recommendations.

Keywords: Allergy, allergen immunotherapy, COVID-19 infection, COVID-19 vaccines

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JOINT SYMPOSIA

JS.7 - Human Inborn Errors of Major Pathways in Immunology: Experiments of Nature

Human type I interferonopathies

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Recognition of foreign nucleic acids represents the primary mechanism by which a type I interferon mediated antiviral response is triggered. Given that our own cells are replete with DNA and RNA, such an evolutionary strategy poses an inherent biological challenge i.e., the fundamental requirement to reliably differentiate self-nucleic acids from non-self. We suggest that the group of Mendelian inborn errors of immunity referred to as the type I interferonopathies relate to a breakdown of self and non-self-discrimination, with the associated mutant genotypes involving molecules playing direct or indirect roles in nucleic acid signaling. This perspective begs the question as to the sources of self-derived nucleic acid that drive an inappropriate immune response, the answers to which will provide fundamental insights into immune tolerance and suggests the possibility to use directed therapies.

Keywords: Interferon, interferonopathy, mendelian

JS.8 - Early Stage Career Russian Scientists Joint Session

Analysing the molecular IgE reactivity profile of russia by microarray technology

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Molecular allergology utilizes allergen molecules for improving the diagnosis of allergy and serves as a basis for the generation of new molecular allergy vaccines. Our study is the detailed analysis of molecular IgE sensitization profiles in a cohort of Russian children using micro-array analysis. There were two groups of age and gender-matched children aged 10-16 years old: group 1 (n=103); with allergy symptoms and group 2 (n=97) without symptoms of allergy according to international ISAAC questionnaire. Children were analysed regarding symptoms of allergy (rhinitis, asthma, atopic dermatitis, food allergy) according to international guidelines and skin prick testing with a panel of aeroallergen extracts. Then sera samples were analyzed in an investigator- blinded manner for IgE specific to more than 160 micro-arrayed allergen molecules using MeDALL allergen-chips which had been developed based on the ImmunoCAP ISAC technology (Thermofisher, Phadia, Uppsala, Sweden). IgE sensitization >0.3 ISU to at least one of the micro-arrayed allergen molecules was found in 100% of the symptomatic children and in more than 30% of the asymptomatic children. Symptomatic and asymptomatic children showed a comparable IgE sensitization profile. Frequencies of IgE sensitization and IgE levels to the individual allergen molecules were higher in the symptomatic children. Sensitization to aeroallergens were dominated by two major allergen molecules, major birch pollen allergen Bet v 1 and the major cat allergen Fel d 1. In the symptomatic children the following allergen molecules which are indicative for certain allergen sources were next in the hierarchy: Major timothy grass pollen allergen Phl p 1, major dog allergen Can f 1, major cypress allergen Cup a 1, major mugwort pollen allergen Art v 1, major plane tree pollen allergen Pla a 2, major cedar pollen allergen Cry j 1, major ragweed allergen Amb a 1 and major house dust mite allergen Der p 2. This study analyzed molecular IgE sensitization profiles n children with and without symptoms of allergy. It detects similar molecular IgE sensitization profiles in symptomatic and asymptomatic children, identifies Bet v 1 and Fel d 1 as the predominant respiratory allergen molecules, PR10 proteins as the major food allergens in Moscow region (Russia).

This work is supported by a Megagrant of the Government of the Russian Federation, grant number 14.W03.31.0024 and by project F4605 from the Austrian Science Fund (FWF).

Keywords: Allergy, component-resolved diagnosis, pollen sensitization, food sensitization, microarray

Engineering of a molecular vaccine for treatment of cat and dog allergy

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Exposure to allergens is an important risk factor for sensitization and respiratory allergic diseases such as asthma and rhinoconjunctivitis. Allergies to furry animals, particularly cats and dogs, have been considered a major risk factor for asthma and rhinitis for many years. The most important cat allergen is Fel d 1, which has been extensively studied regarding its IgE prevalence in different atopic populations. But with the exception of Fel d 1, clinical relevance of other seven cat allergens is not fully clear. We aimed to produce the full panel of cat allergens and test them for their IgE reactivity and allergenic activity in cat allergic patients with different symptoms. Recombinant allergens Fel d 1, Fel d 3, Fel d 4, Fel d 7 and Fel d 8 were expressed and purified from *E. coli* and Fel d 2 and Fel d 6 were obtained as natural purified proteins. Upon biotinilation, 7 cat allergens were bound to straptavidin CAPs and used for IgE testing by ImmunoCAP in cat allergic patients with different symptoms. IgE prevalence in Russian cohorts were as follows: Fel d 1 97%; Fel d 2 30%; Fel d 3 51%; Fel d 4 52%; Fel d 6 33%; Fel d 7 55% and Fel d 8 42%. IgE levels against Fel d 1 correlated highly with IgE against cat extract, but addition of Fel d 4 and Fel d 7 increased this correlation. Regarding allergenic activity, Fel d 1 was dominant allergen but closely followed by Fel d 7 and Fel d 4. Fel d 3 and Fel d 8 also showed high potency even with low IgE levels and low allergen concentrations while Fel d 6 was potent only in higher concentrations. The correlation between the IgE levels to the cat extract and the sum of IgE levels of the sum Fel d 1 - fel d 8 was found. The results showed that sIgE levels for all allergic components depending on symptoms, Fel d 1, Fel d 4 and Fel d 7 are more common in all cases of symptoms and have higher levels of special IgE. It has also been shown that a greater number of symptoms are characterized by sensitization to more allergens and a higher level of sIgE, and the sum of allergic components demonstrates greater sensitivity in diagnosis than cat extract. Although Fel d 1 was the most commonly recognized and biologically most potent cat allergen, other cat allergens like Fel d 4 and Fel d 7 are of high clinical relevance. We found that patients with the most symptoms were sensitized to more cat allergens and had higher sIgE levels. Also, the assessment of the allergy profile for all cat allergens turned out to be more sensitive than for the cat extract.

Keywords: Cat allergy, cat allergens, IgE reactivity, allergy diagnosis, component resolved diagnosis, allergenic activity

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JOINT SYMPOSIA

The role of TH1 and TH2 immunity in rhinovirus infections

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Virus-induced bronchial asthma (BA) exacerbations are a major cause of BA morbidity and mortality. Mechanisms involved are poorly understood. One of a current paradigms is that type 1 and type 2 cytokines polarize alveolar macrophages (Mϕ) into an M1 (classically activated) and M2 (alternatively activated) phenotype respectively and that M2 are increased in the lung of asthmatics. In this study, we aimed (i) to investigate the surface molecule expression of *in vitro* differentiated human M1 and M2-like Mϕ for the identification of subtype-specific markers (ii) to assess Mϕ phenotypes in healthy subjects and people with asthma before and during experimental rhinovirus (RV16) infection *in vivo*. For experiments *in vitro* PBMCs were isolated from blood by Ficoll-Hypaque density gradient centrifugation. The cells were washed, resuspended in Macrophage Serum Free media (Invitrogen) and seeded at 30 × 10^6 cells/dish. The cells were differentiated for 7 days in media containing 10 ng/mL of GM-CSF and penicillin/streptomycin mixture (both from Invitrogen). The mature monocyte-derived macrophages (MDM) were then stimulated overnight with either 2 ng/mL of TNF-α plus 20 ng/mL of IFN-γ (both from R&D Systems) or with 20 ng/mL of IL-4 (Invitrogen) to obtain MDM/TNF/IFN-ψ (M1-like) and MDM/IL4 (M2-like) cells, respectively. Un-polarized control MDM were maintained in culture overnight in MSFM alone. Polarized or un-polarized MDM were then treated with infectious RV16 MOI of 0.001. For experiments *in vivo* with clinical samples RV16 experimental infections were successfully induced in RV16 neutralizing antibody seronegative subjects with moderate (n = 17) and mild (n = 11) atopic asthma and 11 non-atopic age-matched healthy control subjects. Ethics approval was obtained from the local research ethics committee and informed consent was obtained from all subjects. We chose several widely reported M1 (CD14, CD80, CD54 and CD197) and M2 markers (CD36, CD206 and HLA-DR) and quantified their expression *in vitro* polarized MDM and BAL cells from clinical samples by flow cytometry before and after infection with RV16. According to data obtained by flow cytometry we identify M1-like MDM/TNF/IFNg as CD14+CD80+CD197+ cells and analyzed different subsets of Mϕ in this manner. We found that infected and uninfected MDM/TNF/IFN-ψ were characterized by high percentage (10.93±2.179 [media] and 14.29±2.616% [RV16]) of CD14+CD80+CD197+ cells compared to both uninfected MDM and MDM/IL4 (3.82±0.881, 5.298±1.018%, respectively) and infected MDM or MDM/IL4 (4.270±1.082, 4.858±1.081%, respectively) (P = 0.041 to P<0.0001). Next, we analyzed BAL Mϕ to evaluate the CD14+CD80+CD197+ M1-like population. As a result, we found a tendency to increased frequencies of M1-like Mϕ in healthy subjects on day 4 post infection compared to baseline (4.531±1.413, 2.236±0.036%, respectively; P = 0.0655) and a significant decrease of this cell type in the all asthma group on day 4 post infection compared to baseline (3.561±1.589 and 7.396±2.327% respectively, P = 0.0242) and in the moderate asthma group on day 4 post infection compared to baseline (1.867±0.7313 and 5.528±1.099% respectively, P = 0.0157). Furthermore, we assessed changes between baseline and day 4 post experimental infection and found a tendency to decreased frequencies of the CD14+CD80+CD197+ cells in all asthma group −3.977±4.464% (P = 0.0556) and significant decrease of this cell type in the moderate asthma group −3.612±1.788% (P = 0.0205) compared to healthy controls 2.729±1.832%. We found that M1-like MDM/TNF/IFNψ had higher levels of expression of CD14, CD54, CD80 and CD197 compared with MDM/lL4 and RV infection of MDM/TNF/IFNψ did not significantly change their surface levels. At the same time, we found no significant difference in expression of the widely used M2 markers CD206, CD36 and HLA-DR on the surface of human MDM differentiated with GM-CSF and polarized by IL-4 *in vitro*. Then we analyzed BAL cells to identify CD14+CD80+CD197+ positive cells as an M1-like population. We found reductions in numbers of CD14+CD80+CD197+ M1-like Mϕ in asthma patients during virus induced exacerbation compared to baseline. This data suggest that Mϕ polarization is a dynamic process dependent on lung microenvironment and rhinovirus-induced asthma exacerbations in allergic patients is characterized by an amplified Th2 immune response.

Keywords: Macrophage, polarization, rhinovirus, asthma, exacerbation

Acknowledgments: Supported by RSF 19-15-00272.

Tracing cells producing allergen-specific IgE in allergic patients

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The identification of IgE-producing cells in allergic patients is necessary for understanding the mechanisms allergen-specific IgE response to discover new targets for allergy treatment. Allergy to birch pollen is one of the most common form of allergy in the Moscow region of Russia. Due to the high prevalence of birch trees, there is a strong exposure of birch pollen in this region from the beginning of April to the end of May. However, often an allergy to birch pollen is accompanied not only by a reaction to homologous PR-10 allergens, but also to other major allergens. In our study, we developed special flow cytometry approach that makes possible to evaluate the appearance of IgE-positive cells in PR-10 mono sensitized patients before and after the birch pollen season in Moscow. Peripheral blood mononuclear cells were isolated from whole blood by density-gradient centrifugation from 12 PR-10 mono sensitized allergic donors. The presence of PR-10 IgE-specific antibodies was confirmed by ImmunoCAP ISAC chip. IgE+ cells were identified by staining with CD19- Alexa488, Omalizumab-APC, Bet v 1 – PE, CD27-PE-Cy5.5, CD38-PE-Cy7, CD23-PerCP-Cy5.5 and IgD-APC-Cy7. The described staining conditions allowed us to detect an extremely small B lymphocyte subpopulation of IgE+ cells (approximately 0.35%, from B memory cells). In PR-10 allergic patients the identified B lymphocyte subpopulation was low before the birch pollen season and increased after the pollen season in June. For 2 donors Bet v 1 specific IgE+ cells were detected after the pollen season, but their percentage was extremelylow (0.05%, from B memory cells). Our results represent that continuous allergen exposure can provoke the increase of circulating IgE+ memory cells. In this case allergic response is similar to immune response after revaccination, when subjects who underwent vaccination and showed increases of the number of antigen-specific memory B cells indicating stimulation of resting memory cells. The increase of IgE+ B cell subpopulation was evaluated by using specially developed protocol. Our results revealed that circulating IgE+ B cells seems to be included in IgE production in allergic patients after allergen-specific stimulation. Further work will be dedicated to the study of the clonal diversity of IgE+ subset and the role in the secondary allergen-specific IgE response.

This study was supported by a Megagrant of the Government of the Russian Federation, grant number 14.W03.31.0024

Keywords: Pollen allergy, B-lymphocytes, Bet v 1 allergen

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JOINT SYMPOSIA

JS.10 - COVID Vaccines and Immunity

Similarities and differences of COVID-19 vaccines

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COVID-19 vaccines were developed with an unprecedented pace since the beginning of the pandemic. Several of them have reached market authorization and mass production, leading to global application on a large scale. This enormous progress was achieved with fundamentally different vaccine technologies used in parallel. mRNA, adenoviral vector as well as inactivated whole-virus vaccines are now in widespread use, and a subunit vaccine is in a final stage of authorization. All of these vaccines rely on the full-length viral spike protein of SARS-CoV-2 for inducing potently neutralizing antibodies, but the presentation of this key antigen to the immune system differs substantially between the different categories of vaccines. Even within the same category, variations exist that can have an impact on immune responses, protection, and side reactions. In this presentation, I will discuss distinguishing features of current COVID-19 vaccines, highlight their possible influences on protective antibody responses and adverse events, and provide an outlook on potential future developments.

Keywords: COVID-19 vaccines, spike protein, adenovector vaccines, mRNA vaccines, inactivated vaccines, subunit vaccines

JS.11 - Feto-maternal Immune Crosstalk

Innate immune cells in pregnancy

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The decidua is flooded with a variety of leukocytes, most of which are innate immune cells recruited at the implantation site by progesterone. Under the influence of trophoblastic molecules and decidual microenvironmental factors, they differentiate into cells having reduced inflammatory potential and orientation for immunosuppressive/immunomodulatory action. Decidual NK cells (dNK, CD56bright/CD16dim) are the predominant decidual cell population (60–90% of decidual immune cells) from the first stages of pregnancy through the first trimester. Simultaneously with blastocyst implantation, dNK cells become activated and regulate uterine vascular remodeling and trophoblast differentiation and invasion. Furthermore, dNK cell function results in placental augmentation and local immunomodulation. The interactions between the activating and inhibitory dNK receptors with their ligands on the trophoblast provide self-signals to either cytotoxic NK activation (Th1 response) or inhibition of activation and protection of the trophoblast (Th2 response). Among the different NK receptor interactions with their specific counterparts on the trophoblast, the interactions between receptors of the KIR family and their ligands HLA-C molecules appear to be those mainly involved in the function of an NK cell-mediated allorecognition system in pregnancy. Macrophages (M1 and M2 subsets) comprise 20-30% of all decidual leukocytes in early pregnancy. A balance between M1 macrophages promoting Th1 responses and M2 macrophages inducing tolerance is required during pregnancy. M1 macrophages prevail before blastocyst implantation and at the end of pregnancy, have a classical antigen presenting ability, participate in inflammatory clearance reactions of apoptotic and cellular fragments and may be involved in the process of parturition. M2 macrophages prevail after implantation and protect the fetus and placenta by having reduced antigen presenting capacity, producing anti-inflammatory cytokines and exerting immunosuppressive action Dendritic cells (dDC) make up »1% of all decidua's immune cells. The majority of them are immature with CD83-SIGN phenotype associated with reduced classical antigen presenting ability and important regulatory function. During implantation, they secrete IL-15 and recruit NK cells in the endometrium with which they work together to induce a tolerogenic environment, which is enhanced by the interaction of DCs with Treg to which they present allogeneic parental antigens. ψ6 T-lymphocytes consist of the majority of decidual T lymphocytes. They participate in mucosal defense by recognizing peptides and non-peptide antigens without MHC restriction. Most of them are activated, possibly due to the recognition of trophoblastic molecules preserved during evolution. They express receptors for progesterone and secrete PIBF (progesterone-induced blocking factor) that bocks cytotoxic reactions and induces a Th2 response. They carry -preferably - the δ1 TcR chain, which also directs to Th2 response. NKT cells ($CD3^+CD161^+$iNKT) consist 0.5% of decidual NK cells. They recognize glycolipid antigens presented by CD1d trophoblastic molecules. Depending on the glycopeptide they recognize, NKT cells secrete large amounts of either Th1 (IFN-γ) or Th2 (IL-4) cytokines, affecting the Th1/Th2 balance. Innate lymphoid cells (ILC-1, -2, -3) of maternal and fetal origin represent a complex network that play an important role in tissue remodeling, mucosal immunity, homeostasis and tolerance (maternal), fetal lymphatic system development, and defense against intra-amniotic infections (fetal). Disturbances in the functioning of decidual innate immune cells have been associated with pregnancy complications (spontaneous abortions, preeclampsia, fetal growth retardation, premature birth). Apart from their immunoregulatory effect for pregnancy, basic function of the innate cells is to alertly patrol the feto-maternal interface for the presence of endogenous or exogenous antigens, which may endanger pregnancy. Through pattern recognition receptors (PRRs), macrophages and DCs recognize "danger signals" associated with viral and bacterial infections or tissue damage and develop protective immune responses.

Keywords: Innate immune cells, decidual NK cells, pregnancy

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JS.12 - Translational Immunotherapy in Cancer

Tissue-resident macrophages provide a pro-tumorigenic niche to early NSCLC cells

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Macrophages (MF) play a key role in shaping the tumor microenvironment (TME), tumor immunity, and response to immunotherapy, making them an important target for cancer treatment. However, modulating MF has proved extremely difficult, as we still lack a complete understanding of the molecular and functional diversity of the tumor MF compartment. Macrophages arise from two distinct lineages. Tissue-resident MF (TRMF) self-renew locally independent of adult hematopoiesis, while short-lived monocyte-derived MF (Mo-MF) arise from adult hematopoietic stem cells (HSC), and accumulate mostly in inflamed lesions. How these MF lineages contribute to the TME and cancer progression remains unclear. To explore the diversity of the MF compartment in human non-small cell lung carcinoma lesions (NSCLC), we performed single cell RNA-sequencing (scRNAseq) of tumor-associated leukocytes. We identified distinct MF populations enriched in human and mouse lung tumors. Using lineage-tracing, we discovered that these MF populations differ in origin and have a distinct temporal and spatial distribution in the TME. TRMF accumulate close to tumor cells early during tumor formation to promote tumor cell epithelial-to-mesenchymal transition and invasiveness, while also inducing a potent T regulatory (Treg) response that protects tumor cells from adaptive immunity. TRMF depletion reduced Treg numbers and altered Treg phenotype, promoted the accumulation of $CD8^+$ T cells, and reduced tumor invasiveness and growth. During tumor growth, TRMF redistributed at the periphery of the TME, which becomes dominated by Mo-MF in both mouse and human NSCLC. This study identifies the contribution of TRMF to early lung cancer and establishes TRMF as an important target to prevent or treat early lung cancer lesions.

Keywords: Macrophages, NSCLC, EMT, Tregs

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JOINT SYMPOSIA

Antigen targeting to dendritic cells as a way to improve vaccine design

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Conventional dendritic cells (cDCs) are antigen-presenting cells essential for the activation of immune responses. In the last years, different antigens have been successfully targeted to cDCs *in vivo*. This is accomplished by the administration of monoclonal antibodies (mAbs) directed to a cDC endocytic receptor covalently or genetically linked to the antigen of interest. Two examples of such receptors are DEC205 and DCIR2 that are expressed in two different mouse cDCs subsets (cDC1s and cDC2s, respectively). The administration of the chimeric antibodies (anti-DEC205 or anti-DCIR2) in the presence of a cDC maturation stimulus elicits both cellular and humoral immune responses against the coupled antigen. Our group has successfully expressed chimeric anti-DEC205 and anti-DCIR2 mAbs fused to antigens derived from different pathogens, including *Plasmodium* spp, *Trypanosoma cruzi*, dengue virus 2 (DENV2), human immunodeficiency virus (HIV), and human papillomavirus (HPV). Our results show that protection, mediated by a pro-inflammatory Th1 response and by the activation of cytotoxic $CD8^+$ T cells, can be obtained against DENV2 and HPV-induced tumors, respectively, when the anti-DEC205 mAb is used to target cDC1s. On the other hand, the response induced by antigen targeting to cDC2s via DCIR2 induces Tfh cells, germinal center formation, and plasma cell differentiation[4]. A more complete understanding of the immune responses induced when the antigen is targeted to each cDC subset may help the design of more effective vaccines against different pathogens and cancer.

Keywords: antigen targeting, DEC205, DCIR2, dendritic cells, monoclonal antibodies

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The sweet revolution: glycans and glycan-binding proteins in tumor immunity, autoimmunity and inflammation

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The responsibility for deciphering the biological information encoded by the glycome- the whole repertoire of saccharides in cells and tissues- is assigned to endogenous glycan-binding proteins or lectins whose expression is regulated at sites of inflammation and tumor growth. With the overarching goal of identifying novel therapeutic targets, our laboratory investigates the molecular interactions between endogenous galectins and glycans leading to the control of immune and vascular signaling programs. In the past years, we have identified essential roles for galectin-1, a proto-type member of this family, in promoting immune escape in different tumor models and limiting autoimmune inflammation by selectively dampening Th1 and Th17 responses, instructing the differentiation of tolerogenic dendritic cells, promoting the expansion of regulatory T cells and favoring differentiation of alternatively activated ´M2-type´ macrophages. Moreover, we found that glycosylation-dependent interactions between galectin-1 and specific target N-glycans on VEGFR2 can link tumor hypoxia to angiogenesis and preserve vascularization in anti-VEGF refractory tumors. Targeted disruption of galectin-1-N-glycan lattices suppressed aberrant angiogenesis, enhanced tumor immunity in several models and contributed to eliminate resistance to anti-angiogenic and immunotherapeutic modalities. The clinical relevance of these findings as well as those involving other members of the family will be discussed. Thus, galectins may serve as regulatory checkpoints that translate 'sugar codes' into immune and vascular signaling programs in tumor and inflammatory microenvironments.

Keywords: Glycosylation, galectins, inflammation, cancer, autoimmunity

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BRIGHT SPARKS WORKSHOPS

TRACK 1 - CELLULAR IMMUNOLOGY

BS-001

The ability of extracellular vesicle-coating antibody light chains to bind antigenic peptides determines their immune suppressive activity

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The immune activating function of antibody light chains (LCs) has been widely studied, while very little is known on their putative down-regulatory action. Accordingly, we observed that CD8+ suppressor T cells antigen-specifically inhibit delayed-type hypersensitivity (DTH) reaction in mice by releasing miRNA-150-carrying extracellular vesicles (EVs) that are coated with antigen-specific LCs. Therefore, our research aimed at investigating the actual role of LCs in EV-mediated immune suppression. EV-producing suppressor T cells were induced by intravenous administration of a high dose of ovalbumin (OVA)-coupled syngeneic erythrocytes in wild type or immunoglobulin-deficient JH-/- mice. EVs from the latter mice were coated with LCs *in vitro*. The presence of LCs on EVs was confirmed cytometrically, and their antigen-binding ability was tested with ELISA. Finally, the regulatory activity of LC-coated EVs was assessed in an *in vivo* DTH assay. EVs from OVA-tolerized immunoglobulin-deficient mice failed to suppress OVA-induced DTH, unless coated with OVA-specific LCs that were able to bind to native OVA, OVA tryptic peptides, and OVA-323 peptide *in vitro*. Interestingly, preincubation of LC-coated EVs with OVA tryptic peptides blocked their suppressive activity. Criss-cross experiments proved that LCs ensure antigen-specificity of induced immune tolerance. These findings imply that EV-coating LCs specifically target antigen-presenting cells by binding antigenic peptides complexed with MHC molecules. Herein confirmed down-regulatory function of EV-coating antigen-specific LCs expresses great translational potential in designing nanovesicle carriers for specific targeting of desired cells.

Keywords: Allergen-induced immune responses, autoimmunity, cellular interactions, immune communication, immune regulation and therapy, macrophage

BS-002

Characterisation of neonatal innate lymphoid subsets reveals a novel precursor able to generate NKG2A-KIR+ NK cells

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Recent studies suggest that circulating innate lymphoid cells (ILCs) are functionally different from tissue-resident ILCs. We have conducted the first deep characterisation of human umbilical cord blood (CB) ILCs by phenotypic, functional, and transcriptomic analyses. We observed unique gene signatures for each ILC subset, but also nine shared genes including ID3. CB ILCs showed an unusual ID3/ID2 ratio > 1 that was otherwise only seen in CB CD4+ T-cells, but not ILCs or T-cells from PB or other tissues, suggesting a close relationship between CB ILCs and T cells. In line with this, we observed expression of T-cell associated molecules such as CD28, CD5, and CD6 on CB ILC1-like cells. Unlike tissue ILC1, we did not observe effector functions such as IFNγ production. Notably, ILC1-like cells significantly decreased in frequency and total cell count during gestational ageing. Together with the expression of CCR7, CCR4, and CCR9 these observations suggest that ILC1-like cells are only transiently present in the periphery and continue to migrate into organs. Importantly, we show that CB ILC1-like cells are a novel type of CD117- NK cell progenitor that efficiently differentiates on the clonal and bulk level to mature cytotoxic NKG2A-KIR+ NK cells exhibiting a broad KIR repertoire. As CB ILC1-like cells are to our knowledge the first precursor able to efficiently differentiate into mature NKG2A-KIR+ NK cells, these findings provide novel insights into the mechanisms of NK cell maturation and NK cell receptor repertoire formation and may constitute novel aspect for future therapeutic applications.

Keywords: Immune development, innate lymphoid cells, NK cells

BS-003

A type 3 immunity (RORγt+) intrinsic PPARγ regulation contributes to diet-induced obesity

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Recent data indicate that metabolic regulators, such as the nuclear receptor PPAR ("Peroxisome Proliferator Activated Receptor")-γ, directly impact the immune response. PPAR-γ, a key regulatory gene of adipogenesis, lipid and glucose metabolism, is also expressed by immune cells, such as macrophages and ILC2 cells, and is key to their function. Although PPAR-γ activation was described to suppress Th17 differentiation, its contribution to type 3 immunity remains largely unexplored. OBJECTIVE: Herein we aim to investigate the role of PPAR-γ in type 3 immunity (RORγt positive cells) during metaflammation. To study the functional role of PPAR-γ in RORγt positive cells, we conditionally generated PPAR-γfl/fl RORγtCre+ (Tg) and PPAR-γfl/fl (WT) mice that were fed a high fat diet (HFD), which resulted in obesity, insulin-resistance and chronic inflammation. Accumulation of Th17, ILC3 and Treg cells in visceral (VAT) and subcutaneous (SAT) adipose tissues was analyzed by flow cytometry. Our data demonstrate an intrinsic role of PPAR-γ in the RORγt positive compartment during HFD, as deficient mice were protected from weight gain and displayed a lower fasting glucose than control mice. This phenotype was associated with an alteration in the ratio between Th17, ILC3 and Treg cells consequent to an increase in Th17 and ILC3 cell numbers in VAT of mutant mice compared to control mice fed HFD. Metabolic sensing by PPAR-γ influences type 3 immunity by modulating the Th17 and ILC3/Treg balance during metabolic disease.

Keywords: Animal models, chronic inflammation and fibrosis, diabetes, innate immunity, metabolic control of immune responses, regulatory cells

BS-004

The glycolysis intermediate lactate is essential for the suppressive function of human thymus-derived regulatory T cells

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Regulatory T cells (Treg) impede anti-tumor immunity by suppressing the activation of conventional T cells (Tconv). We previously found that human, thymus-derived (t)Treg undergo a glycolytic switch upon tumor necrosis factor receptor (TNFR)-2 costimulation, and as opposed to Tconv, retain lactate intracellularly. AIM: To investigate the role of intracellular lactate retention for Treg suppressive function. Naïve Treg and Tconv were sorted from healthy donor buffy coats by flow cytometry and expanded for one week using CD3/28 agonistic antibodies in the presence of IL-2. Quiescent Tconv and Treg were restimulated by αCD3/28 or αCD3/TNFR2 for RNA sequencing, proteomics and metabolic tracing of ^{13}C-glucose by mass spectrometry. Treg were lentivirally transduced for overexpression of the monocarboxylate transporter (MCT)-4, followed by assessment of their phenotype and suppressive function in a suppression assay. ^{13}C-glucose tracing showed that glycolytic Treg favored net uptake of lactate, while Tconv secrete lactate. MCT1 predominantly mediates lactate influx, while MCT4 mainly mediates lactate efflux. Transcriptome and proteome analyses showed that glycolytic tTreg have higher expression of MCT1 and lower expression of MCT4 compared to glycolytic Tconv. Overexpression of MCT4 by lentiviral transduction of tTreg forced the release of lactate and impaired their suppressive function. MCT4 overexpression did not alter the expression of the key tTreg attributes FOXP3, IKZF2 or CTLA4. These data indicate that tTreg suppressive function is dependent on the glycolysis intermediate lactate. Further research to determine the fate of lactate in Treg should identify opportunities to selectively target these cells.

Keywords: Adaptive immunity, metabolic control of immune responses, regulatory cells

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BRIGHT SPARKS WORKSHOPS

BS-005

Maintenance of longlived memory plasma cells by contact to stromal cells through activation of PI3K/AKT and inactivation of FoxO1/3 prevents activation of caspase 3

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Longlived memory plasma cells secreting protective or pathogenic antibodies are maintained in the bone marrow individually, in close contact to mesenchymal stromal cells. We have established *ex vivo* cell culture conditions mimicking the bone marrow environment of plasma cells, providing them with integrin-mediated cell-contact to cells of a stromal cell line and with the cytokine APRIL. We have shown that both, cell contact and APRIL are required and sufficient to prevent caspase-mediated death of plasma cells. Persistence of plasma cells requires cell contact-induced PI3K signaling and inactivation of FoxO1/3, and prevents activation of caspases 3 and 7. APRIL signaling, via the NF-κB pathway, prevents activation of the endoplasmic stress-associated caspase 12. Thus stromal cells and APRIL ensure persistence of memory plasma cells by complementing each other in providing resilience of memory plasma cells to lethal mitochondrial and endoplasmic reticulum stress. Single cell transcriptomes of stromal cells and memory plasma cells reveal an unforeseen diversity of both, and point to distinct differences in the synapses of different memory plasma cell populations, differences which might impact on their longevity, and which might allow to target them differentially in vaccination or in the therapy of antibody-mediated diseases.

Keywords: Adaptive immunity, B lymphocytes, cell signalling

BS-006

Phenotypic and spatial characteristics of human innate lymphoid cells revealed by highly multiplexed histology

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Innate Lymphoid cells (ILCs) have emerged in the last few years as key players with a role not only in the regulation of immune responses, but also in broader biological processes. Helper ILCs are mainly known to be tissue resident cells, to be particularly abundant in barrier sites and to act as sensors for tissue integrity. In line with these functional characteristics, their microanatomical localization within and across tissues is of particular interest. However, little is known about the precise localization of human ILCs and their interactions with the microenvironment. Based on the tissue-associated features of ILCs, which demand for a histological approach to further understand ILC biology, we have applied a highly multiplexed immunofluorescence technique to several human tissues, allowing us to stain more than 50 markers in the same tissue section. We have combined the image acquisition system with a customized computational analysis pipeline, accessible through open-source software, to unambiguously identify CD127+ ILCs in situ, and characterize these cells and their microenvironments. Thereby, we could pinpoint new markers for human ILC characterization, such as the transcription factor IRF4 and identify stromal landmarks for ILC localization, which are conserved across inflamed tissues. Additionally, we found that CD127+ ILCs share tissue niches with plasma cells in the tonsil. Our work also serves as a resource for future multiparametric histological analysis of ILCs and other immune subsets that will improve our understanding of cell phenotypes in their context.

Keywords: Immunological techniques, innate lymphoid cells, microenvironment

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BRIGHT SPARKS WORKSHOPS

TRACK 2 - MOLECULAR IMMUNOLOGY

BS-007

SARS-CoV-2-induced immunity depends on cytosolic sensors

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Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and is characterized by strong induction of inflammatory cytokines and lung inflammation. Although ACE2 is the primary receptor for SARS-CoV-2 to infect cells, it is unclear what pattern recognition receptors (PRRs) sense SARS-CoV-2 to induce immune responses. Several studies suggest that the Spike (S) protein of SARS-CoV-2 might interact with Toll-like receptor 4 (TLR4) and thereby activateinnate and adaptive immunity. Here we have investigated the role of TLR4 in SARS-CoV-2 infection and immunity using a HEK293 cell line expressing TLR4 (HEK/TLR4) and primary monocyte-derived dendritic cells (DCs). In contrast to LPS stimulation, neither exposure of isolated S protein nor SARS-CoV-2 pseudovirus induced TLR4 activation in HEK/TLR4. Human monocyte-derived DCs express TLR4 but not ACE2. As expected, DCs were neither infected by pseudotyped nor a SARS-CoV-2 primary isolate (Italy variant). Neither pseudotyped nor primary SARS-CoV-2 induced DC maturation or cytokines, indicating that S protein does not trigger TLR4 signaling on DCs. Strikingly, exogenous expression of ACE2 in DCs led to productive infection of DCs as well as the induction of type I IFN and cytokine response. These data imply that intracellular viral sensors are key players in recognizing SARS-CoV-2 as opposed to transmembrane PRRs such as TLR2 and TLR4 or endosomal PRRs such as TLR7 and TLR8. Therefore, further studies to investigate the role of cytosolic PRRs in COVID-19- associated immune responses are required.

Keywords: Dendritic cells, innate host defence, innate immunity, viral infections

BS-008

The role of glucose transporter type 1 in plasma cells

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The formation of antibody-secreting plasma cells is an essential step of the adaptive immune response to control infections with unknown or re-encountered pathogens. To meet the enormous energy demand during antibody-production, abundance of nutrient transporters on the cell surface is increased, mediating a constant e.g. glucose uptake. However, it is still unknown if Glucose Transporter Type 1 (GLUT-1) is essential for the formation of antibody-secreting cells and if it enables plasma cells to acquire the necessary nutrients to produce correctly glycosylated and thereby functional antibodies. To address this question, we used mice with a B cell-specific GLUT-1-deficieny to determine the role of GLUT-1 in the formation and function of plasma cells. In non-immunized and immunized mice, we observed a drastic decline in serum antibody titers and antibody-secreting cell numbers in the spleen and bone marrow of GLUT-1-deficient animals, underlining the importance of GLUT-1 for generating functional antibody secreting cells. As revealed by liquid chromatography–mass spectrometry, serum IgG of GLUT-1-deficient mice showed a truncated glycosylation pattern, indicating the demand of a constant glucose uptake by plasma cells to secrete functional antibodies. These findings elucidate the importance of GLUT-1 for the establishment of long-lived plasma cells and the formation of correctly glycosylated antibodies, providing further insights into the complex mechanisms of adaptive immune responses. Further studies will show if GLUT-1 is also essential for plasma cell survival and the fine-tuning of metabolic processes in plasma cells. This work was supported by IZKF Erlangen (T.B.) and TRR130 (H.-M.J. and W.S.).

Keywords: Adaptive immunity, B lymphocytes, metabolic control of immune responses, molecular immunology

BS-009

Inhibition of drug-induced liver injury in mice using a positively-charged peptide that binds DNA

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Hepatic cell death occurs in response to diverse stimuli such as chemical and physical damage. The exposure of intracellular contents such as DNA during necrosis induces a severe inflammatory response that has yet to be fully explored therapeutically. Here, we sought means to neutralize the ability of extracellular DNA to induce deleterious tissue inflammation when drug-induced liver injury had already ensued. DNA exposure and inflammation were investigated *in vivo* in drug-induced liver injury using intravital microscopy. The necrotic DNA debris was studied in murine livers *in vivo* and in novel DNA debris models *in vitro* by using a positively-charged chemokine-derived peptide [MIG30; CXCL9(74-103)]. Acetaminophen (APAP)-induced liver necrosis was associated with massive DNA accumulation, production of CXC chemokines and neutrophil activation inside the injured tissue. The MIG30 peptide bound the healthy liver vasculature and, to a much greater extent, to DNA-rich necrotic tissue. Moreover, MIG30 bound extracellular DNA directly *in vivo* in a charge-dependent manner and independently of GAGs and chemokines. Post-treatment of mice with MIG30 reduced mortality, liver damage and inflammation significantly. These effects were not observed with a control peptide that does not bind DNA. Mechanistically, MIG30 inhibited the interaction between DNA and histones, and promoted the dissociation of histones from necrotic debris. MIG30 also inhibited the pro-inflammatory effect of CpG DNA, as measured by a reduction in CXCL8 production, indicating that MIG30 disturbs the ability of DNA to induce hepatic inflammation.

Keywords: Innate immunity, cell death, chemokines, neutrophils

BS-010

Effect of microRNA gga-let-7f on chicken macrophage functions in the context of antiviral responses

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Macrophages are the key players of the host innate immune responses during viral infections. They have several important functions, including phagocytosis and cytokine production, which are highly regulated by several host factors. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression of complementary messenger RNA sequences by base pairing, which often result in gene silencing. In this study, we aimed to identify the immunoregulatory roles of specific miRNAs on macrophages. In our previous study, we identified miRNAs with predicted antiviral properties. We hypothesized that one of these identified miRNAs, gga-let-7f, affects chicken macrophage functions. To this end, we transfected a chicken macrophage cell line with 5nM or 25nM of gga-let-7f miRNA mimics using a lipid-based transfection reagent. Following the treatments, transfection efficiency and cytotoxicity of the transfected miRNA were evaluated. Furthermore, the nitric oxide production of macrophages as an indicator of their activity was determined. In order to elucidate the function of the candidate miRNA in the induction of antiviral responses, the expression of some antiviral genes was evaluated. In addition, the impact of candidate miRNA on the replication of avian influenza virus replication in macrophages was determined. The results of this study demonstrated that gga-let-7f has direct effects on macrophage functions and highlighted its possible roles in antiviral response regulation.

Keywords: Macrophage, miRNA, viral infections

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BRIGHT SPARKS WORKSHOPS

BS-011

Distinct transcription factor networks control neutrophil-driven inflammation

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Neutrophils display distinct gene expression patters depending on their developmental stage, activation state and tissue microenvironment. To determine the transcription factor networks that shape these responses, we integrated transcriptional and chromatin analyses of neutrophils during acute inflammation. We show active chromatin remodelling at two transition stages: bone marrow-to-blood and blood-to-tissue. Analysis of differentially accessible regions revealed distinct sets of putative transcription factors associated with control of neutrophil inflammatory responses. Using *ex vivo* and *in vivo* approaches, we confirmed that RUNX1 and KLF6 modulate neutrophil maturation, whereas RELB, IRF5 and JUNB drive neutrophil effector responses, and RFX2 and RELB promote survival. Interfering with neutrophil activation by targeting one of these factors, JUNB, reduced pathological inflammation in a mouse model of myocardial infarction. Our study therefore represents a blueprint for transcriptional control of neutrophil responses in acute inflammation and opens possibilities for stage-specific therapeutic modulation of neutrophil function in disease.

Keywords: Epigenetic control and modulation of immunity, immune regulation and therapy, innate immunity, neutrophils

BS-012

Protection against severe lower respiratory infections in infants by Innate immune training: mechanism-of-action studies

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Birth cohort studies indicate that severe lower respiratory infections (sLRI) in infancy involve a combination of viral and bacterial pathogens. We previously demonstrated that treating infants with a microbial-derived immunomodulator provided significant protection against sLRI. We hypothesised that this protection was mediated through Innate Immune Training (IT) and the aim of this study is to identify the IT-treatment mechanism in high-risk infants. Pre- and post-treatment PBMC from a placebo-controlled trial in which winter treatment with an IT agent reduced infant respiratory infection frequency/duration, were stimulated for 24hrs with the viral/bacterial mimics PolyI:C/LPS. Transcriptomic profiling via RNASeq, pathways and upstream regulator analyses, and systems-level gene coexpression network analyses, were employed sequentially to elucidate and compare responses in treatment and placebo groups. In contrast to subtle changes in antiviral-associated PolyI:C response profiles, the bacterial LPS-triggered gene coexpression network responses exhibited Treatment-associated upregulation of interferon signalling accompanied by rewiring of master regulator IRF7, denoting enhanced innate antibacterial defences; segregation of TNF and IFNg (which potentially synergise to exaggerate Inflammatory sequelae) into separate/independently regulated expression modules; and reduction in size/complexity of the main pro-inflammatory network module (containing IL1/IL6/CCL3 etc.). At the protein level, LPS-induced cytokine production of IL6, Il10 and TNF were reduced in the Treatment group relative to Placebo. Treatment with this IT agent protects against sLRI by primarily modulating gene networks triggered during innate immune responses to bacterial pathogens which typically accompany viral pathogens during sLRI. These changes are consistent with enhanced bacterial pathogen detection/clearance capabilities accompanied by attenuation of potentially pathogenic inflammation.

Keywords: Immune networks, immune regulation and therapy, innate host defence, innate immunity

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BRIGHT SPARKS WORKSHOPS

TRACK 3 - DISEASES AND IMMUNE RESPONSES

BS-013

TIGIT expression differentiates regulatory from inflammatory non-classical Th1-like gut-homing effector CD4+ T cells in inflammatory bowel disease patients

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Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is characterized by intestinal infiltration of pathogenic effector CD4$^+$ T cells. The defects driving loss of T-cell regulation in IBD vary between patients and remain undefined. Previously, we have shown that in human intestine, 20-40% of effector (CD62LnegCD4$^+$) T cells express TIGIT, an inhibitory receptor modulating dendritic cell and T-cell function. In peripheral blood of healthy individuals, TIGIT expression was enriched in gut-homing CD38$^+$ effector T cells while in a subgroup of IBD patients with active intestinal inflammation, frequencies of inhibitory TIGIT$^+$CD38$^+$ effector T cells were decreased and were associated with earlier relapse of disease. This raised the question whether gut-homing effector T cells lacking TIGIT (TIGITneg) are pathogenic mediators of intestinal inflammation in IBD. We monitored TIGIT$^+$ and TIGITneg cells in circulating CD38$^+$ effector T cells and intestinal lamina propria CD4$^+$ T cells of pediatric IBD patients. At diagnosis, approximately 50% of CD patients had strongly reduced frequencies of circulating TIGIT$^+$CD38$^+$ effector T cells compared to UC patients and age-matched healthy controls. As anticipated, absence of TIGIT expression identified CD38$^+$ effector T cells enriched in Ki67, reflecting recent proliferation, and having high expression of chemokine receptors associated with inflammatory non-classical T helper-1 IFN-γhighIL-17low producing cells. Moreover, intestinal TIGITnegCD4$^+$ T cells of IBD patients contained higher frequencies of IFN-γ and IL-17A producing cells than TIGIT$^+$CD4$^+$ T cells. In conclusion, we identify TIGITneg gut-homing effector T cells as potential drivers of intestinal inflammation in a subgroup of CD patients.

Keywords: Regulatory cells, adaptive immunity, monitoring immunity, inflammatory bowel disease

BS-014

Protective role of the nucleic acid sensor STING in idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is the most common type of interstitial lung disease, characterized by progressive lung scarring and a high mortality rate. IPF has no cure and available treatments at best delay disease progression. IPF is largely driven by host-derived danger signals released upon recurrent local tissue damage. We explore the roles of self-DNA and the intracellular DNA sensor stimulator interferon genes (STING), which activation leads to type I/III interferons (IFN) production and autophagy induction. Using a mouse model of IPF by bleomcyin (BLM) instillation, we report that STING deficiency leads to exacerbated pulmonary fibrosis with increased lung collagen deposition and excessive remodeling factors expression. We show that STING-mediated protection does not rely on type I IFN signaling nor IL-17A or TGF-β modulation. Interestingly, we observed persistent airway neutrophilia and decreased type III IFN (IL-28) in lung tissues of BLM-treated Sting deficient (Sting-/-) mice in comparison to their wild type (WT) counterparts. We hypothesize that STING may limit neutrophilic inflammation through IL-28 signaling, downregulating subsequent adaptive immunity, remodeling and fibrosis. In addition, STING displays important anti-inflammatory properties through its phylogenetically conserved autophagy-inducing pathway. Autophagy elicits protective functions in both experimental lung fibrosis and IPF patients. Interestingly, our first results show that lung expression of autophagy related proteins (ATG5/P62/LC3B-II) is reduced in BLM-treated Sting-/- mice in comparison to their WT relatives, indicating that BLM-induced autophagy depends on STING. Together, our data support an unprecedented immunoregulatory function of STING in lung fibrosis that may rely on STING-dependent IL-28 and/or autophagy.

Keywords: Animal models, chronic inflammation and fibrosis, innate immunity

BS-015

High-throughput single-cell analysis revealed transcriptional response of dendritic cell subsets during SARS-CoV-2 infection

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Growing evidence suggests that conventional dendritic cells (cDCs) undergo aberrant maturation during SARS-CoV-2 infection and this negatively affects T cell activation. cDCs are a heterogeneous population originally classified in cDC1s and cDC2s. Recently, single-cell RNA sequencing revealed the complexity of cDC2s, which were stratified into two subpopulations, DC2s and DC3s. Given the pivotal role of these cells in the orchestration of adaptive immune responses, a deep characterization of their transcriptional signatures during COVID-19 may provide novel insights to understand the immune system's reaction to infection. Here, we analysed two available and a newly generated single-cell transcriptomic datasets of peripheral blood mononuclear cells from COVID-19 patients and healthy donors (HD). We observed that, during infection, DC3s showed increased frequencies in patients, which positively correlated with disease severity. When comparing cDCs in severe versus mild patients, we identified an important number of differentially expressed genes. Specifically, inflammatory genes not related to the activation of adaptive immunity, like complement and coagulation factors, were upregulated in cDC2s from severe patients. Conversely, genes encoding MHCII molecules, the costimulatory molecule CD86 and cytokines, showed a progressive downregulation from HD to mild and finally severe patients. Hence, as disease severity increases, cDC2s progressively skew toward inflammatory activities and lose the antigen presenting function. In conclusion, we unravelled the transcriptional signatures, reflecting the functional state, of cDC subsets during COVID-19. Importantly, by inducing the downregulation of crucial molecules required for T cell activation, the virus implements an efficient immune escape mechanism that correlates with disease severity.

Keywords: Dendritic cells, innate immunity, viral infections

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BRIGHT SPARKS WORKSHOPS

BS-016

Tumour derived microRNA-21 reprograms macrophage responses

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Numerous cells of the immune system are present in the tumour microenvironment (TME), with macrophages being the dominant leukocyte population. Dysregulation of the natural immune response against tumours is a now a well-recognized hallmark of cancer, yet the complex signals in the TME which impact on and reprogram immune cells to facilitate tumour growth and dissemination remain unclear. The role of the oncogenic yet anti-inflammatory microRNA-21 (miR-21) in the TME is not clearly defined, despite its upregulation in many cancers. Since re-education of macrophages by tumour derived signals is a central step in cancer pathology, the role of miR-21 in this process was investigated. It was found that co-culture of naïve human macrophages with the Hepatocellular carcinoma derived cell line, HepG2, induces miR-21 expression. This is consistent with a switch from a pro-inflammatory to an immuno-regulatory gene expression profile. Anti-sense technology was used to silence miR-21 expression in macrophages, enhancing the induction of pro-inflammatory mediators such as TNF, boosting the capacity to limit growth of tumour cells. Interestingly, up-regulation of mature miR-21 levels in co-cultured macrophages did not coincide with transcriptional induction of the primary miR-21 transcript and was instead enriched in secreted exosomes from tumour cells, suggesting a novel mechanism employed by tumour cells to re-program macrophages. These findings reveal a key role for tumour derived miR-21 in the re-programming of tumour associated macrophages facilitating tumour growth and highlights a novel target for improving anti-tumour responses *in vivo*.

Keywords: Cancer immunology, macrophage, miRNA

BS-017

Rescue of STAT3 function in hyper-IgE syndrome using adenine base editing

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STAT3-hyper IgE syndrome (STAT3-HIES) is a primary immunodeficiency presenting with destructive lung disease along other symptoms. To date no causative cure of the chronic lung disease is available. CRISPR-Cas guided adenine base editors (ABEs) have the potential to correct one of the most common STAT3-HIES causing heterozygous STAT3 mutations (c.1144C>T/p.R382W). Primary patient fibroblasts and induced pluripotent stem cells (iPSCs) were treated with an ABE system to repair the p.R382W mutation and showed a robust repair efficiency of around 30%. Whole genome sequencing and high-throughput sequencing in fibroblasts showed no off-target effects. Functional STAT3 analysis of DNA-binding activity and target gene expression (CCL2 and SOCS3) revealed significantly improved function in repaired fibroblast single cell clones. Repaired patient iPSCs were successfully differentiated to alveolar organoids after treatment. Our successful proof of concept showed that ABEs are able to functionally correct pathogenic point mutations very specifically and with robust efficiency and thus was an important step towards therapeutic gene correction in STAT3-HIES.

Keywords: Drugs for immune modulation, effector molecules, immune regulation and therapy, immunodeficiency, stem cells

BS-018

Pediococcus pentosaceus derived membrane vesicles modulate inflammation by generating M2-like macrophages and myeloid-derived suppressor cells

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Immunomodulatory commensal bacteria constitutively secrete membrane vesicles (MVs) that modify host immunity through delivery of regulatory microbial-derived products. Pediococcus pentosaceus, one of the human lactic acid commensal bacteria, secrete membrane vesicles that have anti-inflammatory effects. In this study, we aim to investigate Pediococcus pentosaceus MVs anti-inflammatory effect more detailly and test their potential in different models as therapeutic agents. Firstly, we showed that Pediococcus pentosaceus MVs suppressed antigen-specific humoral and cellular responses. MV-treatment of bone marrow derived macrophages and bone marrow progenitors promoted M2-like macrophage polarization and myeloid-derived suppressor cell differentiation, respectively, in a TLR2-dependent manner. Consistent with their immunomodulatory activity, MV-differentiated cells up-regulated expression of IL-10, PD-L1, Arginase-1 and suppressed the proliferation of activated T cells. MVs' anti-inflammatory effects were also tested in acute inflammation models established in mice. In zymosan-induced peritonitis and carbon-tetrachloride-induced fibrosis models, MVs ameliorated inflammation. In the dextran sodium sulphate-induced acute colitis model, systemic treatment with MVs prevented colon shortening and loss of crypt architecture. In an excisional wound healing model, intraperitoneal MV administration accelerated wound closure through recruitment of PD-L1 expressing myeloid cells to the wound site. Collectively, these results indicate that Pediococcus pentosaceus derived membrane vesicles activate suppressor – regulatory cell types and hold promise as potent therapeutic agents for the treatment of inflammatory conditions.

Keywords: Immune regulation and therapy, macrophage, myeloid derived suppressor cells

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BRIGHT SPARKS WORKSHOPS

TRACK 4 - INNOVATIVE TECHNOLOGIES AND IMMUNOTHERAPIES

BS-019

PROTAC-mediated antigen degradation: A novel strategy to enhance CTL responses against cancer

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A prime goal of cancer immunotherapy is to induce specific CD8+ T-cell (CTL) responses. These key immune effectors are able to kill cancer cells upon recognition of antigens presented on major histocompatibility complex (MHC) class I molecules. Cancer cells often downregulate this presentation to avoid recognition by CTLs. Despite of significant progress in cancer immunotherapy, there is currently no therapeutic strategy available to specifically enhance the MHC class I presentation of cancer-associated antigens. We were recently the first to demonstrate that targeted degradation of a model antigen by proteolysis targeting chimeras (PROTACs) can significantly enhance its MHC class I presentation *in vitro*. PROTACs are bispecific molecules that link a specific E3 ligase complex with a target protein that is consequently degraded by the ubiquitin/proteasome system. Here, we exploit PROTACs as a novel tool to improve CTL-mediated cancer cell depletion. We generated murine B16F10 melanoma cells expressing click beetle red luciferase (CBred) in linear fusion with the H-2Kb-restricted CTL epitope SIINFEKL (S8L; from chicken ovalbumin) and the degradation tag FKBP12 (CBred-S8L-F12) to follow PROTAC-mediated antigen degradation *in vitro* and *in vivo*. To date, we could confirm that PROTAC-mediated degradation of CBred-S8L-F12 increases the surface presentation of H-2Kb/S8L, which directly translated to enhanced CTL activation and CTL-mediated killing of melanoma cells *in vitro*. Currently, we are evaluating these findings in pre-clinical mouse models for melanoma *in vivo*. Our results further strengthen the hypothesis that PROTACs could be used to increase the immunological visibility of tumor cells towards CTL-mediated killing in cancer patients

Keywords: Antigen processing and presentation, cancer immunology, drugs for immune modulation, immunotherapy

BS-020

Genetically engineered tolerogenic dendritic cells induce ag-specific CD8+ T cell exhaustion and ag-specific IL-10-produding T cells and modulate Type 1 diabetes *in vivo*

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Tolerogenic DC (tolDC) play a critical role in promoting tolerance and represent the cells of choice to fulfill the goal of promoting/restoring antigen(Ag)-specific tolerance in autoimmunity. Effective Ag-specific DC-based therapy should dampen autoreactive T cell responses, inducing pathogenic T cell exhaustion and restore long-term tolerance via Ag-specific Treg induction. Using state-of-the-art lentiviral vector (LV) technology we generated stable tolDC presenting the encoded Ag to T cells in the presence of sustained levels of IL-10. We transduced murine DC precursors with bidirectional LV co-encoding for a specific HLA-restricted peptide fused to the invariant chain and human IL-10 (DC-Ag/IL-10). We analyzed the phenotype and cytokine profile of DC-Ag/IL-10 and their ability to modulate Ag-specific CD4 and CD8 T cell responses and type 1 diabetes (T1D) development. DC-Ag/IL-10 secreted high levels of human IL-10 and low levels of IL-12. Using ovalbumin (OVA), we showed that DC-OVA/IL-10 reduced proliferation and activation of $CD8^+$ OT-I and $CD4^+$ OT-II T cells *in vitro*. Repetitive *in vivo* DC-OVA/IL-10 injection promoted OVA-specific $CD4^+$ Tr1 cells and $CD8^+$ T cells with an exhausted phenotype. In pre-clinical models of induced diabetes, repetitive administration of DC engineered to co-encoding T1D-related autoAg and IL-10 prevented diabetes development in 50% of recipients. Our results provide a new method to generate IL-10-producing DC able to modulate pathogenic $CD4^+$ and $CD8^+$ T cell responses and to induce Ag-specific Tr1 cells and $CD8^+$ T cells with an exhausted phenotype suitable for cell-based approach to restore tolerance in autoimmune diseases.

Keywords: Autoimmunity, cell based therapies, dendritic cells, diabetes, immunotherapy, regulatory cells

BS-021

The single-cell epigenomic and transcriptional landscape of immunity to influenza vaccination in humans

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Emerging evidence indicates a fundamental role for the epigenome in immunity. In this context, molecular and epidemiological analyses suggest that certain vaccines mediate non-specific protection against a broad range of pathogens by modulating the epigenomic immune cell landscape. This mechanism, often referred to as "Trained Immunity", could be beneficial in situations where specific vaccines are unavailable (e.g., early phase of a pandemic), or less effective (e.g., many Elderly individuals). However, it is currently unclear how different types of vaccines modulate the epigenome, and which immune cell compartments are precisely affected by vaccine-induced epigenomic reprogramming. Aim and Approach: To answer these questions, here, we mapped the epigenomic and transcriptional landscape of immunity to influenza vaccination in humans at the single-cell level using scATAC-seq, scRNA-seq and EpiTOF. Vaccination against seasonal influenza induced persistently diminished H3K27ac in monocytes and myeloid dendritic cells (mDCs), which was associated with impaired cytokine responses to TLR stimulation. Single-cell ATAC-seq analysis revealed an epigenomically distinct subcluster of monocytes with reduced chromatin accessibility at AP-1-targeted loci after vaccination. Similar effects were observed in response to vaccination with the AS03-adjuvanted H5N1 pandemic influenza vaccine. However, this vaccine also stimulated persistently increased chromatin accessibility at interferon response factor (IRF) loci in monocytes and mDCs. This was associated with elevated expression of antiviral genes and heightened resistance to the unrelated Zika and Dengue viruses. These results demonstrate that influenza vaccination stimulates persistent epigenomic remodeling of the innate immune system and reveal AS03's potential as an epigenetic adjuvant.

Keywords: Adjuvants and vaccines, epigenetic control and modulation of immunity, innate immunity, omics technologies, protection, viral infections

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BRIGHT SPARKS WORKSHOPS

BS-022

Inter-donor variability in dendritic cells capacity to respond to stimulation *in vitro* associates with donors gut microbiota composition

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High donor-to-donor variability of immunogenic capacity of *in vitro* derived dendritic cells (DCs) is a reason for reduced therapeutic efficacy of DC-based vaccines. The aim of our study was to analyze whether the variability in the phenotype and function of DCs associates with composition of gut microbiota. Immature (im)DCs (14 healthy donors) were differentiated in the presence of GM-CSF/IL-4 and stimulated with LPS/IFN-γ to obtain mature (m)DCs. Th-polarization capacity of DCs was analyzed in co-culture with allogenic T lymphocytes. DNA isolated from fecal samples were used for 16s rRNA amplicon sequencing. Analyzed by Flow Cytometry, the expression of immunostimulatory and immunosuppressive markers on imDC and mDC, as well as fold change upon LPS/IFN-γ-stimulation varied greatly between the donors. CD1a and IL-10 expression by imDC negatively correlated, and the expression of TNF-α by imDCs positively correlated with the diversity of gut microbiota. Considering HPLC-analysis of SCFA concentrations in feces, and abundances of SCFA-producing bacteria, *Butyricimonas* and *Alistipes*, were negatively associated with the expression of immunogenic molecules, IL-12p70, IL-8, CD1a, CD86 and CD83, and with Th1-polarization capacity of DCs, but positively associated with the expression of immunosuppressive molecules, ILT3 and IL-10. The relative abundance of *Akkermansia muciniphila* negatively correlated with the expression of CD83 by mDCs as well as with Th1-induction ability of DCs. Most importantly, the relative abundance of *Bifidobacterium bifidum* positively associate with the level of CD83 on DCs. These results emphasize a great potential of gut microbiota targeting as a promising strategy for improvement of DC-vaccine therapy.

Keywords: Anti-cancer vaccine, dendritic cells, microbiome and environmental factors

BS-023

TLR9 and STING ligands encapsulated into pH sensitive liposomes protected mice against melanoma tumor growth by elevating Th-1 immune response

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Innate immune system during a pathologic insult activates multiple signaling cascades dependent on simultaneous engagement of different ligands to their corresponding receptors. The cross-talk between each other dictates the magnitude and sustainability of immune response. By this feature, we designed novel liposomal adjuvants which are loaded with multiple nucleic acids synergistically activating innate immune system. We designed pH-sensitive modified sterically stabilized cationic liposomes (SSCL) to co-encapsulate TLR9 and STING ligands. Splenocytes, bone marrow-derived dendritic cells (BM-DCs) and macrophages (BM-DMs) were stimulated with free or liposome-loaded ligands for 24h. Cytokine productions from immune cells were determined. Data implicated splenocytes that were stimulated with liposome co-encapsulating dual ligands boosted IFN-γ and IFN-α/β levels (~60- and ~6-fold, respectively). After stimulation with liposome-loaded dual ligands, BM-DCs and BM-DMs led to significantly higher IL-12 secretion. *In vivo* efficacy was tested on the B16-F10-OVA tumor bearing mice in a therapeutic vaccine system. One week after tumor engraftment, C57BL/6 mice with palpable tumors (~100 mm3) were treated with free or liposome-loaded dual ligands. Dual ligands loaded liposome therapy led to 80% remission of tumors. We detected significantly elevated IgG2c/IgG1 ratio in animals treated with liposomal formulations, indicating the development of antigen specific Th1 biased immunity. Furthermore, liposome co-encapsulating CpG-ODN and CDN significantly elevated antigen dependent IFN-γ producing CD8+ T-cells and significantly decreased M2-type macrophages at the tumor bed. In conclusion, co-encapsulating CDNs and ODNs into pH-sensitive liposomes elicited tumor specific CD8+ T-cells and lead to significant reduction of the established melanoma tumors.

Keywords: Adjuvants and vaccines, immunotherapy, *in vivo* tumor models, innate immunity

BS-024

Inefficient CAR-proximal signaling blunts antigen sensitivity

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Remarkable cancer remission rates (≈ 90%) reported earlier in chimeric antigen receptor T-cell (CAR-T-cell) clinical trials have recently declined to below 50% primarily due to tumor antigen escape. Loss of CAR-T-cell function following tumor-antigen downregulation is hence of grave concern yet poorly understood. We analyzed CAR-mediated antigen recognition on a molecular level via quantitative single molecule live-cell imaging. We found the sensitivity of CAR-T-cells towards antigen is approximately 1000-times reduced when compared to T-cell antigen receptor (TCR)-mediated recognition of nominal peptide/MHC complexes. While CARs outperformed TCRs with regard to antigen binding within the immunological synapse, proximal signaling was significantly attenuated due to inefficient recruitment of the tyrosine-kinase ZAP70 to ligated CARs and its reduced concomitant activation and subsequent release. Our study exposes signaling deficiencies of state-of-the-art CAR-designs, which limit at present the efficacy of CAR-T-cell therapies to target tumors with diminished antigen expression.

Keywords: Cancer immunology, immunotherapy, molecular immunology, visualizing immune responses

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WORKSHOPS

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WORKSHOPS

TRACK 1 - CELLULAR IMMUNOLOGY

OP-025

Mast cell secretory granules are endogenous c-type lectin receptor ligands skewing dendritic cell function towards Th2/Th17 response

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Mast cells (MCs) are known as key effector cells of type I allergy but also play an important role in host defense against pathogens. Despite increasing evidence for a critical impact of MCs on adaptive immunity, the underlying mechanisms are poorly understood. Particularly, the specific relevance of MC granules (MCG) in MC communication with tissue resident immune cells has not yet been studied. We recently reported that dermal dendritic cells (dDCs) engulf MCG exocytosed by MCs upon skin inflammation. MCG-bearing DCs showed an advanced functionality compared to MCG-negative DCs. We consequently highlighted a unique feature of peripheral MCs to impact on adaptive immunity by modifying DC functions. Understanding the uptake mechanism and DC modulating properties of MCG may give rise to therapeutic strategies to either intentionally boost adaptive immunity or dampen elevated immune responses. We found that solely immature DCs take up MCG via macropinocytosis. Nevertheless, the exclusive engulfment of MCG from fully differentiated MCs revealed that sensing of distinct molecular patterns precedes the uptake. We could show that DCs sense and engulf MCG in a Card9-/Syk-dependent manner. Importantly, MCG-bearing DCs modulated their antigen-presenting capacity towards Th2 and Th17 responses. Consistently, Card9 deficiency resulted in a reduced T cell priming/differentiation upon skin inflammation. Questioning the MCG sensing receptors, we identified the c-type lectin receptors (CLRs) SIGNR3 and MCL, which bind to MCG and cooperate in MCG uptake. Collectively, MCG are endogenous CLR ligands that translate danger signals into adjuvant effects promoting lymph node-borne adaptive immunity.

Keywords: Cellular interactions, dendritic cells, immune communication, immune networks, mast cells

OP-026

Directional mast cell degranulation of tumor necrosis factor into blood vessels primes neutrophil extravasation

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Tissue resident mast cells (MCs) rapidly initiate neutrophil infiltration upon inflammatory insult, yet the molecular mechanism is still unknown. Here, we demonstrated that MC-derived tumor necrosis factor (TNF) was crucial for neutrophil extravasation to sites of contact hypersensitivity-induced skin inflammation by promoting intraluminal crawling. MC-derived TNF directly primed circulating neutrophils via TNF receptor-1 (TNFR1) while being dispensable for endothelial cell activation. The MC-derived TNF was infused into the bloodstream by directional degranulation of perivascular MCs that were part of the vascular unit with access to the vessel lumen. Consistently, intravenous administration of MC granules boosted neutrophil extravasation. Pronounced and rapid intravascular MC degranulation was also observed upon IgE crosslinking or LPS challenge indicating a universal MC potential. Consequently, the directional MC degranulation of pro-inflammatory mediators into the bloodstream may represent an important target for therapeutic approaches aimed at dampening cytokine storm syndromes or shock symptoms, or intentionally pushing immune defense.

Keywords: Immune communication, inflammatory molecules, innate immunity, mast cells, neutrophils

OP-027

Single-cell chimerism analysis and transcriptional profiling identify long-term human tissue-resident host T cells in the skin after allogeneic stem cell transplantation

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Tissue-resident memory T cells (TRM) have recently emerged as crucial cellular players for host defense in a wide variety of tissues. Insights into the maintenance and regulatory checkpoints of human TRM cells remain scarce, especially due to the difficulties associated with tracking T cells over time and spatially in humans. We therefore aimed to identify and characterize skin resident T cells in humans defined by their long-term in situ lodgement. Here, we present a clinical model that overcomes previous limitations. Allogeneic hematopoietic stem cell transplantation paired with myeloablative chemotherapy unmasked long-term sequestration of host T cell subsets in the human skin despite complete donor T cell chimerism in the blood. Single-cell chimerism analysis paired with single-cell transcriptional profiling comprehensively characterized these bona fide long-term skin resident T cells and revealed novel properties such as specific TRM cell markers and tissue exit potential with retention of the transcriptomic TRM cell identity. Our data exemplify the power of exploiting a clinical situation as a proof-of-concept for the existence of bona fide human skin TRM cells and reveal long-term persistence of host immunity in the peripheral tissue but not in the circulation or bone marrow for a subset of patients. They provide insights into host TRM phenotypes, functions and regulatory checkpoints that may be harnessed for long-term protective immunity at barrier tissues, for targeting their pathogenic memory in organ restricted autoimmunity or for treating GvHD.

Keywords: Adaptive immunity, immune development, memory

OP-028

Extrinsic factors govern human MAIT cell transcriptional and functional plasticity

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Mucosal-associated invariant T (MAIT) cells are abundant innate-like T cells with a characteristic phenotype. They show considerable changes in frequency and function in numerous human diseases, including COVID-19, and have protective and pathogenic roles *in vivo*. While there is some evidence for phenotypic and functional heterogeneity, whether human MAIT cells comprise multiple transcriptionally distinct subsets remains unknown. To investigate this, we performed single-cell RNA-sequencing (scRNA-seq) of human MAIT cells from matched blood and liver. MAIT cells showed tissue-specific transcriptional profiles, with liver cells exhibiting an activated, tissue-resident phenotype. In contrast, transcriptional variation within each tissue was limited and in contrast with mice, there was an absence of type 1 and type 17 subsets. We next used scRNA-seq and single-cell TCR-sequencing to probe the functionality of MAIT cells at a per cell level following TCR and cytokine stimulation. Pseudotime analysis revealed a branching trajectory, with TCR- and cytokine-stimulated cells deviating early following activation and showing increased transcriptional divergence over pseudotime. While individual cells showed varying degrees of activation, there was no evidence for distinct functional subsets. Moreover, MAIT cells from different TCR clonotypes showed a comparable capacity for activation. Overall, our findings suggest that human MAIT cells fundamentally comprise a single population with a conserved transcriptional programme. Observed heterogeneity reflects differences in tissue localisation and activation state. Our study offers significant insights into MAIT cell biology and provides the most comprehensive analysis of human MAIT cell transcriptional and clonal architecture to date.

Keywords: Big data, RNAseq, MAIT cells

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WORKSHOPS

OP-029

Pre-existing chromatin accessibility and gene expression differences among naïve CD4+ T cells influence effector potential

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CD4+ T cells have a remarkable potential to differentiate into diverse effector lineages following activation. We probed the heterogeneity present among naïve CD4+ T cells before encountering their cognate antigen to ask whether their effector potential is modulated by pre-existing transcriptional and epigenetic differences. Using single-cell RNA-seq, we showed that key drivers of variability were genes involved in T cell receptor (TCR) signaling and chromatin modification. Using CD5 expression as a read-out of the strength of tonic TCR interactions with self-peptide MHC, and sorting on the ends of this self-reactivity spectrum we performed bulk RNA- and ATAC-seq. There were 1006 differentially expressed genes between CD5lo and CD5hi cells, with ~2/3 upregulated in CD5hi cells. Both RNA- and ATAC-seq datasets showed an enrichment of transcriptional regulators and chromatin modifiers in CD5hi cells associated with follicular helper cell (TFH) responses. Moreover, CD5hi cells were enriched for a TFH cell gene signature with increased gene expression of Bcl6, Cxcr5, Pdcd1, and decreased expression of Prdm1 that we showed impacted TFH versus non-TFH effector lineage choice upon infection *in vivo*. Interestingly, T cell deprivation of self-pMHC interactions identified transcriptional and chromatin differences between CD5lo and CD5hi cells that did not rely on continuous self-ligand interactions. Together, our data suggest that transcriptional and epigenetic differences in cell states among naïve CD4+ T cells impacts function upon activation. Ultimately, this may shed light on which pre-existing transcriptome-level differences are accessible to interventions targeting self-pMHC peripheral interactions, compared to others requiring modulation at the chromatin level.

Keywords: Cell signalling, epigenetic control and modulation of immunity, follicular helper T cells, RNAseq

OP-030

Themis1/vav1 signaling hub controls T cell pathogenicity in a mouse model of multiple sclerosis

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Although Multiple Sclerosis (MS) is the leading cause of disability among young adults in the world, the mechanisms underlying its development remain poorly understood. GWAS studies revealed the implication of many genetic factors, the majority of which being immune related. However, genetic factors only account for 20% of MS etiology. This is probably because genetic studies address each gene separately, and not as complexes. In this work, we investigated the implication of two genes, Vav1 and Themis1 (two interacting TCR signaling molecules), in the susceptibility to EAE development, an animal model of MS. To this aim, we used a natural variant of Vav1 discovered as conferring reduced susceptibility to central nervous system (CNS) inflammation in rats, together with a T cell-conditional deletion of Themis1. When taken separately, both mutations triggered an intermediate reduction of EAE severity, whereas this reduction was much stronger when both genes were mutated simultaneously. This was unrelated to the impact of Themis1 on thymic selection, but rather resulted from decreased encephalitogenicity of conventional T cells, characterized by a reduced production of pro-inflammatory cytokines (IFN-γ, IL-17, and GM-CSF) and lowered lymphocytic infiltration in the CNS. Our current work aims as determining the mechanisms involved by analysing the pathways impacted by those mutations. Altogether, our study reveals an epistatic interaction between Vav1 and Themis1, which form a signaling hub controlling Tconv encephalitogenicity. Our work also gives a rationale for examining gene complexes, rather than separated genes, to understand better MS etiology.

Keywords: Adaptive immunity, animal models, autoimmunity, cell signalling, multiple sclerosis

OP-031

Distinct developmental pathways from blood monocytes generate human lung macrophage diversity

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The study of human macrophages and their ontogeny is an important unresolved issue. Here, we use a humanized mouse model expressing human cytokines (called "MISTRG") to dissect the development of lung macrophages from human hematopoiesis *in vivo*. Human CD34+ hematopoietic stem and progenitor cells (HSPCs) generated three macrophage populations, occupying separate anatomical niches in the lung. Intravascular cell labeling, cell transplantation, and fate-mapping studies established that classical CD14+ blood monocytes derived from HSPCs migrated into lung tissue and gave rise to human interstitial and alveolar macrophages. In contrast, non-classical CD16+ blood monocytes preferentially generated macrophages resident in the lung vasculature (pulmonary intravascular macrophages). Finally, single-cell RNA-sequencing defined intermediate differentiation stages in human lung macrophage development from blood monocytes. This study identifies distinct developmental pathways from circulating monocytes to lung macrophages and reveals how cellular origin contributes to human macrophage identity, diversity, and localization *in vivo*.

Keywords: Immune development, innate immunity, macrophage, omics technologies

OP-032

Peripheral mast cell-derived RANKL is a prerequisite for lymphocyte egress from distant lymph nodes

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Mast cells (MC) are key initiators of vasoactivation and immune cell infiltration upon allergic contact dermatitis. We recently identified MCs as a prominent source of receptor activator of NFκB (RANKL), which is best known for its function in bone metabolism but increasingly emerging as an important immune regulator. However, the relevance of MC-derived RANKL in skin inflammation is completely unknown. Using conditional RANKL knockout in connective tissue type MCs, we identified a crucial role of MC-derived RANKL in the onset of skin inflammation and immune cell infiltration upon DNFB challenge. Surprisingly, mice lacking MC-derived RANKL displayed massive lymphocyte hyperplasia in inguinal lymph nodes (LN), 24h after DNFB challenge on ear skin, accompanied by profound blood lymphopenia. LN hyperplasia and blood lymphopenia were found to be restored at later time points. However, despite the increased immune cell infiltration at 48h after challenge, skin inflammation remained markedly dampened. Strikingly, MC depletion and reconstitution with RANKL deficient MCs, only locally in the ear skin, resembled inguinal LN hyperplasia, blood lymphopenia and reduced lymphocyte infiltration to inflamed skin 24h post DNFB challenge as observed in mice lacking MC-RANKL completely. Consequently, RANKL release by peripheral skin MCs upon DNFB challenge exerts a long-distance effect that is a prerequisite for the timely lymphocyte egress from remote LNs, which is in turn critical for the onset and severity of allergic skin inflammation.

Keywords: Allergen-induced immune responses, cellular interactions, cytokines and mediators, effector molecules, immune communication, mast cells

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WORKSHOPS

OP-033

Temporal dissection of the T cell-intrinsic factors that maintain T follicular helper cell identity and germinal centers

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T follicular helper (Tfh) cells are crucial for the establishment of germinal centers (GCs) and potent antibody responses that are elicited during infection and vaccination. Despite their importance for humoral immunity, the T cell-intrinsic factors that are required for the maintenance of already established Tfh cells and GCs remain largely unknown. Here, we used temporally guided, tamoxifen-inducible CD4+ T cell-specific gene ablation to dissect the contributions of various factors, including CXCR5, Bcl6, and mature miRNAs, to the maintenance of Tfh cell function and identity and its impact on B cells in ongoing GCs in viral infection and vaccination models. Induced ablation of Cxcr5 in CD4+ T cells had only minor effects on the identity and function of established Tfh cells. Phenotypical and genome-wide transcriptional analyses revealed that Cxcr5-ablated cells still exhibited most features of CXCR5-positive Tfh cells. In contrast, continued Bcl6 expression was essential to maintain the GC Tfh cell phenotype and GC reaction. CD4+ T cell-specific Bcl6 ablation during acute viral infection resulted in transdifferentiation of "ex-Tfh" cells into Th1 cells. Finally, induced CD4+ T cell-specific depletion of all mature miRNAs resulted in the loss of the Tfh cell phenotype and resolution of GCs. By highlighting the high degree of Tfh cell plasticity, these studies provide novel insights into the mechanisms underlying T-B cell interactions as well as Tfh cell and GC maintenance.

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Keywords: Adaptive immunity, animal models, B lymphocytes, follicular helper T cells

OP-034

Novel players in B cell antigen uptake and trafficking

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Antigen uptake by B cells is critical for high-affinity antibody production. Antigen binding to the B cell receptor (BCR) triggers rapid endocytosis and intracellular trafficking into lysosomal compartments for processing and loading of peptides onto MHCII molecules. Antigen endocytosis critically regulates the number and repertoire of peptide-MHC complexes presented to T cells and impacts germinal centre formation, affinity maturation and antibody production. Further, internalisation of the BCR regulates BCR signalling, and thus modulates B cell activation in normal responses, autoimmunity and malignant transformation. The current model of B cell antigen uptake involves BCR endocytosis by clathrin-coated pits (CCPs) through binding to the clathrin adaptor AP-2. However, depletion of CCP components does not completely abolish BCR endocytosis. We carried out whole-genome CRISPR/Cas9 screens for genes affecting surrogate antigen uptake. Subsequent validation identified over 70 positive and negative regulators, most of which have not previously been associated with B cell endocytosis. These include signalling proteins, ubiquitin ligases, regulators of intracellular trafficking and lysosomal proteins. A large number of these candidates have now been validated in primary cells highlighting the potential of this robust approach for novel discoveries. Interestingly, we detected a role for Endophilin A2, which regulates fast, clathrin-independent endocytosis of ligand-bound receptors. Endophilin A2 was recruited to BCRs in a signalling-dependent manner, and its depletion reduced antigen uptake in primary mouse B cells. Loss of Endophilin A2 resulted in reduced germinal centre responses and serum antibody titres *in vivo*, suggesting a critical role for Endophilin A2 in T-dependent B cell responses.

Keywords: Antigen processing and presentation, B lymphocytes, big data, endo- and exocytic vesicles in immunity

OP-035

Characterization of human innate lymphoid cell subsets in healthy lymph nodes

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Innate Lymphoid Cells (ILCs) include cytotoxic Natural Killer (NK) and helper-type ILCs (ILC1, ILC2 and ILC3). These populations are defined on their developmental transcription factor requirements and cytokine production patterns. Helper ILC (hILC) are present at barrier tissues (intestines, lung and skin) and contribute to tissue integrity and homeostasis. They are also present within all lymphoid tissues but little is known about ILCs in adult secondary lymphoid organs and especially in lymph nodes (LN). We have previously characterized the phenotype and functional capacities of NK cells from mesenteric lymph nodes. Taking into account the definition of ILC subsets, we have undertaken the characterization of donor derived LN ILC including helper type ILCs, gating on Lin-CD7+ cells. We have characterized ILC populations in LN by flow cytometry, unsupervised analyses (viSNE and FlowSOM) and multiplex qPCR. Our results on LN ILCs indicate the presence of three main ILC populations: NK cells (CD56+CD127-), ILC3 (CD56-CD127+CD117+) and a particular "ILC3-like" population expressing both CD127 and CD56. "ILC3-like" cells were shown to express some NK receptors (NKp44, NKp46). Their transcriptomic profile was closed to ILC3 cells, expressing also some NK cell reference genes (EOMES, TBX21, KLRD1, PRF1, GZMB). After IL-2 plus IL-12 activation, these "ILC3-like" cells acquired NK cell functions, degranulating and producing IFN-g in response to the target K562. This results demonstrate the plasticity of the newly characterized "ILC3-like" population under specific cytokine conditions.

Keywords: Innate lymphoid cells, lymphoid organs, NK cells

OP-036

Single-cell transcriptomics reveals discrete steps in regulatory T cell development in the human thymus

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CD4+FOXP3+ regulatory T (Treg) cells control immunological tolerance. Treg cells are generated in the thymus (tTreg) or can derive from mature conventional T cells in the periphery (pTreg). Thymic Treg cells are ostensibly more stably committed to the Treg cell lineage than pTreg cells and are the preferred cell type for adoptive cell therapy (ACT) to treat autoimmune/inflammatory disease. How Treg cells develop, especially in the human thymus, is incompletely understood. Here, we examined the human tTreg compartment through a combination of single-cell transcriptomics and flow cytometry. We delineated three major developmental stages and propose a stage nomenclature similar to that of the conventional B cell lineage. A first stage, which we propose to name pro-Treg, contains cells still expressing lineage-inappropriate genes and exhibits signs of TCR signalling, presumably reflecting recognition of self-antigen. The subsequent pre-Treg stage is characterized by appearance of FOXO1, KLF2 and CCR7, in apparent preparation for thymic exit. This pre-Treg stage can further be refined based on the sequential acquisition of the surface markers CD31, GPA33, and CD45RA. Remarkably, a substantial fraction of thymic Treg cells consists of recirculating, activated mature effector (e)Treg cells, distinguishable from developing Treg cells by expression of inflammatory chemokine receptors and absence of CCR7. The unclear developmental origin of these cells warrants caution when using thymic tissue as a source of stable cells for ACT. These insights help identify fully mature tTreg cells for ACT and can serve as a basis for further mechanistic studies into tTreg development.

Keywords: Adaptive immunity, autoimmunity, biology of the immune system, regulatory cells

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WORKSHOPS

OP-037

Flagellin/TLR5 stimulate myeloid progenitors to enter lung tissue and to locally differentiate into macrophages

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Toll-like receptor 5 (TLR5) is the receptor of bacterial Flagellin. Reportedly, TLR5 engagement helps to combat infections, especially at mucosal sites, by evoking responses from epithelial cells and immune cells. Here we report that TLR5 is expressed on a previously defined bipotent progenitor of macrophages (MΦs) and osteoclasts (OCs) that resides in the mouse bone marrow (BM) and circulates at low frequency in the blood. *In vitro*, Flagellin promoted the generation of MΦs, but not OCs from this progenitor. *In vivo*, intranasal Flagellin inoculation induced recruitment of intravenously transferred MΦ/OC progenitors into the lung and their rapid, local differentiation into MΦs. Recruitment of the MΦ/OC progenitors from the blood stream into the lung was likely promoted by the CCL2/CCR2 axis, since the progenitors expressed CCR2 and type 2 alveolar epithelial cells (AECs) produced CCL2 upon stimulation by Flagellin. Moreover, CCR2 blockade reduced migration of the MΦ/OC progenitors towards lung exudate from Flagellin-inoculated mice. Our study reveals a novel role of the Flagellin/TLR5 axis in recruiting circulating MΦ/OC progenitors into infected tissue and stimulating these progenitors to locally differentiate into MΦs. The progenitor pathway to produce MΦs may act, next to monocyte recruitment, to fortify host protection against bacterial infection at mucosal sites.

Keywords: Bacterial infections, chemokines, immune development, innate host defence, innate immunity, macrophage

OP-038

Developmental bifurcation of human T follicular regulatory cells

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Germinal centres (GC) are anatomic structures where B cells undergo affinity maturation leading to production of high-affinity antibodies. The balance between T follicular helper (Tfh) and regulatory (Tfr) cells is critical for adequate control of GC responses. The study of human Tfh and Tfr cells' development has been hampered due to the lack of *in vitro* assays reproducing the *in vivo* biology, along with difficult access to healthy human lymphoid tissues. We used a single cell transcriptomics approach to study the maturation of Tfh and Tfr cells isolated from human blood, iliac lymph nodes (LN), and tonsils. As independent tissues have distinct proportions of follicular T cells in different maturation states, we leveraged that heterogeneity to reconstruct the maturation trajectory for human Tfh and Tfr cells. We found that the dominant maturation of Tfr cells follows a bifurcated trajectory from precursor Treg cells, with one arm of the bifurcation leading to blood Tfr cells, and the other leading to the most mature GC Tfr cells. Overall, our data provide a comprehensive resource for the transcriptomics of different follicular T cell populations and their dynamic relationship across different tissues.

Keywords: Big data, follicular helper T cells, lymphoid organs, RNAseq

OP-039

MC-derived TNF is crucial for resolution of skin inflammation and tissue remodeling

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Chronic inflammation is often associated with mast cell (MC) accumulation, but it is still unknown whether MCs are cause or consequence of maladaptation. During contact hypersensitivity (CHS) induced skin inflammation, MCs initiate the early disruption of the vascular barrier resulting in edema formation and neutrophil infiltration. While vasodilatation and permeabilization is driven by histamine, we found that MC-derived TNF is essential for the activation of circulating neutrophils and consequently for neutrophil extravasation to inflamed skin. Surprisingly, we found that in absence of MC-derived TNF, the acute phase of CHS is reduced but subsequently prolonged with an increased ear swelling at later time points. Questioning the underlying mechanism, we found that at early time points of CHS not only neutrophils, but also inflammatory monocytes were decreased in the skin, suggesting a role for MC-derived TNF in monocyte recruitment during CHS response. At later time points, the number of alternatively activated macrophages, as well as the production of the anti-inflammatory cytokine IL-10 was reduced in the absence of MC-derived TNF. Intriguingly, the level of the neutrophil-derived matrix metalloproteinase (MMP) -8 and monocyte-derived proMMP-9, which are both crucial for collagen degradation and proper tissue restoration, were significantly reduced. In conclusion, a prompt and sufficient initiation of inflammatory immune responses by MC-derived TNF is crucial for subsequent resolution and tissue regeneration.

Keywords: Chronic inflammation and fibrosis, cytokines and mediators, immune communication, macrophage, mast cells, tissue damage and repair

OP-040

T regulatory cells oscillate between a TNF-producing and a TNFR2-expressing status

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Tumor necrosis factor (TNF) is a pleiotropic cytokine well known for its pro inflammatory effect, though several studies have highlighted its anti-inflammatory and immunomodulatory effects. It has been described that TNF promotes survival, proliferation and effector function of Treg, a CD4 T cell subset with suppressive function that constitutively expresses TNFR2. The aim of this project is to dissect the role of TNF production by Treg on their own expansion and function, especially in tumor, where Tregs are potentially more responsive to this cytokine. We demonstrated that Treg-derived TNF played a role in Treg proliferation thanks to an anti-TNF neutralizing Ab that suppressed Tregs proliferation and TNFR2 expression, suggesting the existence of a positive feedback mechanism. In murine tumors we found that TNFR2 and TNF were both upregulated by Tregs especially at the tumor site; however, TNF and TNFR2 showed a mutually exclusive expression, suggesting the existence of two alternative states or subsets of Tregs that either sense or produce TNF. To investigate the mechanisms behind this dichotomy, we hypothesized a post-translational control of TNF expression in the two Tregs subtypes, since sorted TNFR2+ and TNFR2- Tregs differed for the TNF protein levels but had the same TNF mRNA. Interestingly, TNFR2+ Tregs expressed higher levels of mir146a, which plays key roles in Treg functions and represses TNF synthesis. Overall, our data indicate that Tregs may exist in two functional states, and that internal regulatory mechanisms may dictate the balance between the two.

Keywords: Cancer immunology, *in vivo* tumor models, inflammatory molecules, regulatory cells

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WORKSHOPS

OP-041

Adaptive-like NK cell responses are composed of circulating and resident lineages

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Upon viral infection, natural killer (NK) cells expressing certain germline-encoded receptors are selected, expanded and maintained in an adaptive-like manner. Currently, all NK cells participating in such responses are thought to differentiate along a common pathway. However, by mapping the fate of single NK cells upon murine cytomegalovirus infection, we find that these adaptive-like responses originate from two NK cell lineages that maintain distinct clonal identities throughout infection. One of these is equivalent to recirculating conventional NK (cNK) cells while the other shows splenic residency and selectively associates with dendritic cells (DCs). These spleen-resident NK (srNK) cells are transcriptionally similar to type 1 innate lymphocytes (ILC1s) but capable of vigorous clonal expansion and direct recognition of virus infected cells. These features enable early induction of DC clustering and optimal T cell priming and establish srNK cells as a separate lineage that unites critical features of cNK cells and ILC1s.

Keywords: Innate immunity, innate lymphoid cells, NK cells, viral infections

OP-042

Role of purinergic signaling in regulating IL-1alpha mediated inflammation in chronic kidney disease

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Chronic Kidney disease (CKD) is associated with sterile inflammation where endogenous mediators are able to activate innate immune system and trigger inflammation. The cytokine family IL-1 plays a central role in development of CKD. While the contribution of IL-1ß to CKD is well characterized, the role of IL-1α is much less understood. We identified a novel role of IL-1α as an adhesion molecule mediating immune cells tissue infiltration. The aim of current work is to investigate the role of purinergic signaling in regulation of pro-inflammatory effects of IL-1α. Comparison of monocytes derived from healthy donors and CKD patients showed increased expression of IL-1α on CKD-monocytes without alteration of stoichiometry of monocyte subpopulations. Live cell Ca^{2+} imaging showed that the ATP-induced Ca^{+2} signatures were significantly altered in CKD-monocytes. Analysis of expression profile of purinergic receptors revealed upregulation of P2X7 and P2X4 in CKD compared to healthy donor-derived monocytes. Moreover, inhibition of P2X4 or P2X7 significantly reduced IL-1α release by human monocytes. Furthermore, bone marrow derived macrophages (BMDM) derived from P2X7-/- mice showed significantly reduced IL-1α release. Importantly, P2X7-/- mice were protected against adenine-induced renal injury in a CKD model. Moreover, cardiac myocytes from P2X7-/-mice challenged in a model for myocardial infarction showed a significantly reduced IL-1α accumulation within the infarction areas compared to WT cells. Our results indicate a pivotal role of P2X7 regulating IL-1α mediated inflammation and identify P2X7 as a potential therapeutic target to improved outcome of CKD therapies.

This work is supported by DFG (TRR219-C09 and TRR219-M04).

Keywords: Inflammatory molecules, innate immunity, cell signalling, cytokines and mediators, inflammatory disease, molecular immunology

OP-043

Molecular signature of conventional dendritic cell subtypes in COVID-19 disease

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Myeloid Dendritic Cells (DCs) present in the blood have been classified in cDC1 and cDC2 subsets. Recently, cDC2 has been further separated into two subpopulations: DC2 and DC3. DCs respond early to a viral infection to activate adaptive immunity, to control viral replication and to reduce virus spread from the peripheral site. COVID-19 patients with severe symptoms show immune dysregulation and alterations of lymphoid and myeloid populations in the blood. However, the DCs response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is still undefined. We verified the frequency of the DCs subsets in the blood of COVID19 patients. We found a decrease in the frequency of cDC1 and DC2 in COVID-19 patients, while we observed an increase in the presence of DC3, including the inflammatory DC3, recently identified as CD14+ and CD163+. The scRNAseq analysis revealed that cDC2 subtypes exhibit similar infection-induced gene signatures with the up-regulation of interferon-stimulated genes and IL-6 signaling pathways. Comparing cDCs between severe and mild patients, we find in the former a profound down-regulation of genes encoding molecules involved in antigen presentation, such as MHCII, β2M, TAP and costimulatory proteins, while an opposite trend is observed for proinflammatory molecules. As disease severity increases, cDC2s enhance inflammatory properties and lose antigen presentation capacity. *In vitro*, direct exposure of cDC2s to the virus recapitulates the type of activation observed *in vivo*. Our findings demonstrated that SARS-CoV-2 interacts directly with cDC2s which downregulate crucial molecules required for T cell activation.

Keywords: Dendritic cells, innate immunity, viral infections

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WORKSHOPS

OP-044

Identification and characterisation of novel NK cell-restricted aceNK progenitors

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Recent reports have identified that innate lymphoid cell (ILC) progenitors (ILCPs) retain their potential to develop into Natural Killer (NK) cells, contrary to an earlier proposal that indicated that NK cells differentiate from haematopoietic progenitors before their commitment to ILCPs. Furthermore, we have now found that Lineage-negative Id2$^+$IL-7Rα$^+$CD122$^+$ NKPs, purified using previously reported criteria, show heterogeneity and retain the capacity to differentiate into ILC2s (GATA3$^+$) and ILC3 (*Rorc*$^+$), when used to reconstitute *Rag2*, *Il2rg* double-deficient hosts. To address this inability to accurately define NK-restricted progenitors we used polychromic transcription factor reporter mice and single-cell RNA sequencing to identify and characterise a novel population of bone marrow progenitors exclusively committed to the NK lineage. These NK lineage-restricted NK progenitors are defined as Lineage-negative Id2$^+$IL-7Rα$^+$CD25$^-$Bcl11b$^-$NKG2A/C/E$^+$ (referred here as aceNKPs). *In vitro*, aceNKPs differentiated into NK1.1$^+$NKp46$^+$ innate lymphocytes under neutral conditions with stem cell factor and interleukin (IL)-7, without the requirement for IL-15. Upon maturation, they upregulated EOMES and T-bet expression and produced perforin and interferon (IFN)-γ. Notably, their Bcl11b$^+$NKG2A/C/E$^+$ counterparts acquired a similar phenotype but lacked perforin expression, more closely resembling type-1 helper ILCs. Following reconstitution of *Rag2*, *Il2rg* double-deficient recipients, aceNKPs solely gave rise to mature NK cells regardless of their tissue residence, displayed a cytotoxic phenotype and decreased tumour burden in a model of lung metastasis. In conclusion, our results highlight the multipotency of previously defined NK-committed progenitors and demonstrate that the NKG2A/C/E markers define an aceNKP population in the bone marrow that is exclusively restricted to NK lineage production.

Keywords: Immune development, innate lymphoid cells, NK cells

OP-045

Akt modulates differentiation of CD8+ memory T cell precursor populations but is dispensable for translational regulation

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During acute infection, naive CD8+ T-cells differentiate into short-lived effector cells (SLECs) and memory-precursor effector cells (MPECs) that give rise to long-lived memory cells. We have previously shown that during acute infection Akt signalling favours the generation of SLECs at the expense of MPECs. To better understand how Akt mediates these effects, we carried out scRNA-seq of adoptively transferred OT-1 cells that express wild-type (WT) or a mutant of phosphoinositide-dependent protein kinase-1 (PDK1-K465E) incapable of activating Akt. scRNA-seq of OT-1 cells from *Listeria monocytogenes* infected mice identified two distinct MPEC populations in both the WT and PDK1-K465E OT-1 cells. The MPEC populations differed in the expression of memory, proliferation and metabolism-associated genes. Furthermore, scRNA-seq revealed an upregulation of transcripts encoding ribosomal proteins that correlated with the memory phenotype. To characterise the potential role of Akt in the regulation of mRNAs that encode the translational machinery, we used an established *in vitro* CD8+ T-cell differentiation model with polysome profiling to quantitate total and actively translated mRNAs from WT and PDK1-K465E OT-1 T-cells. Similar to what we observed *in vivo*, PDK1-K465E cells upregulated the expression of memory-associated genes. However, transcriptional upregulation of mRNAs encoding ribosomal proteins was not recapitulated. These data suggest that upregulation of mRNAs encoding ribosomal proteins is a molecular feature of MPECs and not a direct consequence of diminution of Akt signalling in PDK1-K465E OT-1 T-cells. Our findings demonstrate an essential role for Akt in the generation of a continuum of cell intermediates during effector and memory cell differentiation.

Keywords: Cell signalling, costimulatory pathways, immune regulation and therapy, memory, molecular immunology, RNAseq

OP-046

ABO-group and age are individuals factors associated with specific T-cell memory in long-term immunity after COVID-19

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Since December 2019, the coronavirus disease 2019 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spread throughout the world. To eradicate it, it is crucial to acquire a strong and long-lasting anti-SARS-CoV-2 immunity, either by natural infection or vaccination. Therefore, we studied the durability of the antigen-specific response in recovered COVID-19 subjects and possible individual factors related to this response. We collected blood samples 12 – 305 days after positive PCR from health workers in the General University Hospital Gregorio Marañón in Madrid, infected by SARS-CoV-2 between March and December 2020. Peripheral blood mononuclear cells from whole blood were stimulated with SARS-CoV-2-derived peptide pools related to S-protein, N-protein and M-protein SARS-CoV-2 and antibodies anti-S protein were quantified in plasma. After 10 months post-infection, we observed a strong SARS-CoV-2-specific IFN-γ- and TNF-α-producing CD4+ T-cell responses against M-protein, but a decrease in the frequency of these cells against S- or N-proteins. This decrease was parallel to the diminution of the plasma IgG anti-S antibodies as time passes since the infection in recovered individuals. We demonstrated that age and blood group are pivotal factors for the time for viral clearance. A-group subjects presented higher frequencies of IFN-γ- and TNF-α- CD4+ T-cell responses against Pep-M than O-group individuals. Besides, the A-group subjects needed a more extended period to clear the virus than O-group. Therefore, individual factors might determine the specific cellular responses against SARS-CoV-2 and must be considered for future vaccine designs to reach sustained anti-SARS-CoV-2 immunity.

Keywords: Adaptive immunity, infectious disease, viral infections

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WORKSHOPS

OP-047

Unique cellular sources of CXCR3 ligands direct intranodal T cell positioning and effector/stem-like memory fates following viral infection

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The lymph node microarchitecture is a reticular network which provides a mechanical framework and restricts immune cells into structurally distinct regions. Upon activation, the chemokine receptor CXCR3 is rapidly upregulated on the surface of antigen-specific T cells. CXCR3 binds two ligands in C57BL/6 mice, CXCL9 and CXCL10, which are produced in the cortical ridge, interfollicular and medulla regions of draining lymph nodes and provide chemotactic signals to newly activated CXCR3+ T cells. While it has been established that CXCR3+ T cell repositioning within these peripheral regions is required to mount an optimal immune response, the cellular partners that regulate CXCR3+ T cell location during activation and memory formation are still poorly understood. After viral infection, we demonstrated that CXCL9 and CXCL10 chemokines are produced by distinct DC and stromal cell subsets. We have cleared and imaged intact lymph nodes using lightsheet microscopy to determine cell location. Specifically, we showed that CXCL9 is produced by type 1 conventional DCs (cDC1) in the paracortex whereas CXCL10 is produced by type 2 cDCs (cDC2), inflammatory monocytes and stromal cells at the periphery. We demonstrated that CD8+ effector T cell differentiation is associated with positioning at the lymph node periphery driven by CXCL10, while retention in the paracortex is associated with stem-like memory precursor fate and CCR7 expression. This work highlights the finely regulated choreography of T cell migration and fate following viral infection and inform new vaccine strategies.

Keywords: Cellular interactions, chemokines, lymphoid organs, memory, viral infections, visualizing immune responses

OP-048

Copper homeostasis is required for T cell activation

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Copper is elevated in human sera during both autoimmune and infectious disease. Furthermore, copper chelation has shown promise ameliorating disease in mouse models of autoimmunity and infection. However, the mechanisms driving the impact of copper availability on T cells and the effects of copper metabolism on T cell function are not clear. To investigate the role of copper in the activation of primary T cells, we examined T cell stimulation during low, adequate or elevated copper conditions. We found that copper chelation diminished markers of T cell activation and metabolism, as well as cell size and proliferation. Conversely, copper supplementation increased T cell activation markers and cell size. Notably, we found that expression of copper transporters was upregulated in T cells following stimulation and sensitive to copper availability. Additionally, we identified unique transcriptional profiles in T cells treated with chelation or copper supplementation. Specifically, copper availability impacted expression of co-stimulatory markers, transcription factors, and genes involved in metabolism, cytokine production, and the extracellular matrix. Together, our data demonstrate the critical role copper homeostasis plays in proper T cell activation, and reveal new pathways underlying the impact of copper metabolism on T cell function.

Keywords: Autoimmunity, biology of the immune system, infectious disease, cell signalling, immune regulation and therapy, nutrients

OP-049

Most isotype-switched memory B cells reside in bone marrow or spleen

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Memory B cells are an essential component of the reactive immunological memory. However, it remains unclear how B cell memory is organized and maintained over time. Do memory B cells persist mainly as circulating cells or as resident cells of particular tissues, and if so, which tissues? Here we show that in mice, most isotype-switched memory B cells (Bsm) reside either in spleen or bone marrow, while only about 20% are circulating. Single cell transcriptomes and B cell receptor repertoires reveal an unforeseen diversity with distinct subpopulations of isotype-switched memory B cells (Bsm), allowing us to model trajectories and define potential relationships between particular Bsm subsets. Among the resident populations, a population of marginal zone-like Bsm is located exclusively in the spleen, and a population of quiescent Bsm is located exclusively in the bone marrow. In the bone marrow, Bsm are resting in terms of activation, proliferation and mobility, and are individually docked onto VCAM1-positive stromal cells, reminiscent of resident memory T and in particular also memory plasma cells of the bone marrow. The discrete and resident B cell memory of bone marrow may be key to rapid secondary humoral responses to systemic antigens.

Keywords: Adaptive immunity, B lymphocytes, biology of the immune system, memory

OP-050

The rules of engagement of dendritic cells in thymic cooperative antigen transfer

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The presentation of self-antigens to developing T cells by medullary thymic epithelial cells (mTECs) and thymic dendritic cells (DCs) is essential for the establishment of central tolerance. While mTECs produce antigens in a self-autonomous manner, thymic DCs acquire them from mTECs by the process of Cooperative Antigen Transfer (CAT). Given the heterogeneity of mTEC and DC populations, unraveled recently by single-cell RNA sequencing (scRNAseq), the main objective of this study was to establish the rules underpinning CAT between various mTEC and DC subsets. Several Cre-based mouse models and our scRNAseq data analyses were employed to establish flow cytometry protocols to study the participation of various DC subsets in the acquisition of tdTomato neoantigen from distinct mTEC subsets. Using a mouse model in which RFP or YFP were alternatively expressed in mTECs, we tested whether CAT occurs repetitively. We also analyzed whether neoantigens can be transferred across various DC subsets. We found that in regards to CAT: (i) each DC subset preferentially targets distinct mTEC subsets, (ii) the subset of XCR1+ activated DCs is the most potent subset in CAT; (iii) single thymic DC can acquire antigen repetitively, (iv) the monocyte-derived DCs (moDC) were the most efficient in repetitive CAT, and (v) moDCs represented also the most potent DC subset in the acquisition of antigen from other DCs. Thus, our findings outline some of the basic rules of the distribution of mTEC-derived antigens among distinct populations of thymic DCs.

Keywords: Antigen processing and presentation, cellular interactions, dendritic cells, thymic selection

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WORKSHOPS

OP-051

Tissue-resident B cells orchestrate macrophage phenotype and function in non-lymphoid organs

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B lymphocytes play a central role in humoral immunity but have also antibody-independent functions. Studies to date have focused mostly on B cells within blood and secondary lymphoid organs. Here we sought to address the question of whether B cells reside in non-lymphoid organs (NLOs) in steady state and to determine their phenotype and function. Using intravenous labeling and parabiosis, we identified bona-fide self-renewing, tissue-resident B cells (BTR), that included innate-like CD5+ B-1 cells, across murine lung, liver, kidney and urinary bladder. The size and phenotype of BTRs was influenced by genetic background, age, and microbiome, with their expansion in pet-store mice. Although extravascular B cells had less diverse *Igh* repertoire compared to blood, seeding of these cells into NLOs was independent of their BCR specificity. Using genetically modified mice with higher or lower numbers of BTRs in NLOs, we tested their function in the context of urinary tract infection. The number of BTRs was inversely correlated with bacterial clearance suggesting that these cells negatively regulate anti-microbial responses. We observed that BTRs were spatially co-localized with macrophages and had a profound effect on macrophage polarization, promoting an anti-inflammatory M2 phenotype and that this effect was driven, at least in part, via IL-10. Finally, in human organs we found a similar enrichment for non-naïve B cells compared to blood and spleen. In conclusion, our data identify a critical role for tissue-resident B cells in modulating organ immunity, determining the inflammatory 'set-point' of resident and recruited myeloid cells, with important clinical implications.

Keywords: B lymphocytes, innate host defence, macrophage, microbiome and environmental factors, regulatory cells

OP-052

The RNA-binding protein HuR is essential for maintenance of the germinal centre response

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The germinal centre (GC) is essential for the generation of high affinity antibodies and immunological memory. Nowadays we have a good understanding how transcription factors modulate the GC reaction. However, little is known about the post-transcriptional mechanisms that mediate B cell activation, proliferation, selection and differentiation in GCs. Here we show that the RNA binding protein HuR plays an essential role in GC B cells to sustain the germinal centre response. In its absence, the GC reaction and production of high-affinity antibody is severely impaired. Mechanistically, HuR modulates mRNA splicing and stability shaping, qualitatively and quantitatively, the transcriptome of GC B cells. HuR enables the expression of Myc and a Myc-dependent transcriptional program that is essential for GC B cell proliferation and Ig somatic hypermutation. HuR also controls the expression of mRNAs required for entry into and transition through the S phase of the cell cycle and it modulates a gene signature associated with DNA deamination protecting GC B cells from DNA damage and cell death. In summary, HuR is a key component for the post-transcriptional control of the GC reaction allowing expansion and selection of high-affinity, antigen-specific B cells.

Keywords: Adaptive immunity, B lymphocytes, epigenetic control and modulation of immunity, molecular immunology

OP-053

Identification and characterisation of Hofbauer cell progenitors in the first trimester human placenta

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It is well established that tissue resident macrophages can be derived from either primitive or definitive haematopoiesis during fetal development. Despite this, there is still no way to confidently distinguish between primitive and definitive macrophages without murine fate mapping tools, and little is understood about the precursors which give rise to primitive macrophages in humans. Hofbauer cells (HBC) are a population of macrophages found in the human placenta, which arise as early as 3 weeks gestation. We sought to determine if HBC are primitive macrophages, and to identify the population of progenitors which precede HBC. Transcriptomic comparisons of HBC with other human embryonic macrophages revealed they are most similar to primitive yolk sac macrophages, suggesting that HBC are derived from primitive haematopoiesis. We determined that the lack of HLA-DR expression as an intrinsic feature of primitive macrophages, allowing us to identify them in other fetal tissues, and to show that human fetal macrophage dynamics in the liver and brain match established dynamics in the mouse. We used flow cytometry to identify a population of placenta-resident primitive macrophage progenitors (PMP), found exclusively early in gestation, and used single cell RNAseq to investigate the transcriptional mechanisms which underpin PMP-HBC differentiation. Finally we determined the potential of PMP via single cell differentiation assays, revealing that PMP can give rise to HBC-like cells *in vitro*. Our work provides significant additional insight into the nature and ontogeny of human primitive macrophages, and will help future studies clarify the roles of these cells in early embryogenesis.

Keywords: Fetal immunity, immune development, macrophage, myeloid cells, stem cells

OP-054

Cooperation between chemotherapy and immunotherapy to enhance anti-tumour T-cell mediated immunity in oesophageal adenocarcinoma; implications for synergistic combination regimens

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Combining immunostimulatory chemotherapies with immune checkpoint inhibitors (ICIs) to stimulate anti-tumour immunity and prevent immune-exhaustion is an attractive approach to improve outcomes in oesophageal adenocarcinoma (OAC). Immune checkpoint (IC) expression was profiled on circulating and tumour-infiltrating T-cells pre-treatment and correlated with clinical outcomes in OAC patients(n=16). Immunomodulatory effects of chemotherapy regimens (FLOT,CROSS,MAGIC) on T-cells was investigated following 48h-treatment, via assessment of T-cell activation markers (CD69, ICOS), ICs (PD-1, TIGIT, TIM-3, CTLA-4, LAG-3, KLRG-1, PD-L1, PD-L2), T-cell cytokine profiles (TH1:IL-12, IFN-γ,TNF-α, TH2:IL-4, TH17-IL-17A and Treg:IL-10) and T-cell subsets (naïve, effector and central memory) with/without nivolumab and atezolizumab. The effect of chemotherapy regimens on immunogenic cell death (ICD) in OAC cells was also assessed (calreticulin, HMGB1, MIC-A/B, HLA-DR). ICs were increased on tumour-infiltrating T-cells compared with peripheral T-cells. ICs correlated with subsequent worse treatment response and advanced-staged tumours. FLOT and CROSS increased T-cell activation markers (CD69, ICOS), TH1 and TH17 profiles and also increased several ICs. The frequency of effector memory T-cells was increased by FLOT and CROSS and addition of nivolumab/atezolizumab promoted terminal differentiation. FLOT and CROSS induced ICD in OAC cells. Dual nivolumab-ipilimumab significantly enhanced OAC lymphocyte-mediated killing of OAC cells, which was enhanced in the presence of post-FLOT/CROSS tumour cell secretome *ex vivo*. A link between chemotherapy and immune-resistance is highlighted suggesting that ICIs may enhance the efficacy of chemotherapies in OAC patients. As FLOT and CROSS promoted T cell activation and induced immunogenic cell death in OAC cells these regimens may synergise with ICIs.

Keywords: Adaptive immunity, cancer immunology, checkpoint inhibition, immunotherapy

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WORKSHOPS

OP-055

MicroRNA-142 is a critical regulator of group 2 innate lymphoid cell homeostasis and function

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MicroRNAs function as critical post-transcriptional regulators of gene expression, yet microRNA regulation of Innate lymphoid cells (ILCs) remains incompletely understood. Within the immune system, miR-142 plays numerous roles in the regulation of leucocyte development and function. The aim of our study was to investigate whether miR-142 regulates the development and function of ILCs. Utilising constitutive, conditional, and inducible murine models of miR-142 deficiency, peripheral ILCs as well as bone marrow ILC progenitors were immunophenotyped by flow cytometry. Mixed bone marrow chimeras were used to determine the cell-intrinsic nature of miR-142 effects. Proliferative and effector functions of ILCs were investigated in naïve animals and during infection with *Nippostrongylus brasiliensis*. Candidate gene targets of miR-142 isoforms were investigated using RT-qPCR, functional, and molecular reporter assays. Mice deficient for miR-142 demonstrate an ILC2 progenitor (ILC2p)-biased development in the bone marrow and an altered composition of ILC subsets in mucosal tissues including the lung and intestinal lamina propria. In the absence of miR-142, peripheral ILC2s and BM ILC2ps display a greatly altered surface marker phenotype. MicroRNA-142 deficient ILC2s demonstrate dysfunctional proliferative and effector functions during *Nippostrongylus brasiliensis* infection. Mechanistically, Socs1 and Gfi1 expression are among the molecular targets regulated by miR-142 isoforms in ILC2s MicroRNA-142 plays a critical, cell-intrinsic role in the homeostasis and function of murine ILC2s, impacting ILC2 phenotypes as well as the proliferative and effector capacity of these cells. The identification of these novel pathways opens potential new avenues to modulate ILC2-dependent immune functions.

Keywords: Biology of the immune system, innate lymphoid cells, miRNA, parasite infections

OP-056

NKG2D ligand recognition by γδ T cells drives steatohepatitis and fibrosis in MAFLD

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Metabolic-associated fatty liver disease (MAFLD) is considered to be the hepatic manifestation of metabolic syndrome which encompasses liver abnormalities from simple steatosis to non-alcoholic steatohepatitis (NASH). Because of its increasing prevalence worldwide, MAFLD is the topic of intense research, though most studies concentrate on later stages of disease pathogenesis. To investigate the role of early immuno-metabolic sensing in NASH development, we have developed a dietary mouse model (steatosis-steatohepatitis diet, SSD) that closely resembles human pathophysiology. Upon 16 weeks on SSD, mice develop steatosis, fibrosis and hepatitis. SSD drives the early accumulation of γδ T cells in the liver followed by other immune cells. MAFLD-induced γδ T cells were a major source of local IL-17A, which corresponded with their polarization towards a profibrotic $V\gamma6^{+}CD27^{-}CD44^{+}ROR\gamma t^{+}$ phenotype. TCRγδ-/- and AlbCre IL-17Rfl/fl mice showed strongly reduced immunopathology and alleviated fibrosis in comparison to wild type controls after 16 weeks of SSD feeding. Furthermore, metabolic stress upon SSD feeding upregulates surface expression of NKG2D stress ligands on hepatocytes. Using NKG2D deficient mice, we showed that deficiency of NKG2D receptor on immune cells reduces immunopathology and alleviates fibrosis after 16 weeks on SSD. Importantly, γδ T cells of NKG2D-deficient mice have a decreased capacity to secrete IL-17A. Our study supported by Croatian Science Foundation (IP-2016-06-9306) shows a novel role of γδ T cells as hepatic metabolic stress sentinels and inducers of liver fibrosis via the NKG2D/IL-17A axis in context of NASH. Inhibition of this mechanism is therefore a promising target for prevention of MAFLD progression.

Keywords: Gamma-delta T cells, Immune communication, Metabolic control of immune responses, Tissue damage and repair

OP-057

Inflammation induces CCR5-positive pro-NETotic neutrophils via TNFR2 signaling

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Cytokines released during chronic inflammatory diseases induce pro-inflammatory properties in polymorphonuclear neutrophils (PMN). Here we show that *in vitro* cytokine treatment induces the development of a subgroup of human PMN expressing CCR5, termed CCR5+ cytokine-induced PMN (CCR5+ cPMN). Para- or autocrine TNF signaling increases intracellular neutrophil elastase (ELANE) levels and induces NETosis in CCR5+ cPMN. Triggering of CCR5 amplifies NETosis. Membranous TNF (mTNF) outside-in signaling induces the formation of reactive oxygen species, a known activator of NETosis. *In vivo*, we find an increased number of CCR5+ cPMN in the peripheral blood and inflamed lamina propria of patients with ulcerative colitis (UC) but not Crohn´s disease (CD). Notably, failure of anti-TNF therapy is associated with higher frequencies of CCR5+ cPMN. In conclusion, we identify a phenotype of pro-NETotic, CCR5 positive PMN present in inflamed tissue *in vivo* and inducible *in vitro*. These cells may reflect an important component of tissue damage during chronic inflammation and could be of value in inflammatory bowel disease diagnostics.

Keywords: Cell death, granulocytes, inflammatory bowel disease, innate immunity, neutrophils

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WORKSHOPS

OP-058

Enrichment of bioorthogonal antigens from dendritic cells to study antigen processing on peptide level

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Proteolysis is fundamental to many biological processes. In the immune system, it underpins the activation of the adaptive immune response: degradation of antigenic material into short peptides and presentation thereof on major histocompatibility complexes, leads to activation of T-cells. This initiates the adaptive immune response against many pathogens. Studying proteolysis is difficult, as the oft-used polypeptide reporters are susceptible to proteolytic sequestration themselves. We developed a new approach that allows the imaging of antigen proteolysis throughout the processing pathway in an unbiased manner. By incorporating bioorthogonal functionalities into the protein in place of methionines, antigens can be followed and enriched during degradation in antigen presenting cells. Using this approach, we followed and imaged the in-cell fate of the commonly used antigen ovalbumin and found that our bioorthogonal antigen model has advantage over standardly used fluorophore-modified antigens. Additionally, we studied the effect of post-translational modifications, citrullination and carbamylation, on antigen processing with rheumatoid arthritis auto-antigen vinculin. By enrichment of the antigen and subsequent tandem MS-MS analysis, antigen processing can be studied on peptide level. This revealed differences in antigen processing and presentation of PTM modified vinculin. Citrullination and especially carbamylation protect antigen from processing by proteases, which could have an important role in development of rheumatoid arthritis.

Keywords: Antigen processing and presentation, dendritic cells, mass spectrometry, rheumatoid arthritis

OP-059

T cell stemness during chronic infection is maintained by only a subcompartment of TCF1+ cells

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Exhausted CD8 T cell responses to chronic viral infections or tumors are thought to be maintained in a stem cell-like fashion, with TCF1+ precursors (Tpex) continuously replenishing more terminally-differentiated subsets that retain residual effector functions. However, it is currently unknown whether all Tpex are functionally equivalent or whether additional heterogeneity exists within the TCF1+ compartment. By single-cell RNA sequencing focused selectively on Tpex combined with RNA-velocity analysis of in situ developmental dynamics, we identify a small subcompartment among TCF1+ cells that is localized at the origin of differentiation trajectories. Single-cell adoptive transfer and *in vivo* fate mapping reveals key stem cell features restricted to this subcompartment, like self-renewal and the potency to generate diverse effector subsets, but also stable PD1 expression following population re-expansion. Importantly, these T exhausted stem cells (Tsc-ex) retain increased functionality and restore a higher degree of anti-viral protection to immunocompromised hosts than all other Tpex cells.

Keywords: Adaptive immunity, infectious disease, memory, stem cells

OP-060

IL-10 engineered dendritic cells modulate allogeneic cytotoxic CD8+ T cells response *in vitro*

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The ability to control unwanted immune responses and promote tolerance makes tolerogenic dendritic cells (tolDC) a promising candidate for cell-based approaches in T-cell mediated diseases. We developed human tolDC, producing supra-physiological levels of IL-10 (DCIL-10), by genetically engineer monocyte-derived DC with lentiviral vectors (LV) encoding for IL-10 (LV-IL-10). DCIL-10 promote allo-specific Tr1 cells, modulate allogeneic T cell responses *in vitro* and *in vivo*, and are phenotypically and functionally stable. For therapeutic purposes, the ability of DCIL-10 to modulate $CD8^+$ T cell responses should be unraveled. $CD14^+$ monocytes were transduced with LV-IL-10 and cultured with GM-CSF/IL-4 to generate DCIL-10. DCIL-10 were left unstimulated or stimulated with LPS (DCIL-10a). Allo-$CD8^+$ T cells were co-cultured for 14 days with DCIL-10, DCIL-10a, and DCGFP, and T cell proliferation, the expression of activation/exhaustion markers, cytokine production profile and degranulation were analyzed. DCIL-10 and DCIL-10a negatively modulated $CD8^+$ T cell proliferation and activation (i.e., expression of CD71, CD25 and HLA-DR), and promoted a population of allogeneic $CD8^+$ T cells that secrete low levels of IFNγ and GM-CSF. T cells primed with DCIL-10 and DCIL-10a, upon restimulation with mature DC generated from the same donor, degranulated and produce GrzB but at lower extent compared to control T cells. Our data indicate that stable over-expression IL-10 in human DC lead to a population of cells that modulate cytotoxic allogeneic $CD8^+$ T cell responses, overall indicating that DCIL-10 represent a promising cellular product for future clinical applications aimed to induce tolerance after transplantation.

Keywords: Cell based therapies, dendritic cells, immune regulation and therapy, regulatory cells, transplantation

OP-061

Targeting lipid droplet metabolism in tumor associated macrophages as a novel approach to target tumor progression

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The physiology of tumor microenvironment (TME) plays important role in sketching therapeutic response and resistance, thus justifying recent spur to target components of TME for cancer treatment. Reportedly, the presence of tumor associated macrophages (TAMs) in the TME correlates with all stages of tumor progression and poor prognosis. Numerous metabolites viz lipids, amino acids or iron affect polarization and therefore functional properties of macrophages. Our study aims at investigating the role of lipid droplet mediated fatty acid metabolism in TAMs in modulating the immune response. Macrophages differentiated in presence of various fatty acids and treated with range of small molecular inhibitors targeting lipid metabolism. Cells were analyzed by an array of techniques to study the phenotype and functionality using flow cytometry, fluorescent microscopy, metabolism analysis, T cell suppression assay and adoptive cell transfer in murine tumor models. Fatty acid supplementation is known to modulate monocyte derived macrophage differentiation process; long chain fatty acids mediate their differentiation into immunosuppressive M2-like TAMs. It is known that fatty acids are stored into lipid droplet (LDs) organelles. We identified inhibition of enzymes viz DGAT 1&2 involved in the LD storage path, CPT-1a (fatty acid oxidation enzyme) blocks the polarization of these cells into immunosuppressive macrophages and reduces tumor progression in murine models. Considering fatty acid enriched TME, anti-inflammatory phenotype of TAMs, we propose a model wherein extracellular fatty acids polarize monocytes into M2-like pro-tumoral macrophages. Aiming to identify novel drug targets for LD mediated fatty acid metabolism in macrophages to prevent immune evasion.

Keywords: Cancer immunology, macrophage, metabolic control of immune responses, microenvironment, myeloid cells

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WORKSHOPS

OP-062

Inositol 1,4,5-trisphosphate 3-kinase B promotes Ca2+ mobilization and the inflammatory activity of dendritic cells

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The innate immune response to Gram-negative bacteria depends on several membrane receptors that recognize lipopolysaccharide (LPS), such as CD14 and TLR4. While initially described as a molecule capable of facilitating the activation of TLR4, in dendritic cells (DCs) CD14 also has a role in the calcium-mediated activation of NFAT transcription factors, which results in the production of inflammatory mediators. LPS-induced Ca2+ mobilization in DCs involves the production of inositol 1,4,5-trisphosphate (IP3) by phospholipase C γ2 (PLCγ2) and its subsequent conversion to inositol 1,3,4,5-tetrakisphosphate (IP4) by the ITPKB kinase. Surprisingly, the ITPKB kinase also colocalizes in the plasma membrane with CD14 and IP3R3, the receptor of IP3, that is normally involved in the release of calcium from intracellular storages. Interestingly, IP4 facilitates direct entry of extracellular Ca2+ through IP3R3 and the subsequent activation and nuclear translocation of NFAT. In mice, the inhibition of ITPKB reduces tissue swelling caused by LPS and also the severity of inflammatory arthritis, an effect comparable to the inhibition of NFAT by phagocyte-targeting nanoparticles containing an inhibitory peptide. These results underline the role of ITPKB as a candidate target in DC-specific anti-inflammatory therapies.

Keywords: Cell signalling, dendritic cells, innate immunity

OP-063

TET2/IDH mutations in myelodysplastic syndrome patients disrupts Natural Killer cells biology

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Myelodysplastic syndromes (MDS) are a group of heterogeneous clonal disorders associated with a high risk of progression to acute myeloid leukemia (AML). MDS patients can harbor somatic mutations in epigenetic regulators such as *TET2* and *IDH1/2*. Among immune cells, Natural Killer (NK) cells are key players in charge of eliminating tumor cells but displayed impaired phenotype in AML. However no data are yet available on the impact on NK-cells of the mutation status in MDS patients. We hypothesized that MDS patients with *TET2/IDH* mutations could show particular defective NK cell profile leading to the disruption of their anti-leukemic activity. Blood samples from 63 patients with MDS were collected at diagnosis. Mutational status of blood NK cells was analyzed by NGS. NK cells phenotype, function and methylation landscape have been assessed. Finally, NK-cells treated with HMA have been analyzed. Phenotyping of NK cells showed a loss of KIR2D receptors expression counterbalanced by the increase of NKG2A in TET2mut patients. NGS analysis revealed shared mutations between MDS blasts and NK cells. We unveiled the direct binding of TET2 on the KIR promoter region. Genes involved in NK cell physiology were found hypermethylated in the TET2mut NK-cells. Finally, HMA treatment can restore KIR2D, perforin and granzym B expression in NK-cells. Altogether, our findings showed that *TET2/IDH* mutations altered NK-cell biology in MDS patients. This study highlights the potential dual role of mutations in the emergence of MDS and immune defects which could lead to progression to AML.

Keywords: Cancer immunology, epigenetic control and modulation of immunity, NK cells

OP-064

High dimensional analysis of the human lymph node during melanoma progression reveals shifts in myeloid content that relate to differential T cell content

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The sentinel lymph node (SLN) in melanoma represents the crossroads of the initiation of effector T cell responses and of tumor metastasis. In this study we aimed to characterize the alterations in the human LN immune cell network during melanoma progression as these are of particular interest for the development of effective immunotherapeutic approaches for each disease stage. We used mass cytometry (CyTOF) to characterize the alterations in the major immune populations in the human LN. We included LN derived from healthy donors (n=10), tumor-negative (SLN-, n=7) and tumor-positive SLN (SLN+, n=3) and LN metastatic samples (n=4). Our results show that melanoma progression in the LN is characterized by increased relative frequencies of myeloid cells, B cells and NK whereas T cell rates are significantly decreased. More specifically, during early melanoma metastasis we observed decreased frequencies of migratory and of LN-resident cDC subsets. In fully metastatic LN from patients with advanced melanoma, a clear predominance of monocyte-derived subsets was observed. This was accompanied by an increase in CD4+ Tregs and CD8+ effector T cell subsets. Both T cell subsets in LN metastases were further characterized by relatively high expression of PD-1 and TIGIT immune checkpoints. Our results provide insights into the steady-state immune characteristics of the healthy human LN and identify all the changes that accompany melanoma progression through the different stages, giving important clues about possible therapeutic interventions, aiming at immune potentiation of the SLN.

Keywords: Adaptive immunity, cancer immunology, myeloid cells

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WORKSHOPS

OP-065

Proteomic features reflecting unique properties of human thymus-derived and *ex vivo* regulatory T cells are not present in TGF-beta-induced regulatory T cells

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The immune system harbors diverse regulatory T cells (Treg), which counteract the activity of conventional T cells (Tconv) to prevent autoimmunity. Thymus-derived (t)Tregs recognize self-antigen and acquire stable expression of their master transcription factor FOXP3 in the thymus. Peripherally induced (p)Tregs arise from $CD4^+$ Tconvs in the course of a response to foreign antigen and have less stable FOXP3 expression. In *in vitro* studies, human Tregs are often not clearly defined. This leads to discrepancies in literature, which are further complicated by studies on induced (i)Tregs, a common *in vitro* model of Treg function. These iTregs are generated from Tconvs *in vitro* by addition of TGF-beta that induces FOXP3 expression in TCR-stimulated Tconvs. We recently described that naïve and effector Tregs from human peripheral blood have a common proteomic Treg signature, which sets them apart from $CD4^+$ Tconvs (Cuadrado et al., 2018). This core signature reflects unique Treg properties regarding signal transduction, gene expression and cell metabolism. To determine whether iTregs also acquire such properties, we studied human expanded Tconvs, iTregs and tTregs after TCR/CD28 stimulation by global proteomic analysis. We found that iTregs have a protein expression profile that is mostly distinct from both tTregs and Tconvs. Furthermore, the Treg core signature was present in tTregs but was minimally acquired by iTregs. As iTregs share only a minor proportion of the Treg core signature, these cells may not faithfully represent *in vivo* Tregs and therefore experiments with such cells have limited predictive value for the physiological situation.

Keywords: Adaptive immunity, mass spectrometry, omics technologies, regulatory cells

OP-066

Bryostatin exerts anti-tumor effects by reversing T cell exhaustion

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Cytotoxic T cells are crucial components of adaptive immune response, which are responsible for eliminating infected cells or tumor cells. However, in chronic viral infection and tumors, where antigen persists, T cells develop into a dysfunctional state, known as T cell exhaustion. Reinvigorating exhausted T cell function is one of the main strategies in the development of immunotherapies for the treatment of cancer and other diseases. In order to find a potent agent to restore exhausted T cells, we examined the effect of bryostatin, a novel protein kinase C agonist, on reversing T cell exhaustion *in vitro* and *in vivo*. We found multiple inhibitory receptors were downregulated after bryostatin treatment. Simultaneously, TCF-1, an essential transcription factor in progenitor exhausted T cells, could be maintained. Whereas, Tox, a critical marker of programming T cell exhaustion, was reduced. Furthermore, proliferation capacity of exhausted T cells was enhanced. Most importantly, bryostatin upregulated IFN-γ production by exhausted cells as well as the cytotoxicity effects of target cells. Using the B16-OVA tumor model, we showed that bryostatin treatment inhibited tumor growth. Finally, in chronic HIV infected patients, bryostatin could *in vitro* induce HIV-specific CTL expansion in the absence of antigen. Collectively, we propose that bryostatin could rejuvenate CD8+ T cell exhaustion and potentially works as a novel tumor immunotherapy.

Keywords: Cancer immunology, immune regulation and therapy, immunotherapy

OP-067

Dissecting the transcriptional control of airway macrophages: a key role for EGR2

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The field of macrophage biology has undergone somewhat of a renaissance over the last 10-15 years. Technological advances have led to major paradigm shifts in our understanding of tissue macrophage heterogeneity and ontogeny. In particular, it is now clear that there is a huge degree of diversity between macrophages from different tissues and even between macrophages inhabiting distinct niches of the same tissue. Nevertheless, the environmental factors and transcriptional networks that regulate niche-specific differentiation of macrophages and if and how these change in the context of disease remains poorly understood. In our current work, we have focussed on dissecting the transcriptional control of airway macrophages in health and disease. Here we identify the transcription factor EGR2 as a selective and evolutionarily conserved feature of airway macrophages and show that cell-specific deletion of EGR2 in mice leads to defective airway macrophage differentiation. This results in major phenotypic and functional deficiencies, such as reduced capacity to eliminate bacterial pathogens. Mechanistically, we show that EGR2 is induced by GM-CSF and TGFβ, both of which are abundant in the healthy lung, and lies downstream of the 'master' transcription factor PPARγ. As well as controlling macrophage differentiation in health, we show that EGR2 is indispensable for repopulation of the alveolar niche following inflammation and that monocyte-derived, EGR2-dependent macrophages are vital for effective tissue repair and resetting of airway homeostasis. Collectively, we demonstrate that EGR2 is an indispensable component of the transcriptional network controlling the identity and function of airway macrophages in health and disease.

Keywords: Innate host defence, innate immunity, microenvironment, macrophage, myeloid cells

OP-068

HDAC7 controls CD8+ T cell dependent anti-viral and anti-tumor immunity

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Histone deacetylases (HDAC) orchestrate T cell-dependent immune responses via the epigenetic control of genes and via the post-translational modification of cytoplasmic and nuclear proteins, but the contribution of single HDAC family members to T cell differentiation and function remain elusive. To elucidate the role of HDAC7 in T cells, we assessed the immune cell composition of Hdac7flox/floxCd4-Cre mice by mass cytometry under steady state conditions, which revealed a highly pre-activated phenotype within the CD8+ T cell compartment. During memory recall responses, LCMV-Clone 13 infected Hdac7flox/floxCd4-Cre mice failed to clear viral infection due to decreased expansion and increased cellular exhaustion of virus-specific CD8+ T cells. In chronic antigen exposure models of lymphoma, Hdac7flox/floxCd4-Cre mice failed to control tumor growth of syngenic EG-7 tumor cells, as Hdac7flox/floxCd4-Cre mice harbored significantly lower numbers of tumor-infiltrating CD8+ T cells when compared to WT mice. Adoptive CTL transfer experiments revealed that HDAC7-deficiency results in higher expression of inhibitory receptors including Tim-3 and PD-1 as well as increased apoptosis in tumor infiltrating CTLs pointing to their functional exhaustion. Transcriptomic analysis shows that deletion of HDAC7 reduced the survival of CD8+ T cells, due to the deregulation of amino acid metabolism-, inhibitory checkpoint molecule- and apoptosis-regulating genes, partly via increased FasL expression due to disturbed MEF2D binding patterns on FasL promoter. Therefore, we here identify HDAC7 as a key regulator of CD8+ T cell mediated anti-viral and anti-tumor immunity, which controls the survival, metabolism and the exhaustion of CD8+ T cells during chronic infections and in cancer.

Keywords: Cancer immunology, epigenetic control and modulation of immunity, *in vivo* tumor models, memory

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WORKSHOPS

OP-069

CD8+ T cells require signal 3 from non-antigen-presenting cells for optimal clonal expansion

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During viral infection or vaccination, naive CD8+ T cells (TN) are primed by pMHC (signal 1), costimulatory molecules (signal 2) and cytokines (signal 3) to proliferate and differentiate into protective effector CD8+ T cells (TEFF). While individual activated dendritic cells (DCs) are capable of providing optimal signal 1 and 2 levels to interacting TN, it has remained elusive whether "signal 3" cytokines provided during cognate interactions reach saturating levels for exponential TEFF generation. Lack of progress to address this question was mainly due to the difficulty in assessing the precise TN - DC stoichiometry during priming as basis for a correlation with TEFF generation. Here, we used light sheet fluorescence microscopy of entire reactive lymph nodes with single cell resolution, paired with flow cytometry and intravital imaging, to determine the ratio of DCs and T cells required for exponential TEFF generation in a DC vaccination model. Although long-term intravital imaging revealed continuous interactions of individual TN – DC pairs during priming, a 1:1 ratio did not result in strong TEFF expansion. Instead, exponential TEFF generation required a seven-fold excess of co-transferred non-interacting DCs. Bystander DC-derived IL-12 was required for TEFF expansion by acting primarily on host cells and not on antigen-specific CD8+ T cells. Thus, CD8+ T cells not only integrate signals from DCs they interact with but also require a proinflammatory lymphoid microenvironment to generate a substantial TEFF population, presumably as safeguard to avoid unwarranted TEFF generation by occasional "rogue" activated DCs.

Keywords: Adaptive immunity, cellular interactions, cytokines and mediators, microenvironment, visualizing immune responses

OP-070

Targeting the microtubule-network to improve CTL killing capacity in dense 3D matrices

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Cytotoxic T lymphocytes (CTLs) are the key players to eliminate tumor cells. In solid tumors, dense extracellular matrix (ECM) serves as physical barriers to hinder infiltration and dampen functions of CTLs. However, how the killing capacity of T cells is regulated by dense matrices still remains largely unknown. In this work, we analyzed functional changes of primary human CTLs in dense matrices and the underlying mechanisms. More specifically, among all killing related processes, only CTL migration was reduced in dense matrices, leading to impaired killing capacity. Both the pore size and stiffness of the matrices influence CTL migration. The microtubule-network is a negative regulator for CTL migration in dense collagen matrices. Perturbing microtubule integrity by nocodazole or vinblastine (a chemotherapeutic agent) substantially enhanced killing efficiency of CTLs in dense matrices. Our findings will inspire new strategies for tumor treatment, for example combining microtubule-targeting chemotherapeutic agents with CTL adoptive immunotherapy to treat solid tumors.

Keywords: Adaptive immunity, cytoskeleton, microenvironment, visualizing immune responses

OP-071

Protein tyrosine phosphatase non-receptor type 2 and killer cell lectin-like receptor G1 regulate the generation and function of tissue-resident memory T cells in skin

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Tissue-resident memory T cells (TRM) are key mediators of tissue immunity in the context of infection, inflammation and cancer. Here, we investigated the role of the negative regulator of T cell receptor and cytokine signaling, protein tyrosine phosphatase non-receptor type 2 (Ptpn2), in regulating the formation and function of TRM cells in skin. Adoptively transferred, genetically Ptpn2-deficient (Lck-Cre;Ptpn2fl/fl) CD8+ T cells displayed a marked defect in generating epidermal CD69+ CD103+ TRM cells in response to herpes simplex virus (HSV)-1 skin infection. This was accompanied by an early reduction in the proportion of killer cell lectin-like receptor G1 (KLRG1)-negative memory precursor cells in spleen and skin and an overall transcriptional bias towards terminally differentiated effector cells. Of note, forced expression of wild-type but not signaling-deficient KLRG1 was sufficient to impede skin TRM formation. Normalizing memory precursor frequencies by transferring fixed numbers of *in vivo* or *in vitro* activated KLRG1– effector T cells restored the defect in TRM generation seen for Lck-Cre;Ptpn2fl/fl cells, thereby demonstrating that Ptpn2 impacts memory precursor frequencies rather than downstream TRM differentiation in skin. Importantly, Ptpn2-deficient TRM cells generated in this fashion augmented antigen-driven skin inflammation and mediated superior protection from challenge with HSV-1 infection. Thus, our results reveal an important role for Ptpn2 in the establishment and function of TRM cells in response to skin infection and further emphasize that repression of KLRG1 expression is required for optimal TRM formation in this organ.

Keywords: Adaptive immunity, animal models, memory, skin diseases

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WORKSHOPS

OP-072

Dysregulation of ILC3s unleashes progression and immunotherapy resistance in colon cancer

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Group 3 innate lymphoid cells (ILC3s) critically regulate immunity and inflammation, yet their role in cancer remains elusive. Here, we identify that human colorectal cancer (CRC) manifests with altered ILC3s that resemble those found during intestinal inflammation and are characterized by reduced frequencies, increased plasticity, and an imbalance with T cells. We evaluated the consequences of these changes in mice and determined that a dialogue between ILC3s and T cells via major histocompatibility complex class II (MHCII) is necessary to support colonization with microbiota that subsequently induce type-1 immunity in the intestine and tumor microenvironment. As a result, mice lacking ILC3-specific MHCII exhibit increased progression of CRC and develop resistance to immunotherapy. Finally, humans with intestinal inflammation and dysregulated ILC3s also harbor microbiota that fail to induce type-1 immunity and drive immunotherapy responsiveness when transferred to mice. Collectively, these data define a protective role for ILC3s in cancer, and indicate that their inherent disruption in CRC drives dysfunctional adaptive immunity, tumor progression and immunotherapy resistance.

Keywords: Cancer immunology, immune regulation and therapy, immunotherapy, inflammatory bowel disease, innate lymphoid cells, microbiome and environmental factors

OP-073

Functional characterisation and transcriptomic analysis of antigen-specific CD8 T memory stem cells (TSCM) generated from clonally expanded single TSCM cells *in vitro*

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Memory and effector CD8 T cells differentiate from a single progenitor cell through differentiation, and clonal expansion with asymmetric division. Despite numerous mouse studies exploring the characteristics and determinants of the diverse T cell fates, comparable knowledge in humans is limited. A novel *in vitro* single cell colony expansion protocol for generation of human CD8+ T cell clones specific for Cytomegalovirus was established with three donors using cognate peptide, IL-2/IL-15 and autologous B-LCLs for antigen presentation. Single TSCM progenitor cells had superior colony forming capacity over TCM and TEM, with clonality confirmed by TCR sequencing. Phenotypic and functional characterisation of the single TSCM-derived progeny revealed differentiated memory and effector subsets (TCM, TEM, TEFF) and also TSCM, with the latter subset featuring greater multipotency and multiple cytokine production. Single cell transcriptomic analysis of the varied progeny cells (n=509) revealed strong "progenitor-progeny" effects, reflecting heritable genetic traits specific to subjects and progenitors rather than progeny phenotypes. Nevertheless, the progeny formed distinct transcriptomic clusters with genes such as *Sell*, *Tef1*, and *Il7r* expressed only in the TSCM-derived progeny (n=13 clones; n= 231 cells). Further analysis with the transcriptomic dataset partitioned by: subject, phenotype of the progenitor, and by individual clone, revealed a unique molecular signature and enriched gene sets shared by TSCM-derived TSCM-progeny cells across subjects and clones. The TSCM signature included both previously reported and novel genes and signalling pathways. These data will inform studies seeking to induce TSCM cells by immunisation or utilise them in immunotherapy.

Keywords: Adaptive immunity, memory, RNAseq, stem cells, viral infections

OP-074

IL-21 is a key regulator of chronic germinal centre responses in CTLA-4-deficient mice

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Abundant experimental evidence has linked defective T cell and B cell interactions with the development of autoimmunity, highlighting the importance of further investigations into pathways underpinning T cell and B cell collaboration in health and disease. Elevated levels of the archetypal follicular helper T-cell cytokine interleukin 21 (IL-21) have been reported in many autoimmune conditions including type 1 diabetes, rheumatoid arthritis, and systemic lupus erythematosus, and IL-21 has been widely shown to promote pathology in their corresponding animal disease models. However, the underlying mechanisms of IL-21-dependent regulation of humoral immunity as well as their contribution to the development of autoimmunity remain poorly understood. Previous work by our group has demonstrated that, alongside systemic autoimmunity, mice rendered deficient for T-cell peripheral regulator CTLA-4 present with a significant up-regulation of IL-21 as well as a formation of exacerbated germinal centres (GC). Therefore, our current study used this model to home in on the role of IL-21 in the regulation of chronic GC responses. By studying GC in CTLA-4-/- mice that were crossed to IL-21R-/- animals we were able to demonstrate that IL-21 represents a key regulator of light zone GC B cell selection and that IL-21 signalling is essential for an efficient formation of the GC dark zone compartment. Our study provides novel insights into IL-21 involvement in the regulation of GC processes that are central to the development of robust humoral immunity but that may also aid disease pathogenesis in the context of autoimmunity.

Keywords: Adaptive immunity, autoimmunity, B lymphocytes, cytokines and mediators, follicular helper T cells

OP-075

Insufficient up-regulation of YB-1 leads to failed CD4+ T cell survival of patients with SLE

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The importance of cold-shock protein YB-1 for cell homeostasis is well- documented based on prior observations of its association with certain cancer entities. This study was undertaken to explore the role of YB-1 in T cell homeostasis and survival and the potential contribution of YB-1 to the pathogenesis of systemic lupus erythematosus (SLE). Apoptosis was analyzed in T cells *in vitro* after 6 days of stimulation with anti-CD3–coupled or anti-CD3/anti-CD28–coupled microspheres. YB-1 was overexpressed using lentiviral transduction with wild-type green fluorescent protein (wtGFP) YB-1, and knockdown of YB-1 was achieved using specific short hairpin RNA (shRNA) (3-fold reduction; $P < 0.0001$). Knockdown of YB-1 in T cells consequently led to expression of pro-apoptotic molecules and caspase 3 activation (1.6-fold), and subsequently, to apoptosis. Furthermore, YB-1 promoted survival pathways involving enhanced protein expression of the kinase Akt (2-fold) and Bcl-2 (3-fold), even when Fas/CD95 was triggered. YB-1–mediated T cell survival was reversed by Akt and phosphatidylinositol 3-kinase (PI3K) inactivation. In SLE patients, rescue of YB-1 expression strongly promoted survival of T cells and even prevented cell death in T cells that were extremely apoptosis-prone. Our data show that failure of YB-1 up-regulation in T cells from SLE patients led to enhanced apoptosis. These findings imply that YB-1 plays a crucial role in the disturbed homeostasis of activated T cells leading to hematopoietic alterations in SLE. These insights may help facilitate the development of new treatment strategies for SLE.

Keywords: Autoimmunity, cell death, cell signalling, effector molecules

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WORKSHOPS

OP-076

Normality-sensing licenses local T cells for Innate-like tissue-surveillance

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The increasing implications of lymphocytes in basic physiologic processes ranging from dietary regulation to memory raise major questions. Specifically, how might antigen receptors be involved, and how does tissue immune-surveillance distinguish perturbed self from normal self? Here we show in steady-state mouse skin that macromolecular aggregates of intraepidermal γδ T cell receptors (TCRs) both align with and depend upon Skint1, a butyrophilin-like protein expressed by differentiated keratinocytes. Interrupting such TCR-mediated "normality-sensing" did not obviously compromise intraepidermal T cell maintenance, but it altered the cells' phenotype while concurrently dysregulating keratinocyte gene expression and impairing epithelial barrier function. Strikingly, it also limited the capacity of intraepidermal T cells to employ innate TNF-receptor superfamily members to respond rapidly to subsequent tissue perturbation, specifically ultraviolet irradiation. This resulted in atypically sustained DNA damage and inflammation, two cancer-disposing factors. Hence, by constitutive TCR-mediated normality-sensing, mature intraepithelial T cells are licensed to provide innate-like tissue-surveillance.

Keywords: Adaptive immunity, cancer immunology, gamma-delta T cells, Immune communication, RNAseq, tissue damage and repair

OP-077

Relevance of Th17-specific epigenetic signature genes for Th17 differentiation and function

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In recent years, it became evident that T helper (Th) 17 cells, amongst the defined CD4+ Th cell subsets, show a high degree of plasticity and flexibility. A genome-wide methylome analysis carried out in our laboratory confirmed these findings by demonstrating that murine *ex vivo* isolated Th17 cells display the highest number of demethylated regions and share more demethylated regions with naïve CD4+ T cells when compared to Th1 cells. Yet, we also could identify a set of genes that were selectively demethylated in Th17 cells. Amongst the genes that belonged to this newly defined Th17-specific epigenetic signature were *Acsbg1* and *Zfp362*, which both were not reported to be involved in Th17 differentiation and function before. *Acsbg1* (Acyl-CoA Synthetase Bubblegum Family Member 1) plays a central role in fatty acid metabolism by activating long-chain fatty acids. Since T cell differentiation and functionality relies on fatty acid metabolism, *Acsbg1* might be of relevance for Th17 cell development and function. *Zfp362* (Zinc finger protein 362) is believed to act as a transcription factor, however, experimental data on the function of this molecule are completely lacking. Because of their selective demethylation in Th17 cells and the lack of knowledge about their functional role in these inflammatory T cells, we have recently generated conditional knock-out mouse models for both genes and are currently studying the relevance of these genes for the development and function of Th17 cells *in vitro* and *in vivo*.

Keywords: Adaptive immunity, epigenetic control and modulation of immunity, immune development

OP-078

Termination of CD40L signaling drives human naïve B cell differentiation into antibody-secreting cell formation

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Human naïve B cells are notoriously difficult to differentiate into antibody-secreting cells (ASCs) *in vitro* while maintaining enough cell numbers to evaluate the differentiation process. Insights in factors controlling the T cell-dependent differentiation of B cells into ASCs are much needed in order to understand the generation of effective humoral immunity against invading pathogens or to prevent undesired antibody formation in autoimmunity and blood transfusion. B cells require T follicular helper (TFH) cell derived signals like CD40L and IL-21 during the germinal center (GC) response in order to undergo ASC differentiation. However, the cognate interaction between B and TFH cells are short; after TFH contact, B cells migrate away for renewed proliferation to GC dark zones where TFH cells and thus stimulation, is absent. Here we elucidated that enforced termination of CD40L-CD40 stimulation strongly promotes naïve B cell-to-ASC differentiation *in vitro*. Our data show that efficacy of naïve B cell differentiation is dependent on release of CD40 stimulation and is dramatically induced in the appropriate cytokine environment. Using multiparameter phospho-flow and transcription factor (TF)-flow cytometry we show that CD40L blocking, after initial CD40L and IL-21 stimulation, regulates the kinetics of the NF-κB and STAT3 pathways yielding downregulation of the B cell signature TF PAX5 and promoting ASC TFs BLIMP1 and XBP-1s. Our data are the first steps to provide further insights in the regulation of human naïve B cell differentiation to ASCs. This is crucial in improving vaccination strategies and will also aid in the prevention and treatment of autoimmunity.

Keywords: Adaptive immunity, antibody, B lymphocytes, cell signalling, costimulatory pathways, cytokines and mediators

OP-079

Evolution of the SARS-CoV-2 specific B cell and antibody repertoires over time

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The SARS-CoV-2 coronavirus has caused a pandemic for more than a year. Although much effort has been placed in understanding the humoral immune response against SARS-CoV-2, it remains unknown which SARS-CoV-2-specific B-cells and antibodies remain in circulation over time and contribute to long-lasting immunity.

We studied evolution of multiple co-evolving SARS-CoV-2-specific B-cell clones in longitudinal (4 and 8 weeks post symptom onset) blood samples of three RT-PCR-confirmed COVID-19 patients, using high-throughput adaptive immune receptor repertoire sequencing (AIRR-seq) of both single cell and bulk sorted B-cell populations (IgD+CD27-, IgD-CD27±CD38-, and IgD-CD27+CD38+), together with systems phylogeny analysis. Subsequently, we integrated proteomics of affinity purified nucleocapsid and spike protein antibodies in serum, with the CDR-H3 AIRR-seq repertoire. This provides us with insights into antigen specific clonal diversity, selection, and expansion and the relation between the cellular B-cell receptor and plasma Ig-repertoires. SARS-CoV-2-specific B-cell clones were identified for all patients, of which only a small fraction spanned multiple time points. A substantial proportion of these lineages consists of IgM+, near-germline sequences. Interestingly, only small overlap between the cellular AIRR-seq and plasma Ig- SARS-CoV-2-specific repertoires was found for all patients. In addition, few of the SARS-CoV-2-specific antibody peptide fragments mapped to CDR-H3 sequences of other COVID-19 patients and from the Coronavirus Antibody Database. This indicates that a number of antibodies responsive are shared between patients (public clones). In conclusion, our data indicates that the convalescent plasma Ig response to SARS-CoV-2 is oligoclonal, and that only a limited set of antigen-specific B-cells secrete SARS-CoV-2-specific antibodies.

Keywords: Adaptive immunity, antibody, B lymphocytes, mass spectrometry, RNAseq

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WORKSHOPS

OP-080

High-dimensional flow-cytometry and functional evaluation reveals enhanced potency of stem cell-derived dendritic cell bulk vaccination to boost T cell immunity

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Allogeneic stem cell transplantation can be curative for hemato-oncology patients due to powerful graft-versus-tumor immunity. However, relapse remains the major cause of treatment failure. Therefore, adjuvant immunotherapy using dendritic cell (DC) vaccination is highly attractive, as DCs are key orchestrators of innate and adaptive immunity. We developed a clinically applicable culture protocol for large-scale generation of naturally occurring blood DC-subsets from donor-derived stem-cells, including conventional type 1 DCs (cDC1s), cDC2s and plasmacytoid DCs, accounting for approximately 35% of the end-product. Vaccination with the total cultured end-product, also containing the so-called non- DCs, would be highly attractive as clinical translation will be easier and the production process faster and cheaper. Here, using high-dimensional flow-cytometry we characterized the phenotype of the non-DC compartment, and functionally evaluated DC activity in the presence of non-DCs. Flow-cytometry analyses revealed that the non-DC compartment comprises a mixture of CD34+ cells, immature myeloid cells, early DC progenitors and CD14+ cells. Functionally, the presence of non-DCs during toll-like receptor-mediated DC maturation enhanced the expression of co-stimulatory molecules CD80 and CD86, and production of pro-inflammatory cytokines IL-12p70 and IFN-α. Additionally, DC-mediated expansion of antigen-specific T-cells was highly increased in the presence of non-DCs. More importantly, *in vivo* expansion of antigen-specific T-cells using bulk-vaccination was significantly higher after two vaccination cycles compared with cDC1 -or cDC2-vaccination. Together, this data demonstrates that the presence of non-DCs does not hamper, but could even enhance the potency of our stem-cell-derived DC-vaccine, meaning that total bulk-vaccination can be translated towards clinical application for hemato-oncology patients.

Keywords: Adjuvants and vaccines, anti-cancer vaccine, dendritic cells, immunotherapy

OP-081

Systemic hypoxia inhibits T cell response by limiting mitobiogenesis via matrix substrate-level phosphorylation arrest

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Systemic oxygen restriction (SOR) is prevalent in numerous clinical conditions, including chronic obstructive pulmonary disease (COPD), and is associated with increased susceptibility to viral infections. However, the influence of SOR on T cell immunity remains uncharacterized. Here we show the detrimental effect of hypoxia on mitochondrial-biogenesis in activated mouse CD8+ T cells. We find that low oxygen level diminishes CD8+ T cell anti-viral response *in vivo*. We reveal that respiratory restriction inhibits ATP-dependent matrix processes that are critical for mitochondrial-biogenesis. This respiratory restriction-mediated effect could be rescued by TCA cycle re-stimulation, which yielded increased mitochondrial matrix-localized ATP via substrate-level phosphorylation. Finally, we demonstrate that the hypoxia-arrested CD8+ T cell anti-viral response could be rescued *in vivo* through brief exposure to atmospheric oxygen pressure. Overall, these findings elucidate the detrimental effect of hypoxia on mitochondrial-biogenesis in activated CD8+ T cells, and suggest a new approach for reducing viral infections in COPD.

Keywords: Viral infections, adaptive immunity, metabolic control of immune responses

OP-082

Mobilization of tissue-resident memory CD4+ T lymphocytes and their contribution to a systemic secondary immune reaction

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While it is generally accepted that tissue-resident memory T lymphocytes (TRM) protect host tissues from secondary immune challenges, it is less clear whether, and if so, how they contribute to systemic secondary immune responses.Determine the contribution of CD4+ TRM to a systemic secondary immune reaction induced by the measles-mumps-rubella (MMR) vaccine in humans that have an established immunological memory to measles, mumps and rubella viruses. Human subjects, MMR or diphtheria-pertussis-tetanus (DTaP) vaccination, *ex vivo* and *in vitro* studies, including FACS, MACS, ELISA, isolation, expansion and re-stimulation of antigen-reactive memory CD4+ T cell lines, T cell receptor Vβ gene sequencing, reduced representation of bisulfite sequencing, and computational analysis. CD4+ TRM are mobilized into the blood within 16 to 48 hours after vaccination, and then they disappear again, until their progeny reenters circulation from day 4 onwards. The mobilization of TRM is cognate: memory CD4+ T lymphocytes that recognize other antigens, e.g. tetanus toxin, are not mobilized, unless they cross-react with the vaccine. A prominent fraction of newly generated circulating memory CD4+ T cells on day 14 after vaccination express T cell receptor Vβ clonotypes of TRM mobilized on day 1. Notably, mobilized TRM display an epigenetic signature pointing to their former residency in the bone marrow. Tissue-resident memory T cells, here shown for human bone marrow-resident memory CD4+ T cells, are mobilized into circulation and contribute significantly to secondary systemic immune responses against anti-viral vaccines.

Keywords: Adaptive immunity, immune response tracing, memory, monitoring immunity

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WORKSHOPS

OP-083

Investigating the molecular and functional interaction between human macrophages and HiPSC-derived cardiomyocytes by an *in vitro* arrhythmia model

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Recent studies demonstrated a novel role for macrophages in the modulation of cardiac electrophysiology besides their canonical functions. However, the potential role of macrophages in the steady-state or in arrhythmia is largely unknown. To investigate cardiomyocyte-macrophage interactions at the molecular and functional level, we generated human iPSCs-derived cardiomyocytes (hiPSC-CMs) and monocyte-derived macrophages (MDMs) from healthy individuals and congenital arrhythmia patients. The characterization of differentiated cardiomyocytes and macrophages in single cultures or co-cultures was performed comparatively by qRTPCR analysis and immunolabeling for specific markers, such as troponin-T, CD68, CD206, etc. The physical interactions between MDMs and hiPSC-CMs in co-cultures were detected by membrane-staining by WGA. To assess the polarization of MDMs in single or co-cultures, the mRNA expressions of M0 (CD68, CD11B) and M1/M2 (IL-1B, HLA-DRB1, IL-10) markers were analyzed. There were no significant differences in polarization of control or patient MDMs, both exhibiting a naive phenotype *in vitro*. Functionally, Ca2+ transients and contraction rates of patient-derived hiPSC-CMs were found to be statistically higher than those of control hiPSC-CMs confirming the tachycardia phenotype. Interestingly, the presence of MDMs from any origin in co-cultures with healthy hiPSC-CMs significantly increased the contraction rates, demonstrating the positive chronotropic effect of macrophages on cardiomyocytes. Importantly, the interaction with both control and patient-derived MDMs statistically decreased the contraction rates and enhanced rhythmicity of patient hiPSC-CMs, indicating amelioration of the diseased phenotype. Consequently, our results indicate for the first time the functional modulation of macrophages on healthy or arrhythmia-derived iPSC-CMs in a physiologically relevant *in vitro* model.

Keywords: Cardiovascular diseases, cellular interactions, macrophage

OP-084

Distinct roles of Foxp3+ regulatory T cell sublineages in adipose tissue homeostasis and obesity

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While their key role in maintaining immune homeostasis has been firmly established, Foxp3$^+$ regulatory T (Treg) cells have also been implicated in the control of adipose tissue (AT) metabolism, although the underlying mechanisms have remained poorly understood. Here, we have revisited the role of Foxp3$^+$ Treg cells in AT homeostasis, employing the high-fat diet model of obesity and the Foxp3.RFP/GFP mouse line with differential fluorochrome-reporter expression in Foxp3$^+$ Treg cells of thymic (RFP$^+$GFP$^+$: tTreg) and peripheral (RFP$^+$GFP$^-$: pTreg) developmental origin. In otherwise non-manipulated Foxp3.RFP/GFP mice, we found that tTreg and pTreg cells undergo AT-specific and highly dynamic changes in numbers and phenotype during the obesity-mediated transition from type 1 to type 2 immunity. In complementary studies, we took advantage of newly developed Foxp3.RFP/GFP mouse lines, which were genetically modified such that they were selectively deficient in tTreg ('pTreg only') or pTreg ('tTreg only') cells. Unexpectedly, these studies indicated that AT-resident Treg cells can either promote metabolic homeostasis or exacerbate local inflammation and obesity, depending on their developmental origin.

Keywords: Diabetes, immune response tracing, regulatory cells

OP-085

Autoantibodies isolated from systemic sclerosis patients targeting the angiotensin II type 1 and endothelin-1 type A receptor induce endothelial cell activation and pro-fibrotic cellular responses

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Systemic Sclerosis (SSc) is a heterogenous and potentially lethal autoimmune disease hallmarked by dysregulated immunity, vasculopathy and fibrosis. Autoantibodies directed against the angiotensin II type 1 receptor (AT1R) and endothelin-1 type A receptor (ETaR) are present in most patients with SSc and are associated with more severe disease complications. AT1R and ETaR are expressed by endothelial cells (ECs), providing a potential direct link between two major pathophysiological features in SSc. Here, we aim to identify and understand autoantibody-induced endothelial cell damage in SSc. IgG of SSc patient and healthy controls (HC) was isolated and AT1R- and ETaR-mediated effects on ECs were analyzed using specific receptor antagonists. Cytokine responses induced by SSc-derived AT1R and ETaR autoantibody binding to ECs were measured by IL-6, Il-8 and TGF-β ELISA. EC activation was measured by RT-qPCR. We identified that SSc-derived IgG induced upregulation of the EC activation markers MCP-1, ICAM-1 and E-selectin compared to control IgG in an AT1R- and ETaR-mediated fashion. SSc-derived IgG induced AT1R- and ETaR-mediated expression of IL-6, IL-8 and TGF-β, which was not observed with HC IgG. Although higher levels of both autoantibodies were associated with a stronger EC responses, we did not observe EC activation in all patients with AT1R and ETaR antibodies. AT1R- and ETaR-targeting autoantibodies impact ECs resulting in EC activation and pro-inflammatory and pro-fibrotic cytokine release. We have identified a direct pathophysiological mechanism linking AT1R- and ETaR-targeting autoantibodies to EC activation and pro-fibrotic cellular responses in SSc.

Keywords: Antibody, autoimmunity, cytokines and mediators, immune communication

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WORKSHOPS

OP-086

Lack of NFATc1 SUMOylation prevents autoimmunity and alloreactivity

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Post-translational modification with SUMO is known to regulate the activity of transcription factors, but how SUMOylation of individual proteins might influence immunity is mostly unexplored. The NFAT transcription factors play an essential role in antigen receptor-mediated gene regulation. SUMOylation of NFATc1 represses IL-2 *in vitro*, but its role in T cell-mediated immune responses *in vivo* is not clear. To this end, we generated a novel *Nfatc1* transgenic mouse, which prevents SUMO modification of NFATc1. Avoidance of NFATc1 SUMOylation ameliorated experimental autoimmune encephalomyelitis as well as graft-versus-host disease. An elevated IL-2 production promoted Treg expansion and suppressed autoreactive or alloreactive T cells. Mechanistically, increased IL-2 secretion counteracted IL-17 and IFN-γ expression through STAT5 and Blimp-1 induction. Then, Blimp-1 repressed IL-2 itself and the as well induced, proliferation-associated survival factor Bcl2A1. Collectively, we demonstrate that prevention of NFATc1 SUMOylation fine-tunes T-cell responses towards lasting tolerance. Thus, targeting NFATc1 SUMOylation presents a novel and promising strategy to treat T cell-mediated inflammatory diseases.

Keywords: Autoimmunity, inflammatory disease, multiple sclerosis

OP-087

Deciphering the role of cDC2 in Sjögren's Syndrome: transcriptomic profile reveals an enhanced antigen uptake and altered antigen processing associated with IFN-signature

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Primary Sjögren's syndrome (pSS) is an autoimmune disease characterized by lymphocytic infiltration of the salivary glands and prominent B cell hyperactivity. Type 2 conventional dendritic cells (cDC2s) can activate T and B cells, leading to germinal centre formation and autoantibody production. To understand the mechanisms underlying cDC2 function in pSS, we performed RNA-sequencing analysis and functional validation on circulating cDC2s from pSS, non-Sjögren's sicca (nSS) patients and healthy controls (HC). Two independent cohorts were established to identify reproducible gene signatures dysregulated in pSS-cDC2s, which included the interferon (IFN)-signature and antigen processing and presentation pathways. We confirmed by flow cytometry that pSS-cDC2s displayed a higher antigen uptake and altered antigen processing both linked with the presence of anti-SSA autoantibodies. As the majority of the SSA^+ pSS patients exhibit an IFN-signature, we tested whether IFN-α priming of cDC2s, would impact their antigen-uptake capacity. IFN-α priming of HC-cDC2s increased their uptake capacity to levels comparable with SSA^+ pSS-cDC2s. Additionally, pSS-cDC2s showed an increased uptake capacity of apoptotic salivary gland epithelial cells. Furthermore, pSS-cDC2s increased the proliferation of HC $CD4^+$ T cells and the expression of CXCR3 and CXCR5 on the proliferating HC $CD4^+$ T cells, contributing to T cell migration into the inflamed salivary glands. Here we provide the first in-depth molecular characterization of pSS-cDC2s and show that transcriptomic and functional alterations observed in pSS-cDC2s are linked to IFN-signature. In view of its role in pSS immunopathology, delineating the molecular networks that drive cDC2s holds promise for targeting these cells in pSS.

Keywords: Adaptive immunity, antigen processing and presentation, autoimmunity, dendritic cells, innate immunity, RNAseq

OP-088

Cell-derived vesicles present in human milk dampen innate and adaptive immune responses and enhance epithelial barrier function

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Human milk plays a role in the development of the infant's gastrointestinal tract and the immune system. Extracellular Vesicles (EVs) are cell-derived vesicles used for cell-cell communication. Although EVs are general milk components, their role in developmental processes has been poorly studied. We explored the molecular mechanism of milk EV-induced modulation of different cell types present in the oral mucosa. Human milk EVs were purified by differential centrifugation, density gradient floatation and size exclusion chromatography. Effects of milk EVs on epithelial barrier function, Toll-like receptor triggering and T cell activation was studied using different (reporter)cell lines and primary T cells. Functional integrative proteomic analysis was performed based on our previously identified human milk EV proteome. Milk EVs promote migration of oral epithelial cells resulting in enhanced re-epithelialization in a gap closure assay. Functional integrative proteomic analysis unveiled hotspots of regulation in the p38 MAPK pathway targeted by milk EV proteins. Milk EVs also inhibited agonist-induced endosomal Toll-like receptor 3 (TLR3) triggering of these oral cavity epithelial cells, which coincided with reduced full length and cleaved cellular TLR3 levels and inhibition of TLR3 mRNA. Furthermore, milk EVs inhibited αCD3/αCD28-induced CD4+ T cell activation, which could be linked to the presence of EV proteins targeting hotspots of regulation downstream CD28, resulting in cell-cycle inhibition and mTOR stimulation. EVs are bioactive structures of human milk that can modulate canonical signal transduction pathways involved in key processes in the development of the epithelial barrier and the immune system of the infant.

Keywords: Biology of the immune system, cell signalling, immune development

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WORKSHOPS

OP-089

Bacterial lysate enhances protective mucosal immunity via increased expression of antimicrobial peptides

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Polyvalent mechanical bacterial lysate (PMBL) has been reported to be effective in the prevention of common respiratory tract infections. The mechanism of action of PMBL is not yet fully understood. Respiratory tract epithelial cells constitute a frontline physical barrier between the organism and the environment. They are able to sense pathogen associated molecular patterns and secrete a wide spectrum of protective factors. By using primary normal Human Bronchial Epithelial Cells (HBEpiCs), we observed that PMBL can improve epithelial barrier integrity via induction of adhesion molecules. Also, PMBL had a significant effect on epithelial cell proliferation increasing the expression of the autocrine growth factor Amphiregulin (AR). Moreover, treatment with PMBL promoted ex-novo gene expression of human beta-defensin 2 (HβD-2) on HBEpiCs and conferred them a direct antimicrobial activity. Epithelial cells are structural components of mucosal immunity that include also dendritic cells (DCs) and innate lymphoid cell-type 3 (ILC3s). PMBL induced IL-23 and IL-1β secretion by DCs that, in turn, activate ILC3s to produce IL-22, cytokine primarily involved in the induction of antimicrobial peptides (AMPs). Interestingly, IL-23 produced by DCs can also activate epithelial cells and lead to a boost of IL-22 production by ILC3s. Remarkably, HβD-2 and LL-37 AMPs can be induced in the saliva of normal subjects after administration of PMBL. Altogether, these results indicate that PMBL administration might support a critical barrier-protective immune pathway that originates from, and is orchestrated by airway epithelial cells and could be therapeutically exploited for the prevention of airway infections.

Keywords: Effector molecules, innate host defence, innate immunity

OP-090

An integrated multi-omics view on human T cell development

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T cells, the main effectors of the adaptive immune system, originate from thymocyte precursors in the human thymus. These immature cells follow a tediously regulated differentiation process with several critical checkpoints to ensure the generation of a diverse repertoire of fully functional, non-self T cells. In previous studies, we already uncovered part of the regulatory mechanisms that drives this differentiation process in human, which is notably different compared to mouse (De Decker et al., 2020; Dolens et al., 2020; Lavaert et al., 2020; Roels et al., 2020a; Roels et al., 2020b). However, a lot of the regulatory complexities remain unknown. By profiling additional layers of these discrete stages of thymocytes, we here aimed to generate the most complete integrative multi-omics reference map of human T cell development so far. By using a multi-omics factor analysis approach, we combined transcriptomics, epigenomics, proteomics and finally 3D genomics into one model to fully describe the T cell developmental process. We found that human T cell development is equally affected by previously unappreciated drivers, including non-coding and circular RNA, alternative splicing and DNA methylation. While some omics layers show global and genome-wide changes within developing αβ and γδ T cells, the global 3D structure remains very stable throughout all differentiation states. Nonetheless, specific alterations do occur at developmentally important genes, especially at the boundaries of active and inactive compartments. This integrative analysis aids our understanding of immune cell development both in health and disease, and reveals important aspect of lineage differentiation dynamics.

Keywords: Adaptive immunity, epigenetic control and modulation of immunity, gamma-delta T cells, immune development, omics technologies

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WORKSHOPS

OP-091

NKG2A and HLA-E define an additional immune checkpoint axis in bladder cancer

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Anti-PD-1/PD-L1 treatment was recently approved for the treatment of bladder cancer achieving a response rate of 17-26%, thereby suggesting additional mechanisms of resistance. Here we evaluated the potential role of the HLA-E/NKG2A inhibitory pathway in modulating T cell immunity. Mass cytometry was performed *ex vivo* on CD8$^+$ T cells from bladder tumors (n=6), as well as on expanded CD8$^+$ T cells from bladder-draining lymph nodes (n=11) and tumors (n=8). Flow cytometry (n=25) and single-cell RNA sequencing (scRNAseq) (n=13) were performed on cells from fresh bladder tumors. Mechanisms of tumor escape from CD8$^+$ T cell recognition can include impairment of antigen presentation. Accordingly, we found significant reduction of HLA class I expression but retained expression of NK-activating ligands on bladder tumors (e.g. CD112/ CD155/MICA/ULBPs) indicating possible retention of NK cell activation pathways. Using mass cytometry and scRNAseq, we found acquisition of the inhibitory NKG2A receptor on tumor-derived PD-1$^+$CD8$^+$ T cells promoting NK-activating and tissue-residency features alongside diminished CD28 expression and significantly weaker sensitivity to CD3/CD28-signaling. Co-culture with HLA class I-deficient K562 cells activates NKG2A$^+$CD8$^+$ T cells whereas K562 stably transfected with HLA-E maintain inhibition. HLA-E$^+$ bladder tumors render NKG2A$^+$ CD8$^+$ T cells dysfunctional, through HLA-E/NKG2A-mediated inhibition. However, their TCR-/CD28-independent NK-like function is restored with NKG2A-blockade.NKG2A/HLA-E checkpoint axis inhibits CD8$^+$ T cells in head and neck cancer and can be reversed with NKG2A-blockade. Our results describe an analogous mechanism of immune evasion in bladder cancer, providing a strong rationale for NKG2A blockade therapy in bladder cancer.

Keywords: Adaptive immunity, cancer immunology, checkpoint inhibition, immune regulation and therapy, immunotherapy, MHC and polymorphic genes

OP-092

CCR7-guided neutrophil redirection to skin-draining lymph nodes regulates cutaneous inflammation and infection

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Neutrophils are the first effector immune cells to recruit to a site of infection or inflammation, however, uncontrolled neutrophil responses can often cause significant tissue damage. Here, we find that a subset of skin-homing neutrophils express higher levels of C-C-motif chemokine receptor 7 (CCR7), which regulates neutrophil migration to and subsequent clearance in the skin-draining lymph nodes (dLNs). In mouse models of cutaneous *Staphylococcus aureus* infection and Toll-like receptor-induced skin inflammation, neutrophils migrate, in a CCR7-dependent manner, from skin via lymphatic vessels to dLNs where they are phagocytosed by resident phagocytes. Furthermore the immune cells involved in neutrophil clearance produce lower levels of inflammatory cytokines. Disruption of this mechanism by selective CCR7 deficiency leads to increased anti-*staphylococcal* immunity but also aggravates skin inflammation. In summary, neutrophil migration to and elimination in the skin-dLNs regulates cutaneous immunity versus pathology.

Keywords: Innate immunity, lymphoid organs, myeloid cells, neutrophils, phagocytosis, skin diseases

OP-093

Retaining memory: virtual memory CD8+ T cells as an adaptive mechanism of the ageing immune system

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Naïve T cell immunity is impaired during ageing, which impinges immune responses reliant on diversity, including those necessary for efficient vaccinations. As the deterioration of the naïve T cell repertoire is accompanied by an accumulation of virtual memory CD8$^+$ T cells (TVM cells), we aimed to investigate the extent of their contribution to immunity in the elderly. Using CD8$^+$ T cells from P14 young and aged mice, we initially characterized that TVM cells (1) still proliferate upon TCR activation, (2) rely on both glycolysis and OXPHOS and (3) retain their ability to undergo asymmetric cell division, a mechanism known to contribute to T cell memory formation. In adoptive transfer experiments, these features resulted in better survival and re-expansion upon LCMV infection in comparison to their naïve counterparts. Interestingly, the memory pool generated by TVM cells consisted mostly of KLRG1$^+$ CD127$^+$ cells, in contrast to the typical KLRG1$^-$ CD127$^+$ memory derived from naïve T cells. Thus, we compared the transcriptional profile of these two distinct memory populations by RNAseq and observed a unique mixed effector-memory signature in TVM-derived memory cells. Of relevance, these cells were able to survive in absence of antigen and build new memory responses upon antigenic re-challenge. Our results suggest that TVM cells are metabolically adapted to swiftly respond to environmental changes, and might represent an adaptation of the ageing immune system to maintain a memory-like pool of cells in absence of previous antigen encounter, while not compromising effector responses.

Keywords: Ageing, biology of the immune system, immune senescence, memory, visualizing immune responses

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WORKSHOPS

OP-094

Role of Treg/Th17 imbalance at the immunity or tolerance in myasthenia gravis

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T cell-dependent autoantibody production and thymic pathologies are the main features of myasthenia gravis (MG). An increased T helper 17 (Th17) cell activity and dysfunctional regulatory T (Treg) cells have been demonstrated in subgroups of MG. On the other hand, Hypoxia-inducible factor 1 (HIF-1) as a metabolic sensor is implicated in regulating the balance between Treg and Th17 differentiation. We sought evidence for the imbalance of Th17 and Treg cells by expression analysis in thymic tissues. RNA was extracted from thymic tissues of 13 thymoma-associated myasthenia gravis (TAMG), all type B2, 12 thymoma without MG (THY), with type AB, B2 and B1 and 12 MG with hyperplasia (HP-MG) patients. All MG patients had acetylcholine receptor autoantibodies and did not receive immunosuppressive treatment. Relative expressions of TDT, CTLA4, FOXP3, GITR, HIF1A, IL21, RORC, CCR6, IL6, and IL2 were analyzed using RT-PCR. HIF1A was significantly higher in TAMG in comparison to HP-MG (0.82 vs. 0.37, p = 0.03). RORC showed a trend for higher expression in TAMG compared to HP-MG group which may also indicate Th17 pathway activity. IL2 was significantly lower in TAMG compared to HP-MG (1.08 vs. 3.24, p = 0.007) and lower than in THY. The results demonstrated differences probably related to metabolic changes in thymic pathologies. Increased HIF1A implicates Th17 lineage involvement by promoting inflammation through RORγt activation and may contribute to Treg/Th17 imbalance. Lower IL2 expression supports the impairment of Treg maturation in TAMG.

Keywords: Autoimmunity, molecular immunology, neuroimmunology

OP-332

Identification of a CD106+ pericyte stem cell leading to Ly6G+ cell accumulation responsible for resistance to immunotherapy in pancreatic cancer

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We report the identification of a cell population that shares pericyte, stromal and stemness features, does not harbour the KrasG12D mutation and drives tumoral growth *in vitro* and *in vivo*. We termed these cells pericyte stem cells (PeSCs) and defined them as CD45-EPCAM-CD29+CD106+CD24+CD44+ cells. We performed studies with p48-Cre;KrasG12D (KC), pdx1-Cre;KrasG12D;Ink4a/Arffl/fl (KIC) and pdx1-Cre;KrasG12D;p53R172H (KPC) and tumor tissues from PDAC and chronic pancreatitis patients. We also performed single cell RNAseq analysis and revealed a unique signature of PeSC. Under steady-state conditions, PeSCs were barely detectable in the pancreas but present in the neoplastic microenvironment in both humans and mice. The co-injection of PeSCs and tumor epithelial cells into tumor-bearing mice led to increased tumor growth associated with the differentiation of Ly6G+ myeloid-derived suppressor cells and a decreased amount of F4/80+ macrophages and CD11c+ dendritic cells. This population had the potential to induce resistance to anti-PD-1 immunotherapy when coinjected with epithelial tumor cells. Our data reveals the existence of a cell population that instruct immunosuppressive myeloid cell responses to bypass PD-1 targeting and thus suggest potential new approaches for overcoming resistance to immunotherapy in clinical settings.

Keywords: Cancer immunology, cellular interactions, checkpoint inhibition, microenvironment, myeloid derived suppressor cells, stem cells

OP-333

Heterotypic signaling between tumor cells and lipid-loaded macrophages drives tumor progression and resistance to therapy in cancer

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Tumor associated macrophages (TAMs) are correlated with the progression of prostatic adenocarcinoma (PCa). The mechanistic basis of this correlation and therapeutic strategies to target TAMs in PCa remain poorly defined. Here, high dimensional single-cell RNA-seq was used to profile the transcriptional landscape of TAMs in human PCa, leading to identification of a subset of macrophages characterized by dysregulation in transcriptional pathways associated with lipid metabolism. Integrating human and mouse PCa data, this subset of TAMs shows expression of the scavenger receptor MARCO and is characterized by accumulation of lipid droplets. We identified a gene signature derived from MARCO expressing TAMs that correlates positively with PCa progression and shorter disease-free survival. Mechanistically, we unveiled an interaction between cancer cells and TAMs wherein lipid accumulation in TAMs is promoted by cancer cell derived IL-1b and, reciprocally, cancer cell migration is promoted by CCL6 released by lipid-loaded TAMs. Accumulation of lipids in macrophages was found to be dependent on MARCO expression and MARCO blockade hinders tumor growth and invasiveness in models of advanced PCa. Moreover, administration of high fat diet to tumor-bearing mice raises the abundance of lipid-loaded TAMs. Finally targeting lipid accumulation in TAMs improves the efficacy of chemotherapy in PCa, pointing to combinatorial strategies that may influence patient outcomes. Together, our findings identify a heterotypic signaling involving lipid-loaded TAMs and cancer cells that drives PCa aggressiveness and provides novel therapeutic targets for advanced disease.

Keywords: Cancer immunology, *in vivo* tumor models, innate immunity, macrophage

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WORKSHOPS

OP-334

JAK2/STAT5 signaling controls T cell polarization and development of experimental autoimmune encephalomyelitis

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As one of the most important signal transduction molecules that mediate cytokine signals, Janus tyrosine kinases (JAKs) have long been proposed to be critical to T cell responses. However, the role of JAK2 in the function of T cells remains to be elucidated. Here we used an inducible JAK2 knockout mouse model and a cell-specific JAK2 knockout mouse model to dissect the role of JAK2 in the regulation of T cell homeostasis and autoimmune response. Deficiency of JAK2 completely prevents the development of experimental autoimmune encephalomyelitis (EAE). Type 1 helper T cell (TH1) differentiation was dramatically suppressed by the disruption of JAK2, which was subsequently confirmed to be STAT5-dependent. Interestingly, co-transfer of JAK2-deficient (Jak2-/-) T cells decreased the severity of EAE in passive transfer model. By using JAK3 inhibitor on Jak2-/- and STAT5-transgenic T cell, we confirmed that disruption of JAK2 enhances JAK3/STAT5 signaling and thus promotes the differentiation of regulatory T cells (Treg). We then confirmed that deficiency of JAK2 in Treg resulted in improved EAE phenotype by using a Treg-specific JAK2 knockout mouse model (Foxp3Cre x Jak2fl/fl). Therefore, JAK2 may serve as a potent therapeutic for autoimmune disease by regulating the differentiation of TH1 and Treg.

Keywords: Autoimmunity, cell signalling, multiple sclerosis, regulatory cells

OP-337

Metabolic fuel choices control MAIT cell functions at homeostasis and after infection

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Mucosal-associated-invariant-T (MAIT) cells are an innate-like T cell subset that recognizes microbial-derived vitamin B metabolites. In contrast to conventional T cells, MAIT cells have an antigen-experienced phenotype and express memory markers by default. When activated, MAIT cells produce copious amounts of cytokines, resembling IFNγ+ Th1- or IL-17+ Th17 effectors cells. Despite their high abundance in humans and relevance to different diseases, a comprehensive analysis of MAIT cell heterogeneity is missing. Furthermore, while memory-like vs effector-like states in conventional CD4 and CD8 T cells are controlled by mutually exclusive metabolic states, the question remains as to which metabolic programs MAIT cells adopt at steady-state and after infection. Here we integrate scRNA-seq analysis with single-cell metabolic characterization of MAIT cells from humans and mice. We discovered a memory-like metabolic program that is already acquired in the thymus and at steady state in the periphery. We identify a novel cluster of circulatory MAIT cells which is metabolically similar to IFNγ+ MAIT1-like cells and preferentially consumes glucose. MAIT17 clusters, however, uniquely engage in fatty acid uptake and mitochondrial metabolism. Following exposure to bacteria, MAIT cells expand as CD127-KLRG1+ and CD127+KLRG1- populations that adopt divergent transcriptomic and metabolic profiles with enhanced functionality. They remain altered long-term. CD127+, but not KLRG1+ MAIT cells engage in MAIT17-like metabolic and effector pathways and protect mice from lung infection with Streptococcus pneumoniae. In contrast, KLRG1+ MAIT cells depend on Hif1α-driven glycolysis and remain relatively dormant, but more strongly and quickly engage multiple metabolic programs to protect from viral infection.

Keywords: Bacterial infections, cell signalling, MAIT cells, memory, metabolic control of immune responses, molecular immunology

OP-338

Leveraging high-dimensional flow cytometry to assess human immune homeostasis at population-wide scale

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The immune system varies remarkably between individuals. In contrast, the immune composition within an individual remains stable and only shifts over decades. Infections and immune activation cause immune perturbations but upon cleared infection the immune system returns to its pre-infection baseline. This highlights the tight regulation of immune homeostasis during both steady-state and immune activation. More in-depth insights into the regulation of immune homeostasis are needed to better understand immune dysregulation and autoimmune diseases. Here we describe how we combine high-dimensional flow cytometry with genetics, metabolomics, microbiome and systems serology to study mechanisms of immune homeostasis. Our study comprises 3000 individuals including 2000 twins for precise assessment of heritability and genetic regulation. Such large immunophenotyping studies require sophisticated methods and strategies to ensure low interexperimental variation and optimal precision for sensitive measurements. We present our high-throughput flow cytometry pipeline and discuss strategies which enabled us to process 200 samples per experiment while controlling for several sources of technical and experimental errors. Additionally, we discuss analysis strategies including unsupervised clustering algorithm FlowSOM, in view of the fact that most strategies are highly limited for large datasets. Finally, we show some preliminary insights from our comprehensive analysis into the influence of heritability, age and chronic viral diseases on immune homeostasis. Overall, we describe our high-throughput, high-dimensional flow cytometry pipeline to precisely phenotype the immune system in large cohorts. We utilized our pipeline to assess the immune system in 3000 individuals with the goal to reveal mechanisms of immune homeostasis at unprecedented depth.

Keywords: Autoimmunity, big data, biology of the immune system, immune development, omics technologies, visualizing immune responses

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WORKSHOPS

TRACK 2 - MOLECULAR IMMUNOLOGY

OP-095

Mitochondrial arginase-2 is essential for IL-10 mediated metabolic reprogramming of inflammatory macrophages

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Evidence from immunometabolic studies have shown that mitochondrial metabolism and dynamics are important for regulating macrophage polarization. Interlukin-10 (IL-10) is a prominent anti-inflammatory cytokine acting in an autocrine fashion in macrophages to limit inflammatory responses. We demonstrate that Arginase-2 (Arg2), the mitochondrial isoform of arginase, is an IL-10 regulated protein in murine and human inflammatory macrophages. Using approaches such as siRNA-induced knockdown, overexpression plasmids, site-directed mutagenesis, and genetic knockout mice in metabolic flux assays, we aimed to understand the impact of Arg2 on IL10's role as a metabolic rheostat in macrophages to resolve inflammation. We showed that IL-10's enhancement of Arg2 radically promoted a state of 'fusion', an effect that is dependent on both Arg2's catalytic activity and physical presence at the mitochondria. We further demonstrated that Arg2 was critical for IL-10 induced mitochondrial oxidative respiration in these cells. Mechanistically, we illustrated that Arg2 increased complex II (also known as succinate dehydrogenase (SDH)), a bi-functional enzyme that links the mitochondrial electron transport chain (ETC) and the Tricarboxylic acid (TCA) cycle. Moreover, we found Arg2 to be essential for IL-10-regulated decreases in inflammatory mediators succinate, hypoxia inducible factor 1α (HIF-1α) and interleukin 1β (IL-1β) *in vitro*. Accordingly, we observed elevated levels of HIF-1α and IL-1β *in vivo* in $Arg2^{-}/^{-}$ mice in an LPS-induced acute model of inflammation. Altogether, these findings demonstrate a new arm of IL-10 mediated metabolic regulation that works to resolve the inflammatory status of the macrophage.

Keywords: Cytokines and mediators, macrophage, metabolic control of immune responses, myeloid cells

OP-096

Effect of glucose-transporter inhibitors on the function of Natural Killer cells

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Glucose-transporter (Glut) inhibitors effectively inhibit the proliferation of different tumour cells and, as a result, prove to be anti-cancer therapeutics. Therefore, it is important to know, if these inhibitors also affect the anti-tumour function of natural killer (NK) cells. We treated NK cells with the Glut-1/2/3 specific inhibitor Glutor or with Glupin, which inhibits Glut-1 and -3 and analysed the energetic phenotype of these NK cells by Seahorse experiments. Glutor treatment resulted in higher mitochondrial respiration, which could not be detected for Glupin. To examine the effect of Glut-inhibitors on NK cell effector functions, resting or pre-activated human NK cells were stimulated through the activating receptor CD16 in the presence or absence of Glutor or Glupin. Acute inhibition of the glucose-transporters had no significant effect on NK cell cytokine secretion or killing-capacity against different tumour cells. To analyse possible long-term effects, we cultured freshly isolated NK cells for 3 weeks in the presence or absence of Glutor or Glupin. We found a decreased proliferation of Glutor-treated NK cells. Furthermore, long-term treatment with Glutor impaired NK cell cytotoxicity and cytokine secretion, whereas Glupin-treated NK cells showed no difference in comparison to the control treated cells. The analysis of cytokine- and chemokine profile showed an increased spontaneous secretion of MCP-1 and IL-8 after long-term treatment with Glutor. These data show that Glupin is a suitable candidate for cancer therapy, whereas Glutor seems ineligible due to its effect on NK cells.

Keywords: Drugs for immune modulation, immune regulation and therapy, metabolic control of immune responses, NK cells

OP-097

The structure of the marsupial γμ T cell receptor defines a third T cell lineage in vertebrates

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Most T cells found in jawed vertebrates express functional heterodimeric receptors (TCRs) on their surface formed by either α and β or γ and δ chains. Each chain possesses two domains, an amino-terminal variable domain (V) and a constant domain (C) on the carboxy-terminus (V-C pattern). In most cases, the ability of T cells to recognize diverse antigens relies on the surface (or paratope) located within Vα – Vβ or Vγ – Vδ segments. Recent genomic studies of non-eutherian mammals identified clusters of genes that resemble the classical TCR loci but surprisingly contain an additional variable segment. The functional product common for marsupials and monotremes called 'μ chain' was predicted to contain two variable (Vμ and Vμj) and one constant (Cμ) domains. Single cells analysis of blood and spleen from Monodelphis domestica showed that some of the splenic T cells co-express the μ and γ chains suggesting that both polypeptides could form a novel type of T cell receptor, the γμTCR. Using obtained sequences, we generated and structurally characterized two different γμTCRs. Here, we present the novel and unusual architecture of a third lineage of T cell receptor found in marsupials and monotremes.

Keywords: Animal models, biology of the immune system, engineering of antibodies and nanobodies, molecular immunology, veterinary immunology

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WORKSHOPS

OP-098

Sensing low intracellular potassium by NLRP3 results in a stable open structure that promotes inflammasome activation

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The NLRP3 inflammasome is activated in response to a wide range of stimuli and drives diverse inflammatory diseases. The decrease of intracellular potassium concentration is a minimal upstream signal to most of the different NLRP3 activation proposed models. To study the mechanism of NLRP3 activation by low intracellular potassium. Full length and truncations of NLRP3 tagged with YFP and Luciferase was used as a Bioluminescence Resonance Energy Transfer sensor to monitor receptor conformation during activation. Reconstitution of truncated NLRP3 or chimera receptor among NLRP3 and NLRP6 in NLRP3 deficient macrophages to study functionality of the receptors. Reconstitution in NLRP3 deficient mouse was used in uric acid crystal peritonitis model. We found that cellular potassium efflux induces a stable structural change in the inactive NLRP3 promoting an open conformation as a step preceding activation. This conformational change is facilitated by the specific NLRP3 FISNA domain. Then, an unique flexible linker sequence between the PYD and FISNA domains is important to structurally allow the ensemble of NLRP3-PYD into a seed structure for ASC oligomerization. The introduction of the NLRP3 PYD-linker-FISNA sequence into NLRP6 resulted in a chimeric receptor able to be activated by potassium efflux-specific NLRP3 activators and promoted an *in vivo* inflammatory response to uric acid crystals. Our results establish an initial common step involving a conformational change in NLRP3 structure induced by a decrease in the intracellular potassium concentration.

Keywords: Cytokines and mediators, inflammatory molecules, macrophage

OP-099

Characterizing the role of PLCγ2 in autoantibody-induced skin blistering

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Phospholipase Cγ2 (PLCγ2) is a signaling molecule of tyrosine kinase-coupled receptors in cells of hematopoietic origin. Gain-of-function mutations of PLCγ2 have been reported to cause a complex syndrome involving inflammation and bullous skin eruptions in both mice and humans. Our aim was to test the role of PLCγ2 in autoantibody-induced skin blistering. Skin blistering disease was triggered in wild type and PLCγ2-deficient mice by autoantibodies against type VII collagen (C7), a component of the dermal-epidermal junction. Disease course was followed by scoring and histology. Leukocyte accumulation was measured by flow cytometry. Migrating capacity of myeloid cells was checked both *in vitro* and in an *in vivo* competitive migration assay. Autoantibody deposition and the generation of the proinflammatory microenvironment in the skin was analyzed by ELISA and *in vivo* luminescence-based imaging. *In vitro* neutrophil activation was examined on immobilized C7-anti-C7 immune complex surfaces. PLCγ2-deficient mice were completely protected from all clinical and microscopic signs of autoantibody-induced skin blistering. PLCγ2 deficiency prevented the infiltration of neutrophils, eosinophils and monocytes/macrophages to the ear tissue, however the intrinsic migrating capacity of these cells and autoantibody deposition were not affected. The accumulation of various proinflammatory mediators and production of reactive oxygen species were completely blocked both *in vitro* and *in vivo* in the absence of PLCγ2. Taken together, our results identify PLCγ2 as a critical component of autoantibody-induced skin blistering by creating a proinflammatory milieu after autoantibody deposition in the skin.

Keywords: Autoimmunity, cell signalling, neutrophils, skin diseases

OP-100

Activation mechanism and secretome characterization of NLRP3 mutants associated to cryopyrin periodic syndromes

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Cryopyrin-associated periodic syndrome (CAPS) is a hereditary autoinflammatory disorder that results from a gain-of-function (GoF) mutation in NLRP3 gene with overproduction of IL-1β as consequence. However, the activation mechanism of GoF NLRP3 is not well understood. Questions such as how mutated NLRP3 is activated in patients; what molecules beyond IL-1β are implicated downstream NLRP3, and how NLRP3 mutant is post-translational regulated are unknown yet. Nlrp3-deficient immortalized mouse macrophages with a Tet-ON inducible system were used to express several mutated NLRP3, empty vector, and wild type NLRP3 upon doxycycline treatment. Macrophages were treated with LPS, Pam3CSK4, IL-6, S100A9, IL-1α, TNF-α, palmitate, ATP, or uric acid crystals at different concentrations and times in the absence or presence of MCC950. Macrophages were also treated with deubiquitinase (DUBs) inhibitors as PR-619, G5, and b-AP15. The release of different cytokines and alarmins were identified by ELISA. The expression of mutant NLRP3 results in IL-1β release after LPS, Pam3CSK4, IL-6, palmitate and S100A9 treatment. MCC950 was able to block the release of IL-1β, IL-18, HMGB1, IL-1α, P2X7, cystatin B, Annexin 1 but not TNF-α. Deubiquitinase inhibitors PR-619, G5 and b-AP15 were able to reduce released IL-1β and mutant NLRP3 activation without affecting NF-kB activation. The endogenous host-derived molecules IL-6, palmitate and S100A9 lead to the activation of mutated NLRP3, which is in turn inhibited by MCC950. Additionally, the inhibition of deubiquitinases is able to reduce the activation of mutated NLRP3, probably by regulating post-translational NLRP3 modifications.

Keywords: Autoinflammation, cell signalling, cytokines and mediators, drugs for immune modulation, inflammatory disease

OP-101

The natural ompound Beauvericin exhibits immunostimulatory effect on dendritic cells via activating TLR4 signaling pathways

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Our aim is to evaluate the effect of natural compound Beauvericin on conventional dendritic cells and elucidate the underlying mechanism. GM-CSF-cultured bone marrow derived conventional dendritic cells (BMDCs) were generated from C57BL/6, IL-12p40/GFP-reporter mice, MyD88-/-, MyD88/TRIF-/- and TLR4-/- mice. Immune activating capacities of natural products on BMDCs and T cell proliferation were detected by flow cytometry. The effects of Beauvericin on cytokine production were measured by ELISA. The inhibition of Beauvericin on Cathepsin B activity was detected in cell based and cell free assays.By screening a library of natural products derived from endophytic fungi and marine sponges, we found that the cyclic hexadepsipeptide Beauvericin can activate BMDCs to induce IL-12 and IFNβ production, thereby enhancing Th1 cell differentiation and proliferation. However, IL-12 and IFNβ production are not observed in Beauvericin stimulated MyD88-/- and MyD88/TRIF-/- BMDCs. Furthermore, TLR4-/- BMDCs are not responding to Beauvericin treatment, suggesting Beauvericin activates BMDCs via TLR4 signaling pathways. Additionally, Beauvericin significantly suppressed Cathepsin B activity in cellular and cell free inhibition assays.Beauvericin activates BMDCs via TLR4 signaling pathways to increase IL-12 production leading to Th1 cell proliferation. Moreover, Beauvericin significantly and directly suppresses Cathepsin B activity. Interestingly, Cathepsin B has been reported to negatively regulate IL-12 production, suggesting that suppression of Cathepsin B activity by Beauvericin may enhance IL-12 production in Beauvericin stimulated BMDCs. Our observations suggest Beauvericin can be a very promising candidate for immunotherapy of resistant tumors and bacterial infection.

Keywords: Immunotherapy, cell signalling, drugs for immune modulation, dendritic cells

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WORKSHOPS

OP-102

The transcription factor BATF limits the expression of type I interferon in plasmacytoid dendritic cells

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BATF (Basic leucine zipper transcription factors, ATF-like) coordinates multiple aspects of B and T cell function including differentiation, proliferation, metabolism, and effector functions in infection and immunity. In a recent transcriptome analyses we observed *Batf* as differentially expressed in interferon (IFN) β-producing plasmacytoid dendritic cells (pDCs). In pDCs, so far, no expression or function has been described for BATF. In the present study we characterised the implications of BATF in pDCs functions. Using IFNβ/YFP reporter mice we found that BATF is highly expressed in IFN beta-producing splenic and bone marrow (BM) derived pDCs. Upon CpG stimulation the maximum of *Ifnb* expression precedes the maximum of *Batf* expression in these cells. However, *Batf* expression is not dependent on IFNAR signaling in pDCs. In order to evaluate the effect of BATF on the expression of type I IFN we employed *Batf*-deficient mice. In comparison to wildtype (WT) littermates BM-derived pDCs from *Batf*-deficient mice produce increased amounts of IFN α and β at mRNA and protein levels after CpG stimulation. In agreement with the *in vitro* data *Batf*-deficient mice show higher serum levels of type I IFN early after LCMV infection as compared to WT animals. Taken together our data point to a so far unrecognized role of BATF in modulating pDC dependent type I IFN responses. This suggests an important relevance of BATF in anti-infectious immune responses and IFN mediated autoimmunity. Multiomics data suggesting the molecular mechanisms underlying the BATF mediated regulation of type I IFN expression will be presented.

Keywords: Cell signalling, dendritic cells, innate immunity, molecular immunology, viral infections

OP-103

Circadian disruption increases the inflammatory response of lung fibroblasts

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Fibroblasts are stromal cells abundant throughout tissues including the lung and are integral effector cells of the immune response. Circadian rhythms in the lung play a crucial role in mediating immune cell function through control of intracellular metabolic pathways. However, whether circadian regulation of metabolism is important in the fibroblast immune response has yet to be investigated. Evidence shows that fibroblast chemokine production controls immune cell recruitment to the lungs, and that immune cell recruitment to the lungs occurs in a circadian fashion. Moreover, glycolysis is essential for fibroblast activation and responses. We find that upon IL-1β stimulation, lung fibroblasts lacking the core clock protein BMAL1 (*Bmal1*$^{-/-}$ fibroblasts), our model for circadian disruption, differentially express chemokine and growth factor genes in comparison to *Bmal1*$^{+/+}$ fibroblasts. *Bmal1*$^{-/-}$ fibroblasts have significantly higher glycolysis and inhibition of glycolysis significantly reduces chemokine gene expression. By carrying out a migration assay, we observe increased immune cell migration to IL-1β stimulated *Bmal1*$^{-/-}$ fibroblasts in comparison to *Bmal1*$^{+/+}$ fibroblasts. We hypothesise that the composition of this inflammatory infiltrate will differ between *Bmal1*$^{+/+}$ and *Bmal1*$^{-/-}$ fibroblasts due to differential chemokine and growth factor gene expression, which may have implications for the lung immune response in circadian disrupted individuals such as shift workers. These results demonstrate that the fibroblast immune response and metabolism are gated by the circadian clock. These results could reveal new therapeutic targets or improved interventions for the treatment of inflammatory lung diseases that display circadian variation in symptoms including asthma and COPD.

Keywords: Chemokines, cytokines and mediators, innate immunity, metabolic control of immune responses

OP-104

Single-cell profiling of age-associated immunity in murine atherosclerosis

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Aging is one of the most dominant drivers of atherosclerosis, a chronic autoimmune disease caused by accumulation of lipids and immune cells within the arterial wall. Given the demographic shift towards elderly in combination with an increasing life expectancy, elucidating the currently unknown impact of aging on the composition and function of the immune system in atherosclerosis is highly relevant. Here, we provide single-cell protein and transcriptomic insights in age-associated immunity in murine atherosclerosis. Young (3 months old) and aged (18 months old) atherosclerosis-susceptible LDLr-deficient (*ldlr-/-*) mice were fed a chow or Western diet for 10 weeks, after which immune cell frequencies were measured. We report age-associated alterations in our naturally aged atherosclerotic *ldlr-/-* mice, such as elevated percentage of circulating monocytes and a shift from naïve towards effector (memory) T cells with more extreme phenotypes, e.g. elevated IFN-γ and IL-17-producing cells. Using single-cell RNA-sequencing on CD45+ leukocytes from aged *ldlr-/-* aortas we identified 12 distinct immune cell clusters, which in contrast to young aortas, were dominated by T- and B cells, followed by myeloid cell populations and natural killer cells. Subclustering approaches and subsequent flow cytometry revealed an age-associated increase in the frequency of several immune cells, including immature-like CD4+CD8+ T cells. Collectively, we provide comprehensive profiling of aged immunity in atherosclerotic mice and reveal the emergence of age-associated cell subsets in the atherosclerotic aorta. Taking age into consideration will further enhance our understanding of disease etiology and can contribute to diagnostic and therapeutic tools to combat atherosclerosis.

Keywords: Ageing, cardiovascular diseases, immune senescence, inflammatory disease, RNAseq

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WORKSHOPS

OP-105

Pectic polysaccharide RG-I from bell pepper and carrot stimulates protective innate immune responses and modulates gut microbiota in humans

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Respiratory infections are a burden to health. Plant polysaccharides from traditional medicinal extracts like ginseng have been shown to be immunostimulatory with beneficial effects against respiratory (viral) infections. We aimed to identify active polysaccharide constituents from bell pepper and carrot, evaluate the *in vitro* immune- and gut microbiota-modulating effects of a polysaccharide-enriched extract, and then assess innate immune and gut microbiota responses in healthy adults. We used activity-guided fractionation to characterize the nutricophore, *in vitro* immune assays to evaluate cytokine secretion profiles and phagocytic activity, *in vitro* fecal fermentation to determine bacterial metabolic activity and community composition, and a randomized, double-blind, placebo-controlled study to assess immune responsiveness. Rhamnogalacturonan-I (RG-I) was identified as the nutricophore enhancing immune responsiveness and modulating the gut microbiota. Both *in vitro* and human intervention trial data support the biological effect of RG-I. Extracts enriched with RG-I displayed a dual mode of action by exerting 1) an immunomodulatory effect on phagocytosis, and 2) a gut microbiota modulating effect, with concomitant enhanced production of short chain fatty acids. In healthy humans, the RG-I-enriched extract was well tolerated, and it stimulated a dose dependent innate immune response and modulated gut microbiota. RG-I from bell pepper and carrot showed similar immune and gut microbiota modulatory activities and both appear to be efficacious solutions that are safe, sustainable, affordable and that could easily be integrated into food products or dietary supplements aimed to enhance protective innate immune responsiveness.

Keywords: Innate host defence, nutrients, phagocytosis, protection

OP-106

Cells deficient in membrane complement regulators as biosensors of abnormal complement deposition

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Tight regulation of the complement system is critical to avoid tissue damage. Defective regulation of complement components in fluid phase or at the cellular membrane can result in increased complement deposition on cells and ultimately in disease, as observed in atypical hemolytic uremic syndrome (aHUS) or C3 glomerulopathies (C3G). Thus, detection of aberrant complement deposition could be of value for understanding the underlying mechanisms and for the early diagnosis of these pathologies. Our previous work with cells lacking CD46, a membrane complement regulator, failed for sensing aberrant complement deposition, presumably due to expression of other major membrane regulators, such as GPI-linked CD55 and CD59. We thus reasoned that cells simultaneously lacking all these three regulators could be used as biosensors of aberrant complement membrane deposition. To test this hypothesis, gene expression was abrogated by CRISPR/Cas9-mediated disruption of *CD46* and *PIG-A* in K562 cells, and the resulting cell line was tested as biosensor of complement deposition by flow cytometry. Increased surface C3 deposition on the edited cells was readily detected following incubation with sera from aHUS or C3G patients carrying mutations affecting the soluble complement regulators, when compared with sera from normal donors, and with normal serum depleted of Factor H as positive control. Together, the results indicated that the edited KO cell line could serve as a biosensor for improved characterization of diseases related to abnormal complement deposition on cell membranes. We are currently testing patients´ sera to set the signal-to-noise ratio and the global robustness of this novel biosensor.

Keywords: Complement, immunological techniques, myeloid cells

OP-107

The autophagy receptor TAX1BP1 (T6BP) is a novel player in viral antigen presentation by MHC-II molecules

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Antigen presentation by Major Histocompatibility Complex Class II (MHC-II) molecules on the surface of antigen presenting cells (APCs) leads to CD4+ T lymphocyte activation. During viral infection, neosynthesized viral proteins constitute a source of antigens for the less characterized endogenous MHC-II presentation pathway. In fact, depending on cellular localization, trafficking and the nature of the antigen, different pathways might be involved in its degradation and delivery to MHC-II loading compartments (MIIC). Autophagy, a self-eating cellular degradation pathway, has been shown to contribute to the processing of endogenous antigens. Here, we hypothesize that autophagy receptors may contribute at various levels to MHC-II-restricted viral antigen presentation. We show that NDP52, OPTN and p62 do not significantly affect the presentation of an autophagy-dependent viral antigen to CD4+ T cells, while T6BP influences both autophagy-dependent and -independent endogenous viral antigen presentation by MHC-II molecules. By studying the immunopeptidome of MHC-II molecules, the results show that T6BP affects the quantity and quality of peptides presented. T6BP silencing induces mislocalization of MIICs and a rapid degradation of the invariant chain (CD74) without altering expression and internalization kinetics of MHC-II molecules. Finally, to get a hint on possible mechanisms, we defined the interactome of T6BP and identify novel protein partners that potentially participate to the T6BP-mediated regulation of MHC-II peptide loading. Among them, we identified the ER chaperone calnexin. Remarkably, calnexin silencing in APCs replicates the functional consequences of T6BP silencing. Altogether, we unravel T6BP as a key player of the MHC-II-restricted endogenous presentation pathway.

Keywords: Adaptive immunity, antigen processing and presentation, cell signalling, endo- and exocytic vesicles in immunity, innate host defence, viral infections

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WORKSHOPS

OP-108

BamQuery: A new proteogenomic tool to explore the immunopeptidome and validate tumor-specific antigens

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MHC class I–associated peptides (MAPs), collectively referred to as the immunopeptidome, have a pivotal role in cancer immunosurveillance. While MAPs were long thought to be solely generated by the degradation of canonical proteins, recent advances in the field of proteogenomics (genomically-informed proteomics) evidenced that ~10% of MAPs originate from allegedly noncoding genomic sequences. Among these sequences, the endogenous retroelements (EREs) are under intense scrutiny as a possible source of cancer-specific antigens (TSAs). With the increasing number of cancer-oriented immunopeptidomic and proteogenomic studies comes the need to accurately attribute an RNA expression level to each MAP identified by mass-spectrometry. Here, we introduce BamQuery (BQ), a computational tool to count all reads able to code for any MAP in any RNA-seq data chosen by the user, and to annotate each MAP with all available biological features. Using BQ, we found that most canonical MAPs can derive from an average of two different genomic regions, whereas most tested ERE-derived MAPs can be generated by numerous (median of 210) different genomic regions and RNA transcripts. We show that published ERE MAPs considered as TSA candidates can be coded by numerous other genomic regions than those previously studied, resulting in high undetected expression in normal tissues. We also show that some mutated neoantigens previously published as presumably specific anti-cancer targets can in fact be generated by other non-mutated, non-coding, widely expressed RNA-seq reads in normal tissues. We therefore conclude that BQ could become an essential tool in any TSA-identification/validation pipelines in the near future.

Keywords: Anti-cancer vaccine, big data, cancer immunopeptidome, immunological techniques, immunotherapy, omics technologies

OP-109

Discovery of citrullination of MMP-9 in rheumatoid arthritis synovial fluid

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Matrix metalloproteinase-9 (MMP-9) or gelatinase B is a zinc-dependent protease that modulates the extracellular matrix (ECM) in health and disease. In rheumatoid arthritis (RA), matrix metalloproteases have been associated with the breakdown of cartilage and joint inflammation. Evidently, elevated MMP-9 levels were found in RA patients at both mRNA and protein levels. However, since MMP activities greatly depend on posttranslational modifications, such as proteolysis and glycosylation, increased expression of MMP-9 cannot be directly extrapolated to enhanced activity. In light of this, we decided to perform in-depth characterisation of MMP-9 in synovial fluids of arthritis patients. We combined classic immunohistochemical methods (ELISA and Western blot) with orthogonal methods (zymographic analysis) to perform unbiased and detailed MMP-9 profiling. Moreover, we produced a monoclonal antibody specific for modified citrulline, which drastically improved detection sensitivity of citrullinated peptides compared to commercially available anti-citrulline antibodies. proMMP-9 was consistently found in all arthropathies, whereas the active form was observed only in a limited fraction of our patient cohort. Proteolytic fragments of MMP-9 were also detected in specific patients and these included the hemopexin-less form (57 kDa) and a MMP-9 peptide (25 kDa). Importantly, citrullinated MMP-9 peptides of 57 kDa and 25 kDa were detected in RA patients, as opposed to post-trauma samples. We describe, for the first time, the discovery of citrullinated MMP-9 proteoforms in synovial fluid and their apparent unique expression in RA.

Keywords: Autoimmunity, immunological techniques, rheumatoid arthritis

OP-110

Foxp3-specific deletion of CREB generates Th2 biased ST-2 positive regulatory T-cells with enhanced IL-10 production and suppressive capacity

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Regulatory T-cells (Tregs) are characterized by the expression of Foxp3, a master regulator involved in the development and function of Tregs. Foxp3 expression is balanced by the transcriptional activator CREB. We aimed to find out how CREB is regulated in Tregs under Foxp3 specific CREB deficient conditions. Tregs were analyzed by flow cytometric analysis. Cytokine analysis was performed by ELISA and RT-qPCR. Gene expression analysis was performed by ATAC-sequencing, Transcriptome and Methylation analysis. Foxp3creCREBfl/ fl mice showed increased frequencies of Tregs (CD25+/Foxp3+) in Thymus, spleen and peripheral lymph nodes, but decreased Foxp3 expression at the single cell level. In addition, bone marrow chimera mice experiments revealed down regulation of Tregs in CREB-/- CD45.2 Tregs indicating a cell intrinsic mechanism of Foxp3 downregulation by CREB. Despite decreased Foxp3 expression, T-cell suppression assays revealed increased suppressive capacity of CREB deficient Tregs. Upregulation of IL-10, IL-13, IL-4 and IL1RL1(ST2) among other genes was observed in CREB deficient Tregs indicating the induction of a Th2-skewed phenotype in Tregs by CREB deletion. Experimental transfer colitis showed decreased intestinal inflammation and reduced IL-17 and IFN-γ expression in Rag2-/- mice that received Foxp3creCREBfl/fl T-cells. On the other hand, Ova-induced asthma model revealed enhanced levels of IgE in serum and ST2+ Tregs in lungs and BAL of Foxp3creCREBfl/fl mice indicating increased type 2 immune responses. Collectively, our data suggest that CREB expression in Foxp3 cells is of importance in maintaining the balance of Th1 and Th2 responses.

Keywords: Cytokines and mediators, molecular immunology, regulatory cells

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WORKSHOPS

OP-111

MNDA controls the pathogen-stimulated type I interferon cascade in human monocytes by transcriptional regulation of IRF7

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Upon viral infection, pattern recognition receptors (PRRs) such as intracellular DNA sensors activate the innate immune system which triggers the induction of type I Interferon (IFNs) including IFNα. The human PYHIN family comprises five proteins, AIM2, IFI16, PYHIN1, MNDA and POP3, and some members of the family have been shown to contribute to anti-viral immunity. AIM2 and IFI16 function as intracellular DNA sensors, while IFI16 also directly restricts herpesviruses and HIV. The aim of this project was to investigate a potential role for MNDA in innate immune responses. Here we show that MNDA is required for IFNα induction in human monocytes. Unlike other IFI16, this was not due to a pathogen sensing role, but rather MNDA regulated expression of IRF7, a transcription factor essential for IFNα induction. Regulation of IRF7 by MNDA was independent of the cell stimulus used. Mechanistically, MNDA was required for recruitment of RNA polymerase II to the IRF7 gene promoter, and in fact MNDA was itself recruited to the IRF7 promoter after type I IFN stimulation. These data implicate MNDA as a critical regulator of the type I IFN cascade in human myeloid cells and reveal novel insights into the diverse roles of the PYHIN proteins.

Keywords: Immune regulation and therapy, cell signalling, innate immunity

OP-112

Alveolar lipids regulate the response of alveolar macrophages to IL-4

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Activation of macrophages by interleukin-4 (IL-4) is needed for tissue repair, although excessive activation causes pathology associated with fibrosis. We have previously shown that lung surfactant protein A (SP-A) is required for complete IL-4-dependent activation of alveolar macrophages (AMs). However, AMs are less able to respond to IL-4 *in vivo* than macrophages from the peritoneal cavity, due to a still unknown factor of the lung environment. Our objective was to analyze whether pulmonary surfactant lipids, which are continuously endocytosed by alveolar macrophages, modulate IL-4-mediated activation of AMs. Toward that end, isolated rat AMs were pre-incubated with surfactant lipids to allow their endocytosis and then were stimulated with IL-4, in the presence or absence of SP-A. Metabolic profiles of macrophages were performed by using an XF24 extracellular flux analyzer. Analyses of AMs activation, proliferation, and signaling were performed by enzymatic assay, ELISA, and Western blot. We found that SP-A+IL-4 increased mitochondrial respiration and glycolysis in AMs to support cell proliferation and the production of extracellular matrix needed for tissue repair. However, surfactant phospholipids decreased the acquisition of this metabolic profile as well as SP-A+IL-4-dependent alternative activation and proliferation of AMs. Mechanistically, endocytosed lipids decreased IL-4+SP-A-dependent activation of the PI3K-Akt-mTORC1 signaling axis, but lipids did not affect IL-4-induced STAT6 phosphorylation. We conclude that alveolar lipids decrease PI3K-dependent signaling pathways that amplify IL-4 actions in AMs. These results suggest that diminution of alveolar lipid levels in some lung diseases may increase M2 activation leading to fibrotic responses.

Keywords: Cell signalling, innate immunity, macrophage, metabolic control of immune responses, tissue damage and repair

OP-113

Expression of neolacto-series glycosphingolipids by tumors impairs the function of low-affinity immune receptor-ligand pairs

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The transcriptional signature of neolacto-series glycosphingolipid (nsGSL) expression highly associates with patient survival in a number of cancers, such as glioma. We recently identified that nsGSLs on such tumor cells negatively affect immune cell activation *in vitro* (Jongsma et al., Immunity 2020). However, the mechanism underlying this immune suppression is unknown. We discovered in a flow cytometry approach with barcoded cell lines that nsGSLs sterically shield several, but not all, immune cell surface receptors. In depth analyses of shielded receptor properties revealed that they have significantly shorter extracellular domains compared to non-shielded receptors, which may relate to the limited extracellular length of nsGSLs. Secondly, using genome editing and pharmacological inhibitors, we found that negatively charged sialic acids of nsGSLs likely interact with positively charged amino acids of shielded proteins. This interaction inhibited antibody binding to surface receptors, which was highly dependent on affinity as we established with a well-characterized antibody panel against CD147. Consequently, low-affinity interactions of the central immune receptors HLA class I and CD47 with their ligands LIR-1, KIR2DL2 (HLA class I), and SIRP-α (CD47) were largely impaired by nsGSLs. Overall our data strongly indicate that expression of nsGSLs by tumor cells prevents productive communication towards immune cells through charge-based shielding of short receptors from their low affinity ligands. Because the GSL synthesis pathway is safely targeted in lysosomal storage diseases, our data warrant investigations on the efficacy of GSL synthesis inhibition to treat patients with nsGSL-rich tumors.

Keywords: Cancer immunology, cellular interactions, immune communication, immunotherapy

OP-114

The molecular interplay of Ets-2-Tip60 and its role in the pathogenesis of multiple sclerosis (MS)

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In MS, pathogenic effector T helper (Th) cells recognize myelin antigens leading to CNS damage. We previously showed that in naive Th-cells, IL-2 expression is blocked by Ets-2, which binds to the ARRE-2 element of the IL-2 promoter (same binding site of NFAT2 in activated Th-cells). We also demonstrated that Tip60 acts as an IL-2 transcriptional co-activator. In this study, we investigated whether Ets-2, Tip60, and NFAT2 are involved in the transcriptional regulation of IL-2 in MS. Naive and memory effector Th cells were phenotyped and isolated from 12 patients with relapsing-remitting-MS in remission and 12 age/sex-matched controls. Gene expression of Tip60, Ets-2, NFAT2 and IL-2 was determined by real-time qPCR. Naive Th cells were significantly increased in MS patients whereas no significant differences in levels of memory Th-cells or Tregs were observed between MS patients and controls. Significantly higher levels of long-term activated (CD69-CD25-HLADR+) effector Th cells were observed in MS patients. In selected non-activated Th-cells, Ets-2 expression was significantly lower in both naive and memory Th-cells from MS patients compared to controls, while Tip60 expression was significantly higher. NFAT2 expression was similar between MS and control Th-cells. Both naive and memory Th-cells from MS patients expressed IL-2 in contrast to control Th-cells. We suggest that Tip60 overexpression blocks Ets-2 expression in effector Th-cells of MS patients resulting in constitutive IL-2 expression; this may lead to impaired downstream events in Th cell plasticity and the formation of pathogenic Th-clones that mediate CNS damage.

Keywords: Adaptive immunity, cytokines and mediators, epigenetic control and modulation of immunity, molecular immunology

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WORKSHOPS

OP-115

Identification of common metabolic markers of immune aging in western european and african populations

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Impairment of innate and adaptive immune responses with old age leads to higher susceptibility to infections and poor vaccination efficiency. This study aims to identify the metabolic factors affecting the immune responses in the elderly. Metabolomic profiles were measured from the plasmas of a Dutch cohort of 324 healthy individuals (ages 18-71) and a Tanzanian cohort of 323 healthy individuals (ages 18-62). Their whole blood and PBMCs were stimulated *in vitro* with various microbial ligands, and cytokine production was assessed. Age-related defects were observed in IFNγ production in both cohorts. There was also an age-related defect in IL-6 and TNFα production in Tanzanians, but not the Dutch. The metabolome of both the elderly (>50 years old) and younger individuals of the two cohorts were significantly different. Despite distinct metabolic profiles and immune responses, there were commonalities. Out of 1376 metabolites, 61 were significantly more abundant and 81 were diminished in the elderly in both cohorts. The common metabolites were mostly endogenous, rather than food-derived. Several metabolites which were negatively correlated with both innate and adaptive cytokine responses were identified. Two of those, hippurate and 2-phenylacetamide, inhibited IL-6 and TNFα production by human PBMCs upon stimulation with fungal and viral ligands. These results indicate that the immune system ages differently in different populations, possibly due to a combination of genetic and lifestyle reasons. The shared age-related metabolic signatures found in our Dutch and Tanzanian cohorts provide new insights into the mechanisms of immune aging, and identifies new targets to combat it.

Keywords: Immune senescence, innate immunity, metabolic control of immune responses

OP-116

Organ-specific inflammation-driven changes in the epigenetic signature of murine ILC2s

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Epigenetic modifications such as DNA methylation play an essential role in imprinting specific transcriptional patterns in cells. Here, we performed a genome-wide DNA methylation analysis of lymph node-derived innate lymphoid cells (ILC) 1, 2, 3, and lymphoid tissue inducer cells (LTi), which led to the identification of differentially methylated regions (DMRs) and the subsequent definition of epigenetic marker regions in ILC lineages. The epigenetic signature for ILC2 is defined by specific demethylated regions located in *Gata3*, *Il4*, *Il5*, *Bcl11b* and *IL1rl1* formerly identified by chromatin studies, as well as newly identified regions in *Dhx40*, *Ptgir*, *Chdh*, *Nmur1*, *Neb*, *Rem2* and *Ptpn13*. Using models of IL-33-mediated inflammatory challenge in lung and liver, we demonstrate that the methylation status of the ILC2 epigenetic signature remained relatively stable in the lung, while it was affected by the inflammatory stimuli in the liver. In our study, we demonstrate the benefit of epigenetic signatures to investigate phenotypic and functional properties of ILCs under changing environmental conditions.

Keywords: Epigenetic control and modulation of immunity, inflammatory disease, innate lymphoid cells

OP-117

Mass cytometry analysis of the murine hematopoietic system reveals signatures induced by ageing and physiological pathogen challenges

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Mice under 'specific-pathogen-free' (SPF) conditions are frequently used to assess immune-ageing. However, physiological pathogenic challenges are reduced in SPF mice. The question arises to what extent murine experiments performed under SPF conditions are suited to analyze immune-ageing in mice and serve as models for human ageing. We performed mass cytometry assessing splenic and bone marrow leukocytes from young versus aged SPF mice in order to delineate alterations of the hematopoietic system induced during ageing. We then compared young and aged SPF mice to pet shop mice in order to delineate immune-alterations induced by physiological pathogenic challenges and those caused by cell intrinsic or systemic changes during ageing. Notably, distinct signatures were similarly altered in both pet shop and aged SPF mice in comparison to young SPF mice, including increased counts and frequencies of memory T lymphocytes, effector-cytokine producing T cells, plasma cells and mature NK cells. However, elevated frequencies of CD4+ T cells, NK cells, granulocytes, pDCs, cDCs and decreased frequencies of naïve B cells were identified only in pet shop mice. In aged SPF mice specifically the frequencies of splenic IgM+ plasma cells, CD8+ T cells and CD4+ CD25+ Treg were increased as compared to pet shop and young mice. Our study dissects holistically how ageing impacts both innate and adaptive immune cells in primary and secondary lymphoid organs. Secondly, it distinguishes intrinsic immune-ageing alterations from those induced by physiological pathogen challenges highlighting the importance of designing mouse models for their use in preclinical research including vaccines and immunotherapies.

Keywords: Adaptive immunity, ageing, animal models, immune senescence, innate immunity, microbiome and environmental factors

OP-118

Functional T cell diversity drives differences in PD-1 signaling

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Naïve T cell activation by self- and foreign antigens initiates a program of differentiation that generates a continuum of different memory T cell states with diverse functional and molecular profiles. Activated T cells upregulate inhibitory receptors to prevent excessive T cell inflammation and tissue damage. The inhibitory receptor PD-1 is expressed quickly after T cell activation and inhibits T cell functions upon binding to its ligands PD-L1 or PD-L2. Despite its critical role in immune regulation, little is known about the basic biology and contribution of PD-1 signaling in functionally different human T cells. The aim of this study was to determine how the vast functional diversity within the T cell population will direct the cellular responses and molecular networks triggered by PD-1 inhibition. To address this, we identified the gene expression signatures of purified human naive and memory CD4 and CD8 T cells following stimulation in the presence or absence of PD-1 ligation. Our approach allowed us to resolve the T cell subset-specific and PD-1 ligand-triggered signaling pathways engaged by PD-1. We identified that effector function and antigen experience greatly influenced the magnitude of PD-1 inhibition and directed PD-1 resistant and susceptible gene expression signatures. Furthermore, we observed PD-1 ligand-specific functional and gene expression divergence between naïve and memory CD4 and CD8 subsets. Collectively our findings reveal that functional T cell diversity directs the complex immuno-regulatory networks triggered by PD-1. We identified the signaling cascades that underlie these networks and molecular mediators that maybe cultivated in PD-1 targeting immunotherapies.

Keywords: Cell signalling, checkpoint inhibition, costimulatory pathways, effector molecules, immune networks, immune regulation and therapy

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WORKSHOPS

OP-119

DNA-PK is required for viral DNA sensing in human cells

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Host cell pattern recognition receptors (PRRs) are a first line of defence against pathogens and function to generate a productive innate immune response. PRRs sense pathogen-associated molecular patterns (PAMPs), such as viral genomic DNA, which is a major PAMP during DNA virus infection. Viral DNA sensing leads to the activation of the STING-TBK1-IRF3 signalling axis and the induction of a type I interferon response. Previously, our work identified the non-homologous end-joining protein DNA-PKcs, part of the DNA-dependent protein kinase (DNA-PK) complex, as an intracellular PRR for cytoplasmic viral DNA in murine cells. We show that in human cells DNA-PKcs is essential for the production of type I interferon via the STING signalling pathway in response to DNA and DNA virus infection. DNA-PKcs is rapidly activated after stimulation with exogenous DNA and cells that lack DNA-PKcs are deficient in their capacity to mount an immune response. A number of DNA viruses have developed ways to evade the immune response by inhibiting DNA-PK or downstream components of the DNA sensing pathway. Therefore, we make use of attenuated Herpes simplex virus 1 (HSV-1) and Vaccinia virus (VACV) that lack immunomodulatory proteins and are able to drive type I interferon production. Using these viruses, we show that DNA-PKcs-/- cells have a defective innate immune response after infection with attenuated HSV and VACV. Our study demonstrates the role of DNA-PKcs as a viral DNA sensor in human cells and adds to the knowledge of the DNA sensing processes that are essential for anti-viral innate immunity.

Keywords: Cytokines and mediators, infectious disease, innate host defence, innate immunity, molecular immunology, viral infections

OP-120

Defining the rapid actions of glucocorticoids in the lung

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Glucocorticoids (Gc) are potent anti-inflammatory steroids used as first line treatments for pulmonary inflammation including asthma and COVID-19. Gc mediate their cellular effects by binding and activating the glucocorticoid receptor (GR), an intracellular ligand-activated transcription factor. Upon ligand binding, cytoplasmic GR initiates rapid regulation of kinase pathways termed 'non-genomic' signalling. GR then translocates into the nucleus to mediate 'genomic' effects by modulating the transcription of pro- or anti-inflammatory genes. It is not clear whether rapidly induced cytoplasmic GR signalling impacts the transcriptional response. To delineate all rapidly-induced, Gc controlled signalling pathways in lung we completed global phosphoproteomics following acute treatment with the potent synthetic Gc Dexamethasone. We identified over 150 Gc regulated phosphoproteins which included nuclear scaffold proteins, chromatin re-modellers, transcription factors, in addition to cyclin-dependent kinases (CDKs) 12 and 13 and their partner cyclins which regulate the phosphorylation and activity of RNA Polymerase II. This suggested potential crosstalk between rapidly-induced and transcriptional GR effects. Immunoblotting with phospho-specific antibodies confirmed altered phosphorylation of RNA Polymerase II in response to Dexamethasone. The role of CDK12/13 in selectively modulating the GR transcriptional response was also confirmed by PCR using the selective inhibitor, THZ531. RNA-sequencing of lung epithelial cells treated with Dexamethasone alone or in combination with THZ531 showed that CDK12/13 modulate Gc-dependent inflammatory pathways downstream of AP-1. Our data suggests that Gc rapidly change the activity of CDK12/13 which feeds-forward to modulate the downstream transcriptional response. This provides insight into the complex mechanisms by which Gc mediate anti-inflammatory effects *in vivo*.

Keywords: Cell signalling, immune regulation and therapy, inflammatory disease, molecular immunology, omics technologies, RNAseq

OP-121

Multiplexed proteomics of autophagy deficient macrophages reveals enhanced antimicrobial immunity via the oxidative stress response

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Defective autophagy is strongly associated with chronic inflammation. Loss-of-function of the core autophagy gene Atg16l1 increases risk for Crohn's disease in part by enhancing innate immunity through myeloid cells such as macrophages. However, autophagy is also recognized as a mechanism for clearance of certain intracellular pathogens. These divergent observations prompted a re-evaluation of ATG16L1 in innate antimicrobial immunity. In this study, we found that loss of Atg16l1 in myeloid cells enhanced the killing of virulent *Shigella flexneri* (*S.flexneri*), a clinically relevant enteric bacterium that resides within the cytosol by escaping from membrane-bound compartments. Quantitative multiplexed proteomics of bone marrow-derived macrophages revealed that ATG16L1 deficiency significantly upregulated proteins involved in the glutathione-mediated antioxidant response to compensate for elevated oxidative stress, which simultaneously promoted *S.flexneri* killing. Consistent with this, myeloid-specific deletion of Atg16l1 accelerated bacterial clearance *in vitro* and *in vivo*. Pharmacological induction of oxidative stress through suppression of cysteine import enhanced microbial clearance by macrophages. Conversely, antioxidant treatment of macrophages permitted *S.flexneri* proliferation. These findings demonstrate that control of oxidative stress by ATG16L1 and autophagy regulates antimicrobial immunity against intracellular pathogens.

Keywords: Cell signalling, innate host defence, innate immunity, macrophage, mass spectrometry, metabolic control of immune responses

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WORKSHOPS

OP-122

The autophagy core protein ATG5 is essential to maintain intestinal RORgt+ Foxp3+ Treg cells

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Autophagy is an evolutionary conserved catabolic pathway that contributes to the degradation of intracellular components. Peripheral immune tolerance established by Treg cell activity relies on an intact autophagy machinery. However, the autophagy requirement for establishing the intestinal RORγt$^+$ Foxp3$^+$ Treg cell population is poorly understood. The purpose of the study was to elucidate the role of the autophagy-machinery component ATG5 in the maintenance of the Treg cell-mediated intestinal homeostasis. Conditional knockout mice bearing ATG5-deficient Treg cells were used in the study. *Ex vivo* cell analyses by flow cytometry and Western blotting, histological analyses, induction of intestinal pathobiont colonization and intestinal microbial community analyses were performed. We observed Treg cell decline in peripheral lymphoid organs and development of systemic autoimmunity, characterized by premature intestinal inflammation, in mice bearing ATG5-deficient Treg cells. In contrast to the reduced but detectable peripheral Treg cell population, the intestinal RORγt$^+$ Foxp3$^+$ Treg cells were virtually absent. Furthermore, these mice failed to induce RORγt$^+$ Foxp3$^+$ Treg cells upon intestinal pathobiont colonization. Autophagy is required to maintain Treg cell-mediated immune homeostasis. Our study reveals that this process is particularly required to maintain the intestinal RORγt$^+$ Foxp3$^+$ Treg cell population. The immunomodulatory effect of these cells relies on microbiota-derived metabolites to prevent intestinal inflammatory disorders. Since autophagy is connected to the ability to process such metabolites, the deficiency of the autophagy core protein ATG5 may explain the absence of intestinal RORγt$^+$ Foxp3$^+$ Treg cells and the prominent intestinal inflammation described in the mice.

Keywords: Autoimmunity, autoinflammation, inflammatory bowel disease, microbiome and environmental factors, regulatory cells

OP-123

Anti-MDA5 autoantibodies in myositis patients specifically target the Helicase domains of the MDA5 protein

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Idiopathic Inflammatory Myopathies, or myositis, are a group of rare systemic autoimmune diseases, characterized by the presence of specific autoantibodies. Anti-melanoma differentiation associated protein 5 (MDA5) antibodies are associated with rapidly progressing interstitial lung disease (RPILD), which is the main cause of mortality. At present it is unknown which domain of the MDA5 protein elicits an immune response. To determine the main immunogenic domain(s) of the MDA5 protein. IgGs were isolated from MDA5(+) patient plasma (n=9, from France, Sweden and Belgium) by affinity chromatography. An in-house ELISA was developed to measure the reactivity (in OD) against the MDA5 and RIG-I (negative control) proteins and several MDA5 fragments (all produced in E.coli). A depletion ELISA was developed to assess specificity. The reactivity was also assessed in sera from an MDA5(+) dermatomyositis cohort at KI (n=25). All MDA5(+) patients (n=33) showed reactivity towards the helicase domains at the center of the MDA5 protein, but reactivity towards C-terminal domain varied between patients (median OD=2.285 [1.715-3.043]). There was no reactivity towards the RIG-I protein. Reactivity towards the helicase domains persisted after depleting the reactivity to another helicase. Anti-MDA5 antibodies mainly target the helicase domains of the MDA5 protein. Because the reactivity persisted after depletion and there is no reactivity towards the helicase-bearing RIG-I, we conclude the patients have specific antibodies towards each of the MDA5 helicases. The potential usage of anti-MDA5 reactivity as a biomarker for RPILD will be explored in an extended cohort.

Keywords: Antibody, autoimmunity, inflammatory disease, molecular immunology

OP-124

PRINS long non-coding RNA regulates IL-23 expression of keratinocytes

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The PRINS lncRNA is a differentially expressed transcript in psoriatic perilesional epidermis. We have recently shown its role in keratinocytes' inflammatory responses by downregulating the expression of IL-6 and CCL-5 through sequence specific binding to their respective mRNAs. We aimed at identifying additional targets for PRINS-mediated regulation in inflammatory reactions of normal human epidermal keratinocytes (NHEK; n=4). Cytosolic exposure to synthetic analogues of dsDNA [poly(dA:dT)] and dsRNA [poly(I:C)] was used to model psoriasis-associated inflammatory conditions with plasmid-based overexpression or silencing of PRINS. Expression of inflammatory molecules was studied by a real-time RT-PCR-array. Out of 84 studied inflammatory genes 37 and 46 were upregulated in response to the different nucleic acid treatments, including IL23-A, a cytokine playing an important role in psoriasis pathogenesis. Overexpression of PRINS resulted in reduced response of IL-23A expression to nucleic acid induction, but silencing had an opposite effect. In silico analysis revealed two potential interaction sites of PRINS for the IL-23A mRNA, overlapping with the previously described IL-6 interacting site. We observed high intra-individual differences among the NHEK cultures upon PRINS overexpression suggesting the involvement of genetic variants on the polymorphic IL-23A gene. However, we could not identify any IL-23A variants correlating with IL-23A expression-rate, but we noticed a certain threshold of IL-23A expression to be reached for its effective downregulation by PRINS. We propose that PRINS acts as a fine-tuner of inflammatory gene expression in keratinocytes through different lncRNA–mRNA interactions, and altered PRINS expression in psoriatic perilesional epidermis contributes to disease pathogenesis.

Keywords: Cytokines and mediators, inflammatory disease, lncRNA, skin diseases

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WORKSHOPS

OP-125

DDX3X links NLRP11 to the regulation of type i interferon responses and NLRP3 inflammasome activation

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Tight regulation of inflammatory cytokine and interferon (IFN) production in innate immunity is pivotal for optimal control of pathogens and avoidance of immunopathology. The human Nod-like receptor (NLR) NLRP11 has been shown to regulate type I IFN and pro-inflammatory cytokine responses. Here, we identified the ATP-dependent RNA helicase DDX3X as a novel binding partner of NLRP11, using co-immunoprecipitation and LC-MS/MS. DDX3X is known to enhance type I IFN responses and NLRP3 inflammasome activation. We demonstrate that NLRP11 can abolish IKKε-mediated phosphorylation of DDX3X, resulting in lower type I IFN induction upon viral infection. These effects were dependent on the LRR domain of NLRP11 that we mapped as the interaction domain for DDX3X. In addition, NLRP11 also suppressed NLRP3-mediated caspase-1 activation in an LRR domain-dependent manner, suggesting that NLRP11 might sequester DDX3X and prevent it from promoting NLRP3-induced inflammasome activation. Taken together, our data revealed DDX3X as a central target of NLRP11, which can mediate the effects of NLRP11 on type I IFN induction as well as NLRP3 inflammasome activation. This expands our knowledge of the molecular mechanisms underlying NLRP11 function in innate immunity and suggests that both NLRP11 and DDX3X might be promising targets for modulation on innate immune responses.

Keywords: Cytokines and mediators, innate immunity, molecular immunology

OP-126

The inflammatory nature of serum amyloid A (SAA): fact or fallacy?

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Infection, sterile injury, and inflammation trigger the acute phase response with the purpose of re-establishing homeostasis. This response includes the upregulation of positive acute-phase proteins in the liver, including members of the serum amyloid A (SAA) family. Whereas murine acute phase SAAs comprise SAA1-3, in humans, the major acute phase SAAs comprise a group of closely related variants of SAA1 and SAA2. Over the years, SAA has been primarily regarded as a pro-inflammatory mediator with an array of immunogenic functions including the upregulation of cytokines, cell adhesion molecules, reactive oxygen species (ROS), and leukocyte chemoattraction. Nevertheless, emerging reports suggest that the widely described pro-inflammatory functions of SAA might be in part due to the presence of bacterial contaminants in the recombinantly expressed variants. Herein, we biologically characterized recombinantly expressed human SAA1 and murine SAA3 that have been purified to homogeneity using reversed phase-high performance liquid chromatography. In congruence with previous reports, we observed a partial loss of inflammatory function following purification. Indeed, SAA lost the capacity to upregulate the expression of cytokines, ROS and matrix metalloproteinase-9. Conversely, the *in vivo* leukocyte chemoattractant effect of human SAA1 remained intact. Furthermore, homogenously purified human SAA1 and murine SAA3 synergized with the neutrophil chemoattractant CXCL8 to enhance said leukocyte recruitment. Moreover, human SAA1 retained the capacity to enhance monocyte survival. These data imply that if one aims at biological characterization, care must be taken when selecting the production and purification protocol of SAA variants to rule out interference of contaminants in the assay.

Keywords: Chemokines, cytokines and mediators, effector molecules, innate host defence, molecular immunology, neutrophils

OP-127

Small extracellular vesicles obtained from Nrp1+ T regulatory cells favor skin graft tolerance

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Among the mechanisms of suppression that T regulatory (Treg) cells exert to control the immune response, the secretion of small Extracellular Vesicles (sEV) has been lately topic of interest. Previous studies have demonstrated that Treg cells produce sEV to modulate the function of the effector activity of leukocytes, such as dendritic cells (DC), macrophages and CD4+ T cells. Because we have demonstrated that the surface protein Nrp1 is required for Treg-mediated suppression mainly by impacting on the phenotype and function of effector CD4+ T cells, we sought to investigate whether this suppression is mediated by Nrp1+ sEV-derived from Treg cells. sEV were isolated from *in vitro* Treg cultures previously obtained from Foxp3Cre/YFP+Nrp1+/+ and from Foxp3Cre/YFP+Nrp1fl/fl animals, using the ultracentrifugation method. Characterization of sEV was performed by NTA and Western Blot. *In vitro*, sEV were added to CD4+ T cell cultures polyclonally activated. *In vivo*, skin-grafted mice received sEV one day before surgery. Cell phenotype was analyzed by flow cytometry. Our data shows that Foxp3+ Treg cells secrete sEV carrying Nrp1. These sEV modulate effector CD4+ T cells phenotype and proliferation *in vitro* in an Nrp1-dependent manner. Remarkably, we show that the presence of Nrp1 in Treg-derived sEV is required for inducing skin transplant tolerance, which correlates with a greater M2/M1 ratio in the transplanted tissue. Altogether, this study describes for the first time that Treg cells secrete Nrp1+ sEV and that this protein is necessary to promote transplantation tolerance *in vivo* via sEV administration.

Keywords: Immune regulation and therapy, regulatory cells, transplantation

OP-128

HMGCR gene polymorphisms (rs33761740, rs3846662) and promoter DNA methylation association with serum lipids in coronary artery disease

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Coronary artery disease (CAD) is a lipid laden inflammatory disease, has lesions with inflammatory cells. Hydroxy methyl co enzyme A reductase (HMGCR) is a candidate gene in cholesterol biosynthesis. Genetic and epigenetic modifications of HMGCR gene might influence its expression. and modulate the cholesterol lowering and inflammatory response to treatment with Statins. The present study aimed to evaluate the impact of HMGCR genetic polymorphisms (rs33761740, rs3846662) and Promoter DNA methylation status on its expression and serum lipids in CAD patients. The present case-control study included 300 CAD patients and 300 healthy controls. The HMGCR gene rs33761740, rs3846662 polymorphisms were determined in all subjects by PCR-RFLP & As-PCR assay. Promoter DNA methylation status and gene expression were determined using MS-PCR and qPCR respectively. Biochemical parameters are performed using commercially available assay kits. The genotypic distribution of HMGCR rs33761740 revealed that the frequency of CC genotype to be higher in CAD patients when compared to controls. Further, the rs3846662 genotypic distribution revealed that the frequency of GG genotype to be higher in CAD patients. rs33761740 CC- and rs3846662 GG-genotypes have significantly higher TC, TG and LDL-C levels. HMGCR gene expression was found to be significantly higher in CAD patients when compared to controls. Promoter DNA methylation studies of HMGCR have also shown an interesting result. In conclusion, with respect to both the HMGCR single nucleotide polymorphisms and promoter DNA methylation the findings of the present study may open avenues for pharmacogenetics for CAD management.

Keywords: Epigenetic control and modulation of immunity, inflammatory disease, molecular immunology

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WORKSHOPS

OP-129

Pentraxin-3 (PTX3) circulating and synovial expression levels correlate with rheumatoid arthritis severity and tissue infiltration independently of the response to conventional treatments

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Our work aimed to determine the relationship between PTX3 systemic and synovial levels and the clinical features of rheumatoid arthritis (RA) in a cohort of early, treatment naïve patients and to explore the relevance of PTX3 expression in predicting response to conventional-synthetic (cs) Disease-Modifying-Anti-Rheumatic-Drug (DMARDs) treatment. PTX3 expression was analysed in 119 baseline serum samples, 95 paired samples obtained 6-months following the initiation of cs-DMARDs and 43 healthy donors. RNA-sequencing analysis and immunohistochemistry/immunofluorescence for PTX3 were performed to assess synovial PTX3 gene and protein expression. Circulating levels of PTX3 were significantly higher in early RA compared to healthy donors and correlated with disease activity at baseline and with the degree of structural damages at 12-months. Six-months after commencing cs-DMARDs, a high level of PTX3, proportional to the baseline value, was still detectable in the serum of patients, regardless of their response status. Synovial PTX3 expression was observed in numerous cell types and was strongly linked to the degree of immune cell infiltration and the presence of ectopic lymphoid structures. The percentage of PTX3 positive synovial cells was similar in cs-DMARDs responders and non-responders. This study demonstrates that, early in the disease and prior to treatment modification, the level of circulating PTX3 is a reliable marker of RA activity and predicts a high degree of structural damages at 12-months. In the joints, PTX3 associates with immune cell infiltration and the presence of ectopic lymphoid structures. High synovial and peripheral blood levels of PTX3 are associated with chronic inflammation characteristic of RA.

Keywords: Inflammatory joint diseases, innate immunity, rheumatoid arthritis

OP-130

PGE2 signaling reprograms the cDC1 transcription factor network to promote cDC1 dysfunctionality in tumors and limit cancer immune control

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Production of the immunosuppressive lipid prostaglandin E2 (PGE_2) by tumor cells is a key mechanism for cancer immune evasion. However, the consequences of PGE_2 signaling on conventional type 1 dendritic cells (cDC1), critical orchestrators of anti-tumor immunity, have not been investigated so far. Here, we show that tumor-cell derived PGE_2 promotes cAMP-signaling in cDC1, which is associated with cDC1 dysfunctionality within PGE_2-producing tumors. Mechanistically, PGE_2 signaling in cDC1 does not seem to prevent cDC1 activation by TLR-ligands or cytokines per se, but disables activation-induced expression of a distinct set of key genes critical for cDC1-mediated orchestration of immunity in tumors, such as CD40, IL-12 and CXCL9. By using transcription factor network analysis, we further identify that PGE2-mediated cDC1 dysfunctionality depends on the regulation of a set of cDC1-specific transcription factors, including BATF3, SPI1 and IRF8. These alterations can be abolished by ablation of PGE_2 receptor expression in cDC1, rendering cDC1 fully resistant to PGE_2 signaling. *In vivo*, DC-specific PGE_2-receptor deficiency restores cDC1-T cell communication within tumors and enables control of tumor growth. A highly similar dysfunctional program is observed upon PGE_2 receptor signaling in human cDC1 and is associated with poor prognosis in multiple cancer types, suggesting that this mechanism is conserved across species and limits immunity in human cancers. Taken together, our data reveal a novel mechanism responsible for cDC1 dysfunctionality in tumors that could be targeted for cancer therapy.

Keywords: Cellular interactions, cancer immunology, cell based therapies, dendritic cells, immunotherapy, RNAseq

OP-131

Molecular mechanisms of NLRP3 inflammasome activation and its regulation

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NLRP3 inflammasome is a multiprotein complex mediating inflammatory response in a variety of metabolic and degenerative diseases. Upon activation with diverse triggers NLRP3 oligomerizes, recruits adaptor protein ASC and pro-caspase-1. Active caspase-1 processes IL-1β and IL-18 cytokines to their mature form and gasdermin D to a pore-forming protein that induces pyroptotic cell death. Although the role of NLRP3 in various pathologies has been described, not much is known about the molecular mechanism of NLRP3 inflammasome activation. In order to define the role of particular domains of NLRP3 in inflammasome trigger sensing, assembly and autoregulation systematic truncation of NLRP3 and reconstitution of NLRP3 variants in NLRP3-deficient macrophages was performed. We demonstrate that LRR domain is dispensable for NLRP3 activation and self-regulation. A minimal NLRP3 truncation variant was found fully responsive to various canonical NLRP3 activators. Substitution of the pyrin domain of NLRP3 with the CARD domain of NLRC4 or ASC led to a constitutive activation, demonstrating that the pyrin domain restricts NLRP3 in an inactive conformation. NLRC4 inflammasome is formed by self-catalytic polymerization of NLRC4 initiated with bacterial ligand/NAIP complex. We show that pathological mutations of NLRP3 failed to engage wild-type NLRP3 in a self-catalytic oligomerization, demonstrating that the activating signal is not enhanced at the level of NLRP3 oligomerization, representing an additional level of NLRP3 regulation. These results contribute to the understanding of the molecular basis of NLRP3 inflammasome activation and demonstrate the versatility of recognition and regulation mechanisms of the innate immune receptors.

Keywords: Autoinflammation, cell death, cell signalling, innate immunity

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WORKSHOPS

OP-132

Natural human variation in multi-layered cohorts predicts the function of CRELD1: a novel regulator of immune homeostasis in mouse and human

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Within a population of healthy individuals, genetic variation and environmental factors are influencing gene expression. We postulate that variance of gene expression can be exploited by conditional quasi loss-of-function "in population" experiments. Considering this concept, we developed huva (human variation), that takes advantage of population-scale multi-layered data to infer gene function. Within a reference dataset, huva derives two experimental groups, i.e. individuals with LOW or HIGH expression of the gene of interest, enabling the subsequent comparison of their transcriptional profile and functional parameters. With such multi-layered analysis, we identified CRELD1 as an important factor in T cell maintenance. Huva analysis on CRELD1 expression of peripheral blood revealed strong phenotypic and transcriptional differences in individuals with LOW gene expression. Phenotypically CRELD1 LOW individuals have a profile of immunosenescence with a remarked reduction in naïve T cells, especially CD4+. Similarly, the blood transcriptome linked to low level of CRELD1 shows a strong enrichment of an immunosenescence signature. To causally link CRELD1 expression and immunosenescence we developed a T cell–specific Creld1-deficient mouse. In this model, loss of Creld1 was associated with simultaneous over-activation and increased apoptosis both *in vitro* and *in vivo*, resulting in a net loss of T cells with age. Mechanistically Creld1 was linked to both canonical and non-canonical (Ca2+) Wnt-signalling, a result we confirmed by rescue of the phenotype by pharmacological intervention. The role of CRELD1 in immunosenescence was further supported by loss of CRELD1 in diseases such as COPD, for which such immune deviation has been postulated recently.

Keywords: Adaptive immunity, ageing, cell death, immune networks, immune senescence

OP-133

Sibling rivalry among the ZBTB transcription factor family: homo vs heterodimerization

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The BTB domain is an oligomerization domain found in over 200 proteins encoded by the human genome. 49 of these proteins belong to the family of BTB domain and Zinc Finger-containing transcription factors (ZBTB) that are involved in cellular proliferation, immune cell lineage differentiation, and cancer development. The highly conserved structure of the N-terminal BTB domain is the dimerization site for these proteins. Structural information reveals a natural inclination for BTB homodimerization. In this study we investigated the probability of heterodimer formation as a mechanism that would potentially reveal alternative targets of transcriptional regulation. We analyzed the expression profiles of all ZBTB genes in several immune system cell types from microarray and single cell sequencing experiments. We created a matrix of possible correlated expression. We performed in-vitro binding assays with selected BTB domains fused to fluorescent proteins to show homo and heterodimer formation. We show that ZBTB proteins are expressed differently but that in approximately 30% of all sampled cells, they are indeed co-expressed in pairs or more complex combinations. We tested the binding of several BTB pairs, and we were able to confirm the heterodimeric physical interaction between the BTB domains of PATZ1 and PATZ2, previously reported only in an interactome mapping experiment. Finally, we used available structures of BTB domain dimers and newly constructed models in extended (500ns) molecular dynamics simulations to understand the energetic determinants of homo and heterodimer formation. We conclude that heterodimer formation, although frequently described as less preferred than homodimers, is not always unfavorable.

Keywords: Cancer immunology, cellular interactions, immune regulation and therapy, modelling, RNAseq

OP-134

Structural plasticity of KIR2DL2 and KIR2DL3 enables altered docking geometries atop HLA-C

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The closely related inhibitory killer-cell immunoglobulin-like receptors (KIR), KIR2DL2 and KIR2DL3, regulate the activation of natural killer cells (NK) by interacting with the human leukocyte antigen-C1 (HLA-C1) group of molecules. KIR2DL2, KIR2DL3 and HLA-C1 are highly polymorphic, with this variation being associated with differences in the onset and progression of some human diseases. However, the molecular bases underlying these associations remain unresolved. Here, we determined the crystal structures of KIR2DL2 and KIR2DL3 in complex with HLA-C*07:02 presenting a self-epitope. KIR2DL2 differed from KIR2DL3 in docking modality over HLA-C*07:02 that correlates with variabilty of recognition of HLA-C1 allotypes. Mutagenesis assays indicated differences in the mechanism of HLA-C1 allotype recognition by KIR2DL2 and KIR2DL3. Similarly, HLA-C1 allotypes differed markedly in their capacity to inhibit activation of primary NK cells. These functional differences derive, in part, from KIR2DS2 suggesting KIR2DL2 and KIR2DL3 binding geometries combine with other factors to distinguish HLA-C1 functional recognition.

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Keywords: Antigen processing and presentation, innate immunity, NK cells

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WORKSHOPS

OP-135

IgTreeZ: a toolkit for immunoglobulin gene lineage tree analysis

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Somatic hypermutation (SHM) is an important diversification mechanism that plays a part in the creation of immune memory. Immunoglobulin (Ig) variable region gene lineage trees were used over the years to model SHM and the selection mechanisms operating on B cell clones. IgTreeZ (Immunoglobulin Tree analyZer) is a python-based tool that analyses many aspects of Ig gene lineage trees and their repertoires. Using simulations, we show that IgTreeZ can truthfully detect and quantify population transitions in trees, reveal differences in tree topology characteristics, and can be used for mutation and selection analyses. We used IgTreeZ on empirical data, found evidence for different mutation patterns in different B cell subpopulations, and gained insights into antigen-driven selection in COVID-19 patients. Overall, we present a comprehensive lineage tree analysis tool that can reveal new biological insights into B cell repertoire dynamics.

Keywords: Adaptive immunity, antibody, B lymphocytes, big data, RNAseq

OP-136

Signal inhibitory receptor on leukocytes-1 is an inhibitory pattern recognition receptor that recognizes bacterial and endogenous amphipathic α-helical peptides

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Signal Inhibitory Receptor on Leukocytes-1 (SIRL-1) is a negative regulator of myeloid cell function and dampens anti-microbial responses. We found that multiple strains of staphylococci secrete factors that can engage SIRL-1, which we identified as phenol-soluble modulins (PSMs), amphipathic α-helical peptides involved in multiple aspects of Staphylococcus physiology. Human cathelicidin LL-37, a known damage-associated molecular pattern (DAMP), is an amphipathic α-helical peptide that shows structural and functional similarity to S. aureus α-type PSMs. We show that LL-37 also induces SIRL-1 activation. On the basis of structural similarities between α-type PSMs and cathelicidin LL-37 we designed artificial peptides with amphipathic α-helical properties that can engage SIRL-1. None of these peptides induced signaling through FPR2, a known receptor for PSMs and LL-37, identifying them as specific SIRL-1 agonists, with potential therapeutic implication in inflammation and cancer. We propose that SIRL-1 is an inhibitory Pattern Recognition Receptor that recognizes both microbial and endogenous amphipathic α-helical peptides to balance immune responses.

Keywords: Checkpoint inhibition, immune regulation and therapy, innate host defence, innate immunity

OP-329

Systems analysis reveals the unique immune system organization in the long-lived rodent Spalax

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Immunosenescence includes systematic changes in immune cell composition, development, and function and plays a critical role in age-associated disorders. Here we focused on the analysis of the immune system of a unique non-model rodent, Spalax Galili. Spalax has an exceptionally long lifespan (up to 20y vs. 2-3y for mice and rats), is highly resistant to cancer, and has many other evolutionary adaptations allowing an extreme lifespan. We performed an accurate error-corrected immune receptor repertoire analysis from Spalax of a broad age range (0 to 17.5yo). Strikingly, the TCR repertoire diversity remained stable in the advanced Spalax age compared to humans and mice. This stability suggests the restricted accumulation of large, long-lived clones of effector-memory T cells and the steady homeostasis of naïve T cells. The expression of master transcription factors of T-cell differentiation, checkpoint, and cytotoxicity genes was low across ages. The age-associated thymus shrinkage evaluated by MRI analysis was comparable to mice. The B cell receptor repertoire analysis showed stable hypermutation levels and IgM repertoire diversity, which potentially reflects shorter B cell memory. Thus Spalax immune system has a unique structure supporting immune receptor diversity and functionality at an advanced age, which potentially contributes to its extreme longevity.

Keywords: Adaptive immunity, ageing, B lymphocytes, immune senescence

OP-339

The effect of high-density lipoprotein (HDL) on autophagy and mitochondrial dynamics in a monocytic cell line

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Increasing plasma levels of High-density lipoprotein cholesterol (HDL-C) has direct impact on macrophages and play a role in regressing atherosclerotic lesion. Recent studies highlighted the importance of proper mitochondrial function and mitochondrial dynamics in preservation of immune cell phenotypic properties and activity. In this study, we examined the effect of HDL on autophagy and mitochondrial dynamics in a monocytic (U937) cell line. Mitochondrial dynamics, mitophagy, and autophagy levels were analyzed by western blot and flow cytometry in U937 cells treated with various doses of HDL at different time points. Western blot analysis showed that 100 µg/ml of HDL changed the level of LC3B-II protein, a well-known autophagy marker, at 16 hours. We also determined the changes in Pink1, Drp1, Mfn2 protein levels which are related to mitochondrial dynamics. Flow cytometry analysis showed that although mitochondrial mass did not change with HDL treatment, mitochondrial membrane potential (MMP) and mitochondrial superoxide levels decreased in a time dependent manner. In addition, both mitochondrial respiration and glycolysis measured by Agilent Seahorse XFp analyzer decreased in U937 cells. For further evaluation, we are currently examining the expression of M1/M2 surface markers upon HDL treatment. We propose that HDL-C levels may alter mitochondrial dynamics and autophagy. Thereby, these changes might have a direct impact on innate immunity and inflammation in atherosclerosis.

Keywords: Cardiovascular diseases, inflammatory disease, innate immunity, molecular immunology

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WORKSHOPS

TRACK 3 - DISEASES AND IMMUNE RESPONSES

OP-137

Clarithromycin impairs tissue-resident memory and Th17 responses to macrolide-resistant *Streptococcus pneumoniae* infections

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The increasing prevalence of antimicrobial resistance in pathogens is a growing public health concern, with the potential to compromise the success of infectious disease treatments in the future. Particularly, the number of infections by macrolide antibiotics-resistant *Streptococcus pneumoniae* is increasing. We show here that Clarithromycin impairs both the frequencies and number of interleukin (IL)-17 producing T helper (Th) 17 cells within the lungs of mice infected with a macrolide-resistant *S. pneumoniae* serotype 15A strain. Subsequently, the tissue-resident memory CD4+ T cell (Trm) response to a consecutive *S. pneumoniae* infection was impaired. The number of lung resident IL-17+ CD69+ Trm was diminished upon Clarithromycin treatment during reinfection. Mechanistically, Clarithromycin attenuated phosphorylation of the p90-S6-kinase as part of the ERK pathway in Th17 cells. Moreover, a strong increase in the mitochondrial-mediated maximal respiratory capacity was observed, while mitochondrial protein translation and mTOR signaling were unimpaired. Therefore, treatment with macrolide antibiotics may favor the spread of antimicrobial-resistant pathogens not only by applying a selection pressure but also by decreasing the natural T cell immune response. Clinical administration of macrolide antibiotics as standard therapy procedure during initial hospitalization should be reconsidered accordingly and possibly be withheld until microbial resistance is determined.

Keywords: Adaptive immunity, bacterial infections, drugs for immune modulation

OP-138

mTOR inhibitors prevent CMV infection through the restoration of functional gamma-delta and alpha-beta T cells in kidney transplantation

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The reported association of mTOR-inhibitors (mTORi) treatment with a lower incidence of cytomegalovirus (CMV) infection in CMV-seropositive (R+) kidney transplant recipients (KTR) remains unexplained. The incidence of CMV infection and T-cell profile was compared between mTORi-treated and mycophenolic acid (MPA)-treated KTR, and we analyzed mTORi effects *in vitro* on T cell phenotype and functions. In MPA-treated R+ KTR, we showed that both γδ and αβ T-cells displayed a more dysfunctional phenotype (PD-1+, CD85j+) at day 0 of transplantation in the 16 KTR with severe CMV infection when compared to the 17 KTR without or with spontaneously resolving CMV infection. In mTORi-treated patients (n= 27), the proportion of PD-1+ and CD85j+ γδ and αβT-cells decreased when compared to MPA-treated patients (n=44), as well as the frequency and severity of CMV infections. mTORi treatment also led to higher proportions of late-differentiated and cytotoxic γδ T-cells, and IFNγ-producing and cytotoxic αβ T-cells. *In vitro*, mTORi (i) increased proliferation, viability and CMV-induced IFNγ production of T cells, (ii) decreased PD-1 and CD85j expression in T-cells that shifted to a more efficient EOMESlow HOBIThigh profile. In γδ T-cells mTORi effect was related to increased TCR signaling. Our results reveal (i) that severe CMV replication is associated with a dysfunctional T cell profile and (ii) that mTORi improve T-cell fitness in association with a better control of CMV. Dysfunctional T cell phenotype could represent a new biomarker to predict post-transplantation infection and to stratify patients who should benefit from mTORi treatment.

Keywords: Drugs for immune modulation, gamma-delta T cells, monitoring immunity, transplantation, viral infections

OP-139

Second tier testing to reduce the number of non-actionable secondary findings and false positive referrals in newborn screening for severe combined immunodeficiency

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Severe combined immunodeficiency (SCID) is characterized by absence of T-cells. Early identification of SCID in the neonatal phase substantially improves the clinical outcome, because a corrective treatment can be initiated before the onset of life-threatening infections. Newborn screening (NBS) for SCID became recently available and is based on the quantification of T-cell receptor excision circles (TRECs). TRECs are not specific for SCID and also identify other conditions with low T-cells (secondary findings). The high rate of (non-actionable) secondary findings and false-positive referrals raises questions about the harm-benefit-ratio of SCID screening, as referrals are associated with high emotional impact and anxiety for parents. In this study, we performed an alternative quantitative PCR on NBS cards of referred newborns (N=56) and epigenetic immune cell counting was used for relative quantification of CD3+ T-cells (N=59). In addition, retrospective data was used to determine the reduction in referrals with a lower TREC cut-off value or an adjusted screening algorithm. 45% of the referrals had normal TREC levels with the alternative qPCR, including four false-positive cases in which two SNPs were identified. With epigenetic qPCR, 41% of the referrals had relative CD3+ T-cell counts in the range of healthy controls. Lowering the TREC cut-off value or adjusting the screening algorithm would lead to lower referral rates, but would not prevent all false-positive referrals. This study shows lowering cut-off values, adjusting screening algorithms and second tier tests can reduce the number secondary findings and false-positive referrals, while retaining the sensitivity to detect SCID.

Keywords: Adaptive immunity, biomarkers, immunodeficiency, molecular immunology

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WORKSHOPS

OP-140

Differences in the SARS-COV2 IgA and IgG responses to S1, S2, RBD and N regions following a single dose of AZD1222 compared to natural infection

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As the differences in SARS-CoV2 IgA and IgG responses in natural infection compared to AZD1222/Covishield have not been studied, we investigated these responses following immunization and natural infection. SARS-CoV-2 S1, S2, RBD and N specific IgA and IgG antibody responses were measured using multiplex SARS-CoV-2 antigen panels, in those who had mild (n=14), moderate/severe illness (n=20), those who received a single dose of AZD1222/Covishield (n=71) and naturally infected individuals before a single dose of the vaccine (n=20). At 4 weeks, those who had mild illness had significantly lower IgG responses to S1 (p=0.0002), to S2 (p=0.01), RBD (p=0.002) than those who had moderate/severe disease or following a single dose of the vaccine, but significantly higher responses to N than vaccines consistent with the spike vaccine content (p<0.0001). At 4 weeks the vaccinated individuals had significantly lower IgA responses to all the proteins than those with mild or moderate/severe illness. At 12 weeks, except for significantly lower IgG responses to N in the vaccines (p=0.0005), there were no significant differences to the other proteins in the different groups. At 12 weeks, the vaccinated individuals had significantly lower IgA responses to S1, RBD and N protein but not to S2. Individuals who had a single dose of AZD1222 vaccine generate an IgG antibody response to S1, S2 and RBD comparable natural infection at 4 and 12 weeks, although they had significantly lower IgA responses. The significance of these antibody responses against prevention of infection, should be further investigated.

Keywords: Antibody, immune development, viral infections

OP-141

IL-17-producing CD8 T cells associate with poor survival in PDAC and promote carcinogenesis via induction of inflammatory cancer associated fibroblasts

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Pancreatic ductal adenocarcinoma is characterized by highly desmoplastic stroma, suppression of antitumoral immune response and resistance against chemotherapy. Recent publications show a role of pro-inflammatory cytokine IL-17 not only in context of autoimmune diseases and systemic inflammation, but also in the development of pancreatitis and a shift from pancreatic intraepithelial neoplasia (PANins) to the onset of PDACs. Intratumoral CD8+ T-cells are of special interest, either as canonical antitumoral cytotoxic T-lymphocytes (CTLs) or as IL-17 producing Tc17 cells. Modulation of these immune cells in combination with classical antitumor therapies might open up new avenues of treatment. Our data reveal increased intratumoral Tc17 abundance associates with strongly decreased survival and advanced tumor stage in PDAC. Consistently with this finding in patients, also in the mouse model Tc17 cells enhanced pancreatic tumor growth. Mechanistically, Tc17 promoted tumorigenesis in an indirect manner via induction of inflammatory cancer associated fibroblasts (iCAF). IL-17A and F produced by Tc17 cells directed iCAF phenotype, which in turn enhanced tumor cell growth *in vitro* and in the mouse model. Our RNA-seq analysis revealed that Tc17-driven iCAF changed the transcriptional program of pancreatic tumor cells towards expression of genes associated with positive regulation of cell proliferation, cell-cell adhesion, cholesterol biosynthesis and negative regulation of extrinsic apoptosis. These genes were also present human PDAC.

Keywords: Cytokines and mediators, cancer immunology, *in vivo* tumor models, microenvironment

OP-142

Defining the role of microglia in Relapsing-Remitting EAE

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Microglia are the resident mononuclear phagocytes of the central nervous system (CNS) parenchyma, sequestered behind the blood brain barrier (BBB). Exact functional contributions of microglia in CNS pathologies remain unclear due to difficulties in discriminating resident microglia from infiltrating monocyte-derived macrophages. Using the RiboTag strategy, we longitudinally profiled microglial translatomes throughout the stages of a Relapsing-Remitting Experimental Autoimmune Encephalomyelitis (RR-EAE) mouse model of multiple sclerosis (MS) in (SJL*B6) F1 hybrid mice challenged with PLP peptide. We show that microglia translate transcripts related to cell-cell immune interactions, such as MHC-II, ICAM1 and ICOSL, alongside inhibitory molecules such as IL-18bp, Lag3, and PDL-1, at the peak of disease, prior to remission. Depletion of microglia in Cx3cr1CreER:Rosa26iDTR mice led to delayed recovery which was accompanied by accumulation of T cells in the brain, suggesting a protective role of microglia in RR-EAE. Using advanced image-stream analysis, multicolor immunofluorescence and flow cytometry, we found evidence for cognate microglia interactions with T cells, and preferentially T regulatory (Treg) cells. We are in the process to test this hypothesis by generating (SJL*B6) F1 hybrids harboring microglial MHCII and IFNgR deficiencies. Collectively our results suggest that while dispensible for EAE onset, microglia might play a critical role in remission through interactions with Treg cells.

Keywords: Animal models, autoimmunity, innate immunity, macrophage, multiple sclerosis, neuroimmunology

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WORKSHOPS

OP-143

CD160, a CD28 alternative costimulatory molecule of alloreactive CD8 T cells unable to prevent NK cell-mediated mechanisms of rejection

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CD160 is a member of the immunoglobulin superfamily with a pattern of expression mainly restricted to cytotoxic cells. To assess the functional relevance of the HVEM/CD160 signaling pathway in allogeneic cytotoxic responses, exon 2 of the CD160 gene was targeted by CRISPR/Cas9 to generate CD160 deficient mice. Next, we evaluated the impact of CD160 deficiency in the course of an alloreactive response. To that aim, parental donor WT or CD160 KO T cells were adoptively transferred into semiallogeneic F1 recipients. Donor alloreactive CD160 KO CD4 T cells and CD8 T cells clonally expanded less vigorously than WT T cells. This differential proliferative response rate at the early phase of T cell expansion influenced the course of CD8 T cell differentiation toward effector T cells that led to a decreased IL-7Rα / KLRG-1 ratio in CD160 KO CD8 T cells when compared to WT CD8 T cells. Despite these differences in T cell proliferation and differentiation, allogeneic MHC class I mismatched (bm1) skin allograft survival in CD160 KO recipients was comparable to that of WT recipients. The administration of CTLA-4.Ig showed a non-significant trend toward increased survival of bm1 skin allografts in CD160 KO with respect to WT recipients. Finally, CD160 deficient NK cells were as proficient as CD160 WT NK cells in rejecting allogeneic cellular allografts and MHC class I deficient tumor cells. CD160 functions as a CD28 alternative costimulatory molecule that modulates CD8 T cell differentiation with a limited impact in allograft rejection.

Keywords: Animal models, antibody, transplantation

OP-144

Highly efficient CRISPR-Cas9-mediated gene knockout in primary human B cells for functional genetic studies of Epstein-Barr virus infection

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My group has pioneered the genetic analysis of Epstein-Barr virus (EBV) such that all herpesviral genes can be modified. While large panels of EBV mutant viruses are readily available, studies into genetic variants of EBV's genuine human target cells, mature B cells have not been possible. With the advent of the CRISPR-Cas9 technology we have succeeded in genome engineering in primary resting human B cells with a highly efficient, simple and rapid protocol. We demonstrated gene editing of the CD46 locus reaching efficiencies of 85 % and studies into the kinetics of double-strand breaks revealed that they very rapidly appear at the CD46 locus in the majority of cells within hours after nucleofection. Next generation sequencing of CD46 mRNAs metabolically labeled with 4-thiouridine (4sU) documented locus repair within 24h indicating the rapid clearance and active transcription of the edited gene locus. For a functional proof-of-principle, we targeted an EBV relevant cellular gene, CDKN2A, which encodes the cell cycle regulator p16INK4a. Our findings documented that p16INK4a is an important target of the viral EBNA3C repressor protein and the only barrier to EBV-induced B cell proliferation. With this novel technology at hand, we are currently focusing on the role of epigenetic cellular factors that suppress most viral genes in the pre-latent phase of infection as the virus delivers its epigenetically naïve DNA genome free of histones and repressive epigenetic marks. We hope that this knowledge could provide insights critical to the development of epigenetic therapies for both viral and host diseases.

Keywords: B lymphocytes, cell based therapies, epigenetic control and modulation of immunity, infectious disease, viral infections

OP-145

GATA6 deficiency leads to epithelial barrier dysfunction and enhances susceptibility to gut inflammation

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Intestinal barrier dysfunction is a hallmark of inflammatory bowel diseases (IBD), but the mechanisms underlying such a defect are not fully understood. This study was aimed at characterizing the factors involved in the defective barrier function in IBD. Transcriptome analysis was performed on colon samples taken from healthy controls (CTR) and IBD patients. Multiple genes involved in cell commitment/proliferation and wound healing were differentially expressed in IBD compared to CTR. Among these, the GATA-binding factor 6 (GATA6) was significantly decreased in the IBD epithelium compared to CTR. Moreover, decreased expression of GATA6 protein was observed in the intestinal epithelium of mucosal samples isolated from IBD patients compared to CTR. Mice with conditional deletion of Gata6 gene in the intestinal epithelial cells (Gata6del) showed epithelial damage, pronounced inflammatory cell infiltration of the mucosa, altered zonulin-1 expression, increased intestinal permeability, gut dysbiosis, local immune response and enhanced susceptibility to gut inflammation. Antibiotic-driven depletion of gut microbiota abrogated the local inflammatory response in Gata6del mice without changing the main epithelial alterations. Our data suggest that decreased expression of GATA6 contributes to intestinal barrier dysfunction and bacteria-driven immune-inflammatory response in IBD.

Keywords: Animal models, inflammatory bowel disease, innate immunity

OP-146

Trained immunity confers prolonged protection against systemic listeriosis

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Trained immunity characterizes the capacity of memory of the innate immune system. Induction of trained immunity is associated with epigenetic, metabolic and functional reprograming of innate immune cells as well as hematopoietic stems cells (HSCs). We reported that trained immunity protects from lethal bacterial sepsis (Ciarlo, JID 2020), but the length of the protection remains unknown. To determine whether trained immunity confers prolonged protection from lethal infection. Mice (n=8-9/group) were trained with β-glucan and challenged 9 weeks later with *Listeria monocytogenes*. Mice were analyzed by bioluminescence imaging. Blood, bone-marrow, liver and spleen were collected to quantify HSCs, leukocytes (flow cytometry), bacteria, metabolic and antimicrobial activity (SeaHorse and killing assay), and injury (histological examination). Control mice were bacteremic and died from listeriosis, while mice trained 9 weeks earlier controlled bacterial burden and survived infection (P<0.001). Trained mice showed lower inflammation (bioluminescence imaging, systemic cytokines) and liver injury. Listeriosis induced the depletion of blood leukocytes, which was counterbalanced by trained immunity. Trained mice had increased myelopoiesis, and their blood contained 2-fold more Ly6Chigh monocytes and neutrophils (P<0.001), produced more G-CSF, IFNγ, IL-1α, IL-1β, IL-6, IL-10, TNF and CXCL2 in response to LPS, and controlled better the growth of L. monocytogenes. Monocytes and neutrophils showed enhanced glycolytic activity. These results suggest long-lasting protection afforded by trained immunity against lethal listeriosis. We are running experiments to better define the window of protection conferred by trained immunity.

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Keywords: Infectious disease, innate host defence, innate immunity, memory

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WORKSHOPS

OP-147

The role of DYRK1A in B cell immune responses

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Germinal centers are microanatomical sites within lymphoid organs, wherein high affinity antibodies originate through B cell proliferation, insertion of somatic hypermutations and affinity-based selection. The dual-specificity tyrosine-regulated kinase 1A (DYRK1A) is known to participate in neurodevelopment and plays a role as a negative regulator in cell cycle progression. This kinase is encoded in the Down syndrome chromosome 21 resulting in overexpression in this individuals. How this kinase modulates B cell proliferation and functions in the germinal center reaction is still unknown. Here, we show that DYRK1A-deficient mice exhibit dysregulated germinal center size and abnormal B cell proliferation in a time dependent manner following immunization. Gene expression profiles of germinal center B cells are massively altered, especially in the dark zone, depicting cell division genes upregulation in the absence of the DYRK1A kinase. By using single-cell immunoglobulin sequencing we found that although DYRK1A-deficient mice display clonal diversity and expansion comparable to their control counterparts, they lack the ability to generate antigen-specific high-affinity B cells. Clinical data collected from a Down syndrome cohort, revealed that IgA blood levels in Down syndrome individuals are elevated, whereas IgA titer in blood and bone marrow of DYRK1A-deficient mice are reduced compared to the corresponding controls. These shed light on previously unknown functions of kinase phosphorylation during B cell affinity maturation and cell cycle events and may explain immunodeficiency in Down syndrome individuals.

Keywords: Immunodeficiency, proliferative disorders, RNAseq, antibody, B lymphocytes

OP-148

Role of danger and microbial signals in the control of the recruitment of neutrophil subpopulations during infections

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Neutrophils are the first line of defence against bacteria and fungi and help fight parasites and viruses. Recently, neutrophil subpopulations with distinct functions have been reported under homeostatic and pathological conditions, although their role and their mechanisms of recruitment during inflammation have not been clarified. The aim of our project is to establish the mechanism of recruitment and to functionally characterize neutrophil subsets during fungal, Gram-negative and Gram-positive bacterial infections. To this purpose we used a model of skin infection with *Candida (C.) albicans*, *Pseudomonas (P.) aeruginosa* and *Staphylococcus (S.) aureus* and we performed a multi-parametric flow-cytometry analysis. We identified aged and fresh neutrophils as CXCR4+CD62LIow and CXCR4- CD62+/high respectively. Moreover, we employed mice lacking key Pattern Recognition Receptors (PRRs), PRR signalling molecules or alarmins to study the mechanisms of neutrophil subsets' recruitment. Our preliminary data suggest the existence of two waves of neutrophil recruitment, the first mediated exclusively by danger molecules and the second mediated by both danger molecules and PAMPS (Pathogen Associated Molecular Patterns). Our data also suggest an increased accumulation of aged neutrophils during the second wave. An enhanced "effector" phenotype and an increased phagocytic activity *in vitro* has been described for aged neutrophils. Accordingly, our preliminary results indicate that neutrophil aging is not merely a passive mechanism to direct their death in tissues. In fact, aged neutrophils are recruited at the infection site at late time points when fresh neutrophils have confined the infection. This suggests an active role of aged neutrophil in determining pathogen clearance.

Keywords: Ageing, bacterial infections, fungal infections, innate immunity, neutrophils

OP-149

Functional monocytic myeloid-derived suppressor cells increase in blood but not airways and predict COVID-19 severity

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The immunopathology of coronavirus disease 2019 (COVID-19) remains enigmatic, causing immunodysregulation and T cell lymphopenia. Monocytic myeloid-derived suppressor cells (M-MDSCs) are T cell suppressors that expand in inflammatory conditions, but their role in acute respiratory infections remains unclear. We studied the blood and airways of patients with COVID-19 across disease severities at multiple time points. M-MDSC frequencies were elevated in blood but not in nasopharyngeal or endotracheal aspirates of patients with COVID-19 compared with healthy controls. M-MDSCs isolated from patients with COVID-19 suppressed T cell proliferation and IFN-γ production partly via an arginase 1-dependent (Arg-1-dependent) mechanism. Furthermore, patients showed increased Arg-1 and IL-6 plasma levels. Patients with COVID-19 had fewer T cells and downregulated expression of the CD3ζ chain. Ordinal regression showed that early M-MDSC frequency predicted subsequent disease severity. In conclusion, M-MDSCs expanded in the blood of patients with COVID-19, suppressed T cells, and were strongly associated with disease severity, indicating a role for M-MDSCs in the dysregulated COVID-19 immune response.

Keywords: Infectious disease, innate immunity, myeloid derived suppressor cells, viral infections

OP-150

SARS-CoV-2-specific T cell immunity is retained 12 months post-infection and is dependent on CD4 T cells

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The SARS-CoV-2 pandemic has led to around 3.2 million deaths and continues to rapidly infect millions worldwide. T cell responses against SARS-CoV-2 are critical in the development of long-lasting immunity to sufficiently control this infectious disease. The aim of this study was to assess longitudinal SARS-CoV-2 specific T cell responses in COVID-19 patients. To do so, peripheral blood mononuclear cells (PBMCs) were isolated at multiple time points from hospitalized patients during acute infection (enrolled day 2-37 after symptom onset; n=25) and non-hospitalized patients (with PCR-confirmed SARS-CoV-2 infection but asymptomatic prior to enrolment; n=30) and healthy controls (uninfected; n=17). PBMCs were stimulated with SARS-CoV-2 specific peptide pools and the production of IFNγ and IL-5 was measured using a dual-colour ELISPOT assay. We found that all patients retained spike-specific and nucleocapsid-specific T cell responses at 6 and 12 months post-infection. Unlike other viral infections, we did not observe a T cell contraction phase against SARS-CoV-2. Interestingly, the majority of the SARS-CoV-2-specific T cell response was mediated by CD4+ T cells. Vaccination of previously infected patients significantly boosted spike-specific T cell responses. Overall our data shows that SARS-CoV-2 specific T cell responses persist up to at least 12 months post-infection, are further boosted by vaccination and that CD4 T cells play an important role in this response.

Keywords: Adaptive immunity, infectious disease, memory

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WORKSHOPS

OP-151

Frequencies and functional properties of innate lymphoid cells are altered in melanoma patients and modulated by immune checkpoints inhibitors

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The use of monoclonal antibodies (mAbs) targeting immune checkpoints has improved the prognosis of malignant melanoma (MM). Immune checkpoint receptors are expressed by helper innate lymphoid cells (ILCs), innate lymphocytes mirroring T helper cells. Thus, mAbs therapy would likely affect ILC subsets in MM patients. Here, we found a higher frequency of total peripheral ILCs in MM patients compared to healthy donors, with lower percentages of c-Kit+ ILC2 and ILC3 and higher frequencies of ILC1. ILC1 and ILC3 from MM patients also showed an impaired capability to secrete TNFα when activated *ex vivo* with PMA/ionomycin. In addition, therapy with anti PD-1 mAb (Nivolumab) reduced the frequency of total peripheral ILCs increasing at the same time the percentage of c-Kit- ILC2. The treatment also restored the capability of ILC2 and ILC3 to secrete IL-13 and TNFα, respectively, while ILC3 frequency after therapy was positively associated with longer survival. We also observed that all the three ILC subsets were able to respond to melanoma cells by up-regulating TNFα, with ILC2 also increasing the number of IL-13 producing cells. Collectively, data suggest that MM affects the relative frequencies as well as the functional capabilities of ILCs, while Nivolumab treatment is partially able to rescue these alterations. Moreover, activated ILCs are able to secrete cytokines in response to melanoma cells. Thus, strategies aimed to restore ILCs secretory activity and/or modulate their plasticity could represent a novel approach to improve the outcome of current immune therapies.

Keywords: Biomarkers, cancer immunology, innate lymphoid cells

OP-152

Arginase 1 inhibits the resolution of colitis due to the consumption of L-arginine and the expansion of an inflammatory microbiota

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Arginase 1 (Arg1) converts the semi-essential amino acid L-arginine into urea and ornithine which can be further metabolized into polyamines. Besides enzymatic degradation, the availability of L-arginine depends on the diet, intraluminal consumption by intestinal microbiota and uptake in the gut. Furthermore, many disorders alter L-arginine metabolism and Arg1 function. Thus, Arg1 expression and activity are enhanced in colonic tissues of experimental colitis models and patients with inflammatory bowel disease (IBD). Therefore we characterized the impact of L-arginine and Arg1 on intestinal microbiota and the resolution of colitis. Arginine-free chow accelerated DSS-induced colitis while a dietary supplementation of mice with L-arginine promoted the resolution of intestinal inflammation. Unexpectedly, Tie2-Cre+/-xArg1fl/fl mice lacking Arg1 in hematopoetic and endothelial cells recovered faster from experimental colitis than Arg1-expressing littermates. Protection from disease was associated with an accumulation of intraluminal polyamines, decreased inflammatory cytokine production and compositional changes in the intestinal microbiota in L-arginine-supplemented wild-type litters, similar as observed in control chow fed Tie2-Cre+/-Arg1fl/fl mice. Fecal microbiota transfers (FMTs) from wild-type litters supplemented with L-Arginine restored the protective, anti-inflammatory phenotype in recipientssimilar as FMTs from control chow fed Tie2-Cre+/-Arg1fl/fl donors, suggesting the microbiota as source for protective polyamine production. Vice versa, dietary L-arginine restriction abolished the anti-colitogenic effects of Arg1-deletion, suggesting that protection is related to an enhanced availability of L-arginine, subsequent accumulation of polyamines and expansion of an anti-inflammatory microbiota. Due to its high expression and acitivity in colitic tissues, Arg1 might serve as novel target for clinical intervention in IBD patients.

Keywords: Immune communication, inflammatory bowel disease, mass spectrometry, metabolic control of immune responses, microbiome and environmental factors, RNAseq

OP-153

Re-circulating tissue-resident memory T cells are poised for a systemic Th2-driven host-versus-graft reaction after human allogeneic hematopoietic stem cell transplantation

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Tissue-resident memory T cells are sessile cells providing protection against outside pathogens at barrier sites. Recent observations in mouse models indicate that a subset of tissue-resident memory T cells (Trm) may exit the skin and form a discrete circulating T cell population. To explore the existence of a re-circulating Trm population (cTrm) in humans, we characterized circulating T cells with a skin Trm phenotype in the blood of patients after allogeneic hematopoietic stem cell transplantation (HSCT). We found a small and stable population of CD4$^+$CD103$^+$CLA$^+$ T cells in the blood of all patients analyzed and verified their tissue origin by genotyping in sex-mismatched HSCT recipients. Transcriptional analysis on single-cell level revealed their striking resemblance to skin Trm and mathematical modelling of proliferation dynamics suggested renewal via the skin Trm pool. Importantly, blood from patients with GvHD contained elevated numbers of host cTrm producing pro-inflammatory Th2/Th17 cytokines, which highlights the potential of skin Trm to contributeto inflammation on a systemic level. Collectively, our data offers first proof of a distinct Trm-like circulating T cell type, which mirrors cutaneous inflammation and may disseminate disease via the blood circulation.

Keywords: Adaptive immunity, bone marrow transplantation, memory, RNAseq, skin diseases

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WORKSHOPS

OP-154

Sepsis induces long-term reprogramming of human hematopoietic stem cells

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Sepsis is a life-threatening syndrome which is mainly caused by a dysregulated host response to pathogen infection, and affects ~50 million people worldwide every year. Typically, recovered patients exhibit persistent low-grade inflammation and immunosuppression which results in poor functional independence, increased susceptibility to secondary infection and reduced survival. In this study, we collected peripheral blood from sepsis survivors at least 6 months after the recovery from sepsis. Compared to age-matched healthy subjects, sepsis survivors showed an expansion of circulating hematopoietic stem cells (HSCs), myeloid progenitors and immature myeloid cells such as myeloid-derived suppressor cells. Transcriptomic analysis of macrophages derived from HSCs showed that sepsis survivors' HSCs give rise to a myeloid progeny characterised by impaired metabolic activity and rewiring of transcription factor networks, comprising STAT1, IRF9 and IRF3. Further *in vitro* experiments showed that type-I IFN signalling on healthy HSCs affects their proliferation and terminal differentiation of their myeloid progeny. Our data support the idea that long-term reprogramming of HSCs during sepsis, possibly through a type-I IFN axis, might be responsible for the persistent immunosuppression and increased frailty of sepsis survivors and suggest that targeting HSCs differentiation in these patients might improve overall survival and quality of life.

Keywords: Infectious disease, innate immunity, memory, myeloid cells, stem cells

OP-155

Cytokine-producing tumor-infiltrating lymphocyte products can be effectively generated from pre-treated late stage non-small-cell lung cancer (NSCLC) lesions irrespective of location and pre-treatment regimens

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Non-small cell lung cancer (NSCLC) is the second most occurring type of cancer. Because of the high mortality rate with the current treatment regimens, novel therapeutic approaches are warranted. The high mutational rate of the tumors, and the high level of T cell infiltrates should render NSCLC patients eligible for autologous T cell therapy. We recently showed that tumor infiltrating lymphocytes (TILs) from treatment-naive, stage I/II NSCLC tumors can be effectively expanded and reprogrammed into a tumor-reactive T cell product for adoptive T cell therapy. Whether this promising finding translates into late stage NSCLC patients, and whether pre-treatment regimens and location of metastatic lesions influences the generation and quality of tumor-reactive TIL products is unknown, yet a pre-requisite for bringing this treatment regimen to the clinic. Here we analysed TILs isolated from different metastatic lesions. The immune infiltrates from metastatic NSCLC lesions and in particular the CD3 compartment did not substantially differ from early stage primary NSCLC lesions. With the clinically approved TIL expansion protocol, we reached a similar expansion rate as described for early stage NSCLC tumors and metastatic melanoma lesions, demonstrating that obtaining the required cell numbers to generate TIL products for the clinic is feasible. Importantly, the TIL products were not only functional but the majority also displayed anti-tumoral activity after exposure to tumor digest. Our study shows that irrespective of the pretreatment, metastatic NSCLC lesions can provide the source to generate tumor-reactive TIL products for the clinic.

Keywords: Cancer immunology, cell based therapies, immunotherapy

OP-156

Tumor-associated macrophages heterogeneity is driven by distinct niches in breast cancer

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Macrophages represent the most abundant population of the tumor microenvironment. Their accumulation is correlated with bad prognosis in many tumor models. However, tumor-associated macrophages (TAM) constitute a highly heterogeneous population, of which subsets can have antagonistic effects on tumor development. Recently, the concept of niche has emerged to define macrophage populations not uniquely in terms of origin, function or localization but as a scaffold for the macrophage in which there is an equilibrium between the tissue needs and the macrophage needs. However, upon tumor development, resident niches are modified and new niches arise as the organ is growing. In this study, we identify TAM niches in breast tumors using a combination of single cell RNA sequencing, muti-parameters flow cytometry and intra-vital imaging. We highlight specific niches of resident and monocyte-derived TAM that accumulate upon tumor growth while stromal macrophages appear to be excluded from tumor nodules in spontaneous mammary tumors. We show that in absence of initial niches, using an orthotopic tumor model, macrophage subsets harbor similar phenotypes and localization throughout tumor growth. Finally, we compared the signatures of our different TAM niches to published human dataset and found similarities in the subsets suggesting that this heterogeneity also exist in human breast cancer. Our results indicate that TAM niche identification could have major implications for specific targeting in anti-tumor therapies.

Keywords: Cancer immunology, macrophage, microenvironment, RNAseq

OP-157

Anti-inflammatory effect of Crocus sativus extract evaluated in a murine model of osteoarthritis

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Osteoarthritis (OA) is a chronic degenerative disorder of the joint and represents one of the most common diseases worldwide characterized by cartilage breakdown and synovial inflammation. The clinical symptoms of OA are joint swelling, synovitis, and inflammatory pain. Available drugs provide symptomatic relief of pain only, and joint replacement is the only choice, eventually. The aim of our study was to investigate the potential role of saffron extract to the activity and pathogenesis of the CIOA. The collagenase-induced OA mouse model, Balb/c mice, saffron extract, flow cytometry, proliferation, ELISA, histology. The extract of Crocus sativus has shown very good properties as a stimulator of splenocytes proliferation *in vitro*. The flow cytometric analysis of spleen cells from CIOA animals treated with saffron extract showed a decrease in the CD8+ T cell population and an increase in the CD4+ T cells compared to the CIOA controls. T lymphocyte activation markers level were reduced in the treated animals. The extract treatment decreased the percent of activated CD107+ NK cells and increased the CD 11b+ F4/80+ macrophages. The administration of Crocus sativus extract to CIOA mice significantly suppressed inflammation in the joints, reduced bone erosion, and helped the preservation of articular cartilage. The active components of the tested extract affected the destructive action of osteoclasts and reduced the loss of proteoglycans and glycosaminoglycans in the joint. It can be concluded that saffron extract has therapeutic potential for the treatment of patients with OA.

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Keywords: Animal models, inflammatory joint diseases, tissue damage and repair

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WORKSHOPS

OP-158

CD69 expression on regulatory T cells protects from immune-mediated damage after myocardial infarction

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CD69 depletion of the lymphoid compartment promotes a Th17/Treg imbalance and exacerbates the development of atherosclerosis. Since atherosclerosis can trigger myocardial infarction (MI), we have analyzed the role of CD69 in Treg cells after a) permanent occlusion of the left-anterior-descending coronary artery (LAD)-ligation in mice, and b) in two cohorts of patients with acute MI. Our data in mice demonstrate that CD69 expression on Treg cells is critical for maintaining immune homeostasis after myocardial infarction and increases overall survival in mice after LAD-ligation. Cd69-/- mice develop strong IL17A+ gamma delta (gd) T cell responses shortly after ischemia that increases myocardial inflammation and, consequently, worsens cardiac function. CD69+ Treg cells induce apoptosis and decrease IL-17A production in gdT cells by a mechanism dependent on membrane CD39 ectonucleotidase activity. Adoptive transfer of CD69+ Treg cells to Cd69-/- mice after LAD-ligation reduces IL17A+ gdT cell recruitment, resulting in increased survival and improved outcome. Moreover, clinical data from two independent cohorts of patients indicate that increased CD69 expression in peripheral blood after acute MI is associated with a lower risk of re-hospitalization for chronic heart failure. Our data support a role of CD69+ Treg cells in preventing excessive inflammation and myocardial damage after MI, leading to improved cardiac function.

Keywords: Adaptive immunity, animal models, cardiovascular diseases, gamma-delta T cells, inflammatory molecules, regulatory cells

OP-159

Early-life vitamin A treatment rescues neonatal infection-induced long-lastingly impaired lymph node operativeness

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Gut-draining mesenteric and celiac lymph nodes (mLNs and celLN) are important for inducing peripheral tolerance towards food and commensal antigens. We previously reported that the tolerogenic properties of mLNs and celLN are stably imprinted in their stromal cells (SCs) by microbiota and vitamin A (VA), respectively, and that microbiota-mediated imprinting takes place in early-life. Here, we demonstrate that acute *Yersinia pseudotuberculosis* (*cnfY* knock-out strain YP147) infection occurring exclusively during the neonatal phase, but not during adulthood, severely impaired *de novo* Treg induction in celLN in a long-lasting manner. Transplantations of celLN from neonatally infected mice showed permanently abrogated Treg-inducing capacity of celLN SCs post neonatal infection (*p.i.*). Bulk and single-cell RNAseq revealed the durably altered transcriptome of celLN SCs, including differential expression of multiple chemokine/chemokine receptors, and distinct heterogeneity of celLN fibroblastic SCs *p.i.*. In line with abnormal expression of chemokine/chemokine receptor axis, long-lastingly shifted dendritic cell (DC) composition as well as altered transcriptome of migratory DCs were observed in celLN as late as twelve weeks *p.i.*. Remarkably, a transient reduction of VA levels within the first seven days *p.i.* was observed and exogenous VA administration within this period could retrieve VA levels and consequently rescue the superior *de novo* Treg-inducing capacity of celLN. Together, our data demonstrate that neonatal gastrointestinal infections can long-lastingly influence the functional properties of LNs, the key hubs of the immune system, and early-life VA supplementation can rescue the permanently abrogated LN operativeness, highlighting the therapeutic potential of VA treatment post gastrointestinal infections in infants.

Keywords: Bacterial infections, dendritic cells, immune regulation and therapy, infectious disease, regulatory cells, RNAseq

OP-160

High-protein diet and TLR9 knock out effect on the immune responses in the development of colorectal cancer

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A certain type of diet can cause a disruption of gut microbiome, i.e., dysbiosis, which interferes with pathogenesis of many diseases, including colorectal cancer. Recent evidence reveals the over-expression and functionality of receptor of innate immunity TLR9 in the development of colorectal cancer. But the role of TLR9-mediated inflammatory response in this process is not completely clear. In our experiment, we used C57BL/6 wild type (WT) and TLR9 knock out mice to analyze, how interaction between high-protein diet (HPD) and gut microbiota influences the sensitivity to development of tumors in murine model of colorectal cancer. Due to the induction of the disease, we found higher weight loss in mice fed with HPD compared to control groups, in both WT and TLR9 knock out mice. HPD caused a significant increase in number of macrophages, monocytes and neutrophils in spleen and mesenteric lymph nodes. Nevertheless, these values were always lower in TLR9 knock out mice compared to the WT group. Slight increase in the number of regulatory T cells and Th17 cells was observed in both groups of mice with HPD in mesenteric lymph nodes, but decrease was observed in spleen of TLR9 knock out mice. These preliminary data show differences in the immune response between WT and TLR9 knock out mice. Furthermore, interaction between HPD and gut microbiome affects processes of innate and adaptive immunity and it may be involved in the development of colorectal cancer.

This project is supported by a grant from the Czech Science Foundation number 20-03997S.

Keywords: Adaptive immunity, cancer immunology, immune response tracing, *in vivo* tumor models, innate immunity

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WORKSHOPS

OP-161

Novel heterozygous missense mutations in NFkB1 causes immunedysregulation and immunodeficiency

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Investigating the effect of *NFkB1* heterozygous missense mutations in CVID patients. Genetic background was evaluated for 300 primary immunodeficiency patients using targeted sequencing. "Among others, 4 *NFkB1* variants were identified in 5 common variable immunodeficiency (CVID) patients with autoimmunity. pc. DNA3.1(+)-N-eGFP plasmid expressing the wild-type and variants of *NFkB1* were transiently expressed in HEK293 cells. Total protein and RNA were isolated for western blot and qRT-PCR respectively. ‹›Confocal microscopy was performed to investigate the nuclear localization of the *NFkB1* variants and wild-type. Statistical analysis was performed with GraphPad prism. Genetic analysis identified two novel and two already reported heterozygous missense mutations in *NFkB1*, c.1736 G>A, (p.R578K), c.1601G>A, (p.R533H), c.2793G>C, (p.E930D), and c.691C>T, (p.R230). The mutation c.691C>T, p.R230 caused a severe reduction in the expression of p105/50, also confocal microscopic analysis for this variant showed that this mutation restricts the transcriptionally active p50 in the cytoplasm. The other three mutations also impacted the expression of p105 negatively as NFkB1 expression was reduced. Results of qRT-PCR also showed a reduction in the mRNA levels of the variants compared to the wild-type. Investigation of immunological parameters of studied patients suggested a progressive course of immunodeficiency which may partially account for the incomplete penetrance of this inborn error of immunity and suggest the need for closer immunological monitoring of those mutation carriers. The down-regulation in the expression of p105/50 could affect its function and hence cause the hypogammaglobulinemia as observed in our cohorts.

Keywords: B lymphocytes, infectious disease, autoimmunity, immunodeficiency

OP-162

Oncostatin M opens the gate for T helper 17 cells during neuroinflammation

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Blood-brain barrier (BBB) dysfunction is an intrinsic feature of neurodegenerative and -inflammatory diseases, including multiple sclerosis (MS). Oncostatin M (OSM) cytokine levels are elevated in the blood and brain of MS patients. We previously demonstrated that OSM exerts neuroprotective and remyelination-promoting functions after central nervous system (CNS) damage. However, OSM's role in neuroinflammation and BBB function is poorly understood. We hypothesize that OSM protects the BBB while suppressing neuroinflammation. Experimental autoimmune encephalomyelitis (EAE) is induced in wild-type and OSM receptor (OSMRβ) deficient mice. CNS immune cell infiltration and BBB leakage are analysed post-mortem. Effects of OSM on inflamed BBB-endothelial cells (ECs) are investigated *in vitro* using primary mouse brain microvascular endothelial cells and the human cerebral microvascular endothelial cell line (hCMEC/D3). Parameters of BBB activation and integrity are examined. In contrast to our hypothesis, OSMRβ deficient mice show milder EAE symptoms and lack a disease peak which is associated with diminished T helper 17 (Th17) cell infiltration into the CNS and reduced BBB leakage. *In vitro*, OSM promotes secretion of Th17-attracting chemokine CCL20 by inflamed BBB-ECs, whereas it downregulates cell adhesion molecule expression of intercellular cell adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). Moreover, OSM reduces the transendothelial electrical resistance of BBB-ECs under control and inflammatory conditions by downregulating Claudin-5 and VE-cadherin expression. Together, these data suggest that OSM contributes to the observed BBB permeability in neuroinflammatory disorders while recruiting T helper 17 cells through enhanced endothelial CCL20 secretion.

Keywords: Chemokines, cytokines and mediators, multiple sclerosis, neuroimmunology

OP-163

PD-1 expressing regulatory T cells and basophils with an activating profile in infants with moderate-to-severe atopic dermatitis hypersensitized to food antigens

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Infants with severe Atopic Dermatitis (AD) may be sensitized to foods that have not yet been introduced into their diet. The aim of this work was to perform a comprehensive analysis of the sensitization profile in infants with moderate-to-severe AD and to identify cellular and molecular markers predicting food allergy (FA). Blood samples from healthy donors and children with moderate-to-severe AD were studied. Specific IgE to several allergens were measured using ImmunoCAP FEIA system and ISAC technology. Moreover, using flow cytometry-based studies, basophils and regulatory T (Treg) cells were phenotypically characterized. 90% of children with AD were sensitized to food antigens before introducing them into the diet, and 100% developed FA by the age of 2. Phenotypic analysis of Treg cells showed a significantly higher percentage of CTLA-4 and PD-1 expressing cells in AD patients than in healthy controls. Basophils from patients with moderate-to-severe AD exhibited a marked reduction in the expression of the inhibitory receptor CD300a and to some extent higher expression of activating CD300c. Infants with moderate-to-severe AD are at high risk of being sensitized to food allergens. Therefore, to avoid allergic reactions, broad-spectrum sensitization studies are necessary before introducing complementary diet. Increased expression of CTLA-4 and PD-1 suggests a greater suppressive potential of Treg cells in infants with AD than healthy controls. Furthermore, our results also suggest a role for CD300 molecules on circulating basophils as biomarkers for food allergy susceptibility.

Keywords: Allergic disorders, basophils, biomarkers, inflammatory disease, regulatory cells, skin diseases

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WORKSHOPS

OP-164

Mast cell chymase affects the functional properties of primary human lung fibroblast: implications for asthma

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Mast cells have a profound impact on allergic asthma. Under such conditions, mast cell undergo degranulation, resulting in the release of exceptionally large amounts of mast cell-restricted proteases. However, the role of these proteases in asthma is only partially understood. Here we hypothesized that the mast cell proteases can influence the functionality of human lung fibroblasts(HLF). HLFs underwent major morphological changes in response to chymase, showing signs of cellular contraction, but were refractory to tryptase. However, no effects of chymase on HLF viability or proliferation were seen. Chymase, but not tryptase, had a major impact on the output of extracellular matrix-associated compounds from the HLFs, including degradation of fibronectin and collagen-1, and activation of pro-matrix metalloprotease-2. Further, chymase induced the release of various chemotactic activity on neutrophils. Transcriptome analysis revealed that chymase caused a pro-inflammatory gene transcription profile in HLFs, whereas tryptase had minimal effects. Our findings reveal that chymase, but not tryptase, has a major impact on the phenotype of primary airway fibroblasts, by modifying their output of extracellular matrix components and by inducing a pro-inflammatory phenotype.

Keywords: Cytokines and mediators, RNAseq, mast cells

Acknowledgements: This study was supported by grants from The Swedish Heart and Lung Foundation, The Swedish Research Council, The Swedish Cancer Foundation and Knut & Alice Wallenberg Foundation.

OP-165

Reversal clinical and immunological effects of abatacept in patients with LRBA and CTLA4 deficiencies

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LRBA and CTLA4 deficiencies present with susceptibility to infections, autoimmunity and lymphoproliferation. We sought to investigate the role of abatacept in controlling the different disease manifestations along with immune modulation, potentially helpful to understand the expansive effector mechanisms of targeted therapy in LRBA and CTLA4 deficiencies. LRBA- (n=29) and CTLA4-deficient (n=12) patients were evaluated prospectively at different time points (baseline,3rd,8th,12th months) for clinical manifestations, abatacept responses, regulatory T-cells, circulating T follicular helper cells(cTFH), and intracellular T-helper cells cytokine responses. Three major clinical phenotypes of LRBA deficiency were recurrent infections (n=25, 86.2%), immunodysregulation (n=25, 86.2%) and lymphoproliferation (n=21, 72.4%). While CTLA4 deficiency presented with immunodysregulation (n=10, 83.3%), recurrent infections(n=9, 75.0%) and lymphoproliferation (n=7, 58.3%). LRBA-deficient patients showed significantly low age of symptom onset, high rate of pneumonia, and failure to thrive compared to CTLA4 deficiency. The clinical features characterized by lymphoproliferation, Evans syndrome and diarrhea were controlled in the majority of patients on abatacept, and only two patients showed partial responses necessitating transplantation. The quality of life was significantly improved after abatacept. Long-term abatacept usage showed best reversible effect on T-helper cell responses by increasing naïve T-cells, while decreasing memory T-cells and cytokines including IL-17, IFN-g, IL-4, and IL-10. Furthermore, reduced cTFH and Treg numbers were observed on abatacept in both diseases. Abatacept effectively control the disease activity by regulating the effector T-cell functions, which are mainly responsible for the immune dysregulation in LRBA and CTLA4 deficiencies.

This work was supported by TUBITAK (318S202).

Keywords: Drugs for immune modulation, cytokines and mediators, follicular helper T cells, immunodeficiency, regulatory cells

OP-166

Towards the suppression of the disease-relevant brain-homing CD4+ T cell in multiple sclerosis

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In multiple sclerosis (MS), pro-inflammatory $CD4^+$ T cells invade the central nervous system, but it remains elusive which subset contributes to disease activity. Previously, we demonstrated that circulating Th17.1 (CCR6+CXCR3+CCR4-/dim) cells are reduced in patients with rapid disease onset and elevated in patients responsive to natalizumab (anti-VLA-4 mAb). Here, we further explored the pathogenicity of Th17.1 cells in MS by addressing their sensitivity to steroid hormones. Pro-inflammatory $CD4^+$ T-cell subsets including Th17.1 were discriminated based on chemokine receptor expression patterns using FACS. Cytokine production (Luminex), transmigration (human BEC assay) and glucocorticoid sensitivity (proliferation, activation) were determined *in vitro*. Both multidrug resistance 1 (MDR1) and glucocorticoid (GR) receptor expression was measured using qPCR/FACS. Different MS cohorts (pregnant, treatment-naive early-stage, late-stage patients) and compartments (blood, CSF, postmortem tissue) were assessed. Memory $CD4^+$ T cells of MS patients with postpartum relapses displayed increased production of Th17.1-related cytokines IFN-γ and GM-CSF during pregnancy. These patients had decreased frequencies of circulating Th17.1 cells after delivery, suggesting transmigration into the CNS. Indeed, Th17.1 cells predominated the CSF of early MS in contrast to control patients, which was confirmed *in vitro*. This corresponded to a glucocorticoid resistance profile (MDR1highGRlow) that delineated Th17.1 cells present in MS brain tissue. Their glucocorticoid insensitivity was reversed *in vitro* by female hormones and vitamin D3. These results indicate that MDR1+ Th17.1 cells are promising candidate markers for predicting MS disease activity. Furthermore, the interplay between steroid hormones may be used to suppress this brain-homing T cell.

Keywords: Autoimmunity, biomarkers, multiple sclerosis, neuroimmunology

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WORKSHOPS

OP-167

Oral administration of short chain fatty acids to PBMC-engrafted humanized mice prevents allergen-dependent airway and intestinal inflammation

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Probiotic bacteria and their metabolites, particularly short chain fatty acids (SCFA), have been shown to prevent or ameliorate allergic inflammation in OVA- or house dust mice-driven allergy mouse models. The aim of this study was to investigate the role of SCFA and the probiotic formulation BactoFlor® 10/20 in a recently developed humanized mouse model of allergen-induced IgE-dependent lung and gut inflammation. Therefore, immunodeficient NSG mice were injected intraperitoneally with human PBMC from highly sensitized aeroallergen allergic donors together with the respective allergen or PBS as control. Mice were treated with 200 mM sodium butyrate, acetate or propionate in drinking water or with 1x108 cfu of BactoFlor® 10/20 (containing 3 Bifidobacteria, 5 Lactobacilli, Lactococcus lactis and Enterococcus faecium) every other day per gavage. Three weeks later, inflammation of the gut and lung was monitored by video mini-endoscopy evaluating translucency, granularity, fibrin production, vascularity, and stool, or by invasive body plethysmography and histology after rectal or intranasal allergen challenge, respectively. Compared to control mice, allergen-specific human IgE production in mouse sera, being only detectable in allergen-treated groups, was strongly reduced in mice receiving SCFA or BactoFlor® 10/20. Consequently, allergen-induced IgE-dependent intestinal inflammation, airway hyperreactivity and mucus-producing goblet cells were significantly inhibited in these mice. Importantly, numbers of FoxP3+ cells were slightly increased in the colon, lung and spleen. Furthermore, butyrate but not acetate and propionate concentration in stool was enhanced in BactoFlor® 10/20-treated mice. These results confirm the importance of microbiota-related metabolites for tolerance induction and possible therapeutic intervention of allergic diseases.

Keywords: Allergen-induced immune responses, animal models, microbiome and environmental factors, modification allergic responses

OP-168

The T-cells sweet tooth: T-lymphocytes exposed to highly sialylated cancer associated fibroblasts present an exhausted phenotype, which can be reversed through desialylation

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Hyper-sialylation of cancer cells induces an immunosuppressive tumour microenvironment (TME). Immune cell Siglec receptors bind sialic acid, inhibiting immune cell activation. Cancer-associated fibroblasts (CAFs) play a vital role in enhancing a tumour-promoting microenvironment and our aim was to investigate if CAF sialylation contributes to their immunosuppressive properties. Intestinal stromal cells (iSCs) were isolated from colorectal cancer patient biopsies. Tumour-derived iSCs were termed CAFs while control iSCs, isolated from tumour-adjacent non-cancerous tissue, were termed normal-associated fibroblasts (NAFs). NAFs/CAFs were co-cultured with healthy allogeneic PBMCs and their immunosuppressive properties were assessed by flow cytometry. CAFs significantly induced a more exhausted and immunomodulatory T-cell phenotype, as evidenced by increased expression of exhaustion markers, TIM-3, LAG-3 and PD-1, and immunomodulatory receptors Siglec-7 and -9 when compared with NAFs, demonstrating their potent immunosuppressive properties. To elucidate the role of sialylation on CAF-mediated immunosuppression, NAFs/CAFs were treated with the sialyltransferase inhibitor (SI) prior to co-culture. SI-treated NAFs/CAFs were co-cultured with allogeneic T-cells to assess the functional consequences of reduced NAF/CAF sialylation. SI-treated CAFs induced a significantly less exhausted and immunosuppressive T-cell phenotype compared to their untreated counterparts. This effect is reproducible in an *in vitro* mouse model of colorectal cancer and we have also observed changes in the sialylation profile of stromal cells isolated from primary multiple myeloma patients compared to healthy controls. These results demonstrate that stromal cells in the tumour-microenvironment suppress activated T-cells and that this immunosuppressive effect can be significantly reversed through the modulation of sialylation on the stromal cell surface.

Keywords: Adaptive immunity, biology of the immune system, cancer immunology, cellular interactions, immunotherapy, microenvironment

OP-169

Human intrahepatic CD56bright NK cells display a tissue-resident transcriptional profile and enhanced ability to kill allogenic CD8+ T cells

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Natural killer (NK) cells are enriched in the human liver and liver-resident CD56brightCD16$^{+/-}$ NK cells have a phenotype distinct from their blood counterparts. Although functional studies have revealed these cells are capable of rapid cytotoxic effector functions, their role in the liver remains unclear. RNA sequencing was carried out on FACS-sorted NK cells from perfusates of healthy livers undergoing transplantation (n=5) and blood from healthy controls (n=5). Upregulation of genes encoding surface receptors were validated by flow cytometry using matched liver tissue and perfusate samples (n=5). An *in vitro* coculture system of liver perfusate-derived NK cells and blood-derived allogeneic activated T cells was used to assess hepatic NK cell cytotoxicity effect on T cells (n=10-13). Liver-resident CD56brightCD16$^{+/-}$ NK cells upregulate genes associated with tissue residency as well as functional surface receptors such as TIGIT, CD160 and LY9. Coculture of activated PBMCs with hepatic NK cells, not blood NK cells, increased T cell death ($p<0.0032$). CD8$^+$ T cell death was significantly higher than CD4+ T cells ($p<0.0001$). Addition of agonistic mAb against CD160 increased CD8+ T cell death in PBMC ($p<0.0254$) and CD3$^+$ T cell cocultures ($p<0.0098$). FACS-sorted CD56brightCD16$^{+/-}$ but not CD56dimCD16$^+$ NK cells increased overall T cell death ($p<0.0221$). Liver-resident CD56brightCD16$^{+/-}$ NK cells are transcriptionally distinct from liver and blood CD56dim and blood CD56brightCD16$^-$ NK cells. They can regulate allogeneic activated CD8$^+$ T cell populations by directly killing them, a mechanism that can be enhanced by engagement of their CD160 receptor.

Keywords: Cell death, innate host defence, innate immunity, innate lymphoid cells, NK cells

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WORKSHOPS

OP-170

Alk4 disruption in Kras-induced pancreatic cancer remodels mechanical and immunologic properties of the tumor microenvironment

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Pancreatic adenocarcinoma (PDAC) is associated with a vast stromal tumor microenvironment (TME). Here, we report that deletion of the activin receptor Alk4 in the context of KrasG12D mutation massively drives remodeling of the pancreatic TME affecting both the tissue mechanics and anti-tumor immune responses. Noteworthy, Alk4 mutations in PDAC patients are associated with poor outcome. By performing single cell RNAseq and multicolor flow cytometric analysis of tumor tissues from *p48-Cre;KrasG12D* (KC) and *p48-Cre;KrasG12D;Alk4fl/fl* (4KC) mice, we revealed a unique Alk4 signaling-dependent signature of cancer-associated fibroblasts (CAFs) that is driven through pancreatic stellate cell (PSC) instruction by metaplastic cells. While in KC mice CD61$^+$/ PDGFRα$^+$ cells represent the major CAF population, this population loses PDGFRα surface expression *in vivo* due to continuous signaling in the presence of high amounts of PDGF ligands in the pancreatic TME of 4KC mice. Consequently, a unique CD61$^+$/ PDGFRα$^-$ CAF population with increased secretory capacities of extracellular matrix molecules emergences in the context of Alk4 signaling disruption. Moreover, this population has immunosuppressive potential by impacting the Ag-presenting efficiency of CD8α$^+$ dendritic cells. Our data reveal that paracrine signaling in metaplastic cells lacking Alk4 signaling leads to profound modifications of the tissue mechanics and immune responses promoting tumor progression.

Keywords: Cancer immunology, immune regulation and therapy, *in vivo* tumor models, microenvironment

OP-171

Preferential induction of brain-homing T-bet+ B cells by infectious triggers in multiple sclerosis patients

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Epstein-Barr virus (EBV) infection of B cells is associated with increased risk of multiple sclerosis (MS). In MS patients, B cells are prone to infiltrate the central nervous system (CNS), associate with lesions and produce antibodies. Here, we aimed to decipher how infectious triggers such as EBV impacts the development of CNS-infiltrating B cells in MS patients. *Ex vivo* FACS analysis revealed that CXCR3-expressing B cells were enriched in different CNS tissues of MS patients (n=10). CXCR3high IgG$^+$ B cells accumulated in the blood of patients treated with natalizumab (anti-VLA-4 mAb), corresponding to their increased ability to cross CNS barriers *in vitro*. Naive B cells from MS patients were more sensitive to develop into antibody-secreting cells during IFN-γ-containing, follicular T helper 1 (Tfh1)-like cultures. In both human B-LCL and patient B cells, the presence of coding MS risk allele *IFNGR2* increased this sensitivity to IFN-γ. The IFN-γ receptor was constitutively more active in EBV-infected B-LCL carrying the *IFNGR2* risk allele. T-bet was further upregulated through additional TLR9 induction, resulting in enhanced class-switching. In autologous bone-marrow-transplanted MS patients, EBV copy numbers were elevated and positively correlated to CXCR3 and not CXCR4 or CXCR5 expression in switched B cells 3-7 months post-treatment. High EBV copy numbers in memory B cells of natalizumab-treated MS patients corresponded to increased development into antibody-secreting cells under Tfh1-like conditions. These findings implicate that persistent EBV infection potentiates CXCR3(T-bet)$^+$ memory B cells to enter the CNS and be locally re-activated to differentiate into IgG-producing cells in MS.

Keywords: B lymphocytes, chemokines, environmental factors in autoimmunity and allergy, immunotherapy, multiple sclerosis

OP-172

Depending on the tumor microenvironment, immunoproteasomes exert pro- or anti-tumorigenic effects

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In contrast to the ubiquitously expressed standard proteasome, the high immunoproteasome levels are predominately detected in the cells of immune system. On the one side, the immunoproteasome is implicated in chronic inflammation and exaggerated immune responses, which frequently results in the cancer promotion. On the other side, an effective anti-tumor immunity provided by the presentation of cancer antigens by immunoproteasomes is essential for eradication of solid tumors. We here investigated the role of the immunoproteasome in two different cancer types. In melanoma tumors, a possible role of immunoproteasome in shaping the tumor environment is unknown. Our analysis revealed that the increased expression of three immunoproteasome subunits, LMP2, LMP7 and MECL-1, was associated with better overall survival of melanoma patients. Moreover, mice lacking immunoproteasomes displayed an impaired anti-tumor immunity against melanoma tumors as compared to WT animals. In contrary, in an experimental model of inflammation-associated colonic carcinogenesis, the immunoproteasome strongly promoted chronic inflammation and tumor development. Decreased levels of pro-tumorigenic cytokines and chemokines, as well as impaired recruitment of immune cells into the colonic lamina propria were found in the absence of immunoproteasomes. Collectively, the immunoproteasome perpetuates inflammatory reactions in the colon leading to the development of carcinogenesis. In contrast to the intestinal inflammatory milieu, in the microenvironment of melanoma cells, the immunoproteasome has an anti-tumorigenic role by promoting the activity of antigen presenting cells and by supporting T cell-mediated anti-tumor immunity. Thus, the role of the immunoproteasome during development of tumorigenesis depends on the tumor-specific microenvironment.

Keywords: Cancer immunology, *in vivo* tumor models, inflammatory disease

OP-173

Loss of skin CXCR3+CD4+ tissue-resident T cells causes irreversible skin-confined immunodeficiency in HIV late presenters

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People living with HIV (PLWH) show increased risk of human papilloma virus (HPV)-related malignancies of the skin and mucosal membranes despite systemic reconstitution of CD4+ T cells upon antiretroviral therapy (ART). Tissue-resident T cells (TRM) are abundant in skin and mucosal tissue and are thought to participate in cancer protection, including HPV-related malignancy. Here, we investigated the consequences of HIV-infection and ART on cutaneous and mucosal TRM. We collected longitudinal skin biopsies from early-presenting HIV+ individuals before and after one year of ART and compared them to HIV late presenters with initial low systemic levels of CD4+ T cells. Our results show that skin CD4+ TRM are strongly depleted both in late presenters and in newly infected patients, but reconstituted upon ART only in early presenters. Reconstitution of CD4+ T cells following ART is accompanied with an increased diversity of TCR-clones shared between skin and peripheral blood of early presenters, suggesting a systemic origin of the reconstituting CD4+ TRM pool. RNA sequencing in HIV late presenters revealed reduced expression of the chemokine receptor CXCR3 in HIV+ skin, which we confirmed to be expressed on CD4+ TRM cells of the skin and mucosa. Strikingly, we found a stark reduction of CXCR3+CD4+TRM in rectal biopsies of HPV-related intraepithelial neoplasia in PLWH compared to HPV lesions of HIV-negative patients. These results reveal a hitherto unrecognized irreversible loss of CXCR3+CD4+TRM in the skin and mucosa of untreated PLWH, which may be a precipitating factor in the development of HPV-related mucosal cancer in these patients.

Keywords: Immunodeficiency, infectious disease, RNAseq, skin diseases, viral infections

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WORKSHOPS

OP-174

Targeting PD1/PDL1 pathway by endogenous recombinant soluble PD1

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Targeting the immune checkpoint inhibitor, PD1 is a new approach to treat a wide range of cancers as it has the potential to restore patient's anticancer immune response that has been suppressed by the PD1/PDL1 interaction. Several therapies are currently available that can block the PD1/PDL1 pathway. However, these therapies are restricted to a specific subset of patients. PD1 is alternatively spliced to form a soluble variant (sPD1) that can bind to PDL1 ligand and block negative signals from cancer cell to T cells. Splicing in cell is controlled by RNA binding protein called SRSF. Nuclear localisation of SRSF is regulated by SRPK1 phosphorylation in the cytoplasm. Thus, inhibiting SRSF or SRPK may result in alternative splicing of PD1 to sPD1. SRSF binding sites were identified by ESE finder. T cells were transfected with SRSF plasmid and RT-PCR was carried out. Knock down of SRPK1 was undertaken by lentiviral transduction or by using small pharmacological compound. T cells after activation expressed both sPD1 and PD1. sPD1 transfection resulted in increased IL-2 production by T cells when co-cultured with melanoma cells. SRSF1 transfection resulted in reduced sPD1 expression. SRPK1 knock down resulted in increased sPD1 expression and rescued T cell's IL-2 production. SRPK1 phosphorylate SRSF1 and results in its nuclear location where it induces a switch in splicing from sPD1 to fl-PD1. Blocking SRPK1 would thus result in sPD1 that can target the PD1/PDL1 pathways and can be a potential cancer treatment option.

Keywords: Adaptive immunity, cancer immunology, checkpoint inhibition, immunotherapy

OP-175

Stimulation of the PD-1 pathway decreases atherosclerotic lesion development in Ldlr deficient mice

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Signalling through the coinhibitory programmed death (PD)-1/PD-L1 pathway regulates T cell responses and can inhibit ongoing immune responses. Inflammation is a key process in the development of atherosclerosis, the underlying cause for the majority of cardiovascular diseases. Dampening the excessive immune response that occurs during atherosclerosis progression by promoting PD-1/PD-L1 signalling may have a high therapeutic potential to limit disease burden. In this study we therefore aimed to assess whether an agonistic PD-1 antibody can diminish atherosclerosis development. Ldlr-/- mice were fed a western-type diet (WTD) while receiving 100µg of an agonistic PD-1 antibody or control vehicle twice a week. Stimulation of the PD-1 pathway delayed the WTD-induced monocyte increase in the circulation up to 3 weeks and reduced T cell activation and proliferation. More specifically, we observed a 23% decrease in atherogenic IFNγ-producing splenic $CD4^+$ T cells and a 20% decrease for cytotoxic $CD8^+$ T cells, whereas atheroprotective IL-10 producing CD4+ T cells were increased with 47%. Furthermore, we found an increase in regulatory B cells, B1 cells and associated atheroprotective circulating oxLDL-specific IgM levels in agonistic PD-1-treated mice. This dampened immune activation following agonistic PD-1 treatment resulted in reduced atherosclerosis development ($p<0.05$). Our data show that stimulation of the coinhibitory PD-1 pathway inhibits atherosclerosis development by modulation of T- and B cell responses. These data support stimulation of coinhibitory pathways as a potential therapeutic strategy to combat atherosclerosis.

Keywords: Adaptive immunity, animal models, cardiovascular diseases, checkpoint inhibition

OP-176

Critical role of resident M2-like macrophages in healthy subcutaneous adipose tissue expansion

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Storing excess nutrients in subcutaneous adipose tissue (SAT) protects the visceral compartment from detrimental lipid deposition thus reducing the risk for type 2 diabetes. Healthy adipose tissue expansion depends on effective extracellular matrix (ECM) remodeling. This study aims to elucidate the role of macrophages in ECM-remodeling processes during SAT expansion. Male C57BL/6 mice were fed chow or high-fat diet (acute HFD, aHFD) for one week. SAT ECM-remodeling was assessed by histological methods, WB and qPCR. SAT stromal vascular fraction was analyzed by flow cytometry and confocal microscopy. Collagen degrading capacity was determined *ex vivo* with FITC-collagen endocytosis assay on sorted SAT-macrophages and fibroblasts. Local clodronate-liposome injections were used for SAT-macrophage depletion. aHFD provokes significant ECM-remodeling, judged by loss of collagen type 1 (CT1) and increase in CT1-fragments. This is accompanied with increased proliferation of resident, M2-like macrophages (CD45+F4/80+CD11b+CCR2-CD206+), demonstrated by elevated EdU incorporation and unaffected CCL2/CCR2 signaling axis. aHFD SAT-macrophages, but not fibroblasts, show increased collagen endocytosis *ex vivo*. Depletion of macrophages with clodronate-liposomes elicited significant accumulation of CT1-fragments, fibrosis, angiogenesis and inflammation in aHFD SAT compared to PBS-liposome controls. We propose that aHFD provokes adaptive tissue remodeling of SAT, governed by tissue resident M2-like macrophages. These macrophages are pivotal in the removal of CT1-fragments enabling healthy tissue expansion. Further understanding of this process could identify novel targets in obesity related disorders.

Keywords: Chronic inflammation and fibrosis, diabetes, macrophage

OP-177

Innate PD-L1 limits T cell mediated adipose tissue inflammation

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Obesity has become a major health problem in the industrialized world. Immune regulation plays an important role in adipose tissue homeostasis; however, the initial events shifting the balance from a non-inflammatory, homeostatic environment towards inflammation are poorly understood. Here, we investigated how innate immune cells regulate adaptive T cell responses via PD-L1 in the context of obesity. Mice were kept on a high-fat diet to induce obesity and we recorded weight and metabolic state, and analysed adipose-tissue infiltrating cells. Co-cultures were setup to investigate the role of PD-L1 at the DC/ILC2-T cell interface. We observed an important role for PD-L1 in the regulation of adipose tissue inflammation. PD-L1-/- mice showed enhanced weight gain, impaired metabolic function and exaggerated T cell responses. Conditional deletion of PD-L1 on ILC and T cells did not alter disease, while deletion on DC led to increased weight gain and inflammation. In particular, PD-L1 on DC limited adipose tissue Th1 cells and promoted Treg responses. Importantly, adipose tissue samples from people with obesity also show elevated expression of PD-L1. Our results demonstrate that PD-L1:PD-1-interactions play an important role in adipose tissue homeostasis. Expression of PD-L1 on DC limits effector T cell responses and increases Treg polarization *in vivo*. Thus, our findings are not only relevant for our understanding for pathogenicity of obesity, but also for patients undergoing PD-L1/PD-1-checkpoint inhibitor therapy.

Keywords: Adaptive immunity, checkpoint inhibition, dendritic cells, inflammatory disease, innate lymphoid cells

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WORKSHOPS

OP-179

Immune checkpoint inhibitor-induced colitis is mediated by IL23 responsive CD90+ cytotoxic lymphocytes

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Immune checkpoint inhibitors (CPI) have revolutionised cancer treatment, with previously untreatable disease now amenable to potential cure. Combination regimens of anti-CTLA4 and anti-PD-1 show enhanced efficacy but are prone to off target immune-mediated tissue injury, particularly at the barrier surfaces. CPI-induced colitis is a common a serious complication. To probe the impact of immune checkpoints on intestinal homeostasis mice were challenged with combination anti-CTLA4 and anti-PD-1 immunotherapy, manipulation of the intestinal microbiota and antibody blockade/depletion studies. Colonic immune responses were profiled using RNA-sequencing, including high-resolution single cell analyses, and flow cytometry. CPI colitis was dependent on the composition of the intestinal microbiota and was characterized by remodelling of mucosal lymphocytes with induction of polyfunctional, cytolytic responses in T-cells (both CD4+ and CD8+ cells) and innate lymphoid cells (ILCs), all of which likely participated in immune-mediated tissue injury. CD90+ lymphocytes were especially enriched for cytolytic molecules (*Gzmb*, *Prf1*, *Nkg7*), pro-inflammatory cytokines (*Ifng*, *Il22* and *Il17a*), and chemokines (*Ccl3*, *Ccl4* and *Ccl9*). Network analysis of predicted upstream regulators identified multiple potential activators of CD90+ mucosal lymphocytes, including IL23. Functionally, CD90 depletion or IL23 blockade significantly attenuated CPI-colitis. This study provides new mechanistic insights into CPI colitis, identifying IL23 responsive CD90+ lymphocytes with cytolytic and polyfunctional cytokine responses as key mediators of disease. Therapeutic targeting of these pathogenic effector cells likely holds the key to preventing and reversing CPI colitis.

Keywords: Adaptive immunity, cytokines and mediators, immune regulation and therapy, immunotherapy, inflammatory bowel disease, RNAseq

OP-180

Functional monovalency of IgG4 autoantibodies amplifies their pathogenicity in MuSK myasthenia gravis

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In recent years, a new niche of autoimmune diseases was identified with predominant pathogenic IgG4 autoantibodies (IgG4-AID). It is not known why IgG4 dominates in these diseases. IgG4 is a unique antibody subclass as Fab-arm exchange induces random exchange between IgG4 half-molecules, resulting in antibodies with two specificities and, consequently, functional monovalent antigen binding. We hypothesized that autoantibody functional monovalency alters the pathomechanism in IgG4-AID. Myasthenia gravis (MG) with autoantibodies against muscle-specific kinase (MuSK), hallmarked by fatigable muscle weakness, is an archetypical IgG4-AID. To study if functional monovalency alters the pathogenicity of IgG4 MuSK antibodies, we generated recombinant monoclonal MuSK antibodies (i.e. bivalent antigen binding) and bispecific MuSK antibodies (i.e. monovalent antigen binding) from patient-derived antibody sequences. Upon passive transfer to mice, monovalent MuSK antibodies caused progressive myasthenic muscle weakness, whereas the same antibodies in their parental bivalent form were less potent or did not induce a myasthenic phenotype. This can be explained by their respective antagonistic or agonistic effect on the MuSK signalling cascade, as assessed in myotube cultures. Thus, isotype switching to IgG4 and subsequent Fab-arm exchange in an autoimmune disease may be a critical step in development of the disease. These findings may be relevant to the 28 other known IgG4-AID and other disease settings where IgG4 plays a (pathogenic) role.

Keywords: Animal models, antibody, autoimmunity, neuroimmunology

OP-181

Single cell sequencing reveals expanded cytotoxic CD4+ T cells and two states of peripheral helper T cells in synovial fluid of ACPA+ rheumatoid arthritis patients

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Rheumatoid Arthritis (RA) is a chronic autoimmune disease targeting the synovial joints, where CD4+ T cells play a pathogenic role. Anti-citrullinated peptide antibodies (ACPA) are present in two thirds of patients with RA. Cytotoxic CD4+ T cells (CD4+ CTL) and peripheral helper T cells (TPH) have been identified in ACPA+ RA but their characterization is still incomplete. Here, we investigated CD4+ T cells in synovial fluid (SF) of RA patients, using flow cytometry (n=21) and single cell sequencing (n=7), in combination with 5´ TCRαβ sequencing. Flow cytometry experiments show that Granzyme-B+ Perforin-1+ CD4+ CTL are significantly increased in SF of ACPA+ RA patients as compared to ACPA-negative patients. The presence of CD4+ CTL was confirmed by single cell sequencing in SF of ACPA+ RA patients. Moreover, we identified two states of TPH cells, with differential expression of *CXCL13* and *PRDM1*. We also found that the adhesion G-protein coupled receptor GPR56 is selectively expressed on the synovial TPH cell-subset. TCR clonality analysis revealed that most expanded clones in SF are contained within the cytotoxic and the *CXCL13+* TPH CD4+ T-cell populations. Common TCR sequences between the two TPH states and the CD4+ CTL suggest a shared differentiation. Overall our study provides an immunoprofiling map of CD4+ T-cell subsets in ACPA+ RA SF and suggests GPR56 as a potential therapeutic target in RA.

Keywords: Autoimmunity, follicular helper T cells, inflammatory joint diseases, rheumatoid arthritis, RNAseq

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WORKSHOPS

OP-182

A novel role for nutrient receptors in cartilage and joint disease

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Osteoarthritis (OA) is a common joint disease characterized by loss of cartilage. Post-traumatic OA (PTOA) is a specific sub-type caused by joint injury, such as ligament damage. In PTOA, levels of the pro-inflammatory cytokine IL-1β peak within 24-48hrs post injury. Chondrocytes are the main constituent cell type in cartilage, and in healthy tissue produce crucial proteins for the stability and strength of the cartilage extracellular matrix. When IL-1β is present, chondrocytes switch from anabolic to catabolic metabolism with the release of extracellular protease enzymes such as MMP13 which ultimately degrade cartilage leading to PTOA. However, the metabolic pathways in chondrocytes which are affected by IL-1β and lead to this catabolic phenotype are poorly understood. Our data show that IL-1β stimulation of chondrocytes increases glycolytic metabolism and *Mmp13* induction. Induction of *Mmp13* was suppressed by blocking glycolysis with glucose analogue 2-Deoxyglucose (2-DG). Nutrient receptors *Slc7a2* and *Slc7a7*, which transport arginine, were highly induced by IL-1β treatment. The metabolic enzyme *Arginase2* which converts arginine to ornithine was also selectively upregulated with IL-1β, and suppressed with 2-DG. Strikingly, we also showed that IL-1β induced *Mmp13* production could be suppressed by preventing arginine uptake through siRNA silencing of *Slc7a2* and *Slc7a7*. These data show the reprogrammed metabolic pathways in chondrocytes under inflammatory conditions, and the importance of amino acid uptake in the disease process of PTOA. Understanding these pathways will allow us to design new therapeutics or repurpose pre-existing small molecules which target glucose and arginine metabolism, to maintain the anabolic state in chondrocytes.

Keywords: Cytokines and mediators, inflammatory disease, metabolic control of immune responses, tissue damage and repair

OP-183

The role of DRAK2 in graft versus host disease

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Graft versus host disease (GvHD) is the most common adverse event after bone marrow transplantation and is treated with broad immune suppression, which limits the desired graft versus leukemic effect. Despite this aggressive treatment, GvHD remains the second leading cause of death for pediatric bone marrow transplant recipients, after infection. A treatment for GvHD that effectively limits the tissue damage and inflammation of GvHD but does not suppress the immune system's function against pathogens is urgently needed. The tissue damage in GvHD is phenotypically similar to T cell-mediated autoimmune conditions, like type 1 diabetes and multiple sclerosis. A deficiency in the kinase Drak2 inhibits T cell-mediated autoimmune disease without causing global immune suppression in murine models. Therefore, we tested whether Drak2 would be a suitable protein target to treat GvHD without causing generalized immunosuppression. We utilized a murine model of acute graft versus host disease (aGvHD) in which lethally irradiated mice received MHC-mismatched bone marrow and mismatched T cells that were either wildtype or Drak2-deficient. We found that mice that received MHC-mismatched Drak2-deficient T cells had lower clinical disease scores, reduced proportions of effector T cells, and higher proportions of regulatory T cells compared to mice that received wildtype T cells. Together, these data suggest that T cell activation against host tissues early in GvHD development is reduced in the absence of Drak2, thus Drak2 inhibition may be a novel treatment for GvHD. Ongoing experiments are investigating mechanisms in which Drak2 contributes to GvHD.

Keywords: Animal models, autoimmunity, bone marrow transplantation, drugs for immune modulation, immune regulation and therapy

OP-184

Characterization of pre-existing and post-infectious immune responses to SARS-CoV-2 in cancer patients

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Cancer patients are at increased risk for severe COVID-19. We assessed antibody and T-cell responses in unexposed and SARS-CoV-2 convalescent cancer patients to identify immunological parameters contributing to COVID-19. Pre-existing SARS-CoV-2 cross-reactive $CD4^+$ T-cell responses were detected in 78% of unexposed healthy volunteers (HVs, n=94) and 77% of solid tumor patients (n=97) and were substantially reduced in unexposed hematological malignancy (HM) patients (n=99) assessed by IFN-γ ELISPOT using previously defined T cell epitopes (Nelde *et al.*, Nat Immunol, 2021). Increased PD-1, LAG-3, and TIM-3 expression in HMs compared to solid tumor patients and HVs points towards exhaustion as underlying cause for these reduced frequencies. Within SARS-CoV-2 convalescents, antibody titers and recognition frequencies to SARS-CoV 2-specific epitopes were comparable in HMs (n=8), solid tumors (n=9), and HVs (n=67). Recognition frequency to HLA-DR cross-reactive epitopes however was significantly reduced in cancer patients and was again attributed to reduced $CD4^+$ T-cell responses in HMs (38%) compared to solid tumor patients (78%) and HVs (87%). T-cell responses to HLA-DR peptides after 12-day *in vitro* expansion revealed reduced T-cell expandability for 73% of SARS-CoV-2-derived peptides in convalescent cancer patients. Diversity of $CD4^+$ T-cell responses was significantly reduced in HMs (20%) compared to solid cancer patients (35%) and HVs (50%) and, alike in non-cancer convalescents, associated with severe COVID-19 in cancer patients. Together, our results identify impaired T-cell immunity as determinant for dismal COVID-19 outcome in cancer patients, particularly patients suffering from HMs, and guide the development of therapeutic measures and vaccines for these vulnerable patients.

Keywords: Cancer immunology, immunological techniques, infectious disease, monitoring immunity, viral infections, visualizing immune responses

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WORKSHOPS

OP-185

Infiltrating neutrophils present an aged phenotype and an altered recruitment dynamics and functionallity in chronic liver disease

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Alcoholic hepatitis is characterized by the expansion of ductular reaction (DR), which is accompanied by a prominent infiltration of neutrophils. The aims of the present study were to characterize infiltrating neutrophils and to investigate their role in chronic liver injury progression. Infiltration of neutrophils was assessed by immunofluorescence and tissue clearing in stages of alcoholic liver disease progression. Intravital microscopy was performed in mice treated with DDC diet. Infiltrating mouse neutrophils were phenotypically and functionally characterized by qPCR and FACS. Neutrophil impact on disease progression was evaluated in a 3-week DDC mouse model by administration of 1A8 antibody or CXCR1/2 receptor inhibitor. Histologically, DR cells showed direct contact with infiltrating neutrophils, which increased with human disease progression. Alongside, intravital microscopy in a DR mouse model revealed a time-dependent infiltration of neutrophils, which remained static in close contact with DR cells. Infiltrating neutrophils acquired an aged phenotype (CXCR4+ CD62L low Cd11b high). Functionally, neutrophils showed a reduced phagocytic capacity and an exacerbated ROS production when compared with circulating neutrophils. Both inhibition of neutrophil recruitment with CXCR1/2 inhibitor and depletion by 1A8 antibody attenuated DR expansion, liver fibrosis and inflammation. Our study shows that chronic liver disease progression is associated with increasing number of infiltrating neutrophils that remain static at periportal areas, where they acquire an altered functionality and phenotype. Besides, preventing neutrophil infiltration impacts on DR expansion, suggesting that strategies targeting neutrophil recruitment may improve chronic liver injury.

Keywords: Animal models, chronic inflammation and fibrosis, immune regulation and therapy, neutrophils, tissue damage and repair

OP-186

The imbalance of the circulating regulatory B cell subsets in patients with primary immunodeficiencies

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Regulatory B cells (Bregs) are immunosuppressive B cells subsets which produce IL-10, TGF-B and IL-35 and act crucial roles in tolerance to immunopathological conditions such as chronic inflammation, autoimmunity and infection. It is known that Breg cells repress inflammatory T cells such as Th1, CD8+ T lymphocytes and Th17 cells and induce tolerogenic activity of regulatory T cell (Treg) subsets. Distinct Breg subsets arise in different stages of the development of B cells depending on changing microenvironmental status during occurence of the disease. In this study, we aimed to analyze Breg subsets in patients with primary immunodeficiencies (PIDs). We investigated IL-10 expressing Br1, B10, immature B cells and plasmablasts in 36 patients and 18 healthy donors by using flow cytometry. 29 patients had genetic defects including LRBA, DOCK8, PIK3CD, AID, STAT1 LOF, STAT1 GOF, STAT3 LOF, WAS, CORO1A, HAX1, GMAP, IL17RA, ADA and Artemis. Genetic diagnosis could not be detected in 7 patients. We detected significantly reduced Br1 cell ratio compared to healthy controls. In contrast, we showed that significantly increased B10 cells and plasmablasts ratios compared to healthy controls. The patients had significantly reduced Treg cell subsets including Helios+ or - natural Tregs, Tr1 and Th3 cells in contrast to increased T follicular helper (Tfh) cells. We identified a negative correlation between Br1 and Tfh cells in patients. Aberant sub-populations of Bregs and Tregs and altered Tfh levels were related to PIDs. However, there was no association with molecular diagnosis and altered regulatory B and T cell phenotypes.

Keywords: Adaptive immunity, B lymphocytes, follicular helper T cells

OP-187

CHAPLE disease and non-CHAPLE protein losing enteropathies: natural history and immune characteristics

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CHAPLE disease is a newly discovered form of hereditary protein-losing enteropathy (PLE), caused by mutations in the complement regulatory gene, CD55. In this largest cohort of PLE patients world wide, we investigated the natural history, immune and genetic characteristics and treatment strategies of CHAPLE and non-CHAPLE (those with intact CD55) PLEs. We performed gene sequencing, plasma anaphylatoxin measurement, immune phenotyping, and comprehensive clinical lab asssesment to comparatively analyze PLE subsets. We identified eight novel CD55 gene defects, all of which abrogated protein expression. Thirty three CHAPLE patients, compared to forty eight non-CHAPLE PLEs, had unique clinical and lab features. Anaphylatoxin C3a and C5a were higher in CHAPLE; C5-inhibition therapy led to a reduction in C5a but not in C3a. A history of bowel resection surgery, receipt of inflammatory bowel disease medications, thromboembolism, thrombocytosis, and hyperlipidema were more common in the CHAPLE group ($p< 0.001$). While hypoalbuminemia responded to steroids in a subgroup of CHAPLE patients, life-threatening complications persisted despite conventional approaches apart from the C5-inhibition therapy (eculizumab). We also identified a subgroup of non-CHAPLE PLEs with profound immunologic skewing dissimilar to CHAPLE (who had normal immune subsets). CHAPLE disease differs from non-CHAPLE PLEs with respect to the mechanism of intestinal leakage, natural history, and management options. Our findings offer new diagnostic and management strategies towards various forms of PLE.

Keywords: Antibody, autoinflammation, cytokines and mediators, Immunodeficiency, Inflammatory bowel disease

OP-188

Eomes controls central nervous system inflammation by increasing survival of effector CD4 T cells through the regulation of mitochondrial metabolism

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The transcription factor Eomes was mostly described in Natural Killer cells and in CD8 T cells as a key regulator of IFN-γ production and cytotoxicity. Moreover, Eomes was shown to belong to a susceptibility locus associated with Multiple Sclerosis (MS), and an increase of EOMES$^+$ CD4 T cells was reported in patients with a severe form of MS. However, the precise role of Eomes in pathogenic CD4 T cell differentiation and susceptibility to central nervous system (CNS) autoimmunity remains unclear. Using a mouse model of neuroinflammation, we showed that Eomes deletion in antigen-specific CD4 T cells leads to a strong reduction in disease severity. We showed that Eomes-deficient CD4 T cells exhibit a decreased overall survival leading to lesser CNS accumulation ability during the disease progression. RNA-sequencing analyses comparing Eomes-competent *versus* Eomes-deficient CD4 T cells revealed that Eomes strongly impacts mitochondrial metabolism. Indeed, using the SeaHorse oximeter and different dyes to measure mitochondrial activity, we demonstrated thatEomes-deficient cells exhibit reduced oxidative phosphorylation linked to altered mitochondrial respiration. Interestingly, we also observed a downregulation of Romo1 expressionin Eomes-deficient cells, which is a key player in metabolism through its role in maintaining the mitochondrial cristae structure. Altogether, our results demonstrate a link between mitochondrial metabolism, long-term survival and increased pathogenic functions of CD4 T cells that is driven by Eomes. Addressing the implication of Romo1 in those phenomena might open new therapeutic strategies in MS by targeting mitochondrial actors.

Keywords: Adaptive immunity, animal models, autoimmunity, metabolic control of immune responses, multiple sclerosis

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WORKSHOPS

OP-189

Potential pathogenic role for respiratory symbionts in the development of self-reactive Th17 cells

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Self-antigen reactive Th17 cells are important pathogenic effectors in numerous autoimmune diseases, including multiple sclerosis (MS) and the preclinical model experimental autoimmune encephalomyelitis (EAE). Th17 cells are innocuous immediately following differentiation and IL-23 signalling is critical for pathogenicity, associated with increased expression of Tbet-regulated molecules. However, antigen-specific Th17 cells only upregulate IL-23R following differentiation in peripheral lymphoid tissues. When and where self-reactive Th17 cells are exposed to this pathogenic signal is unknown. We hypothesize that the airways are a critical site where respiratory tract bacteria induce IL-23 expression by innate immune cells that confers pathogenic potential on self-reactive Th17 cells. We exposed innate immune cells *in vitro* and *ex vivo* to a range of respiratory symbionts, and examined IL-23 and related cytokine secretion. *In vivo*, we colonised the upper respiratory tract of mice with selected bacteria to determine expression of IL-23 and related cytokines in the airways, and their impact on clinical disease in a Th17-mediated model of EAE. Our data indicates that Proteobacteria species, including *Klebsiella pneumoniae*, *Moraxella catarrhalis* and *Neisseria cinerea* stimulate IL-23 expression by dendritic cells *in vitro* and in the lungs *in vivo*, and co-culture of Th17 cells with dendritic cells exposed to these bacteria promoted conversion to a pathogenic-like ex-Th17 cell phenotype. Crucially, Th17 cell-mediated EAE is exacerbated in mice colonised with these respiratory tract bacteria, and disease susceptibility is partially restored in germ-free mice colonised with *K.pneumoniae*. These findings support the concept that respiratory symbionts regulate Th17 cell pathogenicity in CNS autoimmune disease.

Keywords: Autoimmunity, cytokines and mediators, environmental factors in autoimmunity and allergy, microbiome and environmental factors, multiple sclerosis

OP-190

Deep immune phenotyping of SARS-CoV-2 specific B cell profiles after primary infection

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T cell-driven B cell responses are needed to induce class switched antibodies upon infection or vaccination, thereby establishing long-term protection against subsequent infections. Although SARS-CoV-2 infections and SARS-CoV-2 vaccines indeed induce protective antibodies against the virus, it remains to be elucidated which unique SARS-CoV-2 specific B cell subsets become activated upon primary infection, if the various B cell differentiation pathways induced differ between primary disease severities and how they relate to formation of long-lasting antibodies against SARS-CoV-2. Using multiparameter high-dimensional spectral flow cytometry, we elucidated deep immune profiles of the SARS-CoV-2-specific B cell responses in patients who recovered from COVID-19 with varying degrees of disease severity. We identified distinct populations of antigen-specific B cells targeting Nucleocapsid, Spike and RBD sites on SARS-CoV-2 in these patients when compared to other pathogens. Currently, we are investigating how the SARS-CoV-2 specific B cell subsets observed after three months after recovery of COVID-19 relate to the longevity of induced SARS-CoV-2 antibodies in persons who recovered from COVID-19 with high persistence or quick decay of antibody titers. This study aims to elucidate the ongoing and established B cell responses against SARS-CoV-2 and to establish their role in COVID-19 disease for future therapies and vaccinations.

Keywords: Adaptive immunity, B lymphocytes, immunological techniques, infectious disease

OP-191

Warm autoimmune hemolytic anemia is associated with a regulatory T cell deficiency and a Th17 polarization

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Warm autoimmune hemolytic anemia (wAIHA) is a rare autoimmune disease mediated by antibodies targeting erythrocytes. The role of T helper (Th) lymphocytes in its pathogenesis has been scarcely studied in humans. The skewing to a Th17 polarization and the alteration of Treg functions remain debated in humans. Assuming that an imbalance between the pro- and anti-inflammatory T cell responses could participate to AHAI pathogenesis, we investigate both Th subsets and Tregs in the blood. Newly diagnosed wAIHA patients (n=22) were compared to healthy donors (n=30). Cell quantification and polarization was determined by flow cytometry. Treg functions were evaluated by their capability to inhibit T cell proliferation. Cytokines were measured by luminex assays and RNA sequencing was performed on sorted Treg. The frequency of Treg was lower during AHAI (3.20% vs 4.45%). Moreover, Treg arbored a functional deficiency with a lower ability to inhibit T cell proliferation (51% vs. 73%). Transcriptomic analysis revealed an engagement of TNF signaling in line with the increase in TNF-α in the sera of patients. Although Th polarization did not differ, there was an increase in the Th17/Treg ratio associated with a higher secretion of IL-17 by effector T cells during AHAI. We here showed for the first time the unbalance of the pro-inflammatory over the anti-inflammatory T cell response, associated with a functional deficiency of Treg in human AIHA. Whether these alterations depend on TNF- α and whether this cytokine could become a therapeutic target remain to be determined.

Keywords: Autoimmunity, regulatory cells, RNAseq

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WORKSHOPS

OP-192

Lung functioning and inflammation in a mouse model of systemic juvenile idiopathic arthritis

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Systemic juvenile idiopathic arthritis (sJIA) is an immune disorder characterized by fever, skin rash, arthritis and splenomegaly. Recently, increasing number of sJIA patients were reported having lung disease. Here, we explored lung abnormalities in a mouse model for sJIA relying on injection of IFN-γ deficient (IFN-γ KO) mice with complete Freund's adjuvant (CFA). Monitoring of lung changes during development of sJIA using microcomputer tomography revealed a moderate enlargement of lungs, a decrease in aerated and increase in non-aerated lung density. When lung function and airway reactivity to methacholine was assessed, gender differences were seen. While male mice showed an increased tissue hysteresivity, female animals were characterized by an increased airway hyperactivity, mirroring ongoing inflammation. Histologically, lungs of sJIA-like mice showed subpleural and parenchymal cellular infiltrates and formation of small granulomas. Flow cytometric analysis identified immature and mature neutrophils, and activated macrophages as major cell infiltrates. Lung inflammation in sJIA-like mice was accompanied by augmented expression of IL-1β and IL-6, two target cytokines in the treatment of sJIA. The increased expression of granulocyte colony stimulating factor, a potent inducer of granulopoiesis, in lungs of mice was striking considering the observed neutrophilia in patients. We conclude that development of sJIA in a mouse model is associated with lung inflammation which is distinct to the lung manifestations seen in sJIA patients. Our observations however underscore the importance of monitoring lung disease during systemic inflammation and the model provides a tool to explore the underlying mechanism of lung pathology in an autoinflammatory disease context.

Keywords: Animal models, autoinflammation, cytokines and mediators

OP-193

Tolerance induction with vitamin D3, dexamethasone or sialic acids influences maturation status and metabolic activity in human dendritic cells

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Rheumatoid arthritis(RA) patients are currently treated with a variety of immunosuppressive drugs to dampen the inflammation. Reprogramming immune tolerance is key to cure these patients. *Ex-vivo* tolerogenic dendritic cells(DCs), generated with Vitamin D3(VitD3) or dexamethasone, have shown promising phase-I clinical results. Recently, we showed that binding of sialic acids(sia) to Siglec receptors on DCs can induce tolerance *in-vivo* in mice and *in-vitro* in human moDCs, with decreased effector T cell and increased regulatory T cell differentiation. It is currently unknown how sia-Siglec-instructed tolerogenic DCs(tolDCs) compare to VitD3 or Dexamethasone induced tolDCs. Therefore, we aimed functionally characterized VitD3, Dexamethasone and sia-Siglec induced tolDCs for maturation status, cytokine secretion and metabolic activity. TolDCs were generated in the presence of VitD3, dexamethasone or sia-Siglec from isolated healthy human monocytes and subsequently activated for 24 hours with LPS. Alterations in maturation markers were measured by flowcytometry. VitD3 and Dexamethasone induced tolDCs displayed reduced CD80 and CD86 expression, while sia-Siglec tolDCs had similar expression of these markers as LPS-activated moDCs. All tolDCs had a decreased inflammatory cytokine secretion. Surprisingly, glucose uptake and mitochondrial mass were only increased in VitD3 tolDCs. Basal glycolysis and glycolytic capacity were increased in both VitD3 and sia-Siglec tolDCs and not in Dexamethasone TolDCs. In contrast, basal respiration and ATP production were only increased in sia-Siglec tolDCs. Overall, our findings indicate that the different stimuli to induce tolerance in DCs instruct a distinct maturation and metabolic phenotype and yield functional different tolDCs that can be used to treat auto immune diseases.

Keywords: Autoimmunity, CELL signalling, dendritic cells, drugs for immune modulation, metabolic control of immune responses

OP-194

Identification of a disease-associated network of intestinal immune cells in treatment-naïve inflammatory bowel disease

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Inflammatory bowel disease (IBD) is characterised by chronic intestinal inflammation. Previous studies on IBD indicated alterations in the cellular immune system. However, it has been challenging to interrogate the role of all immune subsets simultaneously. Using mass cytometry, we aimed to identify immune cell types associated with inflammation in IBD. We analysed 192 biopsies (IBD-inflamed, IBD-uninflamed and control) in treatment-naïve IBD patients (n=48) and controls (n=26) from two cohorts. We used mass cytometry (36-antibody panel) to resolve single cells and analysed the data with unbiased Hierarchical-SNE. Imaging-mass cytometry (IMC) was performed to reveal the spatial distribution of the immune subsets in the tissue. We identified 44 subsets from the primary intestinal dataset containing 3.1 million immune cells in a data-driven manner. Correlation analysis identified a statistically significant network of IBD-associated immune subsets, including adaptive (HLA-DR+CD38+ CD4+ TEM cells, T regulatory-like cells, and PD1+CD8+ TEM cells); and innate (neutrophils, CD27+γδ T & NK) cells. All disease-associated subsets were validated in a second cohort. This network was present in a subset of patients and was not associated with IBD subtype, severity or intestinal location. With IMC we revealed the intestinal anatomy and the colocalization of neutrophils, CD4+ TM cells and CD11c+ myeloid cells in the inflamed lamina propria. Our study indicates that a coordinated cellular network of both innate and adaptive immune cells is present in inflamed biopsies from a subset of IBD patients. These results contribute to dissecting disease heterogeneity and may guide the development of targeted therapeutics in IBD.

Keywords: Immune networks, inflammatory bowel disease, omics technologies

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WORKSHOPS

OP-195

Fibre-associated lachnospiraceae reduce colon tumorigenesis by modulation of the tumour-immune microenvironment

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Several studies have highlighted the role of microbiota in colorectal cancer (CRC) progression. Patients with CRC harbour gut microbiomes that differ in structure and function from those of healthy individuals, suggesting this altered microbiome could contribute to carcinogenesis. Despite the increasing evidence implicating the gut microbiome in CRC, the collective role of different microbial consortia in CRC carcinogenesis is unclear. Using a combination of 16S rRNA amplicon sequencing, transcriptomics, and metagenomics, we assessed the capacity of the gut microbiota to shape the tumor microbiome, modulate the immune system and, ultimately, affect tumor growth. Here, we found that tumour biopsy tissue from patients with a "high-risk" Pathogen-type microbiome had a different immune transcriptome from those with a "low-risk" Lachnospiraceae-type microbiome. Transplantation from patients of the two faecal microbiome types into mice with an orthotopic tumour differentially affected tumour growth and the systemic anti-tumour immune response. The differences in tumour volume and immunophenotype between mice receiving the high-risk and the low-risk microbiome correlated with differences in the engrafted human microbial species and predicted microbiome-encoded metabolites in the two groups. These data suggest that the configuration of the gut microbiome may influence colon cancer progression and disease outcome by modulating the anti-tumour immune response.

Keywords: Cancer immunology, microbiome and environmental factors, omics technologies

OP-196

Staphylococcus aureus-induced immunosuppression facilitates persistence during nasal colonisation

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Staphylococcus aureus is an important human pathogen. Despite its' infectivity, *S. aureus* also acts as a commensal, colonising the nasal mucosa of individuals. Little is known about the immune response elicited during *S. aureus* colonisation. Previous studies demonstrate IL-22 and IL-17 producing T-cells are integral to *S. aureus* clearance by promoting phagocytic removal of the bacterium. We hypothesize that *S. aureus* is dampening effector T-cell responses to achieve persistence. The aim of this work is to investigate *S. aureus*- induced immunosuppression during colonisation. Mice were intranasally colonised with *S. aureus*, leading to significantly increased production of IL-10 and IL-27 within their nares.A time-course of IL-10 production was established, with protein levels peaking 24h post-colonisation. Myeloid and B-cells were identified as the major sources of IL-10, increasing their IL-10 gene expression within 6h of colonisation. The impact IL-10 has on the local immune response was next investigated using intranasally colonised IL10-/- mice. Enhanced bacterial clearance was seen alongside elevated levels of IL-17 and IL-22. The principal sources of IL-17 and IL-22 were γδ+, CD4+ and CD8+ T-cells. The function of IL-27 was then determined. *In vivo* blocking of IL-27 prior to and during colonisation reduced IL-10 levels and enhanced bacterial eradication, indicating IL-27 is central to *S. aureus*-induced immunoregulation, acting upstream of IL-10. These results demonstrate *S. aureus* manipulates the local immune response to ensure persistence by impeding protective T-cell responses. The consequence of this commensal-induced immunosuppression may have far reaching negative implications on subsequent *S. aureus* exposures and vaccine efficacy.

Keywords: Bacterial infections, cytokines and mediators, infectious disease

OP-197

Neutrophil-dependent secretion of the biomarker LRG1 in tuberculosis

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), kills 1 million people annually. Neutrophils recruited during infection, partly due to IL-8 secretion by macrophages, are key in TB pathogenesis. Leucine-rich alpha-2 glycoprotein-1 (LRG1) represents a novel biomarker for active TB. However, its role in TB pathogenesis and its cellular source are unknown. Therefore, we investigate whether neutrophils are key regulators of LRG1 secretion during TB. Human monocytes from healthy donors were infected with Mtb at a multiplicity of infection of 1 for 24h. This conditioned media (CoMTb) and media from uninfected monocytes (CoMCont) were filter-sterilised. Peripheral neutrophils from healthy donors were stimulated with CoMTb, CoMCont, phorbol myristate acetate (PMA), or IL-8. Protein and gene expression of LRG1 in neutrophil-derived supernatants or neutrophils were quantified by ELISA, flow cytometry, immunohistochemistry, and qPCR. Immunohistochemistry confirmed that neutrophils express LRG1. LRG1 secretion by human neutrophils increased significantly after 2 hours of CoMTb stimulation compared to CoMCont treatment ($p<0.001$). Stimulation with the agonist PMA also promoted significant LRG1 secretion ($p<0.001$), whilst IL-8, a neutrophil chemokine, only caused moderate LRG1 release compared to unstimulated neutrophils. Flow cytometry confirmed LRG1 surface expression on a larger proportion of IL-8 treated neutrophils compared to resting neutrophils (36% vs. 14%, respectively). In addition, activated neutrophils showed upregulated lrg1 gene expression. Our novel findings show that neutrophils express LRG1 in a monocyte-dependent network in TB and are potentially the main source of this biomarker. Future studies will define the role of LRG1 in TB pathogenesis to lead to new host-directed therapies.

Keywords: Bacterial infections, biomarkers, infectious disease, innate immunity, neutrophils

OP-198

Expression of the coinhibitory receptor TIGIT on memory CD4$^+$ T cells is induced by TCR ligation and retinoic acid co-stimulation and is elicited during microbial colonization of the intestine

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The coinhibitory receptor TIGIT is widely expressed by immune cells, including CD4$^+$ T cells. Previously, we demonstrated that low frequencies of TIGIT$^+$ cells in circulating gut-homing CD38$^+$CD4$^+$ effector T cells associate with more severe inflammatory bowel disease. We hypothesize that TIGIT$^+$CD4$^+$ T cells regulate immune responses to intestinal bacteria. Thereto, we investigated TIGIT expression in subpopulations of intestinal CD4$^+$ T cells and assessed the signals required for its induction. TIGIT was expressed by >50% of CD4$^+$ memory, but not naïve, T cells in mouse and human intestinal lamina propria. Both Foxp3$^+$ and Foxp3neg CD4$^+$ T cells expressed TIGIT. Intestinal TIGIT expression depended on the presence of microbial antigens, as conventionalization of germ-free mice with SPF microbiota dramatically increased TIGIT expression in small intestine and colon. Next, we identified the signals required for TIGIT induction using human healthy donor peripheral blood CD4$^+$ T cells. T cell receptor (TCR) ligation and downstream calcium-dependent signaling were essential to induce TIGIT expression on CD4$^+$ T cells. However, TIGIT expression could only be induced on antigen-experienced CD4$^+$ memory T cells but not naïve CD4$^+$ T cells. Retinoic acid, a metabolite produced by intestinal antigen-presenting cells (APCs), significantly enhanced TCR-induced TIGIT expression. These data argue that locally in the lamina propria of the intestine, resident APCs may favor TIGIT induction on memory CD4$^+$ T cells. In conclusion, intestinal APC-derived signals, including TCR stimulation and retinoic acid production, induce TIGIT expression on CD4$^+$ memory T cells, which may contribute to regulation of immune responses against microbial antigens.

Keywords: Adaptive immunity, inflammatory bowel disease, memory, regulatory cells

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WORKSHOPS

OP-199

TTC7A/PI4KIIIα pathway controls leukocytes' migration

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The actin cytoskeleton is a critical regulator of the immune homeostasis, as it controls cell migration, allowing the orchestrated movement of immune cells between lymphoid and extra-lymphoid tissues. TTC7A has been described in the context of primary immune disorders associated with dysregulation in the actin cytoskeleton dynamics, however the underlying molecular mechanisms, and the consequences in immune homeostasis have not been address. To assess the impact of TTC7A deficiency in leukocytes' migration, we leverage microfluidics to study cell migration reproducing physiologically relevant environments. This approach enables us to determine leukocytes' kinetic parameters at the single cell level. Our data showed that T-cells derived from TTC7A-deficient patients present with altered cell migration caused by an increment in the actin polymerization at the cell rear. Through chemical interventions we have identified the affected molecular pathway controlling cell migration, which depends on the availability of phosphoinositides in the plasma membrane, phosphorylation of AKT triggered under confinement and the regulation of RhoA activity. Moreover, when faced with a constricted pathway, TTC7A-deficient cells are less efficient in passing through than control cells, suggesting alterations in the machinery regulating nucleus compression. Notably, this phenotype associates with an increase in DNA damage and a higher percentage of cell death in cells moving in dense 3D environments. Altogether our results suggest that the defects in cell migration observed in TTC7A-deficient cells are due to a disbalance between actin regulators and could be the cause for the progressive lymphopenia observed in patients.

Keywords: Cell death, cytoskeleton, immunodeficiency

OP-200

Kinetics of peripheral blood neutrophils in severe coronavirus disease 2019

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Emerging evidence of dysregulation of the myeloid cell compartment urges investigations on neutrophil characteristics during disease evolution in coronavirus disease 2019 (COVID-19). Neutrophils were isolated from the blood of COVID-19 patients receiving general ward care and from patients hospitalized at intensive care units (ICU) to explore kinetics of circulating neutrophils and factors important for neutrophil activation and migration. Neutrophil differentiation and activation markers were analyzed by multicolor flow cytometry. Multiplex and ELISA technology were used for quantification of protease, protease inhibitor, chemokine, and cytokine concentrations in plasma. Neutrophil polarization responses were evaluated microscopically. Gelatinolytic and metalloproteinase activity in plasma was determined using a fluorogenic substrate. Co-culturing healthy donor neutrophils with SARS-CoV-2 allowed to investigate viral replication in neutrophils. Upon ICU admission, patients displayed high plasma concentrations of granulocyte–colony stimulating factor (G-CSF) and the chemokine CXCL8, accompanied by emergency myelopoiesis as illustrated by high levels of circulating CD10-, immature neutrophils with reduced CXCR2 and C5aR expression. Neutrophil elastase and non-metalloproteinase-derived gelatinolytic activity were increased in plasma from ICU patients. Significantly higher levels of circulating tissue inhibitor of metalloproteinases-1 (TIMP-1) in patients at ICU admission was associated with decreased total MMP proteolytic activity in blood. COVID-19 neutrophils were hyperresponsive to CXCL8 and CXCL12 in shape change assays. Finally, SARS-CoV-2 failed to replicate inside human neutrophils. Our study provides detailed insights into kinetics of neutrophil phenotype and function in patients with severe COVID-19, and supports the concept of an increased neutrophil activation state in circulation.

Keywords: Chemokines, cytokines and mediators, neutrophils, viral infections

OP-201

Downregulation of A20 promotes immune escape of lung adenocarcinomas

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About 20 % of patients suffering from lung adenocarcinoma (LAC) respond to PD-1/ PD-L1 directed monotherapies, and foreseeing the response of individual patients is difficult. Accordingly, a better understanding of tumor immune evasion is needed to identify novel prediction markers. To investigate the role of the potent anti-inflammatory enzyme A20 in lung tumorigenesis we took advantage of gene expression data and tissue-micro-arrays of LAC patients, genetically engineered mouse models of "K-ras" driven lung tumorigenesis and orthotopic transplantation models of CRISPR/Cas9 modified LAC cell lines. We identified A20 as a potent tumor suppressor in patients and mice. A20 modulates the tumor immune microenvironment, as downregulation of A20 resulted in escape of T cell mediated immune evasion. Intriguingly, A20 knockout tumors exhibited an increased interferon signature, caused by hyper-active TBK1, which triggered a feedback loop leading to elevated STAT1 expression. Deletion of IFNAR restored tumor immune surveillance in A20 deficient LAC, but did not impact the development of A20 proficient tumors. Furthermore, immune evasion of A20 deficient lung tumors was dependent on STAT1 mediated PD-L1 expression. Accordingly, deletion of PD-L1 in K-ras mutated LAC cells rescued the A20 tumor suppressor function in syngeneic orthotopic mouse models and pharmacological blocking of PD-L1 re-established T cell infiltration and reduced tumorigenesis in K-ras mutated A20 knockout tumors. Our results show that reduced A20 levels in LAC enhance a TBK1-STAT1-PD-L1 axis driving immune evasion, but also sensitizing tumors to immune therapy. Therefore, A20 can be a biomarker predicting patient responses to immune checkpoint blockade.

Keywords: Cancer immunology, Cell signalling, Checkpoint inhibition, Immune regulation and therapy, *in vivo* tumor models

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WORKSHOPS

OP-202

Single cell spatial analysis reveals changes in the tissue immune landscape during initiation of colitis associated cancer in patients

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Chronic inflammation of tissue can result in initiation of cancer. Colitis Associated Cancer (CAC) is a dreaded complication of Inflammatory Bowel Disease (IBD). Patients with severe chronic IBD have an increased risk (2-9 fold) of developing CAC. Therefore, these patients are enrolled in Surveillance screening protocols, requiring regular endoscopies and biopsies to remove early stages of CAC (dysplasia). To understand how the chronic inflammatory responses in tissue contribute to cancer initiation, we performed a multiplex spatial protein analysis of the immune response in longitudinally collected tissue biopsies of these patients. We have collected and stored tissue biopsies from IBD patients undergoing yearly surveillance in our Surveillance tissue biobank. From this biobank, longitudinally collected biopsies containing healthy, inflamed and dysplastic tissue were selected. We performed 35-plex Imaging Mass Cytometry and microscopy analysis, using our pipeline 'MATISSE', allowing single cell segmentation of epithelium, stroma, and immune cells. To determine differences in immune cell infiltrate composition and localization, we performed single cell clustering analysis, and neighborhood analysis. Our analyses revealed differences in immune cell subtypes localization between healthy, inflamed and dysplasia biopsies. We observed changed proportions of $CD4^+$ and $CD8^+$ T cells located intra-epithelially versus the lamina propria, and changes in inflammatory cytokines. *In vitro* culture assays of human epithelial organoids showed that inflammatory cytokines, such as IL-17, influence epithelial integrity. Our studies reveal immunological mechanisms that could contribute to cancer initiation. Identified inflammatory factors could serve as future precision treatment targets in patients at risk for CAC.

Keywords: Cancer immunology, inflammatory bowel disease, microenvironment, omics technologies

OP-203

CD28 Superagonist TAB08-mediated activation and expansion of human regulatory T cells might protect patients from acute graft versus host disease

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Acute Graft-versus-Host Disease (aGvHD) remains an often therapy-limiting and life-threatening complication of allogenic hematopoietic stem cell and T cell transplantation (aHSCT) which is driven by allo-reactive T cells contained in the graft. In mice, we had shown that *in vivo* or *in vitro* pre-activation and expansion of CD4+ Foxp3+ regulatory T cells (Tregs) by superagonistic anti-CD28 monoclonal antibody (CD28-SA) stimulation protects aHSCT recipients from aGvHD. Therefore, we now investigated whether the human CD28-SA TAB08 (TGN1412) could also be used to modulate human T cell responses to reduce alloantigen-driven inflammation and facilitate tolerance without abolishing memory T cell responses. After two days of high-density culture, we stimulated human peripheral blood mononuclear cells (PBMCs) with CD28-SA at 0.05 µg/ml to increase the frequency of Tregs among CD4+ T cells. We then tested the inflammatory activity of the pre-cultured T cells in both allogenic mixed lymphocyte reactions (allo-MLRs) and antigenic recall assays. While memory T cell responses against recall antigens remained unchanged after CD28-SA pre-culture, a strong enrichment of Foxp3+ T cells was observed in allo-MLRs together with decreased proliferation of pro-inflammatory Foxp3- T cells, in particular CD8+ T cells. FACS sorting experiments confirmed that the Foxp3+ cells detected at the end of the human allo-MLRs stemmed from pre-existing Tregs. Taken together, our data suggest that similar to our observations in mouse models of aGvHD CD28-SA stimulation of human T cells might be suitable to reduce the risk of aGvHD in humans, while maintaining memory and possibly also Graft versus Leukemia (GvL) responses.

Keywords: Antibody, bone marrow transplantation, immune regulation and therapy, infectious disease, regulatory cells

OP-204

Using multiparameter flow cytometry to understand microbiota-host crosstalk in chronic inflammatory diseases

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Chronic inflammatory diseases are often accompanied by dysbiosis, i.e. alteration in the intestinal microbiota. Next generation sequencing of the 16S rRNA gene has allowed the detailed analysis of the composition of the intestinal microbiota, but fails to capture cellular properties of the bacteria. We analyze cell surface properties of human intestinal microbiota by multi-parameter flow cytometry to gain further insights into the host-microbiota crosstalk. For a better understanding of the recognition of bacteria by the host and to differentiate the immunological context of this recognition, we analyze host immunoglobulin-coating of bacteria, distinguishing the isotypes IgA1, IgA2, IgG and IgM. Further, we use various lectins to characterize surface sugars, which relate to metabolic conditions, adhesion and potential communication of the bacteria with the host. Comparing stool samples of patients with chronic inflammatory diseases, such as juvenile idiopathic arthritis, IgG4-related diseases, rheumatoid arthritis and inflammatory bowel disease, to healthy controls, we found distinct populations of bacteria which displayed increased lectin binding and enhanced host immunoglobulin coating. We hypothesize that the lectin-binding bacteria induce an enhanced immune recognition and are critical in the induction and maintenance of chronic inflammation. The hypothesis will be addressed by analyzing the memory T cell compartment of patients and controls for it's microbiota reactivity. Overall, it is our aim to reconstruct the communication of microbiota and host in the context of autoimmunity.

Keywords: Autoimmunity, immune response tracing, inflammatory disease, memory, microbiome and environmental factors

OP-205

Parallel detection of SARS-CoV-2 epitopes in convalescent COVID-19 patients reveals immunodominance and memory CD8+ T cells

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic leads to global morbidity and mortality. A sustainable solution requires universal vaccines that are effective against the complete spectrum of SARS-CoV-2 variants. Current vaccines aim to induce neutralizing antibodies, but recent data indicate that these vaccines are less effective against some SARS-CoV-2 variants. We aim to unravel whether SARS-CoV-2-specific CD8+ T cells contribute to broad protective immunity. CD8+ T cells can recognize the more conserved internal SARS-CoV-2 proteins, enhance viral clearance and thereby prevent or reduce disease severity. Although several SARS-CoV-2 epitopes have been discovered, they only cover a minor proportion of the viral peptides presented in the total human HLA-pool. We selected 137 potential SARS-CoV-2 peptides covering 11 HLAs based on prediction programs (NetMHCv4.0), literature and homology with seasonal coronaviruses. After confirming binding to the respective HLA, combinatorial encoded HLA class I tetramers were used to screen the peptides in 51 HLA-typed convalescent COVID-19 patients directly *ex vivo*. Combinatorial encoding allowed simultaneous screening of 41 SARS-CoV-2 peptides in a single donor in multiple time points up to 12 months post infection. Our study resulted in the discovery of new epitopes and confirmed previously described epitopes. Furthermore, the parallel tetramer and phenotypic staining allowed us to establish immunodominance, phenotype and longevity of the epitope-specific CD8+ T cells. We identified a minimal set of SARS-CoV-2 peptides which are of interest for the induction of long-lived immune protection in next-generation vaccines which aim to provide protection against current and emerging SARS-CoV-2 variants.

Keywords: Infectious disease, memory, monitoring immunity, viral infections

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WORKSHOPS

OP-206

Src-family kinases in immune complex-mediated glomerulonephritis

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Murine nephrotoxic nephritis is a widely used model of immune complex-mediated glomerulonephritis. In this model, mice are immunized with normal sheep IgG, followed by administration of a sheep antiserum against mouse glomerular preparation (nephrotoxic serum; NTS). This leads to glomerular antibody deposition, severe glomerular inflammation and concomitant albuminuria and increased serum creatinine levels. The Src-family kinases Hck, Fgr, and Lyn are critical for various autoantibody-induced inflammatory disease models. Here we used Hck−/−Fgr−/−Lyn−/− triple knockout mice to test whether they participate in the development of nephrotoxic nephritis. Mice were preimmunized with sheep IgG followed by intravenous injection of NTS or normal sheep serum. Eight days later urine was collected over a 24-hour period. After an additional day blood was collected, the kidneys were removed, and the leukocyte infiltration and the glomerular injury was tested by flow cytometry and light microscopy. Wild type NTS treated mice developed severe albuminuria on the eighth day. In these animals' kidney glomerular hypercellularity, leukocyte infiltration, mesangial expansion and glomerular sclerosis could be also detected by light microscopy in several glomeruli. Serum creatinine levels were higher than in the control group, while serum albumin concentrations decreased due to the disease. In contrast, NTS treated Hck−/−Fgr−/−Lyn−/− animals serum and urine parameters remained in the control range, with no detectable histological differences between the NTS treated and the control group. Our results indicate that Hck, Fgr, and Lyn have a crucial role in the development of immune complex-mediated glomerulonephritis.

Keywords: Animal models, autoimmunity, myeloid cells, neutrophils

OP-207

Liver X receptor activation promotes intestinal repair while limiting tumorigenesis

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Aberrant tissue repair characterized by chronic healing following injury such as inflammation may link to tumorigenesis. Intestine, being exposed to a wide range of potential insults from daily food intake, has a dynamic but controlled epithelial regeneration in response to tissue damage. However, the mechanism behind this fine-tuned proliferation avoiding neoplastic formation has not been clear. Here, we combined two RNAseq datasets from different intestinal damage-repair mouse models and unbiasedly funneled to a mutually enhanced cholesterol metabolism via Liver X receptor (LXR) as an adaption to tissue damage. We demonstrated that through LXR activation in mouse enhanced stem cell activity *in vivo* and boosted crypt domain formation in enteroids via EGFR signaling. Furthermore, Cyp27a1, a LXR natural ligand producing enzyme was found upregulated upon damage in cells surrounding intestinal crypts. In the AOM-DSS colon cancer model, LXR activation resulted in lower tumor counts and slower development while the anti-tumor effect was not observed in Cyp27a1 deficiency. Data together, LXR may play a crucial role in guiding epithelium remodeling away from tumorigenesis risk upon tissue injury.

Keywords: Inflammatory bowel disease, stem cells, tissue damage and repair

OP-208

Regulation of IL-36 receptor driven skin in inflammation in psoriasis

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IL-36 family cytokines play and orchestrating role in driving psoriatic skin inflammation. However, the specific mechanisms through which these cytokines mediate such effects and how they are regulated, have yet to be determined. In this study, we generated a new transgenic mouse lacking Il36r expression specifically in keratinocytes (Il36rδK mice) and demonstrated that these mice are protected against psoriasiform inflammation to the same level as mice with global deficiency in Il36r. After IMQ induced psoriasiform inflammation Il36rδK mice displayed significantly reduced expression of IL-17a, IL-23, IL-22 and CXCL1, alongside reduced infiltration of neutrophils and IL-17A expressing γδ T cells in the inflamed skin. These data identify keratinocytes as the key responsive cell in mediating IL-36 driven skin inflammation. We have also identified Sigirr as an important negative regulator of Il36r signaling in keratinocytes, using Sigirr-/- mice. These mice were found to exhibit enhanced psoriasiform skin inflammation which was successfully reversed upon treatment with an anti-IL-36r blocking mAb. Significantly, an analysis publicly available gene expression databases demonstrated that while the levels of expression of Il36 family genes are elevated in psoriatic lesional skin, expression levels of SIGIRR are reduced, indicating an important regulatory role for this molecule in human psoriatic disease. In conclusion, these data identify keratinocytes as the major cell in orchestrating IL-36 driven psoriatic inflammation and demonstrate that Sigirr plays an important regulatory role in dampening these responses in both mice and humans.

Keywords: Inflammatory molecules, autoimmunity, cytokines and mediators, inflammatory disease, neutrophils, skin diseases

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WORKSHOPS

OP-209

Asthma-associated immunological responses in RSV infections on primary human lung tissue *ex vivo*

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The respiratory syncytial virus (RSV) is the most common cause of severe airway infections in infants and has been linked to the development of asthma in children. The inflammatory response may contribute to the initial disease severity and facilitate consequent asthma onset. However, the exact mechanisms remain unknown. We therefore aim to investigate the pathophysiological mechanisms of RSV infections in a novel human *ex vivo* setting. Disease-free, human precision-cut lung slices (PCLS) containing airways were infected with RSV with and without palivizumab. After three days the virus load was quantified by real-time qPCR. The immune response was determined by measuring secreted cytokines by ELISA. RSV infected human PCLS and led to a considerable virus load in tissue ($1.1 \times 10^6 \pm 5.7 \times 10^5$ copies/well) and supernatants ($8.6 \times 10^5 \pm 7.1 \times 10^5$ copies/well). Imaging showed patches of infected cells in the alveolar region and predominantly in the ciliated airway epithelium. Furthermore, RSV induced the secretion of IP-10 (95.9-fold), IL-5 (5.4-fold), and IL-13 (4.5-fold) compared to uninfected PCLS, which could be prevented up to 77 % by palivizumab pre-treatment. Our results show that RSV infects human PCLS and initiates an anti-viral immune responses in the tissue. Pre-treatment with palivizumab prevented the RSV-mediated cytokine secretion. Moreover, the data suggest specific interactions between virus-induced and allergic inflammatory responses contributing to asthma development. Thus, our *ex vivo* model resembles key features of the clinical situation and can be used to further characterize the immunological mechanisms of RSV-mediated asthma.

Keywords: Allergic disorders, biomarkers, cytokines and mediators, infectious disease, inflammatory disease, viral infections

OP-210

Leukemic extracellular vesicles drive highly suppressive regulatory T cells by modulating mTOR and STAT5 signaling, leading to progression of myeloid leukemia

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Tumor extracellular vesicles (EVs) have been implicated as drivers of regulatory T cells (Treg) in cancer. However, little is known about precise molecular and phenotypic changes in Treg due to cancer EVs, especially in hematological malignancies.

We aimed to study modulation of Treg by chronic/acute myeloid leukemia (CML/AML) EVs, using human *ex vivo* and mouse *in vivo* models, advanced flow cytometry and RNA sequencing. We observed that leukemic EVs induced Foxp3+ iTreg from CD25- Tconv and amplified suppressive activity of mature Treg. EVs-treated Treg maintained demethylated TSDR in *Foxp3* promoter and secreted less of non-Treg-specific IL-6, IFN-γ and IL-17. Downregulation of phopho-mTOR-S6 and upregulation of phospo-STAT5 were identified as molecular pathways modulated by leukemic EVs to drive suppressive Treg. RNA-seq revealed significant remodeling of gene expression and pin-pointed transcription factors engaged in this regulation. Treg phenotype on protein level was studied using 23-color spectral cytometry, revealing upregulation of several tumor Treg molecules (CCR8, CCR4, CD39 and others). Using unsupervised clustering of flow cytometric data, we identified an effector subset of Treg driven by leukemic EVs. Finally, we validated our findings using mouse model of leukemia, induced by wild-type or Rab27a-deficient (releasing less EVs) CML cells. Mice with Rab27a-deficient leukemia had less Treg, less CD44+CD62L- activated Treg and lower Treg expression of suppressive CD39, CD73 and IL-10, in parallel with weaker engraftment of leukemic cells. In conclusion, leukemic EVs significantly drive immunosuppressive Treg, contributing to leukemia progression *in vivo*. We identified signaling and transcriptomic regulatory mechanisms engaged in EVs-mediated tumor-Treg polarization.

Keywords: Cancer immunology, immune communication, *in vivo* tumor models, regulatory cells

OP-211

The BNT162b2 mRNA vaccine against SARS-CoV-2 reprograms both adaptive and innate immune responses

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The mRNA-based BNT162b2 vaccine from Pfizer/BioNTech was the first registered COVID-19 vaccine and has been shown to be up to 95% effective in preventing SARS-CoV-2 infections. Little is known about the broad effects of the new class of mRNA vaccines, especially whether they have combined effects on innate and adaptive immune responses. Here we confirmed that BNT162b2 vaccination of healthy individuals induced effective humoral responses and cellular immunity against several SARS-CoV-2 variants. Fluorescent-bead-based multiplex immunoassay revealed that antibody concentrations elevated significantly, and specific IFN-γ secretion moderately increased following vaccination. Interestingly, however, the BNT162b2 vaccine also modulated the production of inflammatory cytokines by innate immune cells upon stimulation with both specific (SARS-CoV-2) and non-specific (viral, fungal and bacterial) stimuli. Enzyme-linked immunosorbent assay results showed that the response of innate immune cells to TLR4 and TLR7/8 ligands was lower after BNT162b2 vaccination, while fungi-induced cytokine responses were stronger. In conclusion, our data show that the BNT162b2 vaccine induces effects on both the adaptive and the innate branch of immunity and that these effects are different for various SARS-CoV-2 strains. Intriguingly, the BNT162b2 vaccine induces reprogramming of innate immune responses and this needs to be taken into account: in combination with strong adaptive immune responses, this could contribute to a more balanced inflammatory reaction during COVID-19 infection.

Keywords: Adjuvants and vaccines, innate host defence, innate immunity, viral infections

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WORKSHOPS

OP-212

The major peanut allergens Ara h 1, 2, 3 and 6 are taken up by dendritic cells via different routes and receptors

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Peanut allergy ranks amongst the most severe food allergies. Of the major peanut allergens, Ara h 2 is considered to have the highest allergenic potential. We questioned whether differences in allergenicity find their origin during the process of sensitization, i.e. during allergen uptake, processing and/or presentation to T cells. Flow cytometry analysis of fluorescently labeled allergens indicated that the uptake of Ara h 1, 2, 3 and 6 by human dendritic cells is facilitated by general mechanisms as macropinocytosis and phagocytosis. As these processes can involve receptor-mediated uptake, especially via recognition of carbohydrate moieties, we determined glycosylation profiles of these allergens. Ara h 1 proved to be highly mannosylated, and uptake might be mediated via the mannose receptor, because it could be blocked with mannan and EDTA. Surprisingly, the uptake of both highly homologous (59% identity) non-glycosylated 2S albumins, Ara h 2 and Ara h 6, was also abrogated in the presence of EDTA, indicating a role of other transmembrane receptors in this process. Further experiments, determining receptor binding, endosomal and lysosomal co-localization and proliferation of allergen-specific T cell lines might reveal more differences between the allergens. These data will provide new insights in the fundamental processes during sensitization to peanut allergens and could contribute to improved immunotherapy.

Keywords: Allergen-induced immune responses, allergic disorders, antigen processing and presentation, dendritic cells

OP-213

Hyper-inflammation in severely ill COVID-19 is induced by early phase anti-SARS-CoV-2 IgG and can be specifically counteracted by FDA/EMA-approved small molecule inhibitors

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Patients diagnosed with COVID-19 become critically ill primarily around the time of activation of the adaptive immune response. Here, we provide evidence that antibodies play a key role in this worsening of disease at the time of seroconversion. We show that early phase IgG against the Spike protein of SARS-CoV-2 in serum of critically ill COVID-19 patients induces excessive inflammatory responses by human alveolar macrophages. We identified that this inflammatory response is dependent on two antibody features that are specific for patients with severe COVID-19, i.e. extremely high titers of anti-spike IgG, and aberrant glycosylation of the Fc tail of anti-spike IgG, particularly low fucosylation. We identified Fcγ receptor (FcγR) IIa and FcγRIII as the two primary IgG receptors that are responsible for this excessive inflammation. Strikingly, we show that the hyper-inflammatory response induced by anti-Spike IgG can be specifically counteracted by fostamatinib, an FDA- and EMA-approved therapeutic small molecule inhibitor of the kinase Syk, which is now tested in phase III clinical studies involving 42 different hospitals worldwide. In addition, we show that hyper-inflammation induced by low fucose anti-Spike IgG is dependent on a novel interferon-dependent pathway. Finally, we applied this knowledge to identify FDA- and EMA-approved small molecule inhibitors that are even more specific to counteract inflammation in severely ill COVID-19 patients than fostamatinib. These drugs may be highly valuable to treat individuals that have not been vaccinated, or for the treatment of (future) SARS-CoV-2 mutants to which the current vaccines do not provide sufficient protection.

Keywords: Antibody, infectious disease, inflammatory disease, macrophage

OP-214

Assessment of changes in the expression of immunomodulatory miRNAs (miR-20b, miR-155 and miR-221) in workers occupationally exposed to Cadmium

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Cadmium (Cd), a well-known environmental pollutant, can induce several adverse health effects following its accumulation in the human body. Cd is also a potential carcinogen, capable of causing multi-system alterations. The molecular mechanisms by which Cd exerts systemic damage in the human body are under exploration. The present study, aimed to evaluate levels of three candidate miRNAs (miR-20b, miR-155 and miR-221) in individuals exposed to Cd. The study enrolled 110 individuals from handicraft and welding factories of Jodhpur, who were occupationally exposed to Cd and 97 apparently healthy individuals without any history of occupational exposure. Blood levels of Cd were determined by graphite furnace atomic absorption spectroscopy (GFAAS). Circulating miRNA were isolated from serum by Qiagen isolation kit and subsequently converted to cDNA using Qiagen miRCURY LNA RT kit. miRNA expression was assessed using Qiagen miRNA PCR assay in RT-PCR. The median Cd level in the exposed individuals (2.40µg/L) was significantly higher than the non-exposed (0.90µg/L) ($p<0.0001$). miRNA expression analysis revealed a significant upregulation in the expression of miR-221 in the exposed group with a fold change of 3.22, while miR-20b and miR-155 did not differ significantly between the groups. The findings of our study highlight the importance of miRNA dysregulation in Cd exposed individuals that may contribute to systemic effects of Cd toxicity. The role of miR-221 and other miRNAs as a potential biomarker of Cd toxicity needs to be further validated in diverse population groups to highlight the possible molecular pathways through which Cd exerts its toxic manifestations.

Keywords: Chronic inflammation and fibrosis, inflammatory molecules, miRNA

OP-215

Characterising the relation between genetic background and gut immune response against microbiota in a murine model of lupus

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Changes in the gut microbiota has been recently associated with autoimmune disease severity. Whether such changes are the cause or consequence of the disease remains unknown. In systemic lupus erythematosus (SLE), an autoimmune disease characterized by persistent inflammation, the contribution of the gut microbiota is particularly elusive. We have identified the existence of gut inflammation in both a murine model of lupus that is a TLR7 transgenic mouse strain as well as in the imiquimod-induced model. To further analyse the microbiota contribution to disease severity, we fine-tuned a method to measure the IgA bound to faecal bacteria, as the amount of binding can serve as an indicator of gut inflammation and a shifted gut immune response. As a result of this characterization, we have been able to accurately identify a side scatter low bacterial population present only in mice deficient for the B cell scaffold with ankyrin repeats kinase (*Bank1*) gene, but not in WT animals. These bacteria present a characteristic low IgA coating, and seem to be completely altered upon acute inflammation. These results, altogether with those obtained from early 16S-sequencing of the faeces, suggest that microbiota may have a different composition in the absence of *Bank1*, which could, in turn, be determinant in defining the severity of the immune response initiated in the gut. Further studies are needed to elucidate whether this unique population in *Bank1* deficient mice plays a role in disease development or affects the local gut immune response.

Keywords: Animal models, autoimmunity, inflammatory disease, microbiome and environmental factors

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WORKSHOPS

OP-216

Obesity is associated with an altered baseline and post-vaccination influenza antibody repertoire

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As highlighted by the ongoing COVID-19 pandemic, vaccination is critical for infectious disease prevention and control. Obesity is associated with increased morbidity and mortality from respiratory virus infections. While obese individuals respond to influenza vaccination, what is considered a seroprotective response may not fully protect the global obese population. To investigate how obesity and aging impact the IgG and IgA antibody responses to influenza vaccination. We conducted an in-depth analysis of influenza-specific IgG and IgA antibody repertoires of 205 subjects, 100 with healthy weight (HW: Body mass index (BMI) = 18.5 to 24.9 kg/m2), and 105 with obesity (OB; BMI≥30kg/m2) prior to, and 30 days post-vaccination with the 2010-2011 split trivalent inactivated influenza vaccine (TIV). To compare the antibody repertoires of the study groups, we used two types of antigen microarrays (AMs): (a) Flu AMs spotted with whole inactivated viruses recombinant hemagglutinin proteins from 34 influenza strains; and (b) Cal09 peptide AMs spotted with partially overlapping peptides spanning the sequences of the hemagglutinin and neuraminidase proteins of the A/California/7/2009 (A/H1N1; Cal/09) vaccine strain. Baseline immune history and post-vaccination responses were found to significantly differ in obese individuals compared to healthy controls, especially towards the 2009 pandemic strain of A/H1N1 influenza virus. Young, obese individuals displayed responses skewed towards linear peptides versus conformational antigens, suggesting aberrant obese immune response. Overall, these data have vital implications for the next generation of influenza vaccines, suggesting that obese subjects may benefit from a different vaccination strategy.

Keywords: Adaptive immunity, adjuvants and vaccines, antibody, infectious disease, visualizing immune responses

OP-217

IgA immune history profiles predict influenza disease in vaccinated and unvaccinated individuals

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Seasonal influenza viruses are a significant threat to public health and are estimated to cause up to 650,000 deaths per year. The protective effect of seasonal influenza vaccines varies by year due to antigenic drift. A variety of factors impact an individual's heterogeneity in vaccine induced immune responses, such as age, ethnicity and 'immunological history' - the individual's memory antibody repertoire to previously encountered pathogens and vaccines. To study the role of immune-history to previous influenza infections on the efficacy of the seasonal influenza vaccine. We developed Flu Array - a novel influenza antigen microarray spotted with whole-inactivated influenza viruses and recombinant hemagglutinin and neuraminidase proteins from 36 A/H1N1, A/H3N2 and B influenza strains. To profile the effect of immune history on vaccine-induced immune responses and protection from infection, we used samples from an influenza vaccine efficacy trial comparing the trivalent inactivated and live-attenuated vaccines in adults aged 18-65, conducted in 2007-2008. We used Flu Array to compare the baseline and post-vaccination IgG and IgA antibody profiles of subjects who subsequently became infected to those who did not. We identified several novel correlates of risk that were based both on IgG and IgA responses. Surprisingly, the strongest correlate of risk was based on baseline serum IgA antibody levels. Our results highlight the role of immune-history to previous influenza infections as a baseline measurement that may be predictive of vaccine-induced immune responses and vaccine-induced protection.

Keywords: Adaptive immunity, antibody, infectious disease, viral infections

OP-218

Peripheral CD39-expressing regulatory T cell subsets play an age-dependent role in the severity of COVID-19

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COVID-19 is a disease characterized by acute respiratory failure that causes a major global healthcare issue. CD39 is considered a marker of highly suppressor Tregs. Here, we aimed to evaluate the role of CD39-expressing Treg cell subsets in COVID-19 immunopathogenesis and their relationship with disease severity. 190 COVID-19 patients (juvenile and adults) and 31 volunteers as healthy controls were included in our study. Flow Cytometry analysis was performed with a 10-color monoclonal antibody panel from peripheral blood samples. When evaluating CD4+ CD25high CD127low cell percentages in the adult severe and mild cases, a significant increase was observed compared to the control group. Consistently, a significant increase in severe and mild cases was noted in Treg cell subsets expressing CD39. In juvenile patients, the percentages of CD4+ CD25high CD127low FoxP3 + CD39+ cells were lower in the 0-12 age range compared to the 13-18 age range. To conclude, our study reports significant increases in FoxP3+ CD39+ Treg subsets in adult COVID-19 patients. Interestingly, percentages of Tregs co-expressing FoxP3 and CD39 were positively correlated with increasing age in juvenile patients. Our findings provide a better understanding of the possible role of Tregs in the immune response mechanism in COVID-19 cases.

This work was supported by a grant from the Scientific Research Projects Foundation (BAP) of the Bursa Uludag University of Turkey [Project no: OUAP(T)-2020/6].

Keywords: Adaptive immunity, biomarkers, regulatory cells, viral infections

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WORKSHOPS

OP-219

Prevention of *mycobacterium tuberculosis*-induced neutrophil necrosis restricts bacterial proliferation and may represent a target for a host-directed therapy

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Rising multi drug-resistant tuberculosis cases require novel approaches to tackle the epidemic including host-directed strategies. Neutrophils represent the main infected population in tuberculous lungs and drive pathology. We have shown before that *M. tuberculosis* induces necrosis of human neutrophils, a prerequisite for subsequent growth in other phagocytes like macrophages and dendritic cells leading, again, to host cell necrosis and release of a multiplicity of mycobacteria ready to infect new host cells. This scenario likely takes place in lungs of chronic active tuberculosis patients resulting in tissue damage and transmission of contagious aerosol particles. We were able to interrupt this vicious circle of necrosis by pharmacological inhibition of myeloperoxidase (MPO) or scavenging of reactive oxygen species (ROS) by N-acetylcysteine thereby reducing neutrophil ROS production as well as necrosis and mycobacterial numbers in infected neutrophils, neutrophil–macrophage co-cultures, and whole blood assays. Interestingly, ESAT-6-dependent M. tuberculosis-induced neutrophil necrosis could not be prevented by inhibitors of autophagy, necroptosis, ferroptosis, pyroptosis, and compounds that have been described to avert NETosis, such as inhibitors of neutrophil elastase (NE), histone deacetylases, peptide arginine imidases (PAD), and specifically PAD4 that citrullinates histone H3, the only known mechanistic premise to drive NETosis. However, M. tuberculosis-infected, dead neutrophils resemble the morphology of NETotic ones e.g., extracellular NET-like DNA associated with MPO, NE, and the pro-cathelicidin CAP-18. Revealing the exact mechanism of neutrophil necrotic cell death is important to identify putative targets for host-directed therapies for tuberculosis as well as diagnostic markers for bed-side point-of-care testing to monitor therapy outcome.

Keywords: Bacterial infections, cell death, innate host defence, neutrophils

OP-220

The role of interleukin-6 in pathogenesis of apical periodontitis studied by flow cytometry and cell cultures

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Evaluation of interleukin-6 (IL-6) production by cells isolated from clinically symptomatic (IL-6hi producing) periapical lesions (PLs) and asymptomatic (IL-6low producing) PLs *in vitro*; IL-6+cells subsets analysis and investigation of modulatory effect of IL-6 on cytokine production in such cultures depending on their inflammatory status. Inflammatory cells were isolated from 94 human PLs. Multicolor flow cytometry was used for detection of IL-6+ cells. Cytokine levels in cell cultures were determined by ELISA and Flow Cytomix Microbeads. In order to evaluate the role of IL-6 in PLs, mononuclear cells (MNC), isolated from IL-6hi producing PLs and IL-6low producing PLs, were treated with exogenous IL-6 or Tocilizumab (an IL-6 receptor blocking monoclonal antibody). We showed that NKT cells, activated CD8+ T cells and M2 macrophages were dominant IL-6 producers, but CD8+ T cells and monocytes/ macrophages contribute the most to the IL-6 production. IL-6 increased the production of IL-17A, IL-21, RANKL and RANKL/OPG ratio, independently of the PL activation status. The addition of IL-6 to IL-6hi producing cultures decreased IFN-γ, IL-4 and IL-33, whereas Tocilizumab up-regulated IL-10 and TGF-β levels in IL-6low producing cultures. Only high levels of IL-6 were able to suppress Th1 and Th2 responses. Total IL-6 levels in PL cell cultures are mainly derived from macrophages and CD8+ T cells. IL-6 exerts inflammation in PLs through the activation of Th17/Tfh cells, by stimulation of osteodestruction and by suppression of regulatory T cells.

Keywords: Cytokines and mediators, inflammatory disease, macrophage

OP-221

Neuroimmune cardiovascular interfaces in atherosclerosis

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Atherosclerosis is a chronic inflammatory disease of medium- and large-size arteries causing heart attack and stroke. As atherosclerotic plaques involving the intima and media lack nerve fibers, hardwiring and sensing of atherosclerosis by the nervous system has not been probed before. Yet, the nervous system uses the adventitia as the outer connective tissue layer of arteries as their principal conduits to reach peripheral tissues. The discovery of arterial tertiary lymphoid organs (ATLOs) in the diseased aortic adventitia led us to postulate that the peripheral nervous system (PNS) may interact with arteries via adventitial immune cells. In aged hyperlipidemic mice and human atherosclerotic tissue, we found widespread neuro-immune-cardio-vascular-interfaces (NICIs) to form atherosclerosis-brain circuits (ABCs) able of sensing and affecting atherosclerosis: ATLOs interact with the PNS by stimulating axon growth adjacent to atherosclerotic plaques, axon terminals form neuro-leukocyte- and neuro-smooth-muscle-cell-junctions within ATLOs, the adventitia is wired to the brain stem via thoracic dorsal root and perivascular ganglia, advanced atherosclerosis is associated with increased cFos expression in ABC associated brain and spinal cord nuclei and elimination of the sympathetic PNS disrupts ATLOs, attenuates atherosclerosis progression, and reduces plaque vulnerability. Our data demonstrate the pathophysiological relevance of NICIs in atherosclerosis and that the PNS employs NICIs to assemble ABCs. We hypothesize that intervention into neural circuits creates multiple unexpected opportunities to treat atherosclerosis.

Keywords: Cardiovascular diseases, inflammatory disease, neuroimmunology

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WORKSHOPS

OP-222

Single-cell transcriptional and repertoire profiling of CD4+ TILs unveils distinct immune evasion strategies in primary and metastatic CRC

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Colorectal cancer (CRC) is one of the most common tumours with a very limited survival rate for metastatic patients. The immune system has the capacity of killing transformed cells, but tumours have evolved strategies to evade immune surveillance. The quality of T lymphocytes effector responses against tumours is affected by the heterogeneous nature of cancer cells and of the tumour microenvironment. Thus, a better understanding of the heterogeneity of tumour infiltrating T cells (TILs) from different anatomical compartments and of the mechanisms underlying their altered anti-tumour immune response, will help in identifying new targets to reactivate the impaired immunity. To disentangle the complexity of microenvironment-specific phenotypic and functional heterogeneity of TILs, we integrated single-cell transcriptomic and repertoire analyses on $CD4^+$ T cells from primary and metastatic CRC, and their normal adjacent tissues. We identified nine transcriptionally distinct T cell subpopulations with a graded transition from central to effector memory and effector phenotypes paralleled by increasing clonal expansion. Both the frequency and the clonal expansion of the identified Th subsets varied according to the tissue of origin. Focusing on TILs activation profile, tissue-residency, and plasticity we identified distinct immune evasion strategies in primary and metastatic CRC.

Keywords: Adaptive immunity, cancer immunology, molecular immunology, omics technologies, RNAseq

OP-223

Identification of a Kupffer cell subset capable of reverting the T cell dysfunction induced by hepatocellular priming

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Kupffer cells (KCs) are highly abundant, intravascular, liver-resident macrophages long known for their scavenger and phagocytic functions. KCs are also able to present antigens to CD8+ T cells and promote either T cell tolerance or full effector differentiation, but the mechanisms underlying these discrepant outcomes are poorly understood. Here, we used a mouse model of hepatitis B virus (HBV) infection – where HBV-specific naïve CD8+ T cells recognizing hepatocellular antigens are driven into a state of immune dysfunction – to identify a subset of KCs (referred to as KC2) that improves the antiviral function of T cells upon IL-2 administration. Mechanistically, KC2 were found to be both enriched in the IL-2 sensing machinery and poised to cross-present hepatocellular antigens in response to this cytokine. Removing MHC-I from all KCs –including KC2 –as well as selectively depleting KC2 impaired the capacity of IL-2 to revert the T cell dysfunction induced by intrahepatic priming. Together, these findings indicate that, by sensing IL-2 and cross-presenting hepatocellular antigens, KC2 overcome the tolerogenic potential of the hepatic microenvironment and suggest new strategies for boosting T cell immunity in the liver.

Keywords: Adaptive immunity, antigen processing and presentation, cytokines and mediators, immunotherapy, viral infections

OP-224

The Role of Siglec-9 in ANCA-associated vasculitis

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Sialic acid-binding Ig-like lectin 9 (Siglec-9) is constitutively expressed on innate immune cells and is believed to modulate inflammatory responses. The role of Siglec-9 in ANCA-associated Vasculitis (AAV), a group of devastating autoimmune diseases affecting small/medium blood vessels, is yet to be examined. We aimed to characterise Siglec-9 in AAV by examining serum, cellular and tissue expression. Furthermore, we investigated the *in vitro* role of Siglec-9 on key leukocyte functions relevant to the pathogenesis of AAV. Leukocytes and serum were isolated from peripheral venous blood of AAV patients and expression of Siglec-9 was assessed by flow cytometry and ELISA. Immunohistochemistry was performed on kidney biopsies of AAV patients with AAGN and stained for the presence of Siglec-9 in their glomeruli. Healthy donor neutrophils were incubated with Siglec-9 mAb +/- PR3-ANCA to investigate its role in ROS production, Siglec-9 shedding, apoptosis and cell viability. Our data showed increased serum Siglec-9 expression in active AAV compared to remission and this correlates with disease activity. Neutrophils and intermediate ($CD14^+/CD16^+$) monocytes from PR3-ANCA patients have increased Siglec-9 expression compared to MPO-ANCA patients. Siglec-9 is also positively expressed in the biopsies of active AAGN. Siglec-9 in the presence of ANCA was shown to modulate ROS production, siglec-9 shedding and apoptosis. Our data supports a possible role for Siglec-9 in AAV, particularly in PR3-ANCA populations.

Keywords: Antibody, autoimmunity, inflammatory disease, innate immunity, neutrophils

OP-225

Dissecting the role of IFN-ψ in antiviral CD4+ T cell fate

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Although humoral and cellular immunity upon viral infection usually co-exist, sometimes one response emerges and is responsible for most of the antiviral activity. For example, vescicular stomatitis virus (VSV) infection induces early and potent neutralizing antibody (nAb) responses with robust Tfh development, whereas lymphocytic choriomeningitis virus (LCMV) infection induces strong cellular responses supported by Th1 differentiation, but weak nAb responses. We have recently analyzed the transcriptional cell state of VSV and LCMV priming niches, and identified the spatiotemporal regulation of type I IFN expression as a critical regulator of antiviral CD4+ T cell polarization. Although our study links the Tfh phenotype to the early type I interferon sensing, it does not fully explain the strong Th1 differentiation observed during LCMV infection. The cytokine milieu in different infections is a key player in controlling Tfh versus Th1 fate. In this study we aimed to elucidate the role of IFN-γ in CD4+ T cell differentiation upon LCMV infection. IFN-γ has been previously reported to influence T helper cell polarization, however its effect is controversial. We found that IFN-γ plays a key role in early CD4+ T cell differentiation upon LCMV infection, inducing Th1 cell polarization and suppressing Tfh cell development, resulting in a shift in the equilibrium towards Tfh and humoral responses. Future studies will determine the cellular source for this Th1-polarizing cytokine and the mechanism by which IFN-γ exerts its function, possibly unveiling the mechanisms underlying the reduced humoral response in the context of viral infections like LCMV.

Keywords: Adaptive immunity, cytokines and mediators, follicular helper T cells, viral infections

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WORKSHOPS

OP-226

Store-operated calcium entry controls immune cell function and activation in inflammatory bowel disease

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Store-operated calcium entry (SOCE) is the predominant calcium influx pathway in T cells, regulating many of their functional properties. However, it is currently unknown whether the pharmacologic inhibition of SOCE is a suitable drug target in inflammatory bowel disease (IBD), where T and epithelial cells play an important role. Therefore, we aimed to investigate the effects of SOCE inhibitors (SOCEi) on primary lymphocytes and epithelial cells isolated from IBD patients as well as in experimental models of colitis. Peripheral blood mononuclear cells (PBMCs), lamina propria lymphocytes (LPMCs) and epithelial cells were isolated from IBD patients undergoing colon resection. LPMCs were *ex-vivo* stimulated in the presence of SOCEi and subsequently stained with a panel of 37 immunological markers for mass cytometry acquisition. Ca^{2+} influx measurements were performed in order to assess the metabolic status of immune and epithelial cells after SOCE blockade. Finally, murine models of transfer colitis, in which SOCE components STIM1, STIM2 or ORAI1 were conditionally deleted in T cells, were employed in order to investigate the effects of SOCE blockade on the induction of colitis. The inhibition of SOCE attenuated the production of pathogenic cytokines including IL-2, IL-4, IL-6, IL-17, TNF-α and IFNγ by colonic T cells and ILCs, reduced the activation of B cells but also myeloid cells, without affecting the viability of primary human epithelial cells. Using experimental colitis models, we observed that SOCE is required for the induction of intestinal inflammation, suggesting that inhibition of SOCE might serve as a new drug target in IBD.

Keywords: Big data, drugs for immune modulation, inflammatory bowel disease

OP-227

IL-1R8 acts as an immune checkpoint in CD8+ T cells

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IL-1R8 is a member of the Interleukin-1 receptor (ILR) family, acting as a negative regulator of ILR and Toll-like receptor pathways. IL-1R8 deficiency was shown to enhance IL-18-promoted NK cell effector functions restraining tumor metastasis and viral dissemination. IL-1R8 is thus considered a new immune checkpoint. Since NK and CD8+ T share most of the cytokine-related mechanisms responsible for their anti-tumor activity, we characterized IL-1R8 in T cells. Expression profile indicated that IL-1R8 was upregulated during the T cell maturation and its expression was associated with the acquisition of effector markers in physiological and pathological contexts. We demonstrated that IL-1R8 deficiency promoted CD8+ T cell-mediated protection against immunogenic tumors and IL-1R8-deficient mice further benefited from immune checkpoint blockade. Moreover, we observed improved effector functions in IL-1R8-deficient human CD8+ T cells. Surprisingly, we found that IL-1R8 regulated T cells through the integration of two independent cell-autonomous mechanisms. IL-1R8 deficiency promoted Type-1 responses and enhanced IL-18-mediated pathway by inducing T-bet. Furthermore, IL-1R8 regulated the IL-2/EOMES/IL-2R signaling axis affecting T cell maturation, proliferation and cytokines production. Here we show that IL-1R8 acts as a checkpoint for mouse and human lymphocytes and it is regulated during the transition from naïve to mature T cells. Moreover, we describe an unexpected circuit linking the immune checkpoint IL-1R8 and signals from both IL-2 and Type 1 stimuli, with a profound impact on T cell anti-tumor potential. Therefore, our data suggest that IL-1R8 genetic targeting represents a tool for improving the activity of lymphoid cells in cancer immunotherapy.

Keywords: Adaptive immunity, cancer immunology, immunotherapy, *in vivo* tumor models

OP-228

Identification of SARS-CoV-2-cross-reactive resident memory CD8+ T cells in oropharyngeal lymphoid tissue

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A pre-existing cross-reactive immunity directed against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can commonly be detected in unexposed individuals. While previous studies have consistently identified cross-reactive $CD4^+$ T cells in the peripheral blood, a $CD8^+$ T cell counterpart is less frequently found in the circulation. However, as memory $CD8^+$ T cells commonly reside within tissues, it remains possible that cross-reactive $CD8^+$ T cells to SARS-CoV-2 are preferentially located at sites of initial virus entry, such as the oropharynx. Here, we used a flow cytometry-based assay for the highly sensitive detection of SARS-CoV-2-cross-reactive T cells in matched tonsil tissue and blood samples collected prior to the current pandemic. We comprehensively investigated phenotype and function of SARS-CoV-2-cross-reactive T cell immunity in comparison to virus-specific T cells induced by other natural viral infections (Epstein-Barr Virus (EBV), cytomegalovirus, (CMV), human coronavirus (HCoV)-OC43). We found a discordant pattern between tissue and blood, where cross-reactive memory $CD8^+$ T cells to various SARS-CoV-2 antigens were commonly detected in tonsils, but not peripheral blood, in both SARS-CoV-2-unexposed young children and adults. Cross-reactive $CD8^+$ T cells specific to SARS-CoV-2 in tonsils displayed a tissue-resident memory and follicular phenotype – similar to EBV-specific $CD8^+$ T cells – and demonstrated specific functional features, but of somewhat lower polyfunctionality compared to memory $CD8^+$ T cells of other virus specificities. Our findings indicate that previous studies on peripheral blood have missed cross-reactive $CD8^+$ T cells resident in tissues, forming a potential sentinel response against SARS-CoV-2 in oropharyngeal lymphoid tissue.

Keywords: Adaptive immunity, lymphoid organs, memory, viral infections

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WORKSHOPS

OP-229

Single cell RNA sequencing of gluten-specific CD4+ T cells reveals a novel gene expression pattern with NK-like cytotoxicity

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Coeliac disease (CD) is a chronic immune mediated illness with autoimmune-like features triggered by dietary gluten. A gluten-specific CD4+ T cell response is a hallmark of CD and the strong HLA class II genetic association highlights a key pathogenic role of these cells. Despite the strong genetic associations and gluten-specificity, the mechanisms underpinning how these lymphocytes escape tolerance checkpoints and acquire a "rogue" phenotype that contribute to a destructive inflammatory response are poorly understood. Single cell multi-omics analysis was employed to better characterise the phenotypic, transcriptomic, and mutational profile of these rare circulating gluten-specific cells. Gluten-specific tetramer positive cells were identified and parallel sequencing of transcriptome and genome at the single cell level was carried out in five gluten challenged patients. T cell receptor repertoire analysis revealed clonally expanded gluten specific CD4+ T cell subsets, with no lymphoma associated mutations. A distinctive gene signature profile was found compared to the tetramer negative controls, which was indicative of cell-mediated cytotoxicity (PFN, LIME1, KLRB1, IL-32), gut homing (CCR9), stimulator of interferon genes (STING) and exhaustion (CTLA4, PD1). Unsupervised analysis of transcriptomic data revealed 3 distinct clusters showing gene signature of effector memory, activated and a novel cytotoxic NK-like response (NKG7, KLRK1, KLRC2). While this activating NK phenotype is well characterised in disease specific intraepithelial lymphocytes, this study reports for the first time this signature in circulating CD4+ T cells that target gluten. These data implicate a novel mechanism through which gluten specific CD4+ T cells may contribute to disease pathogenesis.

Keywords: Inflammatory disease, molecular immunology, adaptive immunity, autoimmunity, omics technologies, RNAseq

OP-230

Group 1 innate lymphoid cells regulate T cell-mediated liver immunopathology by controlling local interleukin-2 availability

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Besides being important innate effectors, group 1 innate lymphoid cells (ILCs) – which comprise both natural killer (NK cells) and ILC1s – can positively and negatively influence adaptive immune responses. The latter function is generally ascribed to the ability of NK cells to recognize and kill activated T cells. Here, we employed multiphoton intravital microscopy in mouse models of hepatitis B to study the intrahepatic behavior of group 1 ILCs and their cross-talk with hepatitis B virus (HBV)-specific CD8+ T cells. We found that hepatocellular antigen recognition by effector CD8+ T cells triggered a 30-fold and 15-fold increase in the number of hepatic NK cells and ILC1s, respectively. Group 1 ILCs colocalized and engaged in prolonged interactions with effector CD8+ T cells undergoing hepatocellular antigen recognition; however, they did not induce T cell apoptosis. Rather, group 1 ILCs constrained CD8+ T cell proliferation by controlling local IL-2 availability. Accordingly, group 1 ILC depletion – or genetic removal of their IL-2 receptor alpha chain – considerably increased the number of intrahepatic HBV-specific effector CD8+ T cells and the attendant immunopathology. Together, these results reveal a novel role for group 1 ILCs in controlling T cell-mediated liver immunopathology by limiting local IL-2 concentration and have important implications for the treatment of chronic hepatitis B virus infection.

Keywords: Adaptive immunity, inflammatory molecules, innate lymphoid cells, viral infections

OP-231

Aberrant glycosylation of anti-SARS-CoV-2 IgG is a pro-thrombotic stimulus for platelets

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A subset of patients with COVID-19 become critically ill, suffering from severe respiratory problems and also increased rates of thrombosis. The causes of thrombosis in severely ill COVID-19 patients are still emerging, but the coincidence of critical illness with the timing of the onset of adaptive immunity could implicate an excessive immune response. We hypothesised that platelets might be susceptible to activation by anti-SARS-CoV-2 antibodies and contribute to thrombosis. We found that immune complexes containing recombinant SARS-CoV-2 spike protein and anti-spike IgG enhanced platelet-mediated thrombosis on von Willebrand Factor *in vitro*, but only when the glycosylation state of the Fc domain was modified to correspond with the aberrant glycosylation previously identified in patients with severe COVID-19. Furthermore, we found that activation was dependent on FcγRIIA and we provide *in vitro* evidence that this pathogenic platelet activation can be counteracted by therapeutic small molecules R406 (fostamatinib) and ibrutinib that inhibit tyrosine kinases syk and btk respectively or by the P2Y12 antagonist cangrelor.

Keywords: Antibody, cardiovascular diseases, infectious disease, inflammatory disease

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WORKSHOPS

OP-232

Liver resident CD4 T cells in malaria infection

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Malaria is caused by different *Plasmodium* species that can infect a variety of animals including humans and rodents. The life cycle of these parasites is complex, including a liver stage followed by a blood-stage in their vertebrate hosts. While the host's immune response is incompletely understood, CD4 T-cells are known to play an important role in immunity to *Plasmodium* infection during both stages. CD8 Tissue resident memory T-cells (TRM) in the liver have been shown to be highly protective in malaria. However, nothing is known about CD4 TRM cells in this context. This project aims to examine the CD4 T-cell response to a novel MHCII-restricted epitope in *Plasmodium* in C57BL/6 mice, and to characterise the protective capacity of these T-cells. To do this, we used of a recently generated *Plasmodium* specific TCR transgenic mouse line (PbT-II). PbT-II cells were injected into mice that were then primed with either a subunit vaccine (αClec9A-Hsp90) or radiation attenuated *Plasmodium* sporozoites (RAS). Flow cytometric analyses of the liver lymphocytes 4 weeks later revealed the existence of a memory PbT-II cell population that expressed surface markers associated with residency (e.g. CD69, CXCR6). Parabiosis studies confirmed the liver residency of this PbT-II cell population. Furthermore, transcriptional analysis showed that CD4 T-cells expressed a core gene signature similar to that of CD8 resident memory T-cells. Our results highlight that CD4 TRM cells form in the liver during malaria infection and share gene expression profiles with CD8 TRM cells.

Keywords: Adaptive immunity, infectious disease, memory, parasite infections

OP-233

Tumor-infiltrating plasmacytoid dendritic cells are associated with survival in human colon cancer

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Plasmacytoid dendritic cells (pDCs) may profoundly influence tumor progression, adopting either an anti-tumorigenic or pro-tumorigenic phenotype. To gain novel insights into the role of pDCs in colon cancer, we investigated the frequency and clinical relevance of BDCA-2+ pDCs in primary tumor tissues using classical immunohistochemistry. Furthermore, by multiplex immunofluorescence stainings, we evaluated the localization and phenotype of pDCs in stroma and tertiary lymphoid structures (TLS). An increased density of colon-cancer infiltrating pDCs was associated with lower UICC stages. Moreover, a higher pDC frequency was significantly correlated with increased progression-free and overall survival, while a lower density was significantly and independently linked to worse prognosis. Additionally, a proportion of pDCs showed nuclear expression of interferon regulatory factor 7 (IRF7), which is a key transcription factor for an activated phenotype. In various tumor stroma regions, IRF7+ pDCs were located close to granzyme B-expressing CD8+ T cells. Moreover, pDCs were identified as a novel component of the T cell zone of colon cancer-associated TLS, which are major regulators of adaptive anti-tumor immunity. A proportion of TLS-associated pDCs displayed nuclear IRF7 expression and was located nearby CD4+ T cells. These results indicate that higher densities of colon cancer-infiltrating pDCs are associated with prolonged survival and may represent a novel prognostic factor. The co-localization of activated pDCs and T cells in tumor stroma and within TLS may contribute to the correlation between higher pDC densities and better prognosis. Our results may provide a rational for designing novel immunotherapeutic strategies based on targeting colon cancer-infiltrating pDCs.

Keywords: Biomarkers, cancer immunology, dendritic cells

OP-234

Type I interferon receptor-independent interferon-alpha induction upon infection with a variety of negative-strand RNA viruses

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Type I interferons (IFNs) are a first line of defense against viral infections. Upon infection, a first small wave of early type I IFN, mainly IFN-β and particularly IFN-α4, are induced and bind to the type I IFN receptor (IFNAR) to amplify the IFN response. It was shown for several viruses that robust type I IFN responses require this positive feedback loop via the IFNAR. Recently, we showed that infection of IFNAR knockout mice with the orthomyxovirus Thogoto virus lacking the ML open reading frame (THOV(ML-)) results in the expression of unexpected high amounts of type I IFN. To investigate if IFNAR-independent IFN responses are unique for THOV(ML-), we performed infection experiments with several negative-strand RNA viruses using different routes and dosages for infection. A variety of these viruses induced type I IFN responses IFNAR-independently when using the intraperitoneal (i.p.) route for infection. *In vitro* studies demonstrated that myeloid dendritic cells (mDC) are capable of producing IFNAR-independent IFN-α responses that are dependent on the expression of the adaptor protein mitochondrial antiviral-signaling protein (MAVS) whereas pDC where entirely depending on the IFNAR feedback loop *in vitro*. Thus, depending on dose and route of infection, the IFNAR feedback loop is not strictly necessary for robust type I IFN expression and an IFNAR-independent type I IFN production might be the rule rather than the exception for infections with numerous negative-strand RNA viruses.

Keywords: Cytokines and mediators, dendritic cells, infectious disease, innate host defence, innate immunity, viral infections

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WORKSHOPS

OP-235

Identification and mapping of distinct human lymph node stromal cell subsets by combining single cell RNA sequencing with spatial transcriptomics

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Lymph node stromal cells (LNSCs) constitute a crucial immunomodulatory role in human health and disease. Here, we report the profiling of 12,000 LNSCs isolated from a human lymph node (LN) and analysed by droplet-based singlecell RNA sequencing. Uniform manifold approximation and projection analysis (UMAP) revealed 10 major subtypes of human non-endothelial stromal cells (SCs), 4 subtypes of lymphatic endothelial cells (LECs) and 4 subtypes of blood endothelial cells (BECs). Interestingly, detailed SC analysis shed light on unprecedented heterogeneity in human LN fibroblasts. This study comprehensively defined the gene signatures of the 10 identified SC subtypes: NR4A1+ SC, CCL21+ medullary reticular cells, CCL19+ T- zone reticular cells, CD34+ CXCL14+SC, pericytes, Desmin+ medullary reticular cells, LAMP5+ and HLA-DR+ SCs being active in antigen processing and presentation, SEPT4+ smooth muscle cells and GLDN+ neuro-fibroblasts. To localize each distinct subtype within a human LN we transferred the SC-subset-specific gene signatures to a publicly available human spatial transcriptomic data set of a human LN. The results underpin the distinct location of the different identified LNSC subtypes. This study determined the human LNSC landscape and sets the stage for future investigations to learn more about the relationship between their LN niche and immunomodulatory function during health and disease.

Keywords: Lymphoid organs, microenvironment, RNAseq

OP-236

Multi-omics analysis of the age-related effects of SARS-CoV-2 infection in rhesus macaques

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Age is a key predictor of COVID-19 prognosis. In this study, we utilized the established rhesus macaque model to characterize the relationship between age and host immune responses to SARS-CoV-2 infection. Two cohorts of eight older (16-23 years) and younger (3-5 years) rhesus macaques were inoculated with SARS-CoV-2. Four animals per group were euthanized at 7- and 21-days post infection (DPI). Our time-resolved evaluation included viral RNA quantification, clinical observations, pulmonary x-rays, single-cell transcriptomics, multiparameter flow cytometry, multiplex immunohistochemistry, cytokine detection, and lipidomics. Significant differences in clinical scores, viral load, and pulmonary infiltrates were not observed between age cohorts. Moreover, at 3 DPI, gene expression signatures in bronchoalveolar lavage cells were similar across groups. These findings suggest that age does not substantially skew major outcomes of acute disease in this model. However, age-specific divergence of immune responses emerged 7-21 DPI. Older animals exhibited sustained local inflammatory innate responses. Meanwhile, we detected an earlier induction of local and circulating effector T-cell responses in the younger animals. Lung-tissue staining revealed signs of an amplified influx of T cells in the younger versus older cohort, which was efficiently controlled by 21 DPI. Lipid mediator and cytokine levels highlighted increased anti-inflammatory and repair-associated signals in the younger animals, contrasted by persistent pro-inflammatory responses in the older animals 10-21 DPI. In summary, despite uniformity in clinical disease outcomes, multi-omics profiling in SARS-CoV-2-infected macaques suggests that age may impair the induction of anti-viral cellular immune responses and efficient recouping of homeostatic control following acute infection.

Keywords: Ageing, innate immunity, adaptive immunity, animal models, omics technologies, viral infections

OP-237

Glycolysis is a key driver of tissue degradation and remodelling in tuberculosis

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Tuberculosis (TB) is a global infectious threat complicated by rising drug resistance. Clinical TB sequelae result from tissue inflammation, partly driven by matrix metalloproteinases (MMP). TB macrophages exhibit metabolic reprogramming via the Warburg effect. We explored the relationship between innate inflammation and metabolism in TB. Primary normal human bronchial epithelial (NHBE) cells or monocyte-derived macrophages (MDM) were incubated with the hexokinase (HK) inhibitor, 2-deoxyglucose (2DG), or transfected with HK2 siRNA. Cells were infected with *M.tb*, or stimulated with conditioned media from *M.tb*-infected monocytes (CoMTB). Measurements were obtained by ELISA, luminex, zymography, qPCR and DQ substrate assays. HK2 immunohistochemistry was performed in murine and human TB samples. Glycolysis inhibition with 2DG reduced gene expression and secretion of the key collagenase, MMP-1, and gelatinase, MMP-9, in a dose-dependent manner. In CoMTB-stimulated NHBE, there was a 7-fold drop in MMP-1 (from 5852±1090 to 774±17.36 pg/ml; $p<0.0001$) and MMP-9 ($p<0.0001$) secretion, while in *M.tb*-infected MDM, there was a 5-fold drop in MMP-1 ($p<0.0001$) secretion. siRNA HK2 knockdown confirmed reduced MMP-9 gene expression ($p<0.0001$). 2DG decreased the amount of active MMP-9, and functionally downregulated collagen ($p<0.0001$) and gelatin ($p<0.0001$) breakdown. 2DG decreased IL-1β ($p=0.0001$) and IL-10 ($p<0.0001$) and increased TNF-α ($p=0.0012$) secretion. *M.tb*-induced transcription factor HIF-1α expression was attenuated by 2DG. HK2 was highly expressed in areas of central granulomatous necrosis in murine and human TB. We show, for the first time, that glycolysis is a critical immunometabolic modulator of MMP-1 and -9 secretion in TB, and plays a key role in regulating tissue destruction.

Keywords: Innate immunity, bacterial infections, infectious disease, innate host defence, metabolic control of immune responses, tissue damage and repair

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WORKSHOPS

OP-238

Cytomegalovirus subverts macrophage identity

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Cytomegaloviruses (CMV) have co-evolved with their mammalian host for millions of years, leading to remarkable host specificity and high infection prevalence. Macrophages (MΦs), which populate barrier tissues already in the embryo, represent the predominant immune cells at potential CMV entry sites. Here we show that upon CMV infection MΦs undergo a morphological, immunophenotypic and metabolic transformation process with features of stemness, altered migration, enhanced invasiveness and provision of the cell cycle machinery to viral proliferation. This complex process depends on Wnt signalling and the transcription factor ZEB1. In pulmonary infection, mouse CMV primarily targets and reprograms alveolar MΦs, which alters lung physiology and facilitates both primary CMV and secondary bacterial infection by attenuating the inflammatory response. Thus, CMV profoundly perturbs MΦ identity beyond established limits of plasticity and rewires specific differentiation processes, allowing for viral spread and impairing innate tissue immunity.

Keywords: Infectious disease, innate immunity, macrophage, myeloid cells, viral infections

OP-239

Colchicine limited secondary lung injury in an experimental rat sepsis model

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To evaluate the possible protective effects of colchicine on sepsis-related lung injury with an experimental lipopolysaccharide (LPS) induced rat sepsis model. 28 Wistar male rats were randomized and divided into four groups; `Sham` (n=6), `Colchicine` (n=6), `Sepsis` (n=8), `Sepsis + colchicine` (n= 8). In zero time point of the experiment, intraperitoneal % 0.9 NaCl was administered to the `sham` and `colchicine` groups, and intraperitoneal LPS was administered to the `sepsis` and `sepsis + colchicine` groups at a dose of 1-1.2 mg/kg to induce sepsis. At the 90th minute of the experiment, the `sham` and `sepsis` groups were given %0.9 NaCl; and the `colchicine` and `sepsis + colchicine` groups were given 0.6-0.7 mg/kg colchicine by oral gavage. An observational sepsis scoring tool was used to assess the effectiveness of the sepsis model. Rats were sacrificed at the 24th hour of the experiment. Lung tissue was examined histopathologically according to the American Thoracic Society animal acute lung injury assessment report. We successfully induced sepsis in LPS injected rats and sepsis signs were most evident between 4th and 6th hours. As assessed by the total lung histopathological score, 1.lung injury was not different between the `sham` and `colchicine` groups; 2.lung injury in the `sepsis` group was significantly higher compared to all other groups ($p<0.001$); 3.lung injury in the `sepsis + colchicine` group was lower than the `sepsis` group ($p<0.001$). Colchicine limited lung injury secondary to sepsis in an experimental rat sepsis model.

Keywords: Adjuvants and vaccines, animal models, drugs for immune modulation, inflammatory disease, tissue damage and repair, infectious disease

OP-240

Dysbiosis in gut mucosa in relation to CD4 counts at cART onset and CD4 recovery

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In HIV-infected subjects, studies on gut dysbiosis have hardly explored the potential influence of the immune status when initiating antiretroviral-treatment (ART) and mostly rely on fecal samples. Our aim was to explore the microbiome composition in mucosa biopsies of HIV-subjects with different immunological profiles at the ART onset and afterwards. Thirty-five treated-patients were classified as follows: early-treated (ET), with >250 CD4 before ART, late-treated (LT), with <250 CD4 before ART. LT were finally subdivided into high recovery (LT-HR) or low recovery (LT-LR) depending on whether or not recovered >250 after two years ART. Three non-treated elite controllers (EC) and 10 non-HIV subjects were also included. Biopsy samples from terminal ileum and caecum mucosa were analyzed. Then, microbial 16S rRNA was sequenced obtaining different Operational Taxonomic Units (OTUs) whose diversity and abundance parameters were analysed. Microbiome alpha-diversity values were similar among groups and highly correlated between both gut mucosa locations. Beta diversity revealed clear grouping of non-HIV and EC microbiotas, differing from HIV-treated groups. A differential abundance analysis of OTUs among groups showed similar profiles of abundant bacteria in non-HIV and EC subjects, dominated by Ruminococcaceae and Lachnospiraceae members. ET and LT-HR shared almost all their most abundant OTUs. By contrast, LT-LR showed a totally different pattern with greater abundance of the Proteobacteria pathobiont Escherichia. Grade of dysbiosis was similar between those subjects early initiating ART and those recovering CD4, despite late initiating ART. Non-recoverers exhibited specific dysbiosis despite similar alpha diversity than comparison groups.

Keywords: Immunodeficiency, microbiome and environmental factors, viral infections

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WORKSHOPS

OP-241

Afucosylated IgG is naturally produced in response to intracellular pathogens including SARS-CoV-2 and *Plasmodium falciparum* but not by subunit vaccination

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Immunoglobulin G (IgG) plays a crucial role in clearing and protecting against infectious diseases by activation of complement and IgG-Fc receptors (FcγR) on myeloid- and NK cells. A conserved glycan at N297 of IgG CH2 is of major importance affecting the antibody effector functions, and is tuned by altered composition in humans. Without core fucose (afucosylated), IgG has up to 40-fold increased affinity to FcγRIII compared to fucosylated IgG. The vast majority of plasma IgG is fucosylated, but afucosylated IgG is producedin response to alloantigens during pregnancy and blood transfusions, but also against enveloped viruses. We hypothesized that as both types of antigens are expressed on the surface of human cells, this type of antigen-display is a necessity for the formation of afucosylated IgG. The resulting afucosylated IgG, with its enhanced FcγRIII-activation profile, can be beneficial in infectious diseases but detrimental in alloimmune diseases. Previously, we found that strong FcγRIII-provoked reactions caused by afucosylated IgG lead to immunopathology in COVID-19. Using affinity-purified antigen-specific IgG and liquid-chromatography mass spectrometry, we now extend our findings to include central antigens of naturally acquired blood-stage malaria immunity, showing exceptionally strong afucosylation. While causing immunopathology for SARS-CoV-2, those responses correlate with traits of protective malaria immunity. In contrast, immunization with a soluble subunit vaccine based on the same *P. falciparum* antigen resulted in fully fucosylated IgG, highlighting the importance of antigen-context regarding fucosylation levels of IgG. These results have implications for understanding natural and vaccine-induced antibody-mediated protective immunity to malaria and other infectious diseases.

Keywords: Adaptive immunity, antibody, biology of the immune system, infectious disease, mass spectrometry, parasite infections

OP-242

Severe COVID-19 is marked by a dysregulated myeloid cell compartment

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Coronavirus Disease 2019 (COVID-19) is a mild to moderate respiratory tract infection, however, a subset of patients progresses to severe disease and respiratory failure. The mechanism of protective immunity in mild forms and the pathogenesis of severe COVID-19, associated with increased neutrophil counts and dysregulated immune responses, remains unclear. In a dual-center, two-cohort study, we combined single-cell RNA-sequencing and single-cell proteomics of whole blood and peripheral blood mononuclear cells to determine changes in immune cell composition and activation in mild vs. severe COVID-19 (242 samples from 109 individuals) over time. HLA-DRhiCD11chi inflammatory monocytes with an interferon-stimulated gene signature were elevated in mild COVID-19. Severe COVID-19 was marked by occurrence of neutrophil precursors indicative of emergency myelopoiesis, dysfunctional mature neutrophils, and HLA-DRlo monocytes. Our study provides detailed insights into the systemic immune response to SARS-CoV-2 infection and reveals profound alterations in the myeloid cell compartment associated with severe COVID-19.

Keywords: Innate host defence, myeloid cells, myeloid derived suppressor cells, neutrophils, omics technologies, viral infections

OP-243

EGR2 and EGR3 prevent uncontrolled accumulation of B1 and CD21low age-associated B cells

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Egr2 and *Egr3* genes encode early growth response (EGR) transcription factors, act as immediate early genes following B cell receptor (BCR) signalling and are highly up-regulated in anergic mouse B cells. Anergy preserves microbial epitope responsiveness from a finite pool of pre-immune B cells, but is also reversible. This creates a risk of autoimmune disease, or even of pathological proliferation in chronic lymphocytic leukemia (CLL). 3.8% of CLL harbour somatic missense EGR2 mutations that predict very poor prognosis. We analysed B cells in mice lacking one or both alleles of *Egr2* and/or *Egr3* and show that *Egr2* and *Egr3* deletion causes the cell-intrinsic accumulation in spleen, blood and bone marrow of populations enriched for self-reactive BCRs: B1a and CD21low CD23low age-associated B cells. Global single-cell RNA profiling of these expanded populations *in vivo*, in chimeras, demonstrated their differential expression of genes involved in their survival and maintenance, many of which we show by chromatin immunoprecipitation sequencing (ChIP-Seq) are direct EGR2 transcriptional targets in human CLL. This includes several genes crucial to B1 cell survival, differentially expressed in human CLL and that can be targeted with small molecule inhibitors. This is the first report on the roles of Egr2/3 in B cells. We reveal the landscape of genes regulated by an Egr2/Egr3 checkpoint crucial to suppressing the accumulation of B1a and age-associated or atypical memory B cells.

Keywords: Animal models, autoimmunity, B lymphocytes, cancer immunology, immunodeficiency

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WORKSHOPS

OP-244

C5aR2 deficiency ameliorates inflammation in antibody transfer-experimental *epidermolysis bullosa acquisita* and reveals promoting of C5aR1 signaling outcomes

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Epidermolysis bullosa acquisita (EBA) is a rare blistering disease of the skin induced by autoantibodies directed against type VII collagen (COL7). Transfer of antibodies against murine COL7 (mCOL7) into mice mimics the effector phase of EBA leading to a subepidermal blistering phenotype. Activation of the complement system and especially the C5a/C5aR1 axis, as well as the subsequent activation of neutrophils are critical for EBA pathogenesis. The role of the second C5a receptor, C5aR2, in the pathogenesis of EBA is, however, still elusive. Therefore, we sought to elucidate the functional significance of C5aR2 in the effector phase of EBA by injecting C5aR2-deficient (*C5ar2-/-*) mice with anti-mCOL7 antibodies. Here, *C5ar2-/-* mice showed an attenuated disease phenotype, indicating a pathogenic contribution of C5aR2. To decipher the molecular mechanisms mediated by C5aR2, neutrophils from *C5ar2-/-* or wild-type control mice were activated with either immune complexes or C5a *in vitro*, and their biological response was subsequently analyzed. *C5ar2-/-* neutrophils showed significantly lower C5a-mediated activation and migration. Both functions are completely absent if *C5ar1-/-* neutrophils are activated, indicating a promoting interaction between C5aR2 and C5aR1 signaling outcomes. Collectively, we here demonstrate a pro-inflammatory contribution of C5aR2 to the pathogenesis of (auto-)antibody-induced tissue damage in experimental EBA.

Keywords: Autoimmunity, complement, innate immunity, molecular immunology, neutrophils, skin diseases

OP-245

Complementary but distinct protective roles of breast tumor-infiltrating IgG and IgA producing cells

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Antibody-secreting cells (ASC) emerge as critical actors of anti-tumor immunity in various cancers, including breast cancers, but their diversity and clinical impact remain poorly understood. Using fresh tumor samples and flow cytometry, we confirm that multiple B cell differentiation stages infiltrate invasive breast tumors and show that ASC account for about 8% of infiltrating B cells and consist of not only IgG-, but also IgA-, producing cells, the latter being the dominant ASC population in about 15% of patients. In situ tissue imaging and other approaches show that ASC localize mainly in the stroma, mostly consist of plasmablasts, and reveal a dominance of monomeric over dimeric. Strikingly, ASC from in situ carcinomas (early stage) differ from that of invasive carcinomas by an over-dominance of IgA-ASC and a higher proportion of IgA2. In addition, profiling of antibody specificity in serum and tumor indicates that IgG and IgA mostly target different antigens. Importantly, immunofluorescent staining of a triple negative breast cancer cohort and analysis of TCGA database shows that IgA-ASC are associated with better patient prognosis irrespective of IgG-ASC density. Comparison of IgA-ASC rich only tumors (G-Lo/A-Hi) with their G-Hi/A-Lo counterparts reveals increased expression of genes of luminal A tumors, mast cells and neutrophils in IgA-rich tumors, while genes of activated adaptive immune cells are overexpressed in the G-Hi/A-Lo group. These data reveal massive changes of the local antibody response during breast tumor progression and demonstrate that IgA- and IgG-ASC play complementary, yet distinct, positive roles on patient survival.

Keywords: Antibody, B lymphocytes, biomarkers, cancer immunology

OP-246

Characterisation of allergen-specific B cell tolerance in children with cow's milk-oral immunotherapy and natural outgrow of milk allergy

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Understanding the mechanisms of tolerance induction to food allergens is crucial for the development of medical treatments in food allergies. Several immune cells are involved during to food oral immunotherapy (Food-AIT), however,the role of allergen-specific-B-cells in the induction of allergen tolerance remains unclear. Therefore,we aim to demonstrate the role of allergen-specific-B-cells and compare the differences of gene expression in allergic children during Food-AIT and natural tolerance induction. PBMCs from cow's milk allergic children, who followed Food-AIT and who have developed natural tolerance were isolated. αS1-casein-specific-B-cells were purified using dual-color staining with fluorescently labeled αS1-casein-allergen in flow cytometry. The immortalization of αS1-casein-specific-B-cells was transduced with a retroviral vector containing GFP, BCL6, and Bcl-xL and expanded by culturing with CD40L and IL-21. Total and specific IgE, IgG and IgG subclass (IgG1 and IgG4) antibodies from culture supernatants were measured by ELISA. The single-cell/Ultra Low RNA next-generation-sequencing was performed for quantitative transcriptomics. After purification of αS1-casein-specific-B-cells and non-specific-B-cells,we measured the Ag-specific Ig profile to confirm their specificity. Specific IgE, IgG1, and IgG4 production from culture supernatants of αS1-casein-positive-B-cells were significantly elevated compared to αS1-casein-negative-B-cells,while total IgE, IgG1, and IgG4 levels were comparable. The in-depth analysis of gene expression showed significantly different αS1-casein-specific-B-cells of allergic children before and after OIT compared to natural tolerance. After Food-AIT proinflammatory-cytokine-machinery was shut down and tolerance related genes were upregulated. Our data suggest that allergen-specific-B-cells in food allergic children compared to natural tolerance display clearly different gene signatures. The in-depth analysis of significant genes are suggesting the cells migrate to gut with the presence of immunoreguatory molecules and regulate the allergic-related genes.

Keywords: Allergen-induced immune responses, B lymphocytes, RNAseq

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WORKSHOPS

OP-247

Prolonged neutrophil survival at necrotic sites is a fundamental feature for tissue recovery and resolution of hepatic inflammation

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Neutrophils were classically described as powerful effectors of acute inflammation, and their main purpose was assumed to be restricted to pathogen killing through production of oxidants. As consequence, neutrophils also may lead to significant collateral damage to the healthy tissues, and after performing these tasks, these leukocytes are supposed to die within tissues. However, there is a growing body of evidence showing that neutrophils also play a pivotal role in the resolution phases of inflammation, because they canmodulate tissue environment due to secretion of different kind of cytokines.Drug-induced liver injury (DILI) is aworldwide concern being one of the most prevalent causes of liver transplantation, and iswell established that there is an intense neutrophil recruitment into necrotic liver during DILI. However, information if such abundant granulocyte infiltration is also linked to the tissue repairing phase of hepatic injury is still largely elusive. Here, we investigated the dynamics of neutrophil traffickingwithin blood, bone marrow, and liver during hepatic inflammation, and how changes in their gene expression profile could drive the resolution events during acetaminophen (APAP)-induced liver injury. We found that neutrophils remained viable during longer periods following liver damage, because they avidly patrolled necrotic areas and up-regulated pro-resolutive genes, including Tgfb, Il1r2, and Fpr2. Adoptive transference of "resolutive neutrophils" harvested from livers at 72 h after injury to mice at the initial phases of injury (6 h after APAP) significantly rescued organ injury.

Keywords: Animal models, biology of the immune system, cell death, immune networks, neutrophils

OP-248

A two-photon imaging platform for investigating cytotoxic T lymphocyte dynamics in virus-infected explanted lungs

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Cytotoxic T lymphocytes (CTLs) are critical for mediating virus clearance in an antigen- and cell-contact dependent manner, and therefore an attractive target for therapeutic manipulation. During killing, CTLs establish stable and dynamic interactions with virus-infected target cells forming a cytolytic immune synapse that might be stabilized by integrins. In this study, we established an *in vitro* platform to investigate the killing efficiency of CTLs and their interactions with virus-infected cells using two-photon microscopy and explanted lung tissue. We generated mouse cytomegalovirus (MCMV)-infected lung slices via microinjection of MCMV mutants, differing in the expression of immunoevasins and presentation of the artificial SIINFEKL-peptide. These infected lung slices closely represent a relevant *in vivo* environment and were used as scaffolds for testing the migratory behavior and killing capacity of *in vitro* activated OT-I CTLs. Killing of MCMV-infected targets was evident only in the context of cognate antigen recognition and depended on the presence of theintegrin-binding adaptor protein Talin1. Talin1-deficiency in CTLs, or deficiency of Intercellular Adhesion Molecule (ICAM)-1 in infected targets, reduced the frequency and durationof CTL-target interactions needed during the killing process, and changed the CTL migratory behavior. In summary, our proof-of-concept study reveals that two-photon imaging of virus-specific T cells interacting with virus-infected cells in explanted tissue represent a valid tool for the study of CTL-targets dynamics in live tissue. Furthermore, this platform allows the study of different molecules involved in the migration of CTLs and their killing capacity, by generating gene knockouts in CTLs using CRISPR/Cas9 genome editing.

Keywords: Cellular interactions, viral infections, adaptive immunity

OP-249

COVID-19 in patients with primary and secondary immunodeficiencies: a single-center experience

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The consequences of SARS-CoV-2 infection in individuals diagnosed with primary immunodeficiency (PID), or secondary immunodeficiency (SID), have not been thoroughly elucidated. We aimed to present the manifestations, clinical course, severity, and outcomes of COVID-19 in our primary and secondary immunodeficiency patients. Among 168 immunodeficiency patients who were followed up in our clinic, those who had COVID-19 were screened. Demographic featurers, treatment modalities and outcomes were collected from our database. The medical records of the cases treated in different centers were retrieved by means of a questionnaire and based on the reports obtained from the physicians. Total 14 patients were diagnosed COVID-19 documented with SARS-CoV2 -PCR(n:13) or radiological findings(n:1). The mean age was 44,3 years(male/female:10/4). Most patients (n:8) had pre-existing common variable immunodeficiency(CVID), 3 CTLA-4 mutations, 1 hereditary hemorrhagic telangiectasia with CVID pattern, 1 XLA(BTK), and 1 secondary immunodeficiency patient due to Hodgkin lymphoma. 13 patients were on immunoglobulin replacement therapy. 8 patients had mild disease and were treated as outpatients. 6 patients required hospitalization and 4 of them recovered without any complication. 1 patient(P5) needed noninvasive ventilation in the intensive care unit and 2 patients(P2, P3) who required invasive mechanic ventilation were expired. Inpatient mortality in individuals with immunodeficiency exceeded that of the general population. Pre-existing chronic lung disease was prevalent in the hospitalized PID cohort. This and future studies have the potential to suggest the most appropriate immune modulation and treatment options for COVID-19 in patients with immunodeficiency and the general population.

Keywords: Adaptive immunity, antibody, B lymphocytes, immunodeficiency, infectious disease, innate immunity

OP-250

Development and employment of a human cell-based *in vitro Mycobacterium avium* infection model to identify host-directed therapeutics as a novel treatment strategy

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Mycobacterium avium complex accounts for more than 80% of all pulmonary infectious diseases caused by non-tuberculous mycobacteria (NTM), which have an alarming increase in prevalence of currently 0.3-9.8 per 100.000 individuals. Poor clinical outcomes, caused by increasing microbial drug-resistance and low treatment adherence due to drug-toxicities emphasize the need for more effective alternative treatments. Mycobacteria are highly efficient in evading host immunity by modulating host cells. Redressing these processes represents an attractive alternative treatment strategy. Such host-directed therapies (HDT) are unlikely to evoke drug-resistance and are likely also effective against drug-resistant and metabolically-inactive mycobacterial strains. To investigate host-pathogen interactions and to identify potential HDT-compounds in humans, we developed an *M. avium*-human macrophage infection model. Optimization of infection conditions led to infection rates of at least 15% as determined by flow cytometry using a fluorescently-labelled *M. avium* strain. Furthermore, quantitative readouts were developed and validated for the determination of intracellular bacterial load to monitor macrophages' ability controlling the infection and to identify new therapeutics. By testing a library of compounds, ten molecules were identified that reduced the intracellular bacterial load by at least 30% through host-directed but not direct anti-bacterial mechanisms. Using our infection model, the efficacy, safety and potential mechanism of action of promising HDT-compounds are currently being assessed, paving the way for HDT strategies to combat *M. avium* infections.

Keywords: Bacterial infections, drugs for immune modulation, infectious disease, innate host defence, macrophage

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WORKSHOPS

OP-251

Changes in circulating monocytes predict severity during acute COVID-19 and include chromatin modifications still detectable six months after recovery

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We analyzed 131 untreated COVID-19 patients at ER arrival (acute-COVID-19), 52 recovered (6 months, post-COVID-19) and 45 healthy controls (HC). Acute-COVID-19 showed increased classical monocytes with down-regulated HLA-DR and *in vitro* augmented production of M1, pro-inflammatory GM-CSF, IL6, IL8, IL18, CCL2 and reduction of anti-inflammatory IL1ra and IL10. Monocytes HLA-DR expression inversely correlated with augmented circulating inflammatory cytokines. In post-COVID-19, monocytes were similar to HC with recovered HLA-DR and CD86, however, serum G-CSF, IL1b and IL6 were still increased. Acute-COVID-19 showed lymphopenia (72.5%), reduced CD4 and CD8 T-cells proliferation and reduced IFNγ (20%) and IL2 (12.5%) S1-specific cellular response. In post-COVID-19, T-cell proliferation was fully restored and 92,7% and 95.1% showed IFNγ and IL2 S1-specific cellular response respectively. Both T-cell proliferation and SARS-CoV-2 specific response correlated with monocytes HLA-DR and CD86 in acute- and post-COVID-19. Upon ER arrival, low CD33, CD16, CCR2, CCR5, CD86 and HLA-DR in monocytes identified severe patients, with high IL6 and PCR, low CD8+ T-cell proliferation and SARS-CoV-2 specific response, who had higher risk to enter ICU (2.78) or die (3.05). Only IRF1 transcription was significantly diminished in low-expressor, severe patients monocytes. Transcriptomics in acute-COVID-19 monocytes revealed up-regulation of M2, anti-inflammatory, tissue-repairing IL10, BCL6, MAFF, MAFG and AREG, which were still augmented in post-COVID-19. ATAC-seq showed superior chromatin accessibility in acute-COVID-19, intermediate in post-COVID-19 and lowest in HC monocytes. Thus, M1 and M2 features are observed in acute-COVID-19 monocytes at phenotype, functional, transcriptomic and epigenetic levels, some remaining six months after recovery.

Keywords: Innate immunity, omics technologies, viral infections

OP-252

Hyaluronic acid in synovial fluid in inflamed joints inhibits neutrophil activation

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Rheumatoid arthritis (RA) and spondyloarthritis (SpA) patients suffer from tissue damage in inflamed joints, generally thought to be caused by among others activated neutrophils in the synovial fluid (SF). However, surprisingly, we found that in these patients synovial neutrophils are inactive, despite the presence of many neutrophil-activating stimuli in SF. This finding suggests that an inhibitor of neutrophil activation is present in SF. Neutrophils were isolated from healthy donor blood and from SF from RA and SpA patients (n=25) and stimulated with activating agents in the absence or presence of increasing concentrations of SF. Degranulation and ROS production were determined to analyze the neutrophil activation status. Neutrophils derived from SF did not show an activated phenotype. However, activation of SF-derived neutrophils *in vitro* in the absence of SF led to full degranulation and ROS production. Activation of blood-derived neutrophils was dose-dependently inhibited by the presence of SF. This effect is independent of the diagnosis, gender, age, and medication use of the patients from which the SF originates. Furthermore, we showed that when SF was treated with the enzyme hyaluronidase the inhibitory effect of SF on neutrophil activation was reduced, indicating that hyaluronic acid plays a role in the inhibition of neutrophil activation. Together, our data suggest that SF inhibits neutrophil activation and that hyaluronic acid in SF is partly responsible for this. This information may be important for preventing tissue damage in inflamed joints.

Keywords: Autoimmunity, inflammatory joint diseases, neutrophils

OP-253

Intestinal barrier dysfunction plays an integral role in arthritis pathology and can be targeted to ameliorate disease

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Evidence suggests an important role for gut-microbiota dysbiosis in the development of rheumatoid arthritis (RA). The link between changes in gut bacteria and the development of joint inflammation is missing. Here, we address whether there are changes to the gut environment and how they contribute to arthritis pathogenesis. We analyzed changes in markers of gut permeability, damage, and inflammation in peripheral blood and serum of RA patients. Serum, intestines, and lymphoid organs isolated from K/BxN mice with spontaneous arthritis or from wild-type, genetically modified interleukin (IL)-10R−/− or claudin-8−/− mice with induced arthritis were analyzed by immunofluorescence/histology, ELISA, and flow cytometry. RA patients display increased levels of serum markers of gut permeability and damage and cellular gut-homing markers, both parameters positively correlating with disease severity. Arthritic mice display increased gut permeability from early stages of disease, as well as bacterial translocation, inflammatory gut damage, increases in interferon γ (IFNγ)+ and decreases in IL-10+ intestinal-infiltrating leukocyte frequency, and reduced intestinal epithelial IL-10R expression. Mechanistically, both arthritogenic bacteria and leukocytes are required to disrupt gut-barrier integrity. We show that exposing intestinal organoids to IFNγ reduces IL-10R expression by epithelial cells and that mice lacking epithelial-IL-10R display increased intestinal permeability and exacerbated arthritis. Claudin-8−/− mice with constitutively increased gut permeability also develop worse joint disease. Treatment of mice with AT-1001, a molecule that prevents development of gut permeability, ameliorates arthritis. We suggest that breakdown of gut-barrier integrity contributes to arthritis development and propose restoration of gut-barrier homeostasis as a new therapeutic approach for RA.

Keywords: Autoimmunity, biomarkers, microbiome and environmental factors, rheumatoid arthritis

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WORKSHOPS

OP-254

Infection and transmission of SARS-CoV-2 depends on heparan sulfate proteoglycans

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The current pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and outbreaks of new variants highlight the need for preventive treatments. Here we identified heparan sulfate proteoglycans as attachment receptors for SARS CoV-2. Notably, neutralizing antibodies against SARS-CoV-2 isolated from COVID-19 patients interfered with SARS-CoV-2 binding to heparan sulfate proteoglycans, which might be an additional mechanism of antibodies to neutralize infection. SARS-CoV-2 binding to and infection of epithelial cells was blocked by low molecular weight heparins (LMWH). Although dendritic cells (DCs) and mucosal Langerhans cells (LCs) were not infected by SARS-CoV-2, both DC subsets efficiently captured SARS-CoV-2 via heparan sulfate proteoglycans, and transmitted the virus to ACE2-positive cells. Moreover, human primary nasal cells were infected by SARS-CoV-2 and infection was blocked by pre-treatment with LMWH. These data strongly suggest that heparan sulfate proteoglycans are important attachment receptors facilitating infection and transmission, and support the use of LMWH as prophylaxis against SARS-CoV-2 infection.

Keywords: Dendritic cells, infectious disease, viral infections

OP-255

Macrophage innate training induced by IL-4 activation enhances OXPHOS driven anti-mycobacterial responses

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Macrophages are key innate immune cells for determining the outcome of *Mycobacterium tuberculosis* infection. Polarization with IFNγ and LPS into the "classically activated" M1 macrophage enhances pro-inflammatory and microbicidal responses, important for eradicating the bacterium. By contrast, in response to for instance parasitic infection, "alternatively activated" M2 macrophages, polarized with IL-4, oppose bactericidal mechanisms and allow mycobacterial growth. These activation states are accompanied by distinct metabolic profiles, where M1 macrophages favor near exclusive use of glycolysis, whereas M2 macrophages up-regulate oxidative phosphorylation (OXPHOS). Here we demonstrate that activation with IL-4 counterintuitively induces protective innate memory against mycobacterial challenge. This was associated with enhanced pro-inflammatory cytokine responses and killing capacity. Moreover, despite this switch towards a phenotype that is more akin to classical activation, IL-4 trained macrophages do not demonstrate M1-typical metabolism, instead retaining heightened use of OXPHOS. Moreover, inhibition of OXPHOS with oligomycin, 2-deoxy glucose or BPTES all impeded heightened pro- inflammatory cytokine responses from IL-4 trained macrophages. Lastly, this work identifies that IL-10 negatively regulates protective IL-4 training, impeding pro-inflammatory andbactericidal mechanisms. In summary, this work provides new insight into alternative macrophage activation states in the context of mycobacterial infection.

Keywords: Infectious disease, innate immunity, macrophage, memory, protection

OP-256

CD8+ lymphocyte mediated suppression of HIV expression in CD4$^+$ T cells

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The persistence of HIV infection under ART is due to a reservoir of latently infected cells that remain indefinitely despite suppression of virus replication. Defining the mechanisms responsible for the establishment and maintenance of the HIV reservoir is considered the key for HIV eradication. Studies have demonstrated that CD8$^+$ T cells inhibit virus replication during HIV/SIV infection; however, the mechanisms responsible for this antiviral effect remain poorly understood. We used our primary cell based *in vitro* model of HIV latency to examine the CD8$^+$ T cells mediated suppression of HIV expression. Memory CD4$^+$ T cells from HIV-negative donors were infected *in vitro* and then co-cultured with activated CD8$^+$T lymphocytes. We assessed the intracellular Gag expression by multiparameter flow cytometry, and quantified the frequency of integrated HIV DNA by qPCR. High-dimensional single-cell marker analysis were combined with a transcriptomic approach to identify differentially expressed pathways in CD4$^+$T cells modulated by CD8$^+$T cell co-culture. HIV expression in CD4$^+$ T cells was reduced when co-cultured with autologous activated CD8$^+$ T cells an average of 9-fold ($p<0.0001$) and 18-fold ($p<0.0001$) at 1:1 or 1:5 target:effector ratios respectively, without significantly reducing the frequency of HIV-infected cells. Our studies have demonstrated a CD8$^+$ lymphocyte mediated suppression of HIV expression inCD4$^+$T cells in the establishment of latency. Understanding the mechanisms by which CD8$^+$ lymphocytes suppress virus transcription and ultimately promote maintenance of HIV latency in ART-treated HIV-infected individuals may provide critical insight to support the design of new approaches for HIV eradication.

Keywords: Adaptive immunity, cellular interactions, infectious disease, omics technologies, viral infections

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WORKSHOPS

OP-257

cGAS-STING-dependent interferon signalling in a mitochondrial disease

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Definition of the type I interferonopathies (genetic diseases characterized by pathogenic chronic interferon production) has emphasised the importance of discrimination between self and non-self nucleic acid. Mitochondria represent vestigial bacteria in the cytosol of eukaryotic cells, and there is increasing recognition that mis-localised mitochondrial DNA (mtDNA) can drive immune system activation. However, the induction of interferon signalling by mtDNA has not been demonstrated in a Mendelian mitochondrial disease. We identified two patients demonstrating enhanced interferon-stimulated gene (ISG) expression in blood, each harbouring a de novo dominant-negative heterozygous mutation in ATAD3A encoding mitochondrial ATPase family AAA domain-containing protein 3A (ATAD3A). The same mutations have been previously associated with a mitochondrial disorder characterised by severe neurological disease. In five further patients with mutations in ATAD3A, we recorded up-regulated ISG and interferon alpha protein. Interestingly, two patients demonstrated marked features of the autoimmune disorder systemic sclerosis. ATAD3A knockdown resulted in increased interferon signalling, mediated by cGAS and STING. This was abrogated upon mtDNA depletion in ATAD3A-knockdown cells and patient fibroblasts. By immunofluorescence, we detected cytosolic leakage of DNA upon ATAD3A knockdown. There was no major contribution of BAX/BAK macropores or VDAC1 channels in the release of mtDNA. Thus, how ATAD3A prevents mtDNA cytosolic leakage remains to be determined. Our data suggest that ATAD3A dysfunction leads to constitutive activation of interferon signalling through cGAS-STING dependent sensing of mtDNA. Mutations in ATAD3A define a novel type I interferonopathy, raising the question as to the pathological contribution of enhanced interferon signalling in other mitochondrial disorders.

Keywords: Autoinflammation, cytokines and mediators, innate immunity, metabolic control of immune responses

OP-258

Auto-aggressive CXCR6+ CD8 T cells cause liver immune pathology in NASH

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Non-alcoholic steatohepatitis (NASH) is a manifestation of systemic obesity-related metabolic disease, characterized by chronic sterile inflammation that causes liver disease. Tissue-resident T cells are key immune cells for immune surveillance and tissue integrity, but the mechanisms how they contribute to liver damage in diseases like NASH is unclear. Here, we identify murine and human Foxo1lowCXCR6+CD8+ T cells as critical tissue-resident T cells causing liver damage in NASH after IL-15 and metabolic activation. Single-cell and bulk RNA-seq, antibody intervention studies in preclinical mouse models, flow cytometry and cytotoxicity assays of murine and human CXCR6+CD8+ T cells In NASH mice, we detected hepatic accumulation of CD8 T cells with phenotypes that combined tissue-residency (CXCR6) with effector (Granzymes) and exhaustion (PD-1) characteristics. Liver CXCR6+ CD8 T cells were characterized by low FOXO1 transcription factor activity and were abundant in mouse and human NASH. Mechanistically, IL-15 induced FOXO1 down- and CXCR6 up-regulation, which rendered CXCR6+ CD8 T cells susceptible to metabolic stimuli in NASH livers, including acetate and extracellular adenosine triphosphate, and collectively triggered auto-aggression through TNF and FasL. Treatment of NASH mice with antibodies blocking the activity of TNF, FasL or IL-15 significantly ameliorated liver damage. Importantly, CXCR6+ CD8 T cells from mouse and human NASH livers had similar transcriptional signatures and showed auto-aggressive killing of cells in an MHC-class-I-independent fashion after signaling through P2X7 purinergic receptors. Killing by auto-aggressive CD8 T cells fundamentally differed from that by antigen-specific cells, thus mechanistically distinguishing auto-aggressive from protective T cell immunity.

Keywords: Autoimmunity, chronic inflammation and fibrosis, effector molecules, environmental factors in autoimmunity and allergy, metabolic control of immune responses, tissue damage and repair

OP-259

The alternative pathway of complement and long pentraxin PTX3 form a functional axis in the immune response to *Aspergillus fumigatus*

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Aspergillus fumigatus (AF) is the major etiologic agent of invasive aspergillosis (IA), a severe infection amongst immunocompromised individuals. A key role in host resistance to AF is played by polymorphonuclear neutrophils (PMNs) and complement. The long pentraxin PTX3 exerts opsonic activity towards AF conidia, and increases their phagocytosis and killing by PMNs through the alternative pathway (AP) of complement. Here we characterized the molecular crosstalk between PTX3 and AP in opsono-phagocytosis of AF. Complement activation, phagocytosis, and killing experiments were performed using AF conidia and purified human and murine PMNs, and analyzed by Western blotting, ELISA, and flow cytometry techniques. A cohort of 483 patients undergoing hematopoietic stem-cell transplantation (HSCT) and their donors was screened for AP SNPs modifying the risk of IA. We found that PTX3 promoted selective recruitment of C3b onto AF conidia by forming a ternary complex with C3b and factor H (fH, main inhibitor of AP). Consistent with this, complement receptor 1 (CR1, major C3b receptor that is expressed on PMNs) was required for the pro-phagocytic and pro-killing activities of PTX3. Informed by these findings, SNP genotyping of the HSCT cohort showed that both recipients and donors homozygous for the GG allele of rs2230199 in the C3 gene had increased risk of infection. Our study indicates that AP (via fH and C3b) forms a functional axis with PTX3 and CR1 in opsonization, phagocytosis, and killing of AF by PMNs. This is corroborated by genetic evidence from the human pathology.

Keywords: Complement, effector molecules, fungal infections, innate host defence, innate immunity

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WORKSHOPS

OP-260

Circulating immune profile changes reflect memory immune responses in spinal cord injury patients

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Following a spinal cord injury (SCI), an inflammatory immune reaction is triggered which results in advanced secondary tissue damage. This study aimed to extensively analyse the circulating immune cell composition in traumatic SCI patients. High-dimensional flow cytometry was performed on peripheral blood mononuclear cells of traumatic SCI patients and healthy controls (n=18 each). SCI blood samples were collected at multiple time points in the (sub)acute ((s)aSCI, 0-4 days and 3 weeks post-SCI) and chronic (cSCI, 6, 12, 18 and >18 weeks post-SCI) disease phase up to a total of 46 SCI samples. Total and CD4+ T cell frequencies were increased in cSCI patients. CD4+ T cells and B cells were shifted towards memory phenotypes in (s)aSCI and cSCI patients, respectively. Most profound changes were observed in the B cell compartment. Decreased immunoglobulin (Ig)G+ and increased IgM+ B cell frequencies reflected disease severity, as these correlated with American Spinal Injury Association (ASIA) impairment scale (AIS) scores. Post-SCI B cell responses consisted of an increased frequency of B cells and B cell subsets expressing the survival receptor CD74. Expression of CD74 was also elevated on B cell subsets of cSCI but not (s)aSCI patients. In conclusion, post-SCI inflammation is driven by memory immune cell subsets. The elevated CD74 expression on B cells of SCI patients suggests the potential involvement of CD74-related pathways in post-SCI B cell responses. Monitoring of circulating IgM+ and IgG+ B cell levels could aid in the clinical evaluation and prognosis of SCI patients.

Keywords: Adaptive immunity, B lymphocytes, neuroimmunology

OP-261

The long pentraxin PTX3 at the host-pathogen interface in *Staphylococcus aureus*-dependent osteomyelitis

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Osteomyelitis (OM) is a debilitating infection of the bone primarily caused by the opportunistic pathogen *Staphylococcus aureus* (SA). SA exploits several strategies to evade the immune response and subvert bone homeostasis, yet the underlying mechanisms are largely unclear. Here we studied the SA/bone interface with a focus on the soluble pattern recognition molecule long pentraxin 3 (PTX3), which is emerging as a new player in bone physiology and pathology. A murine model of SA intrabone infection was developed that recapitulates surgery/trauma-OM in humans. Local and systemic infection and inflammation were assessed by means of flow cytometry, RT-PCR, ELISA, histochemistry, and microCT. At 14 days from SA challenge, >95% of mice developed infection in the injected limb only, which underwent extensive remodeling with loss of trabecular and apposition of periosteal bone. Larger bacterial burdens were found in the bone of WT than in that of PTX3 KO mice at 6 and 14 days from infection. Accordingly, WT animals had more severe inflammation, in terms of expansion of innate immune cells in the spleen, and increase of inflammatory cytokines in the serum. Also, PTX3 levels were augmented in SA-infected mice in the serum and bone. Genetic deficiency of PTX3 protects from infection in a murine model of locally-induced SA-OM. Our findings point to an involvement of this long pentraxin in the pathogenesis of OM, and open unforeseen windows on the molecular mechanisms of this disease, with therapeutic and diagnostic potentials.

Keywords: Animal models, infectious disease, innate host defence, innate immunity, tissue damage and repair

OP-262

In depth analysis of the bee venom allergen-specific B cell repertoire in immunotherapy-treated venom allergic individuals and naturally exposed healthy beekeepers

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Understanding the mechanisms of tolerance to allergens is critical for development of therapies for the treatment of allergies. Beekeepers, who are frequently exposed to high-doses of bee venom allergens, represent a unique *in vivo* model to study these mechanisms. Here we characterize the allergen-specific B-cell repertoire in beekeepers and venom allergic patients. Blood samples were collected from non-allergic beekeepers before and during the beekeeping season, as well as from venom allergic individuals before and after three months of venom immunotherapy. B-cells specific for the venom allergen phospholipase A2 (PLA) were purified and immortalized through transduction with BCL6 and BCL-XL. Deep sequencing of the B-cell repertoire was performed on expanded PLA-specific B-cells as well as total PBMC. Frequencies of PLA-specific B-cells were higher during the beekeeping season than before. PLA-specific clones were overrepresented within the IgE and IgG4 repertoire compared to other isotypes. PLA-specific clonal lineages had increased V-gene mutations at the end of season. PLA-specific clonal lineages contained members of different isotypes. Within PLA-specific clonal lineages containing IgE, members of other isotypes with the highest similarity to the IgE member were mostly IgG2 or IgG4, suggesting sequential class switch recombination. We also observed that allergen-specific B cell clones can persist for many years. It remains to be determined which features of the B cell repertoire are indicative of allergen tolerance. Comparative analysis with bee venom allergic individuals before and after allergen-specific immunotherapy is currently underway and may help to identify key differences between healthy and allergic B cell responses.

Keywords: Adaptive immunity, allergen-induced immune responses, allergic disorders, antibody, B lymphocytes, memory

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WORKSHOPS

OP-263

The critical expansion of uterine CD8+ T cells in pregnancy is under tight homeostatic control

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In physiological pregnancies CD8+ T cells, the most frequent uterine T cell population, elude inflammatory responses that are otherwise exacerbated in pregnancy pathologies. However, CD8+ T cell tolerance towards the allogenic conceptus remains incompletely understood. Thus, our aim was to characterize the uterine CD8+ T cell-compartment with regards to local cell recruitment and differentiation throughout pregnancy. Leucocytes isolated from uterine decidua, blood, spleen and uterine-draining lymph nodes of Balb/c-mated C57Bl/6 female mice at early, mid and late pregnancy were characterized and quantified by flow cytometry. We used pregnancies in which the conceptus expressed ovalbumin to investigate the migration of fetal-antigen specific OT-I CD8+ T cells to the uterus upon their adoptive transfer to Rag2-/-gc-/- or C57Bl/6 females. Uterine CD8+ T cell counts dramatically increased across pregnancy. This expansion relied at least partly on the recruitment of circulating cells, as adoptively transferred CD8+ T cells were localized in the pregnant uterus of immune-deficient and immune-competent females. Uterine CD8+ T cells presented predominantly effector and memory phenotypes, among which early-activated, effector-memory and tissue-resident memory cells increased remarkably in pregnancy. Comparing to the distal decidua-parietalis, cell activation was enhanced in the decidua-basalis adjacent to the allogenic placenta, underscoring important local antigenic responses. Intriguingly, CD8+CD122+T cells, reported to be immunomodulatory and expressing inhibitory molecules including PD1, were enriched exclusively in the uterus and a balanced CD8+ T cell:CD8+CD122+T cell cotransfer prevented fetal loss in adoptive transfer experiments. Our results suggest that CD8+CD122+T cells maintain a tight homeostatic control of the massively expanding uterine CD8+ T cell-compartment, which is pivotal for pregnancy success.

Keywords: Adaptive immunity, fetal immunity, reproductive immunology

OP-264

Hepatitis B virus negatively impacts the protective immune crosstalk between natural killer and dendritic cells

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Natural killer (NK) cells play a crucial role in the clearance of human viruses but their activity is significantly impaired in patients infected with chronic hepatitis B (CHB). Cooperation with dendritic cells (DCs) is pivotal for obtaining optimal NK cell antiviral function; thus, we investigated whether HBV might impact the ability of DCs to sustain NK cell functions. Human DCs were poor stimulators of interferon-gamma (IFN-γ) production by NK cells when exposed to HBV, while maintaining the capability to trigger NK cell cytotoxicity. HBV prevented DC maturation but did not affect their expression of human leukocyte antigen class I, thus allowing DCs to evade NK cell lysis. Tolerogenic features of DCs exposed to HBV were further supported by their increased expression of IL-10 and the immunosuppressive enzyme indoleamine 2,3-dioxygenase, which contributed to the impairment of DC-mediated NK cell IFN-γ production and proliferation, respectively. HBV could also inhibit the expression of inducible immunoproteasome (iP) subunits on DCs. In fact, NK cells could induce iP subunit expression on DCs, but they failed in the presence of HBV. Remarkably, circulating blood DC antigen1 (BDCA1)+ DCs isolated from patients with CHB were functionally compromised, hence altering, in turn, NK cell responses. The abnormal NK-DC interplay caused by HBV may significantly impair the efficacy of antiviral immune response in patients with CHB.

Keywords: Cellular interactions, dendritic cells, innate immunity, NK cells, viral infections

OP-265

Kinin-B2 receptor signaling in neuroinflammation and innate immune response in Alzheimer's disease

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Neuroinflammation is an inherent process in the pathogenesis of Alzheimer's disease (AD) pathogenesis. Recent data show that the kinin system is stimulated by pathologic amyloidβ (Aβ) in AD patients. Effects of this system primarily mediated by bradykinin (BK) and its kinin-B2 receptor (B2R) in AD are not completely understood. Thus, we determined effects of B2R blockage in different AD models: transgenic triple mouse as a model of the familial AD without B2R expression (APPswe/PS1dE9/B2R-/-) or injection of oligomeric Aβ into a mouse model. Aβ increased blood-brain barrier (BBB) permeability and genetic and pharmacological B2R blockade provided BBB protection in AD mice. The B2R antagonist HOE140 prevented ROS increase in Aβ-treated mouse hippocampus. Besides augmented microglia frequency, the peripheral immune system also presented alterations, modulated by BK/B2R signaling. HOE140 counteracted IL6 serum level increase of Aβ-treated mice and prevented granulocyte death, suggesting dependence of NET formation in AD mouse peripheral blood on bradykinin signaling. Both HOE-140 and B2R(-/-)KO prevented memory loss. Moreover, Aβ oligomers dose-dependently reduced neurosphere diameters, and HOE-140 prevented this effect. Differentiated APPswe/PS1dE9 cells expressed up to 100-times CCL12, CCL5, CCL3, CX3CR1 chemokine and C3, TLR2 and TNF inflammatory mediator levels. Microglial Iba-1 expression was 20-fold upregulated in APPswe/PS1dE9 ¬cells. HOE140 significantly decreased CCL12, CCL5 and C3 levels, while BK increased CCL12, CCL3 and TLR2 levels in APPswe/PS1dE9 ¬cells, suggesting that B2R signaling triggers chemoattraction of inflammatory cells. B2R presents a key role in neuroinflammation observed in AD, from BB disruption and peripheral immune system alterations to memory loss.

Keywords: Animal models, inflammatory disease, innate immunity, memory, neuroimmunology

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WORKSHOPS

OP-266

Hypoxia ameliorates allergic asthma *in vivo* by interfering with immune cell cross-talk

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High altitude climate therapy has been reported to be beneficial in treating allergic asthma. However, the underlying mechanisms still remain unclear. Here we investigate the hypothesis that reduced oxygen concentrations confer this amelioration. Experimental asthma was induced in C57BL/6 mice via intranasal application of House Dust Mite (HDM) over four weeks; mice were co-exposed to 10% oxygen or room air for the final two weeks. Read-outs included airway hyper-responsiveness, mucus hypersecretion and inflammatory cell recruitment. Isolated immune cells from mouse and allergic patients were stimulated *in vitro* with HDM under normal and low oxygen conditions, adaptive immune activation was analysed by flow cytometry and cytokine-ELISA. In mice, low oxygen conditions significantly ameliorated HDM-induced allergic asthma, as shown by reduced mucosal hypersecretion and airway hyper-responsiveness in comparison to normoxic HDM-mice. Immune cell infiltration, especially eosinophilia, and secretion of pro-inflammatory cytokines were also strongly reduced. *In vitro*, hypoxia almost completely suppressed the HDM-induced adaptive immune response in both mouse and human immune cells, however, did not impair a polyclonal T-cell response induced by aCD3/CD28. Hypoxia also led to alterations of antigen presenting cells *in vitro* and *in vivo*, affecting both cell numbers and differentiation. Hypoxia ameliorates allergic asthma via interfering with immune cell cross-talk, resulting in an attenuated TH2 response. We postulate that changes in antigen presenting cell differentiation might confer a longer lasting beneficial effect as observed in patients returning from high altitude.

Keywords: Allergen-induced immune responses, dendritic cells, environmental factors in autoimmunity and allergy, macrophage

OP-267

Hindering triple negative breast cancer progression by targeting endogenous Interleukin-30 requires IFNγ signaling

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Triple–Negative (TN) Breast Cancer (BC) is one of the most aggressive malignancy. IL30 expression by tumor and infiltrating immune cells is frequent in TNBC and is associated with a worse prognosis. Here, we investigate the consequences of targeting host-derived IL30 on TNBC behavior and identify the underlying molecular events. The METABRIC dataset was used to analyze the expression of IL30 in BC and its distribution by molecular subtype. After testing their response to IL30 treatment, TNBC cells were implanted in syngeneic hosts lacking endogenous IL30 (IL30KO) and survival curves were plotted by Kaplan-Meier method. Microarray data, obtained from 1699 BC cases, established a positive correlation of IL30 expression with TNBC. In TNBC cells endowed with IL30 receptor, IL30 boosted proliferation, migration and a broad tumor progression and immune evasion program. Whenimplanted into IL30KO hosts, both IL30-responsive and -unresponsive TNBCs gave rise to poorly vascularized and slow growing tumors with low metastatic potential, which led to increased survival. Intra-tumoral influx of CD3+CD8+ and CD3+CD4+T lymphocytes, NKp46+cells, and their IFNγ production, were the hallmarks of tumor growth inhibition in IL30KO mice. Knocking-out IFNγ gene or blocking IFNγ pathway in IL30KO host, restored tumor vascularization, abolished intratumoral T-cell recruitment and the anti-tumor efficacy due to the lack of endogenous IL30. The ability of IL30 to affect the host environment can circumvent its ineffectiveness on cancer cells and be fundamental to tumor behavior. IL30 is avaluable target to improve immunotherapy and life expectancy in TNBC patients.

Keywords: Cancer immunology, cytokines and mediators, *in vivo* tumor models, microenvironment

OP-268

A rapid screening strategy identifies a pan-coronavirus neutralizing antibody

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The identification of broadly neutralizing antibodies to coronaviruses is of interest to respond to current variants of concerns and generally for pandemic preparedness. Here, we introduce a facile and high-throughput method to rapidly identify memory B cells producing cross-reactive anti-coronavirus antibodies. Using this approach, we isolated 5 monoclonal antibodies (mAbs), from infected and vaccinated individuals, that bind to all human α and β coronaviruses by recognizing the highly conserved fusion peptide region. These memory B cells are extremely rare, and germline reversion suggests these mAbs can be primed by either α or β coronaviruses and acquired affinity and breadth through somatic mutations. Although all of these mAbs target the same region, only one of these mAbs, VN001_H1, is able to neutralize SARS-CoV-2, SARS-CoV-1 and MERS-CoV pseudotyped viral infections by inhibiting viral fusion in a TMPRSS2-dependent manner. Collectively, these findings describes the first pan-coronavirus neutralizing antibody, paving the way for the design of a universal vaccine.

Keywords: Antibody, B lymphocytes, infectious disease, memory

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WORKSHOPS

OP-269

Immune profiling of soft tissue sarcoma: cellular, proteomic and transcriptomic analysis of peripheral blood

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Soft Tissue Sarcoma (STS) have often been the subject of research as a result of advanced disease at diagnosis and therefore its poor prognosis. Clinical trials have incorporated various disease subtypes, despite its heterogeneity. Immunophenotyping and soluble proteome could help to define a better approach for STS therapy. The present study groups consisted of 26 STS patients and 37 age-matched control samples. Extended immunophenotyping (flow cytometry), plasmatic proteome (Luminex®) and transcriptomic (qPCR) analysis was performed. Although STS patients presented a significant lymphopenia, granulocytosis and decrease of CD4/CD8 T cell ratio with upregulation of HLA-DR in both populations. In addition, plasmatic IFN-g, sIL-2R and sCD30 were found in the patient group. Frequency of central memory and effector-memory CD4 T cells were found increased in association with higher soluble TIM-3 levels in STS patients. Th17 and Th1/Th17 as well as Tc1 cells were found increased in STS patients, with significant cell activation. B cells were significantly decreased in STS patients with a significant increase of plasmablasts. STS patients were significantly deficient in cytotoxic NK cells and dendritic cells (mainly cDC2 and pDC). Immunosuppressive cells (Tregs and MDSCs) together with plasmatic VISTA, arginase and TIMD4 were significantly increased in STS patients. Transcriptomic analysis of peripheral blood provide signatures associated with downregulated NK cell-related genes (e.g. NCAM1, PRF1, GZMB, KLRK1) and upregulation of immune suppressor targets (e.g. ARG1, IL10, IL27). Immunophenotyping, gene expression and soluble proteome analysis in STS could help to select the best individual therapeutic approach, ultimately increasing progression free survival.

Keywords: Adaptive immunity, biomarkers, cancer immunology, checkpoint inhibition, innate immunity, myeloid derived suppressor cells

OP-270

First-dose mRNA vaccination is sufficient to reactivate immunological memory to SARS-CoV-2 in recovered COVID-19 subjects

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The characterization of the adaptive immune response to COVID-19 vaccination in individuals who recovered from SARS-CoV-2 infection may define current and future clinical practice. To determine the effect of two doses BNT162b2 mRNA COVID-19 vaccination schedule in individuals who recovered from COVID-19 (COVID-19 recovered) compared to naïve subjects, we evaluated SARS-CoV-2 Spike-specific T and B cell responses, as well as specific IgA, IgG, IgM and neutralizing antibodies titers in 22 individuals who received BNT162b2 mRNA COVID-19 vaccine, 11 of which had a previous history of SARS-CoV-2 infection. Evaluations were performed before vaccination and then weekly until 7 days post second injection. Data obtained clearly showed that one vaccine dose is sufficient to increase both cellular and humoral immune response in COVID-19 recovered subjects without any additional improvement after the second dose. On the contrary, the second dose is proved mandatory in naïve ones to further enhance the immune response. These findings were further confirmed at serological level in a larger cohort of naïve (68) and COVID-19 recovered (29) subjects, tested up to 50 days post vaccination. These results question whether a second vaccine injection in COVID-19 recovered subjects is required and indicate that millions of vaccine doses may be redirected to naïve individuals, thus shortening the time to reach herd immunity.

Keywords: Adaptive immunity, adjuvants and vaccines, infectious disease

OP-271

Fas/FasL contributes to HSV-1 brain infection and neuroinflammation

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The Fas/FasL pathway plays a key role in immune homeostasis and immune surveillance. In the central nervous system (CNS) Fas/FasL is involved in axonal outgrowth and adult neurogenesis. However, little is known about the role of the Fas/FasL pathway in herpes encephalitis. In this study, we used a neuropathogenic clinical strain of herpes simplex virus type 1 (HSV-1) to explore infection-induced inflammation and immune responses in the mouse brain and the role of Fas/FasL in antiviral CNS immunity. HSV-1 CNS infection induced the infiltration of Fas- FasL-bearing monocytes and T cells in the brain and also to an up-regulation of Fas and FasL expression on resident astrocytes and microglia within infected sites. Upon infection, Fas- and FasL-deficient mice (lpr and gld) were partially protected from encephalitis with a decreased morbidity and mortality compared to WT mice. Fas/ FasL deficiency promoted cell-mediated immunity within the CNS. Fas receptor stimulation abrogated HSV-1 induced activation and inflammatory reactions in microglia from WT mice, while lack of Fas or FasL led to a more pronounced activation of monocytes and microglia and also to an enhanced differentiation of these cells into a pro-inflammatory M1 phenotype. Furthermore, the specific immune system was more efficient in Fas- and FasL-deficient mice with significantly higher numbers of infiltrating HSV-1-specific cytotoxic T cells in the brain. Our data indicate that the Fas/FasL pathway leads to excessive neuroinflammation during HSV-1 infection, which is associated with a diminished anti-viral response and an excessive neuroinflammation.

Keywords: Cell signalling, cytokines and mediators, neuroimmunology, viral infections

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WORKSHOPS

OP-272

Role of MHC class I-like XNC4 during mycobacterial infection in two developmental stages in *Xenopus*

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MHC class I-like restricted innate-like (i)T cells (iNKT and MAIT) are involved in early immune responses against *Mycobacterium tuberculosis* in mammals, although their role is not fully understood. For the first time in a non-mammalian species, iT cells have been characterized in the amphibian *Xenopus*. Notably, reverse genetic approaches have revealed that invariant Vα45 T cells interacting with the non-polymorphic MHC class I-like XNC4 are critical for tadpoles' resistance against *Mycobacterium marinum* (*Mm*). Alike human foetus, tadpole immunity appears to be hyporesponsive and biased toward tolerance compared to adult frogs. Here we have further investigated the role of XNC4 in *Xenopus* immune tolerance against *Mm*. Using a combination of XNC4 specific nanobody, fluorescent DsRed+ *Mm*, and recombinant CSF1 and XNC4, we characterized by flow cytometry the recruitment of putative XNC4 presenting cells and XNC4 interacting T cells in tadpoles and adults frogs after *Mm* injection. In addition to the recruitement of XNC4-restricted T cells, XNC4+ cells consist in heterogeneous immune cell populations including macrophages whose recruitment is delayed in tadpoles. Interestingly, infected DsRed+ XNC4+ myeloid cells exhibit higher XNC4 surface expression than DsRed- counterpart, suggesting that *Mm* internalisation stimulates XNC4 antigen presentation. Preliminary results indicate that XNC4 binds unusually long peptides (9-14 mers) and interacts with T cells characterized by a diverse TCR repertoire. Importantly, XNC4+ macrophages have a dominant M2-like phenotype, which reinforce our hypothesis that XNC4 and the associated T cells are important for mycobacterial tolerance in *Xenopus*.

Keywords: Antigen processing and presentation, bacterial infections, immune development, infectious disease, MHC and polymorphic genes

OP-273

CD14low16highHLA-DR+ monocytes are increased in breast cancer patients with good response to neoadjuvant chemotherapy

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Introduction. Tumor-associated macrophages (TAMs) can support or interfere with chemotherapy. For breast cancer (BC) patients, neoadjuvant chemotherapy (NAC) is used before surgery. Monocytes are a major resource for TAMs. Our aim was to examine the relation between monocyte subpopulation and chemotherapy in BC patients. Methods. Flow cytometric was used to identify monocytes expressing CD14, CD16, CD163, HLA-DR in a cohort of 38 BC patients before and after NAC. Seventeen healthy women constituted a control group. Results. The percentage of CD14low16highCD163+ and CD14high16highCD163+ monocytes were higher in BC patients compared to healthy women (99.08% vs 60.00%, p=0,039, and 98.08% vs 86.96%, p=0.046, respectively). The HLA-DR+ monocytes of both CD14low16high and CD14high16high subpopulations were similar in BC patients and the control groups. Patients who responded to NAC had a significantly higher percentage of CD14low16highHLA-DR+ cells than the non-responders' group to NAC (84.62% vs. 55.12%, p=0.005). Conclusion. Our data demonstrated that monocyte content in the blood reflects the presence of breast cancer and also response to NAC. The expression of CD163 on CD14low16high and CD14high16high subpopulations is indicative of the BC. The expression of HLA-DR on the non-classical monocyte subpopulation (CD14low16high HLA-DR+) before NAC is predictive for the response of BC patients to NAC.

Keywords: Biomarkers, cancer immunology, innate immunity, macrophage

OP-274

Dissecting the cellular defects that lead to activated PI3K δ Syndrome (APDS)

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APDS is a rare condition caused by heterozygous gain-of-function (GOF) mutations in *PIK3CD* (APDS1), which encodes the leukocyte-restricted p110-delta catalytic subunit of phosphoinositide 3-kinase (PI3K) or heterozygous loss-of-function (LOF) mutations in *PIK3R1* (APDS2), which encodes the regulatory subunit of PI3K. PI3K is activated downstream of many receptors expressed by T and B cells and has been implicated in the control of lymphocyte activation and differentiation. APDS patients have increased PI3K activity that leads to multiple immune manifestations including lymphoproliferation, respiratory tract infections, Th2-related pathologies, impaired Ab responses and autoimmunity. We previously generated a mouse model of APDS1 (*Pik3cd*-GOF) in order to dissect the cellular changes that lead to disease in these patients. We demonstrated changes in both T and B cells including increased memory T cells, defective Tfh function, decreased isotype switching and a B cell intrinsic break in tolerance and production of autoantibodies. We have now generated a mouse model of APDS2 (*Pik3r1*-LOF) in order to determine whether increased PI3K signalling due to loss of regulatory function causes the same cellular changes. In the *Pik3r1*-LOF mice we observed many of the same changes previously observed in *Pik3cd*-GOF mice, including altered cytokine production and decreased isotype switching. However, we also identified multiple differences between the phenotype of *Pik3r1*-LOF and *Pik3cd*-GOF mice. Together, these studies reveal that although APDS1 and 2 both cause increased PI3K signalling and result in similar clinical phenotypes, there are distinctions between the way these two types of mutations affect cellular function.

Keywords: B lymphocytes, cell signalling, follicular helper T cells, immunodeficiency

OP-275

Circulatory T Follicular Helper cell (cTFH) profile during SARS-CoV-2 infection

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T follicular helper cells (TFH) play an essential role in regulating the germinal centre reaction and, consequently, the generation of high-affinity antibodies and memory B cells. Our aim is to study circulatory TFH (cTFH) in SARS-CoV-2 infection. PBMCs from 71 RT-PCR confirmed SARS-CoV-2 patients were collected upon arrival to the hospital, along with 25 healthy donors (HD). 16 out of these 71 patients had a second sample taken 5 days later. cTFH were identified as CD4+CXCR5+ and cTFH1, 2 and 17 subsets as CXCR3+CCR6-, CXCR3-CCR6- and CXCR3-CCR6+ respectively. Activated cells were those co-expressing ICOS+PD1hi and senescent cells were ICOS-PD1hi. S1-specific IgG and IgA were detected by a semicuantitative ELISA. SARS-CoV-2 infected patients presented higher frequencies of cTFH2, and lower cTFH17, than HD ($p<0.0001$ and $p=0.007$, respectively). cTFH1 and cTFH2 included a higher proportion of activated cells than cTFH17 within their subset ($p<0.0001$ and $p<0.0001$). In comparison to HD, cTFH1 were significantly more activated in SARS-CoV-2 patients ($p<0.0001$). Mild patients presented higher frequencies of cTFH1 ($p=0.02$) and activated cTFH1 ($p=0.007$) than severe patients. On the contrary, severe patients presented higher frequencies of senescent cTFH2 and cTFH17 compared to mild patients ($p=0.02$ and $p=0.01$, respectively). IgA ratio positively correlated with activated cTFH1 and cTFH17 ($p=0.04$ and $p=0.01$) while IgG ratio positively correlated with activated cTFH1 ($p=0.02$). cTFH1 appeared to be involved in anti-S1 IgA and IgG secretion and associated with mild SARS-CoV-2 infection. Studies are ongoing to further dilucidate the role of cTFH subsets in promoting COVID-19 resolution.

Keywords: Follicular helper T cells, immune response tracing, infectious disease, viral infections

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WORKSHOPS

OP-276

Disease severity-specific neutrophil signatures in blood transcriptomes stratify COVID-19 patients

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The SARS-CoV-2 pandemic is continuing to lead to increasing numbers of COVID-19 patients all over the world. While clinical presentations range from asymptomatic, mild respiratory tract infection, to severe cases with acute respiratory distress syndrome, respiratory failure, and death, the latter has been associated with dysregulations in the immune system. In order to dissect COVID-19-driven immune host responses, we profiled whole blood transcriptomes of 39 COVID-19 patients and 10 control donors enabling a data-driven stratification based on molecular phenotype. Neutrophil activation-associated signatures were prominently enriched in severe patients, which was corroborated in whole blood transcriptomes from an independent cohort of 30 patients as well as in granulocyte samples from a third cohort of 16 COVID-19 patients (44 samples) using a combination of conventional and data-driven co-expression network analysis. Comparison of COVID-19 blood transcriptomes with those of a collection of over 3,100 samples derived from 12 different viral infections, inflammatory diseases and independent control samples revealed highly specific transcriptome signatures for COVID-19. Stratified transcriptomes predicted patient subgroup-specific drug candidates targeting the dysregulated systemic immune response of the host, such as dexamethasone to be efficient for only a subgroup of patients with severe COVID-19 and not for patients with mild disease, which was subsequently confirmed by several clinical studies including the large RECOVERY trial. Our study provides novel insights into distinct molecular subgroups or phenotypes that are not simply explained by clinical parameters. Whole blood transcriptomes are extremely informative for COVID-19 since they capture granulocytes, which are major drivers of disease severity.

Keywords: Granulocytes, infectious disease, RNAseq

OP-277

Neutrophils controlling the intestinal microbiota provide protection against colitis and colitis-associated colorectal cancer

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Neutrophils are essential players of immune response and inflammation. Recent evidence highlighted the complexity and heterogeneity of neutrophils in cancer and cancer-related inflammation, including colorectal cancer (CRC). However, their role in inflammatory bowel disease (IBD) and colitis-associated CRC (CAC) is controversial. Using a genetic model of neutrophil deficiency (Csf3r-/- mice) we found that Csf3r-/- mice displayed increased susceptibility to acute and chronic colitis and CAC. Intestinal dysbiosis is associated with the development of IBD and CRC. Metagenomic analysis showed a significant difference in the beta-diversity of faecal microbiota between Csf3+/+ and Csf3r-/- mice, indicating that the microbiota composition was altered in Csf3r-/- mice. Interestingly, broad-spectrum antibiotic administration was sufficient to rescue the susceptibility to colitis and CAC to the level of Csf3r+/+ controls. Reduced number of repairing ulcers were found in colon tissue sections of Csf3r-/- mice, indicating that neutrophil deficiency affected the regenerative capacity of colonic epithelial cells. Accordingly, tissue levels of IL-22 and the activated isoform of its downstream mediator (pSTAT3) were reduced in Csf3r-/- mice. *Ex vivo* stimulation of colonic mononuclear cells showed that γδT cells from Csf3r-/- mice displayed a reduced capacity to produce IL-22 upon stimulation, while the production by other cell types was not affected. In IBD patients, CSF3R and IL-22 expression were positively correlated, and patients with higher CSF3R expression showed the enrichment of epithelial repair gene signatures. Therefore, neutrophils play an essential role in controlling the composition of the intestinal microbiota and in the activation of an IL-22-dependent tissue repair pathway.

Keywords: Cancer immunology, inflammatory bowel disease, innate immunity, neutrophils

OP-278

Immunomodulatory effect of IgM/IgA-enriched high dose intravenous immunoglobulin therapy in COVID-19 patients

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Severe COVID-19 is mediated by host's inappropriate immune response, resulting in auto-destruction of tissues and organs. High-dose intravenous immunoglobulin (IVIG) preparations were shown to exert anti-inflammatory and immunoregulatory effects in life-threatening conditions. Ten COVID-19 patients (age 29-66, 8:2 males/female ratio) were treated with an IgM/IgA-enriched IVIG (Pentaglobin, Biotest, GmbH) when upcoming pulmonary distress was recognized. Controls were age/gender-matched patients who fulfilled the inclusion criteria (n=10). Clinical/biochemical parameters were monitored daily, while immunological parameters were analyzed on Day 0 (pre) and Days 3 and 10 post Pentaglobin infusion. Pentaglobin dose (1g/kg of body weight) and the rate of its administration (35 ml/h) were both well tolerated with no adverse effects observed. The treatment prevented progression from emerging to fully developed acute respiratory distress. There were no deaths in the treatment group, as compared to 20% fatality rate among controls. Levels of inflammatory markers such as C5a, CRP and LDH were reduced post-treatment relative to Day 0, but not in controls. Increased levels of IL-6 were not affected by Pentaglobin infusion. In peripheral blood mononuclear cell isolates from the treated patients, an increase in the number of CD4+ and CD8+ T lymphocytes was observed on Days 3 and 10, without accumulation of monocytic myeloid-derived suppressors cells. Contrary findings were noted in the control group. Our results indicate that treatment with IgM/IgA-enriched IVIG preparation may prevent serious complications and death in Covid-19 by attenuating inflammation and regulating cellular immune responses.

Keywords: Immune regulation and therapy, immunotherapy, viral infections

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WORKSHOPS

OP-279

A colorimetric pseudovirus-based assay using secreted embryonic alkaline phosphatase for analysis of neutralizing activity against SARS-CoV-2

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Previously we reported a feasible method under BSL-2 conditions for detection of neutralizing activity against SARS-CoV-2 in convalescent plasma (CP) samples by using green fluorescent protein (GFP) encoding lentiviral particles pseudotyped with the Spike (S), Membrane (M), Envelope (E) and Nucleocapsid (N) proteins. Although such an approach is efficient in precisely detecting neutralizing activity, the use of flow cytometry-based data acquisition and analysis for GFP expression presents a bottleneck if a high number of samples are to be processed daily. Alternative approaches include pseudovirus particles encoding Luciferase which requires the use special bioluminescence reader equipment. In this study we aimed to increase the throughput of our previously developed method with colorimetric measurements using simple Optical Density readings. For this purpose, we produced lentiviral vector-based SARS-Cov-2 pseudovirus particles encoding Secreted Embryonic Alkaline Phosphatase (SEAP). Detection of SEAP activity in cell culture supernatants proved faster and easier than Flow Cytometry based analysis of GFP expressing cells. Optimized SEAP-based assays showed the best correlation (R^2>0.95) to GFP-based pseudovirus at 90 minutes of incubation time with supernatants from ACE2 expressing 293FT cells with the QuantiBlue substrate. Of note, a medium change at 24 hours following viral transduction was found to be necessary for preventing SEAP overaccumulation which may skew the results. Our findings show that the use of optimized SEAP-based methods can increase the availability of neutralizing assays by overcoming the need for advanced equipment and increase the processing speed of multiple samples in a given lab.

Keywords: Immunological techniques, infectious disease, monitoring immunity, viral infections

OP-280

Increased inflammatory parameters, chemokines and chemokine receptors in obese children

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Obesity represents a state of low-grade chronic systemic inflammation, potentially leading to insulin resistance and endothelial damage, causing metabolic and vascular complications. We aimed to investigate metabolic and inflammatory parameters in obese children, by analyzing laboratory data and peripheral blood chemokine/chemokine receptor profile. Upon Ethical approval, mononuclear cells were isolated from peripheral blood of healthy controls (n=29, 14 M, age 15.46+/-1.51 years) and obese children (n=34, 19 M, age 14.69+/-1.60 years). B-cell (CD19+), T-cell (CD3+) and monocyte (CD14+) frequencies were determined in regard to chemokine receptor expression (CCR2, CCR4, CXCR3 and CXCR4) by flow cytometry. Chemokines (CCL2, CCL5, CXCL10, CXCL11) were determined by LEGENDplex™ and CXCL12 by ELISA. Data were correlated with clinical and laboratory parameters (HOMA-IR, ALT, lipid profile, diastolic blood pressure (DBP), CRP, fibrinogen). Obese children have higher inflammatory markers (CRP, fibrinogen) and parameters of metabolic complications (ALT, LDL-cholesterol, triglycerides, DBP, HOMA IR). Their peripheral blood samples have decrease monocyte expression of CCR4 and CXCR3, and lower concentration of CXCL12. CXCR3+ monocytes showed negative correlation with CRP, fibrinogen, ALT, LDL-cholesterol and HOMA-IR, while CCR4+ monocytes positively correlated with HDL-cholesterol, and negatively with HOMA-IR and triglycerides. We also found correlation between CCR2+ B-cells and HDL-cholesterol, and CCR4+ B-calls and DBP. CCL2, CRP and fibrinogen positively correlated with metabolic parameters. Obesity is associated with systemic inflammation and metabolic complications even in young age. Precise identification of inflammatory molecules associated with these complications may indicate therapeutic targets for preventing obesity related morbidity and mortality.

Keywords: B lymphocytes, chemokines, inflammatory disease, inflammatory molecules, myeloid cells

OP-281

The distribution of mature and/or immature myeloid cells and their role in effective anti-viral immune responses in COVID-19 positive patients

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The elemination of viral infections is depend on the coordination and balance of pro-/anti-inflammation necessary for viral destruction and immunoregulatory mechanisms necessary to prevent host pathology. Evidence is accumulating that mature neutrophils and inflammatory monocytes are play a critical role in the immune system's antiviral responses Moreover, their immature sub-groups such as MDSCs play unique immunoregulatory roles post-infection, and critical for restoring homeostasis. In our study, we focus on the immunophenotypes, morphology, ROS / NO production, T cell stimulation capacity, and cytokine secretion of miyeloid cells (monocytes and neutrophils) which are in mature and immature stages in COVID-19 disease. Leukocytes were obtained from upper or lower 1.077g/mL ficoll phase and whole blood of COVID-19 positive children and adults (mild, moderate and severe disease). The surface molecules (CD45,CD11b,CD66b,CD15,CD14,CD33,CD14,CD16,HLA-DR,CD114, CD62-L,Lox-1,PD-L1,PD-L2,CD80,CD86,CD25,CD154 CD69) were studied with flow cytometry. Dichlorodihydrofluorescein diacetate(DCFDA) and 4,5-diaminofluorescein diacetate(DAF-2 DA) were used to measure ROS and NO production by myeloid cells, respectively. Carboxyfluorescein succinimidyl ester(CFSE) for proliferation analysis of CD4+ or CD8+ T cells obtained from healthy donors in the presence of myeloid cells from COVID-19 positive patients. Haematoxylin-eosin staining of blood smears were used for confirm myeloid cells maturation stages. Cytokines from co-cultures were measured with LEGENDplex (IL-4 IL-2,CXCL10 (IP-10),IL-1β,TNF-α,CCL2(MCP-1),IL-17A,IL-6 IL-10,IFN-γ,IL-12p70,CXCL8 (IL-8),TGF-β1. Percentage and composition of mature or immature myeloid cells were correlated with good or poor prognosis of COVID-19.

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Keywords: Immune networks, innate host defence, innate immunity, myeloid cells, myeloid derived suppressor cells, viral infections

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WORKSHOPS

OP-282

The influence of HLA genotype on the severity of COVID-19 infection

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HLA plays a pivotal role in the immune response to pathogens so HLA variation may be associated with SARS-COV-2 infection. We aimed to assess the association between HLA allele frequency distribution in a group of 72 patients affected by mild and moderate/severe form of COVID-19. HLA-A, -B, -DRB1 typing in 72 COVID-19 patients (males/females: 41/31) and 300 healthy controls by using Sequence-Specific Oligonucleotide (SSO) method were performed. Patients were divided into two groups: Moderate/Severity Group (n=48, males/females: 29/19) included patients who were required hospital care and mild group (n=24, males/females: 12/12) patients who did not have any symptoms or presented with mild disease. The most common antigens in patients with mild symptoms (MS) and healthy controls (HC) were HLA-A*02 (MS: 35.4% - HC: 25,3%), -B*35 (MS: 14.6% - HC: 17,2%), -DRB1*04 (MS: 14.6% - HC: 17,2) while in the patients with moderate/severe symptoms were HLA-A*02 (30.2%), -B*35 (18,8%) and DRB1*07 (20.8%). Compared to healthy subjects, there was a decrease in HLA-DRB1*04 (p=0,028) allele, however increase in HLA-B*50 (p=0,004) and DRB1*07 (p=0,012) alleles in COVID-19 patients. Also, higher rate of the alleles HLA-A*33 (p=0,042), HLA-B*44 (p=0,003), HLA-B*57 (p=0,035) in mild group than in moderate/severe group while higher rate of the allele HLA-B*50 (p=0,009) in moderate/severe group was compared to mild group. Our findings suggest that HLA-DRB1*07 and HLA-B*50 may be associated with COVID-19 disease susceptibility, HLA-B*50 with severe infection, and also HLA-A*33, -B*44, -B*57, -DRB1*04 may be protective against the COVID-19 disease as well.

Keywords: Biology of the immune system, MHC and polymorphic genes, viral infections

OP-283

Determinants of susceptibility in mice infected with mouse-adapted SARS-CoV-2

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Infection with SARS-CoV-2 can cause a broad range of outcomes, ranging from asymptomatic to severe disease and the development of fatal SARS-CoV-2 pneumonia. While known risk factors for severe disease are advanced age or obesity, little is known about the immunologic determinants of COVID19 outcomes. We generated a mouse adapted SARS-CoV-2 virus, named maVie16, which enabled us to study the development of SARS-CoV-2 induced pneumonia *in vivo*. To understand mechanisms of disease susceptibility, we studied and compared two widely used mouse strains upon maVie16 infection. As reported earlier, we noticed significant differences in the susceptibility of BALB/c mice and C57BL/6 mice upon infection. While BALB/c mice quickly succumbed to low dose maVie16 SARS-CoV-2, C57BL/6 animals developed transient pneumonia and survived infections with high viral doses. Unexpectedly, susceptible BALB/c animals exhibited a more pronounced early NK cell response and higher lung IFNγ levels than resistant C57BL/6 mice. In contrast, we found a higher abundance of plasmacytoid dendritic cells (pDCs), which are considered essential antiviral effector cells and potent producers of type I IFN, predominantly in lungs of C57BL/6 animals. Our data suggest NK cells as drivers of detrimental hyperinflammation upon SARS-CoV-2 infection and a fine balance between pDCs and NK cells as potential determinant of disease severity.

Keywords: Infectious disease, innate host defence, innate immunity, NK cells, viral infections

OP-326

The protective role of mast cells against neuroendocrine prostate cancer depends on the release of cytokines mediated by intracellular osteopontin

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Fatal neuroendocrine prostate cancer (NEPC) often emerges in patients relapsing after hormone therapies. Besides, de novo NEPC can rarely occur in treatment-naïve patients. Treatment-related and de-novo NEPC have different genomic alterations but share a common transcriptional profile. Investigating the tumor microenvironment, we recently found that mast cells (MCs) accumulate within hormone-sensitive prostate cancer favoring its growth, whereas are excluded by de-novo NEPC both in patients and in the transgenic TRAMP spontaneous mouse model. TRAMP mice backcrossed with MCs-deficient KitWsh mice showed increased frequency of de-novo NEPC. The frequency of de-novo NEPC similarly raised also in TRAMP mice deficient for the matricellular protein osteopontin (OPN). Reconstituting KitWsh-TRAMP mice with wild type, but not with OPN-deficient, MCs lowered the frequency of NEPC to that of untreated TRAMP mice. We found that MCs stain positive for OPN in tumor sections and *in vitro* cultures, but release a tiny amount of OPN in supernatants if compared to NEPC cells. Notably, OPN has both secreted (sOPN) and intracellular (iOPN) forms; the latter can bind to MyD88 and regulate the signaling downstream toll-like receptors (TLRs). *In vitro*, wild type, but not OPN-/- or MyD88-/-, MCs inhibited the proliferation of NEPC cells. Also, in silico analyses showed that genes related to inflammatory response and TLRs signaling are down regulated in human and murine NEPC. Our data suggest that TLRs/MyD88/iOPN-mediated pathways induce MCs to release factor(s) able to restrain NEPC. Further studies are required to molecularly dissect this novel function of MCs, to identify actionable targets against NEPC.

Keywords: Cancer immunology, cellular interactions, cytokines and mediators, *in vivo* tumor models, mast cells, microenvironment

OP-328

Evolution of enhanced innate immune evasion in lung epithelial cells by the SARS-CoV-2 B.1.1.7 variant

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Emergence of SARS-CoV-2 variants, including the B.1.1.7 lineage, suggests viral adaptations to host selective pressures resulting in more efficient transmission. Although much effort has focused on Spike adaptation for viral entry and adaptive immune escape, B.1.1.7 mutations outside Spike likely contribute to enhance transmission. We have previously shown that early SARS-CoV-2 lineages replicate rapidly in Calu-3 lung epithelial cells despite triggering a robust innate immune response through the activation of cytoplasmic RNA sensors RIG-I and MDA5 signalling via MAVS. These inflammatory epithelial responses can mediate paracrine macrophage activation and further enhance myeloid cytokine production. Here, we probed the innate immune activation by B.1.1.7 in comparison to early SARS-CoV-2 lineages. We used unbiased abundance proteomics, phosphoproteomics, mRNA sequencing and viral replication assays to show that B.1.1.7 more effectively suppresses host innate immune responses in lung epithelial cells. We found that B.1.1.7 isolates have dramatically increased subgenomic RNA and protein levels of Orf9b and Orf6, both known innate immune antagonists. Expression of Orf9b alone suppressed the innate immune response through interaction with TOM70, a mitochondrial protein required for RNA sensing adaptor MAVS activation, and Orf9b binding and activity was regulated via phosphorylation. We conclude that B.1.1.7 has evolved beyond the Spike coding region to more effectively antagonise host innate immune responses through upregulation of specific subgenomic RNA synthesis and increased protein expression of key innate immune antagonists. We propose that more effective innate immune antagonism increases the likelihood of successful B.1.1.7 transmission, and may increase *in vivo* replication and duration of infection.

Keywords: Infectious disease, innate host defence, innate immunity, viral infections

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WORKSHOPS

OP-330

3D human skin models of inflammatory skin diseases psoriasis and atopic dermatitis

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Mouse and human skin differ in cellular architecture and physiology which makes it difficult to extrapolate from mouse studies to humans. 3D human skin models provide an approach to bridge the gap between traditional 2D cell culture and animal models. Recently, our group has established 3D human skin equivalents (HSE) that mimic inflammatory skin diseases psoriasis (Ps) and atopic dermatitis (AD). HSEs are generated out of primary human keratinocytes and fibroblasts derived from fore skin. Inflammatory conditions were induced treating HSEs with cytokine cocktails (TH17: IL-17 and -22 (Ps-model) or TH2: IL-4, -5 and -13 (AD-model)) for 3 days during airlift cultivation. Disease phenotype formation was validated histologically and by qPCR. HSEs consist of a cellular dermis and comprise the epidermis with stratified differentiated keratinocytes and a well-developed stratum corneum. The Ps and AD phenotype is successfully induced by treating HSEs with cytokines. Cytokine stimulation results in abnormal terminal differentiation and induces changes in structural protein expression. In both skin disease models a thickening of epidermis is detectable and elafin expression is clearly increased. In the Ps-model expression of further structural proteins including psoriasin, hBD1, hBD2 and involucrin is increased, while involucrin reduction is detectable in the AD model. Inflammatory HSEs mimic *in vivo* changes quite well and can be used to study pathogenesis and treatment strategies. We will further improve the 3D skin disease models by integrating TH1, TH17 or TH2-polarized T cells to more closely simulate the physiological situation.

Keywords: Cellular interactions, inflammatory disease, skin diseases

OP-335

Cellular response of HaCaT keratinocytes in co-culture with *Staphylococcus aureus* in bacterial cellulose-based antibacterial wound dressings

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3D biomaterial studies are constantly being developed with electrospinning, 3D printing or hydrogel designs.They are involved in many fields of study such as disease and organ models, toxicology, and smart drug designs. In addition, these materials produced according to the studies are used as a mechanical support, as well as with many kinds of materials; biopolymer, polysaccharides, polymers,antibiotics etc. The modified structure can be given specific features due to the applications. In our study, *Gluconacetobacter xylinusis* was used for bacterial cellulose production.Because researches show that the cellulose produced by *Gluconacetobacter xylinusis* is purer and the morphological crystal structure changes according to the culture conditions. In this study, the characterization of bacterial cellulose-based scaffolds, including surface morphology, surface chemical properties, thermal stability, degradation behavior, wettability and water holding capacity, was produced by supporting the production of starch solution in various ratios (0.5%, 1% and 2%) with drug loadings has been made. Drug release was investigated over a 120 hour period with a UV-VIS spectrophotometer at a wavelength of 190-400 nm. In addition to the characteristic analyses, cell viability tests, antimicrobial activities in nanocomposite structure, bacteriostatic effect of *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)* by co-culture were tested to reveal this feature. Finally, human keratinocyte cell and *S. Aureus*bacterial cell were co-cultured on the scaffolds to analyze adhesion, proliferation and cell viability. The results suggest that our drug loaded bacterial cellulose drived hydrogel design is promising to be used as a material in tissue engineering and wound healing applications.

Keywords: Cellular interactions, modelling, tissue damage and repair

OP-336

Novel adjuvants that promote the efficacy of R21 malaria vaccine candidate in a mouse model

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A milestone on the path to controlling malaria infection was recently achieved, with vaccine candidate R21 in Matrix-M adjuvant (R21/MM) demonstrating 77% efficacy in field trials. Here, we present a panel of non-proprietary highly immunogenic adjuvants resulting in protective efficacy comparable to that of R21/MM. The R21 vaccine was combined with adjuvants based on either emulsified squalene oil (S) or liposomes (L), containing saponin immunostimulant QS-21 (Q) and, optionally TLR4 agonist 3D(6-acyl)-PHAD (M), and tested in a well-established malaria mouse model. Following intramuscular prime-boost vaccination, these R21/adjuvant formulations induced sterile protection ranging from 40-80%. The best performing adjuvants, SQ and LMQ matched the efficacy of Matrix M in the same model system. All adjuvants strongly increased the total IgG levels compared to R21 alone. Although IgG titre and avidity were comparable across the adjuvants, overall survival correlated with total IgG titre compared to mice that succumbed to malaria ($p<0.01$). Interestingly, SQ induced an IgG1-dominated response, while LMQ showed a more balanced IgG subclass profile with increased levels of IgG2a, IgG2b, and IgG3. LQ- and SMQ-adjuvanted R21 induced an intermediate profile of subclasses. We demonstrate that these adjuvants shape the antibody response both quantitatively and qualitatively. Broadening the antibody profile could be a prerequisite to enable protection per se, as unadjuvanted R21 induced only IgG1 responses. Our goal is to fully characterise the mode of action of these promising new adjuvants and identify potential correlates of protection in order to facilitate future mechanistically-driven vaccine design for other antigens and disease indications.

Keywords: Adjuvants and vaccines, antibody, B lymphocytes, infectious disease, protection

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WORKSHOPS

TRACK 4 - INNOVATIVE TECHNOLOGIES AND IMMUNOTHERAPIES

OP-178

Combination therapy of CAR-NK-cells and anti-PD-1 antibody results in high efficacy against advanced-stage glioblastoma in a syngeneic mouse model and induces protective anti-tumor immunity *in vivo*

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Checkpoint inhibitors as well as adoptive cell therapy hold great promise for cancer therapy. Encouraging treatment responses have already been demonstrated. Glioblastoma (GB) is the most common and aggressive primary brain tumor. Standard therapy has very limited. Analysis of the tumor microenvironment (TME) has shown prominent immunosuppressive features including expression of PD-L1 on tumor cells. While the surrounding brain is HER2-negative, GB tumors are frequently HER2-positive, suggesting HER2 as a promising target for adoptive immunotherapy. Previous results from mouse glioma models showed efficacy of CAR-NK cells directed against HER2 as monotherapy in small tumors, but not in advanced-stage tumors. The murine glioma cell line GL261 was transfected with HER2. Tumor cells were implanted into C57BL/6 mice and treated with HER2-specific CAR-NK cells and anti-PD-1 antibody. Effects on tumor growth and survival were determined. Lymphocyte infiltration and immunosuppressive TME were characterized via high-dimensional analysis methods. Combined treatment with CAR-NK cells and anti-PD-1 checkpoint blockade resulted in tumor regression and long-term survival of advanced-stage tumor bearing mice. Analysis of TME showed enhanced T cell infiltration and altered profiles of exhaustion markers in tumor and immune cells, leading to altered TME after combined treatment with CAR-NK cells and anti-PD-1 antibody. These data demonstrate efficacy of CAR-NK cells in combination with checkpoint blockade, resulting in successful treatment of advancedtumors. Moreover, the combination therapy induces a cytotoxic rather than immunosuppressive TME. Further, we are preparing a combination therapy cohort as part of our phaseI clinical study (NCT03383978).

Keywords: Cancer immunology, immunotherapy, *in vivo* tumor models, microenvironment

OP-284

Protective long-term effects of mesenchymal stromal/stem cell-based therapy in experimental colitis is mediated by induction of an innate immune memory-like response

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Mesenchymal stem/stromal cell (MSCs) therapy is an innovative cell-based therapeutic approach for treatment of patients with inflammatory bowel disease thanks to their capacity for modulating unwanted inflammatory immune responses. Among the mechanisms participating in the inhibition of intestinal inflammation, the induction of regulatory innate and adaptive immune responses has been involved in the beneficial effects of MSC-based therapy. In this study, we aimed to analyse the long-term effects of MSC-based therapy using an experimental model of colitis. For this purpose, two dextran sulfate sodium (DSS)-cycles three months apart were carried out in C57BL/6J and in RAG-1-deficient mice. A single dose of adipose-derived MSCs (ASCs) was infused intraperitoneally during the 1st DSS cycle. The disease activity index was significantly reduced in ASC-treated colitic C57BL/6J and ASC-treated colitic RAG-1-deficient mice during the 1st and 2nd DSS cycles in comparison to untreated colitic C57BL/6J and RAG-1-deficient mice. Increased levels of $Ly6G^+CD11b^+$ myeloid cells were noticed in the peritoneal cavity, spleen and bone marrow in ASC-treated colitic mice with respect to untreated colitic mice three months after the 1st DSS cycle. Strikingly, during the 2nd DSS cycle, an increase of $IL\text{-}10^+F4/80^+Ly6G^+CD11bdim$ cells and a decrease of $GM\text{-}CSF^+TNF\alpha^+IL\text{-}6^+IFN\gamma^+Ly6G^+/dimCD11b^+$ cells were observed in colon lamina propria in ASC-treated colitic mice compared to untreated colitic mice. These findings suggest that MSC-based therapy can imprint an innate immune memory-like response in mice that protects to recurrent inflammation in the gut in the long-term.

Keywords: Animal models, cell based therapies, immune regulation and therapy, inflammatory bowel disease, memory, myeloid cells

OP-285

Targeting glycosaminoglycan-CCL2 interactions prevents the immunosuppressive polarization of myeloid cells and provides an anti-tumor immune response

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Myeloid cells are guided to the tumor site by solid-phase gradient of chemokines bound by glycosaminoglycans (GAG) on endothelium and extracellular matrix. Being recruited to the tumor microenvironment, myeloid cells significantly contribute to cancer progression via stimulation of angiogenesis and metastatic spread and suppression of adaptive immunity. In murine models of oropharingeal carcinoma and melanoma, we observed the attraction of myeloid cells to the tumor tissue in CCL2-dependent manner and induction of PDL1 proportionally to the CCR2 expression, indicating the significant role of CCL2-CCR2 axis in regulation of immunosuppressive properties of myeloid cells. The analysis of available transcriptome data of human head and neck cancer and melanoma tissue samples confirmed the significant association between CCL2-CCR2 and PDL1-PD1 axis, proving the clinical relevance of the model. We developed C-terminal 65-76 peptide fragment of CCL2 blocking GAG-CCL2 interactions and thus disturbing the guiding chemokine gradient for myeloid cells. The treatment demonstrated excellent safety profile and significantly suppressed tumor growth. Moreover, it was associated with inhibited migration of dendritic cells and macrophages to the tumor site and prevented the upregulation of PDL1 on them, what correlated with decreased amount of T cells with exhausted phenotype (PD1+) at the tumor site and lymph nodes and with decline in expression of IL10 and IFNg as possible mediators of immunosuppression.

In the light of still missing efficient anti-cancer approaches based on the modulation of myeloid cells activity, treatment with GAG-interacting peptides, such as CCL2(65-76), may become a promising therapeutic target to suppress tumor growth and spread.

Keywords: Cancer immunology, chemokines, drugs for immune modulation, immune regulation and therapy, myeloid cells

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WORKSHOPS

OP-286

Personalized molecular portraits of systemic lupus erythematosus patients as key for prognosis and therapeutic decisions

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Systemic Lupus Erythematosus is a complex autoimmune disease that leads to important worsening of the quality of life and significant suffering to those affected. Currently, therapies used are partially inefficient, mainly due to the molecular heterogeneity of the disease, being personalized medicine the big promise for the future of autoimmunity. With this work we intend to take a step further in that direction by developing MyPROSLE, a system capable of measuring the molecular portrait of individual patients.

We defined co-expressed and functionally annotated gene-modules conserved across two longitudinal datasets with 158 and 301 patients. The dysregulation magnitude for each gene-module was calculated at patient level using averaged z-scores. We analyzed the association between gene-modules, clinical manifestations and the evolution of the disease by ANOVA, Student's t-test and Cox proportional-hazard models. Drug response to hydroxychloroquine and mycophenolate was analyzed comparing molecular portraits. A third dataset of 1760 patients was used to measure the response to Tabalumab.

The system allows to quantify the dysregulation of 30 functional gene-modules in individual patient with respect to healthy distributions. We show that dysregulation of certain gene-modules is strongly associated with different clinical manifestations and with predicting the time when remissions and relapses of the disease are to occur in the short time. We also demonstrate how the analyzed drugs act specifically on patients with gene-modules related with dysregulated plasma cells.

MyPROSLE allows to extract information from the patients useful for medical practice and may be a support for more precise therapeutic decisions in the future.

Keywords: Autoimmunity, big data, RNAseq

OP-287

Patient-specific cellular models for Sézary syndrome immunotherapy

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Sézary Syndrome (SS) is an aggressive leukemic form of cutaneous T-cell lymphoma characterized by circulating malignant CD4 T lymphocytes (Sézary cells). Patients with SS have poor prognosis and current treatments show high rates of relapse. Thus, there is an unmet need for an efficient treatment. Sézary cells have unique clonal potentially targetable epitopes, including its TCR, and TCR-, neoantigen- and/or overexpressed proteins-derived HLA-restricted peptides, which we aim to target. However, SS patient-specific cellular models are lacking for screening and targeting purposes, as SS cells do not grow *in vitro* or in mouse models as compared to healthy CD4+ lymphocytes. Our specific aim is thus to develop and validate long-lasting SS patient-specific cellular models. Pure SS cells and non-SS cells were sorted using CD4 and PD-1 and expanded *in vitro* using different immortalization procedures, such as exposure to HTLV-I, HVS, a telomerase vector or irradiated allogeneic feeder cells (PBMC and EBV-B cells from different donors). Feeder SS and non-SS cell lines were observed to grow rapidly, but SS cells' phenotype changed by flow cytometry. A TCR monoclonal antibody screening revealed that UCHT-1, HIT3a, SK7 (anti-CD3), IP26 (anti-TCRα/β) and JOVI.1 (anti-TCR Cβ1) had a dull expression on SS compared to non-SS cells. Only RW2-8C8 (anti-CD3) was bright on SS but not in non-SS cells. Other immortalization procedures are currently under study. We have thus obtained a patient-specific SS feeder cell culture, and found RW2-8C8 to be SS-specific. This cell line is a valuable tool to test SS-patient-specific immunotherapeutic reagents in preclinical settings.

Keywords: Biomarkers, cancer immunology, immunotherapy

OP-288

Avidin-based universal CAR regulatory T cells (UniCAR-Tregs): a versatile approach for future immunotherapy

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A chimeric antigen receptor (CAR) is a synthetic protein composed for an extracellular region which recognizes antigens bound to an intracellular region that promotes T cell activation. To overcome the limitation that new constructs must be designed for each new target, a novel CAR strategy based on the biotin-avidin union has been developed. First, the desired antigens are recognised by biotinylated intermediaries, such as biotinylated antibodies. This biotin is then localized by the avidin-CAR (termed Universal CAR or UniCAR). Thus, a CAR construct can be used for numerous targets, simply by changing the intermediate. Moreover, UniCAR could be used against autoimmune diseases, utilizing regulatory T cells (Treg). This T cell subset maintains the immune homeostasis by inhibiting activated effector cells. In our group, we extract a large quantity of naïve Treg from thymic tissue (thyTreg), an alternative source of Tregs. Hence, the aim of this research was to combine the inhibitory power of thyTreg with the incalculable potential of the UniCAR technology. To study if UniCAR construct was functional, we have transduced thyTreg and peripheral blood lymphocytes (PBLs) with lentiviral vectors encoding for UniCAR. Here, we showed that UniCAR construct could be used in effector T cells and thyTreg without modifying their phenotype nor function stability. Moreover, UniCAR-effector cells respond specifically under the correct biotinylated antibody and the desired antigen, respecting control conditions. To sum up, UniCAR-thyTreg cells are phenotypically stable and specifically functional. Therefore, UniCAR-thyTreg could treat a large quantity of autoimmune diseases, simply by changing the biotinylated intermediates.

Keywords: Cellular interactions, immune regulation and therapy, regulatory cells, thymic selection

OP-289

NK cells release subsets of extracellular vesicles with distinct profiles and tumor-targeting abilities

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Natural killer (NK) cells can broadly kill malignant cells, and their utility for cancer immunotherapy has been shown for hematological malignancies. However, they have a low capacity for eradicating solid tumors due to a low capacity for tumor infiltration. NK cell-based nano-therapies, including NK cell-derived extracellular vesicles (NK-EVs), could be a promising tool for targeting solid tumors. Indeed, NK-EVs that contain cytolytic proteins and tumor-targeting molecules can directly interact with and kill malignant cells. They may also be more efficient at extravasating into the tumor tissue and be less sensitive to the hostile tumor microenvironment. We have isolated EVs from the NK-92 cell line and found that there are at least two distinct populations of NK-EVs based on the difference in density, ranging from 1.09-1.11 g/ml and 1.11-1.14 g/ml. The proteomic analysis supports that these two subpopulations contain different EV functional cargos, which cause cell death of solid tumors, including colon cancer, *in vitro*. According to the EV-associated markers we identified, distinct NK-EV biogenesis pathways may separately contribute to these two EV populations, to mediate death receptor-mediated and/or granule-dependent cytotoxicity. To conclude, we have discovered two functional subtypes of NK-EVs that will be exploited as therapeutic agents against cancerous cells.

Keywords: Cancer immunology, cell based therapies, endo- and exocytic vesicles in immunity, immunotherapy, molecular immunology, NK cells

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WORKSHOPS

OP-290

Selective elimination of allergen-specific B lymphocytes in chronic mouse house dust mite allergy model

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Der p1 is a major allergen of *Dermatophagoides pteronyssinus* (Dpt) which causes house dust mite (HDM) allergy. The production of allergen-specific IgE antibodies by the pathological Der p1-specific B cells mediate most of the hypersensitivity allergic reactions.It may be possible to influence Der p1-specific B cells by administration of chimeric molecule in a mouse model of HDM allergy, containing the 2.4G2 monoclonal antibody which targets the B cell inhibitory receptor FcγRIIb conjugated to a B cell epitope-carrying peptides from the Der p1 molecule. Co-crosslinking of the FcγRIIb receptors and the immunoglobulin receptors by this molecule is expected to deliver higher affinity and suppressive signal selectively silencing these B cells and the subsequent allergic response. Protein engineering, FACS, animal models, ELISpot. The synthetic peptide Der p1 p52-71 and 2.4G2 monoclonal antibody were used for the construction of the protein engineered chimeric molecules, which bind to the inhibitory FcγRIIb receptor on murine splenocytes. A chronic mouse HDM allergy model was established. The chimeric molecules reduce the number of IgE anti-Dpt antibody-producing plasma cells from splenocytes of allergic mice *in vivo.* Allergen-driven proliferation of B and T cells was also reduced in the presence of chimera. The present study explores a different approach for suppression of the pathological Dpt-specific B cells. Targeted elimination of these B cells reduced the number of allergen-specific plasma cells, lead to reduction of allergen-induced lymphocyte proliferation and might result in silencing of the allergic immune response.

Keywords: Allergen-induced immune responses, allergic disorders, animal models, B lymphocytes, engineering of antibodies and nanobodies, immune regulation and therapy

OP-291

Aromatic ketoacids represent novel therapies for inflammatory diseases through activation of the Nrf2/HO-1 pathway and suppression of pro-inflammatory responses in primary human immune cells

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Trypanosoma brucei (*T. brucei*) are parasites best known for causing fatal human sleeping sickness. It has long been noted that Trypanosomiasis is accompanied by the excretion of high levels of trypanosome-derived ketoacids into the host's bloodstream. The aim of this study was to investigate the impact of trypanosome-derived ketoacids on immune-cell fate and function, using a wide range of methods including flow cytometry, western blotting, qPCR, ELISA, FLIM and Seahorse. We demonstrate that *T. brucei* strongly upregulates the stress-response protein Heme Oxygenase 1 (HO-1) in primary murine glia and macrophages *in vitro*. Furthermore, using a novel AHADHin *T. brucei* cell line, we demonstrate that specific aromatic ketoacids secreted by *T. brucei* are potent drivers of HO-1 expression in both glia and macrophages. We also report that *T. brucei* ketoacids are capable of inducing HO-1 in human dendritic cells (DC). Additionally, we present data to support Nrf2 activation as the mechanism of action by which these ketoacids upregulate HO-1 expression. We demonstrate that these ketoacids show immunomodulatory properties in DC by limiting maturation and suppressing production of pro-inflammatory markers, skewing the differentiation of pathogenic T helper cell subsets. Finally, we show that ketoacids are capable of modulating DC cellular metabolism, favouring oxidative phosphorylation. This study therefore reports a novel immune-evasion mechanism potentially employed by *T. brucei* to suppress the host immune response, via induction of the Nrf2/HO-1 pathway. Furthermore, the *T. brucei*-derived ketoacids investigated also represent a new class of HO-1 inducer with therapeutic potential for the treatment of inflammatory conditions.

Keywords: Autoimmunity, dendritic cells, inflammatory bowel disease, molecular immunology, parasite infections

OP-292

Immunotherapy with apitopes® blocks the immune response to S-antigen in HLA-DR transgenic mice

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Uveitis describes a group of diseases associated with inflammation of the inner eye. The cause can be either infectious or autoimmune. Autoimmune uveitis is mediated by autoreactive T and B lymphocytes responding to several autoantigens, including S-antigen (S-Ag, S-arrestin or retinal arrestin). Local inflammation will lead to tissue damage and, when left untreated, can lead to blindness. Present treatments for uveitis include the use of glucocorticoid steroids and other immunosuppressive agents. However, these are not able to interrupt the autoimmune processes in uveitis. In contrast, antigen-specific immunotherapy could be an alternative for long-term cure of autoimmune uveitis. We designed a peptide-based antigen-specific immunotherapy to specifically re-establish immune tolerance to S-Ag through the development of antigen-processing-independent epitopes (apitopes®). Combining MHC binding assays with immunization in human leukocyte antigen HLA-DRB1*0301 transgenic (DR3tg) and HLA-DRB1*1501 (DR2tg) mice, S-Ag immune dominant peptides were identified. *In vivo* MHC-II loading assays confirmed the trafficking of peptides to dendritic cells in secondary lymphoid tissues of DR3tg and DR2tg mice. These S-Ag-derived peptides induced T cell tolerance toward S-Ag in DR3tg and DR2tg mice analysed *ex vivo*. Furthermore, selected peptides were assessed for their antigenicity using PBMC samples from healthy volunteers, confirming them to be relevant human T cell epitopes. Combining several of the peptides in a mixture further increased the tolerogenic potential and HLA binding profile. These results demonstrate that antigen-specific immunotherapy with apitopes® from S-Ag is a promising approach for the treatment of uveitis.

Keywords: Autoimmunity, immune regulation and therapy, regulatory cells

OP-293

Cytomegalovirus vector expressing NKG2D ligand generates superior CD8 T cell response with distinct phenotypical and functional features

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Vaccination strategies aimed at induction of protective humoral immune response have not successfully tackled the problem of several major infectious agents against which T cells play an essential role in acquired immunity. Vaccines inducing a potent CD8 T cell response represent a promising alternative against these microbial pathogens but also have a great potential to serve as therapeutic vaccines against cancers. Live replicating viral vectors, genetically engineered to express foreign epitopes, can generate potent and long-lasting cellular immunity against both infectious agents and malignant cells. In this respect, cytomegaloviruses (CMVs) represent particularly attractive viral vectors due to their large genome and numerous immunomodulatory genes which can be manipulated in order to modulate their vaccine properties. In addition, CMVs induce strong antigen specific CD8 T cell response with gradual accumulation of these cells in latently infected hosts. We have constructed a murine CMV vector expressing an NKG2D ligand RAE-1γ (RAE-1γMCMV). RAE-1γMCMV proved to be highly attenuated compared to the control vector yet induced and maintained several-fold higher CD8 T cell response to vectored foreign epitope. These epitope-specific CD8 T cells had a terminally differentiated, effector-like phenotype, expressing low levels of Tcf1, CD62L and CD127 and high levels of KLRG1. During priming, CD8 T cells activated with RAE-1γMCMV had much stronger TCR signaling and proliferated more abundantly than cells primed with the control vector. Overall, our studies indicate that small genetical changes of viral vectors can lead to gross differences in CD8 T cell expansion, phenotype, and function.

Keywords: Adaptive immunity, anti-cancer vaccine, *in vivo* tumor models, memory, protection

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WORKSHOPS

OP-294

Investigating the role of multicomponent multitarget drug Tr14 in inflammation resolution using in silico perturbation experiments

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Inflammatory responses are complex defense mechanisms, requiring spatio-temporal processes for resolution and return to homeostasis. In such a complex system with a large number of feedback and feedforward loops, generally changes in a single target cannot generate desired clinical outcomes. Phenotypic reversal can be achieved more efficiently by targeting a large number of regulators together to avoid any bypass for therapeutic intervention. Here, we evaluate the effect of a multicomponent multitarget drug Tr14 on resolution of inflammation using an Atlas of Inflammation Resolution (AIR) (http://air.bio.informatics.uni-rostock.de), a comprehensive resource on acute inflammation initiation, transition, and resolution, recently developed by us. We prepared a drug interactome for Tr14 by connecting bioactive compounds in Tr14 to their biological targets present in the molecular interaction map associated with the AIR. Using the in-silico perturbation tools of the AIR plugins, we modulate top biological targets regulated by Tr14 and predicted the impact on inflammation related processes and phenotypes. We found 242 unique compounds in Tr14 targeting 972 biomolecules in the AIR. Our analyses suggest that PTGS2 and IL6 are top downregulated while CASP3 and BAX are top upregulated proteins by compounds present in Tr14. Furthermore, in-silico perturbation experiments using AIR plugins hypothesize that components in Tr14 may downregulate the prostaglandin and thromboxane synthesis and may upregulate apoptotic process and fibroblast migration to resolve the inflammation. These observations may provide additional support to the mode of action of Tr14 in inhibition of inflammation and activation of resolution.

Keywords: Drugs for immune modulation, immune networks, inflammatory disease, modelling, visualizing immune responses

OP-295

The hidden stage of V(D)J-recombination: examination of the first time discovered human TRB D1-D2 rearrangements repertoires

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VDJ-recombination is considered to be a thoroughly studied mechanism of TCR formation. According to the classical view, TCRß chains are formed in two consequential stages: D-J fusion and subsequent V-DJ recombination. Here we discovered a previously unreported additional stage of VDJ-recombination of the TRB locus: D1-D2 (D-D) fusion occurring before D-J2 recombination. Using genomic DNA from PBMC and target high-throughput sequencing we obtained human repertoires of incomplete TRB D-D rearrangements never described before. Being non-functional passenger genomic variations, D-D rearrangements follow clonal expansions of second functional alleles and form repertoires with similar clonal distribution. We detected ~350 unique D-D clonotypes per 5000 T-cells with a major clonotype (up to 25%) containing completely deleted D1 and D2 segments between RSS heptamers. Non-functional rearrangements evading thymic selection reflect the full set of events occurring during VDJ-recombination which makes D-D rearrangements crucial to understanding of the process. D-D rearrangements can contain one, both, or none of D1 and D2 coding segments. Interestingly, D-D junction length has a bimodal distribution (maximums at 5 nt and 24 nt) which probably indicates the existence of two distinct subsets of T-cells precursors with different activity of VDJ machinery components. Furthermore, the results of matching DD junctions from D-D repertoires and D1D2J junctions from D-J repertoires for the same individuals additionally indicate that D-D rearrangements are not random deadlock events but an important part of VDJ-recombination process. This work was supported by RSF grant №20-75-10091 to A.K.

Keywords: Adaptive immunity, immune development, molecular immunology

OP-296

Liposomal vitamin D3 and retinoic acid induce tolerogenic dendritic cells

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Dendritic cells (DCs) differentiated with vitamin D3 (VD3), or retinoic acid (RA) are now applied in *ex-vivo DC* vaccination trials to foster immune tolerance. Contrasting this cumbersome method, a therapeutic vaccine containing DC-targeting liposomes loaded with anti-inflammatory adjuvants has excellent potential to enhance tolerogenic DCs *in vivo*. We examined monocyte-derived DC (moDC) and dermal DC (DDC) uptake of six different liposomal formulations, together with their DC-modulating effect, screening for an optimal vaccine carrier using flow cytometry and ImageStream. Subsequently, we loaded VD3 or RA in two liposomal formulations. We investigated their tolerogenic effect on moDC-mediated naïve CD4+ T cell priming and DC migration upon injection in *ex vivo* human skin. ImageStream measurement revealed that cationic formulations merely adhered to the moDC membrane instead of being efficiently internalized. Moreover, cationic formulations were internalized less efficiently by DDCs than neutral or anionic formulations. None of the formulations yielded significant DC modulation as determined by the upregulation of maturation markers and cytokine production. However, both liposomal RA and VD3 primed-moDCs induced the development of regulatory CD4+ T cells that inhibited bystander memory T cell proliferation. Strikingly, skin injection of both RA and VD3 liposomes selectively stimulated migration of CD14+ DDCs. RA primed moDCs also prompted the development of IL-10 producing and Foxp3+ CD4+ T cells. These results suggest that anionic liposomes would be more suitable as vaccine carriers for dermal application. Most importantly, liposomal VD3 and RA could be used as tolerogenic adjuvants in a DC-targeting vaccine.

Keywords: Adjuvants and vaccines, allergic disorders, autoimmunity, cell based therapies, dendritic cells, regulatory cells

OP-297

Mucosal administration of polyethylenimine in formulation with SARS-SoV-2 spike elicits potent neutralising responses

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Mucosal adjuvant formulations rarely elicit robust immunity while maintaining a favourable safety/reactogenicity profile. Given the ongoing SARS-CoV-2 pandemic, there is a focus on developing adjuvants suitable for the induction of mucosal immunity, including those compatible with intranasal administration to protect the upper respiratory tract. We compared the use of 100 µg polyethylenimine (PEI) or other commonly used mucosal adjuvants (such as 50 µg CpG or 5% chitosan) in an intranasal SARS-CoV-2 Spike-based regimen in mice. Adjuvantation with PEI resulted in superior Spike- and receptor binding domain (RBD)-specific IgG and IgA titers at the lung, compared with other groups. Antibodies raised in mice given PEI further displayed far greater neutralisation efficacy against the "Victoria" strain virus (NT50 = 1315±509), compared with CpG (566±331) or chitosan (96±33). These finding, in combination with data from a chaotropic ELISA strategy, indicates that PEI promotes the maturation of B cell clones of exceptional affinity that consequently offer a potent neutralisation outcome. Given the central role of antibodies in determining vaccine efficacy against respiratory pathogens such as SARS-CoV-2, these findings provide insight into the utility of PEI as a mucosal adjuvant.

Keywords: Adaptive immunity, adjuvants and vaccines, antibody, B lymphocytes, viral infections

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WORKSHOPS

OP-298

Single-cell RNA sequencing reveals striking heterogeneity of mononuclear phagocytes in bovine mesenteric lymph nodes

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Single-cell RNA sequencing allows for unbiased analysis of cell heterogeneity in complex tissues. We applied 10x Genomics to elucidate the composition of FACS-sorted (CD172a$^+$ and/or Flt3$^+$) mononuclear phagocytes in mesenteric lymph nodes of three adult cows, revealing ten dendritic-cell (DC) clusters and seven monocyte/macrophage clusters with potential functional significance. The most striking finding was the clear transcriptomic separation of CCR7$^+$ migratory DC from resident DC subsets (cDC1, cDC2, pDC), offering exclusive insights into differential gene regulation in highly activated DC *ex vivo*. Furthermore, we found clusters of proliferating or cycling DC, which likely represent DC progenitors giving rise to lymph-node resident DC subsets. Our analyses also revealed a cluster of inflammatory cDC2 with close transcriptional similarity to monocyte-derived DC, highlighting the difficulties to delineate these two cell populations. Monocyte-like cells and macrophage-like cells clustered separately from DC and clearly apart from each other, displaying subclustering that appears to be driven by pro- vs. anti-inflammatory expression signatures. Taken together, our dataset is highly valuable to increase our understanding of the mononuclear phagocyte compartment in bovine lymph nodes, and offers new possibilities to study these cells in steady state and infection.

Keywords: Dendritic cells, innate immunity, lymphoid organs, macrophage, myeloid cells, omics technologies

OP-299

Anti-tumor properties of epitope-specific vaccine in murine model of melanoma

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Cancer is one of the major reasons for human death in the last decade. Different cancer vaccines have been developed for treatment of malignant diseases. A group of potential anti-cancer agents are the hemocyanins - oligomeric copper-containing glycoproteins used so far for therapy of superficial bladder cancer and melanoma. The aim of the present work was the selective suppression of tumor progression in a mouse melanoma model by a chimeric protein vaccine that contains a hemocyanin molecule conjugated to a mimotope peptide structurally resembling the tumor-associated carbohydrate epitope GD3. Murine melanoma cell line B16F10 was used for solid tumor establishment in Balb/C mice. The properties of hemocyanins isolated from the marine snail *Rapana thomasiana* (RtH) and the terrestrial snail *Helix aspersa* (HaH) to be used as carrier-proteins in conjugated vaccines, containing tumor-associated ganglioside mimotope GD3P4 were studied in the developed murine melanoma model. Protein engineering, Flow cytometry and Cytotoxicity assays were also performed. Both protein-engineered vaccines exhibited strong anti-cancer effects in the developed murine model of melanoma. The administration of the conjugates RtH-GD3P4 or HaH-GD3P4 suppressed tumor growth, decreased tumor incidence, and prolonged the survival of treated animals. The immunization of experimental mice induced an infiltration of immunocompetent cells into the tumors and generated cytotoxic tumor-specific T cells in the spleen. The protein-engineered vaccines RtH-GD3P4 and HaH-GD3P4 exhibited strong anti-tumor immune response in the B16F10 murine melanoma model. This study demonstrated a promising approach for cancer treatment with potential applications for cancer vaccine research.

Keywords: Anti-cancer vaccine, immunotherapy, skin diseases

OP-300

Pan-cancer survey of tumour mass dormancy and underlying mutational processes

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Tumour mass dormancy is the key intermediate step between immune surveillance and cancer progression, yet due to its transitory nature it has been difficult to capture and characterise. To understand the genomic and cellular context in which tumour mass dormancy may develop, we comprehensively profiled signals of immune and angiogenic dormancy in 9,631 cancers from the Cancer Genome Atlas and linked them to tumour mutagenesis. We find that immunological and angiogenic dormancy are pervasive, non-exclusive programmes in cancer that can be captured in 16.5% of bulk sequenced tumours, with a frequency of up to 33% in certain tissues. Mutations in the CASP8 and HRAS oncogenes were positively selected in dormant tumours, suggesting an evolutionary pressure for controlling cell growth/apoptosis signals. By surveying the mutational damage patterns left inthe genome by known cancer risk factors, we found that ageing-induced mutations were relatively depleted in these tumours, while patterns of smoking and defective base excision repair were linked with increased tumour mass dormancy. Furthermore, we identified a strong link between APOBEC mutagenesis and dormancy, which comes in conjunction withimmune exhaustion and may partly depend on the expression of the angiogenesis regulator PLG as well as interferon and chemokine signals. Tumour mass dormancy also appeared to be impaired in hypoxic conditions in the majority of cancers. The microenvironment of dormant cancers was enriched in cytotoxic and regulatory T cells, as expected, but also in macrophages and showed a reduction in inflammatory Th17 signals.

Keywords: Big data, cancer immunology, immune senescence, molecular immunology, regulatory cells, RNAseq

OP-301

Proteomic analysis of exosomes highlights their role in specific-signaling pathways' modulation during Leishmania infection in macrophages

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Exosomes are known to play a crucial role in intracellular communications in eukaryotic cells, however their role is not yet fully understood. This form of communication can be exploited by microorganisms like Leishmania in order to establish the infection in the host. Leishmania, a eukaryotic parasite which is the causative agent of leishmaniasis, is introduced to the host through a sand-fly blood meal, depositing the infectious metacyclic promastigote form of the parasite in the skin and eventually enter a variety of host cells, mostly macrophages where the parasite replicates intracellularly during chronic leishmaniasis. Like other eukaryotic cells, Leishmania has been demonstrated to release exosomes, while infected macrophages of the host are believed to have leishmanial protein and mRNA cargo. Aim of this study is to investigate the protein cargo of exosomes derived from cell cultures of infected macrophages with Leishmania infantum, in order to evaluate its potential immunomodulatory role during the infection and its possibility to be exploited as a vaccine against the disease. Upon isolation, exosomes were extensively characterized and subsequently analyzed by LC-MS/MS. An enriched network of leishmanial as well as host proteins participating in key role signaling pathways was emerged during the data analysis. Interestingly, proteins belonging in the host's immunology/inflammation pathways (i.e. IL-17 signaling pathway) were highly enriched in the exosomes derived from the infected mammalian cells. This research is co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH –CREATE –INNOVATE (project code:MIS 5031816).

Keywords: Cell signalling, endo- and exocytic vesicles in immunity, infectious disease, macrophage, omics technologies, parasite infections

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WORKSHOPS

OP-302

Immune reconstitution after autologous hematopoietic stem cell transplantation in multiple sclerosis

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Multiple sclerosis (MS) is an autoimmune disease of the central nervous system, with autoreactive CD4+ T cells and memory B cells as key players. Autologous hematopoietic stem cell transplantation (aHSCT) is a procedure, in which patients undergo chemotherapy and thereafter receive back their own hematopoietic stem cells. This procedure blocks MS disease activity efficiently and long lasting, supposedly by the depletion of autoreactive cells and subsequent renewal of the immune system. We aim to understand how the reconstitution of adaptive immune cells after aHSCT takes place and find immunological correlates explaining the excellent efficacy of aHSCT in MS. Since the approval of aHSCT for MS in Switzerland in 2018, more than 30 MS patients were treated in Zurich and donated biomaterials. In the first 27 patients, we studied immune reconstitution using explorative, multidimensional flow cytometry, T cell receptor (TCR) sequencing and telomere length profiling. 3-6 months after the transplantation, naïve T cells were present at very low numbers, while effector memory (EM) T cells quickly reconstituted to pre-aHSCT levels. Using a variety of approaches, we show that early EM T cells partially consist of carry-over T cells, while the renewal of T cells increases greatly one year after aHSCT. These observations refine our current understanding of immune-renewal by aHSCT in MS. Our data supports a renewal of a majority of the T cell compartment late(r) after the transplantation, while carry-over T cells appear to play a substantial role in the early phase.

Keywords: Autoimmunity, cell based therapies, immunotherapy, multiple sclerosis, neuroimmunology, transplantation

OP-303

Transduction of NK cells with TYPOBP gene increases their functional activity and the NKp44 receptor expression level

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TYROBP gene encodes the signaling adapter protein DAP12, which contains the activation motif ITAM and conducts signals from the activating receptors: activating KIRs, NKp44 and NKG2C. DAP12 is the important signal protein in adaptive NK cells, as these cells have an increased expression of NKG2C. We hypothesized that additional copies of TYROBP gene could enhance the antitumor properties of NK cells: cytotoxic activity and activating receptor expression. In this work, we studied the effect of TYROBP gene overexpression on the phenotype and functional activity of NK cells. The TYROBP gene was obtained from RNA isolated from human lymphocytes and cloned into the retroviral vector pBABE-GFP. Viral particles were harvested using RD114 envelope protein. Before the transduction, human NK cells were stimulated for 7 days with IL-2 and IL-21-expressing K562 feeder cells. After the transduction, NK cells were sorted according to the expression of GFP reporter protein. Analysis of natural cytotoxicity showed a 1.6-fold increase in the level of degranulating cells among GFP^+ NK cells compared to GFP^- NK cells. Besides, GFP+ NK cells had an increased expression of the NKp44 receptor. In GFP-positive degranulating ($CD107a^+$) NK cells, the expression level of NKp44 was approximately 1.5 times higher than in GFP-negative cells. Thus, the overexpression of DAP12 in NK cells led to an increase in their functional activity associated with an increase in the NKp44 expression level.

This work was funded by the grant # 075-15-2020-773 from the Ministry of Science and Higher Education of the Russian Federation.

Keywords: Immune regulation and therapy, NK cells, cancer immunology, immunotherapy, molecular immunology

OP-304

Discovery of subtype specific markers through fuzzy logic and non-parametric approaches using Transcriptomic data in immune related Colon cancer

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The heterogeneity of colon cancer complicates the determination of potential treatments. To characterize the disease despite the heterogeneity, recent studies have focused on the molecular subtyping of the colon cancer. Classification of colon cancer patients with four consensus molecular subtypes (CMS1-4) is a recent approach expected to be approved for clinical practice. This recent classification could contribute to the clinical decision support systems by supporting the determination of treatment approaches through predictionof prognosis, hence increasing the interest on translational studies. In the current study, we proposed two novel approaches, fuzzy-based and nonparametric, to identify molecular markers specific to the CMS of colon cancer. Since CMS1 is characterized by high level of immune infiltration, we also investigated immune related markers for this molecular subtype. The proposed approaches have training and validation phases: In the training phase, we preprocessed raw RNA-Seq data from the TCGA-COAD cohort. For each CMS, we apply fuzzy-based and nonparametric approaches to stratify patients into two groups. Next, we identified subtype-specific genes that can significantly differentiate between the 5-year disease specific survival (DSS) of these groups with Kaplan-Meier analysis and log-rank tests. Cox regression analyses of the most significant genes were used to calculate the subtype specific risk scores for predicted prognosis. We verified our results with a validation cohort. Our results show that each CMS has a distinct gene-set for precise prognosis prediction with low false discovery rates. Overall prognosis prediction with a combined gene set was also highly significant in chemotherapy-naïve colon adenocarcinoma patients.

Keywords: Big data, biomarkers, cancer immunology, omics technologies, RNAseq

OP-305

Probing the importance of valency and spatial organization of immune regulator PD-1 with DNA-based nanomaterials

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Dendritic cells (DCs) regulate immune cell priming by expressing programmed death-ligand 1 (PD-L1) and PD-L2, which interact with PD-1 on activated T cells to suppress their effector functions and induce apoptosis. Tumor cells may also overexpress PD-L1 to inhibit anti-tumor T cell responses. Blocking this inhibitory pathway has provided significant benefit in the clinic, although this applies only to a subset of cancer patients. To improve the efficacy of PD-1/PD-L1 blockade, we need an improved understanding of the interaction between PD-1 and its ligands. It is currently unclear what the importance is of spatial organization and valency of PD-1 or how these parameters affect the efficacy of blocking agents. Here, we capitalized on the high spatial addressability of DNA origami to create nanoparticles that present PD-1 proteins at defined valencies (0,1,3 or 6 proteins) at controlled nanoscale distances and patterns. Surface plasmon resonance showed a strong valency-dependent increase in the binding affinity and kinetics of PD-1-functionalized particles to PD-L1. We also observed remarkable differences in binding of particles with varying PD-1 valencies and patterns to murine tumor-derived DCs (mutuDCs). Finally, we co-cultured mutuDCs with antigen-specific T cells to study the valency- and pattern-dependent efficacy of PD-L1/PD-L2 blockade. These findings suggest that the spatial arrangement of PD-1 has a majorimpact on its ligand interaction and may alter the efficacy of blocking this inhibitory pathway. This work furthermore demonstrates the power of DNA origami as a tool to unravel the importance of multivalent patterns of biomolecules in their interaction with ligands.

Keywords: Adaptive immunity, anti-cancer vaccine, cellular interactions, checkpoint inhibition, dendritic cells, immune regulation and therapy

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WORKSHOPS

OP-306

Antioxidant empowerment enhances antitumor functions of HER2-specific CAR T cells

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Chimeric antigen receptor (CAR) T cells achieved remarkable success against B cell lymphoma and leukemia in clinical studies. Yet, against non-hematopoietic solid tumors the therapeutic efficacy of CAR T cells is very limited, at least in part, due to the immunosuppressive tumor microenvironment (TME). One central cause of immunosuppression is the pro-oxidative micromilieu. In a solid tumor, high levels of reactive oxygen species (ROS) are well-tolerated by tumor cells by increasing the expression of antioxidant genes. This is, however, not the case in T cells, which results in T cell hypo-responsiveness at many stages. Thus, to improve the efficacy of CAR T cells in solid tumors, we aimed to empower the antioxidant capacity of CAR T cells against the pro-oxidative TME. We found that antioxidant-empowerment allowed CAR T cells to strongly retain their capacities for cytolytic immune synapse formation, cytokine release, proliferation, and cytotoxicity under pro-oxidative conditions. To provide molecular insight into these findings, we have employed nCounter gene expression analysis and redoxosome analysis by mass spectrometry. Specifically, the analysis of differentially expressed genes by ingenuity pathway revealed that a pro-oxidative micromilieu downmodulated co-stimulatory and cytokine signals required for antitumor functions of T cells. Furthermore, the redoxosome analysis unrevealed 196 oxidized proteins that were regulated by specific antioxidants. The majority of these proteins were annotated for T cell migration, and effector functions. Taken together, our results provide evidence that antioxidant empowerment can increase the efficacy of CAR T cells against solid tumors.

Keywords: Cancer immunology, cell based therapies, immune regulation and therapy, mass spectrometry, microenvironment

OP-307

Modeling of dendritic cell vaccines for ovarian cancer immunotherapy using cell lines of different molecular profiles

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Dendritic cells (DCs) orchestrate the cytotoxic immune response and serve as an attractive component of cell-based anticancer immunotherapies. DC vaccines have shown promising results in various cancers, including ovarian (OC). Usually, DCs are loaded with allogeneic cancer cell line lysate, mimicking the patient's tumor. However, lysates extracted from different cancer cell line models of the same tumor might have a dissimilar effect on DC maturation and surface protein expression. In our study, we aimed to find out whether unique OC cell lysates have an impact on DCs maturation. We characterized four different EOC cell lines (OV7, SKOV3, COV362, A2780) by their stemness-related gene and protein expression, as well as secretory cytokine profile. We next prepared the OC cell line lysates and used them for maturing the DCs, which were later examined for their maturation surface markers level and expression of genes reflecting immunogenic or tolerogenic properties. Our findings confirmed the diversity of transcriptional and secretory profiles among different OC cell lines. Moreover, despite the unique features of each OC cell line, DCs loaded with the mixture of OC cell line lysates had the highest expression of surface markers CD11c, CCR7, CD80, and best immunogenic-to-tolerogenic gene expression ratio in comparison to DCs loaded with individual cell line lysate. After a thorough analysis of DCs loaded with individual cell line lysates, we distinguished several OC cell line properties, such as high expression of epithelial-to-mesenchymal transcriptional orchestrator SNAIL or multidrug resistance pump ABCG2, that could contribute to a better quality of prepared DCs.

Keywords: Cancer immunology, cell based therapies, cytokines and mediators, dendritic cells, immunotherapy, stem cells

OP-308

Functionalization of tolerogenic dendritic cells with iron oxide magnetic nanoparticles for their magnetic retention

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The T cell immune balance can be disrupted in some pathologies such as autoimmunity. Looking for new therapeutical strategies, it has been proposed the cell transference of *in vitro* generated tolerogenic dendritic cells (tolDCs) in order to restore this disequilibrium. Nowadays, this kind of approaches are been tested in clinical trials with promising results. Nevertheless, we still need to overcome the dispersion of the *in vivo*-administered cells. In our laboratory, we aim to address this problem using a nanobiotechnological perspective. We stablished a reproducible protocol to generate functionally and phenotypically different types of DCs *in vitro* from murine bone marrow precursors: tolDCs, mature DCs (mDCs) and immature DCs (imDCs). In parallel, we synthesized and characterized 12 nm coprecipitation iron oxide nanoparticles coated with (3-Aminopropyl)triethoxysilane (APS-MNPs) and studied their biocompatibility by viability assays and location in tolDCs by electronic and confocal microscopy. Finally, we carried out a flow chamber assay to evaluate their potential in magnetic retention of tol-DC. In comparison with mDCs, tolDCs showed lower levels of MHCII, CD80 and CD86 and higher levels of CD73 and PDL-1. After24h incubation, tolDCs treated with 50 μg/mL of APS-MNPs internalized the nanomaterial inside their cytoplasm showing no significant toxicity and without affecting their ability to induce immunosuppression on T cells from MRL/MPJlpr (murine model of lupus erythematosus). Finally, we demonstrated that APS-MNPs-tolDCs can be magnetically retained with a neodymium magnet. In conclusion, we propose the use of biocompatible MNPs to increase the retention of functional tolDCs.

Keywords: Animal models, autoimmunity, cell based therapies, dendritic cells

OP-309

In vitro results of first in class targeted therapy in uveal melanoma suggest immunostimulatory capacity

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Uveal melanoma is the most common primary ocular malignancy in adults and metastases are the main reason for death. A novel photosensitizer, AU-011, a virus-like drug conjugate, shows enhanced tumor-specificity by targeting Heparan sulfate proteoglycans (HSPG) expressed on malignant tumor cells. We hypothesized that treatment with light-activated AU-011-PDT can enhance systemic immunity. The cellular location of AU-011 in UM cells was evaluated by fluorescence microscopy. The EC50 of eight UM cell lines was measured by flow cytometry. The expression of Damage Associated Molecular Patterns (DAMPs) such as Calreticulin (CRT) and HMGB1 was assessed in three UM cell lines by Elisa and flow cytometry. After 4 hours incubation, most AU-011 had moved from the cell surface inside the UM cell. Toxicity of AU-011 in the dark or light only were minimal. Light activation *in vitro* showed strong light- and sensitizer-dependent tumor cell death across all eight UM cell lines with little variability in EC50. As expected, fluence and AU-011 concentration were the most important parameters influencing cell death. Finally, AU-011-PDT strongly enhanced the CRT membrane-exposure and HMGB1 release. The narrow range of EC50 in a panel of UM cell lines suggests that AU-011 is effective against UM in general. After light activation, all cell lines expressed DAMPs on the cell surface and released them into the extracellular space, indicating a potential to induce antitumor immune responses.

Keywords: Cancer immunology, cell death, immunotherapy

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WORKSHOPS

OP-310

Pembrolizumab drug levels and anti-drug antibodies: measurement in two real-world centres

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The development of anti-drug antibodies (ADA) against anti-TNFα therapy agents is associated with reduced therapeutic response in inflammatory bowel disease. Pembrolizumab (anti-PD-1) is widely used to treat melanoma and other cancers, however there is little data reporting therapeutic drug levels or ADA development. Here we measured drug and ADA levels in patients receiving pembrolizumab, to further understand any potential link with clinical outcomes. Commercially available research assays were utilised to measure 1) Pembrolizumab drug levels and 2) Pembrolizumab ADA. These assays were used on the serum of 31 patients with melanoma from Oxford and Liverpool, UK, at three time-points: 1) Baseline (pre-treatment) 2) Prior to Cycle 2 and 3) Prior to last available sample (median cycle #4). There was no detectable pembrolizumab at baseline, but at time-points 2 and 3 median drug levels were 311 and 275 ng/μL respectively. No correlation was found between drug levels and sex, age, weight or body surface area. ADA were measured in 25 patients, detectable at all time-points. Drug levels decreased for 18/41 patients (58%, median -42 ng/μL, Wilcoxon p=0.001) between time-points 2 and 3. Of these patients, 13 (72%) had ADA at any time-point, with 4/18 (22%) displaying both falling drug levels and increased ADA between time-points 2 and 3 (median +2.90 ng/μL, Wilcoxon p=0.07). In conclusion, pembrolizumab and ADA levels can be readily measured in serum. ADA are common, larger studies with longer follow-up may be required to determine effects on clinical outcome.

Keywords: Antibody, cancer immunology, immune regulation and therapy, immunotherapy, skin diseases

OP-311

Low frequency of differentiated CD3+CD27-CD28- T cells at enrollment predicts a favorable response to CAR T cell therapy in patients with DLBCL

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Chimeric antigen receptor T cell (CART) therapy targeting the B cell specific differentiation antigen CD19 had shown clinical efficacy in a subset of relapsed/refractory (r/r) diffuse large B cell lymphoma (DLBCL) patients. So far it is not known if the composition of recipients' immune-cells could predict clinical responses to CART therapy. In this study blood lymphocytes and serum markers along with clinical data of DLBCL patients scheduled for CART therapy were analyzed to search for biomarkers of response. Compared to healthy controls (n=24), DLBCL patients (n=28) showed significant lymphopenia (1003±940 x106/L versus 1785±478 x 106/L, P<0.001), with a lower CD3+CD4+ T helper and CD3-CD56+ NK cell count, while cytotoxic CD3+CD8+ T cell counts were normal. T cells of DLBCL patients were more activated (HLA-DR+: 294±271 x106/L versus 113±116 x 106/L, P<0.004) and patients showed higher frequencies of differentiated CD3+CD27-CD28- (30.2±18.5% versus 6.6±5.8%; P<0.001) T cells at the time of leukapheresis. Twenty-one patients were infused with CARTs at a median of 76.5 days thereafter, 20 of them could be analyzed for month 3 overall response rate (ORR). Univariate and multivariate regression analyses showed that low levels of differentiated CD3+CD27-CD28- T cells (25.0±19.5% versus 37.8±17.0%) were independently associated with OR (n=12). This association was even more pronounced (P=0.001) when patients were stratified for complete response (CR; n=8; versus non-CR, n=12; CD3+CD27-CD28- T cells: 15.1±11.6% versus 40.1±16.1%). The frequency of differentiated CD3+CD27-CD28- T cells at leukapheresis represents an early assessable marker with potential use for prediction of clinical response to CAR-T cell therapy.

Keywords: Biomarkers, bone marrow transplantation, cell based therapies, immune senescence

OP-312

Intravesical bacterial immunotherapy for bladder cancer can elicit tumor-specific immunity and relies on the capacity of the bacteria to infect and persist in the bladder

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Intravesical BCG therapy for bladder cancer was the first immunotherapy approved by the FDA, and despite being highly effective for preventing tumor progression and recurrence, some patients do not respond and its mechanism of action is incompletely understood. Here, we try to improve immunotherapy for bladder cancer using a different tuberculosis vaccine, MTBVAC, and attempt to unravel the mechanism of action of this kind of immunotherapies. First,using the MB49 orthotopic bladder cancer model, intravesical instillations of MTBVAC cured bladder tumors in 20 out of 29 mice compared to BCG, which cured 4/18 mice. Survivors of this experiments rejected MB49 subcutaneous and intravenous tumor rechallenges, except when CD4 and CD8 cells were depleted. We analysed the immune response during tumor rejection and observed that MTBVAC treated animals had significantly higher CD4 and CD8 responses against endogenous tumor antigens compared to BCG and PBS groups. To find out why MTBVAC was more effective than BCG, we made use of MTBVAC KO strains. A KO in Esat-6 and Cfp10, proteins previously implicated in adherence of M. tuberculosis to the lung epithelium, showed diminished ability to infect the bladder and recruit immune cells, which correlated with a lower efficacy in the MB49 model compared to wild-type MTBVAC, and being comparable to BCG, which lacks these proteins. These findings indicate that interaction between the bacteria and the bladder is a key factor in the mechanism of action of bacterial therapy for bladder cancer, and that by improving this step efficient antitumor immunity can be achieved.

Keywords: Adaptive immunity, bacterial infections, cancer immunology, immunotherapy, *in vivo* tumor models

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WORKSHOPS

OP-313

Importance of antibody isotypes in antitumor immunity by monocytes using human immune tumor models

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Monoclonal antibodies (mAbs) have become the keystone in cancer treatment. Despite the success of mAbs, response rates to immunotherapy still fail for many patients. Therefore, there is a need for new strategies to enhance antitumor immune responses. Accordingly, in this work we explored the most efficient mAb isotype triggering antibody-dependent phagocytosis (ADP) by human monocytes in 2- and 3-dimensional (D) cultures of human CD20+ B cell lymphoma. As mAb model, we used anti-CD20 Rituximab (RTX) in different isotype variants (IgG1-4 and IgA1, 2), alone or in combinations. Our findings show differential efficacy by isotypes triggering tumor cell killing by ADP, being IgG3 the most potent isotype followed by the clinical standard IgG1. The isotype pattern capacity to kill, IgG3>IgG1>IgG4>>IgG2, was similar in 2D and 3D cultures, but 3D tumors showed greater resistance to ADP despite presence of infiltrating monocytes. Interestingly, a 2-fold increase of ADP was achieved in 2D cultures when any RTX isotype was combined with the apoptosis-inducing RTX IgG2 isotype. This enhancing effect of ADP was also evident when tumor cells were treated with staurosporine in combination with RTX. Furthermore, we show that RTX IgA efficiently stimulates ADP of 3D tumors by human primary monocytes, representing an interesting alternative therapeutic isotype. These results support a therapeutic strategy of using different isotypes as well as combining it with apoptosis-inducing agent to improve antibody-based immunotherapy. The 3D tumor model demonstrates additionally a great advantage over 2D models to predict mAbs efficacy, as presenting therapeutic resistance similar to cancer in situ.

Keywords: Antibody, cancer immunology, immunotherapy, myeloid cells, phagocytosis

OP-314

A lateral flow assay to detect neutralising antibodies against SARS-CoV-2

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Even with the unprecedented rollout of vaccines against SARS-CoV-2, a thorough understanding of immunity to the virus will be required for a return to pre-pandemic normality. To this end, the ability to measure protective immunity with rapid turnaround and without the need for laboratory facilities will be of great benefit. Current rapid point-of-care tests measure antibodies against the SARS-CoV-2 virus, however, these tests provide no information on whether the antibodies can neutralise viral infection and, therefore, provide protection. Most current neutralising antibody tests require hours or days, venous blood sampling, and access to a laboratory to perform, all of which may be challenging in resource-limited communities. We have developed a lateral flow test that can measure the level of RBD-ACE2 neutralising antibodies from whole blood, including finger-prick samples, with a result that can be determined by eye semi-quantitatively, or quantitatively on a dedicated instrument. Our point-of-care assay shows a high degree of correlation with the gold-standard microneutralisation assay. We also demonstrate that this test is compatible for use with samples from other species, and that this assay is readily adaptable to test for immunity to newly emerging SARS-CoV-2 variants. Finally, using samples from vaccinated humans, we demonstrate that our test correlates closely with microneutralisation assay data, with a sensitivity of 97%. Thus, we demonstrate a COVID-19 neutralising antibody test that can provide a rapid readout of immunity to SARS-CoV-2 that can be used at the point of care.

Keywords: Antibody, immune response tracing, monitoring immunity

OP-315

A supported lipid bilayer platform to study the impact of bi-specific engager architecture on T cell activation

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Bi-specific T cell engagers (TcEs) are artificial antibodies that represent a promising therapy for cancer. In their basic form, they are fusion proteins of the variable domains (VDs) of two antibodies of different specificity, allowing them to link two molecules of choice. Most commonly, this approach is used to link CD3 on T cells with tumour associated antigens (TAAs), in order to re-direct the cytotoxic activity of T cells to kill tumour cells. However, while a plethora of different TcE formats exist that differ in the spacing of their VDs or their valency for CD3 or TAA, we lack the systematic understanding of how these architectural features influence their ability to activate T cells on the cellular and molecular level. To address these questions, we adapted the classic planar lipid bilayer system, developed to study the immunological synapse (IS) of T cells, to present TAA, allowing for imaging in high spatio-temporal resolution. We show that in the presence of TcEs, T cells rapidly cluster TAA and frequently form highly organized synapses. Furthermore, by using a panel of TcEs with different architectures, we show that minimizing the distance between VDs leads to increased TAA clustering and T cell signalling. We suggest that our system will lead to a deeper understanding of the cellular and molecular events that lead to TcE mediated T cell activation and that it might also serve as a platform to screen large numbers of differentTcEs in a fully controlled setup.

Keywords: Antibody, cancer immunology, immune regulation and therapy, immunological techniques, visualizing immune responses

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WORKSHOPS

OP-316

Using spatial proteogenomics to create a practical cell atlas of the murine and human liver

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Despite being the largest solid organ in the body, the precise cellular composition of the liver and the locations of the distinct cell types within the liver remain unclear. This is true of the murine liver but even more so for the human liver, where to date we lack reliable definitions and markers of specific cell types such as Kupffer cells. To overcome this bottleneck, here we present a spatial proteogenomic atlas of both the healthy human and murine liver. Ensuring all hepatic cells were profiled, we compare the cell types isolated using two different tissue digestion techniques as well as single-nuclei RNA sequencing (snRNA-seq) and demonstrate that while snRNA-seq best recapitulates the cellular proportions observed *in vivo*, a combination of approaches is required to thoroughly investigate all cell types. By incorporating CITE-seq into our analysis, we were able to identify reliable surface markers for all cell types including the CD45- structural cells enabling their purification and further study by flow cytometry. Finally, by integrating these datasets with spatial transcriptomics and proteomics approaches, we provide validated strategies to localize all hepatic cells by confocal microscopy. Moving forward, these relatively cheap flow cytometry and confocal microscopy strategies can now be applied enabling hepatic cellular functions to be assessed in disease settings across multiple patient and/or transgenic mouse cohorts.

Keywords: Biology of the immune system, immunological techniques, myeloid cells, RNAseq

OP-317

Single-cell metabolic profiling of macrophage polarization by fluorescence lifetime imaging microscopy (FLIM)

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Classically activated macrophages (M1) may be characterized through their use of aerobic glycolysis as an energy source, whereas alternatively activated macrophages (M2) show a greater usage of oxidative phosphorylation (OxPhos). Microenvironmental cues can promote polarization of macrophages through metabolic reprograming, therefore impacting function. Due to this, there is a growing interest in real time non-invasive monitoring of macrophage metabolism and morphology. FLIM is a non-invasive technique that allows real-time live imaging of important metabolic co-factors such as free/protein-bound nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FAD). However, a FLIM based metabolic analysis of macrophage polarization and immunomodulation has yet to be clearly established. M1 and M2 activation of primary human macrophages was achieved using IFNγ and IL-4 respectively and verified by PCR and ELISA. Extracellular acidification (ECAR) and oxygen consumption (OCR) ratios were obtained and calculated. Here, M1 macrophages revealed higher dependence on glycolysis whilst M2 macrophages shown a more OxPhos profile. FLIM was performed continuously on the same imaging area while following ECAR/OCR cellular treatments. This uncovered distinct fluorescence intensity and lifetime parameters in M1 and M2 macrophages. In order to observe similarities in single-cell response UMAP was used to group M1 and M2 macrophages based on their FLIM parameters. Inhibiting glycolysis and the mitochondrial respiratory chain yield the highest data clustering. Machine learning models were applied to distinguish the different macrophage populations based only on their FLIM parameters. Our results can help establish FLIM as a non-invasive metabolimaging platform and an alternative to endpoint conventional methods.

Keywords: Macrophage, metabolic control of immune responses, modelling, visualizing immune responses

OP-318

Intratumoral co-injection of the poly I:C-derivative BO-112 and a STING agonist synergize to achieve local and distant anti-tumor efficacy

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BO-112 is a nanoplexed form of poly I:C that acting on TLR3, MDA5 and PKR elicits rejection of directly injected transplanted tumors, but has only modest efficacy against distant untreated tumors. Its clinical activity has also been documented in early phase clinical trials. The DMXAA STING agonist shows a comparable pattern of efficacy when used via intratumoral injections. Mice subcutaneously engrafted with bilateral MC38 and B16.OVA-derived tumors were treated with proinflammatory immunotherapy agents known to be active when intratumorally delivered. The combination of BO-112 and DMXAA was chosen given its excellent efficacy and the requirements for anti-tumor effects were studied upon selective depletion of immune cell types and in gene-modified mouse strains lacking BATF3, IFNAR or STING. Spatial requirements for the injections were studied in mice bearing three tumor lesions. BO-112 and DMXAA when co-injected in one of the lesions of mice bearing concomitant bilateral tumors frequently achieved complete local and distant anti-tumor efficacy. Synergistic effects were contingent on CD8 T cell lymphocytes and dependent on conventional type 1 dendritic cells (cDC1), responsiveness to type I IFN and STING function in the tumor-bearing host. Efficacy was preserved even if BO-112 and DMXAA were injected in separate lesions in a manner able to control another untreated third-party tumor. Efficacy could be further enhanced upon concurrent PD-1 blockade. Clinically feasible co-injections of BO-112 and a STING agonist attain synergistic efficacy able to eradicate distant untreated tumor lesions.

Keywords: Dendritic cells, immunotherapy, *in vivo* tumor models

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WORKSHOPS

OP-319

Toll-like receptor ligand inclusion in GM3-containing liposomes induces potent dendritic cell maturation and T cell responses

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Cancer vaccination aims to (re)activate immunity to cancer cells and can be achieved by delivery of cancer antigens together with toll-like receptor (TLR) ligands to antigen presenting cell (APC). Subsequently, matured APC stimulate CD8+ T cell responses. However, translation from preclinical successes to the clinic proved to be difficult and improving vaccine design may augment its efficacy. We have previously shown that liposomal inclusion of the ganglioside GM3, which is an endogenous ligand for CD169-expressing splenic macrophages, led to robust uptake by these cells and enhanced T cell responses. In this study, we have incorporated ligands for TLR 4 (MPLA) or TLR 7/8 (3M-052), alone or in combination with the oxidized lipid PGPC into GM3-containing liposomes. Here, we show that TLR 4- or TLR 7/8-ligand containing liposomes efficiently mature human and mouse dendritic cells *in vitro* and this is largely dependent on the inclusion of GM3 and its interaction with CD169. Furthermore, we show efficient maturation of mouse dendritic cells after *in vivo* administration. Immunization with GM3 liposomes containing TLR4 or 7/8 ligand and immunogenic peptide efficiently stimulated CD4+ and CD8+ T cell responses. In conclusion, the combination of TLR ligand, GM3 and antigen in liposomes results in a vaccine modality that can stimulate robust immune responses.

Keywords: Adjuvants and vaccines, dendritic cells, immunotherapy, macrophage

OP-320

Drafting an evidence-based immune-related adverse outcome pathway of interleukin-2-induced adverse effects on human lung tissue

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The use of high-dose interleukin-2 (hdIL-2) treatment as an anti-cancer therapy has suffered drawbacks due to severe side effects, including pulmonary toxicity. Still, there is a current interest in using IL-2 as an immunomodulatory therapy. To investigate mechanisms of IL-2-induced effects on lung tissue, an evidence-based immune-related adverse outcome pathway (irAOP) was drafted. To examine if tissue cultures can be used to mimic key events included in the irAOP, human precision-cut lung slices (PCLS) were stimulated with IL-2. Based on extensive literature research, we developed an irAOP taking into account possible cellular key events of IL-2-induced lung toxicity. Human PCLS were stimulated with increasing doses of IL-2 (Proleukin®) and immune responses were investigated by measuring cytokine release and immune cell markers. Toxic effects were analyzed by lactate dehydrogenase (LDH) release. Regarding the irAOP, we found proliferation and activation of NK cells and infiltration of eosinophils and neutrophils, among others, as key events in IL-2-mediated lung toxicity. Granulocyte infiltration might be attributed to secondary secretion of chemokines. In line with published *in vivo* animal studies, proportion of $CD56^+$ NK cells of human PCLS stimulated with hdIL-2 were slightly but significantly increased after five days, along with LDH release. Additionally, several pro-inflammatory cytokines were secreted in a dose-dependent manner, including IFN-γ, IL-5, and IL-13. Human PCLS reflect key events of the irAOP leading to IL-2-induced pulmonary side effects. Future studies will elucidate the impact of NK cell activation and eosinophil infiltration on IL-2-induced lung toxicity to fill knowledge gaps of this irAOP.

Keywords: Cytokines and mediators, drugs for immune modulation, immunotherapy, NK cells

OP-321

Phosphonated cellulose nanocrystals potentiate Th1 polarizing capacity of monocyte-derived dendritic cells

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Phosphonates display great potential for the therapy of primary and metastatic bone tumors. Delivery of phosphonates via nanomaterials could potentiate their efficacy without inducing adverse effects, but the immunomodulatory effects of nanoparticle-phosphonates have not been investigated thoroughly. Here we used cellulose nanocrystals (CNC) for the delivery of phosphonate 3-AminoPropylphosphoric Acid (ApA) (ApA-CNC) and tested its immunomodulatory potential in a model of monocyte-derived dendritic cells (moDCs) *in vitro*. The effects on phenotype and functions of moDCs were compared to the effects of soluble ApA and native (n)CNC. We found that non-toxic doses of ApA-CNC, similarly to the effects of higher doses of soluble ApA, but not lower doses, induced the maturation of moDCs, according to their higher expression of CD86, CD83, HLA-DR, and lower expression of ILT-3, ILT-4, PDL1 and CD73. ApA-CNC treated moDCs produced higher level of IL-1β, TNF-α, IL-6, and IL-12p70 compared to control DCs or nCNC-DCs. In co-culture with allogeneic T cells, ApA-CNC treated mature moDCs increased the relative number of Th1 and cytotoxic CD4 and CD8 T cells, while decreasing the number of Th17, Th2, and regulatory T cells, compared to corresponding control DCs. The stimulatory effects of soluble ApA and ApA-CNC on DCs were partially mediated via GABA-B receptor and downstream regulation of cAMP levels in DC, since the blockage of GABA-B-R with CGP55845A blocked some of the effects. Cumulatively, the delivery of phosphonates via CNC could be a useful platform for potentiation of DC-mediated immunological effects in the treatment of bone resorptive diseases and cancer.

Keywords: Cell based therapies, cell signalling, dendritic cells

OP-322

MOG-derived Imotope™, inducing cytolytic CD4+ T cells and reducing EAE manifestations in mice, as novel antigen-specific immunotherapy for multiple sclerosis

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Imotopes™ are synthetic peptides comprising MHC-II T cell epitope of autoantigen associated in flanking region with amino acid motif having thioredox activity. This study aimed to develop human and murine MOG-derived Imotopes™ and to determine their ability to induce cytolytic CD4+ T cells using PBMCs from MS patients and to reduce clinical MS manifestations in EAE model. PBMCs from MS patients were used to isolate naïve CD4+ T cells. These were co-cultured with autologous APCs loaded with human Imotope™ and periodically re-stimulated. Imotope™ ability to generate antigen specific CD4+ T cells was evaluated by flow cytometry and the cytolytic phenotype of these CD4+ T cell lines was evaluated by APC apoptosis. Mice were immunized with MOG35-55 to induce EAE and were therapeutically treated with murine Imotope™. Clinical scoring was performed daily. Inflammation and demyelination were evaluated on spinal cord sections. Neurofilament levels were quantified in serum samples. Imotope™-induced CD4+ T cell lines were generated from several MS patients. Upon Imotope™ re-stimulation, their specificity was detected by upregulation of CD154 activation marker. These Imotope™-induced CD4+ T cell lines were able to drive Imotope™-loaded APCs into apoptosis demonstrating their cytolytic phenotype. Mice treated with Imotope™ showed strongly reduced MS manifestations, highlighted by reduced clinical scores, inflammation, and demyelination status, as well as serum neurofilament levels. MOG-derived Imotopes™ were demonstrated to induce specific CD4+ T cells with a cytolytic phenotype, and to reduce clinical MS manifestations in EAE model. This promises a new immunotherapy with curative potential for MS patients.

Keywords: Autoimmunity, immunotherapy, multiple sclerosis

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WORKSHOPS

OP-323

Microbial short-chain fatty acids enhance CD8+ T cell-mediated cancer immunotherapy

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Recently, we identified the short-chain fatty acid (SCFA) pentanoate as a low-abundant commensal bacterial metabolite. In this study, the effects of the SCFAs pentanoate and butyrate on CD8$^+$ cytotoxic T lymphocyte (CTL)-mediated anti-tumor immunity have been analysed. SCFAs induced the metabolic reprogramming of murine and human CTLs towards elevated glycolysis by increasing the activity of the mTOR complex. Furthermore, the enhanced cytotoxic capacity of CD8$^+$ T cells was achieved by SCFA-mediated inhibition of class I histone deacetylases (HDACs) promoting production of effector molecules such as perforin, granzyme B, TNF-α and IFN-γ by CTLs. Moreover, we showed that pentanoate treatment led to histone hyperacetylation at the promoter regions of the master transcription factors EOMES and T-bet, highlighting that epigenetic modifications were mechanistically involved in inducing the CTL-associated phenotype. Importantly, the SCFA treatment of murine antigen-specific CTLs enhanced their capacity to reduce tumor growth in both melanoma and pancreas tumor models. Finally, we investigated the impact of pentanoate on chimeric-antigen receptor (CAR) T cells targeting the receptor tyrosine kinase-like orphan receptor 1 (ROR1). Of note, pentanoate was capable of enhancing the anti-tumor potential of both murine and human ROR1 CAR T cells. Collectively, we demonstrate that SCFAs as commensal-derived epigenetic and metabolic modifiers have a therapeutic potential in the context of adoptive cancer immunotherapy.

Keywords: Cancer immunology, cell based therapies, immunotherapy, metabolic control of immune responses, microbiome and environmental factors

OP-324

Non-canonical inflammasome activation mediates the adjuvanticity of nanoparticles via IL-1 and IL-18-driven Th1 and CD8 T cell responses

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Vaccination prevents over 6 million deaths per year globally and saves billions in infection-related morbidity/disability. Subunit vaccines based on purified antigens offer a safer alternative to whole-cell/inactivated formulations but require adjuvants to boost their efficacy. Many adjuvants in licensed vaccines effectively promote antibodies but are poor inducers of cell-mediated immunity (CMI), limiting the efficacy of vaccines against intracellular pathogens and cancer. Polymeric particles are promising CMI-inducing adjuvants, but our incomplete understanding of their mechanisms of action (MOA) limits their clinical development. The non-canonical inflammasome sensor caspase-11 and gasdermin D (GSDMD) drive inflammation and pyroptosis, a type of immunogenic cell death that contributes to CMI during cancer and infection. However, the role of these effectors in adjuvanticity remains unexplored. Here we addressed the design principles that link polymeric particle adjuvanticity to CMI and characterized their MOA. Using a preclinical model of intramuscular vaccination with particles and protein antigens, we identified particle size as a key attribute for CMI induction, and nanoparticles of 50-60nm in size as optimal adjuvants for induction of long-lived Th1 and CD8 responses. Caspase-11 deficiency and pharmacological inhibition of GSDMD impaired these responses, whereas pharmacological inhibition of caspase-1 did not. Using IL-1R1 and IL-18 deficient mice, we revealed a division of labor for these cytokines where IL-1 supported Th1 responses and IL-18 promoted CD8 T cell responses. Our work implicates the non-canonical inflammasome sensor caspase-11 and its effectors in the mode of action of polymeric nanoparticulate adjuvants and establishes size as a key design principle of CMI-inducing adjuvants.

Keywords: Adaptive immunity, adjuvants and vaccines, anti-cancer vaccine, cell death, memory, molecular immunology

OP-325

Immunotherapy-on-chip against an experimental sepsis model

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Lipopolysaccharide is commonly used in murine sepsis models, which are largely associated with immunosuppression (incretion of MDSCs cells and Tregs, imbalance of inflammatory/ anti-inflammatory cytokines) and collapse of the immune system. After adapting the LPS treatment to the needs of locally bred BALB/c mice, the present study explored the protective role of Micrococcus luteus peptidoglycan pre-activated vaccine-on chip in endotoxemia. The established protocol consisted of five daily intraperitoneal injections of 0.2mg/g LPS. Such protocol allowed longer survival, necessary in the prospect of the therapeutic treatment application. A novel immunotherapy technology, the so-called vaccine-on-chip consists of a 3-dimentional laser micro-texture Si-scaffold loaded with BALB/c mouse macrophages and activated *in vitro* with 1μg/ml PG, which exert its action upon subcutaneous implantation. The LPS treatment significantly decreased CD4+ and MHC-II+ cells, while increasing myeloid-derived suppressor cells, and CD25+ cells. These results were accompanied by increased arginase-1 activity in spleen cell lysates and production of IL-6, TNF-a, and IL-18, while acquiring severe sepsis phenotype as defined by the murine sepsis scoring. The *in vivo* application of PG pre-activated vaccine-on chip significantly increased the percentage of CD19+ cells, while decreasing the percentage of Gr1+, CD25+ cells and arginase-1 activity in the spleen of LPS-treated animals, as well as IL-6 and TNF-a in the serum, allowing survival to all animals tested and rescuing the severity of sepsis phenotype. In conclusion, these results reveal a novel immunotherapy technology based on PG pre-activated micro-texture Si-scaffolds in LPS endotoxemia, supporting thus its potential use in the treatment of septic patients.

Keywords: Animal models, immune development, myeloid derived suppressor cells

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WORKSHOPS

OP-327

A novel monoclonal antibody (x-mAb) produced by a newly discovered lymphocyte (DE cells) identifies universal autoreactive T cells that are associated with self-related immunity

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We have recently identified a previously unknown lymphocyte that is a dual expresser (DE) of productively rearranged and surface-expressed TCRαβ and BCR (surface immunoglobulin, Ig) (Ahmed et al, Cell, 2019: 177:11583). Importantly, a single immunoglobulin heavy-chain, IGHV clonotype (clone-x) predominates DEs that encodes a potent autoantigen (x-autoantigen) in its CDR3 region. The x-autoantigen (as a soluble intact x-mAb) cross-activate autoreactive-T cells. The goal of this study is to investigate the properties of x-mAb reactive-T cells and examining the mechanisms of how x-mAb recognizes and activates the tolerant autoreactive-T cells in autoimmune diseases particularly in T1D. We used EBV immortalized DE clone as a source of x-mAb and FACS-based protocols to identify x-mAb-responsive autoreactive-T cells and their functional properties. ImmunoSEQ assay used to characterize TCR repertoires. Preliminary data show that x-mAb potentially binds and activates a subset of autoreactive-T cells in T1D compared to HC subjects. Additionally, x-mAb-reactive T cells exhibits an activated and antigen experienced phenotype, including expression of CD45RO, CD44, and CD69. TCRVβ repertoire analysis shows that x-mAb reactive T cells are enriched for public clonally expanded TCRs in T1D patients. Further, x-mAb activates the autoreactive T cell through by crosslinking directly to their T cell receptor (TCR). DE cells in T1D patients secretes a public x-mAb that binds and activate specific subset of autoreactive T cells predominated by few clonotypes that express public TCRs. Our results are revealing previously unknown mechanism that appears to be a play critical role in pathogenesis of T1D.

Keywords: Adaptive immunity, antibody, autoimmunity, biomarkers, diabetes, immune regulation and therapy

OP-331

From bench to bedside – Clinical therapy with T regulatory cells

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Monotherapy with autologous expanded CD4+CD25highCD127- T regulatory cells (Tregs) or rituximab has been documented to slow disease progression in recent-onset type 1 diabetes mellitus (T1DM) patients. Whether a combined therapy including both drugs would further benefit this patients is unknown. We conducted a trial TregVAC2.0 to explore the efficacy and safety of the combined treatment with Tregs and rituximab in pediatric patients with recent-onset T1DM The analysis included also previous studies testing monotherapies with Tregs or rituximab as monotherapies [TregVac1.0 and TN-05, respectively]. The patients were allocated to 4 groups (aged 5-18 years; N=115): Tregs alone (N=25), rituximab alone (N=34), Tregs+rituximab (N=12), and control (N=44). The key primary efficacy analyses were C-peptide levels (mixed meal tolerance test [MMTT]) and the proportion of patients in remission at 12 and 24 months. At month 24, compared to the control, only the combined therapy remained superior in area under the curve of C-peptide MMTT (1.912; 90% CI, 1.112-3.287). Additionally, the proportion of patients in remission was significantly higher in the combined therapy group (54.5%) than in either the Tregs (20.0%, P=0.048) or rituximab (28.1%, P=0.016) monotherapy group. Although adverse events (AEs) occurred in most (79%) patients, the rituximab group had the highest frequency (p<0.001). No AEs led to withdrawal of the study intervention or death. Over two years, combined therapy with Tregs and rituximab was consistently superior to either monotherapy in delaying T1DM progression in terms of serum C-peptide levels and the maintenance of remission.

Trial registration: EudraCT:2014-004319-35

Keywords: Autoimmunity, cell based therapies, diabetes, immune regulation and therapy, immunotherapy, regulatory cells

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POSTER PRESENTATIONS

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POSTER PRESENTATIONS

TRACK 1 - CELLULAR IMMUNOLOGY

P-0001

Proteostasis in dendritic cells is controlled by the PERK signaling axis independently of ATF4

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In stressed cells, phosphorylation of eukaryotic initiation factor 2α (eIF2α) controls transcriptome-wide changes in mRNA translation and gene expression known as the integrated stress response. We show here that DCs are characterized by high eIF2α phosphorylation, mostly caused by the activation of the ER kinase PERK (EIF2AK3). Despite high p-eIF2α levels, DCs display active protein synthesis and no signs of a chronic integrated stress response. This biochemical specificity prevents translation arrest and expression of the transcription factor ATF4 during ER-stress induction by the subtilase cytotoxin (SubAB). PERK inactivation, increases globally protein synthesis levels and regulates IFN-β expression, while impairing LPS-stimulated DC migration. Although the loss of PERK activity does not impact DC development, the cross talk existing between actin cytoskeleton dynamics; PERK and eIF2α phosphorylation is likely important to adapt DC homeostasis to the variations imposed by the immune contexts. This work was financially supported by Maratona da Saúde and by the project PTDC/BIA-CEL/28791/2017 and POCI-01-0145-FEDER-028791, funded by FEDER, through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI), and by national funds (OE), through FCT/MCTES.

Keywords: Cell signalling, dendritic cells, immune communication, innate immunity

P-0002

Effect of cationic nanostructured lipid carriers on the function of antigen-presenting cells

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Use of nanotechnology to deliver therapeutic nucleic acid such as siRNA or mRNA could open up a new avenue to cure genetic disorders. Lipid nanocarriers currently in use for a number of biomedical applications integrate cationic lipids as carriers to form complexes. Although the use of cationic Nanostructured lipid carriers (cNLCs) to deliver therapeutic nucleic acids is extensive however, due to their small size and highly charged nature, these nanocarriers can interact with antigen-presenting cells (APCs), such as dendritic cells (DCs) and macrophages. As this might prove to be a safety concern for developing therapies based on NLCs, we sought to understand how they could affect APCs activity. We examined the roles of non-activated and LPS or IL-4-activated primary bone marrow-derived macrophages (BMDMs) and dendritic cells (BMDCs), as representative APCs, in response to cNLCs (45.18 nm; +45.8 mV). We monitored the effects of cNLCs on BMDMs and BMDCs by assessing pro-inflammatory molecules, including IL-6, TNF-α, MCP-1, Nitric Oxide (NO), and metabolism (glycolysis and oxidative phosphorylation). In case of BMDMs, we observed cNLCs remarkably enhanced the secretion of several molecules (IL-6, TNF-α, MCP-1, NO) and metabolism in non-activated and LPS- or IL-4-activated BMDMs. Whereas in the case of BMDCs, alteration is restricted for a few secretory molecules (TNF-alpha, MCP-1, NO) and no effect on the metabolism is observed for non-activated and LPS- or IL-4-activated BMDCs. Overall, we conclude that macrophages are the most affected cells and that the cationic nanocarrier has a more substantial impact on their function, than dendritic cells.

Keywords: Dendritic cells, drugs for immune modulation, innate immunity, metabolic control of immune responses

P-0003

***In vitro* effects of recombinant Sars-Cov-2; Spike1 (S1) and nucleocapsid (N) proteins on MRC-5 cell line**

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SARS-CoV-2 proteins, including Spike1 (S1) and Nucleocapsid (N) proteins, have a significant role in binding the SARS-Cov2 virus to ACE receptors causing Covid-19 disease. The recombinant SARS-CoV-2 S1 and N proteins were produced by recombinant technology, at the University of Belgrade, Faculty of Chemistry. Aims of this study are to evaluate the effects of S1 and N protein with and without vitamin D supplementation in cell cultures of Human MRC-5 cell (lung fibroblasts) were examined after 24 and 48 hours of in-vitro incubation at 37 °C in the presence of 5% CO^2 in humid air using MTT assay. The definitive concentration added of S1 protein was in the range of 6.0 $x10^{-2}$ ng/µl to 7.5 $x10^{-4}$ng/µl while for N protein it was 12.00 $x10^{-2}$ ng/µl to 15.00 $x10^{-4}$ ng/µl. S1 protein shows greater effects on cellular toxicity compared to the N protein. The results also show a time dependence effects regarding the release of AST from cells (ANOVA, $p < 0.05$). The increased release of AST enzymes correlated with the increase of Potassium in the supernatants of cell cultures. The presence of vitamin D in cell cultures leads to a decrease in the release of potassium and AST enzyme from cells. These preliminary results may indicate that in cell cultures there are complex interactions between S1 and N proteins with MRC-5 cells and that there is likely to be a stronger binding of S1 protein than N protein to lung fibroblast cells and that vitamin D decrease these effects.

Keywords: Cell death, cell signalling, tissue damage and repair

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POSTER PRESENTATIONS

P-0004

Salt generates antiinflammatory Th17 cells but amplifies pathogenicity in proinflammatory cytokine microenvironments

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Th cells integrate signals from their microenvironment to acquire distinct specialization programs for efficient clearance of diverse pathogens or for immunotolerance. Ionic signals have recently been demonstrated to affect T cell polarization and function. Sodium chloride (NaCl) was proposed to accumulate in peripheral tissues upon dietary intake and to promote autoimmunity via the Th17 cell axis. Here, we demonstrate that high-NaCl conditions induced a stable, pathogen-specific, antiinflammatory Th17 cell fate in human T cells *in vitro*. The p38/MAPK pathway, involving NFAT5 and SGK1, regulated FoxP3 and IL-17A expression in high-NaCl conditions. The NaCl-induced acquisition of an antiinflammatory Th17 cell fate was confirmed *in vivo* in an experimental autoimmune encephalomyelitis (EAE) mouse model, which demonstrated strongly reduced disease symptoms upon transfer of T cells polarized in high-NaCl conditions. However, NaCl was coopted to promote murine and human Th17 cell pathogenicity, if T cell stimulation occurred in a proinflammatory and TGF-β-low cytokine microenvironment. Taken together, our findings reveal a context-dependent, dichotomous role for NaCl in shaping Th17 cell pathogenicity. NaCl might therefore prove beneficial for the treatment of chronic inflammatory diseases in combination with cytokine-blocking drugs.

Keywords: Adaptive immunity, autoimmunity, biology of the immune system, cytokines and mediators, multiple sclerosis

P-0005

Sodium chloride is an ionic checkpoint for human TH2 cells and shapes the atopic skin microenvironment

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The incidence of allergic diseases has increased over the past 50 years, likely due to environmental factors. However, the nature of these factors and the mode of action by which they induce the type 2 immune deviation characteristic of atopic diseases remain unclear. It has previously been reported that dietary sodium chloride promotes the polarization of T helper 17 (TH17) cells with implications for autoimmune diseases such as multiple sclerosis. Here, we demonstrate that sodium chloride also potently promotes TH2 cell responses on multiple regulatory levels. Sodium chloride enhanced interleukin-4 (IL-4) and IL-13 production while suppressing interferon-γ (IFN-γ) production in memory T cells. It diverted alternative T cell fates into the TH2 cell phenotype and also induced de novo TH2 cell polarization from naïve T cell precursors. Mechanistically, sodium chloride exerted its effects via the osmosensitive transcription factor NFAT5 and the kinase SGK-1, which regulated TH2 signature cytokines and master transcription factors in hyperosmolar salt conditions. The skin of patients suffering from atopic dermatitis contained elevated sodium compared to nonlesional atopic and healthy skin. These results suggest that sodium chloride represents a so far overlooked cutaneous microenvironmental checkpoint in atopic dermatitis that can induce TH2 cell responses, the orchestrators of atopic diseases.

Keywords: Adaptive immunity, allergic disorders, cytokines and mediators, environmental factors in autoimmunity and allergy

P-0006

Spatial localization of CD4 T cells in tumors is required for potent CD8-mediated tumor elimination

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Adoptive cell transfer has generated considerable excitement, inducing durable remission in a subset of patients and cancer types, yet most patients still fail to achieve long-term responses: despite the infusion of millions of functional, tumor-reactive effector CD8 T cells, tumor-infiltrating CD8 T cells become dysfunctional and/or are not able to persist in the tumor microenvironment. Therefore, understanding how T cell dysfunction can be prevented or reversed and designing effective therapeutic interventions for the treatment of cancers has become the concerted effort of clinicians and basic scientists. CD4 T helper cells have been shown to be critical for robust CD8 T cell responses during infections, and while studies also demonstrated a role of CD4 T cells in the context of tumors, especially for the priming of tumor-specific CD8 T cells (TST), it is currently not known if CD4 T cells within progressing tumors can reprogram dysfunctional TST and mediate tumor elimination. Using a cancer mouse model with defined CD8 and CD4 tumor model antigens, we show that tumor-specific CD4 T cells indeed can prevent TST dysfunction through transcriptional and epigenetic programming, resulting in the destruction of large established tumors. Strikingly we found that for successful TST reprogramming and tumor elimination, CD4 T cells, CD8 T cells and dendritic cells had to form three-cell-clusters (triads) within the tumor microenvironment, and tumors that were unable to form triads did not result in tumor elimination. Here we reveal a novel mechanism of CD4-CD8 T cell cooperation within established tumors leading to potent anti-tumor immunity.

Keywords: Cancer immunology, cellular interactions, immunotherapy, *in vivo* tumor models

P-0007

Memory like ILC1s during repeated liver damage

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Innate lymphoid cells can also mediate memory-like responses. While the liver seems to be involved in memory responses of NK cells, much less is known about memory-like responses of ILC1 during repeated liver damage. To investigate this, we established a mouse model where we injected 1.6g/kg Carbon tetrachloride (CCl_4) to induce liver damage. Acute liver damage upon one CCl_4 injection was quickly repaired and did not result in changes of liver ILCs. In contrast, repeated liver damage by injecting CCl_4 three times in intervals of one month to allow for recovery of the damage in between injections resulted in an increase of IL-7Rα+ CD200r+ ILC1s as detected by flow cytometry. Liver ILC1s were not only increased in absolute numbers but they also showed enhanced IFNγ production in comparison to control groups (untreated, oil and acute CCl_4 control). This increase of more functional liver ILC1s after repeated liver injury was very transient, as it was most prominent 24 hours after the last injection of CCl4. We did not observe increased staining for the proliferation marker Ki67 of IL-7Rα+ CD200r+ ILC1s, suggesting that the increase is not mediated by proliferation of these cells. We hypothesize that ILC1s migrate to the liver after repeated CCl4 treatment possibly from the gut via the liver-gut axis. Supporting this hypothesis, we found that the IL-7Rα+ CD200r+ ILC1s were also positive for IL-18Rα+ and cKit+. However, the exact origin and the functional role of these ILC1s needs to be clarified.

Keywords: Innate immunity, innate lymphoid cells, memory

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POSTER PRESENTATIONS

P-0008

IL-21 and IFN-α have both opposite and redundant role on human innate precursors and memory B-cell differentiation

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Immunological memory is essential for effective immune protection upon antigen rechallenge. Memory B cells encompass multiple subsets, heterogeneous in terms of phenotypes, origins, anatomical localization, and functional responses. B-cell responses are conditioned by micro-environmental signals, including cytokines. This study aimed at investigating the *in vitro* effects of two cytokines implicated in B-cell differentiation, interferon-alpha (IFN-α) and interleukin (IL)-21, on the early functional response of four sorted mature B-cell subsets (IgD^+ $CD27^-$ naive B cells and IgD^+ $CD27^+$ unswitched, IgD^- $CD27^+$ switched and double negative memory B cells). The dual response of naive and memory B cells to IL-21 allowed us to uncover a unique IgD^+ $CD27^-$ $CD10^-$ B-cell population (referred to as $NARB^+$) characterized by the expression of marginal zone B-cell markers CD45RB and CD1c. Similar to memory B cells, $NARB^+$ cells were in a pre-activated state, allowing them to rapidly differentiate into IgM-secreting plasmablasts upon innate signals. In the presence of IL-21, $NARB^+$ B cells were more susceptible to IL-21-induced apoptosis as observed for the naive compartment and adopted pre-germinal center features. Both in-depth phenotypic analysis of circulating B cells and identification of these cells in different lymphoid tissues, supported that $NARB^+$ cells were uncommitted precursors of human marginal zone B cells.

Keywords: B lymphocytes, cytokines and mediators, memory, microenvironment

P-0009

The role of SLy1 in thymocytes' p53 signaling

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SLy1 is a lymphocyte-specific adapter protein necessary for physiological thymocyte development and efficient infection response. Consequences of SLy1 deficiency for the murine immune system are reduced cell numbers in all lymphatic organs as well as elevated proportions of DN and reduced proportions of DP thymocytes. Moreover, an increased tumor susceptibility in SLy1-KO mice was attributed to increased expression of p53 in NK cells. A NK cell specific p53 deficiency restored NK cell dysfunction. This led to a strong interest in analysing p53 signaling in SLy1-KO T cells. Thymocytes from SLy1KO mice were analysed with qPCR and immunoblot regarding their p53 and DDR expression and phosphorylation. Mice with a T cell specific p53 deficiency (p53fl/fl Lck-Cre+/tg) were crossed with SLy1-KO mice to analyse via FACS how thymocyte development and tumor development is affected by SLy1 expression. p53 and DNA damage response signaling is upregulated in SLy1-KO thymocytes matching the reduced cell numbers previously observed. However, regarding cell number and distribution of thymocyte populations, SLy1-KO had the same effect on mice regardless of p53 deficiency in T cells leading us to the conclusion that SLy1 is not reliant on p53 as an effector. Mice with a T cell specific p53 deficiency showed reduced survival rates and immune cancer phenotypes, with longer survival in p53fl/fl Lck-Cre+/tg SLy1KO mice compared to p53fl/fl Lck-Cre+/tg SLy1-WT mice. Since SLy1 deficiency served as a partially protective factor against early death due to lymphatic tumors in p53fl/fl Lck-Cre+/tg mice, we propose SLy1 as (proto)oncogene for T cell tumors.

Keywords: Animal models, immune development, immunodeficiency, *in vivo* tumor models

P-0010

Autoreactive CD4+T-cell phenotypes and TCR repertoires in ANCA associated vasculitis

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ANCA-associated vasculitis (AAV) is an autoimmune systemic disease affecting kidneys, lungs and ear-nose-throat (ENT). One of the ANCA-autoantibodies targets the enzyme proteinase-3 (PR3), and its presence is genetically associated with HLA-DPB1*04:01 genotype. The combination of the autoantibody (PR3+ANCA) and MHC class II (HLA-DP) implicates CD4+T-cell help in the pathogenesis, still our understanding of autoreactive CD4+T cells in PR3-AAV is limited. Our study included 66 patients: 26 PR3+ cases with active AAV, 21 PR3+ with inactive disease and 19 PR3- with inactive AAV. Antigen-specific responses were studied by flow cytometry and fluorospot. Single-cell sequencing of TCR was done by a bare-coded NGS approach. PBMCs from AAV-patients demonstrated HLA-DP dependent cytokine responses to PR3 protein stimulation. This T-cell autoreactivity was confined to a highly differentiated CD4+ T cell population characterized by cytotoxic features (perforin) as well as surface expression of GPR56. Such cells were most prominent in patients with inactive disease, indicating that during active disease such cells home to affected tissues. This phenotype was shared with T cells specific for human cytomegalovirus (HCMV). Still, PR3 and HCMV reactive T cells displayed distinctly different TCR gene usage. We could identify shared (public) PR3-reactive T-cell clones amongst HLA-DPB1*04:01+ patients. The autoreactive CD4+ T cells, present in both active and inactive disease, implicate chronic antigen exposure and the persistence of long-lived pathogenic T-cell clones. The presence of public autoreactive clones suggests for their active role in pathogenesis of AAV, including the high rates of relapse that so far have been difficult to predict.

Keywords: Adaptive immunity, autoimmunity, immunological techniques, inflammatory disease

P-0011

The impact of sphingosine-1-phosphate receptor type 4 (S1P4)-mediated signaling on peritoneal B cell migration *in vivo*

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Migration of peritoneal B cells (pBC) is regulated by the complex interplay of a plethora of biological mediators, including chemokines and lysophospholipids. Recently, we described a substantial reduction of peritoneal B1 B cell populations in S1P4-deficient (*s1pr4–/–*) mice. In this study, the interaction of S1P4- and chemokine-mediated signaling in pBC was analyzed *in vitro*, and the impact of S1P4-mediated signaling on peritoneal B cell migration was characterized *in vivo*. Transwell migration assays were used to assess the interaction of S1P4- and CXCR4/CXCR5-mediated signaling in pBC *in vitro*. Adoptive cell transfer by the i.p. and i.v. route of *wt* and *s1pr4–/–* peritoneal cells was performed to analyze the impact of S1P4-mediated signaling on pBC trafficking, cytokine, and antibody production *in vivo*. S1P4 synergistically enhances migration of peritoneal B cells to combined gradients of S1P with CXCL12 and CXCL13 *in vitro*. *S1pr4–/–* B1a B cells showed both reduced immigration into and decreased emigration from the peritoneal cavity. Similarly, S1P4 deficiency affected pBC tropism to various secondary lymphoid organs, IL-10 levels, and IgM synthesis. *In vitro* proliferation and viability was similar in *wt* and *s1pr4–/–* animals. These findings suggest that S1P4 is a vital receptor modulating chemokine-mediated trafficking of peritoneal B cells, constituting a potential target to modulate B cell function in inflammatory pathologies.

Keywords: B lymphocytes, chemokines, cytokines and mediators, immune networks

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POSTER PRESENTATIONS

P-0012

Expansion of human peripheral blood NK cells in serum- and feeder-free culture

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Natural killer (NK) cells are effectors of innate immunity that secrete cytokines and kill tumor or virus-infected cells. We have developed a culture system that supports the expansion of NK cells, providing a large cell source for researchers. NK cells were isolated from peripheral blood and cultured for 14 days in serum-free medium in plates coated with an optimized expansion coating material. Cells were passaged on day 7 and day 10/11, then harvested on day 14 for characterization. The average frequency of $CD56^+CD3^-$ NK cells was 84% (range 65 - 96%, n=29), 71% (range 20 - 91%) of which expressed CD16, with an average expansion of 85-fold (range 4 - 409). Expanded NK cells were stimulated with phorbol 12-myristate 13-acetate (PMA) and ionomycin, or co-cultured with K562 cells. As detected by intracellular flow cytometry, the average frequency of interferon-γ^+ among NK cells was 68% (range 41 - 90%, n=7) and 43% (range 12 - 55%) when stimulated by PMA/ionomycin or K562 cells, respectively. Similarly, the frequency of degranulated NK cells ($CD107a^+$) was 81% (range 35 - 97%, n=7) and 64% (range 35 - 97%), respectively. The ability of expanded NK cells to kill target K562 cells was visualized by activated caspase in co-cultures with labeled K562 cells using the Incucyte® imaging system. At an effector:target ratio of 1:1, an average of 50% of K562 cells were killed (range 45 - 56%, n=3). These results show that large numbers of functional NK cells can be expanded under serum- and feeder-free conditions.

Keywords: Innate immunity, immunological techniques, immunotherapy, innate lymphoid cells, NK cells

P-0013

Determinants of liver-resident exhausted CXCR6+ CD8 T cells during persistent viral liver infection

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A vigorous CD8 T cell response is required to control viral liver infections such as hepatitis B virus infection. Persistence of hepatotropic viruses, however, is associated with poor T cell functionality. We aimed at defining the exhaustion-mediating mechanism of liver-resident CD8 T cells during persistent viral liver infection. Naïve virus-specific CD8 T cells were transferred before C57Bl6/J mice were infected using an adenovirus-based model system for ovalbumin or HBV genome expression and induction of a resolved or persistent course of infection depending on viral doses. CD8 T cells were re-isolated on d>30 after infection and analyzed by flow cytometry or sorted for RNA isolation and sequencing for subsequent transcriptome analysis. We identified $CXCR6^+CD69^+$ liver-resident exhausted CD8 T (T-LRX) cells during persistent viral liver infection and $CXCR6^+CD69^+CD49a^+$ liver-resident memory (T-RM) cells as well as CX_3CR1^+ circulating memory T cells (T-EM) after resolved viral infection. Liver-restricted T-LRX and T-RM cells both expressed a tissue-residency gene signature but were fundamentally different concerning cytotoxicity towards antigen-bearing hepatocytes. Strikingly, T-LRX were characterized by a single transcription factor, i.e. Crem (cyclic AMP responsive element modulator) compared to T-RM cells. Induction of cAMP signaling in T-RM cells rendered these cells dysfunctional and generated a phenocopy of T-LRX cells. In conclusion, we describe regional exhaustion of liver-resident CD8 T cells that is governed by the transcription factor Crem.

Keywords: Adaptive immunity, memory, molecular immunology, RNAseq, viral infections

P-0014

Effect of cucurbiturils on phenotypic and functional characteristics of T regulatory cells

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Cucurbiturils (CB) have been used for various applications, including drug incapsulation and delivery. The use of delivery systems is one of the topical areas of modern science. However, a comprehensive study is required for new delivery systems before use in clinical practice, including the study of immunotropic properties of these substances. The aim of this study was to assess the effect of cucurbiturils on regulatory T cells. Peripheral blood mononuclear cells (PBMCs) were isolated from the blood of healthy donors (n=10). Regulatory T cells were defined as $CD3^+CD4^+CD25^+FoxP3^+$. For this analysis, PBMCs were cultivated for three days in the presence of different concentrations of CB (CB6, CB7 and CB8).Then cells were stained with the following antibodies: anti-CD3-FITC, CD4-PE/Cy7, CD25-APC, CTLA-4-PerCP/Cy5.5, PD-1-APC/ Cy7, PD-L1-PerCP/Cy5.5 (BioLegend, USA). Intracellular staining was performed using a set of True-Nuclear Transcription Factor buffers, cells were fixed, permeabilized, followed by intracellular staining with anti-Foxp3-PE antibodies (BioLegend, USA). It was found that CB7 and CB8 had no effect on the frequency of Tregs. However, CB[8] increased the number of PD-1^+Treg. CB6 (0.5 mM) decreased the relative amount of Treg in the PBMCs culture. In addition, 0.5 mM CB6 and 0.1 mM CB6 decreased expression of CTLA-4 in Treg. According to these data, CB6 can reduce immunosuppressive activity by decreasing the number of Tregs and affecting their functional properties.

This study was supported by Russian Science Foundation according to the research project №19-15-00192.

Keywords: Checkpoint inhibition, drugs for immune modulation, regulatory cells

P-0015

Engagement of CD56 can stimulate Natural Killer cell responses

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CD56 is expressed on the majority of human NK cells and is widely used as NK cell marker. The functional role of CD56 on NK cells, however, is not yet fully explored. In a previous study, the stimulation with anti-CD56 antibodies led to the activation of pre-activated, but not resting NK cells. Using CD56 knock-out NK cells we show that this activation through an anti-CD56 antibody was specific. Based on alternative splicing, different isoforms of CD56 such as NCAM120, NCAM140 or NCAM180 can be generated. We did not find any differences in the expression of CD56 isoforms between resting and pre-activated NK cells. However, our data show differences in CD56 glycosylation, which might explain the different responsiveness of resting versus activated NK cells. Furthermore, stimulation of resting NK cells with cytokines such as IL-2 + IL-15 or IL-12 + IL-15 + IL-18 can induce their reactivity for CD56 stimulation. To analyse the downstream signalling of CD56, we treated pre-activated NK cells with several inhibitors against different signalling molecules. Inhibition of Syk, PI3K, Erk and src-family-kinases impaired CD56-mediated NK cell stimulation. To analyse the influence of CD56 on the killing-capacity of NK cells, we performed killing-assays of CD56 knockout or wt NK cells against different tumour cells. The killing-capacity of NK cells was not affected by the absence of CD56, demonstrating that the stimulating effect of CD56 on activated NK cells does not have a major impact on their cytotoxic activity.

Keywords: Cell signalling, innate immunity, molecular immunology, NK cells

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POSTER PRESENTATIONS

P-0016

The role of granzymes in NK cell cytotoxicity

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Granzymes (Grz) play an important role in NK cell-mediated cytotoxicity. While the cytotoxic activity of GrzB is well established, the contribution of the other human granzymes is much less studied. Here we use fluorescent localization reporters to detect the activity of Grz A, B, H, K, and M inside tumor target cells during NK cell-mediated killing by live cell imaging. For the NK92 cell line we could show that these NK cells exclusively use GrzB-mediated killing. However, while GrzB dominated most killing events of freshly isolated or activated human NK cells, we also detected the activity of other granzymes. While we did not detect any GrzH activity during NK cell-mediated killing events, GrzK initiated target cell apoptosis was observed for freshly isolated NK cells. Additionally, we found individual killing events of activated NK cells where GrzA or GrzM activity was dominant. Interestingly, the kinetics of granzyme depletion during target cell killing were similar for GrzA, B, and M, whereas we did not detect any depletion of GrzH, consistent with the absence of GrzH activity in target cells. The reporter specificity was confirmed by CRISPR/Cas9 granzyme KO's which further gave us insights into alternative granzyme-mediated NK cell cytotoxicity. Here we observed a better efficacy for GrzB compared to GrzA, and an almost extinguished cytotoxicity when both were absent. Currently, we are investigating the surface receptor phenotype of NK cell subpopulations with differential granzyme expression patterns to isolate and better characterize their functional activity.

Keywords: Cell death, effector molecules, innate immunity, NK cells, visualizing immune responses

P-0017

Lymph node stromal cells influence the development of chronic intestinal inflammation

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The role of lymph node stromal cells on the T cell immune response regulation during the development of chronic intestinal bowel diseases (IBD) is still unknown. Therefore, in this study the influence of mesenteric lymph node stromal cells (mLNSC) on induction of inflammation was analyzed. IBD development was investigated in B6-Il10-/- mice. Therefore, activation of immune cells as well as mLNSC were measured using flow cytometry, and cytokine expression of SC isolated from inflamed and healthy mice were detected by qPCR. *In vitro* T cells were stimulated with cytokines and proliferation and activation were determined. The impact on LN microenvironment was analyzed using a transplantation model, in which the mLN were exchanged with donor mLN or peripheral (pLN). This study showed that IBD development affects the whole intestinal tract including small intestine and colon draining mLN. Podoplanin+ LNSC showed increased surface expression of MHC class II molecules and a strong upregulation of CD106. Furthermore, the expression of cytokines such as Ccl2, Ccl7 and Cxcl16 was increased in inflamed SC. A gut-homing phenotype and proliferation was altered on T cells stimulated with CCL7 and CXCL16 *in vitro*. Moreover, colitis severity was reduced in mice after pLN transplantation. In conclusion, SC are activated during IBD development and cytokines expressed by inflamed SC can influence T cell activation *in vitro*. We hypothesize that especially the fibroblastic reticulum cells can thus directly activate T cells during inflammation.

Keywords: Biology of the immune system, cytokines and mediators, lymphoid organs, transplantation

P-0018

Education of murine Natural Killer (NK) Cells – cell biological and molecular correlates

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Natural Killer (NK) cells express activating and inhibitory receptors recognizing ligands such as MHC class I molecules expressed on target cells. The Ly49 receptor family is responsible for recognition of MHC I molecules in mice. The NK system adapts to self by rendering cells "hyporesponsive", if they do not receive inhibitory signals from surrounding cells, which means that NK cells cannot attack normal healthy cells even if the latter are devoid of MHC class I molecules. Here, we investigated the correlation between responsiveness and intracellular granule patterns in murine NK cells. NK cells were isolated from splenocytes of C57BL/6 (B6) and B6 β2microglobulin -/- (β2m-/-) mice by immunomagnetic depletion. Granzyme content was measured by confocal microscopy. Surface markers were stained for Ly49C, biotin Ly49I, NK 1.1, CD3. NK cell subpopulations were sorted with flow cytometry, followed by intracellular staining of Granzyme A. Granules in hyporesponsive Ly49C+ NK cells subset are smaller, less intensely stained and more numerous. Preliminary data support the hypothesis. Further analyses investigating additional mouse strains and Ly49I interactions with non-MHC class I ligands will provide more robust evidences about the role of education on NK cells responsiveness.

Keywords: Cellular interactions, immune communication, innate immunity, molecular immunology, NK cells

P-0019

Loss of balance between protective and pro-inflammatory synovial tissue T-cell polyfunctionality predates clinical onset of Rheumatoid Arthritis

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This study investigates pathogenic and protective polyfunctional T-cell responses in rheumatoid arthritis (RA) patient, individuals at risk (IAR) and healthy control (HC) synovial-tissue biopsies and identifies the presence of a novel population of pathogenic polyfunctional T-cells that are enriched in the RA joint prior to the development of clinical inflammation. Synovial biopsies were obtained from RA (n=118), IAR (n=20) and HC (n=44) and RNAseq analysis performed. Single-cell synovial tissue suspensions from RA, IAR and HC and paired PBMC were stimulated in-vitro and polyfunctional synovial T-cell subsets examined by flow cytometric analysis, SPICE visualization and FlowSom clustering. Flow-imaging was utilised to confirm specific T-cell cluster identification. Fluorescent Lifetime Imaging Microscopy (FLIM) was used to visualise metabolic status of sorted T-cell populations. Increased plasticity of Tfh cells and CD4 T-cell polyfunctionality with enriched memory Treg cell responses was demonstrated in RA patient synovial-tissue. Synovial-tissue RNAseq analysis reveals that enrichment in T-cell activation and differentiation pathways pre-dates the onset of RA. Switch from potentially protective IL-4 and GMCSF dominated polyfunctional CD4 T-cell responses towards pathogenic polyfunctionality is evident in IAR and RA patient synovial-tissue. Cluster analysis reveals the accumulation of highly polyfunctional CD4+CD8dim T-cells in IAR and RA but not HC synovial-tissue. CD4+CD8dim T-cells show increased utilisation of OXPHOS, a characteristic of metabolically primed memory T-cells. Frequency of synovial CD4+CD8dim T-cells correlates with RA disease activity. Switch from potentially protective to pathogenic T-cell polyfunctionality pre-dates the onset of clinical inflammation and constitutes an opportunity for therapeutic intervention in RA.

Keywords: Autoimmunity, cytokines and mediators, metabolic control of immune responses, rheumatoid arthritis, RNAseq

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POSTER PRESENTATIONS

P-0020

Transcriptome profiling of porcine naïve, intermediate and terminally differentiated CD8+ T cells

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Most data on CTLs come from studies in mice and humans, while detailed information about porcine CD8$^+$ CTLs characterised as T-cells expressing CD8αβ heterodimers is still sparse. The aim of this study was to analyse three subsets with distinct differentiation stages within the porcine CD8β$^+$ T-cell subpopulation by using high-throughput NGS technologies. CD8β$^+$ T cells were enriched from PBMCs using MACS and further FACS sorted based on expression of CD27 and CD11a. RNA from sorted cells was freshly isolated or after stimulation with ConA and PMA/Ionomycin. Subsequently, transcriptional changes were analysed in the respective groups by NGS. Furthermore, gene ontology enrichment and pathway analyses were performed. Within the CD8β$^+$ T-cell subpopulation three subsets could be defined: naïve or progenitors (CD8β$^+$CD27$^+$CD11alow), intermediate differentiated (CD8β$^+$CD27dimCD11a$^+$), and terminally differentiated cells (CD8β$^+$CD27$^-$CD11a^{++}). The highest number of differentially expressed genes (DEGs) was identified in terminally differentiated CTLs when compared to naïve and intermediate differentiated CTLs. Using Venn diagram analysis, the number of unique and shared DEGs between *ex vivo* CTL subsets could be identified. Genes related to early and late stages of CD8 T-cell differentiation were highly expressed in the naïve and terminally differentiated CTLs, respectively. Overall, a higher number of upregulated DEGs was observed in response to ConA and PMA/Ionomycin stimulation. This study is an important contribution to the characterization of porcine CD8$^+$ T cells of a species with the potential to become a highly relevant preclinical model for human diseases, pharmacological questions and transplantation studies.

Keywords: Adaptive immunity, animal models, big data, omics technologies, RNAseq, veterinary immunology

P-0021

The functional role of Tspan2 in anti-infectious immune responses

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Tetraspanins (Tspans) constitute a superfamily of membrane proteins that contain four conserved transmembrane domains and form lateral associations with partner proteins in characteristic Tspan-enriched microdomains within the cellular plasma membrane. So far, Tspan2 has been mainly characterized in oligodendrocytes, but its role in anti-infectious immune responses remains unclear. The cellular expression pattern, molecular interaction partners, and functional roles of Tspan2 in murine infection models will be investigated. Using Tspan2/EGFP reporter mice we analysed Tspan2 expression via FACS after LPS injection *in vivo* and *in vitro* in GM-CSF and FLT3L driven BM cultures after stimulation with Toll-like receptor (TLR) ligands. To define the subcellular localization we generated mammalian expression vectors encoding a Tspan2-GFP fusion protein. We performed a bacterialkilling assay and measured ROS production from bone marrow (BM) neutrophils of Tspan2-/- and wildtype control mice. The major fractions of Tspan2-expressing cells in the spleen constitute neutrophils, conventional and plasmacytoid DCs, all of which exhibit downregulation of Tspan2 after LPS stimulation. After TLR-stimulation *in vitro* Flt3L-derived plasmacytoid and conventional DCs upregulated Tspan2/EGFP expression while its expression was downregulated in GM-CSF-derived DCs. The subcellular localization of Tspan2 is currently under investigation. Neutrophils from Tspan2-/- mice showed reduced ROS production and a tendency towards reduced killing of bacteria. Our findings indicate an involvement of Tspan2 in supporting neutrophil effector functions. Differential regulation of Tspan2 expression in DC populations after stimulation further hints at its potential complex role in anti-infectious innate immune responses.

Keywords: Innate host defence, dendritic cells, neutrophils

P-0022

Laboratory mice with a wild microbiota generate strong allergic immune responses

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Allergic disorders are caused by a combination of hereditary and environmental factors. The hygiene hypothesis postulates that early life microbial exposures impede the development of subsequent allergic disease. However, unambiguous evidence that microbes reduce the development of allergic disorders is still lacking. Recently developed 'wildling' mice are colonized by a rich and diverse repertoire of microbes at barrier surfaces and come into contact with putative pathogens. Here, we probed the hygiene hypothesis by comparing the development of allergic inflammation in wildlings to that of genetically identical mice lacking diverse microbial exposure. We find that wildlings develop strong allergic inflammation in response to house dust mites, characterized by marked goblet cell metaplasia, immunoglobulin production and a T helper 2 (Th2) cell response of greater magnitude than in conventional laboratory mice. Moreover, type 2 innate lymphoid cells (ILC2) were significantly reduced in wildlings while endogenous Th2 cells were present at higher frequencies and were rapidly activated by the alarmin, interleukin-33. In all, the results suggest that high microbial content and diversity potentiates, rather than restricts, Th2 cell-mediated allergic immune responses.

Keywords: Allergen-induced immune responses, cytokines and mediators, microbiome and environmental factors

P-0023

Impact of IgG Fab-glycosylation on transplacental transfer of antibodies and their binding to the neonatal Fc-receptor (FcRn)

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Fc neonatal receptor (FcRn) is crucial for IgG half-life and transplacental transport. Different sites of IgG carry glycans which may affect binding to FcRn. While the effect of Fc- glycans has been investigated, the impact of Fab-glycosylation (present on ~14% of total IgG) on IgG-FcRn interaction remains unclear. Anti-citrullinated protein antibodies (ACPA) of rheumatoid arthritis patients exhibit a remarkably high Fab-glycosylation (~90%), being an ideal model for investigating the effect of Fab-glycosylation on IgG-FcRn interaction. To investigate the potential impact of IgG Fab-glycosylation on its uptake and transplacental transfer mediated by FcRn. To study transplacental transport of ACPA and total IgG, serum of ACPA-positive RA-patients (mothers) as well as of healthy mothers and their respective newborns was analyzed. IgG Fab- and Fc-glycosylation was investigated with liquid chromatography and mass-spectrometry. The direct effect of Fab-glycans on the affinity of IgG glycovariants for FcRn was studied with surface plasmon resonance (SPR) and FcRn affinity chromatography. Fab-glycosylation of IgG antibodies was ~20% lower in cord blood samples of newborns compared to serum of their mothers. This appeared to be a general phenomenon, observed for ACPA and non-ACPA IgG in RA-patients, as well as for total IgG in healthy controls. When the direct effect of Fab-glycans was investigated, SPR, but not affinity chromatography showed that Fab-glycosylation inhibited IgG-FcRn interaction. Our results suggest that Fab-glycans may inhibit IgG-FcRn interaction and thus negatively affect the transplacental transfer of IgG. This may have potential implications for IgG half-life and pharmacodynamics of therapeutic immunoglobulins.

Keywords: Adaptive immunity, antibody, B lymphocytes, biology of the immune system

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POSTER PRESENTATIONS

P-0024

The effects of few-layer-graphene and other 2D-nanomaterials on human macrophage toxicity

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Graphene and other 2D materials, such as molybdenum disulfide, have been increasingly used in electronics, composites and biomedical research. In particular, MoS_2 and graphene hybrids have attracted a great interest for applications in the biomedical research, therefore stimulating a pertinent investigation on their safety in immune cells like macrophages, which commonly engulf these materials. In our study, M1 and M2 macrophage viability and activation were mainly found to be unaffected by few-layer graphene (FLG) and MoS_2 at doses up to 50 µg/mL. The uptake of both materials was confirmed by transmission electron microscopy, inductively coupled plasma mass spectrometry and inductively coupled plasma atomic emission spectroscopy. Notably, both 2D materials increased the secretion of inflammatory cytokines in M1 macrophages. At the highest dose, FLG decreased CD206 expression while MoS2 decreased CD80 expression. Overall, FLG and MoS_2 are minimally toxic in human macrophages even though they were found to trigger cell stress and inflammatory responses.

Keywords: Cellular interactions, cytokines and mediators, macrophage, phagocytosis

P-0025

The potential role of innate lymphoid cells in COVID-19 patients

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In addition to common symptoms of COVID-19 such as fever, cough, dyspnea, and loss of taste, pneumonia, severe acute respiratory tract infection and renal failure may develop in severe cases. Innate lymphoid cells (ILCs) are divided into 3 groups according to the types of cytokines they produce and the transcription factors. ILCs activate the other immune cells by secreting cytokines and regulate the immune response. The role of ILCs has been demonstrated in the pathology of various diseases such as inflammatory bowel disease, infectious colitis. ILC subtypes in COVID-19 patients at different clinical stages were investigated in this study. Peripheral blood mononuclear cells were isolated from heparinized peripheric blood obtained from 34 COVID-19 patients (11 mild stage, 13 moderate stage and 10 severe stage) and 15 healthy subjects. ILCs were detected with anti-Lineage, -CD45, -CD161, -CRTH2, -NKp44, -c-kit and -CD127 monoclonal antibodies by flow cytometry. Compared to healthy subjects ILC2, ILC3 and NKp44+ ILC3 subset were increased in mild patients and ILC1 and NKp44- ILC3 subset were decreased. Similarly, ILC2 and ILC3 cell subsets were increased in moderate patients. In contrast to mild and moderate patients, no differences were seen in severe patients. Our data showed that ILCs were shifted ILC2 and ILC3 in mild and moderate COVID-19 patients. It is observed that ILC percentages back to normal after treatment in severe patients. Our findings suggest the potential role of different ILC subtypes, particularly in the immune response to the SARS-CoV-2 virus.

Keywords: Infectious disease, innate immunity, innate lymphoid cells

P-0026

The role of Interleukin-18 signaling for intratumoral T cell dysfunction in a murine pancreatic cancer model

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Adaptive immune response in pancreatic cancer is characterized by immune escape mechanisms that render intratumoral CD8+ cytotoxic T cells (CTLs) dysfunctional. This phenomenon, also known as exhaustion, is characterized by impairment of cytokine production as well as upregulation of co- inhibitory receptors such as PD1 and TIM3, resulting in loss of CTL effector function. Proinflammatory cytokines, like IL-18, might play an important role in the induction of this dysfunctional state. Here, we investigate the role of NLRP3 mediated IL-18 signaling on cytotoxic T cell responses in a murine model of pancreatic cancer. Antigen specific CTLs were generated from transgenic OT-I mice or from IL-18R-deficient OT-I mice. CTLs were adoptively transferred into mice bearing pancreatic carcinoma cells expressing the model antigen OVA (PancOVA). Effector function of these transferred cells was evaluated by flow cytometry analysis of coinhibitory receptors (PD1, TIM3), transcription factors as wells as restimulation capacity *ex vivo*. RNA-sequencing of adoptively transferred CTLs was performed. Our data revealed an immunosuppressive effect of IL-18 receptor signaling on intratumoral CTLs. Analysis of transferred IL-18 receptor deficient CTLs *ex vivo* showed reduced expression of coinhibitory receptors and improved restimulation capability compared to transferred WT CTLs. RNAseq data indicated that IL-18-induced T cell exhaustion is mediated by the IL-2/STAT5-pathway. T cellular signaling of NLRP3-dependent IL-18 induces T cell exhaustion. Our results indicate that efficacy of checkpoint inhibitor therapy for PDAC patients might be improved by use of concomitant anti- IL-18 treatment strategies.

Keywords: Cancer immunology, cell signalling, cytokines and mediators

P-0027

The role of Gαi2 in thrombocytes and granulocytes in myocardial ischemia-reperfusion injury

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Myocardial infarction is a leading cause of death and disability. Current therapies focus on restoring blood flow. However, the pathophysiological contribution of immune cells migrating into the ischemic tissue is coming into focus. This so-called ischemia-reperfusion injury (IRI) further aggravates cell damage. Gi proteins in platelets and immune cells are assumed critical in leukocyte recruitment and activity. In particular, global Gαi2-deficiency has been shown to influence the severity of IRI in a mouse model. In an acute murine myocardial IRI-model, we investigated the effects of selective Gαi2 deficiency in platelets (*Gnai2*fl/fl-PF4-Cre+) or granulocytes (*Gnai2*fl/fl-LysM-Cre+). To evaluate IRI-severity, we measured troponin-I-levels and analysed hearts for infarct size and PNC accumulation. We then examined an inhibiting anti-Gαi1/2-antibody to treat wild-type mice subjected to the myocardial IRI-model. We found that platelet and granulocyte Gαi2 aggravates IRI development. Accordingly, *Gnai2*fl/fl-PF4-Cre+- and *Gnai2*fl/fl-LysM-Cre+-mice experienced lower troponin-I-levels and smaller infarcted areas compared to non-deficient controls. Fewer PNCs were present in tissue sections of either genotype. However, whereas PNC numbers in blood from *Gnai2*fl/fl-PF4-Cre+-mice were reduced, they were unaltered in blood from *Gnai2*fl/fl-LysM-Cre+-mice as compared to controls. Having established a significant role of Gαi2 in platelets and granulocytes for IRI development, we tested a parenterally injected inhibitory anti-Gαi1/2-antibody. Wild-type mice subjected to the myocardial IRI-model showed lower troponin-I-levels and reduced ischemic areas when treated with the antibody before recanalization. These results emphasize the importance of Gαi2 in the development of IRI and offer a novel approach for treating IRI by a specific anti-Gαi1/2-antibody antibody.

Keywords: Animal models, antibody, cardiovascular diseases, granulocytes, neutrophils, tissue damage and repair

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POSTER PRESENTATIONS

P-0028

Fractalkine (CX3CL1) is a key modulator of natural killer cell migration, phenotype and function in obesity associated cancer

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Oesophageal adenocarcinoma (OAC) is an aggressive obesity associated cancer, with a dismal 5-year survival rate of <20%. OAC patients face poor treatment response rates of <30% and urgently require new therapeutics. Our group have previously shown that natural killer (NK) cells amigrate to omentum in OAC patients where their viability is altered. We propose fractalkine mediates phenotypic effects on NK cells in OAC. This study aims to elucidate the role of fractalkine in NK cell migration and function in OAC. Total and CX3CR1+ NK cells were quantified in OAC patient blood, omentum and tumour by flow cytometry. Blood-derived NK cells were treated with 80μM of fractalkine receptor CX3CR1 antagonist (AZD8798) and their chemotaxis towards adipose conditioned media (ACM) and tumour conditioned media (TCM) was measured using a transwell system. NK cells were treated with 30ng/ml recombinant fractalkine and their surface CX3CR1, CD27 and intracellular TNF-α and IFN-γ expression was measured by flow cytometry. Our data show CX3CR1+ NK cell frequencies are diminished in OAC patient omentum ($p<0.0001$) and tumour ($p<0.0001$), compared to the circulation. CX3CR1 antagonism significantly reduces NK cell migration toward ACM but not TCM ($p=0.04$). Fractalkine exposure significantly reduces CX3CR1+ NK cells ($p=0.03$). Conversely, it increases CD27+ ($p=0.04$), TNF-α+ ($p=0.05$) and IFN-γ+ ($p=0.04$) NK cells. Antagonism of CX3CR1 shows potential to reduce NK cell migration to omentum and prevent their depletion in OAC patients. Targeting the fractalkine pathway may have effects on NK cell function since our data has shown that fractalkine alters NK cell phenotype and function.

Keywords: Cancer immunology, chemokines, innate immunity, NK cells

P-0029

Hobit and blimp-1 instruct the differentiation of iNKT cells into resident-phenotype lymphocytes after lineage commitment

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iNKT cells are CD1d-restricted T cells that play a pro-inflammatory or regulatory role in a broad range of infectious diseases and autoimmune disorders. Development of iNKT cells is initiated in the thymus and their differentiation into distinct iNKT1, iNKT2 and iNKT17 lineages with unique lineage-defining transcription factors and cytokine profiles is completed in the periphery. It has remained unclear whether iNKT cells retain developmental plasticity after lineage commitment. iNKT cells acquire a similar phenotype as tissue-resident memory T cells, suggesting that they also differentiate along a trajectory, which enables them to persist long-term as resident memory lymphocytes in the peripheral tissues. Here, we addressed whether lineage commitment and memory differentiation are parallel or sequential developmental programs of iNKT cells. We defined 3 subsets of peripheral iNKT cells using CD62L and CD69 expression that separate central memory, effector memory and resident memory phenotype cells. The majority of iNKT1 cells displayed a resident phenotype in contrast to iNKT2 and iNKT17 cells that were largely maintained as central or effector memory cells. We observed that Hobit and Blimp-1 instructed the differentiation of central memory iNKT cells into resident memory iNKT cells, but did not impact lineage commitment into iNKT1, iNKT2 or iNKT17 cells. Thus, we conclude that memory differentiation and establishment of residency occurs after lineage commitment through a Hobit and Blimp-1 driven transcriptional program.

Keywords: Biology of the immune system, immune development, memory, NKT cells

P-0030

Histocompatibility response in Planaria, and its mediation by immune cellular activity

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Histocompatibility and allogeneic responses in mammalians are attributed to the cytotoxic T cells and NK cells. Recent progress has revealed the presence of such responses in some colonial marine invertebrates; however, the mechanism through which immune rejections occur remains unclear as this response is mediated by species specific mechanism and may evolve independently. Here, by using diverse in-vivo and *ex-vivo* assays, ranging from cell isolation, cell and tissue transplantation, to genetic chimeras, we show the existence of histocompatibility and allogeneic cellular response in planarian flatworms. Together, our results suggest the cytotoxicity as a mean to modulate cellular rejection in planarian.

Keywords: Innate immunity, phagocytosis, transplantation

P-0031

Self-renewal of double negative 3 (DN3) early thymocytes enables thymus autonomy but compromises the β-selection checkpoint

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T lymphocyte differentiation in the steady state is characterized by high cellular turnover whereby thymocytes do not self-renew. However, if deprived of competent progenitors the thymus can temporarily maintain thymopoiesis autonomously. This bears a heavy cost, as prolongation of thymus autonomy causes leukemia. Here we show that, at an early stage, thymus autonomy relies on double negative 3 early (DN3e) thymocytes that acquire stem cell-like properties. Following competent progenitor deprivation, DN3e became long-lived, were required for thymus autonomy, differentiated *in vivo*, and included DNA-label-retaining cells. At single cell level, the transcriptional programs of thymopoiesis in autonomy and the steady state were similar. However, a new cell population emerged in autonomy that expressed an aberrant Notch target gene signature and bypassed the β-selection checkpoint. In summary, DN3e have the potential to self-renew and differentiate *in vivo* if cell competition is impaired but this generates atypical cells, probably the precursors of leukemia.

Keywords: Cancer immunology, immune development, immunodeficiency

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POSTER PRESENTATIONS

P-0032

Regulatory B cells (Bregs) inhibit osteoclastogenesis and play a crucial role in ameliorating ovariectomy induced bone loss

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Increasing evidences in recent years have suggested that regulatory B cells (Bregs) are one of the crucial modulators in various inflammatory disease conditions. However, no study till date have investigated the significance of Bregs in modulating osteoclastogenesis. To the best of our knowledge, in the present study, we for the first time examined the anti-osteoclastogenic potential of Bregs under *in vitro* conditions and observed that Bregs suppress RANKL induced osteoclastogenesis in a dose dependent manner. We further elucidated the mechanism behind the observed suppression of osteoclasts differentiation via Bregs. Our results clearly suggested that the observed anti-osteoclastogenic property of Bregs is mediated via production of IL-10 cytokine. Next, we explored whether Bregs have any role in mediating inflammatory bone loss under post-menopausal osteoporotic conditions in ovx mice. Remarkably, our *in vivo* data clearly suggest that the frequencies of both CD19+IL-10+ Bregs and CD19+CD1dhiCD5+IL-10+ "B10" Bregs were significantly reduced in case of osteoporotic mice model. Moreover, we also found a significant reduction in serum IL-10 cytokine levels in osteoporotic mice, thereby further supporting our observations. Taken together, the present study for the first time establishes the direct role of regulatory B cells in modulating osteoclastogenesis *in vitro.* Further, our *in vivo* data also suggest that modulations in percentage of Bregs along with its reduced potential to produce IL-10 might further exacerbate the observed bone loss in ovx mice.

Keywords: B lymphocytes, cell based therapies, inflammatory disease, regulatory cells

P-0033

Interleukin 7 receptor drives expansion of progenitors that recently seeded the thymus

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The interleukin 7 (IL-7)/IL-7 receptor (IL-7r) signaling axis is essential for T lymphocyte differentiation where it drives thymocyte proliferation and survival. Here we describe a previously unappreciated role for IL-7/IL-7r signaling in early T lineage progenitors (ETPs). Mice lacking IL-7 or IL-7r have a deficiency in ETPs that was best explained when IL-7 was removed specifically in the thymus and did not depend on the number of niches available for seeding. Specifically, IL-7r-deficient ETPs had impaired proliferation and survival *in vivo*. Interestingly, while IL-7r signaling in ETPs followed the canonical Stat5 pathway, it did not appear to regulate Bcl-2 expression. IL-7/IL-7r signaling was highly restricted to the most immature (Flt3+) population within the ETP, where it promoted expansion without affecting differentiation. Taken together, our data implicates IL-7/IL-7r following thymus seeding, by promoting expansion of the most immature T lineage progenitors.

Keywords: Cell signalling, cytokines and mediators, immune development, immunodeficiency

P-0034

Not all that is sticky is tar: platelets increase B cell proliferation, cytokine production and class switch upon T independent B cell activation

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Platelets are increasingly recognized for their ability to affect the adaptive immune response. B cell-platelet complex formation has been described to be increased in patients with autoimmune diseases, correlating with increased B cell activation marker and IL-10 expression. However, very little is known about the extent of – and mechanisms behind - effects of platelets on human B cell function. In this study, we investigated the effects of platelets on B cell activation, differentiation and class switch in a T dependent as well as a T independent *in vitro* culture. Platelets minimally affected B cell proliferation and differentiation in the T dependent *in vitro* culture. In contrast, platelets and their releasate (soluble fraction secreted by platelets upon activation) increase B cell proliferation and class switch in the T independent *in vitro* B cell culture. Additionally, production of IL-10 by B cells was induced in presence of platelets in these cultures, whereas TNFα production was not affected. In contrast to reports in murine models, platelet-mediated induction of B cell proliferation and class switch is not mediated by platelet-derived (s)CD40L. Using a platelet secretome screen, we are trying to identify platelet-derived factors potentially mediating the effects on B cell activation and class switch. Overall, this study provides us with more insight on how platelets may affect human B cell function.

Keywords: B lymphocytes, cellular interactions, cytokines and mediators, inflammatory disease

P-0035

Altered basal lipid metabolism underlies the functional impairment of naive CD8+ T cells in elderly humans

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Aging is associated with functional deficits in the naive T cell compartment, which compromise the generation of de novo immune responses against previously unencountered antigens. The mechanisms that underlie this phenomenon have nonetheless remained unclear. We identified an age-related link between altered basal lipid metabolism in naive CD8+ T cells and their impaired responsiveness to stimulation, characterized by low proliferative potential and susceptibility to apoptosis. In particular, naive CD8+ T cells from elderly individuals showed increased basal activation and metabolic activity levels, characterized by high fatty acid uptake and storage. Reversal of the bioenergetic anomalies withlipid-altering drugs, such as rosiglitazone, improved, *in vitro*, resistance to apoptosis, proliferative capacity and antigen-responsiveness of naive CD8+ T cells in elderly subjects. Interventions that favor lipid catabolism may find utility as adjunctive therapies in the elderly to promote vaccine-induced immunity against emerging pathogens or tumors.

Keywords: Adaptive immunity, ageing, immune senescence, metabolic control of immune responses

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POSTER PRESENTATIONS

P-0036

Increased cytotoxic activity and granzyme B expression in NK cells cultured in a medium with AM3

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Natural killer (NK) cells are a type of innate lymphocytes specialized in the recognition and elimination of viral-infected and malignant cells. They exhibit cytoplasmic granules that contain granzymes and perforins. Recently, these cells have been proposed to play an important role in the regulation and coordination of the innate and adaptative immune system. Besides, NK cells seem to have an important synergistic effect in vaccination due to their immunoregulatory function mediated by IFNγ. AM3 is an immunomodulator whose active ingredient is a polysaccharide/protein compound purified from *Candida utilis* cell wall. We observed the function of NK cells based in their capacity to generate cytotoxicity and granzyme B expression in 8 healthy volunteers. We studied peripheral blood mononuclear cells (PBMCs) in the presence of five concentrations of AM3 (0.1, 1, 5, 10 and 25 µg/ml) during 6, 18 and 48 hours. Afterwards, we selected NK cells subpopulations and assessed CD107a, granzyme B and perforins expression by flow cytometry. We found AM3 significantly increased CD107a expression at 0.1, 1 and 5µg/ml and produced an increment of granzyme B at 5, 10 and 25 µg/ml after 18 hours of treatment with a slight increase in the expression of perforin. In conclusion, AM3 induces NK cell exocytosis which increases their cytotoxic activity and granzyme B expression. With the previous evidence of AM3 as an effective immunoadjuvant and the current evidence on the regulation of NK cells, AM3 could be a useful tool to obtain a better vaccination response, however, further research will be necessary.

Keywords: Immune communication, immune regulation and therapy, innate lymphoid cells, NK cells

P-0037

A subset of regulatory T cells recognises and functionally interacts with CD1a

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The non-classical antigen presenting molecule CD1a is predominantly expressed on antigen presenting cells (APCs) in the skin. Through CD1a, skin APCs can present lipid antigens to T cells and modulate the immune response. Increasing evidence supports the role of regulatory T cells (Tregs) in the control of skin immune programs, keeping inflammation at bay. It has been recently appreciated that effector T cells can respond to CD1a and contribute to the exacerbation of skin diseases such as psoriasis; however, whether Tregs can recognise CD1a and maintain immune homeostasis remains elusive. Using *in vitro* cell-based and cell-free CD1a-stimulation systems, we show that about 2% of *in vitro* expanded polyclonal $CD4^+CD25^+CD127^{low}Foxp3^+$ Tregs secrete the immunosuppressive cytokine IL-10 in a CD1a-dependent manner, but not other skin-inflammation associated cytokines, such as IL- 13, IL-17A, IL-22, GM-CSF and IFN-γ. To study CD1a-reactive Tregs at a clonal level, CD1a-reactive IL-10-producing Treg clones were isolated and expanded. The Treg clones retained suppressive functionality and the ability to secrete IL-10 upon CD1a exposure. Using FACS analysis and TCR sequencing of the Treg clones, we further demonstrate that IL-10-secreting CD1a-reactive Tregs express a variable α/β T cell receptor repertoire. The work demonstrates that regulatory T cells can recognise and functionally interact with CD1a. This highlights the potential of CD1a in mediating immune regulation in skin-inflammatory diseases where changes in skin lipids influence inflammatory responses. Therefore, CD1a-mediated Treg responses may present a novel therapeutic target for the treatment of skin-inflammatory diseases.

This work is supported by the Wellcome Trust (108869/Z/15/Z).

Keywords: CD1-restricted T cells, immune regulation and therapy, regulatory cells

P-0038

CD209/CD14+ dendritic cells characterization in inflammatory arthritis: activation, synovial infiltration and therapeutic targeting

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Dendritic cells (DC) are a heterogeneous population of professional antigen-presenting cells. A specific subset of DCs, deriving from monocyte, and have a key role in inflammation and infection. To identify and characterize the CD209+/CD14+ DC subset and evaluate their characteristics in the periphery and the site of inflammation of rheumatoid (RA) and psoriatic arthritic (PsA) patients. Peripheral blood and synovial fluid mononuclear cells (PBMC and SFMC) were isolated from healthy subject (HC), RA and PsA patients. Single-cell synovial tissue suspension (ST) was obtained by enzymatic digestion. Flow cytometry was performed to identify the CD209+/CD14+ DC subset, its frequency and co-expression of chemokines receptors (CCR6,-CCR7,-CXCR3,-CXCR4-CXCR5) and activation markers (CD40,-CD80) on the surface of the DC subset. CD209+/CD14+ DC subset development was analysed in patients recruited pre and post Tofacitinib and TNF inhibitors therapy. We identified, for the first time, the CD209+/CD14+ DC population in PBMC of RA and PsA patients, which display increased activation, inflammation and migratory capacity when compared to HC. We observed that the DC subset infiltrate the joint of IA patients, and further activate in response to the joint microenvironment, exhibit a unique maturation and migratory phenotype, with an increased co-expression of chemokine receptors. Finally, we demonstrated that Tofacitinib specifically targets the CD209/CD14+ DC subset, by reducing their inflammatory state and their specific migration to the joint of IA patients. This study identifies a new pathogenic and infiltrating DC subset in RA and PsA patients, which could be specifically targeted for therapeutical purpose.

Keywords: Dendritic cells, drugs for immune modulation, inflammatory joint diseases, innate immunity

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POSTER PRESENTATIONS

P-0039

The influence of maternal allergic sensitization on miRNA transcriptome and T-cell modulatory properties of human milk-derived extracellular vesicles

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Human milk supports infant's postnatal immunity by providing maternal components, including cell-derived vesicles (EVs), cell-to-cell vehicles of biological molecules. It has been demonstrated that milk-EVs contain immune-modulatory miRNAs. Furthermore, we showed that milk-EVs modulate T-cell responses. Since allergic diseases can alter the physiological milk composition, here we investigated whether miRNAs and T-cell modulatory properties of milk-EVs are influenced by the allergic status of the mother. Milk (4-13 weeks postpartum) was donated by non-allergic and allergic mothers (serum IgE≥50kU/ml and/or positive Phadiatop specific IgE). EVs were purified by differential centrifugation, density gradient floatation and size exclusion chromatography. EV functionality was assessed by *in vitro* co-culture with αCD3/αCD28-stimulated human PBMC-derived CD4+T-cells. Quantitative differences in milk-EV miRNAs were identified by smallRNA-seq. CD4+T-cell signaling model based on miRNA-target interactions was built to predict hotspots of milk-EV regulation. Allergic milk-EVs were less capable of T-cell inhibition compared to non-allergic milk-EVs, as indicated by less strong inhibition of activation-triggered genes such as CD25, CDK4, JUN, IL5. Transcriptome analysis revealed 30 miRNAs differentially expressed between non-allergic and allergic milk-EVs. Our prediction model shows that miRNAs over-represented in non-allergic samples favour the attenuation of T-cell activation key processes such as cell cycle progression and STAT pathway, while miRNAs over-represented in allergic samples target pro-apoptotic pathway and negative regulators of cyclins. Milk-EVs from allergic mothers are less potent inhibitors of CD4+T-cell activation compared to milk-EVs from non-allergic mothers and this might be linked to quantitative differences in the miRNA cargo between these EVs.

Keywords: Big data, adaptive immunity, allergic disorders, cell signalling, immune communication, miRNA

P-0040

Dietary β-glucans from different sources show variable levels of trained immunity and dectin-1 receptor activation

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Yeast-derived β-glucans have been shown to possess the potential to functionally reprogram ("train") innate immune cells resulting in an enhanced state of immune responsiveness. Trained innate immunity induced in a preventive or curative setting plays an important role in supporting immune fitness. Likely, trained innate cells can be of benefit for elderly and patients to enhance their protection against infections. We set out to establish primary macrophages as an innate immune cell model to study training effects through continuous exposure to M-CSF and β-glucans, respectively. Using this model, we compared nine different β-glucans for their ability to train macrophages and to activate the pattern recognition receptor Dectin-1. We compared the use of macrophages against the established human monocyte model system and identified benefits of continuous β-glucan exposure. In addition, we identified multiple different β-glucans capable of inducing training, which appears to be linked to insolubility and the activation of Dectin-1b. These results represent a first step towards an innate immune training model to *in vitro* screen immunomodulatory compounds for their immune supporting benefit. Next steps include further exploring the correlation between solubility, dispersibility and activity of immunomodulatory dietary fibers.

Keywords: Innate host defence, innate immunity, macrophage

P-0041

A systems immunology study: AID and Ki67 as interrelated partners in germinal center biology

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Ectopic (or tertiary) lymphoid structures (ELSs) are formed in diseased organs in association with cancer, autoimmune diseases, or infections and play important role in disease progression. For patients with metastatic colorectal cancer, we recently demonstrated prognostic power of lymphoid structures in normal colonic mucosa and their interrelations with ELSs at tumor and metastatic sites. To understand better the biological mechanisms that drive ELS formation, maintenance, and function in disease tissues we applied an integrative systems biology approach for comparing them with active lymphoid structures, known as germinal centers, GCs, which are formed in secondary lymphoid organs and regulated by the B-cell molecule activation-induced cytidine deaminase, AID (gene name AICDA). Meta-analysis of transcriptomic data sets (GENEVESTIGATOR platform) for genes showing co-regulation with AICDA pointed to MKI67. Immunostaining of tonsil tissue sections for AID and Ki67 and the follow-up single cell-based quantitative image analysis (TissueFAXS platform) revealed similarity in staining patterns with pronounced dark zone staining of GCs. In support to staining-derived information, correlation analysis for AID and Ki67 within GCs revealed significant association. Next, we extracted and compared the top 200 co-expressed genes for each molecule using public microarray data sets attributed to lymphoid tissues (GENEVESTIGATOR platform). We found 151 overlapping genes. To delineate the molecular relationships among co-expressed genes we used the Ingenuity Pathway Analysis software. Comparative analysis revealed a strong overall overlap among the identified statistically significant Canonical Pathways. Cumulative data strongly suggest a crosstalk between AID- and Ki67-attributed molecular events and open new perspectives for therapeutic modulation.

Keywords: Adaptive immunity, B lymphocytes, big data, cancer immunology

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POSTER PRESENTATIONS

P-0042

Cross-reactive CD4+ T cells enhance SARS-CoV-2 immune responses upon infection and vaccination

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The newly emerged coronavirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), caused a global pandemic challenging economy and health systems. Initially, it was assumed that SARS-CoV-2 encountered an immunologically unprotected population, however, SARS-CoV-2 displays considerable homologies with endemic, seasonal common cold endemic coronaviruses (HCoVs). We and others could demonstrate the existence of cellular and humoral cross-reactivity to SARS-CoV-2, still the role of cross-reactive immunity in SARS-CoV-2 infection and vaccination is under debate. We comprehensively determined HCoV-reactivity and SARS-CoV-2-cross-reactivity in unexposed individuals and COVID-19 convalescents. Pre-existing cross-reactive memory T cells were efficiently recruited into mild SARS-CoV-2 infections and their abundance correlated with higher S1 IgG and neutralizing antibodies titers. Importantly, the cells were also reactivated after primary BNT162b2 COVID-19 mRNA vaccination in which their kinetics resembled that of secondary immune responses. Our results highlight the functional importance of pre-existing spike-cross-reactive T cells in SARS-CoV-2 infection and vaccination. Abundant cross-immunity may be responsible for the unexpectedly high efficacy of current vaccines even with single doses and the high rate of asymptomatic/mild infection courses.

Keywords: Adaptive immunity, memory, viral infections

P-0043

Uncomplicated oocyte donation pregnancies display an elevated CD163-positive type 2 macrophage load in the decidua, which is associated with fetal-maternal HLA mismatches

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The embryo of an oocyte donation (OD) pregnancy is completely allogeneic to the mother, which may challenge the maternal immune system to tolerize the fetus. Decidual macrophages are essential in maintaining a healthy pregnancy, and type 2 macrophages may exhibit immune suppressive activity. We hypothesized that the composition of decidual macrophages is different between uncomplicated OD pregnancies and non-OD *in vitro* fertilization (IVF) pregnancies, and is related to fetal-maternal incompatibility. Women with uncomplicated pregnancy were enrolled: 25 singleton OD pregnancies and 17 non-OD IVF pregnancies. The extent of immunohistochemical staining of CD14 (pan-macrophage marker) and CD163 (type 2 macrophage marker) in both decidua basalis and parietalis was quantitated by digital image analysis. Maternal and fetal DNA was typed for human leukocyte antigen (HLA)-A, -B, C, -DRB1, and -DQB1, and fetal-maternal HLA mismatches were calculated. OD pregnancies showed a higher percentage of CD163+ staining ($p=0.040$) and higher CD163/CD14 ratio ($p=0.032$) in the parietalis than non-OD IVF. The OD group was separated into a semi-allogeneic group (≤5 fetal maternal HLA mismatches) and a fully allogeneic group (>5 mismatches). The HLA-fully-allogeneic OD group, but not the HLA-semi-allogeneic OD group, showed significantly elevated CD163/CD14 ratio in the parietalis compared with the non-OD IVF group ($p<0.05$). Uncomplicated OD pregnancies display a higher CD163-positive cell fraction in the total decidual macrophage population compared to autologous pregnancies, which may suggest that a local type 2 macrophage-related mechanism is needed to compensate for the higher fetal-maternal HLA mismatch load.

Keywords: Fetal immunity, macrophage, reproductive immunology

P-0044

Effect of storage period of erythrocyte suspensions on the CD4 & CD8 T cells

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The aim of this study was to evaluate whether T lymphocytes that exist within the erythrocyte suspension (ES) have maintained the responsive ability to mitogen stimulation under the storage conditions or not. Six units of whole blood were donated from 6 blood donors. Each ES that were obtained from those whole blood units, were divided into 3 equal parts to provide the samples for day 0, 21, 42. At the related days mononuclear cells (MNC) were isolated from these samples and cultured. Phytohemagglutinin was added into the half of MNC culture wells to obtain the stimulated (STI) and unstimulated (US) wells. Analyses were investigated in samples which were collected from those MNC culture wells. CD4+, CD8+, Th, Tc, Th1, Tc1 and IL-21 expressing cells significantly decreased in both US and STI wells on the days 21 and 42 compared to the day 0. Th2 cells were significantly increased on the day 21 compared to the day 0. In comparison between the STI and US groups, significant differences were found in the increase of TNF-α expressing Th cells, IL-21 expressing Th cells and IL-17 expressing Tc cells in the STI group on day 0). Our results indicate that ES storage conditions lead to a suppression of the T lymphocyte response to stimulation, decrease in Th1 and increase in Th2 cells. These results point out that the effect of triggers which may induce the transfusion-related immunomodulation begins in the ES before allogeneic blood transfusion.

Keywords: Adaptive immunity, epigenetic control and modulation of immunity, immune regulation and therapy

P-0045

Leukocyte reduction system chambers as an alternative source of viable peripheral blood mononuclear cells for EasySep™ and EasySep™ direct cell isolation

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Leukocyte reduction system chambers (LRSC) have been identified as a reliable and cost-effective alternative to peripheral blood or apheresis collections for peripheral blood mononuclear cells (PBMCs). With their increased accessibility and use, there is a growing demand for comprehensive phenotypic characterization and optimized protocols for immune cell subset isolation from LRSC. We have developed two immunomagnetic, column-free approaches to isolate highly purified cells in less than 25 minutes from LRSC. Our EasySep™ and EasySep™ Direct approaches target unwanted cells for removal with antibodies recognizing specific cell surface markers and cross-linking them to magnetic particles. Magnetically labelled cells are easily separated from the untouched cells using an EasySep™ magnet, and the entire procedure can be fully automated using RoboSep™-S. We have phenotypically characterized the relative frequencies of LRSC immune cell subsets, identifying several key differences when compared to other sources of PBMCs. Differences included increased proportions of natural killer (NK) cells, myeloid dendritic cells and decreased proportions of memory CD8+ T cells, plasmacytoid dendritic cells, and neutrophils. Using insight gained from this characterization, LRSC-specific EasySep™ and EasySep™ Direct protocols have been optimized for the isolation (mean ± SD) of T cells (99.1 ± 0.6%; n = 3), CD4+ T cells (92.5. ± 2.6%; n = 7), CD8+ T cells (89.2 ± 2.0% n = 5), NK cells (94.8 ± 1.6%; n = 7), and monocytes (92.3 ± 4.5%; n = 5). Isolated cells are untouched and highly purified, making them an ideal source for downstream applications.

Keywords: Immunological techniques, myeloid cells, NK cells

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POSTER PRESENTATIONS

P-0046

Tec tyrosine kinase regulates Th17 cell differentiation by quenching IL-6 sensing

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CD4$^+$ T helper cells are divided into specialized subsets. Among these subsets, T helper (Th) 17 cells were demonstrated to mediate host defense against pathogenic extracellular bacteria and fungi but are also triggering auto-immune responses. Two independent studies involved the cytoplasmic non-receptor protein tyrosine kinase Tec in the differentiation and function of Th17 cells. However, so far it was not known how Tec regulates Th17 cells on a mechanistic level. Here we show that Tec is specifically expressed in Th17 cells in contrast to other T helper subsets. In addition, we could further implicate Tec in the Interleukin-6 (IL-6) sensing by differentiating CD4$^+$ T cells *in vitro*. We could further demonstrate a role of Tec in regulating intestinal Th17 cells under homeostatic conditions and in T-cell driven colitis model. Thus, our results identified Tec as a fine-tuning cytokine sensor specifically implicated in the control of Th17 cell differentiation *in vivo* and *in vitro*.

Keywords: Adaptive immunity, animal models, cell signalling, inflammatory bowel disease

P-0047

Identification and characterization of antigen specific CD4+ T-cells in patients with Jo1+ myositis

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Myositis is a chronic autoimmune disorder represented by lesions in muscle, skin and lung.The most common autoantibody is anti-Jo1, targeting the histidyl-transfer RNA synthetase (HisRS).Importance of T-cells in disease is established by their presence in muscle and sites of inflammation. We previously showed that upon stimulation of PBMC with HisRS protein, CD4$^+$T cells were activated and produced inflammatory cytokines.Moreover, strong associations of HLA-DRB1*03:01 genotype suggest the existence of autoantigens being recognized by CD4$^+$T-cells.Hitherto the presence of antigen specific CD4+T-cells has not been established in myositis.The main aim of this project is to detect and characterize HisRS$^+$CD4$^+$T-cells using HLA Class-II tetramers, a technology that allows the detection of rare antigen specific CD4$^+$T-cells. HLA-DRB1*03:01 monomers with tetanus and HisRS peptides were produced in-house in E.coli system.The peptides were covalently attached to the N-terminus of the HLA-β-chain via a flexible peptide linker and tetramers were assembled using a fluorescently labeled streptavidin.Tetanus was used as a control to assess the efficacy of the peptide-HLA tetramers.The frequency of tetanus$^+$CD4$^+$T-cells were detected from HLA-matched healthy controls by tetramers, and confirmed by their cytokine secretion. Next, we were able to detect HisRS$^+$CD4$^+$T-cells in PBMC of anti-Jo1$^+$ and HLA-DRB1*0301 patients (n=4) using tetramers with the optimised protocol.We are including more patients to confirm our findings along with functionality assays. Although previous studies indicate the importance of T-cells in myositis, their functionality and role in the disease has not been fully established.Characterization of this population will improve our understanding of the disease pathogenesis and develop better treatment options.

Keywords: Immunological techniques, adaptive immunity, autoimmunity

P-0048

Activated CD38+HLA-DR+ CD4 and CD8 T subsets as sensitive age-related markers in patients with SARS CoV-2 infection

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SARS-CoV-2 causes dysregulation of immune response associated with unpredictable clinical course. Multiparameter flowcytometry is the most accessible method for immune monitoring. Evaluation of a minimal flowcytometry panel for monitoring of SARS-CoV-2 infection, including the major T lymphocyte populations and activated subsets Blood samples from COVID-19 patients (n=77) aged 52 (±16) years, divided in three groups based on clinical symptoms: mild (A, n=16); moderate (B, n=23), severe (C, n=38) were studied. Lymphocyte subset, absolute counts (AC) and percentages were determined using CD3/CD8/CD45/CD4, CD8/CD38/CD3/HLA-DR, TRUCount tubes (BD Biosciences). Proportions of CD3+, CD3+CD4+, CD3+CD8+ T cells, and CD4/CD8 ratio did not differ significantly between groups ($p>0.05$ for all). The only subset significantly increased in group C as compared to A and B were the double positive (CD38+HLA-DR+) activated CD4 and CD8 T cells (5.4vs3.9vs 3.6 and 5.7vs4.5vs3.9 respectively, $p<0.05$). As expected, patients aged over 50 were characterized with a higher proportion and AC of CD8T (33.2vs.28.6%, 327vs.231 cells/μl), a lower CD4/CD8 ratio (1.99 vs.2.85), and a higher share of CD38+CD8T (14.6vs.10.3%), $p<0.05$ for all, associated to age-related immune activation. Unexpectedly however, the share of HLA-DR+/CD38+ CD8 was higher in patients aged below 50 (4.77vs.2.27, $p<0.05$), regardless of the infection duration (7.4vs9.1 days, $p=0.4$). HLA-DR+/CD38+ CD4 and CD8T are sensitive age-related parameters for immune monitoring of COVID-19 patients. The pathogenetic significance of each activated subset needs to be clarified, in the context of a possible suppressive effect of the CD38+CD8T subset.

Keywords: Adaptive immunity, infectious disease, monitoring immunity, viral infections

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POSTER PRESENTATIONS

P-0049

Human T-bet$^+$ B-cell development: association with Bruton's tyrosine kinase and targeting by evobrutinib

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A phase II trial (NCT02975349) showed promising results for Bruton's tyrosine kinase (BTK) inhibitor evobrutinib in multiple sclerosis (MS). Recently, we found that CXCR3(T-bet)$^+$ B cells preferentially infiltrate the MS brain and are induced under IFNGR/TLR9-stimulating, follicular T helper-like conditions. Here, we studied how BTK activity is regulated in B cells during the MS course and whether it contributes to the development of brain-homing T-bet$^+$ B cells using evobrutinib. BTK and pBTK levels in transitional, naive mature, class-switched and unswitched memory B cells were compared between CIS, RRMS, SPMS, PPMS and matched healthy control blood (n=30 per group). The impact of evobrutinib on both differentiation and transmigration of B cells was assessed under T-bet-inducing conditions *in vitro*. In contrast to BTK, pBTK was particularly higher in class-switched memory B cells of RRMS and SPMS as compared to CIS and healthy control groups. In RRMS and SPMS, pBTK was less induced after anti-IgM stimulation. *Ex vivo* pBTK levels positively correlated with both CXCR3 and VLA-4 expression, as well as the clinical response to natalizumab (anti-VLA-4) therapy. *In vitro* experiments demonstrated that pBTK was induced by T-bet-associated triggers (IFN-γ/CpG-ODN) and evobrutinib impaired T-bet-related class-switching, IgG$^+$ recall responses and crossing of human brain endothelial monolayers by CXCR3$^+$ B cells. BTK is more activated in memory B cells of both RRMS and SPMS patients and functionally related to CXCR3(T-bet)$^+$ B cells. This provides key insights into how evobrutinib affects pathogenic B-cell differentiation and can modulate the MS course.

Keywords: B lymphocytes, cell signalling, cytokines and mediators, effector molecules, multiple sclerosis, neuroimmunology

P-0051

Gamma/delta T-cell function and TCR profiling in human pregnancy

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The variety of functions makes gamma/delta (γδ) T-cells a particularly interesting but still enigmatic lymphocytes during human pregnancy. In our previous study we found activated, pro-inflammatory and effector phenotype of decidual γδT-cells at maternal-fetal interface (MFI) compatible with normal human pregnancy. Here we aimed: 1) to investigate the γδT-cells' function as effectors in the context of their cytotoxicity and Th1 cytokines production and 2) to analyze γδT-cells' receptor (TCR) repertoire in human pregnancy. We found that significant proportion of γδT-lymphocytes at MFI (80%) produced granzyme-A regardless their localization and stage of pregnancy. The granulysin-positive γδT-cells were in the same number at MFI but halved (40%) as pregnancy advanced. However, the perforin-positive γδT-cells into decidua during early pregnancy were significantly less than in the blood of the pregnant women (paired samples), suggesting "impaired" γδT-cell cytotoxicity. The number of γδT-cells producing pro-inflammatory and cytotoxic Th1 cytokines such as IFNγ and TNFα was very low at MFI, higher in the blood of pregnant women and as expected very high in the blood of non-pregnant women. The next-generation sequencing (NGS) of CDR3 regions of all γδT-cells TCR gamma (2,3,4,5,7,8,9,10) and delta (1,2,3,5,8) chains, operating at MFI, and in the blood of pregnant and non-pregnant women showed that the TRDV repertoire at MFI is more focused and canonical (not shared in many individuals) than the TRGV repertoire, which is polyclonal and public. We were not able to detect decidua/placenta-based clonotypes specifically enriched by the pregnancy.

This study was funded by Bulgarian Science Fund, contract DN03/5.

Keywords: Effector molecules, gamma-delta T cells, omics technologies, reproductive immunology, RNAseq

P-0052

Mucosal immunity in human nasopharynx to a novel viral vectored-MERS vaccine and inactivated MERS-CoV antigens and anti-MERS-CoV specific IgM and IgG antibodies in acute and convalescent MERS sera

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Although no vaccines have been approved against MERS-CoV infection, viral vector-based MERS-CoV vaccine expressing surface Spike protein has been studied as a promising vaccine candidate. Human nasopharynx-associated lymphoid tissues (human NALT) are part of the mucosal immune system in the upper gateway which may play an important role against airborne infection. We examined 1) mucosal immunity to a novel Chimpanzee adenovirus vectored MERS-CoV vaccine expressing Spike protein (ChAdOx1-MERS-CoV) and inactivated MERS-CoV antigens in human NALT from children and adults, and 2) anti-MERS-CoV specific IgM and IgG antibodies in acute and convalescent MERS patient serum samples. To evaluate mucosal responses to MERS-CoV vaccine and antigens, tonsillar mononuclear cells (MNC) were stimulated with ChAdOx1-MERS-CoV vaccine, Heat Inactivated or Irradiated-MERS-CoV antigens. ELISA was utilised to measure anti-MERS-CoV specific IgG antibody production in cell culture supernatants. ELISA assay was also performed to analyse anti-MERS-CoV specific IgM and IgG antibody titres in serum samples of acute and convalescent MERS patients. Significant mucosal IgG antibody responses was detected following MERS-CoV vaccine and antigens stimulation in human NALT from both children (n=25) and adults (n=13) ($p<0.001$). On analysis of patient serum anti-MERS-CoV antibodies, high levels of anti-MERS-CoV IgM and IgG antibodies in acute (n=23) and convalescent (n=23) MERS-CoV serum samples were observed ($p<0.001$). Mucosal immunisation with ChAdOx1-MERS-CoV vaccine may be a promising strategy against MERS-CoV infection. High levels of anti-MERS-CoV IgM and IgG antibody titres in MERS patients serum samples suggest the development of antibody-mediated immunity in individuals following infection which may protect against future new infections.

Keywords: Adjuvants and vaccines, antibody, immunological techniques, lymphoid organs, viral infections

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POSTER PRESENTATIONS

P-0053

A glucocorticoid-dependent axis orchestrates dendritic cell migration *in vivo*

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Glucocorticoids (GC) are among the factors known to control dendritic cell (DC) functions at steady state, in the context of stress or during immunosuppressive treatment. GC effects in DC are largely mediated by a transcriptional and signalling regulator named Glucocorticoid-Induced Leucine Zipper (GILZ). Our previous works showed that GILZ regulates DC functions at different levels, from favoring actin-dependent antigen macropinocytosis when deleted, to promoting DC commitment to a tolerogenic profile when overexpressed. Here, our aim was to determine whether and, if so, according to which mechanisms GILZ controls the migration of DC. Firstly, using Bone Marrow-derived Dendritic Cells (BMDC), we showed that GILZ promotes immature DC motricity in microchannels, while limiting their macropinocytic activity. Secondly, we established that immature and mature GILZ-KO DC display reduced chemotaxis towards the CXCL12 and CCL21 chemokines, despite normal expression of CXCR4 and CCR7. We could further associate these defects with alterations of Calcium responses in GILZ-KO DC, that were due to a reprogramming of Calcium-Release Activated Channel expression pattern. Finally, we showed that these alterations translate into a delay of cutaneous GILZ-KO DC migration from skin to lymph nodes *in vivo*. Taken together, our results shed light on a GC-dependent axis that controls DC migration at both the motricity and chemotaxis levels, along mechanisms that we are currently investigating.

Keywords: Cell signalling, dendritic cells, molecular immunology, chemokines

P-0055

Metabolic changes of cytokine-induced memory-like human NK cells and glycolytic-dependence of their effector functions

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Natural Killer (NK) cells represent a valuable tool in cancer immunotherapy. A therapeutic strategy is the adoptive transfer of IL-12/15/18-stimulated NK cells, also known as cytokine-induced memory-like (CIML) NK cells. Several studies have shown that NK cells modulate their metabolism following cytokine-stimulation. We have characterized the metabolic changes associated to IL-12/15/18 stimulation immediately after the cytokine stimulation and after a resting period. Moreover, we have analyzed the glycolytic-requirements of different effector functions of CIML NK cells. NK cells from healthy donors were stimulated with IL-12/15/18 for 16-18 hours and then washed and cultured with low doses of IL-15 or IL-2 for 7 days. Cells were re-stimulated with IL-12/15/18 or K562 cell line. Flow cytometry, Seahorse technology, and calcein-based cytotoxicity assays were used for this study. CIML NK cells showed an elevated expression of nutrient and retained a metabolic profile shifted towards glycolysis seven days after cytokine withdrawal. Our data revealed that distinct effector functions have different glycolytic requirements. IFNγ and MIP-1β production are very sensitive to 2-DG-mediated glycolysis inhibition, while TNF production, degranulation and cytotoxic activity are reduced but not entirely inhibited when glycolysis is blocked. Moreover, our data suggests that 2-DG-mediated glycolysis inhibition could be dependent on the stimuli, i.e. target cells vs. cytokine-stimulation.

Keywords: Chemokines, cytokines and mediators, NK cells

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P-0056

Role of Tiam1 in the maintenance of tissue-resident Foxp3+ regulatory T cells

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Foxp3+ regulatory T (Treg) cells are critical to maintain self-tolerance and modulate a variety of immune-mediated conditions. In addition to these immunoregulatory properties, specialized Treg cells in non-lymphoid tissues, the tissue-resident Treg (tisTreg) cells, are important to promote organ homeostasis and tissue repair. Although functionally distinct from conventional T cells (Tconv), also Treg cells require TCR-mediated activation to exert their diverse functions. TCR-mediated activation is controlled by immunological synapses (IS), the key organizing compartments of TCR signaling formed upon contact with antigen-presenting cells. As recently revealed, Treg cells and Tconv display a diverse phosphoproteome upon TCR-mediated activation. Among the proteins showing diverging signaling patterns between Treg cells and Tconv was the guanine nucleotide exchange factor Tiam1. Here, we studied Tiam1-deficient (Tiam1-/-) mice under steady-state conditions and observed significantly increased frequencies of tisTreg cells especially within the colon. Interestingly, these Treg cells showed an activated effector phenotype, and single-cell (sc) RNAseq of Treg cells from naïve wildtype mice revealed that *Tiam1* is exclusively expressed in a cluster of colonic Treg cells expressing a set of major activation markers. To further study the role of Tiam1 as putative checkpoint molecule in the activation of tisTreg cells, we currently employ the *in vitro* tisTreg differentiation of Tiam1-/- Treg cells. In order to elucidate the phosphorylation-dependency of Tiam1, we will perform *in vitro* and *in vivo* suppression assays with Tiam1-/- Treg cells reconstituted with phosphomutants of Tiam1. Together, our studies suggest that Tiam1 plays a functional role for the maintenance of tisTreg cells.

Keywords: Biology of the immune system, cell signalling, molecular immunology, regulatory cells

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POSTER PRESENTATIONS

P-0057

Short-term immunization response after administration of Covid-19 mRNA vaccines in non-infected individuals

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The current Covid-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused more than 3 million deaths and enormous economic and social upheaval internationally. With the aim of curbing mortality, more than 80 vaccine candidates are in clinical development at present. Two of them, BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna), are based on encapsulated mRNA, and they are being massively administrated around the world, reporting efficacy of around 95% preventing Covid-19 illness. However, little is known about how many days are required to generate an efficacious immunological humoral and cellular memory. We performed a prospective observational study in 40 healthy naïve volunteers, 21 of them vaccinated with BTN162b2 and 19 vaccinated with mRNA-1273. We analyzed at days 3, 7 and 14 after completing the vaccination schedule, the induction of specific humoral, measuring IgG antibodies against spike protein of SARS-CoV-2 in plasma, and cellular T-cells response quantifying IFN-γ release after *ex vivo* stimulation of whole blood with a pool of viral peptides. Despite we observed slight differences in the evolution of both vaccines, the short-term induction of specific humoral and cellular responses was comparable for both vaccines. In both groups, specific humoral and cellular immunity values were significantly lower at day 3 than days 7 or 14 post-vaccination. This refractory period in the induction of specific immunity could constitute a window of higher infection risk and explain some emerging cases of SARS-CoV-2 infection after vaccination, but further studies are required to elucidate immunization in the long term.

Keywords: Adaptive immunity, adjuvants and vaccines, memory, viral infections

P-0058

Role of vitamin D and CD226 in a T cell regulatory pathway altered in MS

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Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system resulting from a complex interaction between genes and environment, such as Vitamin D (VitD) deficiency. T cells are key for MS pathogenesis, with reduced regulatory T cell function. The complement regulator CD46 converts inflammatory Th1 into Type I regulatory T cells (Tr1), reducing IFNγ but increasing IL-10 production. This Th1/Tr1 switch requires recruitment of CD46 at the immune synapse (IS). This switch is defective in MS patients but restored by active VitD. GWAS studies have associated a non-synonymous SNP in Cd226 with increased MS risk. CD226 is expressed by Tr1 and its expression is increased by VitD. Our aims are to unravel the molecular mechanisms regulating the Th1/Tr1 switch and the role of CD226 and VitD. Cell phenotype and cytokine secretion by primary CD4+ T cells co-stimulated with CD3 and CD46 and/or CD226 was examined. Recruitment of CD46 and CD226 to the IS was determined in T cells preincubated in the presence or absence of active VitD. Our results show that CD226 regulates the CD46 pathway by reducing activation and modulating the profile of cytokines produced. Furthermore, VitD promotes the recruitment of CD46 and CD226 to the IS. These data suggest a role for CD226 in the CD46 pathway, and for VitD through modulation of recruitment of CD46 and CD226 at the IS. These data provide novel insights into regulation of a dysregulated pathway in MS.

Keywords: Adaptive immunity, autoimmunity, costimulatory pathways, immune regulation and therapy, multiple sclerosis, regulatory cells

P-0060

CD116+ fetal liver progenitors give rise to human alveolar macrophages *in vivo*

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The lung is a vital organ which ensures the uptake of oxygen and forms a barrier against airborne pathogens. Alveolar macrophages are essential for healthy lung function, yet the origin and ontogeny of human lung macrophages is poorly understood due to the lack of experimental systems. We aimed to identify the foetal progenitor of human lung macrophages using human gene knock-in mice named MISTRG, which allows the development of human alveolar macrophages. We performed cell sorting experiments coupled with intranasal and intrahepatic cell transfers to MISTRG mice as well as 28-color flow cytometry analysis of human foetal liver and lung. Due to the critical role of GM-CSF, we predicted that expression of GM-CSF receptor (CD116) marks alveolar macrophage progenitors in human foetal tissue. Consistent with our prediction, we identified CD116-expressing candidate progenitors in human foetal liver. To confirm the progenitor-product relationship *in vivo*, we transplanted foetal liver populations into the airways and the liver of new-born MISTRG recipients. Intrahepatic transplantation experiments revealed that CD34-CD116+ CD64-CD115+ circulating precursors can migrate from the liver to the lung to give rise to functional alveolar macrophage that were able to prevent the development of pulmonary alveolar proteinosis. We showed that similar precursors existed in human foetal lung and expressed the chemokine receptor CX3CR1. Our study provides important information on human lung macrophage development that cannot be obtained with other approaches. We expect that it will lead to important new insights that are relevant to inflammatory lung diseases in humans such as COVID-19.

Keywords: Animal models, fetal immunity, immune development, innate immunity, macrophage, myeloid cells

P-0061

Anti-C1q autoantibodies increase cytokine secretion in PBMCs after T cell activation

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Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease with complex pathogenic mechanisms. Complement C1q plays a major role in SLE and autoantibodies against C1q (anti-C1q) are strongly associated with SLE disease activity. The aim of the present study is to elucidate the role of T cells in the downstream effects of anti-C1q on immune cells. Isolated T cells, monocytes, and PBMCs of healthy donors with or without concomitant T cell activation were exposed to C1q and C1q/anti-C1q (patient-derived) complexes in different experimental settings. CD40 signalling in monocytes was assessed with a CD154 transfected cell line, and JAK3 and TRAF6 specific inhibitors. Cytokine secretion was quantified by ELISA, and surface marker expression as well as T cell activation and proliferation by flow cytometry. Exposure of isolated T cells to C1q or C1q/anti-C1q did not affect their proliferation or activation state, respectively. However, unspecific T cell activation in PBMCs via CD3/CD28 cross-link resulted in an increased IFNγ, TNFα, and IL-10 secretion in the presence of C1q/anti-C1q complexes but not in the presence of C1q alone. As shown by co-culture and inhibition experiments, the inflammatory effect of C1q/anti-C1q on PBMCs was due to a direct CD40-CD154 interaction between activated T cells and C1q/anti-C1q primed monocytes. Specific inhibition of JAK3 and TRAF6 revealed two partially redundant signalling pathways downstream of CD40 responsible for the TNFα secretion by monocytes. Our findings demonstrate that C1q/anti-C1q complexes have a proinflammatory effect on monocytes that is T cell activation dependent and mediated by the CD40-CD154 signalling pathway.

Keywords: Myeloid cells, autoimmunity, complement, cytokines and mediators

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POSTER PRESENTATIONS

P-0063

Differences in BAFFR signaling and responses between naive and switched memory B cells in humans

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The BAFF-receptor (BAFFR) plays a critical role in the development, homeostasis and survival of peripheral mature B cells. Binding of BAFFR by its ligand, BAFF induces several signaling pathways which upregulate protein synthesis, promote metabolic fitness and enhance the expression of pro-survival genes. Despite the similar BAFFR levels in circulating mature B cells, depletion of BAFF with neutralizing antibodies in patients suffering from systemic lupus erythematosus eliminates naive and marginal zone but not switched memory B cells, indicating that peripheral B cell subsets have different needs for BAFF/BAFFR signaling. In this context, we investigated the functional consequences of BAFF binding to BAFFR in human naive and memory B cells, as well as the underlying mechanisms that regulate their responses. Here, we show that following BAFFR engagement both subsets differ significantly at distinct levels: in the activation of the NF-κB2 and PI3K signaling pathways, their transcriptomic profile, and the expression of several proteins involved in cell survival, growth, activation and migration. Moreover, we show that BAFFR differentially co-opts proteins of the BCR complex and signalosome to activate the PI3K pathway in the naïve but not the memory compartment. Inducible inactivation of genes encoding for proteins of the BCR complex and for BAFF-receptors in primary cells showed a cell-intrinsic requirement for the survival of mature B cells. Our results provide new insights into the mechanisms regulating the response of naive B cells to BAFF-induced pro-survival signals, which differ from the BAFF-independent longevity of switched memory B cells.

Keywords: Adaptive immunity, autoimmunity, B lymphocytes, cell signalling, memory

P-0064

Evaluation of the effects of exosomes isolated from adipose tissue mesenchymal stem cell on Treg function in patients with Covid-19 PBMCs

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During the COVID-19 pandemic, much attention was paid to the function of immune cells in the pathogenesis of the disease. During the cytokine storm, which is part of the pathogenesis of this disease, a large amount of inflammatory cytokine is produced, leading to the occurrence of acute respiratory syndrome. Regulatory t cells suppress the immune system cell responses by producing inhibitory cytokines such as IL-10 and TGF-B and cell-to-cell interaction. Since mesenchymal stem cells-derived exosomes have immunomodulatory properties, the use of these vesicles can improve the function of Tregs in COVID-19 patient PBMCs. In this study, MSCs were first isolated from adipose tissue. After culturing these cells, the exosome was isolated from their supernatant by ultracentrifugation. SEM, TEM and DLS confirm isolated exosomes characterization. Then PBMCs isolated from patients with COVID-19 cultured in the presence of these exosomes. The expression of transcription factor related to these cells and the secretion levels of cytokines was assessed by real-time PCR and ELISA, respectively before and after treatment with MSCs exosomes. A significant reduction was observed in the levels of TGF-β, IL-10, and FoxP3 expression in COVID-19 patient PBMCs. Our data revealed the expression of FoxP3, TGF-β, and IL-10, the secretion levels of the TGF-β, and IL-10 in cultured PBMCs are increased in MSCs-derived exosome-treated group compared to the control group. The current study results indicated that AD-MSCs derived exosomes can restore the function of Treg cells of COVID-19 patient isolated PBMCs.

Keywords: Adaptive immunity, cellular interactions, cytokines and mediators, immune communication, infectious disease, stem cells

P-0065

Clues about the role of CD3γ in TCR dynamics revealed by CD3γ/δ chimeras delivery

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Humans lacking CD3γ(γ⁻) suffer immunodeficiency, reduced surface TCR and abolished PMA-induced, but not anti-CD3-induced, TCR down-regulation. We are studying delivery of CD3 chains to treat this disorder and to understand TCR dynamics. The response to PMA maps to the intracellular (IC) domain of CD3γ, whereas the cause of reduced TCR surface expression in γ⁻⁻cells is unknown. We developed retroviral vectors carrying normal CD3γ or CD3δ or several chimeras (EC-extracellular, TM-transmembrane and IC): δECγTMγIC (δγγ for short), γγδ, γδδ and γγ-. γγγ is CD3γ, and --- the empty vector. The constructs were validated in a γ⁻ TCR⁻ T cell line called JGN. The results confirmed that expression of the TCR in JGN was dependent on the EC domain of CD3γ since γγγ, γγδ, γδδ and γγ-, but not δδδ, δγγ or ---, scored positive for this assay and could be analysed further. γγ- induced the highest TCR expression, indicating that IC CD3γ is required for normal TCR dynamics in JGN. All four signalled normally for anti-CD3-induced down-regulation and conformational change, but only γγγ was down-regulated by PMA. In γ⁻ primary T cells, which have reduced surface TCR, the chimeras (including δγγ) confirmed that the response to PMA maps to IC CD3γ. However, γγγ, γγδ, and γδδ, and unexpectedly also δγγ, but not γγ- (or ---), normalized surface TCR levels. Since protein homology explains these results better than domain structure, we conclude that CD3γ contributes conformational cues that improve surface TCR expression, likely at assembly or membrane transport steps.

Keywords: Adaptive immunity, autoimmunity, immune development

P-0066

Age-related peculiarities of the immune system in atherosclerosis

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Age-related declines of the immune system contribute to the maintenance of systemic inflammation in atherosclerosis and other age-associated chronic diseases. This study aimed to investigate the influence of age and coronary atherosclerosis (CA) severity on CD4+ T-cell and monocyte subsets in patients with CA. 229 patients (median age 60 (55;79)) with stable coronary artery disease, different stages of CA and receiving statins were enrolled. Lymphocyte (T-helpers 1, T-helpers 17, regulatory T-cells including naïve and memory populations) and monocyte (classical, intermediate, non-classical) subsets were analyzed depending on the severity of CA and depending on age. The absolute values of circulating regulatory T-cells decreased with age, mainly due to the impaired production of naive CD45RA+ cells, that was not affected by the severity of CA. We did not observe associations between the content of circulating effector T-cells, including T-helpers 1 and T-helpers 17 and age or CA stage. The total number of monocytes decreased with age due to the reduction in the number of classical cells. Advanced stages of CA were associated with the elevated quantity of intermediate monocytes in younger patients (≤60 y.o.) and with the decreased values of classical monocytes in patients of the older age (>60 y.o.). The values of non-classical monocytes were not associated with age or CA severity. We assume age-related patterns of cellular immunity in patients with coronary atherosclerosis. The contribution of immune-inflammatory mechanisms to the development of atherosclerosis may depend on age, which requires further study.

Keywords: Ageing, cardiovascular diseases, immune senescence, regulatory cells

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POSTER PRESENTATIONS

P-0068

Coordinated asparagine uptake and asparagine synthetase activity is required for CD8+ T cell activation

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To support exit from quiescence, activated T cells have increased nutrient requirements that are sustained in part by an increased uptake of amino acids, glucose and fatty acids from the extracellular environment. By contrast, the role of intracellular pathways of amino acid synthesis in T cell activation are less well understood. Asparagine (Asn) is a non-essential amino acid that can be synthesized via the activity of asparagine synthetase (ASNS). To determine the contributions of Asn uptake and ASNS activity in CD8+ T cell activation. Naïve OT-I CD8+ T cells did not express ASNS and, consequently, cell viability and antigen-stimulated growth, IL-2 production, activation marker expression, protein synthesis and metabolic reprogramming were defective under conditions of Asn deprivation. After 24h of activation, antigen-induced mTOR and Myc signalling pathways resulted in upregulation of ASNS expression, that enabled OT-I T cells to function independently of extracellular Asn. Furthermore, using *Asns*-gene trap transgenic mice, we determined that in the combined absence of ASNS and extracellular Asn, CD8+ T cell activation is completely blocked. Our results show that CD8+ T cells coordinate nutrient uptake with upregulated expression of mediators of intracellular amino acid biosynthesis, exemplified by ASNS. This transcriptional programme is required to enable T cells to maximise their nutrient resources following activation.

Keywords: Adaptive immunity, metabolic control of immune responses, molecular immunology

P-0069

Autophagy inhibitors Spautin-1 and VPS34-IN1 differentially modulate the metabolism of plasmacytoid Dendritic cells

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Autophagy and cellular metabolism are deeply interconnected processes that are involved in regulation of the immune system. Autophagy is a cellular housekeeping mechanism that helps maintain survival, homeostasis, proliferation, activation and differentiation of immune cells, including Dendritic Cells (DCs). These cells also go through extensive metabolic reprogramming, which will determine their activation, making cell metabolism key to the cells' plasticity. In this work, we are investigating how autophagy modulation impacts metabolism in a subpopulation of immune cells specialized in anti-viral immunity, plasmacytoid DCs (pDCs). In order to achieve this, a pDC cell line (CAL-1) was treated with the autophagy inhibitors Spautin-1 and VPS34-IN1, followed by analysis of LC3 lipidation by Western blotting, to confirm autophagy inhibition, and ^{1}H NMR metabolomics. Both drugs efficiently inhibited autophagy. However, their effects on the cells' metabolism were quite different. Whereas Spautin-1 appeared to induce glycolysis stimulation, TCA cycle impairment and decreased levels of amino acids, VPS34-IN1 had the opposite effect. These results open new prospects for investigating the functional relevance of such distinct metabolic signatures and how they impact pDCs.

Keywords: Dendritic cells, innate immunity, omics technologies

P-0070

KIRs expression on gamma/delta T cells and HLA-C distribution during human pregnancy

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In previous study we found high cytotoxic potential in gdT cells at the site of implantation (SOI, decidua) which together with their end-stage differentiation suggests immediate cytolytic activity. The modulation of the maternal immune cell cytotoxicity at SOI is a key issue of tolerating the semi-allogeneic foetus without compromising cytotoxicity against pathogens. To evade detrimental maternal immune reaction the embryo-derived extravillous trophoblasts (EVT) express unique pattern of HLA class I molecules – non-classical HLA-E and HLA-G and the classical HLA-C molecule. HLA-C molecule is the only polymorphic molecule that could establish an immune tolerance by interaction with its cognate killer Ig-like receptors (KIRs) on the mother's immune cells. KIRs can function either as inhibitory or activating receptors. In this study we examined 1) the expression of inhibitory KIRs on gdT cells in early- and term decidua (placenta) as well as in the blood of pregnant and non-pregnant women, using flow cytometry; 2) in situ HLA-C distribution using immunohistochemistry. Our results showed differential expression of inhibitory KIRs on decidual and peripheral gdT cells. Higher proportion of gdT cells into early pregnancy decidua expressed inhibitory KIR2DL1 compared to those in the paired blood of pregnant women. Both KIR2DL1 and KIR2DL2/L3 receptors were detected in higher number of gdT cells into early decidua as compared to term placenta. Peripheral blood gdT cells, positive for both receptors, were in comparable numbers in pregnant and non-pregnant women. HLA-C was detected on different EVT subpopulations.

This study was funded by Bulgarian Science Fund, contract KP-06-DV-3 (VIHREN).

Keywords: Effector molecules, gamma-delta T cells, reproductive immunology

P-0071

Immature granulocyte altered oxidative burst induces bacterial SOS response

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In septic patient, emergency hematopoiesis realeases immature granulocytes (IG) in blood. IG are supposed to have decreased phagocytosis and Reaction Oxygen Species (ROS) production which induces the bacterial SOS response responsible for DNA repair and adaptation. We aimed to better characterize the difference bewteen IG and MG using an *in vitro* model of granulocytic differentiation and study if ROS production of both populations could induce bacterial SOS repair pathway. Using a promyelocytic cell line (HL60) and retinoic acid (1µM), we were able to follow *in vitro* the granulocytic differentiation according to their CD11b and CD16 expression. IG (CD16lowCD11b+) and MG (CD16highCD11b+) were sorted and incubated with *E.coli* strain expressing constitutively mCherry and a GFP reporter plasmid under the control of the gene of the *E.coli* SOS regulon for 10, 30 and 60minutes. We assessed the phagocytosis capacities, ROS production and SOS response induction by flow cytometry, flow imager and confocal microscopy. HL60-MG have significant higher phagocytosis capacities than HL60-IG at 30min (31.89% vs 18.22%) and 60min (52.24% vs 37.05%, $p<0.05$). The ROS production was also higher in HL60-MG than HL60-IG but differences were not significant. In co-culture with bacteria, we observed a slight significant increase in GFP expression in HL60-IG phagosome at 30min (20.08% vs 16.04%, $p<0.05$). By microscopy, we observed a co-localization of ROS production and bacterial SOS response induction in the phagosome. Our *in vitro* model allowed us to demonstrate that ROS production by IG is able to induce the SOS response in phagocyted bacteria.

Keywords: Bacterial infections, granulocytes, inflammatory molecules, neutrophils, phagocytosis

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POSTER PRESENTATIONS

P-0072

Remodeling of sialic acid-containing glycans control B-cell responses in the germinal centre

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Germinal centres (GC) are specialized sites where B-cells expand and diversify their antibody genes through somatic hypermutation. Immunogen-specific clones emerging from these events are then selected to differentiate into memory B-cells and long-lived antibody producing cells, which provide long-lasting immunity against recurrent infections. GC B-cells are routinely identified through distinct changes on their surface carbohydrates, known as glycans. One striking modification relates to the monosaccharide sialic acid. In mice, this change is mediated through downregulation of an enzyme called CMAH, which results in a GC-specific loss of preferred ligand for CD22, a member of the sialic acid-binding immunoglobulin-type lectins (Siglecs) and an inhibitory co-receptor of the B-cell antigen receptor (BCR). Since CD22 regulates B-cell function, we hypothesize that this glycan remodeling on B-cells control the GC reaction by modulating the activity of CD22. To test our hypothesis, we developed a transgenic mouse model that can conditionally express CMAH on GC B-cells. Using this model, we identified that glycan remodeling, mediated by downregulation of CMAH, is crucial for the GC B-cell response, egress of memory B-cells and plasma cells, and affinity maturation of antibodies. We also demonstrated that the function of these altered glycans is dependent on CD22, highlighting that coordinated loss of preferred ligands acts to modulates the CD22 activity in the GC B-cells. Overall, our study reveals that intrinsic glycan remodeling functions to optimize the B-cell responses in the GC by controlling CD22.

Funding: National Institutes of Health (R01AI118842)

Keywords: Adaptive immunity, antibody, B lymphocytes, cell signalling

P-0073

Galectin-1 expression in CD8+ T lymphocytes controls inflammation in contact hypersensitivity

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Allergic contact dermatitis, also known as contact hypersensitivity, is a frequent T-cell mediated inflammatory skin disease characterized by red, itchy, swollen and cracked skin. It is caused by the direct contact with an allergen and/or irritant hapten. Galectin-1 is a β-galactoside-binding lectin, which is highly expressed in several types of immune cells. The role of endogenous Galectin-1 in contact hypersensitivity is not known. We found that Galectin-1-deficient mice display more sustained and prolonged skin inflammation than wild type mice after oxazolone treatment. Galectin-1-deficient mice have increased CD8+ T cells and neutrophilic infiltration in the skin. After the sensitization phase, Galectin-1-depleted mice showed increased frequency of central memory CD8+ T cells and IFNγ secretion by CD8+ T cells. The absence of Galectin-1 does not affect migration of transferred CD4+ and CD8+ T cells from the blood to the lymph nodes or to the skin. Depletion of CD4+ T lymphocytes as well as adoptive transfer experiments demonstrated that endogenous expression of Galectin-1 on CD8+ T lymphocytes exerts a major role in the control of contact hypersensitivity model. These data underscore the protective role of endogenous Galectin-1 in CD8+ but not CD4+ T cells in the development of allergic contact dermatitis.

Keywords: Allergen-induced immune responses, inflammatory disease, skin diseases

P-0075

Defective monocyte enzymatic function and an inhibitory immune phenotype in HIV-exposed uninfected African infants in the era of anti-retroviral therapy

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HIV-Exposed Uninfected (HEU) infants are a rapidly expanding population in sub-Saharan Africa and are highly susceptible to disease caused by encapsulated bacteria in the first year of life. The mechanism of this increased risk is still poorly understood. We therefore investigated if HIV exposure dysregulates HEU infant immunity and if this is amplified by human herpes virus infection (HHV). Here, we compared monocyte enzymatic function, innate and adaptive immune cell phenotype, and vaccine-induced antibody responses between HEU and HUU infants. We demonstrate altered monocyte phagosomal function and B cell subset homeostasis, and lower vaccine-induced anti-Haemophilus influenzae type b (Hib)and anti-Tetanus Toxoid (TT) IgG titers in HEU compared to HUU infants. There was no difference in the prevalence of HHV infection between HEU and HUU infants. Our findings suggest that even in the era of antiretroviral therapy (ART)-mediated viral suppression, HIV exposure dysregulates monocyte and B cell function during a vulnerable period of immune maturation in infancy. This may contribute to the high rates of invasive bacterial disease and pneumonia in HEU infants.

Keywords: Adaptive immunity, B lymphocytes, infectious disease, innate immunity, phagocytosis, viral infections

P-0076

Autophagy inhibition impacts on the response of plasmacytoid Dendritic Cells following Toll-like receptor activation

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Plasmacytoid dendritic cells (pDCs), a particular population of dendritic cells (DCs), express Toll-like receptors (TLRs) that enable them to recognize pathogens and trigger an immune response. When activated, pDCs secrete pro-inflammatory cytokines and have the unique ability to produce massive amounts of type-I interferon, which makes them an essential component of antiviral immunity, while also being important contributors to the pathogenesis of some autoimmune diseases. However, the exact mechanisms regulating pDCs function are not yet fully understood. Autophagy is a process responsible for degradation of cellular components and microbes in lysosomes, helping in the maintenance of intracellular homeostasis. In addition, autophagy was recently identified as a regulatory element of innate immunity. Thus, our goal is to investigate the interplay between autophagy and TLR activation, deciphering the signaling pathways involved as well as the relevance of this link to pDCs activity. In this work, we stimulated a human pDC cell line (CAL-1) with a TLR7 ligand, in the presence or absence of the autophagy inhibitors spautin and Vps34-in1. It was found that both inhibitors induced a slight increase in TNF-alpha production, which was further promoted with TLR7 activation. Presently, we are dissecting the molecular players regulating the autophagy – innate activation crosstalk. This functional intersection between autophagy and TLR signaling in human pDCs, may in the future be explored for development of novel therapies to treat autoimmune diseases.

Keywords: Dendritic cells, immune regulation and therapy, innate immunity

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POSTER PRESENTATIONS

P-0077

Characterization of the bone marrow-derived dendritic cell phenotype of Mucin-2 knockout mice

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Mucin 2-deficient mice is a model of inflammatory bowel disease.The aim of this study was to evaluate expression of I-Ab, CD80, CD86 on CD11c+ dendritic cells(DCs), generated from bone marrow of Muc2-/-, Muc2+/+, C57 BL/6 mice. The experimental animals were C57BL/6 mice (n=5), Muc2+/+ (n=5) and Muc2-/- mice (n=5), female, 12-16 weeks old, purchased from the Scientific Research Institute of Neurosciences and Medicine (Novosibirsk). Muc2-/- and Muc2+/+ mice on the C57BL/6 background were bred as Muc2+/- x Muc2+/- and offspring were genotyped. Suspension of cells from bone marrow (from each mice separate) were cultured in the RPMI-1640, supplemented with 10% FCS, GM-CSF (20 ng/ml), IL-4 (20 ng/ml), during 7 days. Then cells were plated into 6-well plates for an additional incubation with LPS (2mkg/ml) for 24h. We used antibodies against CD11c to phenotype DCs, and I-Ab, CD80, CD86 (BioLegend) to evaluate the expression of co-stimulatory markers by flowcytometer FACS CantoII. Immature and mature DCs of Muc2-/- mice characterized by low expression of MHCII (I-Ab) compared to cells of Muc2+/+, C57BL/6 mice (8% vs 18,4%; 18,1% and 11,7% vs 12,7%; 21%). Percentage of CD80+ DCs also were decreased in knockout mice. However, the level of CD86 expression was the same in all groups. Futhermore, CD11c+I-Ab+DCs of Muc2-/- mice expressed approximately in 2 time less CD80 than DCs of control groups. Thus, immature and mature DCs of Muc2-/- mice have a decreased ability for antigen presentation and due to low level of CD80 may induce tolerance.

The study is supported by RSF project No20-64-47020.

Keywords: Animal models, dendritic cells, effector molecules, molecular immunology

P-0078

Parapoxvirus IL-10 homologues differ in their receptor binding, immunosuppressive and immunostimulatory capabilities

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Human IL-10 exerts pleiotropic effects on immune cells through its interactions with IL-10R1. IL-10 genes have been identified in genomes of Parapoxviruses (PPV), which infect ungulates and humans, with Orf virus (ORFV) IL-10 showing very similar immunomodulatory effects to human IL-10. We hypothesised that PPV IL-10s derived from Bovine papular stomatitis virus (BPSV), Pseudocowpox virus (PCPV), Red deer parapoxvirus (PVRD) and Seal parapoxvirus (SPV), which share 61-83% amino acid identity with ORFV IL-10, would act in a similar manner. FLAG-tagged PPV IL-10s were expressed in HEK-293 cells then purified using affinity chromatography. Inhibitory effects were assessed in lipoteichoic acid-treated human THP-1 monocytes using ELISAs. Stimulatory effects on murine MC/9 mast cells were assessed using a metabolic assay. IL-10R1 binding was determined using a competitive displacement assay. BPSV IL-10 inhibited pro-inflammatory cytokine production by monocytes, stimulated mast cell proliferation and bound IL-10R1 to a similar extent as ORFV IL-10. PCPV IL-10 showed reduced binding to IL-10R1 and inhibited production of IL-8 and IL-1β, but did not induce mast cell proliferation. PVRD IL-10 also showed reduced binding to IL-10R1, but induced mast cell proliferation and inhibited production of MCP-1 and IL-1β. By contrast, SPV IL-10 showed negligible activity in each assay. Further investigations are underway to establish whether differences in PPV IL-10 protein structure may account for their varied immune stimulatory and immune suppressive effects. However, these findings provide insight into the role of PPV IL-10s in viral pathogenesis, and the opportunity to elucidate how human IL-10 exerts its pleiotropic effects.

Keywords: Cytokines and mediators, inflammatory molecules, innate immunity, mast cells, viral infections

P-0079

IFN-β mediates the anti-osteoclastic effect of bisphosphonates and dexamethasone

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Multiple Myeloma (MM) is a plasma cell neoplasm characterized by severe bone disease. Management of MM includes zoledronic acid (Zol), a potent Bisphosphonate that inhibits the differentiation of monocytes into osteoclasts, and dexamethasone (Dex), a glucocorticoid that promotes the resolution of inflammation. The molecular mechanism triggered by Zol+Dex on monocyte differentiation is unclear. IFN-β is a pro-resolving cytokine that is well-known as an inhibitor of osteoclast differentiation. Here, we explored whether Zol and/or Dex regulate macrophage osteoclastic differentiation via IFN-β. RAW 264.7 and peritoneal macrophages were treated with Zol and/or Dex for 4-24 hours and IFN-β secretion was examined by ELISA, while the IFN stimulated gene (ISG) 15 expression was evaluated by Western blotting. RANKL-induced osteoclastogenesis of RAW 264.7 cells was determined by TRAP staining following treatment with Zol+Dex or IFN-β and anti-IFN-β. We found only the combination of Zol and Dex increased IFN-β secretion by RAW 264.7 macrophages at 4 hrs, and, correspondingly, ISG15 expression in these cells at 24 hrs. Moreover, Zol+Dex blocked osteoclastogenesis to a similar extent as recombinant IFN-β. Neutralizing anti-IFN-β antibodies reversed the effect of Zol+Dex on ISG15 expression and recovered osteoclastic differentiation. Finally, we found Zol+Dex also induced IFN-β expression in peritoneal resolution phase macrophages, suggesting these drugs might be used to enhance the resolution of acute inflammation. Altogether, our findings suggest Zol+Dex block the differentiation of osteoclasts through the expression of IFN-β. Revealing the molecular pathway behind this regulation may lead to the development of IFN-β-based therapy to inhibit osteoclastogenesis in MM patients.

Keywords: Cytokines and mediators, drugs for immune modulation, innate immunity, macrophage

P-0080

Revealing the molecular basis of WASp in the nucleus of B cells

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Mutations in the WAS gene, encoding for the actin regulator WASp, cause two rare primary immunodeficiency disorders (PIDs): Wiskott-Aldrich syndrome (WAS) and X-linked neutropenia (XLN). WAS is caused by a loss-of-function mutation in the WAS protein (WASp) resulting in immunodeficiency, thrombocytopenia, and eczema. WASp overexpression causes XLN. XLN patients show common features of immune system failure and accumulated cytogenetic abnormalities, leading to a tumor-prone phenotype. WASp is a regulator of the actin cytoskeleton, required for hematopoietic cell functions including effective migration and immune synapse formation. In this context, B cell affinity maturation in the germinal center (GC) relies on processes regulated by the actin cytoskeleton, such as migration, proliferation, and genomic rearrangement. The aim with this project is to reveal the role of WASp in the nucleus driving dynamic processes like DNA synthesis, DNA repair, and chromatin remodeling. Performing nuclear-cytosol fractionation experiments on murine XLN B cells and WT B cells derived from an *in vitro* inducible GC system we are able to detect, through immunoblotting, the presence of WASp in the nucleus apart from its known presence in the cytosol. Flow cytometry analysis is valuable in tracking the differentiation of naïve B cells into plasma cells. Considering the well-known association of N-WASp (a homologous protein of WASp in non-hematopoietic cells) with RNA Polymerase II and of WASp with T cell factor 1 (TCF1) in T cells, further ChIP-Seq experiments will be important to determine the function of WASp in active processes of the nucleus of B cells.

Keywords: B lymphocytes, cytoskeleton, immunodeficiency

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POSTER PRESENTATIONS

P-0081

Functional unit concept of invariant T lymphocytes reveals site–specific immunity between lymph nodes

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The adaptive limb of the immune system consists of antibody-producing B cells and CD4 T helper, and CD8 cytotoxic T cells. Besides these classical lymphocyte subsets, unconventional T cells exist characterized by a more limited repertoire of their TCR chains. Gamma delta (gd), mucosal-associated invariant (MAIT), and natural killer T cells (NKT) are the major cell types comprising this invariant T cell compartment. These cells are found throughout the body in the non- and lymphoid tissues, and they recognize antigens linked to non-polymorphic antigen-presenting molecules like CD1 and MR1. Functionally, these cells typically recognize lipids, small-molecule metabolites, and phosphoantigens that may be pathogen-derived or expressed by tissues in the context of activation or stress responses. Investigating these cell types on a functional level as a group, we found that tissue-derived unconventional T cells constantly migrate like dendritic cells to draining lymph nodes. scRNA-seq revealed transcriptional homogeneity of these subsets and shared functional outputs critical to controlling bacterial infections as a group rather than separate entities. Importantly, since every tissue harbors a unique set of invariant T cells with specific differentiation states (Th1-like, Th2-like, and Th17-like), every draining lymph node is as well populated by a unique composition of such cells. By comparing different lymph nodes using scRNA-seq in an unbiased manner, we could resolve internodal differences and further demonstrate the functional consequences on humoral and cellular immune responses. The discovery that every lymph node mounts a unique immune response, directly impacts vaccination

Keywords: CD1-restricted T cells, gamma-delta T cells, innate immunity, lymphoid organs, MAIT cells, NKT cells

P-0082

Analysis of Tregs' activation potential through pSTAT5 and HLA-DR molecules in women with pregnancy failure

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The population of regulatory T-cells (Tregs) is essential in the establishment of immune tolerance. Deviations in Tregs' percentage and/or function were detected in conditions of impaired immune tolerance e.g. autoimmune diseases, recurrent pregnancy loss (RPL), etc. The aim of the present study is to analyze the expression of pSTAT5 and HLA-DR molecules on Tregs, in women with RPL. PBMCs from 15 healthy parous women (23-40 yrs) and 15 age-matched patients with RPL were stained with anti-CD4/CD3/CD25/FoxP3/pSTAT5/HLA-DR antibodies. pSTAT5 was determined, by stimulating PBMCs with IL-2 (30IU/ml) or anti-CD3/CD28 (20µl/ml). Data collected on FACS Canto II, was analyzed by FlowJo V10 and GraphPad7 software. Differential Tregs/non-Tregs profile was found in women with pregnancy failure (p=0.0001). Patients' Tregs expressing pSTAT5 represent a minor population in comparison to women experiencing normal pregnancy (p=0.03). In the patient group, most of the non-Tregs were pSTAT5 positive (p=0.006). *In vitro* stimulation of isolated PBMCs demonstrated a variable trend towards pSTAT5+ shift in Tregs and non-Tregs, when comparing controls and patients. In healthy women, the expression of HLA-DR+ cells, in both Tregs and non-Tregs, was shown to be elevated. That was not the case in women with RPL (p=0.0001), indicating that the dynamics of pSTAT5 and HLA-DR, might be linked to the activation impairment of Tregs. This potentially impacts immune tolerance in women with RPL.

Keywords: Cell signalling, reproductive immunology, regulatory cells

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P-0083

ABCG1 modulates macrophage polarization in type 2 diabetes

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ATP-binding cassette transporter G1 (ABCG1) moves cellular cholesterol to HDL particles, thereby removing excess cholesterol from the cell. Type 2 diabetes (T2D) resembles a natural human macrophage specific "Abcg1 knockout" model by having low ABCG1 expression in macrophages derived from T2D patients' blood monocytes. The aim of this study is to investigate the impact of ABCG1 on human macrophage polarization using the T2D model. ABCG1 expression was inhibited by RNA-interference in primary macrophages derived from human peripheral blood monocytes. Concurrently, macrophages were derived from peripheral blood monocytes from T2D patients. Later, macrophages were stimulated with LPS/IFNγ for M1 and IL-4 for M2a polarization. Expression of M1/M2 specific markers and ABCG1 was analyzed by qPCR. ABCG1 inhibition significantly increased the expression of M1 markers IDO1, CXCL9, CXCL10 and TNFα after LPS/IFNγ stimulation, and significantly reduced the expression of M2 markers MRC1 and CCL22 after IL-4 stimulation. Our data showed that ABCG1 expression was negatively correlated with the increase in M1 marker expression in human macrophages. Furthermore, significantly lower ABCG1 expression was confirmed in macrophages from T2D patients compared to healthy individuals. Our results indicated that macrophages from T2D patients had tendency towards M1 polarization. ABCG1 deficiency promotes primary human macrophages towards the M1 phenotype. Therefore, ABCG1 may be a potential new target in modulating macrophage polarization for the treatment of T2D and similar diseases, in which macrophages are known to play an important role.

Keywords: Diabetes, innate immunity, macrophage

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POSTER PRESENTATIONS

P-0084

The uteroplacental microenvironment influence on NK-92 transmigration, adhesion and cytotoxicity to choriocarcinoma cells

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Tumors induce NK cells polarizing to tumor-promoting phenotypes, that have many in common with decidual NK cells. To analyse the influence of uteroplacental microenvironment on NK-92 cell activity towards choriocarcinoma cells. NK-92 cells (ATCC) were cultured in presence of choriocarcinoma cells of JEG-3 cell line (ATCC). NK-92 cells phenotype, migration activity and cytotoxicity were assessed using cytometer FACSCanto II (BD, USA). Placentas were obtained: after induced abortion at normal 1st-trimester pregnancy between 9 and 11 weeks (n=20), after cesarean section at normal 3rd-trimester pregnancy between 38 and 39 weeks (n=15). Data were analyzed using Statistica software. The presence of secretory products of third trimester placentas (SP1) resulted in more intense CD29, CD49d, CD11a, CD49d and CD58 expression by NK-92 cells. Migration of NK-92 cells through choriocarcinoma cells in the presence of SP1 was increased compared with the migration level in the presence of secretory products from third trimester placentas Uteroplacental contact zone cytokines were able to enhance choriocarcinoma cells mortality both by themselves in case of IL-1β, IL-6, IFNγ, IL-4, TGFβ, bFGF, and also through increasing the cytotoxic potential of NK-92 cells in case of IL-1β, IFNγ, IL-8, TGFβ, and GM-CSF. PLGF decreased NK-92 cell cytotoxicity for choriocarcinoma cells. SP1 enhanced NK-92 cell cytotoxic potential for JEG-3. Uteroplacental microenvironment modifies NK-92 functions towards choriocarcinoma cells.

The study was supported by: RSF 21-15-00021, RFBR 20-015-00014, the government program AAAA-A19-119021290116-1 and AAAA-A20-120041390033-4, the participation of D.O.B. was funded by grant for PhD students 20-315-90003.

Keywords: Cancer immunology, cellular interactions, cytokines and mediators, microenvironment, modelling, NK cells

P-0085

Transcriptomic signature and metabolic programming of bovine classical and nonclassical monocytes indicate distinct functional specializations

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Similar to human monocytes, bovine monocytes can be split into $CD14^+CD16^-$ classical and $CD14^-CD16^+$ nonclassical monocytes (cM and ncM, respectively). Following bulk RNA- seq of sorted subsets, we have performed an in-depth analysis of their steady-state transcriptomes, revealing pronounced functional specializations. Differential gene transcription indicates that pro-inflammatory and antibacterial responses are effectively carried out by cM, while ncM appear to be ideally equipped for regulatory/ anti-inflammatory functions and tissue repair, as well as for T-cell immunomodulation and antiviral responses. Specialization of cM and ncM for antibacterial and antiviral defense, respectively, is also supported by their responsiveness to TLR ligands, as determined by flow cytometric analysis of p38 MAPK phosphorylation. In line with their pro- and anti-inflammatory gene signatures, we have found that oxidative phosphorylation prevails in ncM, whereas cM are clearly biased towards aerobic glycolysis. Taken together, our data indicate complementary functions and distinct metabolic programming of bovine cM and ncM, thereby offering insights into monocyte biology that are likely applicable across species.

Keywords: Innate immunity, metabolic control of immune responses, myeloid cells, RNAseq, veterinary immunology

P-0087

Phenotype and function of decidual natural killer cells in term labour

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The signals that initiate labour in humans are poorly understood. One hypothesis is that the signal originates with immune cells in the lining of the uterus, known as the decidua. The aim of this project is to define the composition of decidual natural killer (dNK) cells at the end of pregnancy and investigate how they change during the labour process. Immune cells were isolated from the decidua basalis and decidua parietalis from placentas from people having caesarean sections, both in labour and not in labour. The phenotype and function (IFNg, IL-8 and CD107a) of the cells were assessed using flow cytometry. Three subtypes of dNK cells are found in early pregnancy. We find that these three subtypes are still present at the end of pregnancy, but their composition has changed. There is a reduction in the proportion of dNK1 and dNK2 cells and a corresponding increase in proportion of dNK3 cells. At term, dNK1 cells generally produce more IL-8 and degranulate more compared to dNK2 and dNK3. Our preliminary results suggest that all three subsets of dNK cells are more activated, in early labouring than non-labouring samples. These changes in dNK function occur in early labour suggesting they may be involved in initiating labour. In future work, we will establish the downstream impact of these functional changes to try and establish a mechanism for labour initiation. We are now collecting samples from people at pre-term to determine whether similar changes are seen.

Keywords: Cytokines and mediators, innate lymphoid cells, reproductive immunology, NK cells

P-0088

Trophoblast cells influence NK cell phenotype and proliferation

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After migration to decidua at pregnancy, peripheral blood NK cells can have contact with trophoblast cells (TC). Evaluation of peripheral blood NK cell proliferation and phenotype in the presence of TC. The research involved pregnant (n=38) and non-pregnant women in proliferative (n=37) and secretory (n=40) phase of menstrual cycle. Mononuclear cells or isolated with sorter FacsAriaIII (BD, USA) NK cells were cultured in presence of TC of JEG-3 cell line (ATCC). NK cell proliferation and phenotype was assessed using cytometer FACSCanto II (BD, USA). TGFβ in JEG-3 cells and SMADs in NK-92 cells were assessed using WesternBlot. Data were analyzed using Statistica software. In presence of IL-2, Ki-67 expression intensity was elevated in all groups, compared with basic level. NK cells in presence of TC decreased the Ki-67 expression in all groups, compared to basic level. NK cells from PBMC in presence of IL-2 showed elevated Ki-67 expression in all the groups, compared to basic level. The amount of CD16+CD56bright NK cells in all groups was elevated after culturing with TC and IL-2, compared to culturing with TC only. The amount of CD16-CD56bright NK cells in the same conditions was elevated only in pregnant women. TGF-β was detected in JEG-3 cells. TC resulted in elevation of SMAD2/3 and pSMAD2/3 in NK cells. NK cells acquire regulatory phenotype as a result of interaction with TC and additional cytokine regulation.

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Keywords: Cellular interactions, innate immunity, innate lymphoid cells, modelling, molecular immunology, NK cells

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POSTER PRESENTATIONS

P-0089

Carboxylesterase-1 enzyme inhibition promotes monocytes differentiation to highly phagocytic and pro-inflammatory dendritic cells that support T helper-17 cells differentiation

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Lipid metabolism is essential for dendritic cells (DCs) differentiation. Inhibiting fatty acid synthesis or oxidation can either block or modulate DCs differentiation respectively. Carboxylesterase-1 enzyme (CES1) is a known triacylglycerol and cholesterol ester lipase, that's expressed in both monocytes and DCs. This study aims to investigate the role of CES1 in modulating DCs differentiation and functions. Human Blood CD14+ monocytes were differentiated to DCs using IL4 and GMCSF. CES1 was inhibited using WWL113. Flow cytometry is used to assess phagocytosis of labelled E.coli particles, DCs surface markers and T cell cytokines expression. Quantitative PCR and RNAseq were applied to profile generated DCs transcriptome. Cytokine and chemokines secretion were measured by ELISA. Bone marrow derived DCs (BMDCs) were generated from WT or transgenic mice expressing human CES1 under CD68 promotor (hCES1 mice). *In vitro* generated human DCs, under CES1 inhibition, showed stronger pro-inflammatory phenotype compared to DMSO treated control. This was demonstrated by enhanced TNF, IL6, IL8, IL12p40, CCL2 and CCL3 secretion, augmented co-stimulatory molecules (CD86 and CD83) upregulation in response to LPS activation and stronger phagocytic activity towards E.coli particles. In autologous DCs-T cells co-culture assay, these DCs supported more Th17 cells induction. In murine system, BMDCs over-expressing human CES1 (hCES1 mice) secreted less TNF and IL6 in responsive to TLR legends compared to WT BMDCs. In conclusion, CES1 enzyme is important modulator of DCs differentiation. The absence of CES1 activity promotes differentiation of highly phagocytic DCs with strong pro-inflammatory phenotype that support Th17 cell induction.

Keywords: Innate immunity, metabolic control of immune responses, phagocytosis, RNAseq, dendritic cells

P-0090

Mixed-phenotype acute leukemia: flow cytometric analysis in a tertiary care center of Bangladesh

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Mixed-phenotype acute leukemia (MPAL) is a rare but difficult to treat hematologic malignancy with immunophenotypic co-expression of at least two cell lineages, or with rare cases involving all three lineages, e.g. myeloid with B-or T-lymphoid or all three of myeloid, B- and T-lymphoid altogether. Advancement in multiparametric flow cytometry has made its identification easier. This study aims to identify cases of MPAL and to study the incidence, clinical features, hematological profile and immunophenotypic expression profile of MPAL at our center. Four color flow cytometry multiparametric immunophenotyping method was used in EDTA bone marrow and/or peripheral blood taken from all consecutive morphologically diagnosed acute leukemia (AL) patients from November 2015 to October 2020. A panel of fluorochrome-labelled monoclonal antibodies against myeloid, B-cell and T-cell markers were used. Among 616 consecutive AL cases diagnosed and evaluated during this period, 22(3.57%) patients with MPAL were identified fulfilling World Health Organization (WHO) 2008/ EGIL criteria for immunophenotypic characteristics of AL. Fourteen were adults, male: female = 5:2. Eight were children, male: female= 3:1. Median age of this cohort (n=22) was 23.5 years (range: 1 -70 years). Fifteen (68.18%) cases were diagnosed as B/myeloid, 5(22.72%) as T/myeloid and 2 (9.1%) as B/T MPAL. Morphologically MPAL cases were diagnosed as 15 acute myeloid leukemia (AML) and 7 acute lymphoid leukemia (ALL) whereas blast morphology was not predictive of a MPAL. Multiparametric flow cytometry using comprehensive panel of monoclonal antibodies is a valuable tool to diagnose MPAL known to have a poor outcome.

Keywords: Antibody, B lymphocytes, cancer immunology, immunological techniques, myeloid cells

P-0091

Expression and function of TRAIL (TNF-related apoptosis-inducing ligand) and its death receptors on human *i*NKT cells

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Invariant natural killer T (*i*NKT) cells are innate-like T cells that are characterized by their invariant Vα24 TCR-chain, which recognize glycolipids presented by CD1d. Once activated, *i*NKT cells rapidly display effector functions, like cytokine production and cytotoxicity. TRAIL ('tumor necrosis factor-related apoptosis-inducing ligand') was initially recognized for triggering apoptosis preferentially in cancer cells. However, besides apoptosis, TRAIL can also promote survival and proliferation of some target cells. We purified PBMCs from healthy donors and measured the expression of TRAIL, DR4, DR5, and effector cytokines in *i*NKT cells *ex vivo* and in *in vitro* generated *i*NKT cell lines by flow cytometry. While very few *i*NKT cells *ex vivo* expressed TRAIL, DR4, or DR5, all three were highly expressed by the *i*NKT cell lines. However, their expression was found to be mostly intracellular. Adding soluble TRAIL, either overnight or for five days, to the *i*NKT cell lines did not influence their survival, but had subtle phenotypic and functional effects. These results provide the first detailed analysis of the role of the TRAIL/DR-system in *i*NKT cells and expand our understanding of the cytotoxic potential of human *i*NKT cells.

Keywords: Cell death, cytokines and mediators, effector molecules, NKT cells

P-0092

CD3G or CD3D knock-down in mature T lymphocytes similarly cripples the human TCRαβ complex

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The human αβ TCR is composed of a variable heterodimer (TCRαβ) and three invariant dimers (CD3γε, CD3δε and ζζ/CD2472). The role of each invariant chain in the stepwise interactions among TCR chains along assembly are still not fully understood. Despite the high sequence homology between CD3γ and CD3δ, the clinical consequences of the corresponding immunodeficiencies (ID) in humans are very different (mild and severe, respectively), and mouse models do not recapitulate findings in human ID. To try to understand such disparities, we stably knocked down (KD) CD3D or CD3G expression in the human Jurkat T-cell line and analyzed comparatively their impact on TCRαβ assembly, transport and surface expression. The results indicated that TCR ensembles were less stable and CD3ε levels lower when CD3γ, rather than CD3δ, was scarce. However, both defective TCR ensembles were strongly retained in the ER, lacked ζζ/CD2472 and barely reached the T-cell surface (< 11% of normal controls) in any of the CD3 KD cells. This is in sharp contrast to human CD3γ or δ ID, whose mature T cells express higher levels of surface TCR (> 30% vs normal controls). Fetal thymus organ culture CD3 KD experiments showed high plasticity in emerging immature polyclonal T lymphocytes that allowed for the expression of significant TCR levels which may then signal for survival in CD3γ, but not in CD3δ deficiency, and explain the immunological and clinical disparities of such ID cases.

Keywords: Adaptive immunity, autoimmunity, immune development

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POSTER PRESENTATIONS

P-0094

Effect of sex hormones on the dopaminergic pathway in B cells

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Both sex hormones and dopamine can influence peripheral blood mononuclear cells (PBMCs). Previous work indicates that an interplay between them might contribute to rheumatoid arthritis (RA) – which predominantly occurs in women – via affecting B cell maturation and cytokine production. Thus, aim of the study was to investigate a connection between dopamine and sex hormones concerning B cell regulation and especially pathological processes in RA. B cell lines 721.221 ('female') and JY ('male') were stimulated *in vitro* with sex hormone receptor agonists or dopamine receptor DRD1 agonist SKF38393. Expression of DRD1 was analysed via FACS. Basal DRD1 level on B cells and dopamine in PBMCs from female RA patients (n=14-23) and healthy women (n=10-23) were quantified via FACS or ELISA. The study was approved by the ethical committee and all patients gave written consent. Sex hormone receptor-specific stimulations resulted in statistically significant increase of DRD1 on 'female' 721.221 compared to 'male' JY. DRD1 activation via SKF38393 also led to increased DRD1 on 721.221 ($p<0.01$). Furthermore, higher DRD1 expression on B cells ($p<0.001$) and dopamine in PBMCs ($p<0.05$) was found for female RA patients compared to healthy women. Elevated DRD1 and dopamine levels in RA women suggest that the dopaminergic pathway is overactivated in these patients. Sex hormones seem to play a crucial role as they can increase DRD1 expression on B cells. The results also suggest that dopamine itself is involved in regulating DRD1. However, the exact interplay needs to be further investigated.

Keywords: Autoimmunity, B lymphocytes, rheumatoid arthritis

P-0095

The effect of time course on the expression of human macrophage polarization markers

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Macrophages can be polarized into M1 or M2 phenotypes according to the signals they receive from the environment. However, there is no consensus in the literature regarding the stimulation times for macrophage polarization in *in vitro* experiments. Here we report a detailed time course of M1, M2a, and M2c polarization of human macrophages that determined the optimal time points for the expression of polarization markers. Primary macrophages derived from human PBMC monocytes were stimulated with LPS+IFNγ for M1, IL-4 for M2a, or IL-10 for M2c polarization for 4, 8, 12, 24, 48, or 72 hours. The changes in the expression of M1, M2a, and M2c polarization markers were determined at mRNA level by qPCR and at protein level by flow cytometry or ELISA. For M1 polarized macrophages, the mRNA expression of CXCL9, CXCL10, and TNFα peaked 4 hours after the stimulation. In contrast, the protein expression of HLA-DR and CD86 were the highest after 12 hours. For M2a macrophages, the maximal expression of CD206 and TGM2 mRNA as well as CD200R and CD206 protein were seen after 72 hours of stimulation. Finally, for M2c macrophages, the expression of IL-10 peaked at the 4 hours and of CD163 at the 8 and 12 hours time points. Our data establish the optimal time points for the *in vitro* polarization of human M1, M2a, and M2c macrophages, which will greatly facilitate their investigation.

Keywords: Molecular immunology, innate immunity, macrophage

P-0096

Immunomodulatory mechanisms of *Astragalus* saponins

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Astragaloside VII (AST VII), a triterpenoid saponin isolated from *Astragalus* plants shows promise as vaccine adjuvant, as it supports a balanced Th1/Th2 immune response. However, the underlying mechanisms of its adjuvant activity have not been defined. Here we investigated the impact of AST VII and its semi-synthetic analogs on human whole blood cells, as well as on mouse bone marrow-derived dendritic cells (BMDCs). Cells were stimulated with AST VII and its derivatives in the presence or absence of LPS or PMA/ionomycin, and the secretion of cytokines and the expression of activation markers were analyzed by ELISA and flow cytometry, respectively. AST VII and its analogs increased the production of IL-1β in PMA/ionomycin stimulated human whole blood cells. In LPS-treated mouse BMDCs, AST VII increased the production of IL-1β and IL-12, and the expression of MHC II, CD86, and CD80. The strength of the IL-1β boost correlated directly with the hydrophobicity of the AST VII compounds. In mixed leukocyte reaction, AST VII and derivatives increased the expression of the activation marker CD44 on mouse $CD4^+$ and $CD8^+$ T cells. In conclusion, AST VII and its derivatives strengthen pro-inflammatory responses, support dendritic cell maturation, and T cell activation *in vitro*. Our results provide insights into the mechanisms of the adjuvants activities of AST VII and its analogs, which will be instrumental to improve their utility as vaccine adjuvants.

Keywords: Adjuvants and vaccines, immunopharmacology, innate immunity

P-0097

Low-dose LPS exposure induces innate memory in alveolar macrophages

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Environmental triggers, including air pollutants, microbes and pathogen-associated compounds, importantly shape our immune system. Such encounters can induce functional changes in innate immune cells, enhancing their response to secondary pathogenic threats in the form of trained immunity. Being located in the distal airways of the lung, tissue-resident alveolar macrophages (AMs) are in direct contact with inhaled compounds and play a critical role in host defense. In this study, we investigated the effects of low-dose lipopolysaccharide (LPS) exposure on AM immune responses upon secondary challenge with *Streptococcus pneumoniae* (*S. pneumoniae*), the major causative agent of bacterial pneumonia. Intranasal (i.n.) LPS treatment of wild type mice improved bacterial clearance during subsequent *S. pneumoniae* infection six days after pre-treatment. In order to investigate whether low-dose LPS exposure induces AM memory, we isolated AMs six days after *in vivo* treatment and analysed their immune response upon *ex vivo* secondary challenge. Compared to ctrl AMs, LPS-exposed AMs displayed increased immune activity after bacterial stimulation. Aiming to characterize the cellular changes induced by LPS-exposure, we performed genetic, epigenetic and metabolic profiling. LPS treatment profoundly modulated the cellular metabolite and lipid composition of AMs, affecting their metabolic responsiveness during secondary challenge. We could further provide evidence for a critical role of LPS-induced metabolic activation and type 1 interferon signalling in the establishment of AM memory. Our ultimate goal is to further uncover the molecular mechanisms and functional consequences of AM memory in order improve our understanding of trained immunity in the context of pulmonary diseases.

Keywords: Infectious disease, innate immunity, macrophage, memory

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POSTER PRESENTATIONS

P-0098

Functional polarization of naked mole rat bone marrow-derived macrophages

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Naked mole-rat (NMR) is a long-lived rodent highly resistant to cancer and age-associated disorders. Recently, it was shown that the immune system of NMR possesses a unique cellular composition with the prevalence of myeloid cells, and the absence of natural killer cells.Thus, thorough assessment of NMR's myeloid compartment may uncover novel mechanisms of immune regulation. Previously we established higher proportion of $CD14^+$ myeloid cells in the bone marrow of NMR as compared to C57Bl/6 mice. To determine whether this trait correlates with increased potential for inflammatory responses in NMR, we examined expression and metabolic profiles of classically (M1) and alternatively (M2) activated bone marrow-derived macrophages (BMDM). In the presence of murine M-CSF NMR bone marrow cultures displayed expected macrophage-like morphology at day 7. Activation of NMR macrophages under pro-inflammatory conditions led to enhanced glycolysis and expected M1 expression profiles. M0 and M1 NMR macrophages had similar maximal glycolytic capacity, while in mouse system this parameter was higher in M1. Interestingly, expression of M2-characteristic genes was not elevated in NMR BMDMs under conventional anti-inflammatory stimuli, but glycolysis and respiration were suppressed as compared to M0, unlike in the mouse system. Altogether, our results indicate that NMR bone marrow-derived macrophages are capable of transcriptomic and metabolic reprogramming under pro- and anti-inflammatory stimuli. At the same time, NMR macrophages may be initially predisposed to the M1 phenotype, suggesting that transition to M2 is different as compared to the mouse system, which may reflect specific adaptations in NMR innate immunity.

Supported by RFBR grant-19-34-51030.

Keywords: Animal models, innate immunity, macrophage, metabolic control of immune responses

P-0099

The percentage of γδT cells is significantly decreased in chronic lymphocytic leukaemia

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The aim of the study was to assess the percentage of γδ T cells in CLL patients along with analysis of bright and dim subsets and CD4/CD8 expression patterns. This is a preludium to further functional studies. Study included 66 untreated patients with CLL and 21 control subjects. Peripheral blood mononuclear cells were isolated by gradient centrifugation and frozen until further processing. After thawing, cells were stained with anti-CD3 PE-Cy5 and anti-TCRγδ FITC, anti-CD4 PE and anti-CD8 APC. Samples were acquired with Cytoflex LX. Statistical analysis was performed with GraphPad Prism. Kruskal-Wallis test was used to test the statistical significance. Values are presented as median (IQR). The percentage of γδT among total T cells is significantly lower in CLL patients [3.335 (3.19)% vs 4.0 (6.355)%, p=0.0476]. Expression of CD8 did not differ between groups; expression of CD4 was significantly higher in CLL patients [2.24 (4.58)% vs 0.55 (1.03)%, p=0.0004]. Based on TCR and CD3 expression, γδT cells can be divided into two subsets – bright and dim [Paget et al., 2015]. While in healthy subjects dim subset prevailed, the opposite was observed for CLL patients. Previous studies focused on the absolute count of γδT cells, strangely it was significantly higher than in healthy subjects. In our opinion, percentage is a more important marker – we have observed a decrease in γδT percentage. Rise in γδT count suggests that they respond to CLL, but decrease in their percentage implies that it may be to weak.

Keywords: Microenvironment, cancer immunology, gamma-delta T cells

P-0100

Novel insights into immune-independent functions of immune checkpoint inhibitors in oesophageal adenocarcinoma; potential implications for overcoming chemoresistance to first-line chemotherapy regimens

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Immune checkpoint inhibitors (ICIs) reinvigorate anti-tumour immunity in oesophageal adenocarcinoma (OAC). However, emerging studies have identified novel immune-independent functions for immune checkpoints (ICs) in other solid tumour-types, whereby IC signalling in gastric cancer cells confers chemoresistance. This study explores immune-independent functions of ICs in OAC and if therapeutic blockade may enhance chemotherapy toxicity. OAC cells were screened *in vitro* and *ex vivo* for a range of ICs(PD-1,TIGIT,TIM-3,LAG-3,A2aR,PD-L1,PD-L2,CD160) by flow cytometry. The phenotype of OAC cells expressing ICs was also assessed for features of stemness (ALDH, CD54), senescence (β-galactosidase) and invasiveness (vimentin) in the absence and presence of chemotherapy by flow cytometry. The effect of ICIs on viability(CCK-8 assay and western blot for Bcl-xL), proliferation(BrdU assay), chemo-sensitivity (annexin-V propidium iodide assay), metabolism (Seahorse), invasiveness and stemness characteristics (flow cytometry) was assessed in OAC cells. A subpopulation of stem-like, senescent and vimentin+ cells were enriched for ICs, which was enhanced by FLOT and CROSS. Blockade of PD-1, TIGIT, A2aR, TIM-3 and PD-L1 decreased proliferation, induced apoptosis and enhanced toxicity of FLOT in OAC cells. Blockade of TIGIT decreased pro-survival Bcl-xL factor, induced cell death and promoted a more glycolytic phenotype in OAC cells. Several novel ICs have been identified as potential targets to enhance chemotherpay efficacy in OAC. Upregulation of ICs on OAC cells following chemotherapy may represent potential mechanisms of chemo-immune resistance for stem-like, senescent and vimentin+ aggressive cancer cell clones. Combining ICIs with chemotherapy may achieve synergistic effects in OAC patients boosting clincal outcomes and warrants further investigation.

Keywords: Cancer immunology, checkpoint inhibition, immunotherapy

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POSTER PRESENTATIONS

P-0102

The secretome of visceral adipose tissue from advanced stage oesophageal adenocarcinoma patients dampened anti-tumour immunity *ex vivo;* implications for the potential use of immune checkpoint blockade to abrogate these immune inhibitory effects

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Visceral obesity is a key risk factor for development of oesophageal adenocarcioma (OAC). Greater than 70% of OAC patients are resistant to chemoradiotherapy and the visceral fat has been implicated in therapy-resistance, via secretion of tumour-promoting mediators into the periphery enhancing OAC cell survival/growth. This study investigates the effect of the secretome from visceral fat of OAC patients with early versus advanced tumours on anti-tumour T-cell immunity and the role of immune checkpoint blockade (ICB) in enhancing anti-tumour immunity. The effect of visceral adipose conditioned media (ACM) on immune checkpoint (IC) expression was assessed on OAC cells (FLO-1 (non-metastatic) and FLO-LM (metastatic) and healthy donor T-cells by flow cytometry (n=20). Anti-tumour cytokine profiles (IFN-γ, TNF-α, IL-2), cytotoxicity (CD107a), activation markers (CD27, CD69) and T-cell differentiation states(naïve, effector and central memory) and T-cell subsets(TH1, TH17, TH1/17, TREG,) were also investigated following treatment with ACM by flow cytometry (n=16). Anti-tumour cytokine production was enhanced following treatment with ACM, however, ACM generated from patients with early stage tumours enhanced T-cell cytotoxicity more substantially than ACM generated from patients with advanced tumours. Markers of T cell activation were decreased, and ICs were increased by ACM generated from patients with more advanced stage tumours. ACM from patients with more advanced stage tumours exerted a more immunosuppressive effect on T-cells. This highlights the systemic impact of the tumour in establishing a tumour-promoting milieu in distal organs, which may have detrimental effects on anti-tumour immunity and response to immune checkpoint inhibitor therapy.

Keywords: Adaptive immunity, cancer immunology, checkpoint inhibition

P-0104

Phenotypical and functional analysis of circulating and lymph-node resident T follicular helper (Tfh) cells from breast cancer patients

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Tfh cells are essential for maturation of antigen-specific antibody-producing B cells in LN. Functional characterization of Tfh cells in cancer patients' lymph nodes (LN) remain to be better elucidated. Lymphocytes isolated from the patients' LN and peripheral blood were used in phenotypical characterization; CD45, CD3, CD4, CD8, PD1, CXCR5, CCR7, CD45RO, CD127, CD25, TIM-3, LAG-3, CTLA-4 and ICOS expression were analyzed with flow cytometer. Immunofluorescence staining was conducted for CD4, CD19 and BCL-6 on LN sections. For functional analysis, $CD4^+$ T cells were isolated from samples with $CD4^+$MACS, CFSE labeled and stimulated with anti-CD3/CD28 microbeads. $PD1^+/^-CXCR5^+/^-$ cells were analyzed for early activation (CD154, CD25, CD69) and inhibitory (CTLA-4, TIM-3, LAG-3) markers expression in time dependent manner after stimulation. BCL-6, ICOS and TIM-3 immunofluorescence staining and STAT1, NFκB, STAT5, GATA3, IL-21, STAT3, FOXP3, BCL6, T-bet and RORγt RT-PCR were performed on activated $PD1^+/^-CXCR5^+/^-$ cells. Compared to circulating Tfh, $CD4^+PD1^+CXCR5^+$ were at high amounts in LN samples and were of central memory phenotype $CCR7^+CD45RO^+$, and expressed CD127, TIM-3 and ICOS. LN-derived $CD4^+PD1^+CXCR5^+$ cells had higher proliferation capacity. After stimulation, $CD4^+PD1^+CXCR5^+$ cells upregulated early activation and inhibitory markers. Furthermore, sorted $CD4^+PD1^+CXCR5^+$ cells detected as expressing significantly higher amounts of Tfh related BCL-6 transcription factor at mRNA and molecular level which is correlated with molecular expression of ICOS and TIM-3. Our study suggests that Tfh cells mainly detected in LN with higher proliferation capacity and can be characterized as $CD4^+PD1^+CXCR5^+BCL\text{-}6^+ICOS^+TIM\text{-}3^+$ so activation of these Tfh cells may induce cancer specific humoral immune response.

Keywords: Adaptive immunity, cancer immunology, follicular helper T cells, immunological techniques

P-0106

"Ex vivo" T cell responses to dengue NS1 protein in individuals with varying severity of past dengue infection

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Dengue NS1 is a secretory protein, which is associated with severe dengue. As "*ex vivo*" T cell responses to this protein has not been characterised, we sought to investigate them in naturally infected individuals with varying severity of clinical disease. "*Ex vivo*" IFNγ T cell responses to the NS1 overlapping peptides were assessed 25 individuals with past infection. P1 corresponded to the β-roll of NS1 (4 peptides), P2 to the early wing domain (10 peptides), P3 to the late wing domain (11 peptides), P4- early C-terminus (11 peptides), and P5 the late C terminus (11 peptides). Overall the "*ex vivo*" T cell responses to NS1 were of very low frequency in 10/21 seropositive individuals, with individuals having <50 spot forming units (SFU)/1 million cells for the pools. There was no significant difference (p>0.99) in the "*ex vivo*" T cell responses to NS1 in those with past severe dengue (SD) compared to those with non-severe dengue (NSD). The highest frequency of responses was seen to P3 (median 35, IQR 0 to 65 spot forming units/1 million cells) compared to other pools. However, those with past SD had higher responses to P5 whereas those with past NSD had the highest frequency to P3, which was not significant. Individuals with past dengue had a low frequency of NS1 specific "*ex vivo*" T cell responses, which are predominantly directed to the wing domain and the C-terminal domain of the NS1 protein.

Keywords: Adaptive immunity, immunological techniques, infectious disease, viral infections

P-0108

Th9 cell polarization is enhanced by *Staphylococcus aureus* while suppressed by retinoic acid and is possibly altered in allergic individuals

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T-helper (Th) 9 cells, characterized by robust secretion of IL-9, are among the most recently described T-helper subsets and have been shown to play a role in the pathogenesis of allergic diseases. Nevertheless, the biology of Th9 cells remains unclear and how these cells can be modulated is largely unexplored. Here, we aimed to study the effects of *Staphylococcus (S.) aureus*, frequently linked to allergic diseases, and retinoic acid (RA), a potent immune modulator, on human Th9 cell polarization *in vitro*. Using flow cytometry and ELISA, we show that exposure of PBMCs or isolated CD4 T-cells to *S. aureus* cell-free supernatant (CFS) potently enhances Th9 polarization, whereas RA has a strong Th9-suppressive effect. Furthermore, we demonstrate that RA dampens the *S. aureus*-induced Th9 cell polarization and instead promotes a shift towards type 2 responses, by substantially changing the transcriptional program of Th9-polarized CD4 T-cells. At the same time, genes associated with the asthma pathway are downregulated by RA, such as IL-9, IL-3 and FCεR1A. Finally, regardless of the treatment, we observe differences in the cytokine profile of Th9-polarized cells between allergic and non-allergic young adults, where allergic heredity plays an important part. In conclusion, our findings illustrate a strong influence of microbial and dietary factors on Th9 cell polarization and suggest an important role for Th9 cell regulation in allergic diseases, which may be considered for future allergy treatments.

Keywords: Adaptive immunity, allergic disorders, cytokines and mediators, microbiome and environmental factors, microenvironment

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POSTER PRESENTATIONS

P-0109

Influence of iTreg on T cell exhaustion in an adoptive T cell transfer model of pancreatic cancer

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Pancreatic cancer remains one of the most aggressive malignancies with poor prognosis and new therapeutic approaches are urgently needed. Intratumoral $CD8^+$ cytotoxic T cell (CTL) dysfunction, also termed exhaustion, plays a critical role in some subtypes of pancreatic cancer and allows tumor escape from immune surveillance. We have established a model system of adoptive T cell transfer in order to elucidate mechanisms of T cell dysfunction and to investigate ways to disinhibit intratumoral T cells. Based on the OVA model antigen system, antigen-specific CTL were generated from transgenic OT-I mice and transferred into tumor-bearing mice (Panc02 vs. PancOVA). In addition to the therapeutic response, *ex vivo* parameters were characterized, including expression of coinhibitory molecules (PD-1, TIM-3, LAG-3, CTLA-4) and intracellular production of effector cytokines (IFN-γ, TNF). To investigate the influence of induced regulatory T cells (iTreg) on transferred CTL, antigen-specific iTreg were co-transferred. Once growth characteristics of PancOVA and infiltration kinetics of effector CTL were established, induction of T cellular dysfunction was shown to be an active, antigen- and contact-dependent process in this model system. iTreg exhibited low stability of FOXP3 expression in this experimental set-up. T cell receptor (TCR) signaling lead to a gradual loss of their regulatory function *in vitro* and *in vivo*. Future work will focus on stabilizing iTreg via transduction with constitutively active STAT5 and FOXO1. Furthermore, mechanisms of iTreg-induced T cell dysfunction will be investigated using FOXP3-IRES-mRFP OT-II mice in an *in vivo*/situ approach with a dorsal skinfold chamber model for intratumoral T cell visualization.

Keywords: Cellular interactions, animal models, cancer immunology, checkpoint inhibition, *in vivo* tumor models, regulatory cells

P-0110

Regulation of decay accelerating factor primes human germinal center B Cells for phagocytosis

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Germinal centers (GC) are sites for extensive B cell proliferation and homeostasis is maintained by programmed cell death. The complement regulatory protein Decay Accelerating Factor (DAF) blocks complement deposition on host cells and therefore also phagocytosis of cells. Here, we show that B cells downregulate DAF upon BCR engagement and that T cell-dependent stimuli preferentially led to activation of DAFlo B cells. Consistent with this, a majority of light and dark zone GC B cells were DAFlo and susceptible to complement-dependent phagocytosis, as compared with DAFhi GC B cells. We could also show that the DAFhi GC B cell subset had increased expression of the plasma cell marker Blimp-1. DAF expression was also modulated during B cell hematopoiesis in the human bone marrow. Collectively, our results reveal a novel role of DAF to pre-prime activated human B cells for phagocytosis prior to apoptosis.

Keywords: Adaptive immunity, B lymphocytes, cell death, complement

P-0111

Staphylococcal enterotoxin-mediated effects on conventional and non-conventional T cells and NK cells in immune-skewed individuals

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Staphylococcus aureus is one of the most frequent causes of bacterial infection in humans due to its ability to produce virulence factors such as staphylococcal enterotoxins (SE). SE act as superantigens and cause polyclonal T cell activation by crosslinking the MHC-I and the T cell receptor, potentially leading to toxic shock syndrome. We have previously demonstrated that SE are also able to activate γδ T cells, MAIT cells and NK cells. This activation is mediated by conventional T cells and monocytes in an indirect, cell contact-dependent manner. To investigate the effects of SE in allergy and pregnancy, conditions known to skew the immune response. We have stimulated peripheral blood mononuclear cells from allergic and non-allergic individuals, and also from women during and after pregnancy with SEA. We have analysed cytokine production and secretion using qPCR, flow cytometry and ELISA. We show that the secretion levels of IFN-γ and IL-17A upon SEA stimulation were higher in non-allergic individuals compared to the allergic. IFN-γ production was higher in the non-allergic both during and after pregnancy in all the studied cell types, but most notably in unconventional T cells and NK cells. Longitudinal studies show a tendency towards a stronger IFN-γ response to SEA during pregnancy regardless of the allergic status of the individual, compared to post-partum. Altogether, this suggests that unconventional T cells and NK cells are more susceptible to allergic conditions upon SEA, emphasising their indirect activation following conventional T cell activation.

Keywords: Antigen processing and presentation, gamma-delta t cells, innate lymphoid cells, MAIT cells, NK cells

P-0112

SP-A amplifies IL-4 mediated effects on alveolar macrophages by increasing efferocytosis

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Interleukin 4 (IL-4)-mediated activation of macrophages is required for resolution of inflammation and tissue repair. We have reported before that macrophages change to repair mode integrating IL-4 signals and specific signals from the tissue. In the lung, surfactant protein A (SP-A) is one of the factors that enhances IL-4 dependent activation of alveolar macrophages (AMs), through mechanisms that are still unknown. Moreover, to induce the tissue repair program in macrophages, IL-4Rα signaling requires concomitant recognition of apoptotic cells and SP-A assists macrophages in recognizing and clearing apoptotic cells. In this study, we test the hypothesis that SP-A-driven efferocytosis is needed for complete activation of macrophages by IL-4. To this end, we cultured AMs with apoptotic A549 pneumocytes in the presence or absence of IL-4 (1 μg/ml) and low concentrations of SP-A (12.5, 25 μg/ml) and analyzed alternative activation of macrophages. We found that low concentrations of either apoptotic A549 cells or SP-A, administered separately, did not increase IL-4-mediated activation of macrophages. However, both factors in combination synergistically increased alternative activation of AMs in the presence of IL-4. Our results suggest that one of the mechanisms through which SP-A amplifies IL-4 actions is by increasing phagocytosis of dead cells, limiting tissue repair to areas of tissue damage.

Keywords: Cell death, cellular interactions, innate immunity, macrophage, phagocytosis, tissue damage and repair

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POSTER PRESENTATIONS

P-0113

TLR8 agonists improve NK cell function by acting primarily on CD56brightCD16- subset

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Endosomal Toll like receptors (TLRs) are dominantly expressed by the innate immune system cells and are primarily involved in the recognition of conserved pathogen structures (PAMPs), including RNA and DNA sequences derived from viruses. Natural Killer (NK) cells are known to express different endosomal TLRs such as TLR3, TLR7/8 and TLR9. In this study, we explored the capability of human NK cells of responding to various TLR agonists in order to establish which molecule can be potentially used as the best adjuvant in immunotherapy of cancer. Although the endosomal TLRs are expressed on both CD56brightCD16- and CD56dimCD16+ cells subsets, TLR7/8-, TLR3- and TLR9- ligands (R848, Poly I:C and ODN2395 respectively) induce NK cell function only when NK cells were cultured in the presence of suboptimal doses of IL-2 and IL-12. We show that TLR7/8, rather than TLR3 and TLR9 ligands, prevalently activate CD56brightCD16- NK cells resulting in increases of proliferation, cytokine production and cytotoxic activity. Lastly, we show that R848 activates CD56brightCD16- NK cell subset through TLR8. Indeed, TLR8-, but not TLR7- agonists', increase cytokine production and cytotoxic activity in CD56brightCD16- NK cells. Importantly, these effects were also observed in NK cells derived from metastatic ovarian carcinoma, which prevalently belong to CD56brightCD16- subset. These data highlight the potential value of TLR8 as a target for immunotherapy in cancer patients.

Keywords: Adjuvants and vaccines, cancer immunology, innate immunity, NK cells

P-0114

Alterations to Paneth cells in ageing may indirectly impede antigen sampling and mucosal immunity

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The decline in mucosal immunity during ageing increases the incidence and severity of infections in the elderly. We previously showed that this immunosenescence includes reduced M cell maturation in the follicle-associated epithelia (FAE) covering the Peyer's patches, diminishing the ability to sample of antigens and pathogens from the gut lumen. Here, co-expression analysis of mRNA-seq data sets of micro-dissected FAE, villous epithelium and ileum revealed a general down-regulation of most FAE- and M cell-related genes in Peyer's patches from aged mice, including key transcription factors known to be essential for M cell differentiation. The functional maturation of M cells in Peyer's patches could be restored by housing of aged mice on used bedding from young mice, or treatment with bacterial flagellin, resulting in the restoration of IgA responses against a model antigen. Our RNA-seq data sets also showed that expression of key Paneth cell-related genes were reduced in the ileum of aged mice, consistent with the adverse effects of ageing on their function e.g. diminished support for intestinal stem cells. The restoration in M cell maturation after both bedding from young mice or flagellin treatment was associated with increased OLFM4+ stem cells in the intestinal crypt. Therefore, ageing effects on Paneth cells may indirectly impede M cell differentiation. Thus, restoring Paneth cell function may represent a novel means to improve M cell differentiation in the ageing intestine and increase mucosal vaccination efficacy in the elderly.

Keywords: Ageing, immune senescence, innate host defence, lymphoid organs, microbiome and environmental factors

P-0116

IL-13 mediated ILC-DC signalling in naive skin imprints dermal dendritic cells to T helper 2 induction

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Type 2 Dendritic Cells (DC2) play a crucial role in informing the adaptive immune system about infectious, innocuous or self antigens, but display tissue specific signatures. To assess if DC2 adapt to their specific tissue environment and modify their functional characteristics in accordance with the peripheral requirements, we assessed DC2 subsets in different barrier and mucosal sites by spectral flow cytometry and RNA sequencing. We found that a subset of dermal DC2, which expressed low levels of CD11b, displayed a unique signature for STAT6 signalling. Strikingly, dermal CD11b-low DC2 did not develop in STAT6-knock-out (KO) mice, while the development of DC2 precursors and DC2 populations in other tissues was independent of STAT6. Dermal CD11b-low DC2 were also absent from IL-13-KO, IL-13Rα1-KO and IL-4Rα-KO mice, indicating that STAT6 was activated through homeostatic IL-13 signalling in the naïve skin. In naïve skin IL-13 was produced by a population of ICOS+ KLRG1- dermal Innate Lymphoid Cells, which did not express the IL-2 or IL-33 receptor and did not produce IL-13 in response to alarmins or the microbiota. In the absence of dendritic-cell-specific IL-13 signalling, Th2 responses in the skin draining lymph nodes were diminished in several *in vivo* models, while Th17 responses increased, suggesting that CD11b-low DC2 actively control the Th2/Th17 balance in the skin. Our results therefore suggest that the IL-13 mediated development of dermal DC2 fosters a protective non-inflammatory environment at the steady state, but might also contribute to its pro-allergic conditioning upon dysregulation.

Keywords: Biology of the immune system, dendritic cells, innate lymphoid cells, RNAseq, skin diseases

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POSTER PRESENTATIONS

P-0117

Topical application of adenosine receptor agonists to skin reduces migration and activation of skin migratory dendritic cells leading to impaired T cell activation

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We have recently shown that production of Adenosine (Ado) by skin and skin migratory (sm) CD73+ dendritic cells (DCs) is critical for tolerance development in a contact hypersensitivity (CHS) model. Since Ado has well documented immunosuppressive properties, we investigated the use of Ado receptor (AdoR) agonists for treatment of CHS. A2A (CGS21680) and A2B (BAY60-6583) AdoR agonists were epicutaneously applied to skin prior to sensitization and challenge with the hapten DNFB. Ear swelling and the immunologic outcome was analyzed in both skin and draining lymph nodes (dLNs). Animals treated with AdoR agonists showed a reduced ear swelling compared to solvent control. Consistently, fewer activated T cells were found in the skin after challenge while Tregs remain the same within all the groups. Furthermore, higher numbers of T cells expressing anergic markers (Lag-3, CD137, PD-1, CD272 and Tim-3) were found in dLNs in CGS-treated group while no differences in activation markers were observed. In ear tissue, AdoR agonists reduced the production of proinflammatory cytokines and chemokines as well as the infiltration of neutrophils upon sensitization. Moreover, reduced numbers of smDCs, which produced less IL-12 and had a lower expression of CD86, were recorded in dLN. When smDCs from CGS-treated animals were co-cultured with OT-I or OT-II T cells, a reduced proliferation of T cells and proinflammatory cytokines production were detected compared with smDCs from solvent control. Thus, topical application of AdoR agonists to skin prevents sensitization of T cells against haptens by reducing migration and activation of smDCs.

Funded by DFG-TR156

Keywords: Allergen-induced immune responses, dendritic cells, drugs for immune modulation, immune regulation and therapy, skin diseases

P-0118

Chronic and acute stress - regulation of natural killer cell functions by catecholamines

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Natural Killer (NK) cells are involved in the control of viral infection and tumors. They interact with catecholamines of the sympathetic nervous system. Importantly, the crosstalk between the nervous and immune system can influence the outcome of cancer therapies. Aim of this project is to understand how the engagement of catecholamine receptors influences NK cell reactivity and how chronic and acute scenarios alter NK cell responses. Primary human NK cells were isolated from buffy coats or blood of healthy donors and stimulated with catecholamines (epinephrine, dopamine or synthetic agonists/antagonists). Analyzed by Seahorse metabolic profiling, xCelligence impedance-based Real-time cell analysis, FACS based receptor expression analyses and Ligand complex-based adhesion assay, IncuCyte® S3 live NK cell killing analysis, IFNγ ELISA, cAMP ELISA and Western blots for NK cell signaling analysis. Our group already demonstrated that acute epinephrine exposure leads to increased cAMP levels in the cytoplasm, affects NK cell signaling and reduces NK cell activation. Here we show that epinephrine interrupts the protein interaction between LFA-1 and ICAM-1. Additionally, acute epinephrine exposure inhibits mitochondrial respiration and prolongs glycolysis of CD16-activated NK cells. In contrast, exposure to dopamine does not alter the metabolic profile, but was already shown to inhibit the NK cell IFNγ production. Interestingly, chronic exposure to beta2AR agonists do not interfere with beta2AR expression, but completely abolishes the inhibitory effects of acute epinephrine stimulation on NK cell functions. These different responses may explain some effects of acute and chronic stress on the immune system.

Keywords: Cell signalling, NK cells, neuroimmunology

P-0120

Synovial fluid neutrophils from patients with juvenile idiopathic arthritis display a hyperactivated phenotype

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Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in childhood. The predominant subtypes, i.e. oligoarticular and polyarticular JIA, are traditionally considered to be autoimmune diseases with a central role for T cells and autoantibodies. Nevertheless, the original T cell-centered hypothesis has been challenged since it does not cover nor completely explain the full spectrum of immune-pathological phenomena observed in JIA patients. Emerging evidence suggests a potentially important role for neutrophils in JIA pathogenesis. Here, we investigated extensively the phenotypical characteristics of neutrophils present in the peripheral blood and in inflamed joints of JIA patients. Multicolor flow cytometry allowed for in-depth phenotypical analysis of neutrophils, focusing on the expression of adhesion molecules, activation and maturation markers, and chemoattractant receptors. The vast majority of synovial fluid neutrophils displayed a strongly activated, hypersegmented phenotype with decreased L-selectin (CD62L) expression and increased numbers of nuclear lobes, upregulation of adhesion molecules CD66b, CD11b, and CD15, and downregulation of chemokine receptors CXCR1/2. An elevated percentage of CXCR4-expressing aged neutrophils was detected in synovial fluids from JIA patients. Strikingly, significant percentages of synovial fluid neutrophils showed a profound upregulation of markers that are not typically found on neutrophils, including CXCR3, ICAM-1, and HLA-DR. Our data indicate that neutrophils present in inflamed joints of JIA patients are strongly activated cells. This detailed molecular analysis supports the notion that a complex intertwining between innate and adaptive immunity drives JIA.

Keywords: Autoimmunity, chemokines, cytokines and mediators, inflammatory joint diseases, neutrophils

P-0121

Influence of the transcription factor Ets-2 on the expression of apoptotic genes in T-cells compared to HIV-infected T-cells

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Ets-2 regulates the activation of T-cells and HIV-1. Herein we investigated the effect of Ets-2 on the expression of pro-apoptotic genes Bak, Bax, p53, Fas, FasL and anti-apoptotic genes Bcl-2, Bcl-xL, p21, in T-cells versus HIV-infected T-cells. The T-cell lines Jurkat and H938 (contain the complete HIV-LTR) were transfected with increasing amounts of an Ets-2 overexpressing vector (pCDNA3-ets-2) in the presence or absence of mitogens. Ets-2 overexpression and apoptotic gene expression were assessed at the transcriptional level by real-time qPCR and at the protein level by Western blotting. Compared to Jurkat cells, constitutive expression of Bcl-xL and p21 was lower and expression of Bax and Fas was higher in H938 cells. Stimulation of the cells resulted in higher expression of Bax, p53, Fas, FasL, Bcl2, Bcl-xL, and p21 in H938 compared to Jurkat cells. Overexpression of Ets-2 increased the expression of p53, Fas, Bcl-2 and Bcl-xl in both unstimulated Jurkat and H938 cells, whereas it upregulated the expression of Bax and FasL in unstimulated Jurkat cells. In stimulated H938 cells, Ets-2 increased Bak expression and decreased the expression of Bax, Fas, FasL, Bcl-2 and p21. In stimulated Jurkat cells, Ets-2 upregulated the expression of Bax, p53 and Bcl-xL. Ets-2 regulates the expression of apoptotic genes in both cell lines. The observed differences in apoptotic gene expression between Jurkat and H938 cells may be due to the presence of HIV-LTR in the latter, which causes significant perturbations in host cell factor expression following Ets-2 overexpression.

Keywords: Adaptive immunity, cytokines and mediators, epigenetic control and modulation of immunity

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POSTER PRESENTATIONS

P-0122

CD29 enriches for cytotoxic human CD4+ T cells

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$CD4^+$ T cell are key contributors in the induction of adaptive immune responses against pathogens. Even though $CD4^+$ T cells are primarily classified as non-cytotoxic helper T cells, it has become appreciated that a subset of $CD4^+$ T cells is cytotoxic. However, tools to identify these cytotoxic $CD4^+$ T cells are lacking. We recently showed that CD29 (Integrin Beta 1, ITGB1) expression on human $CD8^+$ T cells enriches for the most potent cytotoxic T cells. Here, we questioned whether CD29 expression also associates with cytotoxic $CD4^+$ T cells. We show that human peripheral blood-derived CD29hi$CD4^+$ T cells display a cytotoxic gene expression profile, which closely resembles that of CD29hi cytotoxic $CD8^+$ T cells. This CD29hi cytotoxic phenotype was observed *ex vivo* and was maintained in *in vitro* cultures. CD29 expression enriched for $CD4^+$ T cells, which effectively produced the pro-inflammatory cytokines IFN-γ, IL-2, and TNF-α and cytotoxic molecules. Also, CD29hi T cells were enriched in super-centenarian and during primary CMV infection. Lastly, CD29-expressing $CD4^+$ T cells transduced with a MART-1 specific TCR showed target cell killing *in vitro*. In conclusion, we here demonstrate that CD29 can be employed to enrich for cytotoxic human $CD4^+$ T cells.

Keywords: Adaptive immunity, biomarkers, cell based therapies, cytokines and mediators, effector molecules, monitoring immunity

P-0123

Early emergence of T central memory precursors programs clonal dominance during chronic viral infection

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Chronic cytomegalovirus (CMV) infection leads to long-term maintenance of extraordinarily large CMV-specific T cell populations. The magnitude of this so-called 'memory inflation' is thought to mainly depend on antigenic stimulation during the chronic phase of infection. However, by mapping the long-term development of CD8+ T cell families derived from single naive precursors, we find that fate decisions made during the acute phase of murine CMV infection can alter the level of memory inflation by more than 1,000-fold. Counterintuitively, a T cell family's capacity for memory inflation is not determined by its initial expansion. Instead, those rare T cell families that dominate the chronic phase of infection show an early transcriptomic signature akin to that of established T central memory cells. Accordingly, a T cell family's long-term dominance is best predicted by its early content of T central memory precursors, which later serve as a stem-cell-like source for memory inflation.

Keywords: Adaptive immunity, adjuvants and vaccines, immune response tracing, memory, stem cells, viral infections

P-0124

Analysis of myeloid derived suppressor cells (MDSC) in splenic trauma patients

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MDSCs consist of heterogeneous and suppressive myeloid cell population. Spleen has been shown to be a major reservoir for the MDSCs under chronic inflammation. In this study, MDSCs in the human spleen upon acute trauma were aimed to be analyzed through molecular and functional assays. Spleen tissues and peripheral blood samples of 22 patients who had splenectomy because of spleen rupture were collected. Peripheral blood of healthy donors was used as controls. Splenocytes and peripheral blood were layered over 1.077 g/mL Ficoll, the low-density cells were labelled with anti-CD11b, CD33, CD66b, CD15, HLA-DR, CD14 and CD16 antibodies. CD11b+CD33dimCD66b+ cells over 1.077 g/mL in splenocytes from trauma patients were purified with MACS and FACS. MDSCs were also stained with May-Grünwald Giemsa for morphological analyses. MDSCs were co-cultured with PBMCs obtained from healthy donors under anti-CD3 stimulation to determine their immunomodulatory effects. Real-time PCR and western blot experiments were performed to evaluate the expressions of immune modulatory genes and proteins. In both peripheral blood and spleen samples of the patients, percentage of CD11b+CD33dimCD66b+ cells (PMN-MDSC) was increased compared with the healthy controls. It was found that PMN-MDSCs suppressed T cell responses and immune modulatory genes and proteins were found increased in the spleen. In this study, PMN-MDSCs accumulated in the non-malignant spleen tissue and it was found a positive correlation between spleen injury scores and the percentages of MDSCs in the spleen.

Keywords: Granulocytes, myeloid derived suppressor cells, neutrophils

P-0126

Complex interplays between BTN2A1 and BTN3A1 butyrophilins for triggering the reactivity of Vγ9Vδ2 T cells

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Human Vγ9Vδ2 T lymphocytes enforce a non-stop immunosurveillance to sense and eliminate transformed or infected target cells. This peculiar effector subset is activated, in a TCR-mediated manner, by *Self*-derived non-peptidic phosphorylated antigens, and involves BTN3A1/BTN2A1 butyrophilins, but not MHC molecules. Even if the functional outcome is uncoupled to the formation of a well-structured immunological synapse (IS); it is the first step the cells must take. IS are specialized cell-cell junctions characterized by close apposition of the immune cell membrane with the membrane of another cell, adhesion, stability and directed secretion. This phenomenon has been discovered in the 70s and since then, well characterized for. More recently, a variety in the structure of IS for different types of immune cell interactions have been shown, raising the question about the nature of the IS formed during the activation of Vγ9Vδ2 T lymphocytes. Combining biochemical reconstitution of an IS using glass-supported lipid bilayers with high-resolution microscopy, we were able to visualize and describe this synapse. A particular focus was made on the precise role of the butyrophilins BTN2A1, BTN3A1 and isoforms, and further analyses were performed using the Proximity Ligation Assay approach. Contrary to the initial consensus proposing BTN3A1 as Vγ9Vδ2 TCR ligand and/or phosphoantigen-presenting molecules, direct interactions between those two entities have never been observed. In line with the recent studies of Willcox & Hermann's teams, showing BTN2A1 as the ligand of the Vγ9Vδ2 TCR, our findings propose a deeper view of this complex antigenic activation process, which implicates BTN2A1 & BTN3A1.

Keywords: Cellular interactions, gamma-delta T cells, immune communication, innate immunity, molecular immunology

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POSTER PRESENTATIONS

P-0127

Epigenetic drugs interfere with NK/ILC3 differentiation and proliferation

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Targeting the catalytic subunit of EZH2 or the hypermethylation of CpG DNA islands may arrest tumor growth or restore the tumor suppressor genes transcription. However, these drugs may also affect normal hematopoiesis, interfering with CD34$^+$Hematopoietic Stem Cells (HSCs) capability to differentiate towards potential anti-tumor effector lymphocytes. Given the role of NK cells in tumor immune-surveillance, it would be useful to understand whether epigenetic drugs can modulate NK cell maturation. To this end, human HSCs were cultured in the absence or in the presence of the EZH1/2 inhibitor UNC1999 (1μM) or the hypomethylating agent SGI-110 (0,5 μM). Cells were counted and analyzed at different time intervals for NK-specific surface markers, Ki67 proliferation marker, cytokines production, cytolytic activity and transcription factors expression. UNC1999 increased the recovery of CD56$^+$ while the addition of SG110 led to an impairment of cell proliferation, leading to a limited number of CD56$^+$cells. Of note, UNC1999 favored the differentiation of no-cytotoxic CD56$^+$ILC3 according to the early expression of the AHR transcription factor, while SGI-110 led to the generation of few CD56$^+$ cells with higher cytotoxicity and higher percentages of KIR and CD16 receptors as compared to controls. Thus, the inhibition of EZH1/2 favors differentiation towards CD56$^+$ILC3-lineage. On the other hand, SGI-110 interferes with precursor cell proliferation but leads to the generation of more differentiated CD56$^+$ cells. These results would suggest that, evaluation of the effects of these drugs on immune cells during treatment could help to design protocols that both control tumor progression and favor anti-tumor immune response.

Keywords: Cancer immunology, immune development, immunotherapy, innate lymphoid cells, NK cells

P-0128

Molecular analysis of the transcriptional co-activator BOB.1/OBF.1 and its contribution to the germinal center reaction

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BOB.1/OBF.1 (gene name: Pou2af1) is a lymphocyte-specific transcriptional co-activator of octamer-dependent transcription. In studies of conventional BOB.1/OBF.1 knockout mice a function of BOB.1/OBF.1 in B but also in T lymphocytes was described, whereby the main characteristic of these animals is the complete absence of Germinal Centers (GCs). In humans, BOB.1/OBF.1 has been described to contribute to the pathogenesis of autoimmunity, agammaglobulinemia and lymphoma. So far, the precise contribution of BOB.1/OBF.1 to B and T cell physiology and especially to the GC reaction remained unclear due to the absence of appropriate experimental systems. Therefore, we studied the in vivo function of BOB.1/OBF.1 in distinct B and T cell subpopulations by conditional mutagenesis. We generated mice bearing floxed Pou2af1 alleles and crossed them to B (CD19-, CD23-, Cγ1-Cre) and T cell (CD4-, IL21-Cre) stage specific Cre-lines. Lymphocyte subpopulations in primary and secondary lymphoid organs and GC reaction upon immunization were addressed. Our data reveal a requirement for BOB.1/OBF.1 during both early antigen-independent and later antigen-dependent B and T cell development, and further for efficient GC reaction during complete B cell ontogeny. By specifically deleting BOB.1/OBF.1 in GC B cells, we provide evidence that the failure to form GCs is a GC B cell autonomous defect and not exclusively a consequence of defective early B cell maturation. Moreover, BOB.1/OBF.1 deletion specifically in CD4+ T or TFH cells caused significantly reduced numbers of GCs and TFH cell subsets suggesting additionally a requirement of BOB.1/OBF.1 expression in TFH cells for sufficient GC B cell generation.

Keywords: Adaptive immunity, animal models, B lymphocytes, follicular helper T cells, immune development

P-0129

Neutrophils and neutrophil extracellular traps (NETs) in severe bronchial asthma

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To investigate the roles of neutrophils (PMNs) and Neutrophil Extracellular Traps (NETs) in severe asthma. 24 patients with severe asthma and 17 healthy controls (HCs) were prospectively recruited. PMNs were isolated from peripheral blood and evaluated for ROS production and activation status upon stimulation with LPS (lipopolysaccharide) and fMLP (N-Formylmethionyl-leucyl-phenylalanine). Plasma levels of myeloperoxidase (MPO), CXCL8, matrix metalloproteinase-9 (MMP-9), Granulocyte-Monocyte Colony-Stimulating Factor (GM-CSF) and Vascular Endothelial Growth Factor (VEGF-A) were measured by ELISA. Plasma concentrations of Citrullinated Histone H3 (CitH3) and circulating free DNA (cfDNA) were measured as NETs biomarkers. Peripheral blood PMNs from asthma patients displayed reduced ROS production and activation status compared to HCs upon bacterial stimulation. Asthma patients displayed higher circulating levels of MPO, CXCL8, MMP9 and NETs compared to HCs. Our results show that PMNs of asthma patients display reduced ROS production and activation status upon bacterial stimuli. These patients display higher circulating levels of MPO, CXCL8, MMP-9 and NETs. Collectively, our results suggest that neutrophils acquire an exhausted phenotype in severe asthma. A larger cohort of patients with different phenotypes (T2-low versus T2-high) will allow identifying neutrophil-related markers predictive of disease severity and/or therapeutic response.

Keywords: Cytokines and mediators, inflammatory disease, innate immunity, neutrophils

P-0130

The AP-1 factors *FOSL1* and *FOSL2* regulate transcriptional networks governing early human Th17 cell differentiation

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Th17 cells are key players in providing protection against extracellular bacteria and fungi. Their aberrant activity, however, can lead to inflammatory and autoimmune diseases. Transcriptional mechanisms regulating human Th17 cell-function are largely unexplored. Here, we investigated the role of the AP-1 factors, FOSL1, FOSL2 and BATF in regulating early stages of human Th17 differentiation. Transient perturbation of these factors in combination with transcriptome analysis and genome-wide occupancy studies revealed their directly regulated gene-targets in Th17 cells. Our analysis further demonstrated a functional cooperativity between FOSL1 and FOSL2, while highlighting their antagonism with BATF. Moreover, by using public GWAS catalogue, our study discovered a large number of autoimmune disease-associated SNPs within the genomic binding sites of these AP-1 factors. DNA affinity precipitation assays revealed that many of these SNPs altered the ability of the candidate factors to bind DNA. The findings from our study thus advance our understanding on the transcriptional regulation of human Th17 differentiation, which could further deliver insights into the therapeutic interventions of associated diseases.

Keywords: Autoimmunity, immune networks, immunodeficiency, inflammatory disease, molecular immunology, RNAseq

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POSTER PRESENTATIONS

P-0131

Ovarian carcinoma ascites suppresses CD8+ T cell activation and function through cholesterol

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Ovarian cancer (OC) is the most lethal gynecological malignancy with approximately 60.000 new cases annually in the United States and the European Union. Several observations indicate that the immune system plays a crucial role in OC. Thus, an increased accumulation of intratumoral T cells delays recurrence of the disease. Among infiltrating T cells, CD8+ T cells are associated with a better prognosis. However, we found that OC ascites inhibits the activation and proliferation of CD8+ T cells, which was associated with suppression of genes related with cholesterol and fatty acid synthesis as well as of IRF4, which expression is dependent on the T cell receptor (TCR) signal transduction and RAS/NFKB pathway. Neutralization of cholesterol in ascites with Methyl-β-cyclodextrin (MBCD), rescued the activation and proliferation of CD8+ T cells as well as the expression of IRF4, indicating a crucial role of cholesterol in the suppression of CD8+ T cell activation and function by OC ascites. To understand how cholesterol suppresses CD8+ T cell activation, we applied intermediates of mevalonate pathway as well as palmitate, which are important for post-translational modification and membrane localization of proteins, including molecules involved in TCR signaling. The addition of palmitate rescued at least partially CD8+ T cell activation, proliferation and IRF4 expression in OC ascites. Thus, we show, that increased cholesterol levels in ascites, suppress fatty acid and cholesterol synthesis and thereby probably cause diminished post-translational modification of proteins involved in TCR-signal transduction causing diminished CD8+ T cell activation and IRF4 expression.

Keywords: Cancer immunology, immune networks, immunopharmacology, RNAseq

P-0132

Negative immunomodulatory function of the CD6 lymphocyte co-receptor in CD4+ T cells upon physiological antigenic stimulation conditions

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In order to prevent autoimmune diseases the immune system must be tightly regulated. One mechanism that the immune system uses to prevent autoimmunity is through the use of regulatory receptors on the surface of T lymphocytes. CD6 is a surface co-receptor expressed by all T cells and the B1a cell subset and involved in the modulation of lymphocyte activation, proliferation and survival processes following engagement of the antigen-specific clonotypic T and B cell receptors. However, its ultimate stimulatory/inhibitory role as well as the molecular signaling mechanisms involved is still controversial. Here, we analyze the modulatory role of CD6 in peripheral T lymphocytes under antigen-specific stimulation conditions. To this end, MHC class II-restricted and ovalbumin (OVA)-specific TCR-transgenic mice expressing normal (OT-II *cd6*$^{+}$/$^{+}$) or deficient (OT-II *cd6*$^{-}$/$^{-}$) CD6 surface expression were analyzed. Splenocytes from both mouse lines were cultured in the presence of $OVA_{323-339}$ peptide (ISQAVHAAHAEINEAGR), soluble anti-CD3ε mAb (positive control), and $OVA_{257-264}$ peptide (SIINFEKL; negative control) at different time-points. The results show that OVA-activated CD4^{+} T-cells from OT-II *cd6*$^{-}$/$^{-}$ mice express higher surface levels of T cell activation (CD25 and CD69) and exhaustion (PD-1) molecules. Moreover, cell proliferation and cytokine (IL-2 and IFN-γ) production were found increased in CD4^{+} T-cells from OT- II *cd6*$^{-}$/$^{-}$ mice. Deficiency of CD6 also increased Fas/FasL mRNA, while no significant cell apoptosis differences were observed with regard to OT-II *cd6*$^{+}$/$^{+}$ controls. In summary, *in vitro* evidence supports the negative regulatory role of CD6 in helper lymphocytes on early and late events following antigen-specific lymphocyte activation.

Keywords: Cytokines and mediators, adaptive immunity, animal models, antigen processing and presentation, cell signalling

P-0133

In vivo functions of mouse neutrophils derived from HoxB8-transduced conditionally immortalized myeloid progenitor cells

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While neutrophils play important roles in immunity and inflammation, their analysis is very much hindered by their short-lived and terminally differentiated nature. Prior studies reported a HoxB8 transcription factor-dependent conditionally immortalized progenitor cell line (HoxB8 progenitors) as useful tool in the studies related to neutrophil biology. This approach allowed the long-term culture of mouse myeloid progenitors (HoxB8 progenitors) in estrogen-containing media, followed by differentiation towards neutrophils upon estrogen withdrawal. Since little is known about the *in vivo* functional responsiveness of the resulting differentiated cells (HoxB8 neutrophils) we have addressed this issue by generating HoxB8 chimeras. HoxB8 chimeras were generated via single or repeated transplantations of HoxB8 progenitors into lethally irradiated recipient mice. HoxB8 neutrophils of the HoxB8 chimeras were studied in various single-cell based (e.g.: migration, phagocytosis) and whole neutrophil-population-based mouse models (e.g. reverse passive Arthus reaction and K/B×N serum transfer arthritis) as well. Intravenous injection of HoxB8 progenitors into recipients resulted in the appearance of circulating donor-derived HoxB8 neutrophils. *In vivo* differentiated HoxB8 neutrophils could migrate to the inflamed peritoneum and carried our phagocytosis of heat-killed Candida particles. The reverse passive Arthus reaction could be induced in HoxB8 chimeras but not in irradiated non-transplanted control animals. Injection of arthritogenic K/B×N serum triggered robust arthritis in HoxB8 chimeras but not in irradiated non-transplanted control mice. Taken together, our results indicate that HoxB8 progenitor-derived neutrophils are capable of performing various *in vivo* neutrophil functions, providing a framework for using the HoxB8 system for the *in vivo* analysis of neutrophil function.

Keywords: Animal models, biology of the immune system, immunological techniques, myeloid cells, neutrophils

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POSTER PRESENTATIONS

P-0134

A new optimized cell culture platform to study tissue resident macrophages *ex vivo*

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Tissue-resident macrophages (TRMs) play a crucial role in their host organs from development to adulthood, both during homeostasis and pathology. Each TRM population performs tissue-specific functions depending on its origin, local environment and biological influences such as injury or infections. To study functional properties and signaling pathways of TRMs, various *in vitro* culture systems have been developed often relying on bone marrow-derived macrophages, immortalized cell lines or macrophages derived from induced pluripotent stem cells. Limitations of these models include a contrasting origin to the cells of interest, an artificial activation phenotype or sophisticated differentiation protocols. Attempts to culture primary adult macrophages from different organs often require purification by FACS, are hampered by a low cell yield, lack reproducibility and do not allow long-term cultures. Here we established a protocol to obtain mono-cultures of different TRMs without prior purification. With this new culture protocol we obtained pure primary macrophage cultures from brain, lung, liver and peritoneum. Our approach allowed us to culture TRMs for several weeks by combining specific growth factors, a specialized coating and physiological oxygen conditions. All four TRM populations maintain a specific gene signature and can be used to perform functional and metabolic assays under organotypical conditions. Phagocytosis, metabolic and gene expression analyses after immune stimulation revealed organ specific differences between the TRMs *in vitro*. This new culture protocol provides an ideal platform to compare functional aspects between TRMs and to study their properties during steady state and immune activation under organotypic conditions.

Keywords: Innate immunity, macrophage, metabolic control of immune responses, phagocytosis

P-0135

Characterisation of the immune system of Ellegaard Göttingen Minipigs - an important large animal model in experimental medicine

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Aim of this project was to increase the knowledge about the immune system of Ellegard Göttigen Minipigs (EGMs) representing an important model in experimental medicine. Therefore, we studied the postnatal maturation of their immune system from birth until six months of age and the antigen-specific immune response after vaccination against Porcine Circovirus 2 (PCV-2). EGMs. During postnatal maturation, we analysed the composition of lymphocyte populations of ten individuals with FCM. We followed the presence of monocytes using mAbs against CD172a and CD163 and B cells based on the expression of CD79α. NK cells were distinguished as CD3-CD16+CD8α+/- cells and further subdivided using NKp46 expression. Different phenotypes of TCR-γδ T-cells were determined by their CD2 expression. TCR-αβ T cells were defined by CD4 and CD8β expression, and their differentiation was monitored with mAbs against CD27 and CD8α for CD4+ T cells and perforin for CD8β+ T cells. Cytokine profiles and proliferation capacity of PCV-2 re-stimulated lymphocytes were used for the characterization of an antigen-specific immune response. Monitoring the postnatal maturation of the immune system of EGMs we detected an increase of differentiated CD4 T cells with the phenotypes of central and effector memory T-cells. As expected both subsets showed a clear reactivity in an *in vitro* recall response against PCV2. CONCLUSION: This study on the postnatal development and antigen specific immune response of the immune system of EGMs is an important milestone for the additional use of EGMs for immunological questions in experimental medicine.

Keywords: Adaptive immunity, animal models, follicular helper T cells, gamma-delta T cells, immune development, memory

P-0136

Dissecting antibody-secreting cell differentiation at single cell level

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Differentiation of B cells into antibody secreting cells (ASCs) is a key process during the course of an immune response. Dysregulation of this process can cause severe pathological conditions, such as alloimmunization during blood transfusions or self-reactivity in auto-immune disease. Detailed understanding of the cues that control antibody-secreting cell differentiation is important to devise strategies to modulate antibodies formation in both health and disease. We profiled the transcriptomes of single cells derived from an *in vitro* culture system that differentiate human naïve B cells into ASCs (Unger et al., Cells 2021). We analyzed the B-cell receptors (BCR) and applied RNA velocity to get more insights into, respectively, the receptor editing processes and the different naïve to ASCs cellular transitional stages in our culture system. Publicly available single cell transcriptomics data from bone marrow, peripheral blood and tonsils derived ASCs were further analyzed for comparative purposes. Five transcriptional distinct subsets of cells were detected in our *in vitro* system. Two subsets showed a clear ASCs gene signature, highly comparable to *ex vivo* derived ASCs. Class-switched but unmutated BCRs were detected. RNA velocity identified a pre-ASCs cellular stage and a possible second route of naïve to memory B cell differentiation, including a germinal-center like intermediate phenotype. Taken together, these data demonstrates that our *in vitro* naïve B cell differentiation system highly reflects the *in vivo* situation and can therefore be used to identify previously unknown key regulators of ASCs formation.

Keywords: Antibody, B lymphocytes, big data, immunological techniques, omics technologies, RNAseq

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POSTER PRESENTATIONS

P-0139

Building a platform for generation, isolation and functional characterization of single-domain antibodies for B cell isolation

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Keeping immune cells in their natural state during isolation procedures is a critical step for many downstream applications. Traceless affinity cell selection (TACS), based on the Strep-tag® technology, uses low affinity capture reagents that reversibly capture targeted populations. However, generating low-affinity capture reagents can be very tedious, as nature itself and most technology-based approaches aim to increase the affinity of biological interactions. By developing a Strep-tag® based platform for single-domain antibodies we could generate, isolate and functionally characterize single-domain antibodies reliable and fast. The platform circumvents transfer problems, like time-consuming technology switches. This translates into way faster and cheaper development processes and products. The Strep-tag® technology platform allows the easy isolation of functional antigens for immunization. In a next step the antigens, used prior for immunization of animals, can be used for rapid immuno-affinity chromatography-based isolation of antigen-specific B cells after immunization without sacrificing the animals. Finally, the Strep-tag® platform permits detailed functional characterization of binding affinities using BLI sensors and strep-tagged antigens, leading to an optimally characterized single-domain antibody library. This library contains the low affinity single-domain antibodies needed for traceless affinity selection of cells, but also a variety of other applications, which might require different affinities. We demonstrate the workflow from planning to isolation single-domain antibodies against B cells and the adaption to other targets.

Keywords: B lymphocytes, engineering of antibodies and nanobodies, immunological techniques

P-0140

Is the generation of monocyte myeloid-derived suppressor cells (Mo-MDSCs) by extracellular vesicles from colorectal cancer patients accompanied by a change in bone morphogenetic proteins (BMP) expression?

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Verification whether during the generation of Mo-MDSCs by extracellular vesicles (EVs) from colorectal cancer (CRC) patients occurs changes in the expression of Bone Morphogenetic Proteins. EVs were isolated by sequential centrifugation from plasma from 32 CRC patients and 19 healthy donors (HD), followed by their quantitative and qualitative characterization. Next, EVs were added to monocytes isolated from HD blood and cultured for 24h. Thereafter cells were analyzed for mRNA expression of the BMP family proteins, PD-L1 and iNOS. Simultaneously, a measurement of cell oxygen metabolism using Mito Stress Test was performed. After 24h, the cells with phenotype of Mo-MDSCs developed from monocytes cultured with EVs, were isolated and added to the cultures of stimulated autologous T cells, for assessing their immunosuppressive potential. Peripheral blood of CRC patients contains significantly more EVs than blood of HD. These EVs are positive for the expression of CD9, CD63, Alix, EpCAM, and Her2/neu markers, albeit the expression of the last two was higher in CRC EVs. After the culture with EVs from CRC patients an increased expression of BMP1b, BMP2, iNOS, PDL-1 is detected. Moreover, the observed changes in oxygen metabolism indicate the induction of cells with anti-inflammatory potential. Also, the level of PD-L1+ immunosuppressive Mo-MDSCs increase after culture with CRC EVs but not from HDs. EVs could be responsible for induction from healthy donor monocytes a population of PD-L1+ Mo-MDSCs with suppressive activity. The mechanism of Mo-MDSCs induction may involve the proteins from the BMP family.

Keywords: Cancer immunology, endo- and exocytic vesicles in immunity, myeloid derived suppressor cells

P-0141

IL-1R8 Silencing improves the anti-tumor function of human NK cells

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Among recently identified checkpoint molecules acting on effector cells, IL1R8 appears to play a crucial role in NK cells maturation and function. Indeed, il1r8-/- mice are protected against tumor development suggesting that this inhibitory receptor may be detrimental for NK cell anti-tumor activity. To understand whether inhibition of IL1R8 may be crucial for the function of either freshly isolated (resting) or long-term activated human NK cells. Resting or IL-2-cultured NK cells were electroporated with the Neon Transfection System and small interfering RNA (siRNA) strategy was utilized resulting in a marked il1r8-silencing in NK cells. Expression profile studies were carried out using TaqMan Array Card, while cytotoxicity and cytokine were detected by flow cytometry and ELISA. Some genes encoding for NK cell activating and chemokine receptors were increased in il1r8- siRNA NK cells as compared to control NK cells whereas, downregulation of some genes for inhibitory signaling pathways was observed. The reduction of il1r8 expression levels on freshly isolated NK cells resulted in increases of surface expression of CD69, of cytotoxicity against K562 cell line and cytokine production. On the other hand, no significant effects in both phenotype and function were detected in long-term activated NK cells. These findings indicate that Il1r8 silencing by itself deeply impacts on both the activation status and cytotoxic/secreting function of freshly isolated NK cells. This is crucial for developing novel and more effective immunotherapies for solid tumors able to unleash human NK cells against tumors.

Keywords: Checkpoint inhibition, immunotherapy, NK cells

P-0142

Maintenance of bone marrow-resident memory T lymphocytes by stromal cells

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Some memory T lymphocytes of the spleen, in the absence of antigen, are maintained over time by homeostatic proliferation. As we have shown recently, however, bone marrow-resident memory T lymphocytes are maintained as cells resting in terms of proliferation, individually, in contact to bone marrow stromal cells, resembling the maintenance of memory B cells and memory plasma cells in the bone marrow. To identify the signal pathways maintaining memory T lymphocytes of the bone marrow, I have established hypoxic *in vitro* cell culture conditions for the coculture of ST2 stromal cells and memory T lymphocytes isolated *ex vivo*. In this culture system, survival of the memory T lymphocytes is dependent on the presence of stromal cells, but not on the presence of the cytokines IL-7 and IL-15. Contact to stromal cells activates the PI3K/AKT signaling pathway, and inhibiting this pathway in the memory T lymphocytes kills them. Also *in vivo*, the PI3K inhibitor Wortmannin significantly reduces the number of memory T lymphocytes in the bone marrow of mice. Interestingly, only CD69+ memory T lymphocytes, but not CD69- memory T lymphocytes of the bone marrow are ablated, suggesting that only CD69+ memory T lymphocytes are residents of the bone marrow and only they are maintained by stromal cell contact-induced PI3K signaling.

Keywords: Adaptive immunity, biology of the immune system, cell death, memory

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POSTER PRESENTATIONS

P-0143

Gamma delta T cells and inflammation of bovine mammary gland

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Streptococcus uberis is very important Gram-positive pathogen causing inflammation of bovine mammary gland. These bacteria were used for induction of bovine mammary gland inflammatory response. The experiments were carried out on the clinically healthy Holstein x Bohemian Red Pied crossbred heifers. Phenotype of gamma delta T cells were analysed by flow cytometry (BriCyte E6, Mindray, China) at 1, 2, 3 and 7 days following the induction of inflammation. There was also detected apoptosis of lymphocytes by flow cytometry in the same time points. The stimulation of mammary gland with *Streptococcus uberis* resulted in a gradual increase in apoptotic lymphocytes. The proportion of gamma delta T cells was also gradually increased during one week following the stimulation. There was found out the positive correlation between the apoptosis of the lymphocytes and the proportion of gamma delta T cells. The results suggest that gamma delta T cells play a role in inflammatory response of mammary gland induced by *Streptococcus uberis* in connection with lymphocyte apoptosis.

Keywords: Animal models, Bacterial infections, Cell death, Gamma-delta T cells, Inflammatory disease

Acknowledgement: This research was funded by the Ministry of Agriculture of the Czech Republic (grant number QK1910212).

P-0144

Single-cell fate mapping identifies incomplete recruitment of low affinity CD8 T cells in response to infection

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A key factor that shapes the magnitude of a CD8 T cell response to infection is the binding affinity of a given T-cell receptor (TCR) to its cognate peptide MHC (pMHC). While high affinity TCR-ligand interactions are required to sustain prolonged T-cell expansion, it has been proposed that even low affinity interactions are sufficient to activate naïve T cells, induce rapid initial proliferation and enable differentiation into effector and memory T cells. However, these findings were made by studying the average response behavior of naïve T cell *populations* and not by tracking the actual activation and recruitment of *individual* cells. Thus, whether all naïve T cells exposed to low affinity TCR-pMHC interactions are indeed recruited into the immune response currently remains unknown. Here, we mapped the fate of single T cells, harboring identical TCRs, upon infection with Listeria monocytogenes (*L.m.*) expressing peptide ligands of varying affinity. In addition, we set up an assay to detect unrecruited T cells, expressing TCRs of distinct affinities, in response to the same peptide ligand with single-cell resolution. In contrast to previous studies, we find that T cell recruitment is markedly efficient only upon high affinity TCR ligation. Low affinity TCR-pMHC binding instead leads to partial recruitment of only a third of all antigen-specific naïve T cells. Interestingly, this partial recruitment remains in place upon re-infection. Thus, a substantial fraction of low affinity antigen-specific T cells appears to be permanently left behind within the naïve T-cell compartment.

Keywords: Adaptive immunity, immune response tracing, immunological techniques, memory

P-0145

CD56dimCD57–NKG2C– human NK cells expressing inhibitory KIRs show high proliferative activity and share phenotypic and functional characteristics with adaptive-like NK cells

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NKG2C-positive highly differentiated NK cell subset with adaptive features, usually detected in HCMV-seropositive individuals, is characterized by increased expression of KIRs, in particular, inhibitory KIR2DLs. We compared the expression levels of KIR2DL receptors (KIR2DL2/DL3, KIR2DL1) on the surface of freshly isolated NKG2C-positive and negative NK cells at three differentiation stages: CD56bright, CD56dimCD57– and CD56dimCD57+. NKG2C+ subsets at each differentiation stage had a significantly higher KIR2DL2/DL3 levels and larger proportions of KIR2DL2/DL3+ NK cells compared to the corresponding NKG2C– cells. Similar trends were observed for the KIR2DL1 expression. The expression level of KIR2DL1 in the CD56dimCD57–NKG2C+ subset was comparable to that in the adaptive CD56dimCD57+NKG2C+ subset. Along with the increased KIR2DL expression, both CD56dimCD57–NKG2C+ and CD56dimCD57+NKG2C+ NK cell subsets had lower proportion of NKG2A+ cells, reduced expression levels of FcεRIγ, NKp30, CD16, and CD161, and increased CD44 level. Next, we revealed that CD56dimCD57–NKG2C+ NK cell subset had an increased expansion rate compared to other subsets. Moreover, at least in some individuals, CD57–KIR2DL2/DL3+NKG2C+ NK cell fraction, isolated by cell sorting, proliferated more intensively than the CD57–KIR2DL2/DL3–NKG2C+ or CD57–KIR2DL2/DL3+NKG2C– fractions. In functional tests, CD56dimCD57–NKG2C+ NK cells showed higher natural cytotoxicity compared to the corresponding NKG2C– cells and had similar cytotoxic activity with adaptive CD56dimCD57+NKG2C+ NK cells. Thus, CD56dimCD57–NKG2C+ NK cell subset expressing KIR2DL2/DL3 and/or KIR2DL1 may be considered as less mature adaptive-like NK cells with high proliferative activity.

The work was supported by Russian Science Foundation, grant #19-15-00439.

Keywords: Immune development, innate lymphoid cells, memory, NK cells

P-0146

The phenotypical and functional differences between control DCs and DCs with reduced ZNF366

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Dendritic cells (DCs) in patients with IBD, can induce an aberrant host response to the commensal enteric flora. To mimic this disease response *in vitro*, human monocyte-derived DCs were matured with E.coli causing the cells to express an inflammatory phenotype. Using our in-house designed siRNA-based screening assay, a specific set of siRNAs, targeting genes which were speculated to shift the DC phenotype toward an anti-inflammatory state, were evaluated. We identified the transcription factor ZNF366 as an important player in this process as its knockdown reduced IL-12p70 and increased IL-10 secretion from DCs. To better understand the role of this transcription factor in this process we are currently looking into other phenotypic effects on the DCs such as the expression of maturation markers and inhibitory receptors on the cell membrane. In addition, we are also looking into how DCs with reduced expression of ZNF366 are functionally different from control DCs by evaluating the effects of such DC on T cell responses.

Keywords: Cell signalling, cellular interactions, cytokines and mediators, dendritic cells, inflammatory bowel disease

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POSTER PRESENTATIONS

P-0147

Isolation of bovine myeloid dendritic cells (BDCA-1) using immunomagnetic MicroBeads

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The experiment was focused on possibility of using of immunomagnetic MicroBeads conjugated with mouse anti-human CD19 antibodies for depletion of CD19 positive B cells and immunomagnetic MicroBeads conjugated with mouse anti-human CD1c-biotin for positive selection/isolation of CD1c (BDCA-1) cells from bovine blood. Peripheral blood mononuclear cells were isolated from whole blood of cows using Histopaque (density 1.077). BDCA-1 cells were isolated from the population of mononuclear cells indirectly by immunomagnetic MicroBeads. Then, population of BDCA-1 cells were analysed by optical and fluorescence microscopy for determination of effectivity of those isolation procedures.

Keywords: Animal models, dendritic cells, myeloid cells, veterinary immunology

Acknowledgement: This research was funded by the Ministry of Agriculture of the Czech Republic (grant number QK1910212).

P-0148

The TDS assay: a powerful tool for detecting ongoing T cell responses by peripheral blood sampling

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In the course of a mouse study on T cell response after vaccination, we developed a flow cytometry proliferation assay, based on Ki-67/DNA dual staining and an ad hoc gating strategy for data analysis. Our assay was instrumental to measure vaccine-induced clonal expansion in spleen and lymph nodes, and to discover rare antigen-specific CD8 T cells in S-G2/M phases of cell cycle in mouse peripheral blood at early times after vaccination. While actively cycling CD8 T cells in the blood represented a minor "spillover" from lymphoid organs, their presence offered an opportunity for detecting ongoing T cell responses by peripheral blood sampling. We confirmed the presence of rare T cells in S-G2/M phases of cell cycle in the peripheral blood of human donors, and, on this occasion, refined the gating strategy by adding an additional gate for doublet exclusion, based on Imaging Flow Cytometry results. We defined T cells in S-G2/M phases in the peripheral blood "T Double S" for T cells in S phase in Sanguine ("TDS" cells), and our refined method for their quantification "TDS assay". The TDS assay provided useful information on ongoing T cell responses in Infectious Mononucleosis, Type 1 Diabetes, and COVID-19. Our results argue that the TDS assay can provide a window on immune dynamics in extra-lymphoid tissues, a long-sought potential of peripheral blood monitoring, for example in relation to organ-specific autoimmune diseases and infections, and cancer immunotherapy.

Keywords: Adaptive immunity, immune response tracing, immunological techniques, memory, monitoring immunity

P-0150

End stage renal disease (ESRD) and hemodialysis (HD) may force T lymphocytes towards senescence

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Immunosenescence might be a major key-factor leading to susceptibility of ESRD patients to age-related comorbidities. Aim of the present study is to elucidate whether ESRD is related to T cell phenotype alterations resembling immunosenescence and immunoexhaustion. Flow cytometry was performed to analyze CD45RA, CCR7, CD31, CD57, CD28 and PD1 markers on CD4+ and CD8+ lymphocytes. Based on these findings naïve, central memory(CM), effector memory(EM), TEMRA, recent thymic emigrants(RTEs) and exhausted T cells subsets were defined in 26 dialysis patients and 14 age matched healthy individuals. Differences in particular subsets perceived in Principal Component Analysis could clearly discrete HD patients from controls. Patients showed severe lymphopenia in comparison to controls [1500(500) vs 2100(850)cells/μl, p<0.001), which affected both CD4 and CD8 subpopulations [693(300) vs 1002(408)cells/μl, p<0.001, and 356(353) vs 459(423)cells/μl, p:0.039, respectively). Naïve cells [CD4+; 200(161) vs 427(323)cells/μl, p:0.002, CD8+; 147(180) vs 233(280)cells/μl, p:0.048) and RTEs [CD4+; 132(117) vs 250(239)cells/μl, p:0,027, CD8+; 166(134) vs 230(147)cells/μl, p:0.018) were markedly reduced in patients, affecting both subsets. However, highly differentiated memory cells (CD4+EM, CD8+EM, CD8+ TEMRA, CD4+CD28-CD57+, CD8+CD28-CD57+) were similar in both populations, even in the setting of marked lymphopenia observed in HD patients. Finally, CD8+PD1+ exhausted population was reduced in HD patients compared to controls [76(83) vs 126(100)cells/μl), respectively, p:0.025], whereas no difference was evident in CD4+PD1+ cells. Chronic HD results in marked total and naïve T cell lymphopenia. Nevertheless, highly differentiated, senescence-approaching, and exhausted subsets are not equally reduced. The clinical significance of this perseverance remains to be investigated.

Keywords: Adaptive immunity, ageing, immune senescence

P-0151

Regulation of CD161 expression by TCR triggering and TGFβ1 in HPV16-associated tumors

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Human papillomavirus type 16 (HPV16)+ tumors with an intratumoral type 1 response were highly infiltrated by CD4+CD161+ T cells with an effector memory phenotype and their numbers correlated with improved survival. The aim of this study is to determine how CD161 expression on CD4+ T cells is regulated in the tumor microenvironment (TME). HPV16-specific CD4+ T cell clones or sorted CD161+ and CD161- CD4+ T cells were stimulated via their TCR and/or cytokines present in the TME. CD161 (*KLRB1*) expression was determined by flow cytometry or mRNA analysis. Analysis of single cell RNAseq datasets of head and neck, lung, colorectal and liver cancer revealed that CD161+CD4+ T cells do not represent a transcriptionally distinct cell population. Expression of CD161 by CD4+ T cells is dynamic as stimulation with cognate antigen or CD3/CD28 coated beads resulted in a (dose-dependent) reduction in percentage CD161+ cells and CD161 expression at both protein and mRNA level. Co-stimulation with IL-12 and IL-18 enhanced IFNγ production by CD161+ CD4+ cells, but had no effect on percentage CD161+ cells or CD161 protein expression. Stimulation with TGFβ1, but not other TGFβ superfamily members, resulted in a stronger reduction of percentage CD161+ cells and CD161 expression after TCR stimulation. In contrast to CD161, expression of CD39, PD1 and CD103 was strongly increased by TGFβ1. In conclusion, expression of CD161 on CD4+ T cells is regulated by TCR signal strength and TGFβ1 at both protein and mRNA level and follows a pattern opposite of known co-inhibitory markers PD1 and CD39.

Keywords: Cancer immunology, cytokines and mediators, effector molecules, immune regulation and therapy, microenvironment

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POSTER PRESENTATIONS

P-0152

Dietary fish oil induces recruitment of mature NK cells to the inflamed site in antigen-induced peritonitis

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Resolution of inflammation is pivotal in returning tissues to homeostasis after acute inflammation. Natural killer (NK) cells are potent cytotoxic cells capable of inducing apoptosis in aberrant cells without prior stimulation. Our results show that dietary fish oil (FO) enhances resolution of inflammation and increases recruitment of NK cells to peritoneum of inflamed mice. Additionally, depletion of NK cells abrogated resolution of the inflammation. To determine the effects of dietary FO on the phenotype of NK cells recruited to the peritoneum in antigen-induced inflammation. Mice were fed FO enriched or control (C) diets, immunized, and challenged intraperitoneally with mBSA. Mesenteric lymph nodes and peritoneal lavage was collected. Expression of surface molecules was determined by flow cytometry, apoptosis in lymph nodes by TUNEL staining and concentration of cytokines by Luminex. FO fed mice had higher numbers of TUNEL+ cells in intrafollicular areas of draining lymph nodes than mice fed the C diet confirming enhanced resolution of inflammation. The FO diet increased the number of mature CD11bhighCD27low NK cells but decreased the number of intermediate CD11blowCD27high NK cells. In FO fed mice, NK cells expressed higher levels of the activation markers NKp46 and TRAIL and the chemokine receptor CCR5 and concentrations of CCL5 were higher than that in C fed mice. The additional mature NK cells in the FO fed mice may be recruited to the inflamed site through upregulation of the CCL5-CCR5 axis and may contribute to the enhanced resolution of inflammation.

Keywords: Chemokines, cytokines and mediators, inflammatory disease, innate immunity, NK cells

P-0153

Low-density granulocytes are a distinct and highly activated neutrophil population in COVID-19

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Low-density granulocytes (LDG) represent a sub-population of neutrophils that are expanded in numerous pathophysiological conditions. However, the phenotype and function of these LDG has not been fully characterised in the context of COVID-19. Better understanding of the immune role of LDG will help design therapeutic intervention. Here, we conducted an investigation on phenotypical differences between LDG and normal-density granulocytes/neutrophils (NDG) from 35 SARS-CoV-2 infected patients using unperturbed clinical samples and multi-parametric flow cytometry. LDG and NDG were isolated using gradient centrifugation method of whole blood samples collected in lithium-heparin vacutainers. Phenotype of the two neutrophil population was assessed using a multi-colour immunophenotyping panel consisting of neutrophil activation (CD63, CD62L, CXCR2), maturation(CD10, CD33, CXCR4) and immunomodulatory (PD-L1, LOX-1) surface markers. Our results show that LDG are elevated in COVID-19 patients and comprise of increased immature neutrophil subsets which correlate with disease severity. Furthermore, LDG from COVID-19 have a differential immunophenotype than NDG and represent a highly activated cell population with altered migratory and functional phenotype. Our immunophenotypic characterisation of LDG and NDG from COVID-19 patients has provided crucial details about the differential phenotypic features of both these cell populations. Our findings may implicate LDG as a source of dysfunctional/altered immune cells in COVID-19 which may be relevant to clinical decisions. How these phenotypical features translate to functional diversity and vice- versa deserves further investigation in future studies.

Keywords: Innate immunity, myeloid cells, neutrophils, viral infections

P-0155

B cell activation and auto-antibodies in mitochondria-mediated cardiomyopathy and post-MI heart failure

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Necrotic cardiomyocytes release mitochondrial DNA (mtDNA) that triggers B cell activation, production of pro-inflammatory mediators and antibodies. This contributes to maintaining persistent anti-cardiac auto-reactivity, leading to cardiomyopathy and eventually heart failure (HF). Here, we characterized the transcriptomic profile of the mouse myocardial B cell population post-MI and examined the presence of auto-antibodies in serum and their deposition in mice with mutations which impair mitochondrial dynamic processes of fusion (*Mfn1/2*) and fission (*Drp1*), inducing this way myocardial damage and mtDNA release. Single-cell analysis of previously published data (ArrayExpress: E-MTAB-7895) was used to define myocardial B cells post-MI. ELISA was performed to detect anti-heart IgG using longitudinal serum samples from mice with Mitofusin1/2 or Dynamin1 cardiac-specific tamoxifen-activated deletion. Cardiac sections were stained for IgG deposition. Single-cell transcriptomics data showed that myocardial B cells clustered into immature, plasma, memory and B1 cell subgroups. B cell functional profiling was based on effector molecule expression analysis. Pro-inflammatory proteins (*CCR7*, *IFNG*) and collagens (*COL3A1*, *COL5A1*) were upregulated even 28 days post-MI, along with mitochondria-specific transcripts (*MFN1/2*, *OPA1*, *DRP1*, *ESRRA*, *SDHA*) indicative of metabolic remodelling and B cell maturation.

Mfn1/2(n=2) and *Drp1*(n=3) mutant mice with hypertrophic and dilated cardiomyopathy phenotypes respectively showed increased total IgG antibody levels at 4 weeks after transgene deletion (Mean±SEM: 0.2225±0.02339 (*Mfn1*$^{-}$/*2*$^{-}$); 0.3661±0.03543 (*Drp1*$^{-}$)). IgG deposits were also detected in cardiac sections of all *Mfn1/2* and *Drp1* mutants. B cells and auto-antibodies are implicated in mitochondria-mediated cardiomyopathy underlining their possible role in anti-cardiac autoreactivity and HF progression.

Keywords: Adaptive immunity, antibody, autoimmunity, B lymphocytes, cardiovascular diseases

P-0156

Circulating CD8+CD45RA-CD62L+ T cells provide a source of cytotoxic tissue resident-like CD8+CD103+CD49a+ T cells

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Different populations of tissue-resident memory CD69+ T (Trm) cells provide local protection against pathogens in barrier tissues such as skin, where the integrin CD49a defines epithelial cytotoxic CD8+ Trm cells. During progressive disease, de novo seeding of Trm cells to distal sites from the primary infection is needed for robust immunity and migrational plasticity of Trm cells has been shown in murine models. Through epitome indexed single cell RNA-sequencing (scRNA-seq) of memory CD8+ T cells derived from paired skin epidermis and blood of healthy donors, we revealed higher levels of T cell clonotypes sharing between epidermal CD103+CD49a+ T cells and circulating CD45RA–CD62L+CCR7+ relative to CD45RA–CD62L–CCR7– T cell subsets. Accordingly, blood-derived CD45RA–CD62L+CD8+ T cells, but not naïve or other memory T cell subsets, could generate CD69+CD103+CD49a+ Trm-like cells following TCR engagement in the presence of IL-15 and TGF-β. Such *in vitro* differentiated CD49a+ Trm-like cells expressed high levels of cytotoxic mediators and efficiently produced IFN-γ and TNF. In contrast, blood-derived gut-homing CD45RA–CD62L+CCR9+ T cells exclusively induced CD103 expression upon expansion with IL-15 and TGF-β, congruent with lack of CD49a expression on gut CD8+ Trm cells. Together, we demonstrate that epidermal cytotoxic CD8+CD103+CD49a+ Trm cell clonotypes are represented among different subsets of circulating memory CD8+ T cells, but that memory CD8+CD62L+ T cells are preferentially primed for expression of CD49a and cytotoxic effector molecules. Optimizing CD8+CD49a+ T cell formation targeting CD62L+ memory cells by vaccination we can aid generation of specific effector cells at epithelial sites.

Keywords: Adaptive immunity, immune development, memory, RNAseq, skin diseases

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POSTER PRESENTATIONS

P-0157

Single-cell immune profiling of steady-state CD8+ T cells reveals a novel population of interferon-responsive naïve T cells

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Our immune system can eliminate a diverse spectrum of pathogens thanks to the cooperation of different immune cell types. In the last few years, single-cell RNA sequencing has challenged the traditional view of cell types, revealing their unexpected inner diversity. An exemplary uniform cell type is naïve CD8+ T cells, which stay inactive until they encounter their cognate antigen. The heterogeneity of CD8+ T cells after activation is undebatable – dramatic proliferation gives rise to potent effectors capable of direct killing of virally infected cells as well as different kinds of memory cells. However, whether the cell fate is determined by intrinsic properties of naïve cells, TCR specificity or the environmental stimuli, remains unclear. To reveal the heterogeneity of steady-state CD8+ T cells, we have sequenced the transcriptome and TCR repertoires of more than 50 000 murine CD8+ T cells. Unsupervised clustering revealed established cell subsets (conventional naïve cells, antigen inexperienced memory-like T cells) as well as novel subtypes, such as interferon responsive naïve T cells (IRENA). Here, we show that IRENA cells are stably present in mice of different genetic and hygienic background, have diverse TCR repertoire and show similar activation capacity as naïve cells *in vitro* and *in vivo*. Finally, independent analysis and integration of publicly available scRNAseq datasets of human CD8+ T cells revealed that IRENA cells exist in humans. While human IRENA cells are rare in healthy individuals, their frequency dramatically increases after viral infection, such as SARS-CoV-2, and correlates with the disease severity.

Keywords: Adaptive immunity, big data, biology of the immune system, omics technologies, RNAseq, viral infections

P-0158

Quantifying mast cells in the urinary bladder, small intestine and the lung

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Mast cells are tissue-resident immune cells derived from $CD34^+$ progenitor cells in the bone marrow. Mast cells are thought to be present within most tissues of the body, although their overall distribution is still not fully understood. Flow cytometry was used to assess the prevalence of mast cells in the urinary bladder, small intestine and the lungs in C57BL/6J mice. CD45.2, CD117, CD34, and FcεRIα antibodies were used to identify mast cells. Preliminary data suggests that CD117+CD34+FcεRIα+ mast cells constitute 4.30% of all CD45.2+ haematopoietic cells in the urinary bladder (n=6), 3.24% in the small intestine (n=6) and 0.11% in the lungs (n=6). This suggests that mast cells constitute a greater proportion of haematopoietic cells within the urinary bladder, and a lower proportion of haematopoietic cells in the lungs. This data will be used to further the current understanding towards the distribution of mast cells within these organs. Further studies in this area may also facilitate an enhanced understanding of how the systemic distribution and prevalence of mast cells changes during inflammatory diseases.

Keywords: Animal models, antibody, mast cells, protection

P-0159

Benign multiple sclerosis patients display altered peripheral blood B cell subset ratios and B cell gene expression levels

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The interplay between the immune system, sleep dysfunction and cognitive impairment participates in the progression of disability in multiple sclerosis (MS). Our aim was to identify molecular pathways and B cell associated with separate components of MS disability. Benign MS, non-benign MS patients and healthy controls were recruited. Patients underwent polysomnography and cognitive studies. Microarray and bioinformatics analysis performed using peripheral blood mononuclear cell samples identified B cell-associatedgenes with the most significantly altered expression. Expression levels of these genes were validated by real-time PCR and peripheral blood cell subsets were examined by flow cytometry. Putative correlations among clinical and laboratory parameters were investigated by correlation network analysis. Sleep and cognitive functions were equally impaired in BMS and NBMS. BMS patients showed significantly reduced memory B cell and increased regulatory B cell percentages than NBMS patients. Among genes that were selected by bioinformatics, levels of BLK, BLNK, BANK1, FCRL2, TGFB1 and KCNS3 genes were significantly different among study subgroups. Correlation network analysis showed associations among physical-cognitive disability and sleep dysfunction measures of MS versus expression levels of selected genes. BMS and NBMS differ by physical disability but not cognitive and sleep dysfunction. Different components of disability in MS are associated with peripheral blood B cell ratios and B cell related gene expression levels. Thus, it is likely that altered B cell functions participate in the progression of disability in MS.

Keywords: Autoimmunity, B lymphocytes, multiple sclerosis

P-0161

Expression and regulation of MS4A molecules in myeloid cells

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POSTER PRESENTATIONS

The MS4A gene family encodes 18 tetraspanin-like proteins, most of which with unknown function. MS4A1 (CD20), MS4A2 (FcεRIβ), MS4A3 (HTm4) and MS4A4A play important roles in immunity, whereas expression and function of other members of the family are unknown. The present investigation was designed to obtain an expression fingerprint of MS4A family members, using bioinformatics analysis of public RNA sequencing databases, RT-PCR, RNA sequencing and protein analysis when possible. *MS4A3*, *MS4A4A*, *MS4A4E*, *MS4A6A*, *MS4A7* and *MS4A14* were expressed by myeloid cells. *MS4A6A* and *MS4A14* were expressed in circulating monocytes and decreaseddуring monocyte-to-macrophage differentiation in parallel with an increase in *MS4A4A* expression. Analysis of gene expression regulation revealed a strong induction of MS4A4A, MS4A6A, MS4A7 and MS4A4E by glucocorticoid hormones. Consistently with the *in vitro* findings, MS4A4A and MS4A7 were expressed in tissue macrophages from COVID-19 and rheumatoid arthritis patients. Interestingly, *MS4A3* gene, selectively expressed in myeloid precursors, was found to be a marker of immature circulating neutrophils, a cellular population associated with COVID-19 severe disease. While the functions of most MS4A proteins remains unknown, the results reported here showing a differential expression andregulation strongly support their importance in leukocyte differentiation or function, and call for assessment of their functional role and value as therapeutic targets.

Keywords: Macrophage, myeloid cells, neutrophils, rheumatoid arthritis, viral infections

P-0162

Defining the differentiation of T follicular regulatory cells

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Germinal centers are sites within secondary lymphoid organs where mature B cells undergo affinity maturation and isotype switching. The balance between two T cell subsets was shown to have a significant impact on GC outcome: while T follicular helper (Tfh) cells support the production of high-affinity antibodies, T follicular regulatory (Tfr) cells control the magnitude of the GC response. A greater understanding of the biology of Tfr cells is essential as their dysregulation was implicated in autoimmunity, allergy, and may impact protective immune responses. Murine Tfr cells reach the peak at day 10 post-antigen exposure, similarly to Tfh cells. To explore the differentiation steps of Tfr cells, we generated single-cell RNA-sequencing data from three sorted cell populations (Foxp3-CXCR5+ Tfh cells, Foxp3+CXCR5+ Tfr cells and Foxp3+CXCR5- Treg cells) at three different time points (day 0, 6 and 10 after immunization). We were able to show the heterogeneity of those cell populations at the different time points, which will allow us to use pseudotime algorithms to reconstruct a developmental trajectory of murine Tfr cells during a humoral immune response. In parallel, we are using *in vitro* assays to probe specific peripheral lymphoid niches, defined by distinct cytokines, that are required for human Treg cells to differentiate into Tfr cells. Our data suggest that IL-6 and OX40L signals are essential for the Tfr cell development from uncommitted human Treg cells. Determining the molecular mechanisms underlying Tfr differentiation will be essential to identify novel therapeutic targets to modulate Tfr/Tfh participation in GC reactions.

Keywords: Adaptive immunity, regulatory cells, RNAseq

P-0163

Expression profiliing of KIR+ CD8+ T lymphocytes in cancer immunosurveillance

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Killer-cell immunoglobulin-like receptors (KIR) are expressed by natural killer (NK) and effector T cells. Although KIR+ T cells accumulate in oncologic patients, their role in cancer immune response remains elusive. This study explored the role of KIR+CD8+ T cells in cancer immunosurveillance by analyzing their frequency at diagnosis in the blood of 249 patients (80 melanomas, 80 bladder cancers, and 89 ovarian cancers), their relationship with overall survival (OS) of patients, and their gene expression profiles. KIR2DL1+ CD8+ T cells expanded in the presence of HLA-C2-ligands in patients who survived, but it did not in patients who died within the 93-month follow-up period. In contrast, the presence of HLA-C1-ligands was associated with dose-dependent expansions of KIR2DL2/S2+ CD8+ T cells and with shorter OS. In vitro, KIR+CD8+ T cells expanded preferentially in the presence of HLA-C1 and IL-12. Interactions of KIR receptors with their specific ligands had a profound impact on the expression profiles of these cells, involving multiple signaling pathways, effector functions, the secretome, and consequently, the cellular microenvironment. KIR2DL1/S1+ CD8+ T cells showed a gene expression signature related to efficient tumor immunosurveillance. In contrast, KIR2DL2/L3/S2+ CD8+ T cells showed transcriptomic profiles related to suppressive anti-tumor responses. Altogether, results show that depending on their specific ligand, interactions of KIR receptors on CD8+ T cells determine differential gene expression profiles, which could impact their cancer immunosurveillance capacities. These results could be the basis for the discovery of new therapeutic targets so that the outcome of patients with cancer can be improved.

Keywords: Cancer immunology, Cellular interactions, Innate immunity, Molecular immunology, NK cells

P-0165

Human oral epithelial cells impair T cell responses by PGE2 production

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The oral mucosa is a site of intense immunological activity, where oral epithelial cells (OECs) ought to play an essential role articulating tolerogenic and defensive responses, which are yet poorly understood. Here, we have studied the ability of OECs to modulate CD4 T cells immune responses. We have used the H413 and TR146 oral cell lines and primary OECs for co-culture assays with total CD4 T cells from healthy donors' peripheral blood. Cells were cultured in the presence or not of indomethacin (inhibitory drug of COX-1), PF-04418948 and ONO-AE2-208 (agonist of prostaglandin E2 receptor 2 and 4, EP2, EP4) and Poly(I:C). To elicit CD4 T cell responses, anti-CD3/CD28 antibody stimulation. Cell markers were studied by flow cytometry. OECs in culture with total CD4 T cells stimulated with anti-CD3/CD28 abrogated immediately the release of IFNγ and TNFα. However, when OECs were treated with indomethacin they were unable to inhibit the production of both cytokines. In addition, blockage of PGE2 receptors EP2R and EP4R in T cells with PF-04418948 and ONO-AE2-208, respectively, also prevent the inhibition by OECs. On the contrary, OECs stimulated with poly(I:C) elicited T cell responses. Our data indicate that OECs promoted an anti-inflammatory state blocking T cell activation and impairing Th1 cell responses. Here, we suggest that the main mechanism responsible of this effect is PGE2 production-dependent. We also demontrate that viral stimulation of OECs prevent this T cell inhibition.

Keywords: Biology of the immune system, drugs for immune modulation, immune communication, innate immunity

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POSTER PRESENTATIONS

P-0166

Peripheral monocytes in extremely premature infants display altered functional and phenotypic characteristics but are highly responsive to LPS

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Premature children have a compromised immune system that impose an increased risk of sepsis, necrotizing enterocolitis and pneumonia–common causes of mortality, primarily in the neonates with extremely low gestational age (ELGAN) and extremely low birth weight (ELBW). In this study, both phenotypic and functional characterization of monocyte compartment of ELGAN/ELBW was evaluated in a longitudinal way. Peripheral blood mononuclear cells (PBMC) were collected at day-14, 28 and at PMW 36+0 from ELGAN/ELBW infants, participating in a randomized double-blind placebo-controlled study of probiotic supplementation. PBMCs from 29 full-term (FT) infants at 14 days of age were used as controls. Phenotypic analysis was done by flow cytometry and LPS stimulated PBMC culture supernatants were analyzed for cytokines by Luminex assay. The results revealed that the proportion of CD14+ monocytes were similar in both ELGAN/ELBW and FT neonates. However, the expression levels of HLA-DR, TLR4 and CD80 on monocytes were markedly low in day 14 ELGAN/ELBW infants and remained low during the entire study period. Cytokine profiling of secreted pro- and anti-inflammatory cytokines showed a very low basal production but strong robust responses to LPS in ELGAN/ELBW, resulting in a high stimulated/unstimulated cytokine ration in the preterm neonates up to 28 days of age. Moreover, chorioamnionitis clearly associated with altered HLA-DR and CD80 expression and functional characteristics within preterm group. Here we show that the peripheral monocyte population of ELGAN/ELBW infants is altered during the first weeks of life in a way that may influence how they respond to microbial challenges.

Keywords: Innate host defence, innate immunity, innate lymphoid cells

P-0167

Characteristic analysis of THP-1 cell line as a model of viral- and bacterial-mediated inflammation

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THP-1 is an acute myeloid leukemia cell line, which is widely used in research on inflammation of various etiology in monocytes/macrophages. At the same time, the basic features of THP-1 behavior in pathology, for instance, during viral and bacterial infection, remain unclear. In the present study, we investigated the cellular responses of THP-1 cells infected with the influenza virus H1N1 (strain A/Puerto Rico/8/34) or activated with bacterial lipopolysaccharides (LPS) and addressed the THP-1 pro-inflammatory activities. THP-1 cells were incubated with the influenza virus(MOI=0.1) or LPS for 24 h. Then, the expression levels of the metabolic and pro-inflammatory genes were determined by quantitative real-time PCR (qPCR) analysis. We determined the most stable reference genes as HSP90, RPS18, and HPRT1 during LPS activation and HPRT1, RPS18, and PPIA during viral infection. The mRNA levels of GAPDH or GAPDH and HSP90 were dysregulated in THP-1 monocytes exposed to LPS or virus respectively, thus suggesting novel targets for diagnostics and immunomodulation. Our data confirmed that activated THP-1 cells exhibited morphological alterations (cluster formation) and upregulated mRNA levels of pro-inflammatory markers, IL6, IL1B, CCL2, CXCL10, CD80. The viral infection resulted in cell aggregation suggesting that THP-1 represented a suitable model for virus/bacteria-mediated monocyte activation. In conclusion, THP-1 cells reproduce monocyte/macrophage functional features, including cytokine production and stimulated antigen presentation during pro-inflammatory conditions, and can be successfully used for evaluation of immunogenic and metabolic activities for research purposes and drug screening.

Keywords: Chemokines, cytokines and mediators, inflammatory disease, myeloid cells

P-0168

Quantity and quality of HCoV-mediated SARS-CoV-2-cross-reactivity correlates with age

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The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused a global pandemic and challenges economy and healthcare systems worldwide. Most infections evoke none to mild symptoms, but severe cases disproportionately affect the elderly. While the population was initially assumed to be immunologically unprotected against the novel virus, we and others could demonstrate cellular and humoral cross-reactivity against SARS-CoV-2 in unexposed individuals. Comprehensive investigation of the SARS-CoV-2 orfeome revealed CD4+ T cell reactivity against virtually all SARS-CoV-2 proteins, of which spike glycoprotein displayed cognate, endemic coronavirus (HCoV-) mediated cross-reactivity against the more homologous C-terminal rather than the N-terminal part. Cellular reactivity towards HCoV spike was ubiquitous but decreased with age, accompanied by reduced SARS-CoV-2-cross-reactivity. Moreover, epitope-mapping identified an immunodominant cross-reactive peptide recognized by CD4+ T cells in 20% of unexposed individuals and 50% of SARS-CoV-2 convalescents. Together, our results indicate a role of pre-existing cognate spike-cross-reactive T cells in SARS-CoV-2 infection. Abundant cross-immunity may be responsible for the predominantly asymptomatic/mild disease courses, whereas diminished cross-immunity in the elderly may underlie the higher risk for severe illness.

Keywords: Immune senescence, adaptive immunity, ageing, protection, viral infections

P-0169

CX3CR1 expression levels define human CD8 T cell subsets

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CD8 T cells differentiate into heterogeneous effector and memory populations upon priming. While murine and human CD8 T cell subsets/differentiation states carry overlapping names, the molecular markers that distinguish them are distinct. This complicates translation of findings across species, and underscores the need to identify markers that delineate differentiation states in both mice and humans. We investigated whether CX3CR1, a high-resolution differentiation marker for murine CD8 T cells, distinguishes functionally distinct states of human T cells. By measuring CX3CR1 expression levels in relation to cytokine production, cytotoxic molecules and proliferation, we demonstrated that a graded increase of CX3CR1 expression levels correlated with a graded increase in differentiation state of human blood-derived CD8 T cells. High-dimensional analysis of 8 differentiation-associated markers revealed that the traditional 2-dimensional CCR7 and CD45RA-based subsetting did not correspond to the high-dimensional differentiation states. In contrast, CX3CR1 levels faithfully captured these states and thereby seem more suitable for functionally relevant CD8 T cell subsetting. In line with this, we found that CD8 T cells expressing high CX3CR1 levels, which are the majority among T cells lacking lymph-node homing molecules and are thus believed to survey peripheral tissues, were actually absent in human skin. Instead, human skin harbored CX3CR1-negative tissue resident memory (Trm) cells, and CX3CR1-intermediate cells that lack the Trm marker CD69 and might thus be migratory. In conclusion, CX3CR1 is a high-resolution differentiation marker of human and murine CD8 T cells and improves our understanding of human CD8 T cell differentiation.

Keywords: Adaptive immunity, chemokines, effector molecules, memory, viral infections

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POSTER PRESENTATIONS

P-0170

Age-associated impairment of the PEPITEM pathway: Implications for lymphocyte transendothelial migration

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Ageing is associated with systemic inflammation (inflammageing) and immune system dysfunction (immnosenescence). Leukocyte migration is necessary for effective immunity; however, dysregulated leukocyte trafficking contributes to inflammageing and the development of age-related inflammatory diseases. During acute inflammation, B-cells can regulate T-cell trafficking into tissue. At the heart of this pathway, circulating adiponectin binds adiponectin receptors 1 and 2 (AdipoR1/2) on peripheral blood B-cells, stimulating the release of PEPITEM (PEPtide Inhibitor of TransEndothelial Migration). PEPITEM indirectly inhibits T-cell transendothelial migration via the actions of sphingosine-1-phosphate (S1P). However, the PEPITEM pathway is reportedly suppressed in several inflammatory diseases. Here, we investigate the effects of ageing on the PEPITEM pathway. We measured the expression of AdipoR1/2 on circulating B-cells of young and older men using flow cytometry. We then investigated the effects of adiponectin, S1P and PEPITEM treatment on the transendothelial migration of peripheral blood lymphocytes (PBL), using an *in vitro* assay. We found an age-related reduction in the frequency of AdipoR1+ circulating B-cells. Importantly, we found that PEPITEM and S1P effectively reduced the transendothelial migration of younger and older PBL, whilst adiponectin only reduced the transendothelial migration of younger PBL. Our results indicate an age-related blunting of the PEPITEM pathway, which may contribute to dysregulated PBL trafficking in older adults. Our findings offer a novel avenue of investigation into restoring regulated PBL trafficking in older adults, thus limiting inflammation and lowering the risk of age-related inflammatory diseases.

Keywords: Ageing, B lymphocytes, cellular interactions

P-0172

Influence of galectin-1 on immunomodulatory characteristics of human monocyte-derived dendritic cells

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Evaluation of immunomodulatory properties of monocyte-derived dendritic cells (MoDCs) *in vitro* upon the treatment with different concentrations of Galectin-1 (Gal-1). MoDCs were treated with increasing concentrations of Gal-1 (1, 3 and 6 μg/mL) for 48h, upon which their phenotypic characteristics, cytokine profile, and the ability to direct the immune response in the co-culture with allogeneic CD4+T cells was assessed. All applied concentrations of Gal-1 down-regulated the expression of CD80 and CD86 molecules on MoDCs compared to untreated cells. Production of IL-12 by MoDCs treated with 1 μg/mL and 6 μg/mL of Gal-1 was significantly reduced, whereas the concentration of 3 μg/mL increased the IL-12 production. Gal-1 in all applied concentrations induced a significant increase in the IL-10 production. Co-cultivation of allogeneic CD4+T lymphocytes and MoDCs treated with 3 μg/mL and 6 μg/mL of Gal-1 led to increased production of IL-2 and IFN-γ, whereas the treatment with the lowest concentration of 1 μg/mL reduced IL-17 production in the same co-culture. Our study demonstrated a dual immunomodulatory effect of Gal-1 on MoDCs in terms of immunostimulation and immunosuppression, depending on the applied concentration. Future studies are required for obtaining a deeper insight into the dose-dependent manner of MoDC-modulation by Gal-1, which holds promise for its role as a potential adjuvant in the preparation of DCs for cell therapy in treating different immune pathologies.

Keywords: Cytokines and mediators, dendritic cells, drugs for immune modulation

P-0173

Influence of cucurbit[n]urils on phenotype of human monocyte-derived dendritic cells *in vitro*

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Cucurbiturils (CB[n]) are macrocyclic cavitands that can be used as a drug delivery system. The aim of this study was to investigate the effect of cucurbit[n]urils(CB[n]) (n=6,7,8) on the expression of MHCII and co-stimulatory molecules by immature and mature dendritic cells(DCs). Samples of blood were obtained from 8 healthy volunteers after recieving written consent. DCs were derived by cultering of the adherent fraction of PBMCs with GM-CSF(100ng/ml) and IL-4(50ng/ml) during 5 days. Then immature DCs were transferred into the 6-wells plates with LPS111:B4(10 μg/ml) for maturation and 0.5mM CB[6], CB[7], 0.01mM CB[8], cultured for 2 days. After it, DCs were collected and stained with antibodies specific to CD14, CD11c, CD80, CD86(B7-2), HLA-DR. Analysis of cell phenotype was performed on a cytometer FACSCantoII(BD). To compare groups we used nonparametric methods. We determined DCs as CD11c+CD14low cells. During maturation mature DCs(mDCs) upregulated of MHCII and co-stimulatory molecules compared to immature Dcs(imDCs). CB[7] was inert and did not significantly affect on the DCs in cultures. CB[6] contributed to a slightly decrease in the density of HLA-DR expression on imDCs and mDCs.Under CB[8], the frequency of CD80+ cells and the dencity of CD80 decrased on imDCs, however, the percentage of CD80+CD86+ cells increased. On mDCs CB[8] led to a decrease of the density of HLA-DR and CD80 expression, shifting those towards imDCs, that have tolerogenic capacities. Thus, obtained data indicated that CB[6] and CB[8] altered the phenotype of DCs that can affect on the antigen-presentation function.

The study was supported by RSF (project №19-15-00192).

Keywords: Dendritic cells, drugs for immune modulation, effector molecules, immunopharmacology

P-0174

XCR1+ Dendritic cells are required for intestinal Th1 homeostasis

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The intestinal mucosa contains several classical dendritic cell (cDC) subsets each of which appear to play a non-redundant role in intestinal T cell homeostasis. Intestinal cDC1, are dependent on the transcription factor IRF8 for their development. Utilising CD11c-cre.IRF8fl/fl mice, we have recently provided data to suggest that intestinal cDC1 are important for intestinal Th1 homeostasis and priming. CD11c is however expressed by multiple cell types, including intestinal macrophages, plasmacytoid DC and subsets of B and T cells. To determine whether intestinal Th1 responses are dependent on cDC1 we have generated XCR1-Cre.DTAStopfl/fl and XCR1-Cre.IRF8fl/fl mice, that specifically and selectively lack cDC1. Similar to CD11c-Cre.IRF8fl/fl mice, XCR1-Cre.DTAStopfl/fl and XCR1-Cre.IRF8fl/fl mice lack Th1 cells in the small intestinal LP and displayed markedly reduced numbers of Th1 cells in the colon. In preliminary experiments, we have observed that intestinal Th1 response is abrogated in IL-27 receptor-α (IL-27RA) knock out mice whilst Th1 numbers are normal in IL-12p35 or IFN-α receptor(IFNAR) deficient mice. Moreover, in an antigen dependent manner we confirmed that IL-27RA signalling is required for Th1 induction after immunisation with TLR4 ligand. These results indicate that cDC1 driven intestinal Th1 homeostasis is dependent on IL-27RA signalling but independent of IL-12 and interferon signalling. Current studies are thus focused on identifying other cDC1 derived factors required for intestinal Th1 homeostasis as well as the environmental triggers that promote Th1 development in the intestine.

Keywords: Adaptive immunity, cytokines and mediators, dendritic cells

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POSTER PRESENTATIONS

P-0175

Changes in cellular immunological parameters in children with recurrent respiratory infections

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Recurrent respiratory infections (RRI) represent the most frequent childhood pathology, in conditions of a non-compromised immune system. Due to association with an altered cellular immune response, the aim of the study was to identify possible immunological changes with impact in RRI pathogenesis, by quantifying T, B and NK lymphocyte subsets. Two groups of children (1-7 years) were considered: i) RRI (30) – children with at least 6 episodes of RRI/year; ii) control (10) – clinically healthy children. Serum immunoglobulins IgG, IgA and IgM were assayed by nephelometry and lymphocyte subsets by flow cytometry (BD FACSCanto II) as follow: total T-lymphocytes (CD3+) with T-CD4$^+$, T-CD8+ and double-negative T cells (CD4-CD8-CD1d-) subsets, NKT cells (CD3+CD16/56+CD1d+), NK cells (CD16/56+) and total B-cells (CD19+CD20+) with mature/naive B cells (CD27-IgD+), memory B cells (CD27+) and plasmocytes (CD10-CD27+CD38bright). The most important changes observed in T lymphocytes were the decrease of T-CD8+ (p=0.009) values and the increase of the T-CD4+/T-CD8+ ratio (p=0.002). Although NK cells in RRI group are increased (p=0.003) as compared to control, they are within normal ranges. B cells were decreased in 86% cases,mature/naive B cells shown low values and memory B cells were increased (p=0.027), although Ig values were normal in 70% of cases. Testing of cellular parameters may completethe clinical diagnosis, especially when the humoral parameters are within normal limits. Detection of causes and prophylaxis of RRI can contribute to improving the living conditions of the affected child population.

Keywords: Antibody, B lymphocytes, NK cells

P-0177

Human CD5+ innate lymphoid cells are intravascular precursors that migrate into the lung

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Innate lymphoid cells (ILCs) contribute to immune defense and maintain homeostasis at barrier sites such as the lung. However, due to the lack of suitable *in-vivo* experimental systems, it is poorly understood how human lung ILCs develop and are strategically positioned to maintain lung homeostasis. The present study investigated the ontogeny and migration of human lung ILCs *in vivo*. We used the MISTRG humanized mouse model transplanted with human hematopoietic stem cells as our *in-vivo* experimental system. Human ILCs isolated from humanized mice were characterized by flow cytometry. Intravascular antibody labeling was used to determine ILC distribution between vascular and tissue compartments in the lung. ILC progenitor potential was evaluated with an *in-vitro* co-culture system. We identified a CD5-expressing human ILC population that mainly resided in the lung vasculature and circulation. CD5+ CD7+ ILCs had a distinct ontogeny compared to CD5- CD7+ conventional ILCs because they originated from the spleen instead of the bone marrow. The human CD5+ CD7+ ILC population contained both IFNγ-producing ILC1 and a proliferating ILC precursor with a naïve surface phenotype that was able to generate all mature ILC subsets *in vitro*. Human CD5+ CD7+ ILCs represent novel ILC precursors which, due to their strategic location, could serve as a reservoir of blood-borne sentinels, ready to be recruited into the lung during infection.

Keywords: Animal models, biology of the immune system, innate immunity, innate lymphoid cells

P-0178

Extracellular factors of probiotic bacteria promote immunomodulatory effects of bone marrow mesenchymal stem cells

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Human bone-marrow mesenchymal stem cells (hBM-MSC) having the capacity to differentiate into various lineages has shown to possess broad immunoregulatory capabilities.As it is well known, probiotic bacteria can regulate immunoregulatory processes. In this regard, we aimed to evaluate the immunomodulatory effects of probiotic and pathogenic bacteria on hBM-MSCs. Probiotic (“”*Lactobacillus delbrueckii subsp. bulgaricus and Lactobacillus paracasei subsp. paracasei*””) and pathogenic bacteria strains (“”*Staphylococcus aureus and Enterococcus faecalis*””) were cultured at 37oC. Then, the bacterial supernatants were filtered and serial diluted.hBM-MSCs were cultured with these bacterial supernatants for 72 h.Subsequently, conditional mediums (CM) of control and treated hBM-MSCs were obtained after 48 h.The PGE-2, TGF-β1, IL-10, NGF and Netrin-1 levels of the CM samples were determined by ELISA.In addition, the migration abilities of control and treated hBM-MSCs were analyzed with the *in vitro* wound healing assays. According to our findings, the probiotic soluble factors of Lactobacillus spp. strains increased the production of PGE-2, TGF-β1, IL-10 and NGF levels of hBM-MSCs, but did not change their Netrin-1 production. Also, the probiotic bacterial extracellular factors reduced the migration ability of hBM-MSCs *in vitro*. hBM-MSCs exert their immunesuppressive potential by secreting significant immuneregulatory cytokines.Unlike the pathogenic bacteria, we showed the probiotic bacteria supernatants could regulate hBM-MSCs microenvironment by triggering the production of their immunomodulatory factors. As a result, it could be said that extracellular factors of probiotic bacteria could contribute to immunomodulatory effects of hBM-MSCs in a potential therapeutic direction.

Keywords: Cell based therapies, cellular interactions, immune regulation and therapy

P-0179

Maintenance of bone marrow resident versus circulating memory T cells in humans

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Bone marrow is home to discrete populations of resident and resting memory T lymphocytes, maintaining functional long-term memory to systemic antigens, like viruses. In the (murine) bone marrow, memory T cells are docking onto IL-7-expressing mesenchymal stromal cells, but the molecular signals maintaining the memory T cells over long time-periods remain elusive. Identify the molecular signals maintaining human memory T cells in bone marrow and blood. Human paired blood and bone marrow samples, human stromal cell lines, trans-well assay, *ex vivo* and *in vitro* studies, including FACS, MACS, ELISA, isolation and tissue cultures of memory CD4+ and CD8+ T cells. Human memory T cells isolated from bone marrow survive less well than their blood circulating counterparts in a normoxic *ex vivo* cell-culture system. Their survival is also more dependent on interleukin-7. Signals from stromal cells support the survival of memory T cells by regulating the anti- and pro-apoptotic factors, in a way distinct from that induced by the IL-7/STAT5 cytokine signaling pathway. Memory T cells isolated from blood obviously are maintained by different signals over time, as compared to tissue-resident memory T cells from the bone marrow, which survive in contact to stromal cells providing them with persistent survival signals.

Keywords: Adaptive immunity, immune communication, memory, biology of the immune system, cell signalling, cell death

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POSTER PRESENTATIONS

P-0181

High glucose regulates Ca2+ influx in cytotoxic T lymphocytes

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The killing efficiency of cytotoxic T lymphocytes (CTLs) is tightly regulated by intracellular Ca2+ concentration. Glucose is the key energy source for CTLs, lack of which significantly impairs CTL activation, proliferation and effector functions. The impact of high glucose on Ca2+ influx in CTLs remains largely elusive. In this work, we stimulated primary human CD8+ T cells in medium containing either 25 mM (high glucose, HG) or 5.6 mM glucose (normal glucose, NG). We found that store-operated calcium entry (SOCE) induced by thapsigargin (Tg) is elevated in HG-cultured CTLs compared to their counterparts in NG. Unexpectedly, the Ca2+ influx elicited by recognition of target cells is reduced in HG-cultured CTLs. Under HG condition, STIM1 and STIM2, the calcium sensors in the endoplasmic reticulum (ER), were down-regulated; ORAI1, the main structural component of calcium-release activated channels, remained unchanged, whereas ORAI2 and ORAI3 were up-regulated. The fraction of necrosis of HG-cultured CTLs was enhanced after killing without affecting glucose uptake. Thus, our findings reveal that HG has a distinctive impact on Tg-evoked SOCE and target recognition-induced Ca2+ influx in CTLs and causes more CTL death after killing, suggesting a novel regulatory role of high glucose on modulating CTL functions.

Keywords: Adaptive immunity, cell signalling, diabetes

P-0182

A comprehensive platform for the faithful generation of human innate lymphoid cell lineages from CD34+ hematopoietic progenitors

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Innate lymphoid cells (ILCs) are critical effectors of innate immunity, particularly at mucosal surfaces. Besides fetal lymphoid tissue inducer (LTi) cells, post-natal ILCs can be classified into ILC1, ILC2, ILC3, and NK cells. While *in vitro* generation of T helper lineages has expanded our understanding of developmental requirements, ILC engenderment has not been systematically explored, and previous investigations relied on the analysis of few markers or cytokines, which are suboptimal to assign lineage identity. Here, we present a comprehensive platform which allows the reliable generation of human ILC lineages from CD34$^+$ progenitors derived from cord blood and bone marrow. Validation by global and single cell transcriptome analysis exposes the power and the limitations of the system in engendering mature and functional ILCs which recapitulate their *ex vivo* counterparts. These data represent a resource to aid in clarifying ILC biology and differentiation.

Keywords: Immune development, innate immunity, innate lymphoid cells

P-0183

Human CR2 inhibits the BCR induced B cell activation, proliferation and antibody production

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Human complement receptor type 2 (CR2, CD21) is generally considered as an activating coreceptor for BCR on B cells, despite its contradictory literature and based mostly on mouse experiments. To clarify the function of CR2 on human B cells, we aimed to reinvestigate this important but controversial subject. Human resting tonsillar B cells were treated with streptavidin-linked complexes of C3d (as natural ligand of CR2) and suboptimal doses of anti-IgG/A/M (as weak BCR stimuli) at various concentrations, and the effects of co-crosslinking BCR and CR2 were analyzed on different levels of B cell activation: the immediate Ca^{2+} response, early activation marker (CD69) expression, proinflammatory cytokine (IL-6) release, proliferation and antibody (IgM and IgG) production. We observed that when a suboptimal BCR stimulus (incapable of inducing Ca^{2+} response alone) is crosslinked to C3d, the intracellular Ca^{2+} concentration is significantly enhanced, indicating the initiation of signal transduction. We found that co-ligation of BCR and CR2 significantly and dose-dependently inhibits the BCR induced CD69 expression, IL-6 secretion, proliferation, and both IgM and IgG production. The extent of inhibition was particularly noteworthy at low BCR stimuli. The CR2 induced inhibition is most probably mediated by the formation of CR1/CR2 complexes, as CR1 is a strong inhibitor of BCR induced B cell activation in humans. In conclusion, we demonstrate that CR2 is an inhibitory coreceptor for BCR in humans; therefore, C3d may serve as a molecular adjuvant of humoral immune response only in mice, but not in men.

Keywords: B lymphocytes, complement, molecular immunology

P-0184

Metabolic rewiring tunes dermal macrophages in staphylococcal skin infection

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The skin needs to balance tolerance to the colonizing microflora with high alert for the invasion of potential pathogens. Conceptionally, a flexible response machinery is required to accommodate the dynamic challenges of efficient antimicrobial defence and restoration of tissue homeostasis. Here, we dissected macrophage-intrinsic mechanisms and micro-environmental cues, which tune macrophage signalling in localized skin infection with the skin colonizer and opportunistic pathogen Staphylococcus aureus. We found that early in staphylococcal skin infection GM-CSF produced by γδ T-cells and hypoxia condition the dermal microenvironment, diverting macrophages away from the homeostatic M-CSF- and HIF1α dependent program. Thus, macrophages are metabolically rewired for a glycolytic response, mediated by GM-CSF, and at the same time upregulate the expression of Irg1 and generate itaconate. This multifactorial program of macrophage rewiring is required for both the timely clearance of bacteria, reconstitution of tissue homeostasis, and provision of antibacterial immune memory. In summary, during bacterial skin infection dermal macrophages receive complex exogenous and endogenous cues resulting in immunometabolic conditioning, and allowing for cycling between fierce antimicrobial activity, inflammation control and protection against secondary infections

Keywords: Bacterial infections, memory, innate host defence, macrophage, skin diseases

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POSTER PRESENTATIONS

P-0186

Thymoma associated myasthenia gravis patients have increased Th17 Cells, IL-17 production and ICOS expression on CD4 T cells

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Myasthenia gravis (MG) is an autoimmune disease mediated by autoantibodies mainly against the acetylcholine receptor (AChR). A small subgroup of MG is associated with thymoma and the patients reveal common features with the patients without thymoma. Based on the findings of circulating T follicular helper (cTfh) cells and cytokines in MG, the role of T helper cells has been assessed in thymoma associated MG (TAMG). Clinically diagnosed MG patients with AChR antibodies and pathologically proven thymoma (n: 19) were included. TAMG patients were also grouped according to immunosuppressive treatment as positive (n: 12) and negative (n: 7) and compared with patients without thymoma (n: 54) and age- and sex-matched healthy controls (HC, n: 38). Peripheral blood mononuclear cells were used for *ex vivo* intracellular cytokine measurements. Subsets of CD4+ T were determined phenotypically by the expression of the chemokine and costimulatory receptors. IL-21, IL-4, IL-17A and IL-10 productions of CD4+ T cells were increased, whereas IFN-γ was decreased in TAMG patients compared to HC. ICOS+ and PD-1+ populations in CD4+ T cell subgroups were also higher in TAMG patients despite decreased cTfh (CD4+CXCR5+) cells. Increased Th17/cTfh17 and decreased Th1/cTfh1 cells were detected in this subgroup. In TAMG, Th17 cells and IL-17 as well as ICOS+ CD4 T cells were higher than in the patients without thymoma as well. These findings revealed an activity in TAMG patients similar to MG patients without thymoma. However, increases of IL-17 and ICOS were more pronounced implicating the involvement of thymoma in this activity.

Keywords: Adaptive immunity, autoimmunity, cytokines and mediators

P-0187

Understanding the contribution of affinity vs specificity maturation in B cell self/foreign discrimination in a polyclonal immune repertoire

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B cells possess an exquisite capacity to generate antibodies that neutralize foreign antigens while avoiding binding to near identical self-antigens. Although processes of how germinal center B cells may facilitate this process in transgenic models have been described, the total antigen specific response to antigens closely mimicking a known self-antigen in a physiological polyclonal B cell repertoire has never been explored. In this study we have analyzed the germinal center, memory compartment and serum antibodies of mice immunized with foreign antigens differing from self by one to two amino acids. Germinal center B cells underwent single cell B Cell Receptor (BCR) sequencing and were expressed in their pre- and post-immune states. This revealed an exceptional capacity of B cells to differentiate near identical self-antigens from foreign, even prior to somatic hypermutation. This study forms an important basis towards understanding to how B cells can respond to antigens near identical to self in a physiological setting providing information that could help influence vaccine design.

Keywords: Adjuvants and vaccines, antibody, autoimmunity, immune response tracing

P-0189

Quantification of T-cell dynamics during latent cytomegalovirus infection in humans

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Cytomegalovirus (CMV)-infection has a major impact on the T-cell pool. This is generally ascribed to the presence of a large population of CMV-specific memory CD8 T-cells, suggested to be established through gradual accumulation of long-lived cells. It remains unclear whether the impact of CMV on the T-cell pool stretches beyond the presence of large numbers of CMV-specific T-cells, and whether CMV-infection changes the memory T-cell pool dynamics. We investigated the effect of CMV-infection on T-cell dynamics in healthy older adults, and aimed to unravel the mechanisms of maintenance of CMV-specific CD8 T-cells. We studied the expression of senescence, proliferation, and apoptosis markers and quantified the *in vivo* dynamics of CMV-specific and other T-cell populations using *in vivo* deuterium labelling. The increased expression of late-stage differentiation markers by CD8 T-cells of CMV+ versus CMV- individuals was not solely explained by the presence of CMV-specific CD8 T-cells. The expected lifespans of CMV-specific CD8 T-cells did not differ from those of bulk memory CD8 T-cells, neither from CMV- nor from CMV+ individuals. Memory CD4 T-cells of CMV+ individuals showed increased expression of late-stage differentiation markers, decreased Ki-67 expression, and a trend towards a longer T-cell lifespan compared to memory CD4 T-cells of CMV- individuals. Together, this work suggests that the impact of CMV-infection on the T-cell pool stretches beyond the presence of large CMV-specific CD8 T-cell populations, CMV-infection hardly affects the dynamics of the CD8 T-cell pool, and large numbers of CMV-specific CD8 T-cells are not due to longer lifespans of these cells.

Keywords: Adaptive immunity, ageing, immune senescence, infectious disease, memory, modelling

P-0190

Identification of new biomarkers in RRMS patients under natalizumab treatment to reduce progressive multifocal leukoencephalopathy risk

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Natalizumab (NTZ) administrated in extended interval dose (EID) reduces CD49d receptor occupancy levels (RO) without compromising treatment effectiveness. Moreover, EID schedule suggests lower risk of Progressive Multifocal Leukoencephalopathy (PML) compared with standard dose (SD) in patients with anti-JCV antibodies. To identify specific biomarkers in Relapsing-Remitting Multiple Sclerosis (RRMS) patients under NTZ to establish a personalised dose, reducing PML risk. Ongoing transversal study in 29 RRMS patients under NTZ (SD/EID patients with clinical/radiological activity, and EID patients without activity). Peripheral blood samples are analysed by flow cytometry to determine CD49d-RO in CD4, CD8 and B cells, and the expression of CD49d, CD29 and β7-integrin in minor T and B subpopulations. Each patient is analysed at two timepoints separated by 3 NTZ administrations. Other biomarkers in serum will be also analysed. Of 29 patients, 11 were SD(active) and 18 EID(2 active and 16 inactive). In either SD and EID, no significant differences were observed over time neither for CD49d and β7-integrin expression nor for CD49d-RO. SD patients showed higher CD49d-RO in T and B cells. CD49d expression percentage was decreased in CD8+CD27+ and B cell subsets(Switched, Naïve and Double-negative) in SD compared with EID inactive patients. Regarding β7-integrin, its percentage was increased in CD4+ Central Memory and decreased in CD4+ Naïve in SD patients. CD49d and β7-integrin expression, and CD49d-RO, are stable markers that can be used in NTZ treatment immunomonitoring. Our preliminary results suggest that changes in these subpopulations are related to the dose schedule more than to the disease activity.

Keywords: Autoimmunity, biomarkers, immunotherapy

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POSTER PRESENTATIONS

P-0191

β2-integrins and their roles in receptor recycling and migration in human myeloid cells

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The β_2-integrin family consists of four heterodimeric receptors: LFA-1 (CD11a/CD18), CR3 (CD11b/CD18), CR4 (CD11c/CD18) and CD11d/CD18. Among these receptors CR3 and CR4 show the highest structural homology in their extracellular domains, that led to the notion of their overlapping functions. However the substantial differences in their intracellular tails, that engage in signalling and actin binding, enable their functional divergence. Our group previously proved a division of labour between CR3 and CR4 in phagocytosis and adhesion (Sándor *et al.*, 2016; Lukácsi *et al.*, 2017). The aim of this study was to characterize the role of β_2-integrins in migration and the endosomal recycling necessary for this function. The migratory capacity of macrophages and dendritic cells was measured by transwell assay under both physiological and LPS-induced inflammatory conditions. The effect of LPS on the recycling of LFA-1, CR3 and CR4 was monitored at different time points by flow cytometry and confocal microscopy. The rate of protein degradation was also assessed by confocal microscopy via the evaluation of colocalization between internalized receptors and lysosomes. Our results prove that both CR3 and CR4 participate in the migration of human macrophages and dendritic cells. In support of this function, both showed a fast recycling rate that was significantly enhanced by LPS-activation. In contrast, the internalization of LFA-1 was not followed by the rapid secretion of intracellular receptors, not even in the presence of LPS. The low colocalization rate found between intracellular receptors and lysosomes strengthens our suggestion that cells favour the recycling of receptors to degradation.

Keywords: Complement, dendritic cells, innate immunity, macrophage, myeloid cells

P-0192

Ouabain triggers neutrophil extracellular traps in human neutrophils

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Ouabain is a cardiotonic steroid hormone able to modulate immune system functions, including the inflammatory process. We have demonstrated that ouabain impaired neutrophil recruitment induced by different stimuli. Thus, in this work, we evaluated if ouabain was able to interfere with neutrophil extracellular traps release (NET), an effector function of neutrophils. For that, neutrophils were isolated from human peripheral blood, treated with ouabain (1, 10, and 100 nM) for 20 minutes or 2 hours. PMA (100 nM) was used as a positive control. Additionally, the LDH assay was performed to assess neutrophil viability. RESULTS and DISCUSSION: Treatment with ouabain (100 nM) for 2 hours promoted a 2-fold increase in DNA release measured by Quanti-it™ PicoGreen® when compared to untreated neutrophils. Additionally, ouabain (100 nM) did not increase LDH levels, showing that cells were not necrotic, and suggesting that DNA detected in the ouabain-treated neutrophils were NETs. Several signaling pathways may be involved in this effect, such as increased cytoplasmic Ca2+ concentration, PKC activation, increased ROS production, and Src activation. These stated signaling pathways were previous observed in other cell types after ouabain stimulus. Thus, this effect of ouabain in the release of NETs corroborates its role as a modulator of inflammation. Our findings highlight the importance to better characterize the NET release mechanism and since NETs can sequester and degrade pro-inflammatory mediators determine their pro- or anti-inflammatory properties mediated by ouabain.

Keywords: Immunopharmacology, innate immunity, neutrophils

P-0193

Immunogenic factors in periapical lesions: an analysis in two inbred strain of rats

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To investigate the influence of strain differences in immune responses on the pathogenesis of experimental periapical lesions in Dark Agouti (DA) and Albino Oxford (AO) inbred strains of rats. Periapical lesions were induced in male DA and AO rats by pulp exposure to the oral environment. The mandibles were analyzed radiographically, histologically, immunohistochemically, by real-time PCR and flow-cytometry. Blood samples and periapical lesions supernatant were analyzed for cytokines and oxidative stress marker levels. Statistical analysis was performed by the Mann-Whitney U test. A significant difference was considered when p values were <.05. DA rats developed significantly larger periapical lesions. In DA rats, periapical lesions had a significantly higher percentage of CD3+ cells compared to AO rats. Also, the percentage of INF-γ, IL-17, and IL-10 CD3+CD4+ cells was found significantly higher in DA (p<.05). RT-PCR expression of IL-1β, INF-γ, and IL-17 genes were significantly higher in periapical lesions of DA compared to AO rats (p<.05). The RANKL/OPG ratio was higher in DA compared to AO rats with periapical lesions (p<.05). Systemic levels of TNF-α and IL-6 were significantly higher in DA compared to AO rats (p<.05). Levels of lipid peroxidation measured as thiobarbituric acid reactive substances and reduced glutathione were higher in the supernatant of periapical lesions of DA rats. After pulp exposure Dark Agouti has developed much greater periapical lesions compared to Albino Oxford rats. Genetically determined differences in immunopathology have been demonstrated to be a significant element defining the severity of periapical lesions.

Keywords: Animal models, cytokines and mediators, immune development, inflammatory disease

P-0195

Adipose tissue mesenchymal stem cells-derived exosomes improve regulatory T-cell frequency in COVID-19 patients PBMCs

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After the COVID-19 virus epidemic, many studies were conducted to find the role of immune system cells in this disease. One of these cells is Treg, which is important due to its role in regulating immune system responses' homeostasis. An imbalance occurs in Treg/Th17 cells' ratio during the pathogenesis of COVID-19 and shifts to Th17, resulting in uncontrolled inflammatory responses. Therefore, it can be beneficial to study the factors that can increase Treg responses and differentiation. Since adipose tissue-derived mesenchymal stem cell (AD-MSCs) exosomes have immunomodulatory properties, the use of these vesicles can be an excellent option to reduce inflammatory responses and induce anti-inflammatory responses in COVID-19 peripheral blood mononuclear cells. In the present study, we isolated AD-MSCs exosomes by ultracentrifugation and investigated their immunomodulation effect on peripheral blood mononuclear cells (PBMCs) of covid-19 patients. For this purpose, after culturing the PBMCs with MSCs exosomes, the frequency of Treg lymphocytes was assessed by flow cytometry. PBMCs were washed and incubated with FITC-conjugated anti-CD4, PE-conjugated anti-CD25, and PE-cy7-conjugated anti-CD127 monoclonal antibodies isotype-matched IgG controls before staining. The exosomes were by and characterized by SEM, TEM, and DLS. A significant reduction was observed in the proportion of peripheral Treg cell frequency in COVID-19 patient PBMCs. Our data revealed that the ratio of CD4+ CD25+ CD127− Treg was considerably influenced by MSCs exosomes in all the patients. Current study results indicated that AD-MSCs derived exosomes can restore the frequency of Treg cells of COVID-19 patient isolated PBMCs.

Keywords: Adaptive immunity, infectious disease, stem cells

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POSTER PRESENTATIONS

P-0197

LQB 118 reduces peritoneal inflammation by down-regulation p38 MAPK pathway

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LQB 118 is a synthetic hybrid molecule resulting from the union of two bioactive natural molecule groups, pterocarpans and naphthoquinones. Several biological activities have been reported to LQB 118, including anticancer and antileishmanial effects. Recently, the anti-inflammatory effect of this molecule was demonstrated by our group in a zymosan- induced peritonitis model, in which cell migration impairment to the inflamed site was observed. Thus, the aim of this study was to evaluate LQB 118 effect on the pro-inflammatory cytokines levels and p38 MAPK phosphorylation. Briefly, female Swiss mice were treated intraperitoneally (i.p.) with LQB 118 (10 mg/kg or 20 mg/kg) and zymosan (2 mg/mL) was used to induce peritoneal inflammation. After four hours, peritoneal lavage was collected using cold phosphate-buffered saline (PBS) and centrifuged. Supernatants were separated to cytokines analyses by immunoenzymatic assay (ELISA) and cells were used to evaluated P-p38 MAPK expression by flow cytometer. LQB 118 treatment reduced TNF-α, IL-1β, and IL-6 levels in the peritoneal cavity. Additionally, this substance downregulated p38 MAPK phosphorylation. Taken together, our results suggested LQB 118 treatment reduces pro-inflammatory cytokines levels in the peritoneal cavity by reducing p38 MAPK phosphorylation. Furthermore, these data evidence the potential of LQB 118 as a promising anti-inflammatory substance.

Keywords: Cytokines and mediators, immunopharmacology, innate immunity

P-0198

Expression of CD9 on porcine T and B cells and its relation to their differentiation and cytokine production

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The tetraspanin CD9 has immunological relevance due to its roles in orchestrating the immune synapse, leukocyte migration and activation. Novel monoclonal antibodies specific for porcine CD9 were generated. We analysed the frequency of $CD9^+$ cells within CD4 T-cell populations according to their differentiation status. Naïve T cells contained the least $CD9^+$ cells, whilst CD9 expression was most frequent in central memory cells. We noted that the frequency of $CD9^+$ T cells in the blood positively correlates with the frequency of $CD8\alpha^+CD27^+$ central memory CD4 T cells. Further, it could be demonstrated that long-lived IFNγ and TNFα competent cells which responded to *in vitro* recall antigen from influenza A virus were in their vast majority $CD9^+$. These data suggest that in pigs CD9 expression associates with the presence of long lived central memory CD4 T cells and that this molecule might be exploited to monitor the development of CD4 T-cell immunological memory. We also investigated the utility of our new anti-CD9 antibodies by analysing CD9 expression upon other immune cells. We found that CD9 was most frequently expressed on $FoxP3^+$ cells of the lung. However, there was no association with CD9 expression and $IL10^+CD25\uparrow$ cells. Considering subsets of murine IL-10 producing B cells that express CD9 have been identified, we also investigated whether there is an enrichment of IL-10 producing cells within porcine $CD9^+$ B cells, from healthy pigs, but found no correlation. Nevertheless, CD9 expression may be helpful in the further characterisation of $CD21^-IgM^+CD79a^+$ porcine B1 B cells.

Keywords: Adaptive immunity, antibody, memory, veterinary immunology

P-0199

Regulation of B-1 cell trafficking through the C5a/C5aR1 axis

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B-1 lymphocytes mediate broad antibody-based immune responses against several types of infections. With the constitutive production of natural IgM antibodies (nIgM) B-1 lymphocytes play an important role in innate immunity and contribute as well as the complement system to a rapid first-line defense against invading pathogens. Interestingly, the first receptor for C5a (C5aR1) seems to play a pivotal role in B-1 cell regulation as B-1 cell numbers in the peritoneal cavity of C5aR1-deficient mice are significantly decreased compared to their wildtype counterpart. The decreased number of peritoneal B1 cells in C5aR1-deficient mice was associated with increased numbers of B1 cells in the spleen and elevated serum concentrations of nIgM against PC and pneumococcal polysaccharides. Since homing of B-1 lymphocytes to their reservoirs under steady-state conditions is important for their function, we want to examine the importance of C5aR1 expression for B-1 lymphocyte homing. Therefore, we conduct an adoptive transfer assay injecting CFSE-labeled peritoneal cells from wildtype or C5aR1-deficient mice into wildtype or C5aR1-deficient recipient mice. 24h later samples are taken and surface antigens are stained with specific antibodies to track labeled B-1 cells using flow cytometry. With our results we aim to understand the mechanisms behind the role of C5a in B-1 lymphocyte biology further. In the next steps we want to assess underlying signaling pathways by using western blot analysis.

Keywords: Antibody, B lymphocytes, complement

P-0200

Saving animals and stress while optimizing output in the development of monoclonal antibodies

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Since the original invention by Köhler and Milstein multiple protocols for hybridoma generation have been developed. Even though monoclonal antibodies are essential tools for research and clinic, the use of animals draws more and more criticism and red tape for the respective scientist. Here we report on a protocol keeping possible stresses and strains for the animals at a minimum while at the same time allows for an uttermost time saving development of the needed binders. Animals are immunized only twice. Up to six different antigens are administered at the same time. While peptides administered are coupled to a carrier protein, the identification of hybridoma is based on the respective peptides coupled via spacer to biotin. This allows for efficient presentation via avidin in ELISA. Moreover, analyzing for several antigens simultaneously generates valuable additional data for the rapid selection of useful hybridoma and separation of the respective specificities. Using this protocol a multitude of highly specific monoclonal antibodies have been developed. Nowadays scientist who work in basic research for the benefit of future generations find themselves in terms of animal legislature and the need for highly specific binders between a rock and a hard place. The protocol described and thoroughly optimized in many years of practical work enables scientists to join forces in the generation of monoclonal antibodies. It minimizes stress and strains for animals as well as the administrative burden nowadays connected to the development of monoclonal antibodies.

Keywords: Antibody, B lymphocytes, immunological techniques

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POSTER PRESENTATIONS

P-0201

Characterization of peritoneal macrophages from Mucin-2 knockout mice

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Mucin2-deficient (Muc2-/-) mice is a model of inflammatory bowel disease. Mucin deficiency in the gut epithelial cells promotes direct contact with gut microbiota and results in gut inflammation, elevation of colon macrophages number and thickening of the intestinal wall. The aim of this study was to investigate phenotype, metabolic and functional activity of intact peritoneal macrophages from Muc2-/- mice. Muc2+/+ (n=9) (control) and Muc2-/- (n=11) female 8-12 weeks old mice were purchased from the Scientific Research Institute of Neurosciences and Medicine (Novosibirsk). Mice were sacrificed, peritoneal cells were collected and preplated in DMEM media, supplemented with 10% FCS (Gibco) during 1 hour. Adherent cells were used for further experiments. We used F4/80, CD80, CD38, CD209 antibodies (BioLegend) to phenotype macrophages. Phagocytosis was assessed by flow cytometry after incubation of macrophages with fluorescent latex beads (Sygma) during 1 hour. Cells were analyzed by FACSCantoII cytometer. Metabolic activity was evaluated in MitoStressTest Seahorse XF (Agilent). The percentage of CD80+ macrophages was more than two-fold higher, while CD209 expression was decreased both on CD80+ and CD80- cells in Muc2-/- mice compared to control. There was no significant difference in phagocytic activity between Muc2-/- and Muc2+/+ macrophages. Total CD38 expression was significantly higher in Muc2-/- compared to Muc2+/+ macrophages, and only in Muc2-/- it was detected in intracellular form. Oxygen consumption rate in Muc2-/- macrophages was increased both in basal and stress conditions. Muc2-/- peritoneal macrophages demonstrate pro-inflammatory phenotype suggesting systemic shift to M1 polarization.

Supported by RSF project 20-64-47020

Keywords: Animal models, inflammatory bowel disease, macrophage

P-0202

Exosomes derived from adipose tissue mesenchymal stem cells decrease TH-17 frequency in COVID-19 patients

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After the outbreak of the Covid-19 pandemic, much attention was paid to the responses of immune system cells and their role in the pathogenesis of the disease caused by the SARS-COV-2 viral infection. Impaired balance of the ratio of regulatory T cells to TH-17 is one of the main reasons related to the immune system in the pathogenesis of this disease. Recently, the use of MSCs in treating COVID-19 disease has improved long-term pulmonary function. Still, the specific mechanisms by which MSCs derived exosomes inhibit the severe inflammatory response induced by SARS-CoV-2 have not been elucidated. In this study, we first took 5 ccs of blood from people diagnosed with covid-19. Then PBMCs were isolated from these patients' blood. MSCs isolated from adipose tissue is starved for 3 days to isolate exosomes. Exosomes were then separated from the supernatant by ultracentrifugation. Exosomes were characterized by SEM, TEM, and DLS. PBMCs were then co-cultured these exosomes, and the frequency of TH-17 cells was assessed by flow cytometry before and after treatment by intracellular IL-17A staining. A significant increase was observed in the proportion of peripheral TH-17 cell frequency (p=0.029) in COVID-19 patient PBMCs compared to healthy individuals. Our data revealed that MSCs exosomes considerably influenced the ratio of intracellular IL-17A expressing TH-17 in all the patients. The current study results indicated that AD-MSCs derived exosomes can decrease the frequency of TH-17 cells of COVID-19 patient isolated PBMCs.

Keywords: Adaptive immunity, cytokines and mediators, immune response tracing, infectious disease

P-0204

How new generation antibiotics influence the oxidative burst of neutrophilic granulocytes

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Neutrophilic granulocytes represent the first line of defense against microorganisms by specifically synthesizing and directing oxygen radicals against invaders, thereby highly efficiently killing them. To evaluate new-generation antibiotics for their potential influence on the respiratory burst of neutrophilic granulocytes stimulated by ligation of surface receptors or pharmacological agents that trigger granulocyte signaling. Neutrophilic granulocytes were isolated from peripheral blood of healthy volunteer donors. To evaluate the interaction of antibiotics with neutrophil function, Amplex-red based plate assays and flow cytometry were used along with a collection of granulocyte-stimulating agents. We identified two new-generation antibiotics, the glycopeptide antibiotics dalbavancin and teicoplanin, that inhibited ROS production after granulocyte activation via different signaling pathways in a dose-dependent manner, as well as CD62L shedding after stimulation with PMA. In contrast, the oxazolidinone antibiotics tedizolid and linezolid had no effect on neutrophil function. In addition, we revealed that the combination of ceftazidime/avibactam dose-dependently inhibited the fMLP and cytochalasin B-induced granulocyte burst in a dose-dependent manner. Our results provide important information on how the oxidative burst of neutrophilic granulocytes is affected by novel antibiotics and will help to better assess neutrophil function in clinical practice. In addition, our finding that ceftazidime/avibactam inhibits the respiratory burst will help identify how cytochalasin B contributes to the priming of neutrophilic granulocyte.

This work was supported by the Medical University of Vienna

Keywords: Granulocytes, innate immunity, neutrophils

P-0209

The allergy-protective farm effect revisited: Beta-lactoglobulin induces a Th1-response via lipocalin-interacting-membrane-receptor (LIMR) on innate immune cells

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We have shown previously that the loading status of the whey protein beta-lactoglobulin (BLG) determines the *in vitro* and *in vivo* immune response. In stable dust and ambient air of cattle farms, we found BLG associated with zinc. Here, we investigated the effect of zinc-associated BLG on the cellular immune response, as well as the potential mechanism of action of BLG *per se* and BLG in its cognate matrix milk. PBMCs of healthy donors were incubated with zinc-BLG or BLG depleted of zinc (apo-BLG). The subsequent proliferation and cytokine production were determined by flow cytometry. The expression of the potential receptor for BLG, lipocalin-interacting-membrane-receptor (LIMR), was examined on PBMC subsets. Effects of BLG alone and BLG in different milk samples on LIMR-expression were investigated on THP-1 cells. PBMCs stimulated with zinc-BLG showed lower proliferation and lower release of Th2-cytokines, but higher IFN-γ release than with apo-BLG. LIMR was exclusively expressed on CD3-CD14+ monocytes and CD3-CD14-CD19- NK-cells, with highest expression on CD14dim monocytes and CD56dim NK-cells. Interaction of LIMR+ THP-1 cells was shown with FITC-labelled BLG. Incubation with raw milk, but not lactose-free or whole milk, decreased LIMR-expression on THP-1 in a concentration-dependent manner in preliminary experiments. Zinc-BLG reduced proliferation of PBMCs *in vitro* and favoured a Th1-cytokine milieu. The presumed BLG-receptor LIMR is expressed on innate NK- and monocytic cells. LIMR+ THP-1 cells showed interaction with BLG and raw milk. Our study therefore suggests that zinc-BLG contributes in an antigen-independent way to the allergy-protective farm effect.

Keywords: Allergic disorders, cytokines and mediators, environmental factors in autoimmunity and allergy, immune regulation and therapy, NK cells, protection

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POSTER PRESENTATIONS

P-0211

Chemokines and chemokine receptors in distinct clinical forms of human tegumentary leishmaniasis

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The aim of this work was to study the participation of CCL20 and CCL17, chemokines directed to skin and mucosal tissues in cutaneous (CL) and mucosal (ML) leishmaniasis. We processed peripheral blood samples from patients with CL (n=20), ML (n=15) and healthy subjects (HS, n=10). Plasma levels were measured by ELISA. We evaluated the chemokine receptors CCR4 and CCR6 on CD45RO+ CD4+ and CD8+ T cells by flow cytometry. Also, PBMC cultures were performed (1x106 cells/ml,7 days) in presence of (L. braziliensis) or (L. amazonensis) soluble antigens (20ug/ml). Single nucleotide polymorphisms (SNP) for CCL17 and CCL20 were analyzed by RLP-PCR and gene fragment digestion. Higher plasma levels of CCL20 (p=0.0017) and diminished values of CCL17 (p=0.0023) were found in ML (p=0.0017), indicating that this clinical form presents altered chemokine patterns. In (*in vitro*) assays, proteins of (L. amazonensis) inhibited the supernatant (SN) production of CCL17 (p=0.0085) in ML, while (L. braziliensis) slightly increased the SN production of CCL20 in CL (p=0.038) and ML (p=0.031).We suggest that (Leishmania) spp. could influence the degree of chemokine responses. The number of CCR4+CCR6+ T cells was similar between groupswith the majority of them expressing CD45RO+. Analyzing SNP, the most frequent genotype for CCL17 was CT (CL, 72%; ML, 44%; HS, 80%), while TT genotype predominated for CCL20 (CL,45%; ML,87%; HS,60%). This represents a preliminary approach for chemokine polymorphisms in leishmaniasis. We need further research to determine their correlation with plasma levels and pathology outcome.

Keywords: Inflammatory disease, memory, parasite infections, regulatory cells

P-0212

PI3K inhibition reveals a novel gamma delta T cell-associated transcriptional program during thymic development

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γδ T cells play important roles in immunity against pathogens and tumours which correlate with secretion of cytokines, such as IFNγ and IL-17A. These effector functions are acquired by γδ T cells in the thymus during development. TCRγδ signalling plays a pivotal role in determining the effector fate of developing γδ thymocytes, such that, a strong TCR-mediated signal favours the development of IFNγ-committed (γδIFN) cells, whereas, a weaker signal promotes the development of IL-17-committed γδ (γδ17) T cells. The aim of this study was to understand the underlying mechanisms that determine these effector fates. Specifically, the role of PI3K signalling in the development of γδ T cells. We investigated the role of PI3K signalling in the generation of specific γδ T cell subsets by using PI3Kδ-deficient mice and foetal thymic organ culture (FTOC) where signalling pathways were manipulated by small molecule inhibitors. We used both flow cytometry and single cell RNA sequencing to assess early γδ T cell development and adoption of specific effector potential. Our data demonstrate that weaker TCRγδ-mediated signalling, that promotes the generation of γδ17 cells, is manifest by constrained activity of the TCR-proximal kinase Syk. This, in turn, engages the PI3K/AKT pathway that is necessary for the maintenance of key IL-17-associated master regulators RORγt and c-Maf. We found that perturbation of the PI3K signalling pathway prohibited development of γδ17 cells, but permitted γδ progenitors to adopt a type I IFN gene signature that led to the identification of a novel CD8β+ Ly6a+ γδ T cell subset.

Keywords: Cell signalling, gamma-delta T cells, immune development, RNAseq, thymic selection

P-0213

Histone deacetylase 1 controls CD4+ T cell trafficking in autoinflammatory disease

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Gene expression profiles associated with T lymphocyte subset differentiation and acquisition of effector function are in part regulated and maintained by epigenetic processes. Histone deacetylases (HDACs) and histone acetyltransferases (HATs) are key epigenetic regulators, which mediate dynamic changes in the acetylation status of histones at lysine residues, therefore favoring a closed or open chromatin structure, respectively. Moreover, HDACs and HATs have also non-histone targets and changes in acetylation of these target proteins affect their functional properties in many ways (e.g. intracellular localization, stability, activity). We have shown that the class I member HDAC1 in T cells is key for the induction of experimental autoimmune encephalomyelitis (EAE) in mice and uncovered a novel role of HDAC1 in controlling $CD4^+$ T cell migration and trafficking. HDAC1-deficient $CD4^+$ T cells downregulated genes associated with leukocyte extravasation. *In vitro*, HDAC1-deficient $CD4^+$ T cells displayed aberrant morphology and migration on surfaces coated with integrin LFA-1 ligand ICAM-1 and showed an impaired ability to arrest on and to migrate across a monolayer of primary mouse brain microvascular endothelial cells under physiological flow. Moreover, HDAC1 deficiency reduced homing of $CD4^+$ T cells into the intestinal epithelium and lamina propria preventing weight loss, crypt damage and intestinal inflammation in a model adoptive transfer colitis. Thus, our data reveal that HDAC1 controls T cell-mediated autoimmunity via the regulation of $CD4^+$ T cell trafficking.

Keywords: Adaptive immunity, autoimmunity, epigenetic control and modulation of immunity

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POSTER PRESENTATIONS

P-0214

Antibody responses to human rhinovirus induced by different vaccine platforms

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Human Rhinoviruses are small viruses that cause the majority of common colds and have a huge impact on human health. Currently there is no effective vaccine against these viruses which is largely due to the antigenic diversity amongst approximately 160 serotypes. However, studies have shown that antibodies are able to offer some cross-serotype protection, despite sequence variations in the capsid proteins VP1, VP2, VP3 which form the surface and can vary considerably. Antibodies are known to neutralise rhinovirus through four immunogenic epitopes on these capsid proteins and these regions correspond to the most variable exposed regions. The aim of this research is to investigate the options for a rhinovirus vaccine that induces protective cross-serotype antibody responses to more conserved regions of the capsid. Three immunisation strategies of mice were performed using rhinovirus A16; 1) recombinant subunit protein VP0, 2) inactivated whole virus and 3) mRNA encoding capsid proteins. Antibodies generated were determined for specificity and binding diversity by ELISA and for neutralising activity by plaque reduction assay. VP0 immunisation induced antibodies to rhinovirus A16, recombinant capsid proteins and specific peptides whereas inactivated rhinovirus A16 and mRNA immunisations induced antibodies to rhinovirus A16 but not to recombinant capsid proteins or peptides. Cross reactive binding antibodies to rhinovirus 1B were observed with inactivated virus immunisations only but neutralising activity was not detectable. These immunisation strategies induced specific but non-neutralising antibodies to a variety of rhinovirus A16 peptides and proteins that map to diverse regions of the rhinovirus capsid.

Keywords: Adjuvants and vaccines, animal models, antibody, antigen processing and presentation

P-0215

Defining the role of BANK1 in Age-associated B cells in autoimmunity

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Systemic Lupus Erythematosus (SLE) is characterized by the hyperactivity of B cells and the production of autoantibodies. Previous studies in our group described the genetic association between SLE and the B cell-specific gene BANK1. The role of BANK1 remains unclear. Bank1 deficiency ameliorates lupus phenotype. It also reduces numbers of extrafollicular T helper cells (Tefh), which are involved in autoantibody production. A B cell subpopulation rare in normal mice named Age-associated B cells (ABC) has been implicated in autoimmunity, is thought to be driven by TLR7 signaling, secretes autoantibodies and increases with age. The main objective of this study was to determine the effect of Bank1 deficiency on ABCs in an SLE model and the relationship of ABCs with Tefh. This project aims to define which cells are producing proinflammatory cytokines and autoantibodies and what is the role of BANK1 in this process. In order to do that, we worked with two murine lupus models crossed with the knockout for Bank1: a transgenic model of the TLR7 gene and a TLR7 pathway-induced disease model with Imiquimod. The results showed that Bank1 deficiency decreases the total percentage of ABCs and Tefh with a positive correlation between them. The lack of Bank1 also ameliorates signs of autoimmunity such as splenomegaly and the production of autoantibodies. Besides, it restores to normal the cellular phenotypes in the spleen. Future experiments will be focused on understanding the role of Bank1 on the generation and differentiation of ABCs by *in vitro* differentiation and single-cell transcriptome assays.

Keywords: Autoimmunity, B lymphocytes, cell signalling, cytokines and mediators

P-0216

A bidirectional crosstalk between cDC1s and cytotoxic CD8 T-cells and NK-cells is responsible for their privileged interactions

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Preclinical data has shown that a specific dendritic cell (DC) subset, known as conventional DC type 1 (cDC1), is involved in the induction T-cell infiltration and increased cytotoxic T-cell (CD8+ T-cells) numbers in tumors. NK-cells are one of the first immune cells to infiltrate the tumor and produce chemokines that contribute to cDC1 accumulation and therefore maintaining the positive feedback loop of cytotoxic T-cells infiltration. Tumor-infiltrating cytotoxic T-cells have been shown to be directly related to a better prognosis in many cancers, such as melanoma. However, in humans, cDC1 are rare; a deep *in vitro* characterization by which human cDC1 are attracted by T-cells and NK-cells and vice versa needs to be studied in order to improve the influx of these cells in cancer patients. Our results show that there is a bidirectional cross-talk between cDC1s and CD8+ T-cells and NK-cells. In contrast to the other DCs subsets (i) immature cDC1s uniquely express chemokine receptor XCR1, which is responsible for a specific migration towards chemokine ligand XCL1-secreting activated NK-cells and CD8+ T-cells and (ii) via higher secretion of Chemokine (C-X-C motif) ligand 9 and 11 (CXCL9 and CXCL11), mature cDC1s are able to attract NK-cells and CD8+ T-cells via chemokine receptor type 3 (CXCR3). Altogether, our *in vitro* data suggests that cDC1 play a key role in keeping the positive loop of CD8+ T-cells and NK-cells interaction/crosstalk.

Keywords: Cancer immunology, cellular interactions, chemokines, dendritic cells, immunotherapy, NK cells

P-0217

Early diversification during chronic infection generates exhaustion-receptive and -resistant T cell subsets

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CD8 T cells responding to chronic viral infection acquire an exhausted state that critically limits their effector function, but also prevents immunopathology. It has recently been proposed that exhaustion first emerges in TCF1+ memory precursors (MPs) that later generate exhausted TCF1– effector subsets. However, single-T-cell fate mapping upon infection with acute vs. chronic strains of lymphocytic choriomeningitis virus (Armstrong vs. Clone-13), indicates that selective exhaustion of MPs should be insufficient to curtail the emergence of highly cytolytic TCF1–KLRG1+ terminal effectors (TEs). In fact, single-cell RNA sequencing and early adoptive re-transfers reveal that the bulk of TEs derive from TCF1–KLRG1– early effectors (EE) that have already separated from MPs before the onset of exhaustion. While both MPs and EEs are receptive to T cell exhaustion, mature TEs, generated during Armstrong infection, fail to upregulate the exhaustion-inducing transcription factor TOX and retain antiviral effector functions even when exposed to Clone-13.

Keywords: Adaptive immunity, immune response tracing, infectious disease, memory

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POSTER PRESENTATIONS

P-0219

T-cell dynamics in a more natural mouse model

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Memory T-cells in tissues play a major role, not only in protection against re-infection but also in autoimmune disease. Because most research is based on T-cells in the circulation, i.e. cells isolated from human blood or mouse lymphoid tissues, it remains unclear how these cells maintain themselves in tissues. Are they maintained by self-renewal, by cellular longevity or by input from another source? Obtaining such insights will help the development of potential treatments for diseases like rheumatoid arthritis. SPF mice cannot be used to study the maintenance of these cells, as they mostly lack antigen-experienced T-cells in non-lymphoid tissue. To overcome this shortcoming, we studied T-cell maintenancein wildling mice. These C57BL/6 mice born to wild mice have a natural microbiome, which shifts their naïve immune system to a more natural situation with antigen-experienced T-cells present in tissues. Using in-vivo deuterium labelling, we estimated the lifespan of T-cells in the spleen, lymph-nodes, bone-marrow and lungs of wildlings. While the dynamics of memory T-cells in most tissues were comparable, we found that $CD4^+$ memory T-cells in the lungs of wildlings are longer-lived than those in lymphoid tissues. We also found that $CD4^+$ and $CD8^+$ memory T-cells in lymphoid tissues of wildlings are shorter-lived than in SPF mice, while naive T-cells have similar dynamics in both models. Natural microbial exposure thus not only shapes the immune phenotype but also the dynamics of immune cells, and should be taken into account when studying the mechanisms of memory T-cell maintenance in an animal model.

Keywords: Adaptive immunity, animal models, memory, microbiome and environmental factors, modelling

P-0222

Role and mechanisms of c-MAF in B cells

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The importance of B cells in the immune system is now well established. Among the various actors of the immune system, B lymphocytes are known for their production of antibodies, their memory function. However, the existence of another subcategory of B cells showing regulatory and suppressive functions has been demonstrated in literature. Regulatory B cells have very heterogeneous profiles (plasma cells, plasmablasts, immature B cells) which makes them difficult to characterize. For the moment, there is only the real function which permits to identify them. Moreover, the differentiation pathway by which B cells become regulatory cells is not clearly understood. Actually, there are no molecular factors, like FoxP3 in regulatory T cells to clearly identify regulatory B cells. To better understand differentiation pathways between regulatory and pro-inflammatory B cells, the team has set up a co-culture mechanism to produce regulatory or pro-inflammatory B cells. A comparative RNA-seq analysis of this two distinct B cells has highlighted a transcription factor, c-MAF that could be implicated in the regulatory differentiation of B cells. C-MAF is a transcription factor of the MAF family with a basic leucin zipper domain, known to be implicated in different physiologic process as immune system differentiation. Actually, only one study links c-Maf to IL10 production in murine LBs. No other B studies about c-Maf have been published. Its role in the B cells remains currently unknown. The aim of this project is to identify the role and mechanism of c-MAF in B cells, especially in regulatory B cells.

Keywords: B lymphocytes, biology of the immune system, regulatory cells, RNAseq

P-0223

Diversification of antibodies by gene conversion in the domestic chicken (gallus gallus domesticus)

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Sustainable modern poultry production depends on effective protection against infectious disease and a diverse range of antibodies is key for an effective immune response. In the domestic chicken, somatic gene conversion is the dominant process in which the antibody immunoglobulin genes are diversified. Despite this, little is known of how this mechanism directly alters the sequence of the variable gene. This study aims to use high throughput sequencing to generate and analyse immunoglobulin variable genes to determine how the gene is altered during B cell diversification. Immunoglobulin genes obtained from several immune-associated tissues from six 3-week old VALO SPF white leghorn chickens were sequenced using Pac Bio NGS, resulting in the generation of almost 350,000 sequences. Analysis of putative somatic gene conversion events in the variable region revealed restricted patterns of genetic insertions in both the antibody heavy and light chains. These patterns were homologous with the locations of genetic variability in available pseudogenes. This coupled with biased usage of a limited number of pseudogenes during gene conversion, as well as gene preferences identified during diversity gene selection in VDJ gene rearrangement, suggests that chickens are not taking full advantage of the available genetic diversity during antibody diversification.

Keywords: Adaptive immunity, antibody, B lymphocytes

P-0225

Comparison of Janus kinase and phosphodiesterase 4 inhibition in terms of antiviral responses *in vitro*

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The Janus kinase (JAK) inhibitor tofacitinib and the phosphodiesterase 4 (PDE4) inhibitor apremilast are approved for the treatment of psoriatic arthritis. Both therapies have been associated with nasopharyngitis, and upper respiratory tract infections. Increased incidences of zoster have only been observed under tofacitinib treatment. JAKs are found downstream of the type II cytokine receptor used by Th17 cell-associated cytokines for signal transduction. Inhibition of PDE4 leads to accumulation of intracellular cAMP, and subsequent activation of protein kinase A followed by a reduction of a variety of innate and adaptive immune responses including a lower expression of Th17 cell-associated cytokines. These cytokines induce the secretion of antiviral and antimicrobial peptides by keratinocytes. Primary human keratinocytes were treated with tofacitinib or apremilast and various cytokines and/or bacterial surface proteins and analysed by RT-qPCR. CD69 expression on tofacitinib or apremilast-treated PBMCs was investigated via flow cytometry. We found that in contrast to apremilast, tofacitinib markedly reduced the gene expression of antiviral peptides such as MX1 or ISG15 in keratinocytes *in vitro*. Additionally, JAK inhibition but not PDE4 inhibition reduced the activation of T cells stimulated with viral VZV gE. Using both substances we did not observed significant effects on antimicrobial responses. Concluding, we report that tofacitinib reduced the expression of antiviral peptides as well as the activation of T cells by viral antigens *in vitro* while apremilast did not significantly influence the antiviral immunity. These results are in line with the clinical observation of increased numbers for zoster in patients treated with tofacitinib.

Keywords: Inflammatory joint diseases, bacterial infections, cytokines and mediators, innate immunity, skin diseases, viral infections

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POSTER PRESENTATIONS

P-0226

Prostaglandin E2 differentially regulate the suppressive mechanisms in myeloid derived suppressor cells subsets *in vitro*

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Myeloid derived suppressor cells (MDSCs) emerged as key regulators of immune response in chronic inflammation. Prostaglandin (PG)E2 was shown as critical for MDSCs functions *in vivo*, but it is still unclear how it regulates molecular and tolerogenic mechanisms in major MDSCs subsets. To investigate this, C57BL/6 mice bone marrow cells were differentiated *in vitro* using GM-CSF and IL-6, either in the presence or absence of PGE2, followed by the purification of granulocytic (gr)MDSCs (CD11b+HLA-DR-Ly6ClowLy6G+) and monocytic (mo)MDSCs (CD11b+HLA-DR-Ly6ChiLy6G-) for phenotype and functional analyses. MoMDSCs expressed higher levels of Arginase-1, IL-10, TGF-β and IL-4 than grMDSCs, and PGE2 further up-regulated their expression. In contrast, grMDSCs displayed a higher expression of ILT-3, CD44, CXCR1 and iNOS, which were susceptible to PGE2-mediated up-regulation. MoMDSCs suppressed CD3/CD28-beads induced proliferation of CD4 and CD8 T cells more than grMDSCs, and the effect was potentiated with PGE2 and/or LPS on moMDSC, but only with PGE2 on grMDSCs. Also, moMDSCs showed a stronger capacity to reduce relative number of Th1, Th17 and Th2 cells in co-cultures, as compared to grMDSCs, especially if treated with PGE2. Interestingly, moMDSCs showed a higher capacity to induce TGF-β+FoxP3+CD4+ Tregs, and a lower capacity to induce IL-10-producing Tregs, as compared to grMDSCs. However, when differentiated with PGE2 and/or treated with LPS, moMDSCs showed an increased capacity to induce IL-10-producing Tregs, whereas PGE2-treated grMDSCs were more prominent in inducing TGF-β+ Tregs. These results suggest that PGE2 differently regulate suppressive mechanisms of MDSCs, which could be relevant for further development of MDSCs-based cell-therapies.

Keywords: Cell based therapies, myeloid derived suppressor cells, regulatory cells

P-0227

Impaired IFN-γ-induced PD-L1 expression on human vitiligo melanocytes

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Mounting evidence has shown that the PD-1/PD-L1 axis is involved in tumor immune evasion and impaired PD-1/PD-L1 function has been involved in a variety of autoimmune diseases. It is, therefore, hypothesized that manipulating PD-1/PD-L1 signaling might have therapeutic potential in human vitiligo. This research aimed to study the role of PD-1/ PD-L1 signaling in melanocyte destruction in vitiligo and how interferons can influence this signaling route. PD-1+ T cells were abundantly present in vitiligo skin, but its ligand PD-L1 was expressed limited and, if expressed, only by dermal T cells. More specifically, in situ melanocytes nor other epidermal skin cells expressed PD-L1. For IFN-γ plays a crucial role in vitiligo pathogenesis and IFN-γ can induce PD-L1 expression in human melanoma, we investigated if PD-L1 expression can be induced in melanocytes and other skin cells. Exposure to IFN-γ, but also type I interferons, increased PD-L1 expression in primary melanocytes and fibroblasts, derived from healthy controls. A similar trend was observed for IFN-γ-stimulated HaCat cells (human keratinocyte cell line). Most interestingly, melanocytes derived from non-lesional vitiligo skin showed no PD-L1 upregulation upon IFN-γ stimulation, even though Melan-A- skin cells did induce significant PD-L1 expression after stimulation. Furthermore, PD-L1 was massively upregulated by epidermal cells, except for melanocytes, in a vitiligo skin explant model. The lack of PD-L1 upregulation by melanocytes in the presence of IFN-γ-producing T cells shows that melanocytes do not confer protection against T cell attack during vitiligo pathogenesis. Manipulating PD-1/PD-L1 signaling, therefore, may be a therapeutic option for human vitiligo.

Keywords: Autoimmunity, checkpoint inhibition, skin diseases

P-0228

Comparative analysis and the optimization of protocols used to generate iPSC-derived human macrophages

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Generation of macrophages from human induced pluripotent stem cells (iMphs) represents a novel and highly promising approach to study macrophage biology and develop macrophage-based therapeutic techniques. The principles of iMph generation are based on a stepwise differentiation of induced pluripotent stem cells (iPSCs) into mesoderm, hemogenic endothelium, hematopoietic progenitors, monocyte-like cells and iMphs. However, existing protocols are highly diverse raising questions on the identity of the resulting iMph populations and requiring protocol standardization. We report the results of the comparative analysis of different protocols and suggest protocol modification allowing reliable and continuous generation of iMphs. Mphs were generated using: (i) spontaneous differentiation of iPSCs into 3-dimension embryoid bodies followed by the induction of hematopoietic differentiation using only two cytokines, IL-3 and M-CSF (EBS-Mphs); (ii) the differentiation of iPSCs in 2-dimension cultures driven by a large number ofsequentially added exogenous factors (2DF-Mphs). EBS-Mphs and 2DF-Mphs had typical macrophage morphology, expressed macrophage markers CD14, CD11b, CD45, had similar CD115+CD64+CD16+/low CD80low CD86low HLA-DRlow CD163+CD206+MARCOlow phenotype and were highly phagocytic. However multiplex and transcriptomic analyses revealed differences in iMph inflammatory profile (lower in 2DF) and inter-protocol differences in the dynamic changes of gene expression during the process of iMph differentiation. Because original 2DF protocol (Takata et al., 2017) implied one-off collection of iMphs, we developed a modification allowing continuous and high-yield generation of 2DF-Mphs. The data for the first time document differences between iMph generated using different protocols and suggest an optimized protocol for iMph generation.

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Keywords: Immune development, macrophage, RNAseq, stem cells

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POSTER PRESENTATIONS

P-0230

Identification of functional subsets of T lymphocytes based on the expression of immune-checkpoints, in Juvenile Idiopathic Arthritis

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Juvenile idiopathic arthritis (JIA) is a persistent arthritis of unknown cause in children. T cells are certainly involved in the pathogenesis. Nevertheless, functional analyses of T cells subsets in patients with JIA are needed to better characterise T lymphocytes role in disease development. T cells gene expression in JIA samples was investigated trough single-cell RNA sequencing (scRNA-seq) analysis. Moreover, Peripheral Blood (PB) and Synovial Fluid (SF) T cells from 12 children with JIA were analysed by flowcytometry, for the expression of immune-checkpoint (IC) molecules and for cytokine production profile. Data obtained from scRNA-seq analysis showed that T cells of PB and SF samples clustered separately and among the genes differentially expressed between clusters emerged TIGIT and PD1. These two ICs identified four different subsets. To correlate T cells functionality with ICs expression each subsets were checked for cytokine genes expression. The TIGIT-PD1+ subset exhibits the highest expression of cytokines, followed by TIGIT+PD1+, TIGIT-PD1- and TIGIT+PD1-. Moreover, pseudotime analysis data highlighted that at the beginning T cells highly express PD1, and subsequently start to express TIGIT. ScRNA-seq data were confirmed by flowcytometry analysis. Obtained data suggest that in SF recently activated T cells acquire an higher effector function and express PD1. The persisting stimulation induces on T cells the expression of inhibitory molecules (TIGIT) decreasing T cells effector capacity. PD1 is one of the mainly expressed molecules by active cells in SF, suggesting that it can be considered as a valid therapeutic target in chronic inflammation.

Keywords: Adaptive immunity, autoinflammation, inflammatory joint diseases, omics technologies

P-0232

Effect of microtubule inhibitors on danger-associated molecular patterns (DAMP) on cancer cells

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The cytostatic agents vinorelbine and colchicine leads to cell cycle arrest in G2/M phase by preventing the polymerization or depolymerization of microtubules. This study aims to test if DAMP levels are increased upon the stress caused by cell cycle arrest in cancer cells. Fibroblast and cancer cell lines (NIH/3T3, L929, 4T1, B16-F10) were exposed to sub-toxic concentrations of vinorelbine and colchicine with or without serum starvation, which were determined by MTT, and the cell cycle analyses were performed by flow cytometric DNA content analysis. The change in certain DAMPs' gene expression (by qPCR) and subcellular compartment replacement were assessed (by ELISA and immunofluorescence). Non-toxic concentrations of vinorelbine and colchicine disrupted microtubule polymerization, causing cell cycle arrest in G2/M phase. The cell cycle arrest tended to increase the expression DAMP genes *Hmgb1*, *Hspa1b*, *Hsp90aa1*, *Nlrp3*, *H3f3a*, *IL33* and *Ppia*. Translocation HMGB1 out of the nucleus and extracellular levels of HSP70 was increased. Our results indicate that not only cell death but also arrest in the cell cycle can upregulate DAMPs which indicate disruptions in homeostasis and may eventually alert the immune system.

Keywords: Innate immunity, cancer immunology, immunotherapy

P-0233

Lymph node-derived fibroblastic stromal cells support pre-DC maturation into DC-like cells via CSF1

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In the bone marrow, conventional dendritic cells (cDCs) develop from fate-committed precursor dendritic cells (pre-DCs) that continuously migrate to peripheral tissues where they undergo a constant turn-over. Several factors are involved in the generation of DCs from pre-DCs and further up-stream progenitors: FLT3L (Fms related receptor tyrosine kinase 3 ligand), the most important cytokine for cDC development, as well as SCF (stem cell factor) and CSF1 (macrophage colony stimulating factor 1) were demonstrated to induce pre-DC development into DC-like cells. However, after their arrival in lymphoid and peripheral tissues, it remains incompletely understood which cell types instigate and facilitate pre-DC development into DCs in their local microenvironment. Yet, stromal cell lines were shown to foster the generation of DC-like cells. Thus, we here analyzed the influence of lymph node (LN) fibroblastic stromal cells (FSCs) on this maturation process in detail. LN FSCs co-cultured with pre-DCs induced the generation of DC-like cells, capable of inducing the proliferation of naïve T cells. We could further demonstrate that CSF1 plays a pivotal role in the LN FSC-mediated generation of DC-like cells, since blockade of CSF1 abrogated development of pre-DCs into DC-like cells. Moreover, FSCs from mesenteric LNs (mLNs) and axillary and inguinal peripheral LNs (pLNs) yielded nearly identical DC-like cells, which showed similarity to both *ex vivo* isolated DCs as well as macrophages on the basis of their transcriptome. In summary, we could identify LN FSCs as contributors to DC maturation via provision of CSF1.

Keywords: Biology of the immune system, cellular interactions, dendritic cells, lymphoid organs

P-0234

Innate lymphoid cells (ILC) are less responsive to immunosuppressants than T lymphocytes

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Pre and protransplantational drugs are effective prophylactic and therapeutic drugs against graft versus host disease (GvHD). However, it is unknown whether immunosuppressive drugs work singularly by eliminating alloreactive T cells or also by maintaining IL-22 producing type 3 innate lymphoid cells (ILC3). We studied the effect of immunosuppressive drugs on the survival, proliferation, activation and function of ILC3 *in vitro*. Tonsil derived activated ILC3 and T cells were cultured in the presence of commonly used immunosuppressants. Our data demonstrate that commonly used immunosuppressive drugs directed at suppressing T cell function and applied to prevent and treat GvHD have different effects on ILC activation, proliferation and function. Most immunosuppressive drugs did not affect ILC3, while they suppress T cell survival, proliferation and activation. None of these immunosuppressants were able to suppress ILC3 activation. The differential effect of drugs between ILC3 and T cells may be related to the increased expression of ATP-binding cassette transporters B1 (ABCB1) on ILC3. These findings will help to develop strategies to maximize the use of the protective function of ILC in addition to suppressing T cell alloreactivity, in the prevention and treatment of GvHD.

Keywords: Bone marrow transplantation, drugs for immune modulation, inflammatory disease

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POSTER PRESENTATIONS

P-0235

Activation and functional reponsesivness of macrophages cells to aluminum adjuvanted tetanus-diphtheria vaccine

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The purpose of this study is to evaluate the impact of aluminum-adjuvanted vaccines on macrophage activation and functional responsiveness. Murine monocyte/macrophage J774. A1 cells were exposed to two different dilutions of aluminum-adjuvanted tetanus-diphtheria vaccine (Td) in the presence or absence of LPS. The change in macrophage maturation (morphology and marker expression), activation (ROS, NO and phagocytosis) and viability were determined by flow cytometry. Exposure to aluminum-adjuvanted Td vaccine supported F4/80 and CD11b and MHC-II maturation markers either in LPS-stimulated or control macrophages. CD80 expression was increased dramatically by LPS treatment however negatively correlated in combinations with Td. A positive trend was observed in cellular granularity and Td concentration with or without LPS combination. The phagocytosis of latex beads was decreased significantly by increasing Td amount in all conditions. ROS production capacity was increased in both Td and LPS administrations whereas NO production was decreased with combination of LPS and Td vaccine. Our preliminary results may be useful for understanding the impact of aluminum adjuvants on macrophage activation.

Keywords: Adjuvants and vaccines, infectious disease, macrophage, phagocytosis

P-0236

CD28 individual signaling up-regulates IL-22 expression and IL-22–mediated effector functions in human T lymphocytes

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IL-22 is a member of the IL-10 cytokine family involved in host protection against extracellular pathogens, by promoting epithelial cell regeneration and barrier functions. Dysregulation of IL-22 production has also frequently been observed in acute respiratory distress syndrome (ARDS) and several chronic inflammatory and autoimmune diseases. We have previously described that human CD28, a crucial co-stimulatory receptor necessary for full T cell activation, is also able to act as a TCR independent signalling receptor and to induce the expression of IL-17A and inflammatory cytokines related to Th17 cells, which together with Th22 cells represent the main cellular source of IL-22. Here we characterized the role of CD28 autonomous signalling in regulating IL-22 expression in human CD4+ T cells. We show that CD28 stimulation in the absence of TCR strongly up-regulates IL-22 gene expression and secretion. As recently observed for IL-17A, we also found that CD28-mediated regulation of IL-22 transcription requires the cooperative activities of both IL-6-activated STAT3 and RelA/NF-κ transcription factors. CD28-mediated IL-22 production also promotes the barrier functions of epithelial cells by inducing mucin and metalloproteases expression. Finally, by using specific inhibitory drugs, we also identified CD28-associated class 1A phosphatidylinositol 3-kinase (PI3K) as a pivotal mediator of CD28-mediated IL-22 expression and IL-22-dependent epithelial cell barrier functions.

Keywords: Cell signalling, cytokines and mediators, molecular immunology

P-0237

Phenotypical and functional characterization of dapsone treated-neutrophils *in vitro*

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Clinical experiences confirm that dapsone is effective in treating inflammatory diseases, characterized by neutrophil-rich infiltrations. We examined different aspects of reshaping neutrophil's function by dapsone. Human neutrophils were isolated from the venous blood of healthy donors. Neutrophils were treated with dapsone (10μg/mL; 5μg/mL or 2,5μg/mL), and stimulated by phorbol-12 myristate-13-acetate (PMA), N-formyl-L-methionyl-L-leucyl-L-phenylalanine (fMLP) or calcium ionophore (CaI). In some experiments, dapsone-primed neutrophils were treated with interferon-gamma (IFNγ) or granulocyte-macrophage colony-stimulating factor (GM-CSF). Phenotypical characterization and apoptosis were measured by flow cytometry. Oxidative burst and NETosis were determined by a multimode microplate reader using luminol and Sytox Green, respectively. Production of IL-8 was measured by ELISA. We found that dapsone did not change the rate of spontaneous apoptosis after 6h and 24h. Dapsone at all tested concentrations significantly decreased oxidative burst of neutrophils activated by all stimuli compared to control. The highest concentration of dapsone inhibited the CaI-triggered NETosis. Also, dapsone increased the expression of CD16, CD15, CD62L on resting neutrophils and suppressed down-regulation of these markers in the presence of fMLP. The opposite effect of dapsone was seen with CD11b which was up-regulated by both resting and fMLP-treated neutrophils. The production of IL-8 was decreased in both, stimulated (fMLP) and unstimulated conditions. INFγ and GM-CSF increased oxidative burst of neutrophils in stimulated and unstimulated conditions, while priming with dapsone reduced the oxidative burst of these cells. Our results indicate the complexity of dapsone action on neutrophils with the predominance of anti-oxidative mechanisms.

Keywords: Drugs for immune modulation, granulocytes, neutrophils

P-0238

Myelodysplastic syndromes and acute myeloid leukemia display distinctive patterns of bone marrow NK cell maturation and KIR expression

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Myelodysplasic syndromes (MDS) are pre-malignant hematological disorders characterized by ineffective hematopoiesis that frequently progress towards acute myeloid leukemias (AML). Natural Killer (NK cells) are key antitumor elements of the innate immune system and understanding the differences in the NK functioning between MDS and AML is crucial in predicting the progression of MDS to AML and defining the corresponding therapy. The bone marrow aspirates of newly diagnosed patients with MDS (n=25), AML (n=8) and controls (normal bone marrow – NBM, n=30) at the Lucien Neuwirth Institute of Cancerology (Saint-Priest-en-Jarez, France) were collected between March-July 2020, and the resident NK cells were investigated by flow-cytometry. Based on the expression of CD56, CD94, CD16 and CD57 molecules, the three distinct maturation NK subsets were isolated (immature NK as CD56brightCD94hiCD16−CD57− cells, mature as CD56dimCD94medCD16+CD57- cells, and hypermature as CD56dimCD94lowCD16+CD57+ cells) and analyzed for the expression of inhibitory KIR receptors: NKG2A, CD158a, CD158b, and CD158e1. The NK/T cell distribution was impaired and accompanied by an increase in the immature NK subset in the bone marrow of MDS and AML cases compared to the NBM settings. Furthermore, the KIR expression was differentially expressed on the three distinct NK subsets in AML compared to MDS and NBM. This study provides important evidence for the undermined NK cell antitumor response in AML compared to MDS and NBM and explains the failure of this arm of the immune response during the pathogenesis of myeloid malignancies.

Keywords: Biology of the immune system, cancer immunology, innate lymphoid cells, NK cells

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POSTER PRESENTATIONS

P-0239

Functional abnormalities of circulating neutrophils in children with cystic fibrosis

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Cystic fibrosis (CF) is a genetic disease manifested by mucus hypersecretion predominantly in lungs and gut. CF is characterized by impairment of local immune responses including alterations of neutrophil functions. However, little is known whether similar abnormalities of neutrophils are present in blood. In our study we compared neutrophil function of 19 children with CF (mean age 11.65 ± 4.30 years) with healthy age matched controls. Oxidative burst and NETosis were determined by luminol and Sytox Green, respectively. For stimulation of neutrophils phorbol-12 myristate-13-acetate (PMA), N-formyl-L-methionyl-L-leucyl-L-phenylalanine+ lypopolysaccsharide from E.coli, opsonized zymosan (OpZy), calcium ionophore or heat killed Pseudomonas aeruginosa (PA) were used. Production of IL-8, MCP-1, IL-18 and neutrophil elastase were measured by ELISA. Apoptosis was measured by flow cytometry. We showed that the oxidative burst of both PMA and OpZy-treated neutrophils in CF patients were lower compared to healthy controls. Both unstimulated and stimulated cultures from CF patients produced lower levels of IL-18. Apoptosis of CF neutrophils was lower in unstimulated and PA-stimulated cultures. PA-induced NETosis was higher in CF patients with severe pulmonary manifestations. These findings were accompanied by lower elastase levels. No other functions were altered. In conclusion, our results suggest that circulating neutrophils from CF patients are primed for better survival, release higher quantity of NETs which are deficient in elastase activity and produce less oxidative species and IL-18. Most of these parameters are visible in the presence of PA and these findings could be associated with frequent PA infections in these patients.

Keywords: Innate immunity, cytokines and mediators, neutrophils

P-0240

Role of calcium ions released by a calcium alginate dressing on NK cell activation

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Wound healing is a physiological process involving 4 major stages, with an inflammatory phase to eradicate causal aggression. The wound bed is infiltrated by inflammatory cells from the innate response, including NK cells within the first 24h. Calcium alginate dressings including Algosteril® (Algo) are widely used in wound management. They locally release calcium ions in the exudates. Calcium is a key factor in wound healing process and a well-known activator of NK cells. Our aim was to study the effects of Algo on the activation of the cytotoxic capacities and the activation of NK cells. NK cells from healthy donors were incubated with Algo conditioned media (ISO10-993 standard) *in vitro* for 18h. A control 3 mM CaCl2 solution was used as a supply of exogenous calcium. The cytotoxicity and activation of NK cells were assessed by flow cytometry and the calcium channels involved were evaluated using specific calcium channel blockers. Algo significantly stimulated the NK cytotoxicity against K562 cells (x1.3 at 10NK/1K562 ratio). It also induced a significant increase in the cytotoxic CD56dim/CD107a+ NK cell percentage and in the IFN-γ expression in CD56bright NKs. Incubation with EDTA (calcium chelator) inhibited the Algo stimulatory effect on NK cytotoxicity by 69% at the 10/1 ratio. SOCE calcium channels are the major one involved in NK stimulation by the dressing-released calcium ions. Our results demonstrate the major role of calcium in innate response during wound healing and the interest in using the calcium alginate dressing Algo to control infection.

Keywords: Tissue damage and repair, NK cells, innate lymphoid cells

P-0241

Evaluation of expression of hla-dr in monocytes by flow cytometry as an indicator of inflammatory and infectious disease

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This study aimed investigate the potential use of HLA-DR molecule expressed in monocytes quantify by flow cytometry method as a biomarker to systemic inflammatory response syndrome (SIRS) or bacterial sepsis (BS) diagnostic in outpatients and intensive care unit (ICU) patients. Peripherical blood samples collected between May and July of 2018 were analyzed using a flow cytometer. Firstly, the value of immature granulocyte % (IG%) ≥0.65% was used to describe the outcome cases (inflammatory disease). Cut-off of HLA-DR mean fluorescence intensity (MFI) were compared with others laboratory variables commonly changed in inflammatory disease. After, the HLA-DR MFI and cut-off of HLA-DR MFI obtained previously, were analyzed to detect the outcome SIRS or BS. 78 patients were included. Firstly, receiver operating characteristic (ROC) analysis revealed that cut-off of HLA-DR MFI to detect the outcome was ≤1379, with sensitivity of 63.6% and specificity of 97.1%. Area under curve (AUC) was 0.833 (95% confidence interval (CI) 0.746-0.921) and p <0.001. This MFI value was associated with band cells count >10% (p<0.001); leucocytes count >11000 cells/µL (p<0.001); IG% ≥0.65% (p<0.001) and "flags" detection (p=0.001). At the second time, ROC analyses demonstrated a cut-off of HLA-DR MFI to detect SIRS or BS ≤1996 with sensitivity of 96.0% and specificity of 43.4%. For cut-off of ≤1379, sensitivity was 60.0% and specificity was 73.6%, AUC to both was 0.718 (95% CI 0.601-0.835) and p=0.002. Quantification of HLA-DR expression in monocytes by flow cytometry method presents potential to be used as screening test to detect SIRS or BS.

Keywords: Biomarkers, infectious disease, inflammatory disease, myeloid cells

P-0242

Post-pregnancy modifications in peripheral natural regulatory T cells in healthy women

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Natural Regulatory T-cells (nTregs) control the immune response against self-antigens during the extreme endocrine changes in pregnancy and afterward. ERK1/2, an important signal molecule is involved in T-cell proliferation and differentiation. Its phosphorylation is associated with estrogen receptor signaling as shown in hormone-sensitive cancers. The present study is aimed at evaluation of ERK1/2 in nTreg subset in the light of parity. 32 healthy women (23-40 years) were separated as follows: without (Gr.0), with 1 (Gr.1) or more (Gr.2) successful deliveries (at least one year after). Flowcytometric analysis was done with anti-CD3/CD4/CD45RA/CD25/FOXP3/pERK1/2 MoAbs. Data were processed with FlowJo V10 and GraphPad Prism7. The overall analysis revealed that among Gr1./Gr.2 and Gr.0 the percentage of total Treg and nTregs particularly (FoxP3+CD45RA+) was comparable (p> 0.05). In Gr. 0, a positive age-related trend of the CD25+nTreg subset was found (r= 0.82, p= 0.002), that was not the case in the group of women with one successful delivery (r= -0.71, p= 0.02). The percentage of pERK1/2+ CD25+nTreg cells was higher in Gr.0 than in Gr1&Gr.2 (p< 0.05). Our results suggest that the immune-endocrine crosstalk may affect the subset of nTregs. The extreme hormonal dynamic during pregnancy and the postpartum period could exert long-lasting impact on the subset of CD25+nTregs a probably associated with ERK1/2.

Keywords: Adaptive immunity, cell signalling, regulatory cells, reproductive immunology

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POSTER PRESENTATIONS

P-0243

The role of regulator of G-protein signaling (Rgs)-1 in CD8+ TRM-cell mediated intestinal immunity

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The gene encoding regulator of G-protein signaling 1 (Rgs1) is consistently one of the most up-regulated genes in tissue-resident TRM cells. Rgs1 inhibits signal transduction by increasing the GTPase activity of the Gαi protein subunit, which attenuates chemokine receptor-mediated immune cell trafficking. There is a striking genetic association of RGS1 SNPs with altered incidence of T cell-mediated autoimmune disorders in humans (e.g. celiac disease, multiple sclerosis). The precise functions of Rgs1 in the differentiation of T cells, however, remain ill-defined. Herein, we generated a Rgs1-tdTomato reporter mouse, and confirmed the remarkable Rgs1 signature in intestinal resident unconventional and conventional T-cell subsets during homeostasis, and the rapid induction of Rgs1 expression in antigen-specific T cells following local infection with a pathogen. We used an adoptive co-transfer of congenic Rgs1-/-, and Rgs1+/+ OT-I CD8 T cells into recipient mice and infection with L. monocytogenes-OVA to monitor the impact of Rgs1 on the generation of CD8 TRM cells. Accordingly, antigen-specific Rgs1-/- OT-I T cells showed an impaired differentiation into memory precursor effector cells (MPEC) in the intestinal mucosa upon infection. As a consequence, Rgs1-/- OT-I TRM cells are underrepresented in the intestinal mucosa at day 30 post-infection. Furthermore, in contrast to intestinal Rgs1+/+ OT-I TRM cells, Rgs1-/- OT-I TRM cells fail to efficiently mediate pathogen clearance following local re-infection. These experiments revealed the critical requirement of Rgs1 for the accumulation of CD8 TRM cells, and for the efficient immunoprotection from systemic dissemination of the pathogen upon reinfection.

Keywords: Adaptive immunity, animal models, inflammatory bowel disease, memory

P-0245

The modulation of TLRs/CD44 axis regulates clinical features of experimental and human multiple sclerosis

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We investigated a new mechanism through which pathogens directly modify trafficking properties of activated T lymphocytes impacting on the alternative splicing of the adhesion molecule CD44. By isolating murine and human antigen-specific activated T cells and stimulating them *in vitro* with different Toll-Like Receptors (TLRs) ligands, we found that TLR activation impact differently on the ability of T cells to migrate; also, their migration is CD44-dependent. Furthermore, qRT-PCR analysis of CD44 isoforms (CD44vv) of different brain areas, obtained from SJL/J mice affected by Experimental Autoimmune Encephalomyelitis (EAE) during onset of the disease, revealed the enrichment of the specific isoform CD44v9-v10 in T cells infiltrating the forebrain, in association with the distribution of the active lesions. Expression of CD44v9-10 is specifically upregulated in T cells by TLRs ligands, in SJL mice. Analogously, mRNA analysis of cells obtained from the CSF of Multiple Sclerosis patients showed an association between enrichment of CD44v7 and presence of gadolinium- enhancing lesions. Collectively, these data suggest that TLRs represent a pathway through which pathogens and commensals modify trafficking of antigen activated T cells by modulating CD44 alternative splicing. Specifically, TLR2 activation modulates the relative ratios of CD44vv to induce or inhibit T cells enter in specific areas of the CNS. In perspective, these molecules may represent potential targets for drug therapies to provide new clinical tools for disease activity evaluation and treatment efficacy.

Keywords: Autoimmunity, biomarkers, environmental factors in autoimmunity and allergy, multiple sclerosis, neuroimmunology

P-0246

DEK level was reduced in the serum of patients with COVID-19

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DEK, an important protein in inflammation and cancer, is mainly bound to chromatin in the nucleus and post-translational modifications facilitate its release from the chromatin and cell. DEK was associated with the neutrophil extracellular traps (NETs) in juvenile idiopathic arthritis (JIA) and autoantibodies against DEK exist in the synovial fluid of these patients. Since prolonged NETs formation and increase of proinflammatory cytokines are related to COVID-19, here we investigated the level of DEK in the serum of patients with COVID-19 by using ELISA. We found that DEK level in the serum of patients was significantly reduced at both acute (n=23) and convalescent stages (n=44) of the disease (mean±SEM of acute and convalescent phase samples, 920±66 pg/ml, 1070±45 pg/ml, respectively) compared to the healthy controls (n=38) (mean±SEM 1270±55 pg/ml). Consistent with the literature, there was a gradual increase at the level of IL-6 and IL-8 in patients' serum samples, convalescent patients still exhibiting significantly the highest level of both cytokines. Interestingly, double immunofluorescence staining showed that like neutrophil elastase (NE), DEK densely locates in the granules but not in the nucleus of intact neutrophils. Although NE densely located on the NETs, only small amount of DEK was rarely appeared as dotted stains on the NETs which was induced by incubation of healthy control neutrophils with patient's serum. Reduction of DEK level in the patients' serum suggest a possible role for DEK in COVID-19 related inflammation. This work was supported by the Scientific and Technological Research Council of Turkey (TÜBİTAK) (grant 120S979).

Keywords: Cytokines and mediators, innate immunity, neutrophils, viral infections

P-0248

Investigation of SARS-CoV-2 non-structural and accessory proteins effect on host cell inflammasome activation and IFN antagonism

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SARS-CoV-2 RNA genome expresses eight accessory (ORFs) and sixteen non-structural proteins (NSPs) with distinct roles in virus life cycle and pathogenicity. In this study, the role of some of these NSPs and ORFs in modifying inflammasome and type I interferon signaling pathways were investigated. THP1-Dual Wild Type cells were transduced with lentiviral vectors to encode SARS-CoV-2 NSP or ORF related proteins. Cells were then primed with PMA over-night and then further stimulated with dsDNA/dsRNA analogs and different inflammasome activators. IL-1β released into culture supernatant was measured by ELISA, whereas type I IFN production was assessed using the reporter function of THP-1 cells. Mitochondrial integrity of the cells was also assessed by JC-1 staining. Ratio of JC-1 aggregates/monomers were evaluated using flow cytometry and fluorescence microscopy. All results were compared with WT and lentivirus infected control cells. ORF3a and ORF8 proteins have been implicated in inflammasome activation. We found that ORF3a protein had a role for modifying NLRC4 inflammasome activation and IL-1b production but had no effect on AIM2 and/or non-canonical inflammasome activation. Moreover, NLRP3 inflammasome mediated IL-1b production was almost 10-fold higher in ORF3a expressing cells. Furthermore, ORF3a and ORF8 proteins were found to antagonize IFN production in cells stimulated with nucleic acid ligands. JC-1 staining patterns of ORF3a, ORF3b and ORF9b expressing cells also showed some differences, suggesting interference with mitochondrial functions. These results indicate that SARS-CoV-2 accessory proteins may modify anti-viral host immune responses, warranting more detailed studies.

Keywords: Cytokines and mediators, immune development, inflammatory molecules

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POSTER PRESENTATIONS

P-0249

Investigating the potential of BCG vaccine and CpG ODNs to induce trained immunity in the context of antiviral immunity

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Trained immunity has been defined as the non-specific memory generated via metabolic and epigenetic reprogramming of innate immune cells after a primary stimulus such as infection or vaccination. BCG vaccine is a known inducer of trained immunity, whereas CpG-ODNs possess stand-alone immunoprotective activity that confer resistance against a variety of microbial infections. Herein, we investigated the potency of BCG vaccine Russia strain, K- and D-classes of CpG-ODNs to induce trained immunity particularly in the context of antiviral immunity. PBMCs isolated from healthy donors were incubated with either BCG, K3-ODN, D35-ODN or RPMI as negative control for 24 hours. Cells were then washed and left to rest for 6 days, followed by stimulation with viral or bacterial ligands. Cytokines released were measured from cell culture supernatants by ELISA and intracellular ISG15 levels of cells were assessed via flow cytometry. Type I IFN production was indirectly evaluated by Lucia Luciferase activity of the reporter THP-1 Dual cell line. PBMCs of 9 volunteers vaccinated with BCG vaccine were isolated prior to and 4 weeks after BCG vaccination to be subjected to functional assays and gene expression analysis using NanoString PanCancer immune-profiling panel. *In vitro* training with BCG and CpG-ODNs increased intracellular ISG15 levels, secondary IP-10 and type I IFN production of hPBMCs stimulated with viral ligands. Similarly, BCG vaccination enhanced anti-viral recall responses of individuals 4 weeks after vaccination both at protein and mRNA levels. Our findings suggest that BCG and CpG-ODNs could reprogram hPBMCs and increase their responsiveness to potential viral infections

Keywords: Adjuvants and vaccines, cytokines and mediators, innate immunity, memory, viral infections

P-0252

Comparison of SP-D and MBL serum levels between COVID-19 convalescent patients and healthy control subjects

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The COVID-19 pandemic continues to be the focus of global research. In this study, we compared serum levels of the innate humoral factors surfactant protein D (SP-D) and mannose-binding lectin (MBL) in human sera from COVID-19 convalescents with those from a healthy control group to determine whether differences in steady-state levels of SP-D or MBL have an impact on the susceptibility or progression of COVID-19. Serum SP-D and MBL levels of 141 convalescent COVID-19 patients and 98 healthy controls were determined by sandwich ELISA and compared for their association/correlation with cellular, humoral, clinical, and demographic parameters. Serum SP-D and MBL levels were not predictive of susceptibility to SARS-CoV-2 infection in the entire study population. However, we found in severely affected hospitalized COVID-19 patients that SP-D serum levels correlated positively with CD45RO+CCR7-CD8+CD3+ memory T effector cell count and monocyte counts. In the same group, SP-D serum levels correlated negatively with the number of CD27+IgD+CD19+ non-class-switched memory B cells and IgM-CD38+CD19+ plasmablasts. Moreover, SP-D serum levels were highly predictive of myalgia occurrence in this group of patients. In contrast, MBL serum levels correlated positively with the number of CD45RO-CCR7-CD8+CD3+ effector memory T cells and plasmablasts. SP-D and MBL serum levels reflect different courses of severe COVID-19 disease.

Keywords: Infectious disease, innate host defence, innate immunity

P-0254

The epithelial barrier damaging effects of professional dishwasher rinse aid on Caco-2 gastrointestinal epithelial cells

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Given the recent pandemics of food allergy and common use of dishwasher-detergents, we investigated the effects of professional dishwasher-detergents, including sodium/potassium-metasilicate, potassium-hydroxide, potassium-carbonite and sodium-hypochlorite, and rinse-aid, including alkylalkoholalkoxylat, citric-acid and sodium-cumenesulfonate, on cytotoxicity and barrier function of gastrointestinal epithelial cells. Cytotoxicity was evaluated by LDH release. Enterocytic liquid-liquid interfaces were established on permeable supports. Detergent and rinse-aid were added to the apical compartment, and then the transepithelial-electirical-resistance (TER) and paracellular FITC-Dextran-flux was measured. Immunofluorescence staining of occludin, ZO-1 and claudin-1 and quantitative real-time PCR (RT-PCR) experiments were performed. Rinse-aid showed dose-dependent cytotoxicity on Caco-2 cells between the concentrations of 1:2500 and1:20000 v/v dilutions. In contrast, there was no toxicity of detergent at all concentrations. We did not observe any disruption of the monolayer integrity, as indicated through maintenance of TER with detergent at all concentrations. In contrast, rinse-aid induced a dramatical decrease in TER at dilutions of 1:2500, 1:5000 and 1:10000 after 72 h. A disrupted epithelial barrier function was found with increased paracellular-flux in Caco-2 cells exposed to rinse-aid at the same dilutions. Irregular and stratified staining of occludin, ZO-1 and claudin-1 were found in Caco-2 cells exposed to rinse aid at concentrations of 1:2500-1:20000. RT-PCR results showed that rinse-aid significantly downregulated the expressions of sealing proteins claudin-4 and occludin, pore-forming proteins claudin-2 and claudin-15, and adaptor protein ZO-1 at the dilutions of 1:10000 and 1:40000. Our data demonstrated that rinse-aid which shows high cytotoxicity and directly impairs barrier integrity of gut epithelial cells.

Keywords: Allergen-induced immune responses, cell death, molecular immunology

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POSTER PRESENTATIONS

P-0255

RIG-I-induced reactive oxygen species drive metabolic changes that modulate the antiviral immune response in hepatocytes

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Hepatocytes continuously engage in diverse homeostatic activities including processing of gut-derived products, regulation of body metabolism and detoxification events. Hepatocytes also play a major role in host defence by mediating systemic inflammation and innate immunity through the production of acute phase proteins and complement components, and responding locally to infection by hepatotropic viruses via pattern recognition receptor activation, such as RIG-I and TLR3. *Bona fide* immune cells alter their metabolism to meet the metabolic demands of an induced immune response. Reactive oxygen species (ROS), generated through a metabolic switch, have been identified as a key link between the immune response and the metabolic state of the cell. Whether this process also occurs in other cell types, such as hepatocytes, remains to be established. In this study we transfected two hepatoma cell lines, HepG2 and Huh7, with polyI:C, to understand the link between the metabolic state of hepatocytes and their ability to mount an antiviral immune response. Following treatment with polyI:C, we observe an increase in both gene expression and protein production of CXCL10 and IFN-β. To assess the metabolic state of the polyI:C treated cells, we employed fluorescence lifetime imaging microscopy (FLIM) and Seahorse real-time cellular flux analysis. Following treatment with polyI:C cellular oxygen consumption was decreased. Using carboxy-H2DCFDA as ROS-fluorescent probe, ROS levels increased after treatment with polyI:C. Applying a ROS scavenger (N-acetyl cysteine), CXCL10 and IFN-β gene expression and protein levels were decreased, indicating a role for ROS and metabolism in shaping the antiviral immune response in hepatocytes.

Keywords: Cell signalling, innate immunity, metabolic control of immune responses, viral infections

P-0257

Phenotypes and functions of low(er)-density neutrophils (LDNs) in early childhood and children

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Young infants respond relatively poorly to many infections and vaccines, but the basis of reduced immunity in infants is not completely defined. Mature neutrophils and granulocytic-myeloid derived suppressor cells (G-MDSC) can express similar cell surface markers. However, G-MDSCs are functionally distinguished from neutrophils by its immunosuppressive capacity on T cell responses. The literature regards G-MDSCs as low density cells and indicate that they accumulate to upper 1.077g/mL Ficoll fraction. In this study, we aimed to investigate low and high density of neutrophils that were accumulated both upper and lower phase of 1.077g/mL Ficoll phase Leukocytes were obtained from upper or lower 1.077g/mL ficoll phase of children (age range from 5 days to 15 years).High and low density neutrophils were positively selected using with CD66b microbeads by magnetic activated cell sorting.Different CD66b surface expression levels were detected (dim, mod and high) and purified by floresen activating cell sorting (FACS).These subpopulations surface molecules expression (CD15,CD14,CD33,CD16,HLA-DR,CD114,CD62-L,Lox-1,PD-L1,PD-L2,CD80,CD86) were studied with flow cytometry.Dichlorodihydrofluorescein diacetate(DCFDA) was used to measure ROS production.Carboxyfluorescein succinimidyl ester(CFSE) for proliferation analysis of CD4+ or CD8+ T cells obtained from healthy donors in the presence of low or high density of neutrophils from healthy children.Haematoxylin-eosin staining of blood smears were used for confirm maturation stages of neutrophils. Peripheral blood low-density neutrophils in early childhood stimulate T-cell responses as much as high-density neutrophils

This study is being supported by The Scientific and Technological Research Council of Turkey (TUBITAK), Project no. 120S653

Keywords: Biology of the immune system, myeloid cells, myeloid derived suppressor cells, neutrophils

P-0258

Monitoring osteoclast development with a fluorescence-based method

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Osteoclasts are multinucleated syncytia of myeloid origin which are responsible for bone resorption under various physiological and pathological conditions including autoimmune arthritis. The objective of our study was to develop fluorescence-based methods for efficient, real-time monitoring of the development of osteoclast cultures.

Our osteoclastogenesis assays are based on two genetic modifications in mice. The cathepsin K cre (Ctsk-cre) mutation results in the osteoclast-specific expression of the cre recombinase, while the mTmG mutation is a cre-responsive reporter switching from red to green fluorescence upon recombination. First we crossed Ctsk-cre and mTmG-carrying mice to obtain a Ctsk-reporter strain. This resulted in robust green signals in osteoclast cultures. Green fluorescence emerged in both mononuclear pre-osteoclasts and multinuclear osteoclasts. Then, we set up a second assay where Ctsk-cre cells were co-cultured with mTmG cells as a fusion-specific reporter. Here only upon cell-to-cell fusion could cre access its mTmG substrate and thus activate green fluorescence. This time a less robust green fluorescence could be detected that seemed to be confined to multinuclear syncytia but not to mononuclear cells. Parallel macrophage cultures were devoid of green fluorescence, indicating the osteoclast-specific nature of both assays. We also confirmed the working principle of our assays at genomic, transcriptional and protein levels. In summary, we successfully established two fluorescence based assays that enabled us to follow real-time changes in cell cultures differentiated towards osteoclasts. The included control cultures confirm a high degree of specificity for osteoclastls.

Keywords: Animal models, cellular interactions, myeloid cells

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POSTER PRESENTATIONS

P-0259

Macrophage polarization capacity of peripheral blood monocytes and monocytic cell line THP-1 in response to secreted factors from COVID-19 patients

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Regarding on the microenvironment conditions, peripheral blood CD14+ monocytes differentiate into different macrophages subgroups and these cells display phenotypic and functional heterogeneity. THP-1 cell line is generally used as a monocytic model to eliminate donor-derived heterogeneity in peripheral blood monocytes. In this study, we focus on differences between monocytic differentiation into macrophages, in response to secreted factors from COVID-19 patients. Sera samples obtained from both COVID-19 patients that were diagnosed with different clinical stages and healthy donors (n=40) and these specimens were pooled using with the same volume. These pooled samples (diluted, 1:10) were added onto PBMC (1x106) (from healthy and COVID-19 positive patients) or THP-1 cells (25x104) and incubated for 9 days. Surface markers (CD68, CD80, CD86, PD-L1, PD-L2, CD206, CD163, CD11b, HLA-DR, CD14) were evaluated by flow cytometry. Morphological differences were detected by DiffPlus (Diff Quick) staining. Carboxyfluorescein succinimidyl ester(CFSE) for proliferation analysis of PBMC obtained from healthy donors in the presence of these macrophages differentiate under different serum conditions. M1/M2 polarization is correlated to COVID-19 patients clinical outcome. Monocytes differentiated into activator macrophages phenotypes (CD86+, CD80+, CD206low) when added serum from patients with severe COVID-19 disease. These results may explain over immune responses when monocytes migrate into lung tissue and cause damage.

This study is being supported by The Scientific and Technological Research Council of Turkey (TUBITAK), Project no. 120S653

Keywords: Inflammatory disease, macrophage, myeloid cells

P-0260

Investigation of immunomodulatory efficacy of β-glucan and cephalaria spp. saponin formulation

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Food supplements are widely used to support the treatment of certain diseases such as infections, diabetes, cardiovascular disease and cancer. β-glucan is a chemically heterogeneous polysaccharide found in the innermost layer of the Saccharomyces cerevisiae cell wall and constitutes 30-60% of the dry weight of the cell wall. They β-glucans possess immunostimulant, anti-inflammatory, antimicrobial, antiviral, antitumoral and cholesterol-lowering. Saponins are sterol glycosides and triterpene glycosides, which are the active components of plants used in many traditional medicines. Saponins have a wide range... spectrum of their biological potentials such as immunomodulatory, anti-inflammatory, antifungal, anti-tumor, anti-diabetes, anti-oxidative, anti-apoptotic. Saponin structure promotes Th1 and Th2 responses. It is known that the β-glucan have both of Th1 and Th2 responses. The aim of the study is to regulate the Th1 / Th2 response by formulation of β-glucan and saponin. In this study, monocyte THP-1 cells were–stimulated with Phorbol 12-myristate 13-acetate (PMA) for 48 h at 37 °C to differentiate the macrophage cells (M0). Then, THP-1 macrophage cells were treated with β-glucan- saponin formulation and incubated for 24 h.. Following the incubation, macrophages cells were stained with FITC conjugated antibodies against CD80 and CD163 and analyzed by flow cytometry. Additionally, treated macrophages cells supernatants were collected and IFN-γ, IL-1β, TNF-α, IL4 and TGF-β cytokines levels were determined with ELISA. According to the results, β-glucan and Cephalaria spp. saponin formulations were synergisticly increased the immunomodulation by regulating the Th1/Th2 response balance.

Keywords: Adaptive immunity, innate immunity, immune regulation and therapy

P-0261

The TBK-1 antagonist BX-795 differentiates T cells towards a Th-IL-2 phenotype and alleviates type 2 immune responses *in vitro* and *in vivo*

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Interleukin-2 has been shown to be highly important for Treg polarization and function *in vivo*. Identification of small molecules that interfere with T lymphocyte signaling pathways and have IL-2- and Treg-inducing potential. Analysis of changes in secreted cytokine levels, expression of activation markers and transcription factors of T cells upon stimulation in the presence or absence of small molecule inhibitors by cytokine multiplexing and flow cytometry. Gene expression profiles were determined in sorted allergen-specific T cells by RNA-seq. Effects of treatment with small molecules were studied in mouse models of allergic asthma. BX-795 increased IL-2 mRNA and secreted levels of IL-2 in Jurkat T cells, human PBMCs and allergen-specific mouse T cells upon TCR-dependent stimulation. Additionally, BX-795 inhibited Th2 cytokine secretion by human PBMCs and murine allergen-specific T cells. RNA-seq analyses revealed close similarity between BX-795 differentiated allergen-induced T cells and TGF-β differentiated allergen-induced iTregs. Compared to iTregs, BX-795-induced T cells did not express the Treg signature transcription factor Foxp3 but overexpressed IL-2 and were therefore named Th-IL-2 cells. Increased IL-2 was relevant for elevated adenosine-generating 5'-nucleotidase CD73 expression which was shown to contribute to Treg function. When applied locally *in vivo*, BX-795 reduced allergen-extract-induced IL-13$^+$T cells, CD4$^+$GATA3$^+$ T cells and eosinophils in two lung models of allergic asthma. BX-795 potently increases IL-2 secretion and downregulates type 2 inflammation *in vitro* and *in vivo*. Thus, BX-795 and analogues thereof may be useful for the treatment of type 2 inflammatory disorders such as asthma.

Keywords: Adaptive immunity, allergen-induced immune responses, cytokines and mediators, modification allergic responses, regulatory cells, RNAseq

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POSTER PRESENTATIONS

P-0262

Effects of immunosuppressive treatment on thymocyte maturation in Myasthenia gravis patients

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Myasthenia gravis (MG) is an autoimmune disease with antibodies against acetylcholine receptor (AChR). T cell dependent antibody production and thymic pathologies are associated with MG. Immunosuppressive treatment (IS) have profound effects on thymus. The aim of the study was to elucidate the effects of IS on the development of thymic dysregulation in MG. Thymocytes were isolated from thymi of AChR positive MG patients (AP) with/without (P/N) IS treatment (23 APP, 27 APN) and MG patients with thymoma (TAMG, 13 TAMGP, 14 TAMGN). Isolated cells were analyzed by flow cytometry and compared. With the effect of IS treatment, CD4+CD8+ double positive (DP) T cells were decreased both in APP (49.8% vs. 7.6% p<0.0001) and TAMGP (35.4% vs. 18.1%, p= 0.029). In contrary, CD4+CD8- single positive (SP) T cells in APP (35.4% vs. 25.9%, p= 0.003) and CD4-CD8+ T cells in TAMGP (28.0% vs. 14.0%, p= 0.003) were increased. CD25 expression was induced by treatment in APP on thymocytes (6.6% vs. 1.7%, p= 0.046) and on double negative (DN) T cells (14.2% vs. 9.2%, p= 0.017) and this effect is not detected in TAMG. CD27+ thymocytes were also higher in APP than APN (50.1% vs. 25.8%, p= 0.01). In TAMGP, CD8+ SP T cell proportion was higher than TAMGN (62.0% vs. 34.7%, p= 0.037). Increasing the maturation of thymocytes and abnormal induction of CD25 and CD27 at the different thymocyte maturation stages is associated with IS treatment and may interfere with the abnormal intrathymic T-cell differentiation in MG.

Keywords: Autoimmunity, drugs for immune modulation, thymic selection

P-0263

Florescence based osteoclast development fusion assay and the effect of inhibitors in osteoclast development

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Osteoclasts are specialized macrophages that are critically involved in bone resorption under physiological and pathological conditions, including autoimmune arthritis. The aim of our study was to assess the effect of different RANKL concentrations and various inhibitors on *in vitro* osteoclast development using TRAP staining and fluorescent osteoclast assays. Osteoclasts were differentiated from mouse bone marrow cells in the presence of 50 ng/ml M-CSF and various concentrations of RANKL. The osteoclast development kinetics were visualized with histochemically stained osteoclast-specific TRAP enzyme and the nuclei with Hoechst staining. Also, mTmG and Ctsk-Cre based red-to-green conversion fluorescent osteoclast assays were carried out to test osteoclast-specific gene expression and preosteoclast fusion. We performed numerous quantification approaches to assess various aspects of osteoclast development. Our fluorescent assays sensitively reflected changes in the key osteoclastogenic cytokine RANKL. Green fluorescence was seen at as little as 1-5 ng/ml RANKL, whereas 50 ng/ml RANKL seemed to saturate the assay. The Abl/Src-family tyrosine kinase inhibitor dasatinib, the PLC inhibitor U73122 and the proposed fusion inhibitor lisophosphatidylcholine decreased the formation of mature osteoclasts in both the TRAP staining and the fluorescence-based assays in a concentration-dependent manner. The changes observed in the two assays were mostly parallel and well comparable. Our fluorescence-based assays are capable of detecting very low RANKL concentrations and can be used to replace TRAP-based assays to test the effect of various inhibitors on osteoclast development.

Keywords: Animal models, cellular interactions, drugs for immune modulation, myeloid cells

P-0264

Human blood pDC and cDC2 cross-present antigens from live cells more efficiently than cDC1

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Antigens (Ag) from apoptotic HIV-1-infected cells are cross-presented by human conventional and plasmacytoid dendritic cells (cDC, pDC) to CD8+ T cells. We have previously shown that Ag from live cells were also cross-presented by human monocyte-derived DC (MDDC) and by murine bone-marrow-derived DC, leading to protection of mice from tumoral challenge. We explored the mechanism and tested whether human blood DC would also cross-present antigens from live cells. We used MDDC, FACS-purified DC, anti-Gag77-85 HLA-A2+-restricted CD8+ T cells clones, HLA-A2-HIV-infected H9 (H9HIV) cells, ZVAD to avoid caspase-mediated cell death, saquinavir to inhibit HIV replication. Gag p24 antigen was taken up from live H9HIV cells by MDDC into EEA1+ endosomes within 1 hour, requiring energy and actin polymerization. Gag p24 uptake was faster and higher in cDC1 than in cDC2, and in cDC2 than in pDC. However, HLA-A2+ pDC cross-presented Gag from live H9HIV cells without microbial stimulation (Wilcoxon test vs uninfected H9 cells p=0.016, n=7). Conventional DC2 stimulated by LPS cross-presented HIV-Gag from live H9HIV cells (p=0.03, n=6). Conventional DC1 (n=4) stimulated by poly-I:C sometimes stimulated this cross-presentation, but less efficiently than cDC2 or pDC (median: 3% IFN-γ+ CD8+ T cells, vs 6% and 18%, respectively, Mann-Whitney test p= 0.04). Cross-presentation occurred from live cells, and not after cytotoxicity of DC against H9HIV cells. This cross-presentation from live cells has been underestimated in the past, but it will be further studied as a tool to eradicate HIV reservoirs with low HIV expression levels, or residual and metastatic live tumor cells.

Keywords: Adaptive immunity, antigen processing and presentation, dendritic cells, immunodeficiency

P-0265

IgD-CD27- double negative (DN) B cells of multiple sclerosis patients are mature memory cells that can migrate towards pro-inflammatory chemokines

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Pro-inflammatory age-associated IgD-CD27- double negative (DN) B cells are abnormally elevated in the peripheral blood and cerebrospinal fluid of multiple sclerosis (MS) patients. This study aimed to investigate the developmental and migratory phenotype and function of DN B cells in MS. Expression of developmental markers was determined on DN, IgD-CD27+ class-switched memory (CSM) and IgD+CD27- naive B cells of healthy controls (HC, n=48) and MS patients (n=96) by flow cytometry. Pro-inflammatory chemokine receptors and the transcription factor T-bet, previously described in another pathological age-associated B cell subset, were measured on B cell subsets of HC (n=25) and MS patients (n=49). Using an *in vitro* chemotaxis assay, migration of MS (n=7) B cell subsets was studied. DN B cells are mature antigen-experienced cells as indicated by low CD5, CD10 and CD38 expression, and IgG or IgA expression in the majority of cells. However, IgA+ and activated CD95+ cells were less frequent in DN versus CSM B cells. DN B cells showed the highest T-bet expression and similar expression of chemokine receptors CXCR3 and CXCR5 compared to naive and CSM B cells, respectively. MS DN B cells further showed a high migration capacity towards CXCL10 (CXCR3 ligand) and CXCL13 (CXCR5 ligand) that was similar to CSM B cells. DN B cells resemble CSM B cells but are at an earlier maturation state. Their potential importance in MS pathology was underlined by their migration towards chemokines important for B cell migration through the blood-brain barrier in MS.

Keywords: B lymphocytes, chemokines, multiple sclerosis, neuroimmunology

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POSTER PRESENTATIONS

P-0275

Activation of hematopoietic stem cells by immune stimuli

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Hematopoietic Stem Cells (HSCs) are the source of blood and immune cells. Immunological stimuli may or may not affect HSCs' phenotype, functions, and potency. We had recently demonstrated improved identification of mouse HSCs using combination of surface markers and HSC-reporter mouse Fgd5-mCherry. We further discovered HSC-activation markers, including CD69 and CD317, which were recently confirmed and demonstrated to play a functional role *in vivo*. HSC activation markers reveal they can all sense and respond to immune stimulation rapidly. Surprisingly, however, various types of stimulation are having vastly different impacts, and even lack of impact, on the BM HSCs. We suggest for systemic fit of HSCs response to stimulation with the immunological challenges. We find the defined population of Lineage-cKit+ Sca1+CD150+Fgd5mCherry+ HSCs do not change by numbers or frequencies following acute immune-stimuli. On the other hand, chronic long-term immune stimulation might exerts deleterious effects on HSCs, and predispose toward malignancy. The Fgd5mCherry reporter can help again to better identify HSCs out of the naïve-state. Deficiencies of DNA-repair might gain further clonal-advantage during chronic-stimulation. We also develop specific interest in the impact of chronic bacterial-infection of the gut on the BM-HSCs, revealing non-trivial changes of function and potency. Our data further suggest for possible modulations of our own HSCs – to improve their function throughout life and reduce risks of malignancies.

Keywords: Ageing, immune communication, inflammatory molecules, stem cells, viral infections

P-0286

The core clock protein BMAL1 regulates dendritic cells function by altering cellular calcium to control mitochondrial morphology

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Immune responses to infection and vaccination vary depending on time-of-day. Dendritic cells (DCs) of the innate immune system have a robust molecular clock. Although immune responses to infection and vaccination vary depending on time-of-day, the role in which the DCs molecular clock plays in this process is unclear. We observed that wild type mice immunized with OVA and Pertussis vaccine mount a greater response depending on time-of-day of vaccination. Mice lacking myeloid Bmal1-/- displayed a diminished immune response to this vaccine regime. Bone marrow-derived DCs synchronized to induce circadian rhythms *in vitro* displayed a circadian rhythm in antigen processing, mitochondrial morphology and metabolism. When mitochondria were elongated, this was accompanied by higher mitochondrial metabolism and antigen processing. DCs Bmal1-/- displayed no rhythm in antigen processing, with fragmented mitochondria and consistently lower metabolism. Mdivi-1, a compound which promotes mitochondrial fusion, was able to rescue the deficit in antigen processing in Bmal1-/- DCs. Circadian mitochondrial morphology changes were dependent upon Bmal1 control of calcium localization. The molecular clock causes an increase in mitochondrial Ca at distinct times-of-day to enhance mitochondrial metabolism and antigen processing. FK506, an inhibitor of calcineurin, rescued the deficit in antigen processing in Bmal1-/- DCs. Our results demonstrate for the first time that the DC clock regulates antigen processing in DCs by determining the location of calcium to control mitochondrial morphology and bioenergetics. This discovery provides novel insights into how the DC clock might control infection and provide novel approaches to harness the DC clock to design next-generation vaccines.

Keywords: Adjuvants and vaccines, antigen processing and presentation, dendritic cells, metabolic control of immune responses

P-0325

HIC-1 interactome reveals the molecular mechanism through which it regulates human regulatory T cell differentiation and function

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A transcriptional repressor, hypermethylated in cancer 1 (HIC-1) is involved in several biological processes including tumor repression, immune suppression, embryonic development and epigenetic regulation. In the previous study, we have proposed and demonstrated that HIC1 is an important contributor to regulatory T cell development and function. However, a mechanism by which it regulates Treg development is poorly understood. In this regard, we present the systematic characterization of the HIC-1 interactome by affinity-purification mass spectrometry in regulatory T cells. Further, we report for the first time that HIC1 is a part of a protein complex that regulates Treg signature genes and is indispensable for the suppressive function of FOXP3+ regulatory T cells. Other HIC-1 interacting proteins were linked with protein transport, mRNA processing, and ncRNA transcription evaluated by gene ontology studies. Altogether, interaction network highlights the essential role of HIC1 in the development and suppressive function of regulatory T cells.

Keywords: Autoimmunity, inflammatory disease, mass spectrometry, regulatory cells

P-0331

Tocilizumab modulates activation and homing receptor of peripheral memory B cell subsets in rheumatoid arthritis patients

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Memory B cells have been shown to play important role in the pathogenesis of rheumatoid arthritis (RA). With the emergence of B cell targeted therapies, the modulation of memory B cells seems to be a key target. Human peripheral memory B cells can be distinguished by the phenotypic expression of CD27 and IgD defining the three major B cell subpopulations: CD27+IgD+ pre-switch, CD27+IgD- post-switch and CD27-IgD- double negative memory B cells. We evaluated different memory cell populations for activation (CD95 and ki-67) and homing (CXCR3/4) expressions in active RA and under IL6-R blockade by Tocilizumab. Memory B cells were analyzed from RA patients at baseline, week 12 and week 24 under tocilizumab treatment. The phenotypically analysis of RA patients (n=80) compared with healthy donors (n=40) indicated that the memory B cells are activated and have a higher expression of CXCR3. Surface and intracellular staining of B cells showed a significantly higher percentage of CD95 (p=0.01), ki-67 (p=0.03) and CXCR3 (p= 0.03) expression in RA. CD95 & ki-67 expressions were highest in post-switch memory B cells while CXCR3 expression was highest in pre-switch. Based on our findings, we conclude that the three major peripheral memory B cell populations, pre-, post-switch, and double-negative B cells, are activated in RA, demonstrating enhanced CD95 and Ki-67 expressions, and varied expression of CXCR3 and CXCR4 chemokine receptors when compared with healthy individuals. This activation can be efficaciously modulated under cytokine inhibition *in vivo*.

Keywords: Autoimmunity, B lymphocytes, biomarkers, chemokines

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POSTER PRESENTATIONS

P-0388

Immune checkpoint blockade limits the immunosuppressive effects exerted by key features of the hostile tumour microenvironment on T cells in oesophageal adenocarcinoma

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This study aimed to investigate the effect of the hostile tumour microenvironment(TME) including acidity, nutrient deprivation and hypoxia on T-cell anti-tumour immunity in oesophageal adenocarcinoma(OAC) and whether immune checkpoint blockade(ICB) could attenuate these immune-inhibitory effects. PBMCs isolated from treatment-naïve OAC patients were activated for 7-days with anti-CD3/28 and subsequently cultured for 24h under hypoxia, serum-deprivation, glucose-deprivation or acidosis(n=6). Immunophenotyping was conducted by flow cytometry and included assessment of T-cell activation markers (CD69,CD27), ICs(PD-1,TIGIT,TIM-3,CTLA-4,LAG-3,KLRG-1,PD-L1,PD-L2), T-cell cytokine profiles(TH1:IFN-γ,TNF-a,IL-2, TH2:IL-4, and Treg:IL-10) and T-cell subsets (naïve, effector and central memory) with/without nivolumab and ipilimumab. Dual hypoxia and glucose-deprivation or acidic conditions upregulated CTLA-4 on T-cells. However, combination hypoxia and serum-deprivation significantly upregulated A2aR whereas, acidic conditions decreased A2aR expression on T-cells *ex vivo*. Dual hypoxia and glucose-deprivation and acidosis decreased the frequency of central memory T-cells. Under acidic conditions but not hypoxia/nutrient deprivation ICB attenuated this reduction in frequencies of central memory T-cells *ex vivo*. Hypoxia enhanced the production of IFN-γ and IL-10 by T-cells and nivolumab treatment decreased the production of IL-4 under normoxia and hypoxia *ex vivo.* ICB increased T-cell production of IFN-γ under moderately acidic conditions (pH 6.6) but not severe acidic conditions (pH 5.5) and decreased IL-10 production by T-cells under severe acidic conditions. ICB skewed T-cell cytokine profiles toward an anti-tumour phenotype under conditions reflective of a hostile TME, highlighting the ability of ICB to help T cells overcome the immunosuppressive effects of the TME and their promising potential to combine with standards of care in OAC.

Keywords: Adaptive immunity, cancer immunology, checkpoint inhibition

P-0424

Aging promotes mast cell activation in atherosclerosis

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Aging is an independent and dominant risk factor for atherosclerosis. In atherosclerosis, mast cells accumulate upon disease progression and are associated with plaque instability. However, it is currently unknown whether the age-induced pro-inflammatory environment affects mast cell phenotype in the atherosclerotic plaque. Age-induced phenotypic changes were investigated in human and mouse (8-12 weeks vs. 78-86 weeks male LDLr-/-) models of atherosclerosis, both *in vitro* and *in vivo*. We detected an elevated basal activation status in aged bone marrow-derived mast cells (young: 0.13±0.006% vs. old: 3.98±0.1% $CD63^+$ mast cells; P=0.05), indicative of intrinsic age-associated alterations. Using flow cytometry, we found a 2.1-fold increased number of IgE^+CD63^+ mast cells in the aged atherosclerotic aorta (young: 37.5±12.5% vs. old: 79.1±2.1%; P=0.007), suggesting that the classical IgE pathway is the most prominent mechanism of activation. Furthermore, we demonstrated an increased number of $MHCII^+$ mast cells in the aged atherosclerotic aorta (young 0.9±0.3 vs. old 46.9±10.8; P=0.004). Single-cell RNA sequencing showed mast cell-specific expression of *H2-Ab* in aged mouse atherosclerotic aortas and of *HLADR* in human plaques. *In vitro*, aged mast cells induced a significantly increased proliferation of OTII $CD4^+$ T cells upon ovalbumin treatment (young: 35.2±2.7% vs. old: 45.8±2.6% proliferated $CD4^+$ T cells; P=0.0002), further indicating that mast cells may act as antigen-presenting cells in the aged plaque. In this study, we identified age-induced phenotypic changes of mast cells, regarding both activation status and potential antigen presenting capacity. Targeting the aged mast cell could become a promising therapeutic intervention to overcome future clinical events.

Keywords: Ageing, antigen processing and presentation, cardiovascular diseases, mast cells

P-0434

The role of PIM kinases in human Th17 cell regulation

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The family of serine/threonine-specific proviral integration site for moloney murine leukemia virus (PIM) kinases consists of three members (PIM1, PIM2, PIM3) that are abnormally expressed in cancers, especially in hematologic malignancies. Due to their importance in cancer progression, PIM kinases are a promising target for antitumor drug discovery. To overcome drug resistance and improve tumor control, a combination of PIM inhibitors have been developed. However, their effects on the immune microenvironment, and how PIM kinases participate in immune regulation remain poorly understood. We have previously reported that PIM genes promote human Th1 cell differentiation through the IL12/STAT4-pathway and upregulation of Th1 specific key regulators. However, the role of PIM kinases in human Th17 cell regulation has yet to be studied. In this study, we used a CRISPR/Cas9-based knockout approach to investigate the role of PIM kinases in transcriptional gene regulation during early human Th17 cell differentiation. Moreover, using an *in vitro* kinase assay followed by mass spectrometry, novel substrates of PIM kinases in Th17 cells were screened. Our results revealed the upregulation of all three PIM kinases in Th17 cells, differentiated from naïve human CD4+ cells. In addition, over 30 phosphorylated protein targets of PIM kinases were uniquely associated with the Th17 cells. Simultaneous knockout of all three PIM kinases, using the CRISPR/Cas9 approach, lead to impaired Th17 cell function. These results indicate that PIM kinases regulate human Th17 cell differentiation, thus contributing to the selective development of human T cell subsets.

Keywords: Immune regulation and therapy, cancer immunology, molecular immunology

P-0437

Memory-like responses in human dendritic cells

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Immunological memory is a hallmark of adaptive immunity, but recently, enhanced and attenuated recall responses were also discovered in innate cell populations including monocytes, macrophages and natural killer cells. Although dendritic cells (DCs) link innate and adaptive immunity due to T cell activation, induction of memory-like responses in human DCs has received much less attention. To investigate if human monocyte-derived (mo-DCs) and blood DCs display memory-like responses *in vitro* and if retinoic acid (RA) is capable of hampering subsequent functional properties of DCs. Secondary responses in DCs were investigated at the epigenetic, metabolic and protein level using chromatin immune precipitation and ELISA. We show that monocytes priming with β-glucan mediates enhanced secondary responses in mo-DCs, while priming of monocytes or blood DCs with a secretome from the gut bacterium *Lactobacillus (L.) reuteri* induces tolerance. RA does not affect recall functional properties of neither mo-DCs or blood DCs, however, it reduces overall pro-inflammatory cytokine production and histone modifications enrichment at the promoter of distinct genes in mo-DCs when monocytes are primed with *L. reuteri*-CFS. Moreover, we found that the supernatant from *L. reuteri*-primed mo-DC cultures skews T helper cell differentiation pathways. In summary, we demonstrate that apart from classical training agent β-glucan, a gut commensal-derived secretome induces DC tolerance, which could be beneficial where excessive inflammatory responses need to be dampened.

Keywords: Dendritic cells, innate host defence, innate immunity, memory

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POSTER PRESENTATIONS

P-0439

Postnatal development of lymph node stromal cells revisited

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Lymph nodes (LNs) provide the infrastructure and microenvironment to tissue-specifically tailor immune responses. The induction of peripheral tolerance towards food and commensal antigens mainly takes place in gut-draining LNs. We previously reported that the tolerogenic properties of gut-draining mesenteric LNs (mLNs) are stably imprinted in their stromal cells by microbiota within the first ten days after birth. With the advantage of scRNAseq, we could further assign these tissue-specific tolerogenic features imprinted during the neonatal phase to CD34-expressing fibroblastic stromal cell (FSC) subsets. Accordingly, CD34highFSCs were exclusively found in mLNs, and not in skin-draining LNs (pLNs). Here, through detailed kinetic analysis of mLN ontogeny we further revealed that mLN stromal cells are highly proliferative during the neonatal phase, highlighting the opportunity for commensals to impinge onto epigenetic modifications at an early developmental stage. In addition, we identified transcriptional changes in LN FSCs driven by the absence of microbiota on a single-cell level. Remarkably, the frequency of CD34highFSCs drastically increases post birth, but undergoes massive reduction after weaning, while the expression of other location-specific transcriptional signatures (*Cd55, Ly6C, Timd4, MHCII*) remains largely unchanged during mLN ontogeny. To more easily dissect the postnatal development of stromal cells and to explore how neonatal LN stromal cells respond to inflammatory perturbations, we are currently establishing postnatal LN organ cultures (PNLNOCs). Overall, a better understanding of the changes initiated in LN FSCs through the microbiota and inflammatory cues will further elucidate their role in orchestrating immunity.

Keywords: Immune development, lymphoid organs, microbiome and environmental factors

P-0440

Fantastic three: the role of CD16, NKG2D and Nkp46 as ''master regulators'' of NK cell activation

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The activation of NK cells depends on the shift in balance of signals from inhibitory and activating receptors, in favor of the latter. In contrast to most other receptors on these cells, for the ligands of NKG2D, NKp46 and CD16 no inhibitory receptor is known to be expressed on NK cells. Both NKG2D and Nkp46 recognize stress-induced ligands and play an important role in the fight against different types of infections and tumors, whereas CD16 recognizes Fc tails of cell-bound antibodies and is responsible for induction of antibody-dependent cellular cytotoxicity (ADCC). We hypothesize that these three receptors are ''master regulators'' of which a signal is requirerd in order to gain maximal NK cell reactivity. Our preliminary results endorse our hypothesis, as mice lacking both NKG2D and Nkp46 showed reduced survival in murine lymphoma model in comparison to wild type animals. Mice lacking both NKG2D and antibodies, and therefore ADCC, showed further reduction of survival compared to animals lacking NKG2D alone in the B16 model of murine melanoma. This indicates that Nkp46 and CD16 are required for control of B16 by NK cells. Mice lacking all three of these receptors show increased expression of DNAM-1 and reduced expression of TIGIT receptor, probably as form of compensating mechanism. In this project, we want to further elucidate how the lack of NKG2D, NKp46 and CD16 influences effector functions of NK cells and their ability to fight tumors and viral infection. This project is supported by Croatian Science Foundation, project UIP-2019-04-9390.

Keywords: Animal models, biology of the immune system, *in vivo* tumor models, innate immunity, innate lymphoid cells, NK cells

P-0453

Investigating the role of the complement system in the radioresistance of rectal cancer

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Poor pathological response to neoadjuvant chemoradiation therapy (neo-CRT) is a clinical problem in rectal cancer. There is a need to determine molecular factors influencing response to neo-CRT. Evidence supports a role for the complement system in tumourigenesis and therapeutic response in cancer. Radiosensitivity of colorectal cancer (CRC) cell lines (HCT116, SW837, HRA-19, SW1463) was assessed by clonogenic assay. Complement gene expression was assessed by qPCR. Complement protein and anaphylatoxin production was assessed by ELISA. Expression of complement regulatory proteins and receptors was determined by flow cytometry. Circulating C3a levels were assessed by ELISA (n=39). HRA-19 cells are significantly more radioresistant compared to SW837 and HCT116 cells, whilst HCT116 cells are the most radiosensitive. Complement proteins (C3, C5) and anaphylatoxins (C3a, C5a) were produced by CRC cells, with significantly lower levels produced by HCT116 cells. Complement protein production positively correlated with surviving fraction at a clinically-relevant dose of 1.8Gy X-ray radiation. CRC cells expressed complement regulatory proteins (CD46, CD55, CD59) and receptors (C3aR, C5aR). C3a levels were elevated in pre-treatment sera from rectal cancer patients with a subsequent poor pathological response to neo-CRT, when compared to those with a good response (p=0.039). Complement is produced by CRC cells, with increased gene expression and protein levels associated with radioresistance. Increased C3a levels in pre-treatment sera was associated with subsequent poor responses to neo-CRT in rectal cancer, suggesting complement plays a role in the radioresponse. This highlights complement as a potential biomarker predicting response to neo-CRT in rectal cancer.

Keywords: Cancer immunology, complement, innate immunity

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POSTER PRESENTATIONS

P-0466

Non-canonical human CD45- and CD56+ bone marrow plasma cells contribute to systemic IgG production

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Specific serum antibody titers are regulated at the level of plasma cells (PC) residing in the bone marrow (BM). We previously showed that human BMPC comprise a static CD19- subset, potentially containing long-lived PC. We here characterize novel non-canonical human PC independent of CD19- PC, i.e. PC lacking CD45 or expressing CD56 as an indication of a high degree of PC maturation. These subsets comprise approximately 35% and 20% of total BMPC (N=19), and were significantly enriched among CD19- BMPC. High intracellular expression and secretion of immunoglobulins confirmed their PC identity and revealed their enrichment for IgG expression, underlining their relevance for systemic immunity. CD19- CD56+ BMPC transcriptomes were enriched for genes encoding cell adhesion molecules (e.g. ITGA8, CD276) and mediators of autophagy (e.g. LAMP3, SORT1), suggesting improved viability and tissue retention of this subset. Immunohistochemical studies demonstrate heterogeneous spatial organization of PC in human BM. 40% reside in perivascular clusters (N=8), and perivascular clusters were significantly enriched for CD45- BMPC. By contrast, only very few, if any, circulating plasmablasts lack CD45 or expressed CD56, suggesting that CD45- and CD56+ PC phenotypes are acquired in situ and do not represent recently immigrated PC. Our data establish an unexpected phenotypical heterogeneity of human BMPC that is associated with PC localization, IgG secretion, and traits of tissue retention and unfolded protein response stress resilience. These findings are consistent with the differential regulation of serological memory on the levels of phenotypically distinct PC subsets.

Keywords: Adaptive immunity, antibody, B lymphocytes

P-0506

An integrative, multi-modal approach to the human thymus and self-tolerance

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Thymic T cell development is highly dependent on spatial context and on cell-to-cell signalling. Immature T cells migrate through distinct thymic microenvironments in a programmed pattern, encountering signalling cell populations at specific developmental time points. Epithelial and antigen-presenting cell populations mediate stringent selection checkpoints in order to prevent egress of potentially self-reactive T cells to the periphery. Impairment of this process is implicated in autoimmune disease. We aim to expand current knowledge regarding human thymic cell populations, and have performed CITE-seq (N=5) and ATAC-seq (N=5) on paediatric thymic samples at single cell resolution. In addition to full cell suspensions, we have optimized enrichment protocols in order to cover scarce populations with important antigen-presenting functions. The resulting data include, but is not limited to, thymocytes at distinct developmental stages, B cells, fibroblasts, endothelial cells, and several subpopulations of dendritic and epithelial cells. We confirm previous findings, such as the presence of myeloid and neuroendocrine epithelial cells, and XCL1 expression by GNG4$^+$ CD8αα cells. CellphoneDB predicted interaction between CLEC9A$^+$ dendritic cells and GNG4$^+$ CD8αα cells through XCR1-XCL1. We also observe expression of Autoimmune Regulator (AIRE), a transcription factor inducing expression of tissue-restricted antigens in medullary thymic epithelial cells, in a small subset resembling activated dendritic cells. As structural and functional differences between thymic compartments are highly relevant for successful thymocyte selection and maturation, we are currently expanding our dataset to include spatial transcriptomics. We expect this integrative, multi-modal approach to provide novel insights into the establishment of self-tolerance during T cell development.

Keywords: Antigen processing and presentation, autoimmunity, cell signalling, immune development, thymic selection

P-0551

Exploration of innate T-cells in the Multiple Myeloma tumoral niche

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Multiple myeloma (MM) is an uncurable plasma cell malignancy. Restoration of immune surveillance could lead to deep and sustained responses. Yet, the interactions between tumor plasma cells and the bone marrow (BM) microenvironment has to be further elucidated. In addition to the traditional immune system components, cancer immune surveillance involves innate T-cells, including iNKT cells and innate (panKIR(+)NKG2A(+)) CD8 T-cells. Recently, we have shown that iNKT cells and innate CD8 T-cells, are involved in the anti-tumoral response in chronic myeloid leukemia and solid tumors. Here, we sought to explore innate T-cells (MAIT, γδ$^-$T cells, innate CD8 T-cells) in MM tumoral niche and analyze their exhaustion mechanisms. Using spectral flow cytometry, we analyzed innate T-cell subsets in BM samples from MM patients at diagnosis or relapsing and from healthy donors (HD). All innate T-cell subsets analyzed are constitutively present in the BM microenvironment of MM patients. No difference in subset frequencies between HD and MM BM samples is observed except for γδ$^-$T cells, the frequency of which is decreased in MM at diagnosis. CD69, an early marker of lymphocyte activation and tissue residency, is overexpressed in all the innate T-cell subsets at diagnosis, with a diminution at relapse. Expression of exhaustion markers, PD-1 and TIGIT, is higher in γδ$^-$T cells from MM patients, especially at relapse. These results suggest that innate T-cells are resident cells in the BM niche of MM and raise the question of the particular role of γδ$^-$T cells in this niche.

Keywords: Cancer immunology, gamma-delta T cells, microenvironment, monitoring immunity

P-0572

Cell population data and serum polyclonal immunoglobulin free light chains in the assessment of COVID-19 severity

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A challenge for the healthcare system is to distinguish between severe and non-severe COVID-19 to provide sufficient help for patients and keep adequate efficiency. The aim of this study was to assess the usefulness of complete blood count parameters together with analysis of free light immunoglobulin chain serum concentration in risk stratification of COVID-19. CBC was analyzed in 735 COVID ICU, COVID nonICU, and nonCOVID ICU. Free light immunoglobulin chain concentration was analyzed in the group of 133 COVID ICU, COVID nonICU, and nonCOVID ICU hospitalized patients included in the study. COVID ICU patients had the biggest size, granularity and nucleic acid content in neutrophils and lymphocytes comparing with other groups. Significant difference in the concentration of κ and λ FLC was shown between SARS-CoV-2 infected and uninfected patients hospitalized in intensive care units. However, no difference was found in the κ/λ ratio between these two groups, and the ratio stayed within the reference value, what indicates the presence of polyclonal FLC. Significant difference in λ and κ/λ ratio between COVID nonICU and nonCOVID ICU was found. Free light chain κ measurement has significant power to distinguish between severe COVID-19 and non-severe COVID-19 (AUC=0.7669), with a sensitivity of 86.67% and specificity of 93.33%. The odds ratio for κ coefficients was also estimated for 3.0401. It can be concluded that the parameters obtained from as well as the measure of free light immunoglobulin concentration in serum are useful in determining between severe and nonsevere COVID-19.

Keywords: Antibody, granulocytes, infectious disease, inflammatory disease, viral infections

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POSTER PRESENTATIONS

P-0589

The role of SORLA in B cell antigen trafficking

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Antigen presentation by B cells is critical for priming a robust immune response to vaccination or infection. B cells internalise, process and load antigen onto MHC-II for presentation to cognate T cells. This interaction induces B cell somatic hypermutation and class-switching to create a high-affinity antibody repertoire. B-cell receptor (BCR)-antigen complex internalisation is complemented by a signalling cascade of phosphorylation and ubiquitylation leading to B cell activation. The mechanistic details of BCR-antigen internalisation have yet to be elucidated. Understanding this basic biology will offer insights into B cell responses to infection, malignancy, and autoimmune disorders associated with dysregulated B cell antigen presentation. A recent CRISPR knockout screen for BCR internalisation has highlighted a potential new role for the sorting and trafficking receptor, SORLA. SORLA is best described in neurons where it traffics amyloid precursor protein into recycling pathways, with mutations in the SORL1 gene associated with Alzheimer's disease. Using CRISPR-mediated knockout of SORL1 we have confirmed the BCR-antigen internalisation defect in Ramos B cells. In contrast, SORLA deletion did not affect uptake of transferrin receptor, highlighting receptor-specific mechanisms. We have also shown SORLA localises to endocytosed antigen clusters within 5 minutes of antigen binding. Additionally, SORLA interacts with other trafficking proteins, several of which undergo post-translational modification upon BCR antigen binding. Examples include GGA2 and PACS1, which are phosphorylated during BCR signalling, and control trafficking between trans-Golgi networks and endosomes. In conclusion, we report a novel role for SORLA-mediated BCR trafficking during B cell activation.

Keywords: Adaptive immunity, antigen processing and presentation, B lymphocytes, cell signalling

P-0619

Neutrophil subsets in ANCA vasculitis and Covid-19: a disease specific response

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A population of granulocytes appear in the PBMC layer of density separated blood and are termed Low Density Granulocytes (LDGs). These are seen in many conditions including cancer, sepsis, autoimmunity and pregnancy. We previously identified LDGs in acute and remission ANCA vasculitis (AAV) and hypothesise that these LDGs are also present in Covid-19 (C-19) and our aim is to phenotype these cells and determine whether LDGs are a disease specific cellular response to inflammation. Of particular interest is the expression of intracellular Arginase 1 (Arg-1), an enzyme linked to T cell suppression in many disease situations. LDGs were isolated using a modified percoll preparation and analysed by both traditional and imaging flow cytometry, in patients with active and remission ANCA vasculitis, in patients with severe moderate and mild C-19 and in healthy controls. The phenotyping panel included CD14, CD15, CD16, CD10, CD33, CD62l. Intracellular Arg-1 was stained following permeabilisation with saponin. We identified extensive populations of LDGs in both AAV and Covid-19 peripheral blood. LDG levels are associated with disease severity. Arginase 1 is differentially expressed in neutrophils from AAV and C-19. In C-19 Arginase levels are correlated to disease severity suggesting that Arginase release may be associated with favourable outcome. Interestingly, all neutrophil fractions show lower levels of Arginase in C-19 patients whereas in AAV only LDGs have lower levels. Healthy controls have high Arginase expression. Neutrophil subsets display disease specific responses in C-19 and AAV demonstrating their plasticity in inflammatory settings and warrant further investigation.

Keywords: Autoimmunity, granulocytes, innate immunity, myeloid derived suppressor cells, neutrophils, viral infections

P-0624

Metabolic alterations in synovial fibroblasts underlies disease pathogenesis in rheumatoid arthritis

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Cellular metabolism has been studied in fibroblast-like synoviocytes (FLS) of rheumatoid arthritis (RA) and osteoarthritis (OA) patients and raises the question whether observed metabolic alterations appear in response to chronic inflammation or whether primary changes in cellular metabolism might underlie disease pathogenesis. Do we observe metabolic changes in FLS already before onset of clinical disease? We included individuals with arthralgia who were autoantibody positive but without any evidence of arthritis (RA-risk individuals), RA patients, OA patients and seronegative controls. All individuals (n=12) underwent mini-arthroscopic synovial tissue sampling of a knee joint. Subsequently, FLS were cultured from collected synovial tissue biopsies. Cellular metabolism was assessed by the XFe96 Analyzer and mitochondrial β-oxidation was measured using tritium-labelled oleate. Basal respiration is decreased in FLS from RA-risk individuals, RA and OA patients compared with FLS of seronegative controls. Cellular respiration of all FLS largely depended on fatty acid oxidation, whereas glucose is only highly used by RA-FLS. Mitochondrial β-oxidation is impaired in RA-risk individuals and RA patients compared with FLS of seronegative controls. In this exploratory cohort, metabolic alterations are already detected in FLS from RA-risk individuals compared with seronegative controls, suggesting that these alterations start before clinical manifestation of disease and contribute to disease pathogenesis.

Keywords: Inflammatory joint diseases, metabolic control of immune responses, rheumatoid arthritis

P-0628

Rationalized approach to T cell activation - directional magnetic bead-antibody coupling technique

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Hybridoma technology is a commonly used approach for monoclonal antibody production. This system has multiple drawbacks, including genome instability, inconsistent expression and limited possibilities for molecule modifications. Recombinant system has been used as an alternative approach enabling optimization of expression and providing vast opportunities for antibody modifications. To activate and expand T cells, hybridoma produced mouse anti-CD3 and anti-CD28 antibodies are coupled to magnetic beads. Current methodology of antibody conjugation lacks specificity: random protein orientation on the bead surface renders part of immunoglobulins unable to bind their targets. As a result, excess of antibodies must be used to warrant the final product functionality. To address these concerns, hybridoma anti-CD3 and anti-CD28 antibodies were converted to recombinant format. Variable domains were fused with mouse γ1 heavy or κ light chain constant domains via overlap extension PCR. Recombinant antibodies were expressed in ExpiCHO™ cells and shown to maintain their functional activity. Next, SpyTag/SpyCatcher technology was applied to ensure proper molecule orientation on magnetic beads. SpyTag sequences were introduced into the C-terminus of antibody Fc domains. These tags form a spontaneous covalent bond with SpyCatcher coupled to magnetic beads, ensuring directional immobilization of antibodies. Functional testing of antibody-bead conjugates showed that tagged versions had matching cell activation capacity compared with hybridoma produced immunoglobulins. Application of the SpyTag/Spy Catcher system in protein conjugation provided a more controlled platform for surface functionalization. This research has demonstrated how recombinant systems enable implementation of selected improvements of antibody characteristics that would not be achievable using hybridoma systems.

Keywords: Antibody, cell based therapies, engineering of antibodies and nanobodies

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POSTER PRESENTATIONS

P-0659

Salmonella lipopolysaccharide specifically diminishes pro-B cells and CXCL12-expressing stromal cells in the bone marrow

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Salmonella enterica serovar Typhimurium is a gram-negative bacterial pathogen and causes serious infectious disease in animal and human. However, no vaccine against the bacteria is currently available, suggesting that Salmonella escape from humoral immunity. We here show that lipopolysaccharide (LPS) from Salmonella interferes the development of humoral immunity. We have first analyzed the immediate effect of Salmonella LPS on the several stages of B cell development. Salmonella LPS, but not E. coli LPS, specifically reduced the number of IgM^-IgD^- but not IgM^+IgD^- and IgM^+IgD^+ B cells in the bone marrow, in particular pro-B cells in a TNFα-independent manner. Furthermore, Salmonella LPS induced the specific diminishment of CXCL12-expressing stromal cells in the bone marrow in a PI3K-dependent way. Since Salmonella LPS directly failed to kill pro-B cells, the diminishment of pro-B cells is likely caused by the depletion of pro-B cell-supporting niches, CXCL12-expressing stromal cells. These data indicate that Salmonella, but not E. coli, interferes the residence of a subpopulation of mesenchymal stromal cells, inhibiting the provision of a humoral immunity's source.

Keywords: Adaptive immunity, B lymphocytes, bacterial infections, infectious disease

P-0710

Distinct regulation of NK cell-mediated cytotoxicity by regulatory B lymphocyte subpopulations isolated from patients with chronic lymphocytic leukemia

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Regulatory B lymphocytes (Breg) maintain pathological immune tolerance in chronic lymphocytic leukemia (CLL), and, simultaneously, NK cell dysfunction is often observed. To further characterize the potential interplay between these immunocytes, we isolated by FACS $CD24^{hi}CD27^+$ and $CD24^{hi}CD38^+$ subpopulations of $CD19^+$ B lymphocytes from peripheral blood mononuclear cells of CLL patients and healthy donors. To assess the immunosuppressive properties of these primary Breg subpopulations we co-cultured them with NK-92 cells for 24 h and then added these NK cells to pre-seeded breast cancer cells (MCF7). The influence of Breg subpopulations on NK-mediated cytolysis was dynamically monitored using the xCELLigence real-time cell analysis system, with simultaneous evaluation of IL-10 production by Bregs. Both subpopulations of Bregs from CLL patients demonstrated significant suppression of the NK-mediated cytolysis of cancer cells, albeit activated $CD24^{hi}CD27^+$ cells secreted more anti-inflammatory IL-10 than $CD24^{hi}CD38^+$ cells. Our results suggest that $CD24^{hi}CD38^+$ Bregs may perform their immunosuppressive function during CLL progression not only through the secretion of the major anti-inflammatory cytokine but via other mechanisms such as direct contact-dependent inhibition. This work is supported by the Russian Science Foundation (grant #21-74-00106).

Keywords: B lymphocytes, cancer immunology, cellular interactions, NK cells, regulatory cells

P-0723

Identification of immunomodulatory properties of Pyrazinib, a novel small molecule analogue of Quininb, with metabolic reprogramming abilities, and its potential as an adjuvant to immunotherapy in Oesophageal Adenocarcinoma

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The incidence of oesophageal adenocarcinoma (OAC) is increasing rapidly, with a 5 year overall survival rate of ~20%, falling to 3% for patients who are diagnosed in late stages. Therapeutic avenues are limited to chemoradiotherapy and surgery, to which approximately 70% of patients will not respond. Immunotherapy, namely the immune checkpoint inhibitors, has resulted in increased survival across a number of poor outcome malignancies, however in OAC immunotherapy has only been approved in the 3rd/4th line setting. Resistance to immunotherapy is a significant clinical challenge, is multi-faceted and not yet fully understood. One potential mechanism is the suboptimal metabolic environment, which tumour infiltrating lymphocytes (TIL's) face upon migration to the tumour. TIL's compete with tumour cells for nutrients and are subject to nutrient starvation in poorly perfused areas of tissue resulting in diminished anti- tumour immunity. Novel strategies targeting cancer metabolism or bolstering TIL metabolism may improve the efficacy of immunotherapy by promoting anti-tumour immune-metabolic pathways. The small molecule Pyrazinib, previously identified as a radio-sensitising agent in OAC, has metabolic reprogramming properties. Here we screen a panel of Quininb analogues including Pyrazinib, for their effects on T-cell biology. Pyrazinib promotes T cell activation shown by increased activation markers compared to other analogues and to vehicle control, treatment did not affect T cell proliferation. Pyrazinib treatment did not significantly alter the expression of IFN-γ, IL-4, IL-10 or Perforin, however treatment in a direct co-culture model increased the cytotoxic capabilities of patient PBMC's against OAC cell lines, radiosensitive OE33P and radioresistant OE33R.

Keywords: Cancer immunology, checkpoint inhibition, drugs for immune modulation, immunotherapy

P-0737

In vitro analysis of the effect Quinton® isotonic solution on the Treg cell population of psoriatic arthritis patients

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Immunonutrition includes different aspects related to Nutrition, Immunity, Infection, Inflammation and Injury (tissue damage). Quinton® isotonic solution consists of ultrafiltered diluted seawater with a final concentration of 9 g/l of NaCl, obtained from nutrient-rich areas (marine vortices), and containing most of the mineral elements necessary for the proper functioning of the body's cells. Among these bioelements, some minerals of recognized immunomodulatory capacity are particularly noteworthy. Psoriatic Arthritis (PA) can be defined as an inflammatory arthropathy associated with psoriasis, usually seronegative for rheumatoid factor. The aim of this study was to analyse the effect of Quinton® isotonic solution (ISO) on Treg of patients with psoriatic arthritis and healthy controls. Peripheral blood mononuclear cells were isolated by Fycoll-Hypaque. Cells were cultured in different ratios of RPMI:ISO and RPMI:PBS 1X (as control). After 48h CD4, CD25 and FoxP3 expression was analysed. Our preliminary results show that Quinton® solution increases regulatory T cells ($CD4^+CD25^+FoxP3^+$) in healthy individuals and in PA patients. In patients with PA a significant increase is observed for 25% and 50% concentrations. In addition, it is observed that the percentage of $CD4^+CD25^{high}FoxP3^+$ cells in patients with PA is increased at the 50% concentration. In conclusion, Quinton® isotonic solution seems to exert a positive modulation on Treg cells. Further experiments on the effect of this solution on secreted cytokines are being carried out to demonstrate the immunomodulatory effect at different levels, complementing our previous studies showing the effect on cell proliferation, haemoglobin release and cytokines *in vivo*.

Keywords: Autoimmunity, nutrients, regulatory cells

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POSTER PRESENTATIONS

P-0756

Investigating the indirect interactions between synovial fibroblasts and neutrophils in experimental autoimmune arthritis

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Synovial fibroblasts and neutrophils are crucial players in the pathogenesis of rheumatoid arthritis. However, little is known about the relationships between these two cell types, while neutrophils can be found at high numbers in the arthritic synovial fluid, to where they need to migrate through the synovial fibroblast-rich synovial tissue. Here, we investigated the indirect interactions between mouse synovial fibroblasts and neutrophils. Mouse synovial fibroblasts were derived and cultured from the arthritic joints of mice treated with K/BxN serum, while neutrophils were isolated from the bone marrow. Synovial fibroblasts and neutrophils were activated by cytokines, arthritic synovial fluid or the cell-free supernatants of stimulated neutrophils or synovial fibroblasts, respectively. Interactions were tested in the presence of dasatinib, a tyrosine kinase inhibitor mainly targeting the Abl and the Src family kinases. Massive activation could be observed in the superoxide release, intracellular p38 phosphorylation and IL-6 production of arthritic synovial fibroblasts activated by TNFα-, IL-1β- or arthritic synovial fluid-stimulated neutrophil supernatants, pointing at possible indirect interactions between the two cell types. Interestingly, these interferences were not reduced by the treatment with dasatinib. On the other hand, neutrophil migration was augmented by the cell-free supernatants of cytokine-stimulated synovial fibroblasts. Our results indicate that there are bidirectional indirect interactions between arthritic synovial fibroblasts and neutrophils, which seem to be Abl-/Src-independent from the synovial fibroblast side. The identification of the interactions could help us to better understand the pathogenesis of autoimmune arthritis and could lead to the recognition of novel drug targets.

Keywords: Adaptive immunity, autoimmunity, cellular interactions, neutrophils, rheumatoid arthritis

P-0785

Dynamics of tissue seeding and compartment-specific distribution of innate lymphoid cells within the developing and adult mouse CNS

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Innate lymphoid cells (ILCs) are important modulators of tissue homeostasis and the immune response, generally present along different organs including the central nervous system (CNS). ILCs are categorized into five main subsets (cytotoxic NK cells, helper-like ILC1s, ILC2s, ILC3s and lymphoid tissue inducers (LTi)). We have recently reported the presence of CNS tissue resident group 1 ILCs (NK cells, ILC1s, and ex-ILC3s). To elucidate the infiltrating dynamics of group 1 ILCs into the CNS and to determine their distribution and functionality in the developing and adult mouse brain. Lineage-committed ILCs start seeding the CNS at embryonal days E17-E18. ILC numbers increase as the CNS develops (ILC-absolute counts=118.5 cells/brain (E18), 75.54 (P1), 134.4 (P9) and 235,4 (Adult)). ILC seeding occurs in a subset-specific manner. Group 1 ILCs are detected in the CNS prenatally, together with a RORγt$^+$ population (ILC3s and Lti) that disappears during adulthood; while ILC2s arrive after the first week of life. Both in female and male adults, ILCs are present along all brain compartments, but not within the spinal cord. Only NK cells seem to enter the adult CNS from the periphery, meanwhile ILC1s and ILC2s appear as tissue resident cells with low local proliferation (%Ki67$^+$ ILC1s:14.3±4.05% and %Ki67$^+$ILC2s:15.1±1.6, n=3). Within the CNS, ILC numbers progresively increase after first infiltrating the brain early during development as lineage-committed cells and in a subset specific manner. Brain ILCs represent a heterogeneous population of mainly quiescent resident ILCs, with a strict tissue compartmentalization.

Keywords: Immune development, innate immunity, innate lymphoid cells, neuroimmunology

P-0797

Exosomes derived from adipose tissue mesenchymal stem cells decrease TH-17 function in COVID-19 patients

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The study of immune cell responses in the pathogenesis of Covid-19 disease is of particular importance. The production of inflammatory cytokines by these cells and some other cells in the body leads to a cytokine storm. This overproduction of cytokines is responsible for a part of the pathogenesis of a disease called acute respiratory distress syndrome (ARDS), which involves the lung and impairs its function. TH-17 cells are responsible for at least part of the production of cytokines in ARDS. Modulating the responses of these cells can help prevent disease progression. Due to their immunomodulatory properties, mesenchymal stem cells (MSCs) can suppress the inflammatory response in PBMCs isolated from patients with Covid-19. However, due to the prohibitions on the use of cells as a treatment, their exosomes can be used as a substitute. For this purpose, blood samples were first taken from patients with Covid-19, and their PBMCs were isolated. PBMCs were cocultured with MSCs exosomes, and the TH17 cells were evaluated before and after treatment. The expression of the transcription factor, associated cytokines, and their concentration by real-time PCR and ELISA, respectively. Our data revealed that MSCs exosomes considerably influence The expression levels of RORγt ($p < 0.0001$) and IL-17 ($p=0.0044$), and also secretion level of IL-17 ($p=0.0011$) was decreased in the treated group compared to the control group. The current study results indicated that AD-MSCs derived exosomes can decrease the function and cytokine production of TH-17 cells in COVID-19 patient isolated PBMCs.

Keywords: Adaptive immunity, cytokines and mediators, infectious disease, stem cells

P-0798

The Importance of patient clustering by immunophenotyping and biomarker populations in the diagnosis of interstitial lung disease and other complex pulmonary pathologies

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Interstitial lung disease (ILD) comprises a multitude of clinical entities posing significant difficulties regarding a prompt and accurate diagnosis. The continuously evolving classification systems for ILD are supported by ongoing advances in biomarker and predisposing factor research. We developed a model for patient clustering constituting a supportive tool to increase the accuracy of diagnosis in ILD and other complex pulmonary pathologies. The model is based on surface phenotyping of over 40 markers on immune cells isolated from bronchoalveolar lavage (BAL) in combination with extensive clinical data. Based on the marker expression pattern we constructed an immune cell profile for every participant. We then merged the profiles to create a global atlas of the lung microenvironment in various pathologies. The contribution on each participant to the global atlas was assessed with the aid of various dimensionality reduction tools and the ensuing similarity between samples was calculated. We collected 59 consecutive samples from 55 patients undergoing bronchoscopy due to a clinical suspicion or diagnosis of ILD. The most frequent diagnostic group was that of idiopathic interstitial pneumonia (32.2%) and the initial diagnosis was certain in 69.5% of cases. Our model enables two distinct approaches. Firstly, assessing the cell population landscape similarity between patients within a diagnostic group allows rapid identification of outliers, which is particularly helpful for cases with uncertain diagnoses. Secondly, sample clustering is based exclusively on the calculated similarity of the immune cell atlas, thus removing any physician bias and introducing the concept of cellular “nearest neighbour”.

Keywords: Biomarkers, immune networks, immune response tracing, inflammatory disease, visualizing immune responses

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POSTER PRESENTATIONS

P-0805

SARS-CoV-2-specific T cell immunity in pediatric patients with chilblain lesions during the COVID-19 pandemic

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Clinical manifestations associated with SARS-CoV-2 in humans are mainly respiratory and gastrointestinal. However, several publications have described diverse skin manifestations appeared during the pandemic, although the causal association with SARS-CoV-2 has not yet been clearly demonstrated. To evaluate immune response and antigen-specific lymphocyte proliferation against SARS-CoV-2 in pediatric patients with skin manifestations with negative PCR and negative serology against the virus. We prospectively collected clinical and immunologic data of 30 pediatric patients with acral cutaneous lesions during the months of March and April 2020. The SARS-CoV-2 PCR was negative in all cases and serological studies were positive in only one patient. We performed a dye-based proliferation assay to evaluate the Ag-specific T-cell response against SARS-CoV-2. We analyzed 16 patients with skin manifestations and used 7 patients with systemic COVID-19 as positive control and 7 non COVID-19 patients as negative controls. Leucocytes subpopulations were also analyzed by flow cytometry. 13 out of 16 patients (81.3%) showed specific lymphocyte proliferation against SARS-CoV-2 peptides. In the systemic COVID-19 control group, 6 out of 7 patients (85.71%) resulted positive in the proliferation assay whereas in the negative COVID-19 group 2 out of 7 were positive (28.57%). Moreover, cutaneous lesions patients presented a characteristic leucocyte profile with low percentages of Tfh and Th2 and high levels of pDC and CD38+Th cells. The evaluation of specific cellular immune response to SARS-CoV-2 is useful for the diagnostic of patients presenting non-classical COVID-19 symptoms with negative PCR and serology against SARS-CoV-2.

Keywords: Adaptive immunity, dendritic cells, follicular helper T cells, monitoring immunity

P-0816

SARS-CoV-2 epitope-specific CD8+ cells persist 6 month post-infection despite decrease in the overall T-cell reactivity

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T-cells alongside antibodies play a crucial role in viral clearance. Early reports showed that SARS-CoV-2-specifc T-cell responses and antibody levels decreased but were still detected at 6 month after the disease. It remains unclear if the decrease in T-cell reactivity is accompanied by reduction of the number of recognized epitopes. We examined the immune response in 59 COVID-19 convalescent patients. Paired blood samples were collected shortly after infection and approximately 6-month after it. We compared anti-RBD antibody levels and the number of Membrane, Nucleocapsid and Spike-specific T-cells (IFN-γ ELISpot). To evaluate the stability of epitope-specific CD8+ T-cell memory, a panel of 18 MHCI SARS-CoV-2 epitopes were synthesized. Patients were genotyped for HLA and their PMBC from both time points were *in vivo* expanded in the presence of the relevant epitopes. Specific T-cells were further detected by MHC-tetramer staining. As expected, both antibody level and the magnitude of T-cell response diminished over time. 37.5% of donors had no detectable antibodies and 22,5% had no T-cell response to any of all three structural antigens at 6-month post-infection, compared to 12.5% and 2% respectively at the first time point. 7 CD8+ epitopes were immunogenic in 14 donors: 37 total responses, 29 (78,4%) of which were still present at 6 month post-infection. Our data demonstrates that despite the overall decrease of T-cell reactivity most epitopes are still recognized due to the ability of memory SARS-CoV-2-specific CD8+cells to survive over prolonged periods of time.

The work was supported by the Russian Science Foundation grant 20-15-00395.

Keywords: Immune response tracing, infectious disease, memory, monitoring immunity, viral infections

P-0852

Stress and SARS-CoV-2: acute activation of stress response systems to the rescue

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The COVID-19 pandemic strongly affects people with health disadvantages, a population that is also less responsive to vaccination. This may contribute to a long-lasting COVID-19 burden on medical systems and societies worldwide. Stress-related factors such as socio-economic status, prior mental health issues and stress perceived in everyday life may additionally impact infection and severity of disease. Present psychoneuroimmune concepts provide a bio-psychosocial knowledge framework to tackle the question, if stress-associated neuroendocrine-immune mechanisms can possibly contribute to SARS-CoV-2 infections. From this viewpoint, stress may not always be detrimental in that some types of stress could attenuate infection-risk and –progression. We intend to motivate future research efforts to clarify whether neuroendocrine stress-response regulating interventions have the potential to optimize immune responses against respiratory viral infections during and beyond the COVID-19 pandemic. Mediators such as cortisol, (nor)adrenaline, neuropeptides and neurotrophins and how they shape the immune defense against viral diseases will be discussed. Based on this understanding, we describe how psychoneuroimmune knowledge may be used to improve adequate care for COVID-19 and other patients with viral infections.

Keywords: Biology of the immune system, immune communication, neuroimmunology, viral infections

P-0855

Impact of iron overload on the immune and tissue cell crosstalk in skin

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The pathophysiology of chronic venous diseases has been associated with venous hypertension that results in extravasation of erythrocytes, brown colouring of the lower legs due to a local iron-overload in the skin, events that predispose to ulcer formation. Considering little is known on the mechanisms whereby iron affects steady state of skin, which then lead to ulcer, in this project we aim to investigate the pathologic effect of iron overload on resident immune and tissue cell cross-talk. To dissect the response of macrophages to iron-overload we first mimicked erythrocyte accumulation in the skin "*in vitro*" by co-culture of macrophages with autologous erythrocytes. Our results show a clear shift in M2-like macrophages towards a pro-inflammatory phenotype, which was confirmed by TNF, IL6 and IL12 release and by gene expression analysis. Moreover, when we transferred conditional medium of erythrocyte-fed macrophages to fibroblast culture we found an increase in their proliferation accompanied with a decreased gene expression of pro-fibrotic ECM markers. Thereafter, we generated a mouse model of local iron overload in the skin via intradermal injection of iron-dextran. We confirmed that iron induces an increase in immune cells, a pro-inflammatory activation in the skin with a shift in skin resident macrophage subtypes. In addition, we observed increased cellularity of the lower dermis, which we link to an expansion of the fibroblast population in the iron-overloaded skin. Consistently with our "*in vitro*" data, we found ECM genes were downregulated in the dermis, which may explain changes in the skin architecture of these mice.

Keywords: Adaptive immunity, animal models, biology of the immune system, cellular interactions, chronic inflammation and fibrosis, macrophage

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POSTER PRESENTATIONS

P-0858

A novel orthotopic TCR transgenic mouse model dissects the role of antigen receptor specificity for γδ T cell development and function

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γδ T cells are the third lymphocyte lineage undergoing VDJ rearrangement to generate their antigen-receptors. In contrast to B cells and αβ T cells, a conclusive concept of what drives the activation of γδ T cell receptors (TCRs) under physiological conditions remained unresolved for a long time. Recently, a series of publications resolved the unconventional binding modalities of γδ TCRs members to the B7-like family of butyrophilin molecules. Nevertheless, it is unclear how and to what extent the TCR shapes the differentiation and effector function of γδ T cells *in vivo* during steady state and upon activation. Similar questions in the αβ T cell field were addressed by investigating TCR transgenic mouse models. Therefore, we generated a novel mouse model harboring an orthotopic pre-rearranged VDJ cassette within the endogenous TCRD locus using a CRISPR/Cas9 mediated knock-in approach. Interestingly, despite exhibiting a vast increase in γδ T cell numbers, γδ T cell development was conserved in mice expressing semi-invariant γδ TCRs. This independency of the TCR specificity for development and functional programming of γδ T cells was confirmed by using paired transcriptome, TCRγ and TCRδ sequencing on the single cell level. However, in combination with a Vγ1-containing TCRγ, the transgenic TCRδ chain selectively facilitated the rapid activation of γδ T cells upon mCMV infection. Thus, this model provides unparalleled opportunities to dissect the role and function of the γδTCR. By resolving the unparalleled modes of activation of γδ T cells this will ultimately contribute to their application as innovative immuno-therapeutics.

Keywords: Biology of the immune system, gamma-delta T cells, immune development, molecular immunology, thymic selection

P-0895

There Is strength in numbers - episode 2: a comprehensive quantitation of Fc gamma receptors on murine tissue-resident macrophages

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Besides their function as essential mediators of immunological defense mechanisms and their clinically use as therapeutic agents, immunoglobulins are also relevant in various immune-mediated disorders. Many of the biological functions of IgG antibodies depend on their binding to activating and inhibitory Fc-gamma receptors (FcγR). From a qualitative point of view, expression patterns of FcγR on various immunologically relevant cells have been well-characterized, but surprisingly, for a long time only quite limited information about actual receptor quantities was available. Macrophages represent a very important but also very heterogeneous group of highly specialized immune cells. They are involved in detection, phagocytosis, and destruction of pathogens, modulate the activity of other immune cells and represent a bridge between innate and adaptive immunity. Binding of IgG or IgG-containing immune complexes to FcγRs on macrophages mediate diverse effector functions including cytotoxicity, phagocytosis of opsonized targets and cellular activation. In addition, uptake of IgG complexes can modulate antigen processing and presentation, and thereby also affect T-cell responses. In succession of our recent report on how many individual FcγRs are expressed on peripheral blood leukocytes of mouse and man, we here extend these studies to their quantification on tissue resident macrophages in murine liver, lung, kidney, brain, skin, and spleen under steady state conditions. We depict a pronounced heterogeneity between expression patterns of the different tissue macrophages which may reflect their specialized functions at their unique anatomic locations. Concomitantly, we provide strategies for isolation and identification of tissue macrophages and hints on potential pitfalls in their characterization.

Keywords: Animal models, antibody, immunological techniques, innate immunity, macrophage, myeloid cells

P-0898

Cell-mediated and humoral adaptive immune responses to SARS-CoV-2 are lower in asymptomatic than symptomatic COVID-19 patients, both during infection and after recovering

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The characterization of cell-mediated and humoral adaptive immune responses to SARS-CoV-2 in the acute and early convalescent, as well as in recovered individuals, is fundamental to understand COVID-19 progression and the development of immunological memory to the virus. Multiparametric flow cytometric characterization of antigen specific T cells response and Ig specific serum levels were evaluated in 22 SARS-CoV-2 infected patients during the infection phase, in 30 recovered patients after 5 months from SARS-CoV-2 infection and in 15 uninfected healthy controls. We detected T cells reactive to SARS-CoV-2 proteins M, S and N, as well as serum virus-specific IgM, IgA, IgG, in nearly all SARS-CoV-2 infected individuals, but not in healthy donors. More importantly, symptomatic patients displayed a significantly higher magnitude of both cell-mediated and humoral adaptive immune response to the virus, as compared to asymptomatic. We found a heterogeneous magnitude of immunological memory at five months post infection since 20% of the subjects displayed a weak cellular and humoral memory to SARS-CoV-2. Individuals with an history of symptomatic COVID-19 was associated to higher levels of SARS-CoV-2 reactive CD4+ T cells and specific antibody levels compared to asymptomatic individuals. The different levels of both cell-mediated and humoral immune responses to SARS-CoV-2 in symptomatic versus asymptomatic patients, suggest that a possible dysregulation of adaptive immunity in COVID-19 that could be related to different immunopathology. On the other hand, the divergence in antigen specific immune response observed in recovered patients might reveal subjects with higher risk of reinfection.

Keywords: Adaptive immunity, antibody, infectious disease, memory

P-0910

NK cell degranulation responses are potentiated in non-Hodgkin lymphoma patients for at least three months after treatment with rituximab

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Rituximab therapy depletes B cells in non-Hodgkin lymphomas (NHL) by several mechanisms, including antibody-dependent cellular cytotoxicity (ADCC) by NK cells and monocytes, complement-mediated lysis, and directly inducing apoptosis. Immune phenotype and NK cell function were monitored in peripheral blood of 75 B-cell NHL patients and 54 healthy volunteers. Thirteen NHL patients were treated with rituximab and provided blood samples immediately before the first treatment, during treatment and three months after treatment. PBMC were stained for NK cell biomarkers and NK cell degranulation was measured toward 721.221 target cells. Degranulation responses by NK cells did not differ significantly between healthy donors or pretreatment samples from NHL patients. When assayed under natural cytotoxicity conditions, however, CD56dim NK cells from rituximab-treated patients exhibited a significant increase in degranulation response between the pretreatment and during treatment samples ($p<0.001$). This increase from baseline response was retained for at least three months after the end of therapy ($p<0.01$). We also provide evidence that the long-lasting potentiation of NK cell viability and function in post-rituximab treated blood samples was not the result of transfer of rituximab in the blood of patients to the *in vitro* experiments. Granzyme B expression in NK cells declined after rituximab therapy, but CD27 and CD16 expression were unchanged. Our results provide evidence that a treatment course of rituximab can result in enhanced degranulation responses by peripheral blood NK cells under natural cytotoxicity conditions for at least 3 months after the end of therapy.

Keywords: Antibody, cancer immunology, immunopharmacology, NK cells

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POSTER PRESENTATIONS

P-0912

Comparison of SARS-CoV-2 antibody levels in convalescent and vaccinated plasma donors

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The COVID-19 pandemic has had a big impact on society and the healthcare system provoking an unprecedented vaccine development and left many questions. We measured and tracked antibody levels of vaccinated and convalescent plasma donors to determine antibody levels and the course of these levels over time. Antibody levels of vaccinated and convalescent subjects show a large individual variability. The initial anti SARS-CoV-2 activity after vaccination is around 10 x higher than for convalescent subjects. For convalescent as well as vaccinated subjects the anti SARS-CoV-2 specific IgG titer declines over time, but the initial decline is faster for vaccinated than for convalescent subjects: decline to a median of 62% in week 3 based on initial titer for vaccinated compared to 84% for convalescent subjects.The convalescent subjects can be subdivided into groups, based on the antibody activity. The subgroup with the highest antibody activity shows the slowest decrease in antibody activity over time, while the subgroup with the lowest antibody activity shows the fastest decline in antibody activity based on an observation period of 10 weeks. Vaccination gives rise to much larger antibody levels than recovery of an active infection underlining the high potency of the current vaccines to induce antibody generation. The decrease is relatively fast and comparable to the decrease observed with other vaccines. Further research on the long term persistence of the antibody response and the role of the cellular immunity is needed in order to determine the need for vaccination to keep protective immunity.

Keywords: Adaptive immunity, antibody, infectious disease, monitoring immunity, viral infections

P-0915

The functional state of the liver in patients suffering from atopic dermatitis

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In the last 30 years, the prevalence of Atopic Dermatitis (AD) has increased on average by 2-3 times and reached 15-30% of children and 2-10% of the adult. Most patients (70–85%) suffer from an allergic form of AD characterized by a connection with the ingestion of exoallergens, positive skin tests, and increased serum IgE.. AD in adults is more often the result of the persistence of a disease that began in childhood, no more than in 5-10% of cases. It is the result of a late onset of the disease. 60-70% of patients suffering from AD have the classic allergic form.. Recurrent dermatitis is often associated with or is caused by metabolic disorders, diseases of the gastrointestinal tract or organs of the hepatobiliary system. Disorders of the functional state of hepatocytes are characterized by changes in blood biochemical parameters and occur in 60-65% of patients with AD. In particular, a low level of urea, cholesterol, albumin-globulin coefficient was determined in patients' blood serum against the background of an increased concentration of ammonia. As a rule, the severity of metabolic disorders correlated with the severity of the course of the underlying allergic disease. Not only the level of IgE, but also the concentration of ammonia correlated with the severity degree equally well. Thus, the assessment of the functional state of the liver and metabolic hepatoprotection can be promising in clinical management of patients with AD.

This paper has been supported by the RUDN University Strategic Academic Leadership Program.

Keywords: Allergen-induced immune responses, allergic disorders, immunotherapy

P-0920

SARS-COV-2 PCR positive bronchial asthma exacerbation rate with different comorbidities during pandemic period

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We started to evaluate hospitalization rate of Asthma patients with comorbidities or with single disease who had diagnosed only Asthma (from age 18-85 y). Study was done in two regions of Georgia (Tbilisi and Kutaisi, different geographical climatic conditions) We performed cohort study Studded group of 187 adults from 18 to 85 (women – 67.3%; men – 32.7%). First part of the study was interviews with patients and questionnaires to understand details about diagnosis and history of comorbidities. Second part was history of severe exacerbations and hospitalizations due to SARS-COV-2 confirmed by PCR positive test. After all data was collected we used mathematical-statistical data processing provided by software SPSS/V12.5. Among 187 adults 48.2% had known history of comorbidities. The analysis showed a 9.2%($p<0.001$). decreased in hospitalization rate of asthma exacerbation, during lockdowns. Most of the patients were using there maintenance medications everyday (compared to non pandemic period) and preventive recommendations. Patients with older age > 60 y and minimum one comorbidity, using more then 5 medications daily was associated an 21% higher hospitalization rate due to asthma exacerbations with SARS COV2 PCR positive results and to any comorbid disease. ($p<0.01$). Because of climate conditions hospitalizations rates due to asthma exacerbation were more in Kutaisi, west Georgia 2.5%($p<0.001$). Patients age > 60y, with bronchial asthma and more than one comorbidity was associated with increased hospitalization rate in ICU and use of long term oral corticosteroid need compared with young asthmatics without comorbid diseases.

Keywords: Allergic disorders, infectious disease, viral infections

P-0922

Status of immunity in patients with positive COVID-19 infection

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We study aimed at evaluating immune status and determining correlation with Vitamin D deficiency in patients with positive COVID -19 infections in Western Georgia. To achieve the study objective, 89 patients with positive COVID-19 infection (45 women and 44 men, 18 - 70 of ages). The I group involved 31 COVID- 19 positive patients, who revealed vitamin D deficiency. The II - III group composed of 28 COVID -19 positive patients with normal vitamin D level was referred to as the control group. It should be emphasized that among 89 patients, the potential toxicity of vitamin D.. At the next stage, immune status, common level of monoclonal antibodies in the blood was assessed in all three groups of the patients with COVID-19. Analysis of the results obtained after studying the immune status showed a significant decrease in the number of CD3, CD4, CD8 T-lymphocytes ($P> 0.05$) in patients with positive COVID -19 infection of group I with vitamin D deficiency in comparison with the control group III. Correlation analysis has proved a high correlation ($r = 0.4$-0.6) revealed between vitamin D and immunological markers such as: CD3, CD4; CD8. Permanent monitoring of vitamin D levels and assessment of immune status in patients with positive COVID -19 infection will provide the opportunity to apply targeted preventive measures to prevent immunodeficiency and a number of associated diseases that undoubtedly might be considered as an important issue of modern medicine. This paper has been supported by the RUDN University Strategic Academic Leadership Program.

Keywords: Immunodeficiency, infectious disease, viral infections

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POSTER PRESENTATIONS

P-0944

Immunogenicity of tumor cell lysates in dendritic cell-based cancer vaccines is enhanced by the atmospheric plasma treatment

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Active immunotherapy of cancer based on dendritic cells (DCs) vaccines emerged one of the most promising way of immunotherapy, especially for cancers with poor immunogenicity. Tumor lysates are mostly used in clinics as a source of tumor antigens in DCs vaccines, but increasing data suggested that the some tumor lysates can exhibit adverse suppressive effects on DCs maturation, thereby limiting the efficacy of DCs cancer vaccines. Here we found that atmospheric plasma-activated medium (PAM) significantly increases the immunogenicity of tumor cells and tumor lysates, by inducing immunogenic cell death characterized by the induction of intracellular ROS, autophagy, heat-shock proteins expression, release of IL-1β, apoptosis and necrosis. DCs loaded with PAM-lysates, and stimulated with LPS/IFN-γ, showed an increased maturation potential, higher production of IL-12 and attenuated expression of PDL1 and ILT-4, as compared to DCs treated with tumor lysates prepared by standard freezing/thawing (F/T) method. Moreover, PAM-lysates-loaded mature DCs showed an increased capacity to stimulate proliferation of both allogenic and autogenic T cells, and increase the proportion of CTLs and IL-17A-producing CD4+ and CD8+ T cells in co-cultures, as well as to preserve the Th1 polarization compared to antigen-naïve mature DCs. Unlike DCs loaded with PAM-lysates, F/T tumor lysates-loaded DCs increased the frequency of Th2 cells, and subtypes of regulatory CD4 and CD8 T cell subtypes, suggesting their potentially adverse effects in tumor therapy. These results suggest that PAM could be used in a novel method for preparing immunogenic tumor lysates suitable for improved DC-based immunotherapy of cancer patients.
Keywords: Adjuvants and vaccines, anti-cancer vaccine, dendritic cells

P-0978

Th1/Th2 cytokine profiles in type-2 -diabetes mellitus patients

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Clinical and experimental studies demonstrated the regulatory role of pro-inflammatory cytokines in the pathogenesis and progression of Type-2 Diabetes Mellitus (DM). Chronic inflammation has now been identified as an important component of DM. Apart from the metabolic risk factors of DM, such as obesity, hypertension, dyslipidemia, chronic inflammation is one of the as a major risk factors. The key role of the pro-inflammatory cytokines DM patients as well as in insulin resistance has been well established. In the present study, the serum levels of both T helper cells cytokines have been assayed. Pro- and anti-inflammatory cytokines in sera of 32 DM patients and 10 healthy volunteers have been assayed by ELIZA and Multiplex assays using Luminex xMAP technology. It have been demonstrated an increase in γIFN and IL-18 concentrations more than 2 times in comparison to control healthy donors. The concentrations of IL1 receptor antagonist, IL-4 and IL10 were higher than in control donors from 30 to 48% ($p<0.05$). Clinical and laboratory studies demonstrated the existence of high association between clinically relevant factors, like insulin resistance, glycated haemoglobin and C-reactive protein in patients with DM as well as pro-inflammatory serum cytokine profile. In patients with DM the concentration of pro-inflammatory is highly increasing, indicating on an important role of cytokines in the pathogenesis of Type-2 diabetes.
This paper has been supported by the RUDN University Strategic Academic Leadership Program.
Keywords: Cytokines and mediators, diabetes, immune regulation and therapy

P-0981

Relationship between immunological indicators and the course of liver fibrosis in patients co-infected with human immunodeficiency viruses, hepatitis C and B viruses

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We study the immune response on the order of infection of pathogens in triple infection with human immunodeficiency viruses (HIV) and hepatitis C (HCV) and B (HCV) viruses. 84 people were under observation. HIV infection was at stage 4A-4B, and patients had liver fibrosis at different stages of development. The key principle for assessing the course of chronic hepatitis B and C was the change in liver elastometry indicators, with the determination of a progressive, stable or regressive course, as well as the immunological changes. The proportion of patients who were infected with HIV earlier than HCV and HBV accounted for 65%, and 35% were patients with earlier infection with hepatitis viruses than HIV. In cases where the first pathogen was HIV, progressive liver fibrosis was observed in 36% of cases and only 10% of patients showed regression of fibrotic changes. If the first pathogen was HBV and HCV, then progressive fibrosis occurred with approximately the same frequency (33%), while regression of the fibrotic process was in 50% of patients. This was largely accompanied by a different nature of immunological changes, primarily on the part of CD8+ T-lymphocytes, the number of which showed a negative correlation between elastometry indicators in patients primarily infected with HIV, and positive correlations in primary infection with HBV and HCV. Thus, the order of infection with HIV, HCV, HBV has a significant impact on the prognosis of co-infection course and requires consideration.

This paper has been supported by the RUDN University Strategic Academic Leadership Program.

Keywords: Immunodeficiency, infectious disease, viral infections

P-0992

Functional iron-deficiency and a distinct metabolite profile in allergics

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Th2-bias, iron-deficiency as well as microbial changes are described in allergic individuals. 27 non-anemic, female allergic and 22 non allergic subjects completed a dietary questionnaire to assess their weekly dietary iron-intake. Urine and blood samples were collected after overnight fasting and complete blood cell count and iron metabolism markers were assessed. Serum and urine hepcidin were determined by ELISA. Trace elements in serum, urine and stool samples were determined via Inductively Coupled Plasma-Sector Field Mass Spectrometry (ICP-SFMS) and correlated with blood parameters. Metabolic profile of allergic and non-allergic subjects was analyzed by Liquid Chromatography Time-of-Flight Mass Spectrometry (LC-TOFMS). Low molecular weight features were correlated with bacterial communities characterized with 16S rRNA amplicon sequencing. Dietary iron intake did not differ between allergic and no-allergic subjects. Allergics had reduced serum iron, but increased serum ceruloplasmin as well as urinary hepcidin. Metabolites in urine, serum and stool extracts differed in the patterns of allergic and non-allergic subjects. Allergic subjects had lower serum iron-levels and elevated serum ceruloplasmin levels, despite comparable intake of dietary iron with non-allergics. The elevated hepcidin-levels may reflect decreased iron absorption in allergics. Differences in metabolite abundances were associated with the atopic state of allergics. Further investigations will aim at the identity confirmation of relevant biomarkers via LC-QTOFMS.

Keywords: Mass spectrometry, metabolic control of immune responses, microbiome and environmental factors

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POSTER PRESENTATIONS

P-1071

Lymphocyte subpopulations analysis in psoriatic patients treated with biological drugs

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Psoriasis is an autoimmune disease characterized by scaly and itchy skin lesions. In recent years the emergence of new biological drugs has revolutionized its treatment especially in the most severe cases of the disease. These new drugs work by blocking the major cytokines involved in the inflammatory response of psoriasis, thereby achieving a drastic reduction in lesions and improving patients' quality of life. To analyze changes in T-cell subsets in peripheral blood of patients with psoriasis treated with biological drugs. Using flow cytometry, we evaluated effector and regulatory T cells subpopulations in fresh peripheral blood. We analyzed patients with psoriasis treated with Adalimumab (anti-TNFα, N=20), Ustekinumab (anti-IL23, N=26) Secukinumab (anti-IL17, N=9), patients with active psoriasis without biological treatment (N=14) and controls without psoriasis (N=21). Patients with active psoriasis presented a lymphocyte profile characterized by decreased levels of Th1 central memory (CM), Th2 CM, and regulatory T cells with respect to treated patients and controls without psoriasis. Whereas patients treated with Adalimumab and Ustekinumab showed T-cell profiles similar to controls without psoriasis. Moreover, patients receiving Secukinumab had a lymphocyte profile similar to controls but with a significant decrease of the number of Th17 effector memory (EM) and Th1 / Th17EM subpopulations. These findings provide an overview of the effects of the biological treatments over the lymphocyte subsets and can serve as a starting point for the selection of the most appropriate drug and for the evaluation of the risk of opportunistic infectious diseases in psoriatic patients.

Keywords: Autoimmunity, biomarkers, cytokines and mediators, drugs for immune modulation, immunotherapy, skin diseases

P-1103

Chitosan nanospheres in anti-tumor immunotherapy of melanoma

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Currently, antigen encapsulation in biomaterials has been proposed as a promising strategy to increase immunogenicity in antimoral vaccination with CDs. Among the biomaterials that have been used is chitosan as a material. In addition, they have been reported to have a significant adjuvant effect in stimulating innate immune responses. Although nanospheres with tumor antigens have been used to stimulate CD receptors such as CLEC-9, CD40 among others in particular, nanospheres with tumor cell lysates and ligands that stimulate all of them together and in particular that stimulate CLEC-9 have not been used. CLEC-9 is a C-type lectin receptor that has been shown to be very efficient in CD activation. To elaborate chitosan nanospheres, which have a size between 80-100 nm and are uniform. The nanospheres were prepared using the simple emulsion and cross-linking method, with 2% chitosan and 0.1% albumin in distilled water for 24 hr. Subsequently, 100 ml of mineral oil were mixed with 1% SPAN 80, agitated at 700 rpm for three minutes and washed with 25% glutaraldehyde; later, their morphology was evaluated by scanning electron microscopy. Some nanospheres with a regular morphology have been obtained, however their size is between 100-300 microns, so they are not yet ready to be used in culture or to add lysates or peptides to them, so feedback on the procedure should be made.

Keywords: Anti-cancer vaccine, dendritic cells, immunotherapy

P-1108

Ground control to major T-Cell: Assessing immune dysfunction under simulated space conditions with T-cells

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The space environment consists of a series of stress factors, physical (cosmic radiation and altered gravity) and psychological (stress). Space-related dysfunction of the human immune system has been shown during and after spaceflight, indicating a persistent nature. Dysregulation of the activation profiles of T-Cells is present in the form of altered cytokine profiles, cytoskeleton alterations and gene expression dysregulation. However, the exact underlying molecular causes are not identified yet. In order to enable long-duration space missions foreseen in the future, it is necessary to explore these underlying mechanisms of space-induced immune dysfunction and develop successful countermeasures. Activated Jurkat cells were exposed to simulated space conditions: ionizing radiation (either 1Gy of X-rays or Carbon ions), hydrocortisone (1μM) and altered gravity levels (e.g.: Moon and Mars gravity). IL-2 levels were assessed in the supernatant and cells were collected for quantitative polymerase chain reaction (qPCR) analysis. In order to investigate a broader cytokine profile, blood will be collected from healthy volunteers in the second phase of this project in order to extract CD4+ T-cells. These cells will be exposed to the aforementioned spacestressors and investigated with transcriptomic, proteomic, flow-cytometric and cytokine-profiling approaches. An understanding of the molecular mechanisms underlying alterations of the T-cell population when exposed to the combined space environment can potentially enable the development and implementation of space-induced immune dysfunction countermeasures and also prove valuable for the treatment of known immune-related pathologies which occur on earth.

Keywords: Adaptive immunity, cell signalling, cytokines and mediators

P-1118

Bcl-3 as a regulatory mechanism of the tight junction structure at intestinal epithelia

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The Irritable Bowel Syndrome (IBS) is a brain-gut communication disorder which is characterized by a systemic low-grade inflammation. An increased IL-6 plasma levels is observed in IBS patients associated to a high intestinal paracellular permeability, due to an altered structure of the tight junction (TJ) complex. Bcl-3 is a transcriptional regulator of genes activated by NF-κB, whose expression is elevated in the intestinal epithelium of IBS patients. The regulatory role of Bcl-3 in TJ proteins-complex structure is unknown. We aim to evaluate *in vitro* the Bcl-3 role in TJ proteins expression/distribution in intestinal epithelium. A human colorectal adenocarcinoma cell line DLD-1 was stimulated with IL-6 and the Bcl3 expression modulated by transfection with plasmid DNA containing Bcl3 and silencing (siRNA). The expression of Bcl-3, claudin-2 (Clau-2), myosin light chain kinase (MLCK) and pMLC/MLC ratio was determined by q-PCR and western blot, and ZO-1 intracellular distribution, by immunofluorescence. We observed that Bcl-3 expression was early induced by IL-6 in the time course (3h), reached its maximum at 12h. Similarly, Bcl-3 was upregulated by its overexpression using expression vector, being this reduced by its silencing. An increased expression of Clau-2 and MLCK; and pMLC/MLC ratio were observed for Bcl-3 overexpression, being the expression of these proteins unchanged by its silencing. A cytoplasm relocation of ZO-1 was observed under Bcl-3 overexpression, but no changes were observed with Bcl-3 SiRNA. Our results suggest that Bcl-3 has a regulatory role of the structural composition of TJ complex at intestinal mucosa. Financed by FONDECYT 1181699.

Keywords: Cytoskeleton, cell signalling, cytokines and mediators

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POSTER PRESENTATIONS

P-1126

Cellular dynamics of immune evasion during leishmania major infection

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Leishmania major parasites elicit a strong T cell response yet persistently infect a small pool of cells, a mechanism which remains unclear. Understanding of the persistence mechanisms is lacking, but Leishmania major driven induction of the immunosuppressive microenvironment through recruitment of regulatory T cells is proposed to prevent parasite clearance *in vivo*. We used a novel TCR transgenic mouse model, where $CD4^+$ T cells recognize an immunodominant peptide derived from Leishmania- glycosomal phosphoenolpyruvate carboxykinase (PEPCK), to visualize the dynamics of anti-L. major $CD4^+$ T cell responses and characterize mechanisms which restrain their effector function. We show that monocyte-derived macrophage:T cell interaction dynamics were transient at steady-state, but prolonged upon antigen recognition. This activation leads to a production of high levels of IFNγ and can be significantly suppressed by PEPCK-specific Tregs *in vitro*, as compared to polyclonal Treg controls. Co-culture of PEPCK-specific $CD4^+$ T cells, L. major-infected monocyte-derived macrophages, and Tregs shows that antigen activation leads to a substantial increase in IL-10 levels, while decreasing IL-12, TNF, and IL-2 production in the culture. Intravital microscopy studies characterizing PEPCK-specific $CD4^+$ T cell migration dynamics and tissue localization within skin lesions directly in live mice show a significant recruitment of adoptively transferred effector T cells, displaying behaviours consistent with antigen recognition. We are characterizing whether effector T cell responses are altered in healed lesions. Our findings show for the first time that Leishmania-specific Tregs influence $CD4^+$ T cell responses and this could be a mechanism that derives antigen persistence in L. major infection.

Keywords: Adaptive immunity, cellular interactions, infectious disease, parasite infections, regulatory cells, visualizing immune responses

P-1141

Granzyme A and CD160 expression delineates ILC1 with graded functions in the mouse liver

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Type 1 innate lymphoid cells (ILC1) are tissue-resident lymphocytes which provide early protection against bacterial and viral infections. Discrete transcriptional states of ILC1 have been identified in homeostatic and pathological contexts. However, whether these states are associated with different functional properties remains to be evaluated. The aim of our study is to deconvolute ILC1 heterogeneity by employing transcriptomic approaches and to evaluate functional diversification among liver ILC1. We observed that liver ILC1 display a heterogeneous expression of distinct effector molecules and surface receptors, such as granzyme A (GzmA) and CD160, as assessed by flow cytometry. $GzmA^+$ ILC1 reside mainly in the liver of adult mice and represent the main hepatic ILC1 population, at birth. $GzmA^+$ ILC1 differ from NK cells for the requirements of JAK/STAT signals and the transcription factor Nfil3. Moreover, by employing Rorc(γt)-fate map (fm) reporter mice, we established that ILC3-ILC1 plasticity contributes to delineate the heterogeneity of liver ILC1, with $Roryt\text{-}fm^+$ cells skewed towards a $GzmA^-CD160^+$ phenotype. Finally, we showed that ILC1 defined by the expression of GzmA and CD160 are characterized by a graded cytotoxic potential and ability to produce IFN-γ. Our study provides evidence for a spectrum of phenotypically and functionally distinct liver ILC1 subsets. Understanding how liver ILC1 contribute to the immune response can be of relevance in the context of liver pathology.

Keywords: Animal models, cytokines and mediators, innate lymphoid cells, NK cells

P-1143

Human CD4+ T-cell clone expansion leads to the expression of the cysteine peptidase inhibitor cystatin F

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The existence of CD4+ cytotoxic T cells (CTLs) at relatively high levels under different pathological conditions *in vivo* suggests their role in protective and/or pathogenic immune functions. CD4+CTLs utilize the cytotoxic effector mechanisms also utilized by CD8+CTLs and natural killer (NK) cells. During long-term cultivation, CD4+ T cells also acquire cytotoxic functions. In this study, CD4+ human T-cell clones derived from activated peripheral blood lymphocytes of healthy donors were examined for the expression of cytotoxic machinery components. Cystatin F is a protein inhibitor of cysteine cathepsins, that affects the cytotoxic efficacy of CD8+CTLs and NK cells by inhibiting the major progranzyme convertases cathepsins C and H as well as cathepsin L, which is involved in perforin activation. Here, we show that human CD4+ T-cell clones express the cysteine cathepsins involved in the activation of granzymes and perforin as well as both the inactive, dimeric and active, monomeric form of cystatin F. As in CD8+CTLs, cysteine cathepsins C and H were the major targets of cystatin F in CD4+ T-cell clones. Furthermore, CD4+ T-cell clones expressed the active forms of perforin and granzymes. The levels of the cystatin F decreased with time in culture concomitantly with an increase in the activities of granzymes. Therefore, our results suggest that cystatin F plays a role in regulating CD4+ T cell cytotoxicity. Since cystatin F can be secreted and taken up by bystander cells, our results suggest that CD4+CTLs may also be involved in regulating immune responses through cystatin F secretion.

Keywords: Adaptive immunity, cancer immunology, NK cells

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POSTER PRESENTATIONS

P-1144

T cell asymmetry regulates fine-tune metabolism of human CD4 T cells during immune synapses

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T lymphocyte activation by antigen-presenting cells (APC) leads to the reorganization of both cells to mount immune synapses (IS). These cell-cell communication structures depend on the dynamics of membrane receptors, signaling scaffold molecules, microfilaments, and microtubules. The conformation of the IS fine-tunes the potency of T cell activation and subsequent immune response. We have observed that the centrioles re-orientate inside the centrosome through cryocorrelative microscopy by using soft-X-Rays. Resonant scanning-based confocal analysis of CD4 cells forming IS showed that this arrangement of centrioles allows polarized polymerization of microtubules towards the IS, even in absence of translocation of the centrosome to the IS. The cytosolic chaperonin CCT (chaperonin-containing TCP1) helps the folding of protein partners coming from new synthesis. CCT regulates the changes in the reciprocal orientation of the centrioles and polarization of the tubulin dynamics induced by T cell receptor in T lymphocytes forming an IS. CCT also controls the mitochondrial ultrastructure and the metabolic status of T cells, regulating the 'de novo' synthesis of tubulin through mTOR. These changes ultimately determine the function and organization of the mitochondria, as shown by analysis of mitochondria respiration and three-dimensional reconstruction of resting and stimulated primary T cells using cryo-soft x-ray tomography and STED microscopy. Through this mechanism, CCT governs T cell activation and polarity.

Keywords: Cell signalling, immune communication, metabolic control of immune responses

P-1145

Macrophages are polarized by their aged microenvironment in the skin

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Aging is a multifactorial process that affects the entire body, and manifests itself in many phenotypical changes, which are mainly caused by impaired tissue maintenance and regeneration. Macrophages are a subset of phagocytes known to be critical regulators of tissue homeostasis and to be involved in aging-associated changes. Dermal macrophages are either fetal liver derived tissue-resident macrophages or have matured from bone marrow-derived monocytes (MDM). Interestingly, it has been suggested that embryo-derived macrophages are progressively substituted by MDMs with age. Although MDMs can develop more complex phenotypes *in vivo*, they are typically classified into pro-inflammatory (M1) and anti-inflammatory (M2) macrophages. It is reported that aging affects the macrophage phenotype and function, but it is not fully understood whether these changes are caused by intrinsic or extrinsic factors. Therefore, this study investigated the differentiation ability of human MDMs from elderly and younger donors and analyzed the impact of the aged skin microenvironment on their phenotype. We could show that elderly MDM expressed M1 and M2 markers to a similar extend on protein and mRNA level than young MDMs, suggesting that monocytes are intrinsically able to differentiate to both pro- and anti-inflammatory macrophage phenotypes during aging. However, we found that co-culture with elderly fibroblasts increases the expression of pro-inflammatory markers in MDMs compared to their young counterparts. Together, these results demonstrate that monocytes are able to differentiate to M1 and M2 macrophages independent of their age, but that an aged microenvironment causes the macrophages to display more M1 characteristics.

Keywords: Ageing, cellular interactions, cytokines and mediators, innate immunity, macrophage, microenvironment

P-1146

Tocilizumab in COVID-19: an integrated approach to evaluate treatment response

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Some patients with Covid-19 develop hyperinflammatory syndrome. In March 2020 tocilizumab - anti-IL-6 receptor monoclonal antibody - was proposed as immunomodulatory treatment in severe Covid-19. IL-6 levels are correlated with SARS-CoV-2 viral load, disease severity, and prognosis. However, studies on the efficacy of tocilizumab in patients with Covid-19 are controversial. We compared in terms of clinical, laboratory, and radiologic findings 20 severe Covid-19 patients receiving tocilizumab in addition to standard-of-care (SOC) with 13 severe Covid-19 patients receiving only SOC. In 5 patients treated with tocilizumab we characterized via flow cytometry immune cell subsets in peripheral blood and in 13 patients we analyzed via 1H-NMR spectroscopy the metabolomic and lipidomic profiles. Clinical respiratory status, inflammatory markers and vascular radiologic score significantly improved after tocilizumab. Conversely, these parameters were stable or worsened in patients receiving only SOC. Patients treated with tocilizumab displayed increased expression of both perforin and granzyme A in NK cells, as well as partial reversion of metabolic alterations due to SARS-CoV-2 infection. Improvement of respiratory status (i.e. alveolar-arterial oxygen gradient) and vascular radiologic score might explain improved pulmonary vascular perfusion, as IL-6 mediates endothelial dysfunction and promotes prothrombotic state. Tocilizumab restored the cytotoxic potential of NK cells and reverted metabolic alterations. Consequently, blocking IL-6 axis could account for rapid pulmonary vascular improvement and recovery of protective antiviral potential in severe Covid-19. Immunopathology plays a crucial role in Covid-19, thus evaluation of response to immunomodulatory therapies should be based on an extensive approach integrating clinical, laboratory, pathologic, and radiologic features.

Keywords: Cytokines and mediators, immunotherapy, inflammatory disease, viral infections

P-1148

MicroRNA 221 and 222 safeguards hematopoietic stem cell quiescence

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Every single cells of long term quiescent hematopoietic stem cells (HSCs) and their activated and cell cycle active progenies (MPP1 and MPP2) express the micro RNA gene cluster 221/222 in the bone marrow of mice. Vav promoter driven deletion effectively abolishes the expression of miR-221/222 in the earliest stem cell compartments, leading to a 3-5 fold reduction in the number of BM-resident quiescent HSC, and a 1.5-2-fold increase in activated, cycling MPP1 and MPP2. This indicates, that miR-221/222 contributes to the maintenance of quiescence of HSC. Single cell (sc) RNA-seq on HSC, MPP1, and 2 revealed 17 target genes, whose upregulations abolish HSC quiescence. They are involved in nuclear RNA synthesis, pre-mRNA processing, transfer of ribosome precursor complexes to the cytoplasm, protein synthesis and folding in the cytoplasm, energy metabolism in mitochondria (glycolysis), cell cycle control at the M to G1/G0 and G0 to G1 phases and HSC-MPP niche formation in bone marrow. Full-length cDNA sequencing of miR 221/222 deficient and proficient HSC and MPP1 populations detects in quantity and quality an altered activation of quiescent stem cells.

Keywords: Immune development, miRNA, RNAseq, stem cells

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POSTER PRESENTATIONS

P-1151

Profiling of B cell subsets in the draining lymph nodes of head and neck squamous cell carcinoma: B cells with regulatory phenotype were associated with good prognostic factors

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Despite their diverse roles in the immune responses, B cells are mainly overlooked in the investigation of the antitumor immunity in head and neck squamous cell carcinoma (HNSCC). Here, we investigated the expression of co-stimulatory, inhibitory and immune checkpoint molecules in B cells derived from tumor-draining lymph nodes (TDLNs) of HNSCC. Using flow cytometry, we assessed the expression of CD39, CD73, CD80 and CD86 on B cells in 26 LNs obtained from patients with HNSCC. We also assessed the expression of PD-1 and PD-L1 on B cells in unstimulated state and after 6 hours stimulation with PMA/Ionomycin. Results showed that the frequency of CD19+ B cells was higher in patients with stage III+IV compared with stage I+II (P=0.035), and showed a direct correlation with the tumor size. The frequency of PD-L1+ B cells increased rapidly upon a brief stimulation, however, in both stimulated and unstimulated conditions, the percentage of PD-L1+ B cells showed inverse correlations with the tumor size. Besides, the frequency of CD73-expressing B cells showed a decreasing trend in grade II+III compared with grade I (P=0.051). A greater percentage of B cells expressed CD86 in comparison with CD80, and the frequency of CD80–CD86+ B cells was higher in the TDLNs of tongue than in laryngeal carcinoma(P=0.047). CD86+ B cells showed a relatively higher frequency in the lower stages(P=0.071). In conclusion, the percentage of CD19+ B cells showed association with poor prognostic indicators of HNSCC, however the frequency of B cells with regulatory phenotypes showed associations with good prognosticators.

Keywords: Adaptive immunity, B lymphocytes, cancer immunology, regulatory cells

P-1157

Expansion of myeloid derived suppressor cells contribute to platelet activation by l-arginine deprivation during sars-cov-2 infection

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A massive platelets activation and thrombotic events characterize severe COVID-19, highlighting their critical role in SARS-CoV-2-induced immunopathology. Since the expansion of MDSC in severe COVID-19, herein we evaluated their possible role in platelet activation during SARS-CoV-2 infection. During COVID-19, a lower plasmatic L-arginine level was observed compared to healthy donors, which was correlated with MDSC frequency. Additionally, PAC-1 expression was higher on platelets from severe COVID-19 patients compared to healthy controls, and inversely correlated with L-arginine plasmatic concentration. Notably, MDSC were able to induce platelet PAC-1 expression *in vitro* by reducing L-arginine, indicating a direct role of PMN-MDSC in platelet activation. Accordingly, we found a positive correlation between *ex vivo* platelet PAC-1 expression and PMN-MDSC frequency. Overall, our data demonstrate the involvement of PMN-MDSC in triggering platelet activation during COVID-19, highlighting a novel role of MDSC in driving COVID-19 pathogenesis.

Keywords: Infectious disease, innate immunity, myeloid derived suppressor cells, viral infections

P-1161

Characterization of novel human regulatory epitopes and evaluation of their immunomodulatory properties

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Tregs suppress immune responses against self-antigens, food antigens and allergens. They are CD4+ CD25+ FoxP3+ T cells and require antigen-specific stimulation through their T cell receptor (TCR) to exert their suppressive function. Tregs can be stimulated to expand and respond with epitopes known as Tregitopes. The general objective of this project is to identify human Treg epitopes (hTregitopes). Clearly, the identification of Tregitopes is relevant to the development of new immunosuppressive therapies. By using bioinformatics tools, we predicted ~200 Treg epitopes in thymus-specific antigens, anticipating peptides that can bind with high affinity to class II human leukocyte antigen molecules (HLA II). Of those we synthesized 4 epitopes predicted to bind to various HLA II molecules that are expressed with high frequency in the general population. These peptides were pooled and subjected to functional assays. By FACS analysis, we first found that stimulation of PBMCs with the Treg pool leads to the IL-10 production by CD4+FoxP3+CD25+ T cells. In contrast, this population was insignificant when using media alone or a control peptide pool (CEF pool). Subsequently, we verified that incubation of PBMCs with LPS in combination with the Treg pool reduced the IFNγ detected in cell culture supernatants by half compared to that obtained when the cells were incubated with LPS alone. Using intracellular cytokine assays, we also found that the Treg pool inhibits the antigen specific production of IFNγ by both CD4 and CD8 T cells mediated by peptide pools consisting of experimentally verified CD4 and CD8 cell epitopes, respectively.

Keywords: Adaptive immunity, immune regulation and therapy, regulatory cells

P-1164

Characterization of regulatory T cells in the IDH1 mutant and wild type glioblastoma tumor microenvironment

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An activating mutation in isocitrate dehydrogenase (IDH1),R132H, plays critical roles in glioblastoma(GBM) tumorigenesis and shapes tumor immunity. In the present study, we aimed to characterize Treg cells in the tumor microenvironment and peripheral blood of IDH1 R132H mutant (muIDH)(n=6) and IDH1 wild type(wtIDH)(n=28) and healthy donors (n=20) Treg cells were stained with surface and intracellular antibodies and examined by FACSAriaIII.The phenotype of Treg cells, differentiated from CD4+T cells isolated from healthy donors in the presence of wtIDH and muIDH U87MG isogenic cell lines, was also characterized. In the GBM tissue of muIDH, frequency of Treg cells, LAG3, PD1, GARP subsets were significantly higher;LAG3, KLRG1, GARP,TIGIT mean expression levels were also elevated on Tregs of the muIDH patients compared with wtIDH. Production of IL-17A, IFN-γ and IL-10 by Treg cells was comparable between the patient groups.The frequency of Treg cells and their CTLA4,LAG3,PD1,GARP and TIGIT levels were elevated in the peripheral blood of GBM patients compared with those of healthy donors.Peripheral blood Treg GARP expression was significantly lower in the muIDH patients compared with wtIDH while the rest of the suppression markers remained comparable.In contrast to primary tissue data, *ex vivo* generated Treg cells expressed more PD1 and LAG3 and CD25 in cocultures with wtIDH U87MG cells compared with muIDH1. Tumor microenvironment may harbor factors that are absent in the culture conditions or in the peripheral blood that may contribute to differential reprogramming of Treg cells

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Keywords: Adaptive immunity, checkpoint inhibition, immune regulation and therapy, regulatory cells

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POSTER PRESENTATIONS

P-1165

Lack of protective effect of CCR3 blockade during experimental colitis may be related to colonic Tregs expressing CCR3

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The C-C Motif Chemokine Receptor CCR3 that binds to eotaxin, RANTES and many other ligands, is considered as a potential therapeutic target in inflammatory bowel diseases (IBD). In this study, we investigated the consequence of CCR3 pharmacological blockade or deficiency during experimental colitis. Colitis was induced by dextran sulphate sodium (DSS) in CCR3 deficient mice (CCR3 KO) or in GW766994 (10mg/kg/day), a selective CCR3 antagonist, treated mice. CCR3 expression was investigated in colonic leukocytes by flow cytometry. Both CCR3 deficiency and pharmacological blockade, aggravates DSS-induced colitis and aggravates clinically DSS-induced colitis. Strikingly, CCR3 blockade or genetic disruption leads to an unbalanced Treg/Th17 cells ratio in colonic Lamina propria and mesenteric lymph nodes (mLN) during colitis. We demonstrated that a subpopulation of colonic RORγt$^-$ FoxP3$^+$ (Tregs) express high levels of CCR3. CCR3 blockade did not affect Treg proliferation whereas treatment with FTY20, used to abrogates Treg tissue recruitment, resulted in decreased Ki67$^+$ proliferating Tregs, and colitis exacerbation. Although therapeutically promising, our data indicate that CCR3 inhibition is not protective during DSS-induced colitis. Expression of CCR3 by a portion of colonic RORγt$^-$ FoxP3$^+$ Treg population, and its involvement in Treg cell homing/recruitment, might explain this lack of beneficial effect.

Keywords: Animal models, chemokines, immune regulation and therapy, inflammatory bowel disease, regulatory cells

P-1169

Investigation of the expression of IL-21 and its receptor in peripheral B and T lymphocytes of patients with breast cancer

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IL-21 is a pleotropic cytokine. It is mainly produced by NKT and activated CD4+T cells, and its receptor is expressed by B, T, NK and other myeloid cells. In this study we examined the frequency of IL-21 receptor (IL-21R)+CD19+B and IL-21+CD4+T cells in the peripheral blood of patients with breast cancer, and compared them with healthy controls. Blood samples were obtained from 35 patients and 10 healthy individuals. Isolated lymphocytes were stained for CD19 and IL-21R. Lymphocytes were activated with PMA/Ionomycin for 6 hours in the presence of Brefeldin A. Cells were then stained for CD4 and IL-21 and finally were subjected to flow cytometry. The frequency of CD19+B cells did not show significantdifferences in patient and control group (10.1±4.6% and 9.4±4%, respectively). However, the percentage of CD4+T cells was significantly lower in the peripheral blood of patients incomparison with controls (48.3±7.3% and 58.4±8.5%, respectively, P=0.002). Moreover, the frequency of IL-21R-expressing B cells was significantly lower in patients compared with controls (64.1±13.9% and 75.5±11.5%, respectively, P=.019). But, the percentage of IL-21+CD4+ T cells was not significantly different in the two groups. We did not find any significant association between the frequencies of B and T cells or their IL-21R- and IL-21-expressing subsets and different clinico-pathological characteristics of breast cancer. The frequency of CD4+T cells and IL-21R+B cells were significantly lower in patients with breast cancer in comparison with healthy controls, however, larger sample size is needed to confirm these findings.

Keywords: B lymphocytes, cancer immunology, cytokines and mediators

P-1171

Individual Th1 cells maintain quantitative differences after chronic viral challenge infection

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In acute viral infections, Th1 cells arise as the predominant CD4 T cell subtype. We have shown that individual Th1 cells, sorted according to the amount of their interferon-gamma production, can maintain a quantitative cytokine memory for multiple weeks *in vitro* and *in vivo*, which correlates with the expression levels of T-bet. However, during chronic viral infections, T cells undergo phenotypical changes to adapt to the persistence of the pathogen. Here, we investigated if the quantitative differences of individual Th1 cells can be stably maintained during a chronic infection. T-bet-ZsGreen reporter mice were used to sort virus-specific Th1 cells according to their T-bet brightness early after clearance of an acute LCMV infection. The sorted Th1 cells were transferred into new recipients and after 2 weeks of resting, the mice were challenged with a chronic LCMV infection. The differentiation, exhaustion and function of transferred Th1 cells were analyzed by flow cytometry. After the chronic challenge infection, the progeny of the T-bet-high, -low and -mock sorted cells showed no difference in cell survival. They maintained their differential ZsGreen levels and gradients of markers associated with Th1 cells (Ly6C, IL-18R, Interferon-gamma) in secondary lymphoid organs, while upregulating exhaustion markers. The quantitative T-bet differences of Th1 cells seem to withstand a chronic rechallenge at early stages. This observation supports our previous findings that T-bet levels are stably imprinted and suggests that neither persistent TCR stimulation nor the chronic infection cytokine milieu can overwrite them.

Keywords: Adaptive immunity, immune response tracing, memory, viral infections

P-1172

D-lactate enhances T cell functionality by limiting mitochondrial ROS production during activation

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In nature lactate can be found in two enantiomeric forms, D- and L-lactate. Recently, several reports have shown L-lactate´s ability to govern differentiation processes of distinct T cell subsets. However, the role of D-lactate in the immune system has remained ill defined. Hence, we investigated whether D-lactate can impact T cell biology. FACS-sorted naïve CD4+ T cells were differentiated into Th1 cells by IL-12 either with or without 20mM of either D- or L-lactate for 5 days *in vitro*. Then T cell differentiation, function and some metabolic parameters were analyzed by flow cytometry. T cell metabolism was tracked via SeaHorse measurements of OXPHOS and glycolysis. Th1 cells differentiated in the presence of D-lactate showed a strong enhancement in IFN-ψ production when compared to the L-lactate- and control-treated samples. The boosting effect depended on D-lactate's ability to limit mitochondrial ROS production when the cells were stimulated. Additionally, D-lactate treatment enhanced the glycolytic flux resulting in higher levels of ATP production and mTOR signaling. Conversely, L-lactate treatment boosted mitochondrial ROS levels and failed to induce mTOR signaling. Both lactate treatments downregulated glucose uptake in Th1 cells. Modulation of mitochondrial ROS production in the different experimental groups was enough to alter the overall cytokine production levels. The observation that D-lactate augmented the glycolytic activity of the cells while decreasing glucose uptake, suggests that T cells can metabolize D-lactate to sustain their bioenergetic demands.

Keywords: Effector molecules, epigenetic control and modulation of immunity, metabolic control of immune responses

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POSTER PRESENTATIONS

P-1173

Staphylococcal enterotoxin B (SEB) activates TCR- and CD28-mediated inflammatory signals in the absence of MHC class II molecules

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The inflammatory activity of staphylococcal enterotoxin B (SEB) relies on its capacity to trigger polyclonal T-cell activation by binding both T-cell receptor (TCR) and costimulatory receptor CD28 on T cells and MHC class II and B7 molecules on antigen presenting cells (APC). Previous studies highlighted that SEB may bind TCR and CD28 molecules independently of MHC class II, yet the relative contribution of these interactions to the pro-inflammatory function of SEB remained unclear. Here, we show that binding to MHC class II is dispensable for the inflammatory activity of SEB, whereas binding to TCR, CD28 and B7 molecules is pivotal, in both human primary T cells and Jurkat T cell lines. In particular, our finding is that binding of SEB to B7 molecules suffices to trigger both TCR- and CD28-mediated inflammatory signalling. We also provide evidence that, by strengthening the interaction between CD28 and B7, SEB favours the recruitment of the TCR into the immunological synapse, thus inducing lethal inflammatory signalling

Keywords: Adaptive immunity, biology of the immune system, cell signalling, molecular immunology

P-1175

The human placenta induces a pregnancy-specific maternal macrophage population during early pregnancy

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Decidual macrophages play a vital role in maternal tissue homeostasis during pregnancy. The question whether macrophages at the maternal-fetal interface (decidua basalis; DB) differ from those in areas not affected by placentation (decidua parietalis; DP) has not been comprehensively addressed. We aimed to investigate the differences between these populations and study possible effects of placental extravillous trophoblasts (EVTs) on macrophage polarization. Macrophages in human first trimester DB and DP were extensively characterized by flow cytometry, *in situ* immunofluorescence staining, and EdU-labeling for analysis of *ex vivo* proliferation. Macrophages were isolated from patient-matched decidual tissues and analyzed by RNAseq, Luminex, and cytological staining. Isolated macrophages were treated with EVT supernatants and gene expression was monitored using SLAMSeq, specifically detecting newly transcribed RNA. EVT supernatants were also used to treat DP tissue explants for further analyses. Macrophages in DB and DP showed major differences in frequency, appearance, and proliferation, with macrophages being significantly more abundant, more granulated, and more proliferative in DB. More than 700 genes were differentially expressed, and a selection of these was confirmed on protein level. SLAMSeq revealed upregulation of more than 400 genes involved in immune processes upon stimulation with EVT supernatants. The secretome of EVTs also induced proliferation of DP macrophages. We identified a pregnancy-associated maternal macrophage signature and defined a central role for invasive trophoblasts in the induction of this signature. Together these data provide evidence for a placenta-guided adaptation of the immune microenvironment at the early maternal-fetal interface.

Keywords: Immune communication, macrophage, microenvironment, reproductive immunology, RNAseq

P-1176

IL-10 receptor-expressing B cells in the draining lymph nodes of breast cancer

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IL-10 is a pleiotropic cytokine mostly known for its immune suppressing functions however, it plays an important role in plasma cell differentiation and antibody production. IL10 receptor (IL-10R) is expressed on many cell types such as mast cells, DC, T and B cells. Herein, we investigated the expression of IL10R on CD19+ B cells in breast tumor draining lymph nodes (TDLN). We prepared mononuclear cell suspension from 46 axillary LN samples and stained the cells with antibodies against CD19 and IL10R and examined by flow cytometery. B cells comprised 34.5±14.1% of lymphocytes in the breast TDLNs and of them 42.6±15.8% expressed IL-10R without significant differences in involved and uninvolved LNs. Most (70.4±18.7%) IL-10R+ B cells showed CD24hiCD27+, active/memory, phenotype. In patients without LN involvement, the percentage of B cells was significantly higher in those with tumor sizes>2 cm (P=0.011) and correlated directly with the tumor size (R=0.8, P<0.0001). The frequency of IL-10R+ B cells did not show significant association with breast cancer parameters, however it exhibited a nonsignificant increasing trend in stage III compared with stage I+II. Nearly half of the B cells in the involved or uninvolved breast cancer draining LNs express IL-10R without significant association with tumor size, LN involvement and cancer stage.

Keywords: B lymphocytes, cancer immunology, cytokines and mediators, lymphoid organs

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POSTER PRESENTATIONS

P-1181

Accelerated aging of immune cells in childhood and adolescent cancer survivors

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The therapy of neuroblastoma, the most frequent extra-cranial solid tumor in early childhood, is targeting important functions of tumor cells. While replication arrest and induced differentiation of these cells are the therapeutic goals, the damages of other cell types form an eventual risk in long-term perspective. Senescence of immune cells is characterised by decline of a plethora of immune cell functions including adaptive as well as innate responses and is associated with a number of pathologies linked to aging, such as higher susceptibility to infections and cardiovascular diseases. Moreover, frailty, a condition linked to immunosenescence and accelerated aging, has been already described in childhood cancer survivors. Therefore, we hypothesize that intensive therapy and/or inflammatory burden caused by acquired comorbidities, serve as inducers of accelerated aging of immune system. In this study we focused on immunosenescent phenotype in high-risk neuroblastoma patients. The analysis of immunosenescent phenotype in high-risk neuroblastoma patients revealed a senescence-like phenotype in CD8 T cell subsets early after therapy but this phenotype normalized in later follow-up. Nevertheless, the monocyte activation status and phagocytosis remain heightened in the later follow-up. Although only transient senescence-like phenotype was detected in CD8 T cells in high-risk neuroblastoma patients, remaining alterations to monocytes and phagocytes may represent a long-lasting burden that might potentially immunocompromise childhood cancer survivors later in life.

Keywords: Ageing, cancer immunology, immune senescence, myeloid cells, phagocytosis

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POSTER PRESENTATIONS

TRACK 2 - MOLECULAR IMMUNOLOGY

P-0137

Expanding the repertoire of HLA class I-restricted minor histocompatibility antigens for immune monitoring and modulation after allogeneic stem cell transplantation

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Allogeneic stem cell transplantation is given as curative treatment for hematological malignancies, but patients face the risk of relapse of their malignancy as well as Graft-versus-Host Disease. After transplantation, donor T cells recognize polymorphic peptides presented by HLA surface molecules on the patient's cells. These polymorphic peptides, designated minor histocompatibility antigens, are caused by genetic differences in single nucleotide polymorphisms (SNPs) between patient and donor. Dependent on whether the antigen is presented on tumor cells or healthy non-hematopoietic tissues of the patient, donor T cells may induce the favorable Graft-versus-Leukemia effect or Graft-versus-Host-Disease, respectively. Using an optimized approach for whole genome association scanning, more than 80 new minor histocompatibility antigens have been found that are presented by seven common HLA class I molecules. The extended repertoire was investigated for the tissue distribution of antigen-encoding genes, pointing towards several antigens as potential targets for immunotherapy. Furthermore, antigens targeted in immune responses after transplantation were recurrently found in multiple patients, and 30% of antigens were translated in- or outside coding regions in other reading frames than the functional protein. In conclusion, despite many SNP mismatches between patients and donors, our data demonstrate that the repertoire of minor histocompatibility antigens is confined. As the antigens were identified by an unbiased forward strategy (T cell-to-antigen), our collection provides relevant insight to predict immunogenicity of (neo)antigens by reverse strategies (antigen-to-T cell). Furthermore, the antigens are fundamental to predict, follow or manipulate immune responses after allogeneic stem cell transplantation to improve clinical outcome of transplanted patients.

Keywords: Antigen processing and presentation, bone marrow transplantation, cancer immunology, MHC and polymorphic genes

P-0154

Downregulation of leukocyte LKB1/AMPK signaling is associated with the severity of Guillain-Barre syndrome

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Guillain-Barré syndrome (GBS) is an immune-mediated peripheral polyneuropathy with unknown etiopathogenesis. The intracellular energy sensor AMP-activated protein kinase (AMPK) and master metabolic regulator the mechanistic target of rapamycin (mTOR) regulate immune cell function through various mechanisms, including autophagy. We analyzed the LKB1/AMPK/mTOR complex 1 (mTORC1) signaling pathway and autophagy in peripheral blood mononuclear cells (PBMC) of GBS patients at transcriptional and protein level, as well as their correlation with the severity of the disease. The study included 23 newly diagnosed GBS patients and 20 age/sex-matched control subjects. The activation status of LKB1, AMPK, and mTORC1 in PBMC was assessed by immunoblot, while the expression of mRNA for autophagy regulators was analyzed by RT-qPCR. Our results demonstrate downregulation of leukocyte LKB1/AMPK signaling pathway in GBS, which was not associated with dysregulation of mTORC-1 or autophagy. The impairment of LKB1/AMPK pathway correlated with the disease severity, thus indicating its potential usefulness as a biomarker and/or therapeutic target in GBS.

Keywords: Autoimmunity, biomarkers, cell signalling, neuroimmunology

P-0171

RORα regulates trained immunity response induced by BCG vaccination

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Trained immunity is the long-term reprogramming of the innate immune cells induced by specific pathogens and vaccines, such as BCG. Trained immunity response varies depending on age, sex, and genetic variants. Among the genetic factors, RORα is known for its role as a regulator in the inflammatory response in monocytes and cholesterol metabolism. Preliminary analyses showed several SNPs associated with the RORA gene alter pro-inflammatory cytokine production induced by pathogens *in vitro* and immune cell training after BCG vaccination. With this in mind, we investigated the potential role of RORα in BCG-induced trained immunity. Human monocytes were primed with BCG, the RORα inhibitor SR3335, or both. On day 6, cells were re-challenged with LPS, and cytokine levels were measured by ELISA. ROS assay was performed using opsonized zymosan with the monocytes trained with BCG and SR3335. ATAC-sequencing was performed with PBMCs, monocytes, and NK cells from individuals before and 3 months after BCG vaccination. RORα inhibition by SR3335 strikingly increased TNF and IL-6 production in BCG-trained monocytes. Even in the absence of BCG, pre-exposure of monocytes to the RORα inhibitor significantly enhanced pro-inflammatory cytokine production. SR3335 elevated ROS production of BCG-trained monocytes. Lastly, BCG vaccination modified the availability of chromatin regions regulating the RORA gene transcription, with the most extensive changes in NK cells. Collectively, RORα was identified as a modulator of BCG-induced trained immunity in monocytes. Future studies will be focusing on whether RORα inhibition regulates monocyte and NK cell metabolism and epigenome.

Keywords: Immune regulation and therapy, innate host defence, innate immunity, memory

P-0180

Interleukin-34 expression by keratinocytes is downregulated in inflamed psoriatic skin lesions: toward an immunoregulatory role in skin inflammation

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Interleukin-34 (IL-34) expression has been widely reported in the epidermis, where the cytokine contributes to Langerhans cells homeostasis. However, little is known about its expression, regulation and role during skin inflammation. By contrast to numerous cytokines which are upregulated in inflamed skin, we show that IL-34 is downregulated in human psoriatic lesions, and restored by a successful anti-TNFα biotherapy. This finding raises the hypothesis that IL-34 could exert immunoregulatory properties in the skin, as it has been already described in other physiopathological conditions. In this study, we first present the expression profiles of IL-34 and their receptors by epidermal keratinocytes and/or dermal fibroblasts cultivated in monolayer (2D) or in three-dimensions (3D). *In vitro*, we show that, unlike fibroblasts which do not express the cytokine, IL-34 expression is higher in 3D models of human reconstituted epidermis than in 2D monolayer cultures of keratinocytes, demonstrating that IL-34 expression is tightly associated with the level of epidermal differentiation. After stimulation by the proinflammatory cytokine oncostatin M (OSM), IL-34 expression was strongly downregulated in 2D and 3D keratinocyte cultures. This could explain our observations in psoriasis patients where an altered epidermal differentiation characterizes skin lesions. Finally, we show that IL-34 decreases OSM signaling and proinflammatory effects on the expression of target genes involved in epidermal differentiation such as cytokeratin 10 and filaggrin. Together, these results demonstrate that IL-34 is mainly expressed by differentiated keratinocytes in healthy condition but strongly downregulated during psoriasis skin inflammation and suggest its potential immunoregulatory role in the skin.

Keywords: Cytokines and mediators, inflammatory disease, inflammatory molecules, skin diseases

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POSTER PRESENTATIONS

P-0194

IFN-γ produced in infection down-regulates PPAR-γ to modulate adipose tissue biology

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Adipose tissue is a major lipid storage organ which releases and distributes lipids to maintain energy homeostasis. In context of metabolic disease, adipose tissue was shown to closely interact with the immune system as obesity drives inflammation in this organ which alters local and systemic regulation of metabolism. However, how immune cells interact with adipocytes in context of viral infection is largely unknown. Here, we investigated the impact of virus-induced activation of the immune system on adipose tissue metabolism and the underlying benefit of these changes to the organism. In an *in vitro* model of adipocyte differentiation, we could show that the pro-inflammatory cytokine IFN-γ significantly reduces cellular lipid content. High-throughput transcriptome analysis of these cells demonstrated that IFN-γ mediates down-regulation of PPAR-γ, a master regulator of adipocyte tissue metabolism, as well as many of its downstream targets, causing a net efflux of nutrients. Infection of mice with cytomegalovirus induced a striking reduction of adipocyte cell size and induced a change in the transcriptional profile of these cells corresponding with an IFN-γ imprint. Accordingly, infection caused a systemic increase of adipose tissue derived nutrients, such as free fatty acids in circulation. Importantly, our results indicate that these nutrients promote the acute lymphocyte response to viral infection. Our findings suggest that cytokines produced in response to viral infection can modulate adipocyte and systemic metabolism to benefit the immune response to infectious disease.

Keywords: Cytokines and mediators, metabolic control of immune responses, nutrients, viral infections

P-0205

Glycan-dependent signalling routes and transcriptional programs in human dendritic cells after triggering the C-type lectin receptor MGL

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C-type lectin receptors on dendritic cells can shape the immune response to bacteria, viruses and tumour cells. Signalling through the macrophage galactose-type lectin (MGL) reduces the glycolytic capacity of monocyte-derived dendritic cells (moDCs) and increases their IL-10 and TNFα production, which stimulates a Th2 and Tr1 response. Recently, several MGL ligands were discovered to induce different conformations of the MGL carbohydrate-recognition domain but the effect on the moDC maturation and functionality was only investigated for two of these MGL ligands. We will investigate the transcriptional programs that are induced in response to five different MGL ligands, which we coupled to dendrimers to increase their polyvalency. Furthermore, we will determine which cytokines, co-stimulatory and inhibitory receptors, and signalling molecules are associated with the transcriptional programs triggered by the five MGL ligands. Up to now, MGL stimulation resulted in a higher CD11c and CD14 co-expression by moDCs. The five MGL ligands also displayed a different capacity to increase IL-10 secretion. In the future, MGL-mediated ligand-specific transcriptional programs could be exploited to manipulate immune responses in human DCs.

Keywords: Cell signalling, dendritic cells, molecular immunology

P-0206

Novel aryl hydrocarbon receptor antagonist promotes macrophage pro-inflammatory phenotype

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Aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that responds to various aromatic compounds, both endogenous such as kynurenine and exogenous such as natural plant flavonoids, polyphenolics and indoles. AhR has been recently identified as the regulator of immune cells function. The activation of AhR generally leads to the attenuation of the immune response, while its inhibition promotes the opposite effects. In this study we have selected several plant-derived indol derivatives and tested them for their AhR ligand activity. A potent AhR antagonist was identified (code C46) and further evaluated on mouse peritoneal macrophages for its ability to modulate macrophage function. Macrophages were exposed *in vitro* to compound C46 in concentrations ranging from 250 ng/ml to 1000 ng/ml for 48 h. Flow cytometry analysis showed that C46 significantly and dose-dependently up-regulated the proportion of M1 macrophages (F4/80+CD40+). Interestingly, C46 influenced only M1 macrophages, as the proportion of M2 (F4/80+CD206+) remained stable upon the exposure to C46. In addition, C46 increased the cytocidal function of macrophages by increasing the content of nitric oxide as determined by DAF-FM staining. Similarly to *in vitro* effects, intraperitoneal C46 administration up-regulated the proportion of M1 macrophages in the peritoneum, 72 h after the treatment. In conclusion, blocking of AhR pathway by C46 potentiates pro-inflammatory function of macrophages and it may represent a promising approach for future testing in animal models of cancer.

Keywords: Biology of the immune system, immune regulation and therapy, macrophage, molecular immunology

P-0218

Generation and intial characterization of a SARM epitope-tagged mouse

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Sterile alpha and HEAT/Armadillo motif–containing (SARM) protein is a member of Toll/IL-1R (TIR) domain protein family and is highly conserved in evolution with a role in innate immunity in diverse organisms. A major recent discovery was that the TIR domain of SARM actually has NADase enzymatic activity which is required for SARM-dependent neuronal cell death. Although most research to date on SARM relates to its function in axon degeneration, SARM likely has non-neuronal functions too, and we showed that SARM is a regulator of inflammasome responses and pyroptosis in murine macrophages. Studies of non-neuronal SARM are hampered by the fact that immunodetection of the protein outside the brain is challenging. To address this, here using CRISPR technology we generated a mouse expressing epitope-tagged SARM whereby the protein contains a triple-Flag tag and Strep-tag II on its C-terminus (SARM-FS). We confirmed this SARM-FS mouse shows the same level of *Sarm1* mRNA expression compared to the wild-type mouse and also discovered that the mouse expresses SARM protein not only in brains but also in various tissues such as the liver, spleen and kidney, albeit at a lower level than in the brain. Furthermore, we established immortalised BMDMs isolated from the SARM-FS mouse and confirmed SARM protein expression in these cells. These data suggest that SARM has roles in various tissues apart from the brain and pave the way for further work to examine the broader contribution of the SARM TIR domain and NADase activity to normal physiology and the immune response.

Keywords: Animal models, macrophage, molecular immunology

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POSTER PRESENTATIONS

P-0231

VPA-driven oxidative stress alters eosinophilic immune responses

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Targeting epigenetic modifications are one of the therapeutic approaches to treat various diseases like cancer, epileptic seizures and bipolar disorders. However, these treatments can also have toxic effects on normal healthy cells. Studies show that valproic acid (VPA), as an HDACi, may also create oxidative stress in cells and as a result cells increase the expression of antioxidant target genes by transcription factor Nrf2 to reduce toxicity and to provide homeostasis. Effects of such treatments are largely unknown when it comes to eosinophils which have critical roles in fighting against parasites and allergens via PRRs. Therefore, we aim to investigate the pathways that modulate oxidative stress caused by VPA in eosinophil-like cells. On one side, we induced oxidative stress by culturing EoL-1 cells in serum free media and by H2O2 treatment as a positive control and on the other side, we treated EoL-1 cells with VPA to evaluate its ability to induce oxidative stress. First-off, we determined the optimum time (24 hours post stimulation) and dose (5mM) for VPA to induce oxidative stress and its impact on the PRRs mediated inflammation. We compared the percentages of EoL-1 cells that undergone oxidative stress measuring the ROS activity by flow cytometer, NRF2, PRRs expression by western blotting. Next, we, evaluated the proinflammatory cytokine release by ELISA. As a HDACi, VPA induced oxidative stress in a dose dependent manner and increased the Nrf2 at mRNA and protein levels however reduced the acetylated Nrf-2, TLR2, TLR5, NLRC4, and NLRP3 and inflammation, exerting anti-inflammatory properties.

Keywords: Eosinophils, innate immunity, molecular immunology

P-0244

Psoriasis-related miR203 activates NK cells by a complex pDC/monocyte/NK cell crosstalk

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We have recently described that exosomal GU-rich microRNAs (GU-miRNAs) activate plasmacytoid dendritic cells (pDCs) by triggering TLR7 and that pDCs may enhance Natural Killer (NK) cytotoxicity and tissue damage in Lupus Erythematososus. Because both pDCs and NK cells infiltrate psoriatic lesions, we asked whether deregulated miRNA secretion may foster psoriatic tissue damage by activating pDCs and the crosstalk with NK cells. Following extraction from healthy and psoriatic skin, the expression of GU-miRNAs was investigated by RT-PCR. Synthetic miRNAs were used to stimulate purified NK cells, pDCs, peripheral blood mononuclear cells or co-cultures of these cells. Cell activation was assessed in terms of cytokine secretion and target cell killing. Inhibitor experiments were performed to demonstrate TLR activation by miRNAs. Several GU-miRNAs were upregulated in psoriatic skin lesions, with miR203 being the most expressed. pDCs but not purified NK cells were fully activated by miR203. Contrastly, when miR203 was used to stimulate PBMCs, NK cells were able to produce IFN-g and to kill target cells. Co-culture experiments of purified cell populations revealed that both pDCs and monocytes were required for NK cell activation by miR203. This action was dependent on a TLR7/8-mediated release of IFN-g, IL-12 and IL-18 which were responsible for licensing NK cell response to miR203. pDC/monocyte-licensing of NK cell response to TLR7/8-ligands adds a further level of complexity to innate immune cell crosstalk. Deregulated exosomal miRNAs potentially activate a tissue-damaging innate immune crosstalk in psoriasis and may represent a novel mechanism involved in pathogenesis.

Keywords: Autoimmunity, dendritic cells, NK cells

P-0247

Cell activation and expression of immune modulatory factors (RAGE and IDO) in PBMC treated with MAGE or conventional AGEs

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MAGE is the model counterpart of AGEs found in blood. AGEs binding with RAGE (receptor for AGEs) initiate expression of proinflammatory factors and might enhance RAGE expression in some cells. Kynurenine pathway initiated by the indoleamine 2,3-dioxigenase 1 (IDO1) is well accepted contributor to the proinflammatory microenvironment. Previously we have shown that MAGE (AGE derived from melibiose) has unique structure and biological properties being a weak activator of the proinflammatory cytokines. In this study we tested the effect of MAGE and conventional AGEs on activation of blood cells to learn about RAGE, IDO expression and activity of cells. The study was performed on PBMC isolated with the modified Ficol gradient method from blood of healthy volunteers. Cells were cultured in the FBS supplemented RPMI 1640 medium and stimulated with 250 μg/ml AGEs (MAGE, GA-AGE, Glc-AGE) for 24-48 h. RAGE+ and IDO1+ cells were determined by FACS (BD Facs Fortessa) in monocytes, macrophages (M1, M2), T lymphocytes, NK, and NKT population identified with the population-specific markers. MAGE decreased RAGE expression on monocytes, had no effect on lymphocytes B, T, and NK cells, and enhanced expression in NKT cells with initially low RAGE expression. IDO1 was not affected by MAGE, but GA-AGEs and Glc-AGEs increased IDO1 expression in monocytes and NK cells. MAGE increased number of CD107a+ T lymphocytes, NK and NKT cells. All AGEs induced monocyte differentiation into M1. MAGE in contrast to conventional AGEs contributes to T lymphocyte activation and has no effect on IDO1 expression.

Keywords: Immune regulation and therapy, inflammatory molecules, macrophage, microenvironment, NK cells, NKT cells

P-0250

Histone deacetylase 7 (HDAC7) regulates cell survival and amino acid metabolism of CD8+ T cells

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Histone deacetylases (HDACs) canonically suppress transcriptional activity via modulating the epigenetic landscape and also serve as therapeutic targets of multiple inhibitors for treating various types of cancer including T cell lymphoma. However, the biological functions of individual HDACs in immune cells, especially tumor-specific CD8+ cytotoxic T lymphocytes (CTLs), remain ill-defined. Previous studies in our group showed that CTLs derived from *Hdac7* knockout mice exhibit impaired anti-tumor ability and elevated cell death. Given that HDAC7, a member of class IIa HDACs, possesses nuclear export and import signal peptides, we analyzed the subcellular localization of HDAC7 in activated CTLs using immunofluorescence staining. The translocation and enrichment of HDAC7 in the cytosol upon stimulation also implies its potential role on modulating non-histone proteins. Moreover, the result of transcriptional analysis via RNA sequencing acquired from activated wild type and *Hdac7* knock out CTLs reveals altered expression of genes involved in cellular metabolism such as mTOR and numerous amino acid transporters, including *slc1a2* and *slc1a4*. Using metabolic and proteomic analyses, we observed an increased glutamine consumption and reduced mTOR activity in *Hdac7* deficient CTLs. As the mTOR pathway is known to integrate the environmental nutrient signals and acts directly upstream of autophagy induction, we found that *Hdac7* defective CD8+ T cells display altered cellular autophagic level and mitochondrial dynamics. Taken together, our finding suggests HDAC7 is a key regulator of cell survival and amino acid metabolism in CTLs.

Keywords: Cell signalling, molecular immunology, cell death

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POSTER PRESENTATIONS

P-0266

Endogenous 5-methoxytryptophan influences the metabolic state and function of human macrophages

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Factors influencing the immunometabolism can provide insight into the initiation and progression of diseases. In this study we examined the effect of 5-methoxytryptophan (5-MTP), an indole compound present in serum reported to inversely correlate in disease progression of chronic kidney disease and fibrosis, on the metabolism of human macrophages. We found that inflammatory activated macrophages, but not inactivated or alternatively activated macrophages, can degrade 5-MTP by elevated expression of indoleamine oxygenase (IDO), a potent degrading enzyme of indoles. Compounds similar to 5-MTP have been suggested to have high membrane affinity and have been suggested to passively insert into the membranes of mitochondria. Indeed, using the novel FRET-probe Mito-Flipper, we demonstrate that macrophages differentiated with 5-MTP have higher mitochondrial membrane tension. Concomitant with these membrane changes, the mitochondria also change in morphology and function; they become more branched (3D microscopy) and have stronger membrane depolarization but less ROS production upon LPS stimulation. These changes in mitochondria are accompanied by changes in the macrophage metabolism, as the macrophages shift more toward glucose uptake and show less to lipid uptake. Finally, macrophages differentiated with 5-MTP might have a stronger phenotype associated with alternatively activated macrophages as the mannose-receptor (CD206) is upregulated more upon IL-4 stimulation compared to macrophages differentiated without 5-MTP. Finally, as collagen uptake is also increased in 5-MTP macrophages, which is anti-fibrotic. This work demonstrates that 5-MTP is a contributing factor in macrophage anti-inflammatory polarization by influencing the metabolism of macrophages towards glucose and could play a role in disease progression.

Keywords: Macrophage, metabolic control of immune responses, microenvironment

P-0267

Calciprotein particles activate the NLRP3 inflammasome in human monocytes in a CaSR-dependent manner by entering the endolysosomal pathway

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In rheumatoid arthritis joints are affected by erosion which leads to increased extracellular calcium ions, phosphate ions and fetuin-A concentrations and therefore the formation of so-called calciprotein particles (CPPs). Increased extracellular calcium ions trigger CPP uptake through macropinocytosis in human monocytes, which in turn causes the activation of NLRP3 inflammasome and IL-1b release. However, the exact mechanism of CPP-induced NLRP3 inflammasome activation remains unknown. To verify the role of Calcium-sensing receptor (CaSR) in macropinocytosis during NLRP3 inflammasome activation a CaSR-deficient monocytic THP-1 cell line was established using CRISPR-Cas9 technology. A decrease in CPP uptake was detected in comparison to wildtyp cells. In addition, ELISA was used to measure the IL-1b release after calcium+CPPs stimulation and was found to be decrease in CaSR-deficient cells, elucidating the role of CaSR in calcium mediated macropinocytosis and the subsequent NLRP3 inflammasome activation. To follow CPP trafficking within the cell, Imaging Flow Cytometry was used. Freshly isolated human blood monocytes were stimulated with calcium and calcein-stained CPPs while lysosomes were stained with LysoTracker Deep Red. An increase from 5% to 25% of CPPs and LysoTracker colocalization was seen in monocytes incubated with calcein-CPPs in the presence of calcium from 1h to 3h, demonstrating that CPPs enter the endolysosomal pathway. Furthermore, monocytes incubated with no increased extracellular Ca2+, showed only 0.3% of colocalization. In conclusion, our results demonstrate that human monocytes engulf CPPs in a calcium- and CaSR-dependent manner and intracellularly CPPs enter the endolysosomal pathway resulting in the activation of NLRP3 inflammasome.

Keywords: Innate immunity, molecular immunology, rheumatoid arthritis

P-0268

Glucagon-like peptide-1 receptor regulates macrophage migration in monosodium urate-induced peritoneal inflammation

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Macrophage is a critical effector cell in the pathogenesis of inflammatory arthritis such as gout. Whether Glucagon-like Peptide-1 Receptor (GLP-1R) is involved in gout by regulating macrophage migration remains unclear. Both wide-type (WT) and GLP-1R-knockout (KO) C57BL/6 mice were administered an intraperitoneal injection of 3 mg monosodium urate (MSU) to induce peritonitis, which was widely used as an experimental model of acute gout. Due to the lack of the specific antibody against GLP-1R, we used transgenic mouse model which expressed EGFP under the control of GLP-1R promoter to confirm the expression of GLP-1R by macrophages and found that the expression of GLP-1R was greater on M2 macrophages than on M1 macrophages. Its expression was not affected by exposure to an agonist (Ex-4) or antagonist (Ex-9-39). Moreover, Transwell® assay indicated that GLP-1R expression was greater on the migrated macrophages than on cells remained in the upper chamber, with similar results for M1 and M2 macrophages. The proportion of M2 macrophages was significantly greater than that of M1 macrophages among the migrated cells. Interestingly, GLP-1R deficient macrophages displayed a reduced migration ability and an enhanced expression of interleukin (IL)-6, while the expression of IL-1β was not affected. Furthermore, the number of macrophages recruited to peritoneal cavity was also markedly decreased in GLP-1R KO mice treated with MSU, as compared with WT mice. Our data demonstrate that GLP-1R plays an important role in MSU-induced inflammation by promoting macrophage migration.

Keywords: Animal models, inflammatory joint diseases, inflammatory molecules, macrophage, molecular immunology

P-0271

Heterogeneity in clearing uropathogenic *E. coli* by macrophages

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In all humans, Escherichia coli inhabits the gastrointestinal tract, where it is part of the healthy commensal microbiota. However, certain pathogenic strains, called uropathogenic E. coli (UPEC), are derived from this commensal E. coli but account for >80% urinary tract infections (UTIs). These are the most common infections; as >50% of women suffer from it at least once during their lifetime. UTIs are often difficult to treat due to the formation of intracellular E. coli colonies within bladder epithelial cells (BECs) which confer antibiotic resistance. These hidden colonies are a source of recurrent infections in 20-30% of patients. Tissue-resident macrophages (Mø) are among the first immune cells to respond to infected bladder tissue and comprise ~40% of all CD45+ cells in the murine bladder. Infected BECs secrete CCX2 chemokine for the recruitment of CD14+ monocytes from blood, which also differentiate into Mø and supplement the tissue-resident Mø. At the infected site, Mø clear UPEC by phagocytosis and cytotoxicity mechanisms. Following phagocytosis, captured UPEC are contained in membrane-bound phagosomes, which undergo a series of progressive fusion steps with early/late endosomes and lysosomes to deliver bactericidal V-ATPase. In this presentation, I will discuss multiple mechanisms of how different UPEC strains prevent their immune clearance by dysregulating membrane trafficking, down-regulation of the activity of the V-ATPase, and induction of apoptosis in Mø.

Keywords: Bacterial infections, macrophage, molecular immunology, phagocytosis

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POSTER PRESENTATIONS

P-0272

Anti-inflammatory activities of plant derived-lipids on human skin cell

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Cutaneous protection against external threat is provided by the distinctive lipid composition of the stratum corneum, these lipids are orchestrated in a brick-and-mortar system. The hydrophobic barrier created by lipids minimize water loss and conjointly impede the doorway of microorganisms. Thus, depletion of these lipids could result in various skin diseases. Folch's technique was employed for the extraction of lipids and the fatty acid characterization was conducted via liquid chromatography in tandem mass-spectroscopy (LC-MS). Phorbol-12-myristate-13-acetate (PMA) was used to induce inflammation in human adult dermal fibroblast. Furthermore, the determination of pro-inflammatory cytokines (IL-6 and TNF-α) were conducted by ELISA. Additionally, Griess assay was employed in the determination of nitric oxide (NO) released by the inflamed cell, while qRT-PCR was conducted to elucidate the genes (TLR-2, NF-KB, and NOS-2) involved in this immuno-modulatory activity. Crude lipid extracts offers protection against the cell death of PMA and significantly dampens inflammatory mediators (IL-6, TNF-α, and NO). Meanwhile, this effect could be traced to the down-regulation of TLR-2, NF-kB, and NOS-2 genes in various pathways. In corollary, the promising anti-inflammatory activities of crude lipid extracts from locally sourced plant species as a result of suppression of pro-inflammatory cytokines and down-regulation of inflammatory genes could be attributed to the rich composition of various saturated fatty acids (SFA) and poly-unsaturated fatty acid (PUFA), thus warranting their application in derma-cosmetic formulations for various inflammatory diseases pertinent to the the skin barrier.

Keywords: Cell death, inflammatory disease, mass spectrometry, cytokines and mediators, skin diseases

P-0273

The MALAT1/miR-30e/YKL-40 axis is dysregulated in systemic sclerosis

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Systemic sclerosis (SSc) is an autoimmune disease with unclear etiology, which possess treatment difficulties. Expression of both protein coding and non-coding RNAs are dysregulated, during disease development, thus affecting its pathophysiology. The aim of our study was to examine a possible regulatory axis through lncRNAs and miRNAs, implemented in the control of YKL-40 in SSc. A panel of 8 miRNAs and 3 lncRNAs possibly involved in the control of YKL-40 were selected on the basis of an *in silico* analysis. qPCR was used to evaluate the expression levels of both miRNAs and lncRNAs in white blood cells (WBCs) and plasma obtained from 40 female patients with SSc and 14 healthy controls. Among the 8 screened miRNAs, miR-30e (p=0.04) and miR-30a (p=0.01) were significantly downregulated in WBCs and plasma of SSc patients, respectively. In contrary, the expression of the metastasis associated lung adenocarcinoma transcript 1 (MALAT1) in WBCs was significantly up-regulated compared to the controls (p=0.03). Increased levels of MALAT1 could be associated with downregulation of miR-30e and miR-30a expression in WBCs and plasma. We hypothesize that MALAT1 could possibly act as a miR-30e and miR-30a decoy, and this sequestration leads to high serum protein levels of YKL-40 in SSc patients.

Keywords: Epigenetic control and modulation of immunity, autoimmunity, lncRNA, miRNA

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P-0274

Investigating the role of vesicle transport-associated proteins Sec22b and Stx4 in the cross-presentation of PLGA microsphere-encapsulated antigens *in vitro*

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Biodegradable poly-(lactic-co-glycolic acid) microspheres (MS) are a promising antigen delivery system for cancer vaccination. MS are phagocytosed by professional antigen presenting cells (APCs) – such as dendritic cells (DCs) and macrophages – that can display exogenous antigens on major histocompatibility complex (MHC) class I molecules. This process is referred to as cross-presentation(XP) and essential to prime cancer-specific cytotoxic T-lymphocytes (CTLs). Although incompletely understood, XP of MS-encapsulated antigens is thought to follow a cytosolic route involving antigen translocation to the cytosol and proteasomal processing. This pathway requires trafficking of endoplasmic reticulum (ER)-derived proteins to antigen-containing phagosomes via the ER-Golgi intermediate compartment (ERGIC), a process recently shown to depend on the vesicle transport-associated SNARE protein Sec22b. We investigated the role of Sec22b and its interaction partner sytaxin-4 (Stx4) in the XP of MS-encapsulated antigens. Using CRISPR/Cas9-mediated genome editing, we generated homozygous knockout (KO) clones for respectively Sec22b and Stx4 in two mouse APCs lines. While XP of MS-encapsulated ovalbumin(OVA) mainly depended on the cytosolic pathway, we found no evidence for an essential role of Sec22b in our system. This supports a report by Wu and colleagues showing Sec22b-independent XP of OVA-based antigens. Interestingly, while KO of Stx4 did not affect XP of MS-encapsulated OVA in MutuDCs, we observed reduced XP in BMC-2 macrophages lacking Stx4. This project contributes to characterizing the XP of MS-encapsulated antigens and adds to the discussion about the role of Sec22b and Stx4 in XP. Our data point towards SNARE protein redundancy in the cytosolic pathways of XP.

Keywords: Anti-cancer vaccine, antigen processing and presentation, dendritic cells, macrophage, molecular immunology

P-0276

Mitochondrial complex II shapes dendritic cell metabolism and cytokine expression

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The expression of cytokines involved in the polarization of the immune response can be shaped by energetic metabolism. Although prevalent views emphasize the role of glycolysis, mitochondrial respiration and tricarboxylic acid (TCA) cycle activity are also key players. Breaks at citrate and succinate levels characterize the TCA cycle in activated immune cells. Succinate dehydrogenase (SDH, complex II) catalyses the oxidation of succinate to fumarate and yields reducing equivalents in the form of $FADH_2$ to the electron-transport chain (ETC). Thus, a key point in the connection between TCA cycle and ETC. Therefore, SDH function has been scrutinized in response to LPS, but its role in response to fungal patterns remains unclear. The aim of this study was addressing the role of TCA cycle intermediates on cytokine expression in human monocyte-derived dendritic cells (DCs), focusing on succinate metabolism using as a stimulus the fungal surrogate zymosan. To further address this issue, experiments were carried out in the presence and absence of succinate precursors L-Glutamine, sodium succinate and the cell-permeant analogue of succinate (DMS); using inhibitors of the different SDH subunits and the cell-permeant analogue of fumarate (DMF). Collectively, the data reveal the distinct mechanisms whereby SDH activity fine-tunes cytokine expression. And consistent with its anti-inflammatory effects, rewiring TCA cycle by the increase of fumarate intermediates has a reduced effect on cytokine expression. Further research on immunometabolism may yield drugs with a wider prospect of application rather than the biological therapies used to inhibit the effect of pro-inflammatory cytokines.

Keywords: Cytokines and mediators, dendritic cells, fungal infections, innate immunity, metabolic control of immune responses, phagocytosis

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POSTER PRESENTATIONS

P-0277

Absence of functional Treg in scurfy mice leads to pathogenic autoantibody targeting Type VII collagen

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Dysfunction of regulatory T cells (Treg) contributes to the development of different autoimmune diseases. Previously, we have shown that Treg-deficient scurfy mice develop high titers of autoantibodies with reactivity to structural skin proteins including a pathogenic autoantibody to BP230 leading to a bullous pemphigoid-like phenotype. Given the polyclonal nature of the autoimmune disease in scurfy mice, we screened for autoantibodies against other potential pathogenic target sites. Indirect immunofluorescence (IF) microscopy yielded a murine IgG1 autoantibody (H510) with a distinct different linear basement membrane staining. H510 binds to the blister floor on murine salt-split skin and is pathogenic *in vivo* as it induces subepidermal blisters in neonatal mice, implying type VII collagen (Col7) as a potential autoantigen. The binding of H510 on WT skin was significantly reduced by pre-incubation with murine von-Willebrand-factor-A-like domain 2 (mvWFA2) of Col7. Western blot analysis and indirect IF using skin from *Col7* knock-out mice confirmed Col7 as the target antigen of H510. In further mapping work-up mvWFA2 of Col7 was confirmed as the antigenic epitope. In summary, we here introduce a recently identified, pathogenic autoantibody derived from Treg-deficient mice with reactivity against Col7 as useful tool for the induction of EBA in mice.

Keywords: Adaptive immunity, animal models, antibody, autoimmunity, skin diseases

P-0278

Identification of a novel conserved signaling motif in CD200 receptor required for its inhibitory function

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The inhibitory signaling of CD200 receptor 1 (CD200R) has been attributed to its NPxY signaling motif. However, NPxY-motifs are present in multiple protein families and are mostly known to mediate protein trafficking between subcellular locations rather than signaling. Therefore, we investigated whether additional motifs specify the inhibitory function of CD200R. We performed phylogenetic analysis of the intracellular domain of CD200R in mammals, birds, bony fish, amphibians and reptiles. Indeed, the tyrosine of the NPxY-motif is fully conserved across species, in line with its central role in CD200R signaling. In contrast, P295 of the NPxY-motif is not conserved. Instead, a conserved stretch of negatively charged amino acids, EEDE279, and two conserved residues P285 and K292 in the flanking region prior to the NPxY-motif are required for CD200R mediated inhibition of p-Erk, p-Akt308, p-Akt473, p-rpS6 and LPS-induced IL-8 secretion. Altogether, we show that instead of the more common NPxY-motif, CD200R signaling can be assigned to a unique signaling motif in mammals defined by: EEDExxPYxxYxxKxNxxY.

Keywords: Biology of the immune system, cell signalling, molecular immunology

P-0279

Glucose promotes viral replication after conversion into lactate by inhibiting type-I interferon production

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Viral infection has a major impact on systemic metabolism. Previously, we showed that strong viral infection results in relative hypoglycemia (RHG). However, the molecular mechanism(s) underlying the RHG and its beneficial effect remain unclear. Recently, lactate was shown to impair IFN-I production in response to viral infection by inhibiting RIG-I. We observed *in vivo* that viral titers are higher in fasted mice that were drinking glucose-laced water in comparison to animals drinking normal water. To determine whether glucose availability regulates IFN-I production through a lactate-dependent mechanism, infected mouse embryonic fibroblasts (MEF) and seminal vesicle epithelial cells (SVEC) were cultivated under low- or high glucose concentrations and supplemented with sodium oxamate, a specific LDHA inhibitor. Low glucose concentrations, as well as sodium oxamate, significantly reduced the level of viral replication in both SVEC and MEF. In addition, we supplemented the low glucose medium with different nutrients such as citrate, acetate, and galactose, yet none of them managed to compensate for the effect of limited glucose. Finally, infected cells cultured under high-glucose conditions in the presence of oxamate showed significantly increased IFN-β production as determined by ELISA. To summarise, we showed that sodium oxamate suppresses the level of viral replication *in vitro* by reducing lactate levels. Our results confirm that glucose promotes viral replication through direct inhibition of IFN-I secretion by lactate, rather than by promoting catabolic metabolism. Overall, these findings bring us one step closer towards elucidating the metabolic pathway through which RHG promotes IFN-I production.

Keywords: Metabolic control of immune responses, molecular immunology, viral infections

P-0280

Investigating the discrete roles of canonical NF-κB subunits as master regulators of conventional CD4+ T cell function in response to cancer

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Conventional CD4+ T cells (Tconv) play a pivotal role in cancer immunity and often impact cancer outcomes; the identification of moleculear pathways orchestrating their biology is thus of utmost interest for the discovery of novel biomarkers and immunotherapeutic targets. Here we explored the specific contributions of RELA and C-REL, two canonical transcription factors of the NF-κB family, in Tconv biology. Transcriptome analyses of Tconv cells isolated from mice carrying conditional ablation of each gene in T cells, demonstrated a dramatic effect of RELA ablation on the expression of cytokines and surface receptors, while C-REL ablation led to more subtle effects. Mechanistically, RELA modulated Tconv identity both by directly binding to important genes and through regulation of other master transcription factors. In line with this, RELA was required for the optimal proliferation and effector function of Tconv *in vitro*. In the context of cancer, acute ablation of RELA in Tconv increased tumor burden and reduced Tconv function, suggesting a major role for RELA in the orchestration of cancer immunity. Finally, in an effort of translational research, we showed that CRISPR/Cas9-mediated ablation of RELA and C-REL in human primary Tconv, led to defective proliferation and cytokine secretion, associated with impaired gene expression profiles comparable to those observed in mouse cells. Together, we demonstrate that RELA and C-REL are nexus regulators of Tconv effector functions and that their modulation represents strong candidates for cancer immunotherapy.

Keywords: Adaptive immunity, animal models, bone marrow transplantation, cancer immunology, *in vivo* tumor models, omics technologies

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POSTER PRESENTATIONS

P-0281

Highly sensitive C Reactive protein (hs-CRP) as a marker of cardiovascular risk in patients with pre-hypertension

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The concept of Pre-hypertension, defined as a systolic blood pressure of 120-139 mmHg and/or a diastolic blood pressure of 80-89 mmHg, was introduced as the new guideline for the management of blood pressure by the seventh report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure. Hs-CRP has been studied extensively as marker of cardiovascular risk, however its role as a marker of cardiovascular risk in pre-hypertension is not well defined. The aim of this study was to explore the role hs-CRP cardiovascular risk marker in patients of pre-hypertension. 50 adult patients, above 19 years of age,with diagnosed pre-hypertension and 50 age and sex matched healthy controls were studied in a tertiary health care centre in New Delhi, India, over a period of 6 months. The serum levels of hs-CRP was measured by ELISA and routine lipid profile was measured by automated analyzer. Framingham Risk scoring scoring was also done for all the patients. Data is presented as Mean±S.D. and relationships were determined by Pearson correlations. The mean age of the patients was 51±6.5 years (72% men, 28%women). The mean serum Hs-CRP levels [5.40±2.51 mg/l] for pre-hypertension were significantly higher than in controls [0.91±0.76 mg/l] [p<0.001]. Framingham Risk score was higher for patients with pre-hypertension than controls. Higher hs-CRP values correlated with higher Framingham risk score. Our results suggest that hs-CRP is a marker of Cardiovascular Risk in patients of pre-hypertension.

Keywords: Biomarkers, cardiovascular diseases, inflammatory disease, metabolic control of immune responses

P-0282

Activation of the pyrin inflammasome during sepsis

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Sepsis is the leading cause of death in critical care units and it is defined by a systemic inflammatory response which is followed by an immunosuppression of the host. The inflammasome is an important effector mechanism of the innate immune response involved in sepsis. While the function of the NLRP3 inflammasome is involved in sepsis, the function of other inflammasomes, like Pyrin, is not well known. To analyse the function of the Pyrin inflammasome in blood samples and PBMCs of septic patients and compare with the activation of NLRP3. Individuals with intra-abdominal origin sepsis (n=16), abdominal surgery individuals that had not developed sepsis (n=11), and a healthy control group (n=10) were recruited. NLRP3 inflammasome was stimulated by LPS+ATP and Pyrin inflammasome with LPS+TcdB. Flow cytometry was used to determine ASC specks formation in monocytes and ELISA to determine cytokine secretion. We observed a decrease in ASC specks formation and the release of IL-1β, after NLRP3 stimulation in 6 individuals that also had some biochemical and clinical scores of disease severity above the average of septic patients (SOFA score or days in critical care unit among others). We next found that while those septic patients were immunocompromised for NLRP3, the activation of the Pyrin inflammasome was not impaired. The impairment of the NLRP3 inflammasome in septic patients might serve as an early indication of immunosuppression in critical patients, while the Pyrin inflammasome emerge as a positive control of inflammasome activation.

Keywords: Biomarkers, cytokines and mediators, inflammatory disease

P-0283

Immunometabolism in atherosclerosis: Investigating cholesterol crystals as potent drivers of metabolic reprogramming and M1 macrophage polarisation

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Atherosclerosis is a leading cause of heart failure, and a chronic inflammatory disease predominantly mediated by classically activated M1 macrophages. Cholesterol crystals, which are found to accumulate at both early and advanced stages of atherosclerosis; are known to drive inflammatory responses in macrophages. However, the impact of cholesterol crystals on macrophage polarization has not yet been examined. Moreover, it has been demonstrated that macrophages in atherosclerotic plaques have a highly glycolytic profile which correlates with decreased plaque stability and increased incidence of rupture and thrombosis. There are, however, no studies to date linking cholesterol crystals to metabolic reprogramming. Therefore, the aim of this study is to examine the impact of cholesterol crystals in metabolic reprogramming and polarization of macrophages. Primary human macrophages were treated with cholesterol crystals (500 μg/ml) over 24 hours in the presence/absence of the glycolytic inhibitor, 2-deoxyglucose (25 mM). mRNA expression was assessed by qPCR and cytokine production was assessed by ELISA. Macrophage metabolism was examined using fluorescence lifetime imaging microscopy (FLIM) and Agilent Seahorse assays. Mitochondrial dynamics was assessed through confocal imaging. Cholesterol crystals were observed to skew macrophage polarization towards an M1 phenotype. Seahorse and FLIM analysis revealed that cholesterol crystals drive metabolic reprogramming towards glycolysis with increased expression also observed ofq surrogate markers of glycolysis. Finally, cholesterol crystal induced inflammatory responses were attenuated upon inhibition of glycolysis. This study demonstrates for the first time that cholesterol crystals alter macrophage metabolism and drive M1 polarization in primary human macrophages.

Keywords: Cardiovascular diseases, immune regulation and therapy, inflammatory disease, macrophage, metabolic control of immune responses

P-0287

A trimeric recombinant fragment of pulmonary surfactant protein SP-A is sufficient to neutralize cytotoxic and pro-inflammatory effects of cathelicidin on alveolar epithelial cells

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Human cathelicidin (LL-37) is a host defense peptide with antimicrobial activity. However, LL-37 also can trigger tissue injury through binding to host membranes, causing cytotoxic or proinflammatory effects. LL-37 is secreted by epithelial and immune cells of the skin, intestine, ocular system, and lung. LL-37 levels rise in airways of chronic obstructive pulmonary disease patients, contributing to chronic inflammation. Surfactant protein SP-A is secreted by pneumocytes and has essential immune functions in the lung. It is a large oligomeric protein assembled in multiples of three subunits, containing a globular recognition domain contiguous to a collagen-like domain. Our objective was to investigate whether either human SP-A or a trimeric recombinant fragment of the protein, which lacks most of the collagen domain (rfhSP-A), is involved in local regulation of LL-37 activity. We studied the interaction of LL-37 with SP-A and rfhSP-A by tryptophan fluorescence and the effects of these proteins on LL-37 antimicrobial and cytotoxic activities. We found that both SP-A and rfhSP-A bound to LL-37 with high affinity (Kd = 0.45 ± 0.01 nM and Kd = 1.22 ± 0.73 nM respectively). Such interactions result in reduction of LL-37-induced cytotoxicity and inflammation in pneumocytes. However, LL-37 antimicrobial activity against respiratory pathogens was not affected by either SP-A or rfhSP-A. These results demonstrate that SP-A plays a protective role in reducing LL-37's cytotoxic and inflammatory actions, which depends on SP-A's globular/neck domains. Our studies also suggest a potential therapeutic effect of rfhSP-A on chronic inflammatory lung diseases characterized by elevated LL-37.

Keywords: Bacterial infections, cell death, inflammatory disease, innate host defence, innate immunity

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POSTER PRESENTATIONS

P-0288

Regulation of gingival keratinocyte MCPIP-1 and MALT-1 expressions by periodontal bacteria and IL-1β

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To evaluate oral bacteria- and interleukin (IL)-1β-induced protein and mRNA expression profiles of monocyte chemotactic protein-1-induced protein (MCPIP)-1 and mucosa-associated lymphoid tissue lymphoma translocation protein (MALT)-1 in human gingival keratinocytes. Human gingival keratinocytes (HMK) were incubated with Porphyromonas gingivalis (ATCC 33277), Fusobacterium nucleatum (ATCC 25586), P. gingivalis LPS and IL-1β for 2 h, 6 h and 24 h. The protein levels of MCPIP-1 and MALT1 were examined by immunoblots. The mRNA levels of MCPIP-1 and MALT1 were examined by qPCR technique. Statistical analysis was performed by using the One-way analysis of variance (ANOVA) followed by Tukey's correction. After 2 hours of incubation MCPIP-1 mRNA levels were increased by F. nucleatum (MOI 1:50 & MOI 1:100) whereas MCPIP-1 and MALT1 protein levels were suppressed by F.nucleatum (MOI 1:100). MALT1 synthesis was suppressed by F. nucleatum (1:50 & 1:100) and by IL-1β after 6 hours. After 24 hours an increase in MCPIP-1 mRNA expression was observed in F. nucleatum treated HMK cells (1:50&1:100). P. gingivalis degraded MCPIP1 and MALT1 at all time points. LPS did not have any effect on tested proteins. MALT-1 protein expression profile in human gingival keratinocytes seem to be prone to oral bacterial and IL-1β activation, while MCPIP-1 expression is less affected. These findings indicate that oral infection and inflammation regulate anti-inflammatory mechanisms in gingival keratinocytes.

Keywords: Cell signalling, infectious disease, inflammatory disease, inflammatory molecules

P-0290

Identification of a pro-tumorigenic role for IL-36 signalling in colorectal cancer

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The IL-36 cytokines are a recently described subset of the IL-1 family of cytokines, shown to play a role in the pathogenesis of intestinal diseases such as Inflammatory Bowel Disease (IBD). Given the link between IBD and colitis –associated cancer, as well as the involvement of other IL-1 family members in intestinal tumorigenesis, the aim of this work was to investigate whether IL-36 cytokines play a role in the pathogenesis of colon cancer. Expression of IL-36R agonists mRNA and protein were found to be significantly increased in colorectal cancer tissue compared to adjacent non-tumour tissue whilst expression of both the IL-36R and IL-36R antagonist was unchanged. *In vitro* assays showed IL-36R agonists to drive the pro-tumorigenic phenotypes of increased cellular migration, invasion and proliferation in both 2D and 3D models. In addition, the IL-36 cytokines induced strong expression of pro-inflammatory chemokines in both human and murine cell lines. Intraperitoneal injection of the IL-36RN was seen to significantly reduce tumour burden using the subcutaneous CT26 tumour model in syngeneic Balb/c mice. Whilst observable change immune cell infiltration within tumours was seen, a decrease in Ki-67 expression was shown in tumour cells in the IL-36RN treated group relative to untreated group, suggesting the inhibition of the pro-proliferative signalling of IL-36 agonists resulted in the decreased tumour size. Taken together, this data suggests that targeting IL-36R signalling may be a useful targeted therapy for CRC patients with IL-36R+ tumour cells.

Keywords: Immunological techniques, immunotherapy, inflammatory bowel disease, inflammatory molecules, cancer immunology

P-0291

Epigenetic analysis reveals aberrant chromatin organization in T cells from multiple sclerosis patients

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The molecular mechanisms underlying breakage of immune tolerance triggering multiple sclerosis (MS) remain mainly obscure. Since deregulation in epigenetic control has been associated with immune cell alterations and autoimmunity, we hypothesized that aberrant chromatin organization in CD4+ T cells, main players in the disease, may play a pathogenic role in MS. To address this hypothesis, we studied epigenome and transcriptome of RRMS and healthy control (HC) CD4+ T cells. By unsupervised clustering analyses of RNA-seq and ATAC-seq data, we found that epigenome discriminated pathological and healthy conditions better than transcriptome, with consistent differences in accessible chromatin regions but not in transcribed genes between MS and HC cells. We integrated our data with publicly available datasets and gene enrichment analysis tools and found that inaccessible regions in MS CD4+ are enriched for T cell specific enhancers, while MS-specific accessible regions might represent atypical enhancers or genomic elements controlling chromatin structure. To define this issue, we are being mapping 3D interactions. Preliminary Hi-C data suggest altered chromatin conformation in MS when compared to HC CD4+ cells. Overall, chromatin analysis of MS CD4+ cells allowed identification of broad molecular alterations never identified before by other approaches. We are currently studying their nature and their possible pathogenic role in the disease. Moreover, we are analyzing Rheumatoid Arthritis CD4+ cells to assess if observed defects are specific of MS or share by Th1/Th17 triggered autoimmune diseases. By characterizing pathogenic molecular aberrations, our studies may help on the long-term designing targeted therapies for autoimmune diseases.

Keywords: Autoimmunity, epigenetic control and modulation of immunity, multiple sclerosis, omics technologies, rheumatoid arthritis

P-0292

The impact of gamma irradiation on exosome profiles and electrolyte levels in apheresis platelet concentrates

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For some patient groups, blood components should be gamma-irradiated (γ-IR) in order to prevent transfusion associated-graft versus host disease. The aim of this study was to investigate the impact of γ-IR on the exosome and electrolyte profile within the apheresis platelet concentrates (aPC). Eight units of aPC were included in this study. These were divided into four equal parts. While two parts of these were irradiated before storage, other parts were not. Thus, irradiated (IR) and non-irradiated (NI) aPC samples for storage days 0 (D0) and 5 (D5) were obtained. Initially, the electrolyte levels were measured, then exosomes were isolated from these samples via a commercial kit. Isolated exosomes were conjugated with carboxyl latex beads that were coated with anti-CD9 antibody. Exosome-bead conjugates were analyzed via fluorochrome-conjugated monoclonal antibodies in flow-cytometry device and exosomes were evaluated with respect to their parent cells. Na-K-Ca-Mg-Cl levels increased in both NI-aPCs and IR-aPCs in D5 compared to D0, but γ-IR had no effect on electrolyte levels. Platelet-derived exosome (PD-EX) levels decreased in D5 compared to D0 in NI-aPC, while granulocyte derived exosome (GD-EX) levels increased. The mild increasing trend in PD-EX levels during the storage period in IR-aPC was significant, compared to their decreasing levels in NI-aPC. It is detected that γ-IR has no impact on electrolyte and total exosome levels, but it has the opposite effect on platelets and granulocytes in terms of exosome expression and the storage period has an inductive effect on electrolyte levels.

Keywords: Granulocytes, molecular immunology, endo- and exocytic vesicles in immunity

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POSTER PRESENTATIONS

P-0293

Development and validation of novel specific monoclonal antibodies against members of the factor H protein family

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Members of the Factor H (FH) family are seen as fine tuners of the alternative pathway of the complement system. The family consists of FH, factor H like-1 (FHL-1) and five factor H related proteins (FHRs). Recent studies suggest FH family members are key players in diseases characterized by complement dysregulation. While the function of FH and FHL-1 are well described, controversies exist about the functional role and quantification of the FHR members of the family. Development of specific monoclonal antibodies (mAbs) will facilitate quantitative and functional assessment and could pave the way for better diagnostics and therapeutics. Here, we present two promising anti-FHR3 mAbs. Anti-FHR3 mAbs are generated by immunising BALB/c mice via an intraperitoneal injection of recombinant FHR3 at a four-week interval. After the final booster, hybridomas are formed by fusing spleen cells with mice SP2-0 cells. The presence of specific anti-FHR3 antibodies in cell culture supernatant is screened using ELISA. Following candidate selection, antibodies are made monoclonal via several limiting dilutions, purified and validated for native FHR3 specificity. Five anti-FHR3 positive hits were initially identified. After a cross-reactivity assay, two specific anti-FHR3 antibody candidates remained which were made monoclonal and classed as IgG1. Lastly, both antibodies showed affinity towards native FHR3 present in serum. We have identified two promising anti-FHR3 antibodies that show specificity for recFHR3 and affinity towards native FHR3. Use of these novel antibodies will replace current non-specific antibodies and permit further characterisation and exact quantification of FHR3 in complex matrices.
Keywords: Animal models, antibody, complement

P-0295

Modulation of macrophage polarization by TNF-related apoptosis-inducing ligand

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TNF-related apoptosis-inducing ligand (TRAIL) is a homotrimer molecule that can trigger apoptosis or induce survival by binding to DR4/DR5 death receptors in human cells. Macrophages can be polarized into two main phenotypes; pro-inflammatory M1 and anti-inflammatory M2. The impact of TRAIL on macrophage polarization is unknown. The purpose of this study is to investigate this impact. Primary human monocyte-derived macrophages were pre-stimulated with TRAIL and subsequently polarized into M1 with LPS+IFNγ, M2a with IL-4 and M2c with IL-10. Moreover, macrophages were polarized into M2a and then stimulated with TRAIL. The changes in M1/M2 markers in macrophages were analzyed by RNA-seq, qPCR, ELISA and flow cytometry. RNA-seq analysis followed by qPCR confirmation revealed that TRAIL increased the expression of certain M1 markers, while decreasing the expression of certain M2 markers. Furthermore, the classical M1/M2 markers, which were not determined as significantly different by RNA-seq were analyzed. TRAIL pre-stimulation in M1 macrophages, increased the expression of "TNFα/IDO1/CXCL10/CXCL9" M1 markers at mRNA level and also the production of "CXCL10/TNFα/IL-1β/IL-12p70/CD64/CD86/HLA-DRα" M1 markers at protein level. TRAIL pre-stimulation in M2 macrophages, decreased the expression of "MRC1/CD23/TGM2/CD163/IL-10" M2 markers while increasing the expression of "IDO1/CXCL10" M1 markers at mRNA level and also the production of "CD86/HLA-DRα/CD64" M1 markers at protein level. Moreover, TRAIL stimulation shifted M2 macrophages towards M1. In conclusion, TRAIL polarizes primary human macrophages into pro-inflammatory M1 phenotype. Our study sheds new light onto mechanisms of macrophage polarization by defining TRAIL as a new modulator.
Keywords: Cell death, innate immunity, macrophage

P-0296

Epitope characterization for murine autoantibody 20B12 inducing bullous pemphigoid-like phenotype

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Autoimmune bullous dermatoses (AIBD) are severe skin diseases mediated by autoantibodies reacting against certain structural proteins of the skin. Treg-deficient scurfy mice show increased levels of autoantibodies in their sera. 20B12, one scurfy-derived antibody, binds to the epidermal side of the basement membrane zone and induces blisters *in vivo* after injection into newborn mice. Further analyses identified the hemidesmosomal protein BP230 (Bullous Pemphigoid 230 or BPAG1-e), a cytoplasmic protein in basal keratinocytes, as the target antigen of 20B12. To characterize the exact binding site (epitope) of 20B12 we first tested binding to the three different BP230 domains (N-, Rod-, C-terminal domain). Western Blot analysis confirmed binding of 20B12 to the N-terminal domain of BP230; while epitope mapping yielded five putative binding sites within this domain. As the murine and human BP230 protein show a high degree of homology and 20B12 displays cross-reactivity to human skin we further analyzed epitope binding of 20B12 with human BP230 protein fragments. Using fragments of a defined length, covering the five possible epitopes, indicates binding of 20B12 to the Spectrin repeat 9 (SR9) in the N-terminal domain of BP230. In summary we located the binding site of 20B12 in the N-terminal domain of BP230. The finding that this autoantibody 20B12 binds to a cytoplasmic antigen will help to facilitate further investigation of the pathomechanism of blister formation after binding to the target antigen.

Keywords: Animal models, antibody, autoimmunity, skin diseases

P-0297

Non-immune functions of IL-6 produced by microglia

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Microglia are resident macrophages of the central nervous system (CNS) that may orchestrate both homeostasis and pathology in CNS. Activation of microglia *in vitro* leads to the production of various pro-inflammatory cytokines, including IL-6 which is known to control CNS functions under homeostatic, as well as inflammatory conditions. However, specific contributions of IL-6 produced by microglia to CNS maintenance and disease pathogenesis *in vivo* remain unclear. To ascertain the physiological role of IL-6 derived from microglia, we generated mice with microglia-specific IL-6 inactivation using tamoxifen-inducible *Cx3cr1*-CreER genetic system (*Cx3cr1*-CreER:*Il6* fl/fl). At steady state, inducible deletion of IL-6 in adult CX_3CR1^+ microglia led to decreased number of $CD45^+CD11b^-$ cells in CNS. Moreover, IL-6 ablation in microglia at steady state resulted in impaired memory, as revealed by the Morris water maze memory test. During systemic bacterial inflammation modeled by LPS challenge IL-6 derived from microglia mediated recovery from the sickness behavior, as established in Marble burying test and anxiety assessment. At the same time, microglial IL-6 was dispensable for disease pathogenesis in MOG-induced experimental autoimmune encephalomyelitis (EAE). Thus, we concluded that microglial IL-6 may contribute to CNS maintenance and cognitive functions under both homeostatic and inflammatory conditions, however, in case of autoimmune pathology IL-6 produced by microglia appears to be redundant.

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Keywords: Autoimmunity, cytokines and mediators, inflammatory disease, macrophage, neuroimmunology

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POSTER PRESENTATIONS

P-0298

Control of IL-7 responsiveness through IL-7R subunits balance in effector T cells and analytic expressions for amplitude and EC50

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Effector T cells rely on the cytokine IL-7 to receive receptor-mediated signalling for their survival. The IL-7 receptor (IL-7R), composed of the common gamma chain and the specific alpha chain, is also associated with the kinase JAK3, which triggers its signalling pathway. Recently, study of cell-to-cell variability and flow cytometry data yielded a seemingly paradoxical observation: increased expression of gamma chains reduces the IL-7 response. We introduce a mathematical model of cytokine IL-7 and IL-7R signalling that provides an explanation for this empirical inhibitory activity. Our results show the formation of dummy complexes (those receptors that are bound to ligand but not to the JAK3 kinase, and are thus, unable to signal) and indicate that the balance between the number of IL-7R subunits in one cell is crucial for optimal signalling. We also present an algebraic method to compute exact analytical expressions for the maximum IL-7 response (or amplitude) and for the half-maximal effective concentration of ligand (EC50). While predicted amplitudes agree with the experimental data, measurements of EC50 exhibit more complicated behaviour than we have managed to capture with a variation of our IL-7R model.

Keywords: Cell signalling, cytokines and mediators, modelling

P-0301

Pathogenic properties and glycosylation profile of autoantibodies persisting in the serum of pemphigus patients in clinical remission after treatment

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Pemphigus Vulgaris is an autoimmune disease associated with pathogenic autoantibodies that recognize skin adhesion proteins called desmogleins. Treatment of patients with pemphigus with systemic corticosteroids and/or immunosuppressive drugs improve their condition, which is usually associated with a decrease in anti-desmoglein autoantibody titers. However, few patients still have persistent anti-desmoglein antibodies after treatment, even though they are in clinical remission. The purpose of this study was to evaluate whether anti-desmoglein autoantibodies detected in the serum of patients in clinical remission were as pathogenic as autoantibodies of the active phase. If they were not pathogenic, we hypothesized that there might be a change in IgG glycosylation. Therefore, we longitudinally studied the IgG glycosylation profile of pemphigus patients with high levels of anti-desmoglein autoantibodies who experienced different clinical phases such as active phase, remission or relapse. The pathogenic activity of autoantibodies was measured *in vitro* on immortalized keratinocytes by immunofluorescence and dissociation assays whereas glycosylation was measured by mass spectrometry. We show that the pathogenicity of the antibodies is not always associated with the clinical status of the patients. When patients are in remission, their IgG may or may not be pathogenic. The glycans on IgG vary with time and patient, independently of clinical status. Thus, the pathogenicity of IgG *in vivo* does not appear to be due to variations in glycosylation upon treatment.

Keywords: Mass spectrometry, antibody, autoimmunity, skin diseases

P-0302

HLA B*57.01 prevalance in HIV infected individuals: a university experience

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In individuals infected with HIV, the detection of the HLA B*57.01 allele before the addition of a reverse transcriptase inhibitor Abacavir(ABC) to the antiretroviral treatment(ART) regimen is important in terms of preventing hypersensitivity reactions(HSR) that may occur with many symptoms. The aim of this study is to reveal the HLA B*57.01 allele prevalence in HIV-infected individuals. HLA PCR-SSO Luminex typing method was used to determine HLA B alleles (Immucor-SSO, Luminex®).HLA B*57 positive DNA samples were confirmed for the HLA B*57.01 allele by working with the Olerup SSP® HLA-B*57.01 high resolution test kit. It was determined that 2692(91.4%) of 2946 HIV-infected patients were male and 254(8.6%) were female. The three highest alleles found in patients are HLA B*35 (1090(37%)), HLA B*51 (868(29.5%) and HLA B*18 (360(12.2%)) alleles. HLA B*57 allele was detected in 96(3.2%) of a total of 2946 patients and 85(2.9%) of these 96 patients had the HLA B*57.01 allele. In our study, 90(51%) female-86(49%) male, 176 healthy control group were also included. The three highest alleles in the control group are HLA B*35 (74(42%)), HLA B*51 (43(24.4%)) and HLA B*44 (33(18.7%). HLA B*57 allele was detected in 4(2.3%) of the patients. Determining the combination of ART regimen to be initiated in these patient groups diagnosed with HIV after screening for the presence of the HLA B*57.01 allele is important in order not to ignore the risk of hypersensitivity. Data on the prevalence of HLA B*57.01 in our country are limited and more studies are needed.

Keywords: Immunodeficiency, MHC and polymorphic genes, molecular immunology

P-0303

Immune synapse instructs epigenomic and transcriptomic functional reprogramming in dendritic cells

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Understanding the fate of dendritic cells (DCs) after productive immune synapses (postsynaptic DCs) with T cells during antigen presentation has been largely neglected in favor of deciphering the nuances of T cell activation and memory generation. Here, we describe that postsynaptic DCs switch their transcriptomic signature, correlating with epigenomic changes including DNA accessibility and histone methylation. We focus on the chemokine receptor Ccr7 as a proof-of-concept gene that is increased in postsynaptic DCs. Consistent with our epigenomic observations, postsynaptic DCs migrate more efficiently toward CCL19 *in vitro* and display enhanced homing to draining lymph nodes *in vivo*. This work describes a previously unknown DC population whose transcriptomics, epigenomics, and migratory capacity change in response to their cognate contact with T cells.

Keywords: Omics technologies, dendritic cells, epigenetic control and modulation of immunity, immune communication, RNAseq

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POSTER PRESENTATIONS

P-0304

Cytokine synergy used to promote aggressive phenotype in fibroblasts in children with Down's syndrome-associated arthritis

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Down's syndrome associated-arthritis (DA) is a more common, clinically distinct and more aggressive disease compared to juvenile idiopathic arthritis (JIA). This study aims to identify the underlying mechanisms involved in driving synovial fibroblasts (FLS) activation and destructive capacity. Primary DA-FLS were isolated from synovial biopsies from children with DA. DA-FLS were cultured with IL-17a, IFN-γ and GM-CSF in the presence or absence of TNF-α. Culture supernatants were harvested and IL-6, IL-8, MCP-1 and RANTES quantified by ELISAs. Leukocyte adhesion was assessed by leukocyte-DA-FLS adhesion assays. Flow-cytometric analysis was used to examine DA-FLS adhesion molecules (VCAM-1, ICAM-1), chemokine receptors (CCR3, CXCR4) and IFN-γ receptor expression. TNF-α, IL-17a and IFN-γ induced IL-6, IL-8, RANTES and MCP-1 with no effect observed for GM-CSF. TNF-α, IL-17a, IFN-γ and GM-CSF increased leukocyte adhesion to DA-FLS. TNF-α and IFN-γ upregulated cell-surface expression of ICAM-1, VCAM-1, CXCR3 and CXCR4 with no effect observed for IL-17a and GM-CSF. IFN-γ potentiated the effects of TNF-α on IL-6 and MCP-1 while decreasing IL-8. This synergy was also demonstrated for ICAM-1, VCAM-1, CXCR3, CXCR4 expression. No synergistic relationships were observed for either IL-17a and GM-CSF. Finally, IFN-γ downregulated IFN-γ receptor expression with no effect observed for TNF-α, IL-17a and GM-CSF. DA is a more common and aggressive form of inflammatory arthritis compared to JIA. DA-FLS function is regulated by differential cytokine stimulation, with TNF-α and IFN-γ demonstrating potent synergistic induction of adhesion, inflammatory and chemokine receptor expression, suggesting complex cytokine signalling pathway mediate these effects.

Keywords: Autoimmunity, chronic inflammation and fibrosis, cytokines and mediators, inflammatory joint diseases, molecular immunology

P-0305

Characterization of LAG-3 ligands and function

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LAG-3 is a co-inhibitory receptor which synergizes with PD-1 to suppress immune responses against self- and tumor-antigens. Therefore, it is a promising target in cancer immunotherapy. Although antibodies targeting LAG-3 are already used in clinical trials, little is known about the signal transduction pathways that are activated by LAG-3, which lacks prototypic inhibitory motifs. Like its homolog CD4, LAG-3 binds MHC class II molecules, but other binding partners such as LSECtin and FGL1 have also been proposed. We have established a cell-cell-interaction assay with a flow cytometry based readout to study the binding of LAG-3 to MHCII and other putative binding partners. Using this assay, we tested the influence of LAG-3 mutations on the binding between LAG-3 and MHCII. Moreover, we analyzed the capacity of several LAG-3 antibodies to block the interaction. To examine LAG-3 signaling, we utilized a reporter assay based on Jurkat cells expressing an NF-κB::eGFP reporter gene. Binding of LAG-3 to MHCII inhibits NF-κB activation in these cells. Three intracellular motifs have been suggested to play a role in LAG-3 signaling. By deletion, mutation and duplication of individual motifs, we could show that only one of those three domains is necessary for the inhibitory effect of LAG-3 and that the mutation of single amino acids is sufficient to abolish LAG-3 function. Using the reporter assay, we plan to identify intracellular interaction partners of LAG-3. Understanding the signal pathways utilized by LAG-3 will help to fine-tune future cancer immunotherapeutics.

Keywords: Checkpoint inhibition, costimulatory pathways, immune regulation and therapy, molecular immunology

P-0306

Characterization of innate lymphoid cells cultured with Idh1 Wt U-87 MG and Idh1 mutant-U-87 glioblastoma cell lines

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Gliomas, the most common primary brain tumors, arise from supporting cells called glia in the central nervous system. The IDH1 R132H mutation is one of the most common mutations in glioma, an important diagnostic marker in patients at various disease stages. Based on cytokine profiles and the transcription factors they express, ILCs are divided into three separate subclasses: ILC1, ILC2 and ILC3. We aimed to characterize ILCs cocultured with wild type or IDH1R132H mutant isogenic U-87 MG cell lines. Lymphocyte isolation was performed from the tonsillar tissues of patients diagnosed with recurrent tonsillitis. Peripheral blood ILCs were sorted with FACSAria III and were cocultured with GBM cell lines in the presence and absence of ILC-activating cytokines. The expression levels of CTLA-4, KLRG-1, PD-1 on ILCs were analyzed. In addition, proliferation of ILCs were measured by CFSE staining. ILCs significantly increased expression of CTLA-4, KLRG-1 and PD-1 after coculture with both U-87 MG and IDH1R132H mutant-U-87 cell lines. In addition, coculture of ILCs with WT U-87 MG led to significantly higher expression of checkpoint inhibitors than that of IDH1R132H mutant-U-87 cells. In addition, the proliferation of ILCs in cocultures with WT U-87 MG and without cytokine added were significantly less compared to those with IDH1 mutant. In this study, we characterized proliferation and surface markers of ILCs cultured with human GBM cell lines with and without IDH1 mutation and report that absence of (R132H) IDH1 mutation is associated with elevated checkpoint molecule expression and reduced ILC proliferation.

Keywords: Cancer immunology, innate immunity, innate lymphoid cells, proliferative disorders

P-0308

Expression analysis of immune-regulatory molecules HLA-G, HLA-E and IDO in cervical cancer

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Together, human leucocyte antigen (HLA)-G, -E and indoleamine 2,3 dioxygenase (IDO) are key molecules involved in immune tolerance. HLA-G and HLA-E are negative regulators of immune responses. IDO enzyme acts by depleting the surrounding microenvironment of the essential amino acid, tryptophan, thereby inhibiting T-cell proliferation.We investigated the expression of these molecules in cervical cancer and their association with clinical outcome. Cervical cancer biopsies were collected from 91 patients with cervical cancer. HLA-G, HLA-E, and IDO expression was determined by immunohistochemistry. Expression levels were classified semi-quantitatively based on immunoreactive cells percentage. Overall survival (OS) and disease-free survival (DFS) were examined using Kaplan-Meier survival curves with log-rank test. Patients age range was of 23 to 87 years (mean = 54 years). Normal cervical tissue was observed in 17 patients. HLA-G and HLA-Ewere expressed significantly in malignant tissues compared with non-malignant tissues (MannWhitney U test: p< 0.0001). At 5 years, the cumulative survival rates was of 44.4% in patients with HLA-Glow expression vs 0% in those with HLA-Ghigh expression. Patients with HLA-Ghigh expression were at a significantly reduced overall survival (OS) and disease-free survival (DFS) rates (P = 0.001 and 0.003, respectively). HLA-E and IDO expression doesn't reduce OS and DFS. The overexpression of the three molecules together worsen survival rates of cervical cancer patients (OS: p = 0.05, DFS: p = 0.02). Altogether, our results showed that HLA-G, HLA-E, and IDO may represent novel candidate markers for patients' prognosis and potential targets for cervical cancer therapy.

Keywords: Microenvironment, monitoring immunity, biomarkers, cancer immunology

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POSTER PRESENTATIONS

P-0309

Indoleamine 2-3 dioxygenase expression in vulvar squamous cell carcinoma predicts unfavorable clinical outcome

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Indoleamine 2,3-dioxygenase (IDO) is a tryptophan-catabolizing enzyme with immune-regulating activities in many contexts in particular cancer progression. We aimed in this study to investigate the expression and the prognostic significance of IDO in vulvar squamous cell carcinoma. IDO immunohistochemical expression was examined in 56 surgical specimens obtained from patients with vulvar cancer treated with radical vulvectomy. IDO cytoplasmic staining was evaluated by a scoring system based on the percentage of the stained tumor cells. IDO expression was considered low (IDOlow) when stained cells were ≤ 70%. Otherwise, IDO expression was considered as IDOhigh. Association with clinicopathological factors and survival were analysed. IDO was highly expressed in tumor cells in 34 cases (60.7%). IDO high/low expression has a borderline correlation with extranodal spread (p=0.06), but not with stage, tumor size, depth of invasion, lymph nodes metastasis and tumor resection margins. Using Kaplan–Meier analysis, we found that patients with IDOhigh expression had significantly reduced overall survival and disease-free survival (log-rank:p=0.09 and p=0.027, respectively) compared to patients with IDOlow expression. The 5-year OS/DFS rates for the IDOhigh expression were 54% and 27.8%, respectively. High IDO expression is associated with an unfavourable clinical outcome in patients with vulvar squamous cell carcinoma. These findings suggest that IDO expression should be investigated for the monitoring of patients.

Keywords: Biomarkers, cancer immunology, microenvironment

P-0310

Indoleamine 2,3-dioxygenase expression is associated with advanced stage in primary vaginal carcinoma

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Primary carcinoma of the vagina (PCV) accounts for approximately 1–2% of all gynaecological malignancies. Indoleamine2,3-dioxygenase (IDO) have potential regulatory properties for immune escape in cancer. Indeed, IDO could inhibit alloreactive T lymphocytes proliferation by local depletion of the tryptophan. In this study, we aimed to analyse the association of IDO expression with clinicopathological factors in PCV. IDO expression was analysed by immunohistochemistry. Twenty-eight surgical specimens were obtained from patients with PCV. Association with clinicopathological factors was also checked. All patients were staged according to the International Federation of Gynecology and Obstetrics (FIGO) criteria. IDO was detected in the cytoplasm of cancer cells while it was very low or absent in the adjacent tumor stromal cells and in normal vaginal epithelium (p=0.05). IDO expression was positively correlated with advanced stage (stage III and IV) (p=0.005) and with poorly differenciated histological subtypes (p=0.04). Concerning the other clinicopathological characteristics (tumor size, lymph node metastasis and lymph-vascular space invasion) no significant associations were demonstrated. These results proved that IDO expression correlated with PCV progression suggesting that it might be a novel post-operative prognostic indicator and a target for future therapy against vaginal cancer.

Keywords: Biomarkers, cancer immunology, microenvironment

P-0311

SARS-CoV-2 specific antibody responses in COVID-19 infected and uninfected individuals

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To determine the level of virus-specific developing immunoglobulin subtypes, SARS-CoV-2 specific IgG-IgA, and IgM antibody levels in COVID-19 infected-patients in different time points and uninfected-subjects before and after-vaccination were investigated. The study included 50-SARS-CoV-2 infected and 70-uninfected subjects. The detection of anti-SARS-CoV-2 specific IgG-Nucleocapsid, IgG-Spike, IgM- Nucleocapsid, and IgA-Spike antibody in sera samples of baseline, 1st, 3rd, 6th month and post-vaccination were evaluated by ELISA (Euroimmun-Germany) In asymptomatic individuals (n=7), compared to baseline there was no difference in IgG-Nucleocapsid levels in 6th month, however decreased IgG-Spike and increased IgA levels were detected compared to other sampling times. In symptomatic patients (n=43), both IgG-Nucleocapsid and -Spike levels decreased in the 6th month compared to baseline, in contrast IgA levels were increased. When asymptomatic individuals were compared with symptomatic patients, increased IgG-Spike, IgG-Nucleocapsid and IgA levels were observed in the baseline. While no difference was observed for IgG-Spike and IgA levels at 6th months, however IgG-Nucleocapsid levels were increased in symptomatic patients. After 2nd vaccination, IgG-Spike levels were increased in symptomatic, asymptomatic, and also in uninfected subjects (n=70) compared to pre-vaccination levels. Post vaccination, SARS-CoV-2 infected individuals have approximately double folded IgG-Spike levels compared to uninfected subjects. Our findings show that while antibody levels are higher in the baseline in symptomatic patients, this difference disappears in the following months.IgG levels were decreased in all individuals at the 6th month. Antibody screening will also be important in evaluating population immunity in patients who are known to have had the disease.

Keywords: Antibody, B lymphocytes, immune development, protection

P-0312

Soluble HLA-G is associated with chronic hepatitis infection: evidence from a meta-analysis

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Human leukocyte antigen-G (HLA-G), is a non-classical major histocompatibility complex class I (MHC I) antigen. It is secreted under different physiological conditions and is detected in pathologies including cancer, inflammatory and autoimmune diseases. In the context of viral infection, we performed a meta-analysis of case-controls studies to investigate soluble HLA-G (sHLA-G) levels linkage to chronic hepatitis B (CHB) and chronic hepatitis C (CHC). A comprehensive systematic search was performed to investigate the association between sHLA-G and the chronic hepatitis infection. We identified five studies with a total of 646 patients (447 CHB and 199 CHC) patients and 536 healthy controls (HC). The study of sHLA-G levels association to chronic hepatitis infection is determined by standardized mean differences (SMD) and its corresponding 95% confidence interval (CI). Soluble HLA-G levels were significantly higher in cases than in HC under random effects model (SMD= 6.638, 95% CI= 4.308-8.968, P=0.000). However, subgroup analysis showed a strong difference between CHB and HC without significance (SMD=1.532, p=0.113) as well for CHC vs. HC (SMD=17.208, p=0.238). These results should be taken with cautious because of reduced meta-analyzed studies for subgroups. Our meta-analysis revealed increased sHLA-G levels in patients with chronic hepatitis compared to HC. Our findings suggested the potential use of sHLA-G in infected hepatitis patients monitoring. Further larger studies still needed to consolidate our findings.

Keywords: Biomarkers, microenvironment, viral infections

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POSTER PRESENTATIONS

P-0313

Meta-analysis of the association between HLA-G +3142 C/G in HPV+ cervical squamous intraepithelial lesions

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HLA-G +3142 C/G polymorphism has been linked to many cancers particularly to cervical squamous cell carcinoma (CSCC). In this meta-analysis, we studied the real implication of HLA-G +3142 C/G polymorphism in precancerous lesions positive for human papillomavirus (HPV). A comprehensive systematic literature search in Pubmed, Medline, Cochrane, Embase, and Web of Science databases was performed to look up for relevant studies. We identified three studies with a total of 28 patients with HPV+ cervical squamous intraepithelial lesions (HPV+ CSIL) and 470 healthy controls. The study of HLA-G +3142 C/G association to HPV+ CSIL is determined by the calculation of the odds ratio (OR) and the corresponding 95%confidence interval (CI). Pooled analysis showed a statistically significant association between HLA-G +3142 C/G polymorphism and HPV+ CSIL risk. Indeed, a significant association was observed between HLA-G +3142 C/G and HPV+ CISL risk under allelic (G vs. C) model (OR=2.856, %95CI=1.441-5.661, p=0.003) and under recessive (GG vs GC+CC) genetic model (OR=3.113, %95CI=1.384-7.003, p=0.006) by random effects model. No heterogeneity was revealed (Allelic model: I-squared=0%, Tau-squared=0, p=0.437; Recessive model: I-squared=0%, Tau-squared=0, p=0.733). No publications bias were revealed (Allelic model: pBegg=0.929; Recessive model: pBegg=0.602). However, thisresult should be taken with cautious because of reduced meta-analyzed studies. Our preliminary meta-analysis showed a significant association of HLA-G +3142 C/G polymorphism with HPV+ CSIL susceptibility. Our pooled results may suggest the critical role of HLA-G polymorphism in progression to CSCC. Further studies still needed to clearly establish HLA-G molecule as a CSCC progression biomarker.

Keywords: Cancer immunology, MHC and polymorphic genes, molecular immunology

P-0314

Regulatory effects of RNA Polymerase III activity in Dendritic Cells

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Dendritic cells (DCs) play an important role in initiating adaptive immune responses against pathogens and cancer. They modulate their ability to prime either effector or regulatory T cells due to their antigen presentation capacity and regulatory elements, thus playing a crucial role in the establishment of central tolerance/immunity. DCs process intracellular or captured antigens for T-cell priming and can mature in response to various stimuli, to produce pro or anti-inflammatory cytokines that modulate the outcome of the immune response. DCs change their gene expression pattern rapidly after activation by microbes and are key immune regulators. We have shown that tRNA de novo production is necessary to maintain translation and proper DC function upon stimulation. tRNA dynamics are highly regulated during immune activation and have the potential to support the immune gene expression program driven by TLR or interferon signalling. Modulation of protein synthesis during DC activation is a determinant of proper function. In response to TLR stimulation by lipopolysaccharide (LPS) and polyriboinosinic:polyribocytidylic acid (poly(I:C)), RNA polymerase III (Pol-III)-dependent transcription and tRNA expression is strongly induced in DCs. This is caused by the phosphorylation and nuclear export of the Poll-III repressor Maf1. The resulting tRNA expression is necessary to augment protein synthesis and favors translation of DC-specific mRNA. Indeed, protein synthesis regulation and tRNA abundance regulation are vital for DC activation, cytokine production and T cell priming. Therefore, we believe regulation of protein synthesis quality can affect DC fitness and immune function.

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Keywords: Cell signalling, dendritic cells, molecular immunology

P-0315

Itaconate and dimethyl itaconate promote LPS-induced expression of IL-6 in white adipose tissue

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Specific metabolites may play a profound immunoregulatory role in inflammatory conditions. One of such metabolites is itaconate that was shown to regulate cytokine production. In particular, IL-6 secretion by macrophages in response to LPS is decreasing in the presence of itaconate. However, most of the effects of itaconate and its derivatives were extensively studied in macrophage cell cultures *in vitro*, whereas their functions *in vivo* remain poorly understood. In the current study, we investigated the effects of itaconate and its derivative dimethyl itaconate (DI) on cytokine production during inflammation *in vivo*. Surprisingly, both itaconate and DI led to increased IL-6 levels in the blood of LPS-treated mice, demonstrating the opposite effects of itaconate and its derivatives *in vitro* vs *in vivo*. In addition, administration of both itaconate and DI resulted in increased production of IL-10 and CXCL2, as well as in suppressed IFNγ accumulation in the blood of LPS-treated mice. Furthermore, we have found that under the inflammatory conditions itaconate and DI-dependent increase in *Il6* gene expression specifically occurred in white adipose tissue. Altogether, we showed that the effects of itaconate and its derivative, dimethyl itaconate, on inflammatory response may differ *in vitro* and *in vivo*. While the role of itaconate in suppressing IL-6 production was clearly demonstrated using BMDM cultures, we observed an opposite impact of itaconate and dimethyl itaconate on IL-6 expression in the mouse model of inflammation. We conclude that physiological functions of itaconate as well as potential therapeutic implications of its derivatives warrant further investigation.

Keywords: Inflammatory molecules, innate immunity, biology of the immune system, cytokines and mediators, macrophage

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POSTER PRESENTATIONS

P-0316

The impact of ER stress on plasmacytoid dendritic cells activation

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Upon accumulation of unfolded proteins, several events are initiated in order to restore endoplasmic reticulum (ER) homeostasis and cell survival through the unfolded protein response (UPR). ER stress may activate the innate immune system, leading to pro-inflammatory cytokines production, even in the absence of an infection. Plasmacytoid dendritic cells (pDC), a DC subset specialized in anti-viral responses, have a prominent ER due to their high secretory capacity, and are described to be involved in certain autoimmune diseases. To unravel the impact of ER stress on pDC activation a systematic analysis of two UPR pathways – double-stranded RNA-activated protein kinase (PKR)-like endoplasmic reticulum kinase (PERK) and inositol-requiring enzyme 1 (IRE1) – has been performed. Combining the use of pharmacological inducers and inhibitors of ER stress with TLR stimulation we found that triggering ER stress with subtilase cytotoxin (SubAB) promotes pDC activation in a PERK dependent manner. These results were verified also in cells with genetic deletion of key UPR genes. Currently, we are dissecting the molecular mechanisms leading to pDC activation and consequent type I interferon (IFN-I) production at these conditions. With this work, we expect to contribute to the development of new drugs that may be useful in the fight against infection, as well as for the treatment of autoimmune diseases such as systemic sclerosis and systemic lupus erythematosus.

Keywords: Biology of the immune system, dendritic cells, innate immunity

P-0317

The role of mitochondrial permeability transition pore in NET formation of human neutrophils

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At the sites of infection, neutrophil protective weapons are activated, in particular phagocytosis, oxidative burst, exocytosis of various granule types, and release of DNA-based extracellular traps (NETosis). The initiation of NETosis after recognition of pathogens by specific receptors is mediated by increase in intracellular Ca2+ concentration, therefore, the use of Ca2+ ionophore A23187 can be considered as semi-physiological model of NETosis. Induction of NETosis by various stimuli depends on reactive oxygen species (ROS) produced by enzymatic complex NADPH oxidase, however NETosis induced by Ca2+ ionophores was suggested to be mediated by ROS produced in mitochondria (mtROS) or without any ROS in general. A wide inhibitor analysis has been used as well as luminol-amplified chemiluminescence assay and fluorescence detection of neutrophil extracellular traps. Using mitochondria-targeted antioxidant SkQ1 and specific inhibitors of NADPH oxidase, we showed that both sources of ROS, mitochondria and NADPH oxidase, are involved in NETosis induced by A23187 in human neutrophils. In support of critical role of mtROS, SkQ1-sensitive NETosis was demonstrated to be induced by A23187 in neutrophils from patients with chronic granulomatous disease (CGD). We assume that Ca2+-triggered mtROS production contributes to NETosis either directly (CGD neutrophils) or by stimulating NADPH oxidase. The opening of the mitochondrial permeability transition pore (mPTP) in neutrophils treated by A23187 was revealed using electron transmission microscopy as a swelling of mitochondrial matrix. Using specific inhibitors, we demonstrated that mPTP is involved in mtROS production, NETosis, and the oxidative burst induced by A23187.

Keywords: Cell signalling, innate host defence, neutrophils

P-0318

Investigating microbial keratitis: a porcine model

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Infection of the cornea (microbial keratitis) is a major problem in less developed countries that if treated incorrectly can lead to corneal blindness. It can be caused by many types of pathogens such as bacteria, viruses, fungi or protists. Our group is investigating microbial keratitis using human cell lines, human corneas surplus to those required for transplantation and we have also recently established a whole porcine corneal model of infection. We have previously shown that reagents that target host tetraspanin proteins can inhibit the adhesion of bacteria that cause keratitis to human cells. Such reagents may be useful in treating corneal infections as an alternative to antibiotics. This project aims to establish a cell model of corneal infection using pig corneal cell lines and microbial keratitis pathogens and examining the expression of tetraspanins by these cell lines. This study investigated the role of tetraspanins in Staphylcoccus aureus infection of a porcinel epithelial cell line. Our hypothesis is that similar reagents that target tetraspanins may be effective in preventing/ reducing the adhesion of infection of the cornea pig cells, as useful alternative model for human cells. In addition, we are also investigating the ability of IgA antibodies isolated from milk on the host cells and to test their ability to hinder bacterial pathogenesis.

Keywords: Animal models, antibody, bacterial infections, biology of the immune system, immune communication, infectious disease

P-0319

The relationship between MUC1, MUC4, MUC16 molecules and treg cells and epithelial-mesenchymal transmission in pancreatic cancer

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Pancreatic cancer has a poor prognosis due to its increased invasion and metastatic capacity. Typically transmembrane mucins protect the epithelial tissues, but their expression level can increase in tumors. Epithelial-mesenchymal transition (EMT) also plays an essential role in tumor progression and metastasis. The present study evaluates the relationship between transmembrane mucins (MUC1, MUC4, MUC16) and the expression levels of EMT molecules and FOXP3+ Treg cells in the pancreatic cancer microenvironment. 50 (18F/32M) patients with pancreatic cancer were enrolled in the study. The formalin-fixed paraffin-embedded (FFPE) normal and tumor tissues were used for qPCR and immunohistochemistry. Gene and molecule expression levels and their relations with clinicopathological findings were evaluated. There was no significant difference in the expression levels of MUC1 in the tumor. Gene and molecule expression levels of MUC4 and MUC16 were found to be increased ($p < 0.05$) with a positive correlation($p < 0.001$) in tumor tissue. FOXP3 gene and molecule expression levels were also significantly increased in tumor tissues ($p < 0.05$). E-cadherin gene and molecule levels were found to be decreased, whereas N-cadherin and TWIST expression levels were increased in the tumor ($p < 0.05$). Significant correlations were found between the FOXP3 and MUC16 levels in the tumor ($p<0.001$). Their expression levels were also remarkably high in the early stages, according to the TNM stage. In twenty-four EMT-active patients, increased FOXP3 gene expression levels were noted. We conclude that an increase in MUC16 expressions and Treg cell numbers might be a support to EMT in pancreatic cancer.

Keywords: Cancer immunology, immune communication, molecular immunology, regulatory cells

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POSTER PRESENTATIONS

P-0320

TSLPR and IL7Rα expression on Langerhans cells determine TSLP responsiveness in mice

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Langerhans cells (LCs) are specialized antigen presenting cells (APC) located in skin epidermis. They are critical immune response initiators by integrating signals such as microbes and cytokines. The integration of these signals influence LCs activation that further determine T cell phenotype when LCs migrate to local lymph nodes and present antigens. Thymic stromal lymphopoietin (TSLP) is a proinflammatory mediator released by keratinocytes sensed by LCs and associated with type 2 immune response (Th2). Unbalanced Th2 response is found in pathologies such as atopic dermatitis or allergic asthma. TSLP signaling requires the assemblage of TSLP receptor (TSLPR) and IL7Rα. Interestingly, IL7Rα is not commonly expressed on APCs. Our previous studies have shown that in splenic dendritic cells (DCs), cellular activation and subsequent up-regulation of IL7Rα regulates TSLP sensitivity *ex vivo*. How the receptor expression is regulated in LCs and its biological implications has not been elucidated. Here we isolate LCs from mice to assess IL7Rα using flow cytometry and its functionality measured by phospho-STAT5 activation to understand if LCs are responsive to TSLP and IL-7. Our results will elucidate whether IL7Rα and TSLPR, are simultaneously expressed in LCs and how this compares to splenic DCs. These results will show if either TSLPR or IL7Rα expression is needed for tuning TSLP response. Anatomical differences in expression of TSLP and IL7 between spleen and skin, and differential regulation of the shared receptor chains (TSLPR and IL-7Rα) might suggest spatial-temporal regulation of proinflammatory signal achieved by regulation of TSLPR and IL-7Rα expression on LCs.

Keywords: Allergic disorders, cytokines and mediators, dendritic cells, innate immunity

P-0321

New transgenic mice with overexpression of human interleukin-6 in myeloid cells

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IL-6 is a pleiotropic cytokine involved in regulation of inflammation, metabolism, tissue homeostasis and repair. Recently, IL-6 was identified as one of the prime candidates for mediating inflammation in COVID-19 patients and as a key player in the cytokine storm, a condition caused by extensive activation of the immune system. However, long-term consequences of systemic IL-6 elevation were not yet systematically studied. Therefore, mice with cell type-specific overexpression of IL-6 provide a useful model for evaluating the effects of chronic overabundance of this cytokine. Using loxP-Cre technology, we generated mice with transgenic overexpression of human IL-6 and reporter fluorescent protein EGFP in cells of myeloid lineage. These mice (hIL-6 Tg LysM-cre) showed retardation in the postnatal growth and reduced lifespan as compared to littermate control mice. Elevated serum level of human IL-6 was accompanied by an increase in GM-CSF, IL-17, IL-15 and CXCL10. Transgenic hIL-6 Tg LysM-cre mice developed hyperinflammation with extreme splenomegaly, lymph node hyperplasia, and neutrophilia of vital organs at the age of 8-10 weeks. In addition, hIL-6 Tg LysM-cre mice demonstrated erythrocytopenia in the bone marrow and showed blood clotting perturbations, thus, displaying disorders characteristic of acute cytokine release syndrome with high levels of IL-6, as in case of severe COVID-19. New transgenic mice with regulated overexpression of human IL-6 in specific cell type should be useful for studying and mechanistic understanding of severe inflammatory syndromes. They can also be used as preclinical models for testing novel therapeutic approaches.

The work is supported with RSF grant 19-75-30032.

Keywords: Animal models, cytokines and mediators, inflammatory disease, myeloid cells

P-0322

Has-miR-141 downregulation in prostate cancer provides survival benefit via innate immune cell signaling and mitophagy modulation

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Hsa-miR-141 is downregulated in prostate cancer, the second cancer in prevalence. We used a rescue and enhanced phenotype study to find miR-141 role in innate immune signaling and related to it mitophagy in LNCaP and PC3 metastatic prostate cancer cell lines. Using poly(A) enriched nanopore based RNA-seq of intact, miR-141 mimic and inhibitor transfected PC3 cells a we found autophagy related TAK1 pathways, TAK1-NFkB and TAK1-p38MAPK1 interactions and NLR innate immunity pathways enrichment after miR-141 rescue, and mostly non-immune pathways affected after miR-141 inhibition. Therefore, we investigated the rescue effect on autophagy markers ATG16L and LC3, autophagy flux and mitophagy (using DOJINDO specific dyes MtPhagy/Lyso and DAPGreen) in intact, starved or autophagolysosome blockade conditions. We also studied how MAPK1 silencing affected this. Hsa-miR-141 rescue induced macroautophagy similar to starvation in LNCaP in MAPK1 dependent way that was lost in PC3. MAPK1 silencing did not affect mitophagy in LNCaP, but it suppressed it in PC3. miR-141 rescue restored mitophagy induction in starving LNCaP, but only partially in PC3. As MAPK1 is potential miR-141 target, the suppression of later in some cancers (breast) increases MAPK1 and attenuates mitophagy, resulting in an increased ROS, Nlrp3 activation, metastatic promoting cytokines secretion and bone metastasis. We found similar phenomenon here, in prostate cancer, as miR-141 rescue reactivated NLR pathways and also MAPK1 had mitophagy suppressing effect in more metastatic PC3 cells, suggesting for potential treatment scenarios.

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Keywords: Cancer immunology, innate immunity, miRNA, omics technologies

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POSTER PRESENTATIONS

P-0323

Allelic and genotypic distribution of genetic polymorphisms rs12472244 (A/G) and rs1776531 (A/C) in the Tunisian general population

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A total of 115 Tunisian (North Africa) were genotyped for two single nucleotide polymorphism (SNP) rs12472244A/G and rs17765311A/C located respectively in USP40 and USP3 genes using High resolution melt (HRM) method. The aim of this work is to determine genotypic and allelic distribution of two SNPs: rs12472244A/G and rs1776531A/C in Tunisian general population. The 115 participants will serve as control population when studying the association of these two polymorphisms with ulcerative colitis in Tunisia.. All participants recruited in this study were from Tunisian descent. The study was approved by the local ethics committee and all enrolled individual gave their informed consent to participate. Blood samples were collected from 115 healthy individuals on tubes containing an anticoagulant (EDTA) at the outpatient clinic of Pasteur Institute of Tunis (Tunisia, North Africa). Allelic and genotypic frequencies of two SNPs rs12472244A/G and rs1776531A/C located respectively in ubiquitin specific peptidase 40 (USP40) and ubiquitin specific peptidase 3 (USP3) genes were determined. DNA sequencing method was used to solve HRM genotyping ambiguity. Using HRM technique, we genotyped 2 SNPs from 115 individuals, all of Tunisian origin. The two SNPs tested were in Hardy-Weinberg equilibrium.. Our data demonstrated that A allele for rs177653111A/C and rs12472244A/G are predominant with respective frequencies of 0.566 and 0.590. This work provides SNP genotype data suitable for disease association studies in Tunisia.

Keywords: Biology of the immune system, inflammatory bowel disease, molecular immunology

P-0326

Protective role of rs401502 and rs11575934 SNPs, localized in the IL-12 beta 1 receptor gene, against colorectal cancer in Tunisia

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Colorectal cancer (CRC) is a major public health problem worldwide and in Tunisia. Indeed, it ranks first in terms of incidence and cancer-related cause of death. Its pathogenesis is currently retained as multifactorial involving genetic and environmental factors. Interleukin-12 (IL-12) is a pro-inflammatory cytokine that plays an important role in the anti-tumor response. Hence, the interest of studying the association between polymorphisms within the IL-12 and its receptor IL-12RB1 gene and colorectal cancer risk. A total of 110 Tunisian patients with CRC and 141 healthy control subjects were included in this study. Genotyping was performed by High-Resolution Melting (HRM) analysis. All results were confirmed by direct DNA sequencing or PCR-RFLP methods. Later, the allele frequencies and genotype distribution were established and compared between control group and CRC patients. The two SNP were in Hardy Weinberg Equilibrium (HWE) in both patients and controls. According to the dominant genetic model, the studied rs401502 SNP showed a significant association (p=0.038; OR=0.577; 95% CI [0.343-0.972]). The minor allele frequencies of rs401502. were 24% in control group vs 16% in patient group. Protective role was also showed in studied rs11575934 SNP (p= 0.02; OR= 0.547; 95% CI [0.32-0.91]) with 30% of healthy individuals have a minor allele versus 21% among patients. This study showed a potential protective role of rs401502 and rs11575934 SNPs in colorectal cancer.

Keywords: Cancer immunology, cytokines and mediators, molecular immunology

P-0327

Altered glycosylation patterns in inflamed synovial tissue of rheumatoid arthritis patients: a role for immune modulation

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Glycosylation is a posttranslational modification that conjugates glycans to proteins and lipids, that may alter during inflammation and resolving phase in tissues. Through their interaction with glycan binding receptors expressed on immune cells, such as C-type lectin receptors and Sialic acid-binding immunoglobulin-type lectins (Siglecs), they modulate cell communication. The expression of glycan binding receptors on a wide variety of immune cells have illustrated that specific glycan-glycan binding receptor interactions can induce either tolerogenic or immunogenic signaling pathways within immune cells. Aberrant glycosylation patterns show to have pathophysiological effects, however, little is known how glycosylation is altered in autoimmune diseases, such as rheumatoid arthritis (RA). With the data analysis platform R2, we analyzed genes encoding for the enzymes and enzymatic processes responsible for the glycosylation patterns of cells derived from inflamed or non-inflamed synovial joints of RA patients. In the publicly available data set of M.J. Townsend (GSE48780) we compared 27 RA patients with an inflamed joint to 16 RA patients with a non-inflamed joint, using a specific glycan related gene list (303 genes). Our analysis showed that 29 glycan related genes were significantly differentially expressed between the inflamed and non-inflamed groups. One group of genes that was changed, pointed towards lower sialic acid expression in inflamed joints of RA patients. Sialic acids are recognized by various immune inhibitory Siglecs present on immune cells. These finding hint to a role for the loss of inhibitory sialic acids-Siglec interactions in the synovial joints with persistent inflammation, which is characteristics for RA pathology.

Keywords: Big data, cell signalling, immune communication, inflammatory joint diseases, rheumatoid arthritis, RNAseq

P-0328

Immunoglobulin A and G present in human blood carry MAGE modification

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Glycation is a multi-step process leading to Advanced Glycation End-products (AGEs) and disrupting tissue homeostasis. Structural changes of plasma proteins induced by AGEs contribute to functional abnormalities observed in several diseases, including diabetes and cancer. In our previous studies, we have demonstrated presence in human body the novel AGE antigen that model structural analog was obtained by protein glycation with melibiose in anhydrous conditions (MAGE). In here our goal was to identify the blood proteins modified by the naturally occurring MAGE antigen. The *in vivo* analog of MAGE was isolated from the human plasma sample obtained from a healthy donor using immunoprecipitation on MAGE-resin with immobilized the specific anti-MAGE antibody. Proteins bound to MAGE-resin were analyzed by mass spectrometry. Western blot experiments with anti-MAGE and other target-specific antibodies were used to confirm the results generated by mass spectrometry. The fraction obtained after MAGE-specific immunoprecipitation contained two proteins with molecular weight of 61.67 and 49.33 kDa that displayed the MAGE epitope on the immunoblotting with anti-MAGE antibody. Among different proteins present in the immunoprecipitate the mass spectrometry analysis identified immunoglobulin A heavy chain constant region and immunoglobulin G1 heavy chain with the score of 352 and 2510, respectively. Western blot with specific anti-human IgG and anti-human IgA antibodies confirmed identity of these proteins in the analyzed sample. We found the presence of MAGE-modified immunoglobulin G and A in healthy human blood. This modification impacts protein structure and can affect antigen binding or contribute to autoimmunogenicity.

Keywords: Antibody, biomarkers, molecular immunology

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POSTER PRESENTATIONS

P-0329

NFATc1/αA and Blimp-1 support the follicular and effector phenotype of murine Tregs

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CD4+CXCR5+Foxp3+ T follicular regulatory (TFR) cells control the germinal center responses. Like follicular helper T-cells, they express high levels of Nuclear Factor of Activated T-cells c1, predominantly its short isoform NFATc1/αA. Ablation of NFATc1 in Tregs prevents upregulation of CXCR5 and migration of TFR cells into B-cell follicles. By contrast, constitutive active NFATc1/αA defines the surface density of CXCR5, whose level determines how deep a TFR migrates into the GC and how effectively it controls antibody production. NFATc1/αA is necessary to overcome TFR-expressed B lymphocyte-induced maturation protein (Blimp-1), which can directly repress *Cxcr5*. Blimp-1 then reinforces the recruitment of NFATc1 to *Cxcr5* by protein-protein interaction and by those means cooperates with NFATc1 for *Cxcr5* transactivation. On the contrary, Blimp-1 is necessary to counterbalance NFATc1/αA, which strengthens the follicular development of Tregs, but bears the inherent risk of causing an ex-Treg phenotype

Keywords: Cell signalling, follicular helper T cells, regulatory cells

P-0330

Influence of bFGF and IL-17 on endothelial differentiation potential of mesenchymal stem cells from human exfoliated deciduous teeth

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An indispensable part of the regeneration process is the appropriate vascularization of the damaged tissue. Therefore, one of the most promising strategy in the tissue engineering is development of pre-vascularized tissue constructs. Various strategies to obtain endothelial cells (EC) are available including EC differentiation from the adult stem cells (SCs) such are mesenchymal SC (MSCs). Particularly attractive sources of MSCs are dental tissues, whereas minimal invasiveness and accessibility is characteristic of SCs from human exfoliated deciduous teeth (SHEDs). Regarding the frequent exposure of dental tissues to the inflammatory conditions that significantly affect MSCs functionality, this study aimed to investigate the influence of inflammatory niche mediators, interleukin-17 (IL-17) and/or basic fibroblast growth factor (bFGF), on SHEDs endothelial differentiation potential through analysis of endothelial markers expression (vWF and CD31), as well as tubulogenesis. Our results revealed that SHEDs have intrinsic potential to differentiate toward EC progenitors as sprouts and tubules can be noticed in the control group. On the other hand, IL-17 did not affect basal endothelial differentiation of SHEDs, but it did slightly annulled stimulative effects of bFGF on the size of sprouts. On gene and protein level these factors did not affect vWF expression, while CD31 expression was stimulated in the presence of IL-17 or/and bFGF. Interestingly, both factors also stimulated gene and protein expression of IL-6 implying its involvement in IL-17 or bFGF actions as important angiogenic modulators. All these findings are encouraging particularly in the context of IL-17 and bFGF potential to modulate dental tissues vascularization and regeneration.

Keywords: Cytokines and mediators, inflammatory molecules, microenvironment, stem cells, tissue damage and repair

P-0332

Epigenetic regulation of T cell responses by Lamin A/C

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Nuclear envelope protein lamin A/C in CD4+ T-cells enhances T-cell activation and differentiation towards T helper 1 phenotype while reduces Treg polarization. Since epigenetic regulation is a key determinant of the Th fate, our objective is to analyze the capacity of lamin A/C to modulate epigenetic changes of the master transcription factors of Th polarization. To achieve this, we analyzed post-translational histone modifications on the promoters of the genes encoding these transcription factors by ChIP-qPCR in *in vitro* activated WT and *Lmna−/−* CD4+ T-cells. In addition, we performed *in vitro* transduction with EZH1 and RNAi retroviruses in *in vitro* Th1 and Treg differentiated WT and *Lmna−/−* CD4+ T-cells. ChIP-qPCR analysis of the *Foxp3* promoter revealed no differences for the studied modifications (H3K4me3, H3K27me3 and H3K4me1) while *Lmna−/−* T-cells had significantly fewer H3K4me1 marks on the *Tbx1* promoter than WT cells. Regarding the RNAi experiments, we observed that EZH1, but not EED, downregulation abolished *Tbx21* mRNA differences between WT and *Lmna−/−* CD4+ T cells, and also eliminated the differences in T-bet-regulated *Ifng* mRNA. Our findings suggest that lamin A/C contributes to the regulation of T-bet expression during Th1 commitment, at least in part through an epigenetic mechanism. In contrast, lamin A/C-dependent FOXP3 regulation does not involve the same epigenetic changes than Tbet. In conclusion, knowledge of the mechanisms that define differentiation towards a specific Th or Treg phenotype may be of interest for some diseases, such as inflammatory bowel disease, where modified cells could be used as therapy.

Keywords: Epigenetic control and modulation of immunity, immune regulation and therapy, inflammatory bowel disease

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POSTER PRESENTATIONS

P-0333

Blocking of chemokine receptors suppresses migration of arthritic human and mouse osteoclast progenitors

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Enhanced local and systemic bone resorption by osteoclasts is a hallmark of rheumatoid arthritis (RA). Osteoclast progenitors (OCPs) are susceptible to chemokine signals, which mediate their migratory and homing properties. We aimed to explore the effect of chemokine receptor blocking on migration of mouse and human OCPs in murine collagen-induced arthritis (CIA) and human RA samples. Peripheral blood was collected from RA patients. Periarticular bone marrow (PBM) was harvested from mice with CIA. FACS sorted OCPs were exposed to a chemokine gradient (CCL2 and CX3CL1 for mouse and CCL2 and CXCL10 for human OCPs) in a Transwell culture system. Chemoattraction was blocked either by small-molecule inhibitor (SMI) or siRNA knock-down of the respective chemokine receptor. The research was approved by the Ethics Committee. Both mouse and human OCPs (CD45+CD11bloCD115+ and CD45+CD11b+CD14+ respectively) express high levels of selected chemokine receptors (CCR2 and CX3CR1 for mouse and CCR2 and CXCR3 for human). CCL2 chemokine gradient significantly enhanced migration of OCPs through the Transwell membrane compared to unstimulated cultures. Blocking of CCR2 by SMI significantly lowered the number of migrated OCPs in both human and mouse cultures compared to vehicle controls. In addition, downregulation of CCR2 and CXCR3 by siRNA significantly suppressed OCP migration in mouse and human cultures respectively. Chemokine signals significantly increase migration of human and mouse OCPs, possibly contributing to enhanced bone-surface homing in arthritis. Blocking of chemokine receptors may provide an important additional therapeutic option to suppress enhanced bone resorption in arthritis.

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Keywords: Animal models, autoimmunity, chemokines, myeloid cells, rheumatoid arthritis

P-0334

Healthy cells functionally present TAP-independent SSR1 peptides: implications for selection of clinically relevant antigens

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Tumors with an impaired transporter associated with antigen processing (TAP) present several endoplasmic reticulum-derived self-antigens on HLA class I (HLA-I) which are absent on healthy cells. Selection of such TAP-independent antigens for T cell-based immunotherapy should include analysis of their expression on healthy cells to prevent therapy-induced adverse toxicities. However, it is unknown how the absence of clinically relevant antigens on healthy cells needs to be validated. Here, we monitored TAP-independent antigen presentation on various healthy cells after establishing a T cell tool recognizing a TAP-independent signal sequence receptor 1- derived antigen. We found that most but not all healthy cells present this antigen under normal and inflammatory conditions, indicating that TAP-independent antigen presentation is a variable phenomenon. Our data emphasize the necessity of extensive testing of a wide variety of healthy cell types to define clinically relevant TAP-independent antigens that can be safely targeted by immunotherapy.

Keywords: Antigen processing and presentation, cancer immunology, cancer immunopeptidome, immunotherapy

P-0335

The use of chemical tools to reveal kinetics and subcellular routing of antigen cross-presentation

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Cross-presentation is essential for the activation of CD8 T-cells. However, the biochemical processes underpinning this even, remains complex and poorly understood; with different subcellular routings proposed for the process (Cruz, Colbert, Merino, Kriegsman, & Rock, 2017). Precise information on the actual routing of cross-presented antigen *in vivo* is still a valuable prospect. Furthermore, the kinetics by which the antigen undergoes this routing is also under-investigated. Here, I will present a new *in vivo*-compatible (van der Gracht et al., 2018) toolkit to study the both the subcellular routing of antigen, as well as the kinetics thereof. *By using so called click-to-release chemistry we can control the point in time and space by which an antigen is activated.* In this project the attachment of a small molecular group to an antigen prevents CD8 T-cell recognition, upon the uncoupling by the addition of tetrazine, the antigen can induce CD8 T-cell activation again. We have used this technique to determine the speed of processing of antigens, study the persistence of antigen processing, and – by using organelle-restricted activation – we are beginning to study the contribution of the various proposed sub-routes to the overall cross-presentation efficiency. I will share with you the recent kinetic data, which shows some surprising aspects in the speed and persistence in murine iCD103+ (Mayer et al., 2014) DCs.

Keywords: Adaptive immunity, antigen processing and presentation, dendritic cells

P-0336

From ELISA to immunosorbent tandem mass spectrometry proteoform analysis: the example of CXCL8/interleukin-8

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With ELISAs one detects total immunoreactivities in biological samples. For biomolecules undergoing proteolysis for activation, potentiation or inhibition, other techniques are necessary to study biology. Here, we introduce methodology that combines immunosorbent sample preparation and nano-scale liquid chromatography-tandem mass spectrometry for proteoform analysis (ISTAMPA) and apply this to the aglycosyl chemokine CXCL8. CXCL8 is the most powerful chemokine with neutrophil chemotactic and -activating properties in humans, and occurs in different NH_2-terminal proteoforms due to its susceptibility to site-specific proteolytic modifications. Specific CXCL8 proteoforms display an up to 30-fold enhanced potency to attract and activate neutrophils. Our ISTAMPA technology allows for simultaneous detection and differential quantification of full-length CXCL8(1-77), elongated CXCL8(-2-77), and all naturally occurring truncated CXCL8 forms in biological samples. ISTAMPA was validated and applied to synovial fluids of arthritis patients. For the first time, we prove and quantify proteolytic activation of CXCL8 in human body fluids.

Keywords: Autoimmunity, chemokines, immunological techniques, inflammatory joint diseases, mass spectrometry, neutrophils

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POSTER PRESENTATIONS

P-0339

The effect of Ca2+ at a physiological concentration on primary human macrophage polarization

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Ca2+ at a physiological concentration is well known for enhancing the production of TNF-α/IFN-γ by T cells and the quantity of immune cells positive for IL-10, IL-22, IL-17, TNF-α, and IFN-γ, yet its effects on primary human macrophage polarization remain to be elucidated. Macrophages can polarize into two main classes, M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages. Furthermore, M2 macrophages are divided into 4 subsets. Here, we reported the effect of Ca2+ at a physiological concentration on primary human M1, M2a, and M2c macrophages. In this study, primary human macrophages were cultured in regular RPMI (containing 0.8 mM CaCl2) or 1mM CaCl2 added RPMI which had the final concentration of 1.8 mM-. physiological concentration. The cells were differentiated into M1 with LPS+IFN-γ, M2 with IL-4, or M2c with IL-10. The M1 and M2 markers were analyzed by flow cytometry. Polarized macrophages cultured in Ca2+ at a physiological concentration demonstrated an enhanced level of cell death in a manner directly proportional to duration of the treatment. After Ca2+treatment, although the expression of the M1 markers (HLA-DRα/CD86/CD64/TNFα/CXCL10) by M1 macrophages did not change, the expression of HLA-DRα/CD86 was enhanced in M2 macrophages In addition, the expression of the M2 markers (CD206/CD200R) was decreased in M2 macrophages. Likewise, the expression of the M2 marker CD163 was decreased, while, the expression of the M1 marker CD86 was increased in M2c macrophages. In summary, our findings suggest that the macrophages tend to shift towards an M1 phenotype in the presence of Ca2+ at a physiological concentration.

Keywords: Cytokines and mediators, innate immunity, macrophage

P-0340

Soluble CEACAM1 induces suppressive Tregs by binding to CD5

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Carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is an immune checkpoint regulator that controls immunity via self- or heteroligation. Soluble CEACAM1 (sCC1) is elevated in the serum of patients with obstructive and autoimmune liver diseases. Ceacam1-/- mice exhibit exacerbation and persistence of Concanavalin A hepatitis due to quantitative and qualitative regulatory T cell (Treg) impairment. T cell activation and Treg induction require the short CEACAM1 isoform (CEACAM1-S), whereas its ITIM-bearing long isoform (CEACAM1-L) limits effector responses. Recently, we identified the Treg regulator CD5 as a binding partner for sCC1. To reveal the role of the sCC1-CD5 axis in Treg-induction. sCC1 was detected in Western Blots in diseased humans and mice. sCC1 binding to CD5 was identified by LC-MS/MS. Tregs were induced with or without addition of sCC1, sCD5, or αCC1 and αCD5 antibodies. Cellular signaling (pSTAT5, Foxp3, pSmad2/3, mTOR) was analyzed in FACS and Western Blots. Induced Tregs were subjected to suppression assays. Results. sCC1 was detectable in sera of patients and mice with advanced PSC or ConA hepatitis. Interaction between CEACAM1 and CD5 was confirmed by LC-MS/MS and cell binding assays. sCC1 binding to activated T cells induced pSTAT5 and Foxp3, but reduced mTOR activation. These effects were sensitive to addition of αCD5 antibodies. sCC1-induced Tregs were capable to suppress effector cell proliferation. Addition of sCC1 to T cells supports Treg induction by acting upstream of CD5mediated mTOR inhibition. Currently, the relevance of the CEACAM1-CD5 interaction for Treg homeostasis is under validation.

Keywords: Immune regulation and therapy, autoimmunity, regulatory cells

P-0341

Understanding the molecular pathogenesis of primary autoimmune thrombocytopenic purpura. The role of transcription factor Ets-2

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In primary idiopathic/autoimmune thrombocytopenic purpura (pITP), autoreactive B and T cells initiate and sustain platelet destruction in a milieu of T helper (Th-1) and Th17 effector cell polarization, whereas regulatory Th cells (Tregs) malfunction and fail to maintain tolerance. We recently showed that in controls IL-2 is repressed in naive Th cells by the transcription factor Ets-2. In this work, we examined Ets-2 and cytokine gene expression in naive and memory Th cells (Teffs) and Tregs isolated from pITP patients and controlsto investigate the Ets-2 role in pITP pathogenesis. Blood samples were collected from 6 pITP patients and 6 age/sex-matched controls. Naive (CD4+CD45RA+CD25-) and memory (CD4+CD45RO+CD25-) Teffs and Tregs (CD4+CD25+) were isolated. Phenotypic analysis revealed increased levels of naive Teffs and decreased levels of memory Teffs and Tregs in pITP patients versus controls. Bioinformatic analysis revealed multiple Ets-2 binding sites at the promoters of Th-cell signature cytokines. In pITP naive Teffs, Ets-2 mRNA and protein synthesis were significantly lower than controls. pITP naive Teffs constitutively expressed IL-2 and IFN-γ and memory Teffs, IL-17. pITP Tregs constitutively expressed IL-2 and IFN-γ, whereas control Tregs did not constitutively express these cytokines. Compared to control, pITP Tregs constitutively expressed lower IL-10 mRNA levels. Our results suggest that Ets-2 low expression and synthesis in naive Teffs of pITP patients leads to impaired downstream events in Th cell plasticity. This manifests as high constitutive gene expression of Th1/Th17 cytokines in Teffs and abnormal cytokine gene expression in Tregs.

Keywords: Autoimmunity, epigenetic control and modulation of immunity, molecular immunology

P-0343

The influence of human endogenous retroviruses and associated transcripts on colony stimulating factor 1 (CSF1/MCSF) expression in Hodgkin lymphoma cells

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Germline infections by retroviruses during evolution caused the integration of viral DNA into the human genome, which today consists of about 8-10 % of human endogenous retroviruses (HERVs). Few HERVs have open reading frames for the formation of proteins. In varying diseases, HERVs and related long terminal repeat (LTR)-elements have been observed to affect the expression of neighboring genes. We analyzed Hodgkin lymphoma (HL) cells to find HL-associated HERV that may play a role in pathogenesis. We analyzed cDNA libraries, DNA microarrays and RNA sequencing data from HL cells. Using various computational and molecular biology approaches, we identified expressed HERV in HL cells. We discovered novel HERV-related transcripts derived from the chromosomal region directly upstream of the colony-stimulating factor 1 (CSF1/MCSF) gene. The first exon of these *T*ranscripts from *HO*dgkin *L*ymphoma c*E*lls (THOLE) belongs to the LTR8 family of HERV. We detected different THOLE-initiated CSF1 transcripts in HL cell lines. High expression of THOLE was observed only in HL cell lines. Activation of THOLE and other HERVs/LTRs with subsequent transcription of HL-associated genes like CSF1 might explain the specific gene expression profile of HL cells.

Our study is supported by grant ZS/2018/12/96228 from the European Fund for Regional Development (EFRE) within the local program "Sachsen-Anhalt WISSENSCHAFT Schwerpunkte".

Keywords: Cancer immunology, cytokines and mediators, molecular immunology, omics technologies, proliferative disorders

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POSTER PRESENTATIONS

P-0345

Molecular mechanisms underlying anti-cancer immune escape activity of high mobility group box 1 (HMGB1)

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High mobility group box 1 (HMGB1) is a protein of non-histone type, which is predominantly localised in the cell nucleus. Cancer and immune cells were found to release HMGB1 when triggered by a variety of endogenous as well as exogenous stimuli. When released, HMGB1 acts as so called "alarmin" which was reported to promote the ability of cancer cells to escape host immune surveillance, however the molecular mechanisms underlying these processes remain largely unknown and thus we aimed to investigate them. We found that the anti-cancer immune escape activity of HMGB1 is determined mainly by Toll-like receptor (TLR) 4, which recognises HMGB1 as an endogenous ligand. We found that if cancer cells express TLR4 (e. g. several types of acute myeloid leukaemia cells), HGMB1 induces TLR4-mediated production of transforming growth factor beta type 1 (TGF-β) which then displays autocrine and paracrine activities. TGF-β induces expression of immunosuppressive protein galectin-9 in cancer cells. In T cells, TGF-β induces expression of V-domain Ig-containing suppressor of T cell activation (VISTA), which, together with another galectin-9 receptor Tim-3 (T cell Ig and mucin domain containing protein 3), mediates galectin-9-induced suppression of cytotoxic T lymphocyte activities. In TLR4-expressing cancer cells, HMGB1 triggers formation of an autocrine loop inducing galectin-9 expression. In TLR4-negative cancer cells, HMGB1 still triggers similar effects, but indirectly. It induces TGF-β production in TLR4 expressing cells present in the tumour microenvironment (e. g. tumour-associated macrophages) and secreted TGF-β can then trigger galectin-9 expression in cancer cells and VISTA expression in T lymphocytes.

Keywords: Cancer immunology, cell signalling, immune networks, molecular immunology

P-0347

Exploring the intracellular functions of the alarmin HMGB1 in LPS-stressed monocytes using BioID

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We aimed to explore the HMGB1 interactome in the myeloid cell line THP1 using BioID. In addition, we opted to investigate the differences between a proinflammatory and a resting environment. HMGB1 plays a crucial role regulating inflammation and is one of our most studied alarmins. It is well established that HMGB1 is involved in the pathologies of several inflammatory diseases. Today, research has mainly focused on the extracellular functions of HMGB1 as an alarmin. Interestingly, HMGB1 has been suggested as a cytosolic sensor of cellular stress, which is a process far less defined. We screened for potential interaction partners of HMGB1 in resting and LPS-activated THP1 cells using BioID. Briefly, HMGB1 was fused to a biotin ligase introducing biotinylation of proximal proteins in live cells. Biotinylated proteins were extracted and identified by LC-MS. Our results showed differences in the HMGB1 interactome between resting and LPS-activated THP1 cells whereof cytosolic hits significantly increased following LPS-stress. We found several hits that were differently abundant, namely Hsp27, Cactin, Gna12, EIF4G3, Tada2b, TPD52L2, J3QQQ9 and MTHFD1L. Remarkably, Hsp27 detected in resting THP1 cells was completely lost upon LPS-stress. Hsp27 was found to interact with HMGB1 in resting THP1 cells and to dissociate upon LPS-stress. Interestingly, Hsp27 has as well as HMGB1 been described to be involved in inflammation and to play a role in inflammatory diseases. Therefore, it is of high importance to continue studying the role of the Hsp27-HMGB1 complex and why it dissociates following TLR-activation.

Keywords: Inflammatory disease, inflammatory molecules, innate immunity, molecular immunology, myeloid cells, omics technologies

P-0348

Analysis of TAM receptor expression in patients with primary Sjögren's syndrome

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Receptor tyrosine kinases Tyro3, Axl, Mer and their ligand Gas6 are involved in the efficient phagocytosis of apoptotic cells, and defects in their signaling have been linked to various autoimmune diseases. Their association with primary Sjögren's syndrome (pSS) has also been investigated but their exact involvement remains elusive. In this study, we examined the plasma concentrations of the soluble forms of Tyro3, Axl, Mer and free Gas6 from pSS patients and age-matched healthy controls by ELISA. Next we analyzed their mRNA expression levels in peripheral blood mononuclear cells (PBMCs) from the same cohort by RT-PCR. To confirm the mRNA data and define subpopulations of PBMCs, we analyzed TAM receptor expression by PBMCs using flow cytometry. While there were no significant differences in their soluble forms in plasma, a significant decrease in the mRNA levels of Tyro3 and Mer were observed in patients. This could not be confirmed at the protein level using flow cytometry, even though patients tended to have lower expressions of Tyro3 and Mer in most cell populations analyzed. In addition, we observed a significant reduction in the frequencies of different dendritic cell subsets in patients. In summary, we found alterations in TAM receptor expression in pSS patients compared to healthy controls, but further research is needed to understand their role in the pathogenesis of the disease.

Keywords: Autoimmunity, immune regulation and therapy, phagocytosis

P-0350

Control of antigen-independent IFN-ψ production through mitochondrial ROS and Fas in CD4+ T cells

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IFNψ plays an important role in autoimmunity development. CD4+ T cells produce IFNg in response to antigen-specific TCR engagement, or through a mechanism involving synergistic effect of IL12 and IL18 independently of TCR. Recent data reveal that Fas-deficient CD4+ T cells show uncontrolled IFNψ production driven by IL12/IL18 under non-apoptotic conditions, generating a loop that propagates autoimmunity. Although the molecular events leading to Fas-induced apoptosis are well defined, little is known about the role of Fas in non-apoptotic signaling pathways acting independently of TCR. Here, we studied IL12/IL18-induced IFNψ production by naïve and *in vitro*-differentiated memory-like CD4+ T cells from parental (C57BL/6 and MRL/MpJ) and Fas-deficient mice. We analyzed mitochondrial activation and mitochondrial reactive oxygen species (mROS) production, as well as the signaling events linking mROS and IL12/IL18-induced IFNψ production. After IL12/IL18 treatment *in vitro*-differentiated memory-like CD4+ T cells showed faster and higher mROS production in comparison with naïve cells or in response to TCR stimulation. mROS inhibition using mitochondria-targeted antioxidant or different mitochondrial inhibitors significantly downregulated the activation of IL12/IL18 downstream signaling pathways leading to IFNψ production. We identified increased mROS as key drivers of disease-associated CD44hiCD62Llo memory phenotype of Fas-deficient cells and their IFNψ hyperproduction compared to parental T cells. These findings uncover a previously unidentified apoptosis-independent Fas role in regulating mROS production by memory-like T cells and pinpoint mROS as central regulators of TCR-independent signaling.

Keywords: Animal models, autoimmunity, cell signalling, cytokines and mediators

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POSTER PRESENTATIONS

P-0351

RNA cap methyltransferase RNMT is required for IL7R expression and naïve T cell homeostasis

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The m7G RNA cap is a ubiquitous modification on RNA polymerase 2-transcribed RNAs including messenger RNA (mRNA). RNA cap binding proteins which bind to the m7G cap promote transcript stability, processing and translation, thus the cap is integral to mRNA function. We investigated the function of the RNA cap N-7 methyltransferase (RNMT) by generating T cell-specific *Rnmt* conditional KO mice. Transcriptomics analysis revealed that *Rnmt* cKO T cells had reduced expression of mRNAs encoding ribosomal proteins. We found that RNMT was essential for the increase in ribosome biogenesis and protein synthesis following T cell receptor stimulation. Whilst there were large proteomic changes following activation, the differences between control and *Rnmt* cKO naïve CD4 T cell proteomes were much more subtle. Expression of the IL7R was reduced, and IL7R signalling, determined by phospho-STAT5 was decreased in *Rnmt* cKO T cells. Accordingly, the survival and proliferation of *Rnmt* cKO T cells cultured with IL7 was decreased and *Rnmt* cKO T cells performed poorly in competitive chimeras. On the contrary, expression of transcripts induced by IL7R signalling were unchanged or increased in *Rnmt* cKO T cells, and expression of BCL2 protein was increased in *Rnmt* cKO T cells both directly *ex vivo*, and after four hours IL7 stimulation. Thus it appears that in *Rnmt* cKO mice, gene expression downstream of IL7R signalling might be modulated to enhance the survival of the stressed RNMT-deficient T cells. This might involve changes in the transcript stability or protein stability of IL7R targets.

Keywords: Cell signalling, epigenetic control and modulation of immunity, immune development, molecular immunology, omics technologies, RNAseq

P-0352

FOSL1 and FOSL2 centered protein interactome in human Th17 cells

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Th17 cells are crucial for host immunity at mucosal barriers, but their dysregulated activity has implications in autoimmune diseases. The development and functions of Th17 cells are driven by a complex network of transcription factors. Recent studies have revealed the role of the AP-1 family proteins, FOSL1 and FOSL2 in controlling Th17 responses. Though functions of AP-1 TFs are majorly governed by their protein-protein interactions; the interactomes of FOSL1 and FOSL2 are still poorly characterized. In the present study, we identified the putative interactors of FOSL1 and FOSL2 for the first instance in human Th17 cells, using affinity purification-tandem mass spectrometry. Our analysis revealed the unique as well as shared binding partners of FOSL1 and FOSL2, which included several key regulators of Th17-fate. The selected hits of our MS-based identifications were further validated using parallel reaction monitoring targeted mass-spectrometry and immunoblotting. Collectively, our study provides new insights into the molecular mechanisms of FOSL1 and FOSL2 that conceivably regulate Th17 cell-responses and associated diseases.

Keywords: Adaptive immunity, autoimmunity, cell signalling, immune networks, molecular immunology

P-0353

Investigation of Toll Like Receptor-7 Gene (TLR-7) Mutations in COVID-19 Patients

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To detect TLR-7 mutation status in patients hospitalized with COVID-19 infection and to correlate clinical disease severity with our findings. A total of 169 patients hospitalized for COVID-19 and stratified according to disease severity (48 severe, 34 medium severity, 87 mild) were enrolled in the study. Sanger sequencing was performed on DNA samples obtained from peripheral blood samples of patients following PCR amplification with specific primers to analyze the c.2129-_2132del (p.Gln710Argfs*18), and c.2383G>T (p.Val795Phe) mutations. Sequencing results were visualized on Chromas program and sequences were aligned to the reference genome sequence. To our knowledge ours is the first study to evaluate TLR-7 status in a large group of patients. A prior preliminary report consisting of four patients suggested a correlation between c.2129¬2132del and c.2383G>T mutations of TLR-7 gene with severe COVID-19. In our cohort, we did not detect these mutations in any of the three disease severity groups. The COVID-19 pandemic has affected the human population since December 2019. Information about the pathogenesis of the disease is evolving everyday emphasizing a heterogeneous clinical manifestation of the disease. Our study involving such a large cohort did not confirm the previous results and is valuable in itself on the context of factors playing role on disease pathogenesis and severity.

Keywords: Immune regulation and therapy, infectious disease, innate host defence, molecular immunology, viral infections

P-0355

TGF-β inhibits the expression of a T cell activation-induced lincRNA

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Long intergenic noncoding RNAs (LincRNAs) regulate biological processes in health and disease. However, little is known about the contribution of individual lincRNAs in T cell activation. Here we found that a lincRNA is induced several fold upon T cell activation. We confirmed the transcript using rapid amplification of cDNA ends (RACE) followed by PCR. The transcript was primarily found in the cytoplasm, suggesting that it may function in cellular signalling. Although the lincRNA does not significantly regulate the T cell transcriptome, it appears to be a subtle modulator of interleukin-2 secretion and T-cell proliferation. The anti-inflammatory cytokine TGF-β negatively regulates its expression.

Keywords: Adaptive immunity, cell signalling, omics technologies, RNAseq

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POSTER PRESENTATIONS

P-0356

The kinetics of anti-drug antibody formation against anti-TNF biological adalimumab

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Antibodies form the backbone of various treatments for rheumatoid arthritis (RA) however also form the essence of its downfall. Adalimumab is such a treatment for RA, consisting of a fully human IgG1 directed against TNF. Although adalimumab reduces symptoms in RA, its administration is associated with development of anti-drug antibodies (ADA). These ADA are directed at adalimumab and both block antigen-binding to TNF and facilitate drug clearance. This will result in reduction of free serum adalimumab, hampering treatment efficacy or leading to treatment resistance, immediately stating the importance of understanding the kinetics of ADA development against adalimumab. Previous data indicates that this ADA response is skewed towards an IgG4 phenotype. Although not completely understood, IgGs minority subclass IgG4 is associated with pathogenic autoantibodies and induction of tolerance. The kinetics of the IgG4 response remain unclear, but seem to be delayed and require repeated antigenic stimulation. Measurement of different ADA isotypes in patient serum is hindered by complex formation between drug and ADA. Our group has developed assays to circumvent this issue of drug interference, for instance with an acidic dissociation step to release ADA from the drug-ADA complex. Here we describe the time course of IgM, IgG1, and IgG4 ADA formation in cohorts of healthy individuals and patients that receive adalimumab. The cohorts contain early and late timepoints after treatment initiation, allowing us to describe the kinetics of ADA development against adalimumab, particularly the development of the IgG4 antibody response, possibly leading to new insights on prevention of ADA formation.

Keywords: Antibody, autoimmunity, inflammatory disease, molecular immunology, rheumatoid arthritis

P-0357

Expression of complement regulators changes during erythropoiesis, leading to a loss of CD46 expression

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The complement system is tightly regulated by complement regulatory proteins, of which each cell type expresses a specific combination on their membrane. Although widely expressed on other cell types, red blood cells (RBCs) do not express CD46, while they originate from hematopoietic stem cells that do express CD46. Here, we aim to understand the differential expression of complement regulators during erythropoiesis and the effects on complement regulation. Blood from healthy donors was used to track differentiation from hematopoietic stem and progenitor cells to RBCs, using a 3-phase *in vitro* culture system, in which peripheral blood mononuclear cells were expanded until only pro-erythroblasts remain. These enter the differentiation phase, during which they were tested for surface expression of CD35, CD46, CD55, CD59. In addition, these cells are incubated with serum of autoimmune haemolytic anaemia (AIHA) patients that harbour antibodies against RBCs, to determine C3b deposition by FACS. We observed that complement regulators CD55 and CD59 are expressed from the pro-erythroblast stage onwards and remain stable during further differentiation. CD46 is only expressed during the first days, disappearing completely between day 2 and 7. CD35 expression reduces towards final differentiation. Preliminary data showed that pro-erythroblasts are more prone to complement activation induced by AIHA patient serum. CD35 and CD46 are differentially expressed during differentiation of erythroblasts to mature RBCs, which have lost CD46 expression completely. Future research will look into the triggers of CD35 and CD46 downregulation and the effects of the expression of different regulators on complement activation on humane RBCs.

Keywords: Complement, immune regulation and therapy, innate immunity

P-0358

MiRNA post-transcriptional modification dynamics in T cell activation

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T cell activation leads to extensive changes in the miRNA repertoire. Although overall miRNA expression decreases within a few hours of T cell activation, some individual miRNAs are specifically upregulated. Using next generation sequencing, we assessed miRNA expression and post-transcriptional modification (PtM) kinetics in human primary CD4+ T cells upon T cell receptor (TCR) or IFN I stimulation. This analysis identified differential expression of multiple miRNAs not previously linked to T cell activation. Remarkably, upregulated miRNAs showed a higher frequency of 3' adenylation. TCR stimulation was followed by increased expression of RNA modifying enzymes and the RNA degrading enzymes Dis3L2 and Eri1. In the midst of this adverse environment, 3' adenylation may serve a protective function that could be exploited to improve miRNA stability for T cell-targeted therapy.

Keywords: Adaptive immunity, RNAseq, miRNA

P-0359

Human cytomegalovirus (HCMV) infection and colorectal cancer in Tunisia

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Human cytomegalovirus (HCMV) infection have been suggested as a factor associated with the progression of colorectal cancer (CRC). AIMS: The aim of this study is to explore the associations of HCMV virus infection with CRC in Tunisia. For Association study between HCMV and CRC, Nested PCR was performed for HCMV in its latent form (amplification of the UL138 gene) and virulent form (amplification of the UL55 gene). In this purpose, 40 Tumor and 35 peri-tumor tissues from CRC patients and 100 blood samples from healthy subjects were used. Our results show that HCMV is active in 92.5% of patients compared to only 65% in controls. This difference was statistically significant between the two groups (p = 0.01, OR = 6.64, 95% CI 1.01-1.9). CONCLUSION: Ultimately, our results showed that HCMV seems to play a role in the progression of CRC

Keywords: Cancer immunology, molecular immunology, viral infections

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POSTER PRESENTATIONS

P-0360

Elite breath-hold diving causes reduced cytotoxicity and increased cytokine production of peripheral blood lymphocytes

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Extreme exercise is associated with increased susceptibility to infection in cyclists and marathon runners but the underlying mechanism is not known. In breath-hold diving, athletes are acutely exposed to extreme physical stress under conditions of hypoxia. The purpose of this study was to elucidate whether breath-hold diving also impairs immune cell function. Blood samples of fifteen elite breath-hold divers were collected for haematological, biochemical and flow cytometry analysis before and after diving. After diving, a significant increase in lactate dehydrogenase and cortisol concentrations, as well as a significant increase in leukocyte count were observed. Elite breath-hold divers showed only minor differences in immune cell populations and no difference in cytokine production compared to non-diving controls. We observed a significant reduction in granzyme B production by $CD8^+$ and $V\delta1^+$ γδ T cells. Conversely, production of IFN-γ from NK cells, $CD8^+$ and $V\delta2^+$ γδ T cells, and TNF production from NK cells and $V\delta2^+$ γδ T cells were all significantly increased after diving. Acute extreme physical stress causes important shifts in immune cell functionality, which is comparable to prolonged physical stress.

Keywords: Cytokines and mediators, gamma-delta T cells, metabolic control of immune responses, NK cells

P-0361

HLA variants differ in binding preferences of self-peptides from proteins with specific molecular functions

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Presentation of foreign and self peptides to T cells by human leukocyte antigen (HLA) has a key role in adaptive immune responses. Interactions between peptide and HLA binding groove residues shape HLA ligandome favoring binding of peptides having a certain sequence motif. HLA genes are highly polymorphic and the polymorphisms in peptide-contacting sites result in distinct sets of peptides presented by different HLA variants. This may lead to enrichment or depletion in HLA ligands for some proteins, and such preferences may differ between HLA alleles. In this study, we investigated peptide-binding preferences of different HLA variants in terms of functions of the presented proteins by statistical analysis of in silico predicted HLA ligandomes. Our results demonstrate that HLA have a tendency to present peptides derived from proteins with specific molecular functions and these preferences are similar between the alleles with similar anchor residue preferences.This may be explained by preferential HLA presentation of the proteins enriched in amino acids that are favourable anchor residues for that allele. We demonstrate that these differences lead to differential presentation of HIV, Ebola, influenza virus, SARS-CoV-1 and SARS-CoV-2 proteins by various HLA alleles. Finally, we show that the reported HLA presentation bias may be compensated for in haplotypes to increase the size of the immunopeptidome presented in each individual. Our observations can be extrapolated to explain the protective effect of certain HLA alleles in infectious diseases. We hypothesize that they can also explain susceptibility to certain autoimmune diseases and cancers.

Keywords: Adaptive immunity, antigen processing and presentation, infectious disease, MHC and polymorphic genes, modelling

P-0362

Functional study of a late-onset autoinflammatory disease due to somatic NLRC4 mosaicism

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Autoinflammatory diseases (AIDs) are inherited disorders of innate immunity that usually start during childhood. However, several studies have recently reported an increasing number of patients with AID starting in adulthood. This study was undertaken to characterize the cause underlying a patient with a somatic mosaicism due to the post-zygotic p.Ser171Phe NLRC4 variant. Human peripheral blood mononuclear cells (PBMCs) were collected from the patient with NLRC4 mosaicism, four patients with Cryopyrin-Associated Periodic Syndromes (CAPS) and control individuals (n=4-6), cultured and stimulated to activate NLRP3 and NLRC4. Cell-free supernatants were collected to quantify secreted IL-1β, IL-18, IL-6 and TNF-α by ELISA. Intracellular ASC-speck formation was evaluated by flow cytometry. Wild-type and mutant NLRC4 expression vectors were generated by overlapping PCR and expressed in HEK293T cells to quantify NLRC4 oligomerization with or without expression of ASC. *In vitro* studies expressing NLRC4 in HEK293T cells show a higher percentage of cells with oligomers of p.Ser171Phe NLRC4 than wild-type, both in presence and absence of ASC.*Ex vivo* analyses stimulating blood samples of the patient with the somatic NLRC4 mosaicism confirmed a higher percentage of monocytes with ASC specks, suggesting a higher activation of NLRC4-inflammasome, with a subsequent increase in IL-18 but not in IL-1β when compared to controls. We have identified that the post-zygotic p.Ser171Phe NLRC4 variant could be a plausible cause of the late-onset AID in the studied patient, since functional studies support the gain-of-function behaviour of the mutation similarly that in previously reported NLRC4 pathogenic variants.

Keywords: Autoimmunity, autoinflammation, cytokines and mediators

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POSTER PRESENTATIONS

P-0364

AhR sensing bacterial quorum during infection

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The interaction between a bacterial pathogen and its host can be viewed as an "arms race" in which each participant continuously responds to the evolving strategies of the other partner. A mechanism allowing bacteria to rapidly adapt to such changing circumstances is provided by density-dependent cell-to-cell communication known as *Quorum Sensing* (QS). We hypothesised that if a host sensor can detect and differentiate between bacterial QS molecules and their expression patterns, it will allow hosts to customise their immune responses according to the stage and state of infection. Our results demonstrate that infected hosts show differential modulation of host Aryl Hydrocarbon Receptor (AHR) signalling over the course of *Pseudomonas aeruginosa* (*P.aeruginosa*) infection in zebrafish, mice, and human cells. Further, modulation of AHR signalling depends on the relative abundances of several classes of *P. aeruginosa* QS molecules. *In vitro* and *in vivo* studies show that the AHR not only detects *P. aeruginosa* QS molecules in a qualitative way but also quantifies their relative abundances. Quantitative assessment enables the host to sense bacterial community densities that may have distinct gene expression programs and infection dynamics, and thereby to regulate the scale and intensity of host defense mechanisms, which can range from induction of inflammatory mediators to immune cell recruitment and bacterial clearance. We propose that by spying on bacterial *quorum*, the AHR acts as a major sensor of infection dynamics, capable of orchestrating host defence according to the status quo of infection.

Keywords: Bacterial infections, infectious disease, inflammatory molecules, innate host defence, innate immunity

P-0365

Initial properdin binding promotes alternative pathway activation on necrotic cells

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Properdin is a positive regulator of the complement system stabilizing the alternative pathway (AP) C3 convertase, but has also been proposed to act as a pattern recognition molecule. Activation of the AP is associated with spontaneous hydrolysis of C3 into C3-H_2O. It is not clear how this so called tick-over is translated to surface deposition. Here we investigated the requirements for properdin binding to necrotic cells and the functional consequence for complement activation. Both serum purified, recombinant and size fractionated oligomeric forms of properdin were shown to bind to necrotic Jurkat cells. Binding was also shown to necrotic HAP-1 cells deficient for C3, excluding a possible role for endogenous C3. Binding could be prevented by preincubation of properdin with the tick protein Salp20 and with sulfated forms of polysaccharides. Exposure of properdin-bound necrotic Jurkat cells to 10% serum resulted in deposition of C3 and C5b-9. This occurred in the presence of MgEGTA, but was inhibited by EDTA, confirming AP activation. When CFSE-labeled and properdin-opsonized necrotic Jurkat T cells were mixed with non-opsonized cells, complement was preferentially deposited on the opsonized cells. In conclusion, we demonstrate that binding of properdin prepares these surfaces for subsequent AP activation. This is especially relevant with the notion that properdin is produced by myeloid cells within tissues. Moreover, biochemical experiments have shown that intact C3 can interact with properdin with low affinity, but considering the high concentration of C3 in plasma will thereby favor the tick-over of C3 in the neighborhood of properdin-opsonized surfaces.

Keywords: Cell death, complement, innate immunity

P-0367

The evaluation of immunogenic and tolerogenic gene expression with different length Larifan fractions matured dendritic cell

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Double-stranded RNA (dsRNA) of different lengths can activate distinct immune system components. Larifan, a mix of natural-origin dsRNA (50-5000 bp) and thus potential adjuvant in developing anti-cancer vaccines, was extracted from f2 bacteriophage-infected E. coli cells and chromatographically separated by length into fractions. We aimed to investigate the biological activity of dsRNA different fraction as an adjuvant and select the potential candidates for anti-cancer dendritic cell (DC) therapy. The immunogenic (DECTIN1, IL-12, TNF-α, XCR1, ICOSL, CD209a, CCR7) and tolerogenic (IDO, PDL1, TGF-β) gene expression of DCs maturated with dsRNA fractions of different lengths was compared with the gene expression of DCs matured with LPS adjuvant (control group). The following dsRNA fractions were used for DCs maturation: long FR3 (500-1000 bp), medium-length FR9 (200-500 bp), short FR15 (50-200 bp) and unfractionated mixture dsRNA mix. DCs were differentiated from healthy C57BL/6 mice bone marrow. Gene expression was evaluated by PCR. dsRNAs mix, FR3 and FR9 resulted in a statistically significant increase of the genes DECTIN1, IL-12, TNF-α, XCR1, ICOSL, CD209a expression in mDCs compared with the LPS maturated control DCs. A statistically significant increase in DECTIN1 and ICOSL gene expression was also observed in the FR15 of mDCs. The expression of genes reflecting tolerance decreased in all fractions mDCs, and reliable differences were found in maturation with the FR15. In conclusion, dsRNAs fractions of different lengths have a higher adjuvant anti-cancer potential than LPS. Larifan fractions might be more suitable candidates for the development of effective DC vaccines used in anti-cancer immunotherapy.

Keywords: Cancer immunology, dendritic cells, immunotherapy

P-0368

Sex-specific transcriptional profile of peripheral blood neutrophils in COPD patients: a pilot study

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Chronic Obstructive Pulmonary Disease (COPD) is recognized as a systemic disorder, characterized by high levels of pro-inflammatory molecules and alterations in circulating leukocytes. Despite lungs are the primary site of the disease, neutrophilia and alteration of neutrophil functions are generally recognized as systemic hallmark of COPD. Hitherto considered as a disease predominantly affecting men, COPD is currently increasingly being viewed as a disease that greatly impact women as well. Despite sex-specific phenotype characterizing COPD clinical manifestation has been defined, only few studies addressed the molecular differences characterizing immune cells in male and female COPD. With this background, the aim of this study is to characterize the sex-specific transcriptional profile of peripheral blood neutrophils in COPD patients. Circulating neutrophils were purified from whole blood of 3 male and 6 female stable COPD patients and sex- and aged-matched control donors. Transcriptomic analysis was performed using the SmartSeq2 RNA sequencing, differential expression analysis was performed using DESeq2. 232 and 504 genes are differentially expressed (DEG) in COPD as compared to controls in male and female, respectively. Among these, only 22 genes are commonly deregulated in COPD as compared to controls independently of sex, whereas the majority of the DEGs are regulated in a sex-specific manner. Nevertheless, GO term enrichment analysis emphasized that sex-specific transcriptomic alterations of neutrophils from COPD patients underneath the regulation of the same biological processes. In silico analysis aimed at a better characterization of sex-specific alteration in neutrophil from COPD are ongoing.

Keywords: Innate immunity, neutrophils, RNAseq

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POSTER PRESENTATIONS

P-0369

Suppressed expression and incomplete maturation of HERV-K and HERV-Fc1 envelope proteins in mammalian cells

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The human genome contains 8% sequences of retroviral origin, so-called human endogenous retroviruses (HERVs). Some of these mostly defective or silenced proviruses can form viral transcripts upon activation, which can be observed in certain inflammatory diseases, such as multiple sclerosis. Because the consequences of provirus activation in the human body are largely unknown, the present study investigated selected envelope proteins of HERV associated with inflammatory diseases, namely HERV-K18, HERV-K113, and HERV-Fc1, for their protein-forming ability. Subsequent to transfection of mammalian cell lines with wild type and codon-optimized HERV envelope expression vectors, protein formation was analyzed using western blot, quantitative real time PCR, immunocytochemistry, flow cytometry and mass spectrometry. Although the formation of glycosylated envelope proteins could be demonstrated in transfected mammalian cell lines, protein maturation appeared to be incomplete, as no transport to the plasma membrane was observed. Instead, the proteins remained in the endoplasmic reticulum, where they induced the expression of genes involved in the unfolded protein response. Low expression levels of the native envelope proteins were increased by codon optimization, affecting both transcriptional and translational levels, and presumably due to single rare t-RNA pools and mRNA structure, among other factors. In summary, the formation of certain HERV proteins is possible in principle. However, their full maturation and thus full biological activity appears to depend on additional factors that may be disease-specific and remain to be elucidated in the future.

Keywords: Antigen processing and presentation, molecular immunology, viral infections

P-0371

Effect of B-cell activating factor on helicobacter-activated B cell survival and differentiation

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BAFF (B cell-activating factor) has been previously shown to induce B cell maturation and survival. Recently, we showed that *Helicobacter felis* (*H.felis*) antigen induces both IL-10- and TGF-beta- producing regulatory B cells (Breg) *in vitro*. However, Bregs have a short life-span in culture. We investigated whether addition of BAFF would improve *H.felis*-antigen-induced- Breg survival and influence its differentiation. We magnetically sorted B cells with >95 purity from spleen of C57BL/6 mice. After that, we incubated these B cells with *H.felis* antigen (10 ug/ml), TLR2-ligand PAMCy3 (5 ug/ml) or TLR4-ligand LPS (10 ug/ml) in the presence or absence of recombinant BAFF (rBAFF) for 6h, 24h, 48h, and 72h. We assessed B cell viability using 7-aminoactinomycin (7AAD) and annexin V (AnnV) in flow cytometry. Additionally, we assessed IL-10- production by IL-10 ELISA, and Breg-expressing molecule, CD9 and costimulatory molecule, CD86 by flow cytometry. Our preliminary results confirmed that rBAFF increased B cell viability, which was mostly noticiable after 48 h time point(from 30% to 50%). However, rBAFF decreased IL-10- production from *H.felis* antigen, PAMCy3, and to a lesser extent LPS-treated B cells. No difference could be shown for expression of CD9 and CD86 between groups. BAFF decreased the percentage of IL-10-producing Breg cells. Further studies are necessary to investigate the influence of BAFF on TGF-beta-producing Breg cells, and effector B cells.

Keywords: Adaptive immunity, B lymphocytes, regulatory cells

P-0372

Evaluation of indoleamine 2, 3 dioxygenase *(IDO)* gene polymorphisms in COVID-19

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COVID-19 disease is a global problem and has been reported as a pandemic.The responses of the human immune system to SARS-CoV-2 are not still well understood. In this study, it was aimed to investigate the effects of IDO (indoleamine 2-3-dioxygenase),which is an important immunomodulator, on COVID-19. It has been previously reported that polymorphisms in IDO gene regions may be associated with inflammatory conditions. In this direction, single nucleotide polymorphism (SNP) analysis was performed with melting curve analysis for 40 patients in two separate SNP gene regions determined for IDO-1 and IDO-2. In addition, some Th1, Th2, Th17-related cytokines as well as IDO-1 concentrations were detected in serum samples of 40 selected patients and healthy controls by ELISA, and the correlations of cytokines with IDO-1 levels were examined for 160 patients. As a result of our single nucleotide polymorphism study, it is concluded that the IDO-1 rs7820268 SNP region is associated with susceptibility to COVID-19 disease. However, apart from susceptibility we did not find any association of this SNP with disease course. In the rs4503083 SNP region examined for IDO-2,no significant difference was found patient and healthy control groups. According to the ELISA results,lower levels of IDO-1 concentration were seen in intensive care patients than in asymptomatic. Also, it was observed that there was a negative correlation among IL-6, TNF-α, and IDO-1 levels. Importantly, it has been found that the SARS-CoV-2 seems to suppress IDO-1 unlike other viral infections. This study suggests that IDO may be a therapeutic target for COVID-19.

Keywords: Immune regulation and therapy, inflammatory disease, molecular immunology, viral infections

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POSTER PRESENTATIONS

P-0373

Dynamic RNA binding protein interactions to cytokine mRNA govern human T cell effector function

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T cells are critical in killing infected and malignant cells. The clearance of target cells depends on the capacity of T cells to produce ample amounts of effector molecules, including the key pro-inflammatory cytokines IFN-γ, TNF-α and IL-2. We recently showed that the production levels and kinetics of these three cytokines rely on post-transcriptional mechanisms, a feature largely defined by RNA-binding proteins (RBPs). Which RBPs modulate the cytokine production in T cells is however not well understood. Here we employed an RNA-aptamer-based capture assay with human T cell lysates to map RBP interactors with the 3'untranslated regions (3'UTRs) of IFNG, TNF and IL2. We found both promiscuous and cytokine-specific binding of RBPs. Intriguingly, the composition of RBP binding to cytokine 3'UTRs altered upon T cell activation. Genetic deletion of confirmed mRNA-binders in primary T cells uncovered RBP-specific activity in modulating the protein output in response to target tumor cells. For instance, the RBPs ZFP36L1, ATXN2L and ZC3HAV1 dampen the production of all three cytokines, whereas HuR enhances the protein production. Intriguingly, only ZFP36L1 destabilizes cytokine mRNA. ZC3HAV1 and ATXN2L employ an mRNA degradation-independent mechanisms to block cytokine production. In fact, ZFP36L1 and ATXN2L double deletion shows synergistic effects on the protein production. In conclusion, identifying the RBPs that fine-tune cytokine production in T cells should help define novel targets to improve T cell responses against pathogens and malignant cells.

Keywords: Adaptive immunity, cancer immunology, cytokines and mediators, effector molecules, mass spectrometry, molecular immunology

P-0374

Quantitative mass spectrometry provides insight on mechanisms governing cell cycle regulation of expansion phase CD8+ T cells

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The expansion phase of CD8+ T cell activation is characterised by T cells entering a series of rapid proliferations, with doubling times measured up to 2-4 hours. This provides a large pool of short lived effector T cells (SLECs), and a smaller pool of memory progenitor cells (MPECs). SLECs and MPECs progress through the cell cycle at different rates. SLECs rapidly re-enter S phase following mitosis, while MPECs stall prior to S phase, demonstrating differences in cell cycle regulation between the two. Compared to epithelial cells, T cell cycle regulation is more reliant on Cyclin D2/3 isoforms than cyclin D1, is particularly sensitive to p27 knockout, and resistant to CDK4/6 inhibitors. To better characterise cell cycle regulation in CD8+ T cells we devised a method whereby asynchronous activated T cells were sorted into cell cycle phases by staining DNA, p-Rb, and p-Histone3 and analysing their proteomes by quantitative mass spectrometry. Protein fluctuations across the cell cycle identified four patterns of behaviour: G0/earlyG1 peaking, lateG1 peaking, G0/earlyG1 low, and M phase peaking. 90% of G0/earlyG1 peaking and 74% of lateG1 peaking proteins did not contain cell cycle associated GO terms suggesting these proteins were not previously associated with cell cycle regulation. Amongst these groups we identified many anaphase promoting complex cyclosome (APC/C) substrates peaking within lateG1, and increased expression of APC/C inhibitor Emi1, while APC/C coactivator Cdh1 was raised within G0/earlyG1. These data identify a novel cell cycle regulation pathway in CD8+ T cells that may influence their expansion and differentiation.

Keywords: Adaptive immunity, big data, biology of the immune system, cell signalling, omics technologies

P-0375

Evolution of IL-17 family of cytokines and receptors: possible role in development of eutherian pregnancy

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IL-17 cytokines are an unusual family of immune molecules with six interleukins and five receptors, which are structurally and functionally different compared to other cytokines. IL-17A was recently found to be upregulated during mid-luteal phase in women undergoing unsuccessful Assisted Reproductive Technologies (ART). In the grey short-tailed opossum, upregulation of IL-17A occurs during implantation stage. Given their peculiarity, the evolution of this cytokine family was analyzed in mammalian subgroups, to identify possible involvement in pregnancy establishment. Multiple sequence alignments (MSA) were performed on IL-17 cytokine family and their receptors protein sequences retrieved from eutherian mammals, marsupials and monotremes. Search comparisons, reciprocal best hits analysis and synteny of their genomic location were carried. Analysis found that all IL-17s were conserved in Eutheria, Marsupials and Monotremes. Each human IL17 protein had an orthologue in Marsupials and Monotremes, as confirmed by reciprocal best hit analysis and by the synteny of their genomic organization. The MSA identified several amino acids that were consistently different between Eutherian mammals and either marsupials or monotremes; these are located mainly in regions not involved in any specific function. Monotreme IL-17s are more different than those in marsupials, confirming their larger distance on the evolution scale. The IL-17 family of cytokines and receptors are conserved in Eutheria, Marsupials and Monotremes and, in both human and opossum, IL-17A is upregulated before implantation. This suggests a common role in pregnancy establishment, despite the major differences that characterize gestation in these mammalian groups.

Keywords: Immune communication, cytokines and mediators, molecular immunology

P-0376

Impact of previous COVID-19 infection and vaccination status on secreted phospholipase A2 group IIA levels and activity

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We have recently demonstrated that circulating levels of secreted phospholipase A2 group IIA (sPLA2-IIA) are highly associated with severe COVID-19 progressing to mortality. Here, we investigate whether levels and activities of sPLA2-IIA in plasma are impacted by COVID-19 disease history or vaccination status. This study examined a total of 39 subjects (age=33.8 ± 7.7 y, 24 men) out of which 14 had a history of COVID-19 and 9 were vaccinated (two dose Pfizer mRNA vaccine or J&J). Their blood was drawn and plasma levels and activities of sPLA2-IIA were measured in microplates by ELISA (Cayman Chemicals) and spectrophotometrically based on a chromogenic substrate, respectively. Levels of sPLA2-IIA (3.02 ± 1.43 ng/mL for the complete sample) were virtually higher in subjects with a history of COVID-19, although failing to reach a statistical significance. Nine vaccinated subjects presented, however, with lower levels of sPLA2-IIA in comparison with non-vaccinated counterparts (2.11 ± 0.87 vs 3.30 ± 1.46 ng/mL, p=0.027). The enzyme activities (8.24 ± 0.83 microM mL-1 h-1 for the complete sample) did not differ across the groups. Only in subjects with a history of COVID-19 was there a direct correlation between the sPLA2-IIA level and activity (r=0.687, p=0.007), while in vaccinated subjects the relationship tended to be inverse (r= -0.628, p=0.070). Given the prominent role we have previously demonstrated for sPLA2-IIA in severe and lethal COVID-19 subjects, the current data suggest diminishing the sPLA2-IIA enzyme activity might be a protective mechanism associated with the vaccination.

Keywords: Biology of the immune system, infectious disease, metabolic control of immune responses, molecular immunology, viral infections

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POSTER PRESENTATIONS

P-0377

Comparision of panel reactive antibodies (PRA) of patients in kidney transplantation waiting list by two different methods:Luminex -PRA and Flow-PRA

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PRA (Panel Reactive Antibody) tests are performed with two different assays called as screening and identification. Screening is a qualitative test for the detection of the presence of PRA.Measurement of PRA is performed by complement dependent cytotoxicity, Enzyme Linked Immunoassay (ELISA), flow cytometry and luminex methods.The aim of this study was to compare the effectiveness two methods Luminex-PRA and Flow-PRA together with two analysis screening in PRA detection in patients waiting for kidney transplantation. Patients displaying Class I and Class II (n:50,female: 20,male:30) were tested by the 2 different methods,using antigen coated beads. According to analysis of serum samples of 50 patients applied to our laboratory, 74% harmony observed between results of Luminex and Flow for screening analysis. Both Luminex-PRA and Flow-PRA are based on similar bead based principles. We speculated that the low concordance ratios may relate to differences in the antigen coating of the beads, possibly changing epitopes and decreasing concordance between Luminex-PRA and Flow-PRA.In conclusion, both Luminex-PRA and Flow-PRA were sensitive methods to detect class I and class II PRA. We showed that using multiple methods to characterize the immunologic status of patients can better enlighten their clinical status.

Keywords: Autoimmunity, transplantation, antibody

P-0378

MHC-II-dependent antigen presentation in acute myeloblastic leukemia cases with low HLA-DR expression

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Acute myeloid leukemia (AML) is a malignant disease characterized by aberrant blast proliferation and a high rate of relapses. AML blast cells behave as antigen presenting cells able to modulate the T CD4+ cells activity. The antigen presentation process relies on multiple molecules involved in MHC class II groove editing, such as HLA-DM, CD74 (invariant chain), CLIP peptide, and B7 ligands such as ICOS-L, B7.2, PD-L2, B7 -H3. A group of 30 patients, newly diagnosed with non-promyelocytic AML at the Regional Institute of Oncology Iasi, as well as a control group consisting of 4 healthy volunteers, were investigated by flow-cytometry for the expression of both MHC-II groove editing markers and bone marrow blasts B7 checkpoint ligands: HLA-DR, HLA-DM, CD74, CLIP, ICOS-L, B7.2, PD-L2, B7-H3. All patients expressed increased levels of HLA-DM and CD74 compared to the control group. 23% of patients expressed low HLA-DR levels, forming a distinct group of patients when compared to those with normal HLA-DR expression. They displayed different expression levels of CLIP, co-inhibitory (PD-L2, B7-H3), co-stimulatory (ICOS-L) or B7.2 dual function molecules on the bone marrow tumor cells. Thus, the increase in CLIP and co-stimulatory molecules, together with the decrease in co-inhibitory molecules correlated with an unfavorable ELN (European Leukemia Net) risk for patients in the low HLA-DR group.

Keywords: Antigen processing and presentation, checkpoint inhibition, molecular immunology

P-0379

Short-term rituximab and methylprednisolone therapy led to restoration of TNFR1 density level on TNFR1+TNFR2+ double-positive T cells in rheumatoid arthritis reflecting the effectiveness of treatment

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Co-expression parameters of type 1 and 2 TNFα receptors (TNFR1/TNFR2) was shown to be associated with rheumatoid arthritis (RA) presence. However, the possibility of modulating the level of receptor expression through effective therapy is not clear. To assess stability and variability of TNFR1/TNR2 co-expression parameters in RA patients with high disease activity receiving different types of therapy. Patients underwent effective therapy with significant decrease of DAS-28 with rituximab (n=14) or methylprednisolone (n=16). Control group consisted of 46 healthy donors. The expression levels and TNFR1/TNR2 co-expression on T cell subsets were assessed. For Tregs, rituximab group showed decreasing of %TNFR1+ cells and increasing of %TNFR2+ cell after treatment, while the opposite trend was observed in methylprednisolone group. Therapy led to increase %TNFR1+TNFR2+ cells among naive cytotoxic T cells and to decrease in the proportion of cells expressing only one type of receptors (for both TNFR1/TNFR2). Effective therapy led to the restoration of TNFR1 density almost to healthy donors level without significant positive changes in TNFR2 density. Significant associations between different parameters of TNFR1/TNR2 expression on double-positive cells with disease activity parameters (radiological stage, degree of erosive damage, rheumatoid factor and disease duration) were found. Obtained data confirmed key role of balance in quantitative expression of TNF receptors on double-positive cells for cell response reactions. In RA, expression parameters were associated with both indicators of activity and severity of the disease and with level of response to therapy during short-term course of treatment with rituximab.

Keywords: Autoimmunity, biomarkers, cytokines and mediators, drugs for immune modulation, immune regulation and therapy, regulatory cells

P-0380

A novel role for neutrophils in anti-viral immunity

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Pathogen recognition receptors (PRRs) are a class of germline encoded receptors that recognise pathogen associated molecular patterns (PAMPs). Activation of these receptors results in the activation of the innate immune response through the production of proinflammatory and anti-viral cytokines. Neutrophils are the first immune cells recruited to the site of infection, however, while their protective anti-bacterial and anti-fungal roles are well characterised, little is known about their anti-viral role. Therefore, this project aims to ascertain the role of neutrophils during a viral infection and specifically investigate the pathogen recognition receptor and pathways in primary human neutrophils challenged with viral stimuli. Using Western Blotting and qRT-PCR, we measured levels of interferon stimulated gene (ISG) induction (ISG15/MxA/Viperin) in primary human neutrophils from healthy individuals. We observed that neutrophils responded to ssRNA viruses via TLR8 and induced the early expression of ISGs independent of type 1 IFNs. Additionally, we investigated the inflammatory and immunomodulatory capacity of neutrophils through their secretion of cytokines including IL-6, IL-8, TNF and IL-1 in a TLR8 dependant manner. We show that through the expression of TNF, virally activated neutrophils drive the maturation of dendritic cells, which subsequently promotes a strong Th1 CD4 T-cell response, highlighting a novel mechanism by which neutrophils control cellular immune responses during a viral infection. These findings not only identify neutrophils as immediate responders to viral stimuli, but also reveal them to be key in controlling a wider anti-viral immune response, via cellular crosstalk, processes that may be harnesses in future anti-viral treatment.

Keywords: Infectious disease, innate host defence, innate immunity, neutrophils, viral infections

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POSTER PRESENTATIONS

P-0382

Identification of a calcineurin-derived peptide essential for the interaction with nuclear factor of activate T cells

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Due to the pivotal role of calcineurin (CN), a Ca^{2+}-dependent serine/threonine phosphatase, in various biological functions, CN inhibitors are used to treat several diseases, particularly to attenuate transplant rejection. However, their usage is often associated with a variety of serious side effects, because more than 50 substrates are regulated by CN. Therefore, selective regulation of a part of CN substrates is important for decreasing the side effects of CN inhibitors. Through the development of a new means for analyzing the interaction between proteins, we herein investigated the detailed binding mode of CN and its representative substrates, the nuclear factor of activated T cells (NFAT) family transcription factors. A catalytic subunit of CN (CNA) bound four NFAT isoforms, NFATc1-NFATc4, with the same affinity. Previously identified N-terminal CN-binding region (CNBR1) and C-terminal CNBR (CNBR2) contributes to the interaction with CNA in NFATc1, NFATc3, and NFATc4, though CNBR1 but not CNBR2 was involved in CNA/NFATc2 interaction. Furthermore, we identified a new CN-binding region (CNBR3) in NFATc1 and NFATc4 located between CNBR1 and CNBR2. With the usage of mass spectrometry with photoaffinity technology, we found that CNA (Asn^{77} to Gly^{89}) was responsible for the interaction with CNBR3. The introduction of NFATc1-CNBR3 suppressed nuclear translocation of NFATc1 but not NFATc2, suggesting that CNA (77-89) is a promising target for regulating a part of CN substrates. This strategy may be useful for the development of a new CN inhibitor that induce less side effects than existing CN inhibitors.

Keywords: Allergic disorders, cell signalling, drugs for immune modulation, immune regulation and therapy, molecular immunology, transplantation

P-0383

Tyrosine kinase pathways in monosodium urate crystal-induced inflammatory responses

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Deposition of monosodium urate (MSU) crystals in the joints or other tissues is a hallmark in the pathogenesis of gout. The urate crystal-induced inflammation is known to be mediated mainly by neutrophils besides monocytes and macrophages. Although MSU crystal-mediated signal transduction is in the focus of recent investigations, the molecular mechanism is only partially characterized. In this study, we investigated the role of Src family kinases in MSU crystal-induced neutrophil activation *in vitro* and in experimental model of gout *in vivo*. Bone marrow isolated neutrophils from wild type and triple Src family kinases-deficient (Hck–/–Fgr–/–Lyn–/–) mice were stimulated with MSU crystals. Cell responses (such as ROS-production, cytokine and chemokine release and phagocytosis) were followed. Gouty arthritis was induced by injection of MSU crystals into the hind paws of the experimental mice and the clinical signs of arthritis were assessed. The MSU crystal-induced superoxide release, cytokine production and the crystal-phagocytosis were abrogated in Src family kinases-deficient murine neutrophils. In contrast to wild type animals, Src family kinases-deficient mice showed significantly decreased paw swelling and neutrophil accumulation at the site of inflammation. In line with this, the synovial levels of interleukin-1β and CXCL2 were also strongly reduced in Src family kinases-deficient mice compared to wild type animals. Based on our findings Src family kinases play an important role in MSU crystal-induced neutrophil cell responses *in vitro* and in gouty arthritis *in vivo*. Identification of these key players in urate crystal-induced intracellular signaling pathways leads to a better understanding of the pathogenesis of gout.

Keywords: Inflammatory disease, innate immunity, neutrophils

P-0384

Induction of OCT2 contributes to regulate the gene expression program in human neutrophils activated via TLR8

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Neutrophils are key cellular players of the innate immune system. In fact, they not only perform frontline defence against pathogens, but are also able to exert an array of complex functions involved in immunity, which require changes of their gene expression profile. Nonetheless, the transcription factors (TFs) that in activated neutrophils regulate inducible genes are not yet completely characterized. In this study, we performed RNA-seq experiments in neutrophils and autologous $CD14^+$-monocytes stimulated with R848, a TLR8 ligand, and identified remarkably distinct gene expression programs between the two cell types. In accordance, the genomic distribution of the histone modification H3K27Ac, as well as of PU.1 and C/EBPβ [i.e., two myeloid lineage-determining TFs (LDTFs)], significantly changed in human neutrophils treated with R848. Interestingly, differentially acetylated and LDTF-marked regions revealed an over-representation of OCT binding motifs that we subsequently demonstrated to be selectively bound by OCT2/POU2F2. The, analysis of OCT2 genomic distribution in primary neutrophils, and of OCT2-depletion in HL-60 differentiated neutrophils, uncovered the requirement of OCT2 in contributing to promote, along with NF-κB and AP-1, the TLR8-induced gene expression program in neutrophils. Altogether, our data demonstrate that neutrophils, upon activation via TLR8, profoundly reprogram their chromatin status, ultimately displaying cell-specific, prolonged, transcriptome changes. Data also uncover an unexpected role for OCT2 in amplifying the transcriptional response to TLR8-mediated activation.

Keywords: Cytokines and mediators, epigenetic control and modulation of immunity, neutrophils, omics technologies, RNAseq

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POSTER PRESENTATIONS

P-0385

Select hyperactivating NLRP3 ligands enhance the TH1- and TH17-inducing potential of human type 2 conventional dendritic cells

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The detection of microorganisms and danger signals by pattern recognition receptors on dendritic cells (DCs) and the consequent formation of inflammasomes are pivotal for initiating protective immune responses. Typically, the activation of inflammasomes leads to IL-1β secretion accompanied by pyroptotic cell death. However, dependent on the cell type and the inflammasome ligands used, some cells can survive inflammasome activation and exist in a state of hyperactivation (defined by IL-1β secretion from living cells along with other pro-inflammatory cytokines). Here, we report that the conventional type 2 DC (cDC2) subset is the major human DC subset that is transcriptionally and functionally able to induce inflammasome formation and enter a state of hyperactivation. When cDC2 were stimulated with ligands that relatively weakly activated the inflammasome, the cells did not enter pyroptosis but instead secreted IL-12 family cytokines together with IL-1β. Hyperactivated cDC2 induced prominent T helper type 1 (TH1) and TH17 responses that were superior to those seen in response to Toll-like receptor (TLR) stimulation alone or to stronger, classical pyroptosis-inducing inflammasome ligands. These findings not only define the human cDC2 subpopulation as a prime target for the treatment of inflammasome-dependent inflammatory diseases but may also enable new approaches for adjuvant and vaccine development.

Keywords: Adjuvants and vaccines, cell signalling, cytokines and mediators, dendritic cells, inflammatory disease, innate immunity

P-0386

Evaluation of cytotoxicity and regeneration activities of collagen-based biomaterials

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Our aim was to evaluate *in vitro* cytotoxicity, immunotoxicology and regenerative effects of novel collagen-based biomaterial (NCBM), obtained through innovative biotechnological processes. NCBM, compared to commercial collagen (CC) was investigated using *in vitro* methods regarding cytotoxicity, immunotoxicology and regenerative activities. Human fetal osteoblastic (hFOB), monocytes (CRL9855), human chondrocytes (HC), and human fibroblast cell line (HS27) were used. Real-time monitoring of cellular behavior and regenerative effects were achieved with xCELLigence platform (cell adhesion and proliferation) and BioStation IM (timelapse videomicroscopy). xMAP array was used to evaluate molecules release after cell treatment: cytokines, chemokines, and growth factors. Two collagen-based biomaterials were investigated, on monocytes, and did not express significant cytotoxic effects. Our results showed comparative effects of NCBM and CC. In order to ensure a normal healing process and no adverse reactions, classical biocompatibility tests have been extended with complex experiments performed on real-time monitoring. xCELLigence measurements on hFOB cells showed a more pronounced proliferative effect/ cellular index in the case of treatment with NCBM, compared to CC. Nikon BioStation IM used to monitor a wound via the scratch assay, revealed that our NCBM has comparable effects to CC in HS27, HC and hFOB cells. xMAP array revealed an increased level of IL-8, TNFa and MIP-1a in HC cells supernatants, emphasizing the role of these molecules in bone regeneration. Our results confirmed that this NCBM could be used in a wide variety of medical fields including regeneration and wound healing, due to collagen excellent biocompatibility and weak antigenicity.

Keywords: Biomarkers, cytokines and mediators, inflammatory molecules

P-0387

CXCR6 expression depends on the genetic polymorphisms in the 3p21.31 locus enhancer region in CD4+ T cells

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Chemokines and their receptors are crucial for recruiting effector immune cells to the site of inflammation which is a critical component of response to respiratory pathogens. In the case of COVID-19, an immune mystery of current interest, CXCR6 chemokine receptor which is expressed at a lower level in lung T cells is associated with the severity of the disease. To assess *CXCR6* gene regulation, we have focused on the analysis of the potential enhancer (chr3:46097189-46099620; GRCh38/hg38) in the 3p21.31 locus and on the *CXCR6* gene promoter. We used in luciferase reporter assay in Jurkat cell line and in primary CD4+ T cells to examine the influence of the SNPs in the enhancer associated with severe COVID-19 on *CXCR6* promoter activity. We observed a 3-fold increase in the *CXCR6* promoter activity in the presence of a frequent allele of one of the examined SNPs. Using in-silico approaches, we have identified a potential transcription factor that binds to the SNP region and demonstrated its differential binding to more active variant of the *CXCR6* promoter using the DNA pull-down method. This work is supported by grant 19-14-00341 from Russian Science Foundation.

Keywords: Chemokines, infectious disease, molecular immunology

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POSTER PRESENTATIONS

P-0389

Interleukin-6 trans-signaling during Behçet's disease

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Behçet's disease (BD) is an inflammatory multisystem disorder characterized by oral and genital aphthous, uveitis and thrombophlebitis; it can involve several organs. Interleukin-6 (IL-6) is a pleotropic cytokine that signals through two different pathways: classical signaling via the membrane bound IL-6R to induce anti-inflammatory responses and IL-6 trans-signaling via soluble IL-6R (sIL-6R) to promote inflammation. It has been shown that IL-6 trans-signaling is critically involved in chronic inflammatory diseases. For that, we aimed to explore if IL-6 trans-signaling is implicated during BD. Fifty six BD patients and 15 controls were enrolled in this study. Freshly collected blood samples were used. The samples were centrifuged then supernatants were conserved at -80°C. IL-6 and sIL-6R levels were measured by ELISA (Invitrogen for IL-6, eBioscience for sIL-6R). Statistical analyses were performed by Mann Witney test for group comparison while Spearman test was used for correlation analyses. We observed a significant increase of IL-6 serum levels during BD in comparison to controls ($p<0.0001$). However, we noted non-significant differences of sIL-6R levels in patients compared to controls and in patients with active stage versus inactive phase ($p>0.05$). In the other hand, although no correlation was observed between IL-6 and sIL-6R in patients ($r=-0.082$; $p>0.05$), the study of relationship between IL-6, sIL6R levels in the different clinical manifestations showed a significant negative correlation in neuro-Behçet ($r= - 0.6153$; $p<0.05$) whereas non-significant correlation was recorded with all the other manifestations ($p>0.05$). Our results suggest that IL-6 trans-signaling may be involved during the neurological manifestation during Behçet's disease.

Keywords: Autoimmunity, cytokines and mediators, molecular immunology

P-0390

Maturation of monocyte-derived DCs leads to increased cellular stiffness, higher membrane fluidity, and changed lipid composition

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Dendritic cells orchestrate the induction of protective immune responses and the perpetuation of tolerance. These functional abilities render them as ideal targets for immunotherapeutic approaches. However, translation and optimization of DC-based approaches, e.g. autologous monocyte-derived dendritic cell (moDCs) transfer, from bench-to-beside require profound knowledge of multiple aspects of DC biology. While with the onset of single-cell-RNA-sequencing the DC transcriptome is intensively characterized, the role of cell mechanics and the lipidome are poorly understood. By employing cutting-edge techniques including real-time deformability cytometry, conventional flow cytometry, confocal microscopy and shotgun lipidomics, we assessed the impact of maturation on cell mechanics and the lipidome utilizing human moDCs. Maturation increased the cellular stiffness boosting the resistance to mechanical forces, enhanced membrane fluidity and remodeled the overall lipid composition of moDCs. Finally, we found that the donor phenotype was imprinted into the moDC lipidome what was associated with distinct serum lipid species. In summary, these findings might indicate a decisive role for cellular stiffness, membrane fluidity and overall lipid content for DC maturation and the included processes comprising migration, antigen processing and cross-talk to T cells. By decrypting the described phenotypic changes to distinct immune response outcomes, engineering of membrane stiffness, fluidity or overall lipid content might serve as attractive strategy to optimize DC-based immunotherapeutic approaches. Modeling of immature or mature phenotypes might switch gears in the induction of tolerance or immunity. Furthermore, the lipidome or select lipid species within the donors´ serum might serve as biomarker predictive for DC functionality in the future.

Keywords: Biomarkers, cell based therapies, dendritic cells, omics technologies

P-0391

Thymic tissues and thymocytes from thymoma and hyperplasia associated with myasthenia gravis exhibit different patterns of expression

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Myasthenia gravis (MG) is an immune-mediated neuromuscular disease frequently associated with thymic changes and autoantibodies against the acetylcholine receptor (AChR). The most common pathology accompanying MG is thymic hyperplasia. A small subgroup of MG is associated with thymoma and presents with features indistinguishable from MG without thymoma. In this study we assessed global gene expression in thymoma and hyperplasia from MG patients. Thymic samples from thymectomized MG patients with AChR antibodies and pathologically proven thymoma (n= 7) or hyperplasia (n= 8) were included. Half of the patients had immunosuppressive treatment. Thymocytes from 6 hyperplasia and 3 thymoma patients were also compared separately. Tissue and thymocyte derived RNA samples were sequenced and differentially expressed genes (DEG) determined. Among 51 DEG found the tissue samples, 24 were higher in TAMG, whereas 27 genes increased in hyperplasia samples. Gene enrichment analysis of these findings implicated ESR1 (estrogen receptor alpha), NFKB (nuclear factor kappa B), HNF4A (hepatocyte nuclear factor 4 alpha), and EGLN3 (PHD3, prolyl hydroxylase enzymes) related activities as increased in TAMG patients compared to hyperplasia. Comparisons between thymocytes revealed 16 DEG not overlapping with tissue samples. Increases of IRS4 (insulin receptor substrate 4), RYR3 (ryanodine receptor 3) and RNR2 (RNA, ribosomal 45S cluster 2) in thymoma and an increase of NCF1 (neutrophil cytosolic factor 1) in hyperplasia samples were observed. These findings revealed differential gene expression in thymic tissue between thymoma and hyperplasia subgroups. Further differences between thymocytes emphasized differential contribution of lymphoid and stromal cells that has to be further elucidated.

Keywords: RNAseq, autoimmunity, antibody

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POSTER PRESENTATIONS

P-0392

Immune responses of MIS-C and severe COVID-19 patients

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SARS-CoV-2 infection causes different severity and disease progression from asymptomatic to severe symptoms among individuals including children. The SARS-CoV-2 infected children are observed to be mostly mild and asymptomatic, but in some rare cases multisystem inflammatory syndrome in children (MIS-C) emerges following infection. Although the relevance of MIS-C to COVID-19 and its pathogenesis remains elusive, MIS-C patients are shown to have an elevation in inflammation markers, multiple organ dysfunction and high fever. Herein, we study antibody responses against receptor binding domain (RBD), spike S1 subunit (S1) and nucleocapsid (N) proteins in order to compare IgM, IgG and IgA levels in 30 healthy controls, 30 MIS-C and 30 severe COVID-19 patients. We aim to determine the severity of COVID-19 and MISC-C patients based on secretory IgA secretion since elevated IgA levels is a significant marker for MIS-C. The concentrations of IL-1β, IL-4, IL-5, IL-6, IL-10, IL-17, TNF and IFN-γ will be measured by stimulating peripheral blood mononuclear cells (PBMCs) isolated from patients and healthy controls which will reveal the cytokine markers that are distinctive for MIS-C.

Keywords: Antibody, cytokines and mediators, molecular immunology

P-0393

Cytokine synthesis in professional athletes

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Exhaustive endurance exercises induce similar to those observing in typical inflammatory processes of various origins. Increased physical and emotional stress in sports causes changes in immunoregulation mediated via synthesis of multiple cytokines. The aim of this study was to assess the impact of various endurance exercises on the synthesis of the main pro- and anti-inflammatory cytokines. The concentration of cytokines (IL-4, IL-6, IL-10, IL-18 and γ-IFN) have been assayed in sera of 158 professional athletes with different energy expenditure specialized in bullet shooting, biathlon, bobsled, hockey and basketball by ELIZA and immunofluorescence method using a commercial multiplex assay kits. Energy expenditure of athletes ranged from 3358 ± 312 to 6120±468 kcal. It have been demonstrated the increase in the concentration of all studied cytokines, especially IL-6, IL-10 and IL-18 in 2.98, 5.0 and 1.46 fold, respectively. The study demonstrated the direct concentration dependency from muscle mass index as well as energy expenditure of athletes and their physical activity. Here was no correlation between basal metabolic rate of sportsmen and the concentration of studied cytokines. The study of the concentration of cytokines can be useful biological marker of the state of physical activity and body composition of professional athletes.

Keywords: Cytokines and mediators, inflammatory molecules, metabolic control of immune responses

P-0394

Mapping RNA-binding proteins in human B cells and T cells upon differentiation

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B cells and T cells are key players in the defence against infections and malignancies. To exert their function, B cells and T cells differentiate into effector and memory cells. Tight regulation of these differentiation processes is key to prevent their malfunction, which can result in lifethreatening disease. Lymphocyte differentiation relies on the appropriate timing and dosage of regulatory molecules, and post transcriptional gene regulation (PTR) is a key player herein. PTR includes the regulation through RNA-binding proteins (RBPs), which control the fate of RNA and its translation into proteins. To date, a comprehensive RBP expression map throughout lymphocyte differentiation is lacking. Using transcriptome and proteome analyses, we here provide an RBP expression map for human B cells and T cells. We observed that even though the overall RBP expression is conserved, the relative RBP expression is distinct between B cells and T cells. Differentiation into effector and memory cells alters the RBP expression, resulting into preferential expression of different classes of RBPs. For instance, whereas naïve T cells express high levels of translation-regulating RBPs, effector T cells preferentially express RBPs that modulate mRNA stability. Lastly, we found that cytotoxic CD8+ and CD4+ T cells express a common RBP repertoire. Combined, our study reveals a cell type-specific and differentiation-dependent RBP expression landscape in human lymphocytes, which will help unravel the role of RBPs in lymphocyte function.

Keywords: B lymphocytes, mass spectrometry, molecular immunology, RNAseq, adaptive immunity

P-0395

Polymorphism in the ERAP1 gene and its association with prognosis and treatment of cervical carcinoma

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Nanopore sequencing of components of the antigen processing and presentation (APP) pathway, specifically endoplasmic reticulum aminopeptidase 1 (ERAP1), could provide a new tool for identifying those women at 'high risk' of developing cervical cancer following persistent infection with Human Papillomavirus (HPV) and treat them earlier than we do now. ERAP1 edits the peptide repertoire presented to cytotoxic T lymphocytes (CTLs) through N-terminal trimming of peptide precursors to the optimal length for stable MHC I binding prior to presentation. Single nucleotide polymorphisms (SNPs) in ERAP1 exist in multiple combinations that form distinct haplotypes that when expressed as allotypes, alter ERAP1 trimming function. Individual ERAP1 SNPs have been associated with increased cervical cancer risk in GWAS studies, however a cause and effect relationship between ERAP1 allotypes and cervical cancer development has not been established. Here, the ERAP1 allotypes from a cohort of 74 patients at varying stages of cervical carcinoma were identified using MinION, a third generation sequencing device that enables acquisition of full length reads of the 2.7kb-long ERAP1 gene in real time. Identification of ERAP1 allotypes of HeLa cervical cancer cell line and 293T human embryonic kidney cell line using MinION, enabled the establishment of a methodological pipeline, including optimisation of each step of the protocol, identification of the limitations of this technology and the development of a bioinformatics analysis pipeline. Accuracy of MinION sequencing was confirmed by comparison with Sanger sequencing data making MinION a suitable technology for identifying known and novel ERAP1 allotypes in cervical cancer patients.

Keywords: Antigen processing and presentation, cancer immunology, cancer immunopeptidome, viral infections

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POSTER PRESENTATIONS

P-0409

Quantitative analysis of human CD4+ T-cell differentiation reveals subset-specific regulation of glycosphingolipid pathways

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T-cells are sentinels of adaptive immune responses. T-cell activation, proliferation and differentiation involves metabolic reprogramming involving the interplay of genes, proteins and metabolites. Here, we aim to understand the metabolic pathways involved in the activation and functional differentiation of human CD4+ T-cell subsets (Th1, Th2, Th17 and iTregs). We combined genome-scale metabolic modeling, gene expression data, targeted and non-targeted lipidomics experiments, together with *in vitro* gene knockdown experiments and showed that human CD4+ T cells undergo specific metabolic changes during activation and functional differentiation. In addition, we identified and confirmed the importance of ceramide and glycosphingolipid biosynthesis pathways in Th17 differentiation and effector functions. Through *in vitro* gene knockdown experiments, we substantiated the requirement of serine palmitoyl transferase, a de novo sphingolipid pathway in the expression of proinflammatory cytokine (IL17A and IL17F) by Th17 cells. Our findings may provide a comprehensive resource for identifying CD4+ T-cell-specific targets for their selective manipulation under disease conditions, particularly, diseases characterized by an imbalanceof Th17/nTreg cells.

Keywords: Mass spectrometry, modelling, omics technologies, regulatory cells

P-0421

Mitochondrial ROS are involved in chemoattactant-induced oxidative burst and degranulation of human neutrophils

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Activation of neutrophils is accompanied by the oxidative burst, exocytosis of various granule types (degranulation) and a delay in spontaneous apoptosis. The major source of reactive oxygen species (ROS) in human neutrophils is NADPH oxidase (NOX2), however, other sources of ROS also exist. Although the function of ROS is mainly defensive, they can also play a regulatory role in cell signaling. However, the contribution of various sources of ROS in these processes is not clear. A possible role of mitochondria-derived ROS (mtROS) in the regulation of neutrophil activation induced by chemoattractant fMLP *in vitro* was investigated. Using the mitochondria-targeted antioxidant SkQ1 (plastoquinone conjugated with decyltriphenylphosphonium), which was developed at Moscow University, the implication of mtROS in the oxidative burst caused by NOX2 activation as well as in the exocytosis of primary (azurophil) and secondary (specific) granules, was demonstrated. Scavenging of mtROS with SkQ1 slightly accelerated spontaneous apoptosis and significantly stimulated apoptosis of fMLP-activated neutrophils. According to the data obtained, mtROS play a critical role in signal transduction that mediates the major neutrophil functional responses in the process of activation. Scavenging of mtROS with the mitochondria-targeted antioxidants may be envisaged as a novel strategy for treating a variety of diseases associated with excessive activation of neutrophils.

Keywords: Cell death, cell signalling, innate immunity, neutrophils

P-0423

The impact of CD95 activation on primary human macrophage polarization

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Expression of the CD95 ligand (CD95L) is increased in inflammatory diseases, tumor microenvironment and patient sera. The binding of CD95L to its receptor CD95, a member of the TNF receptor superfamily, increases the expression of some pro-inflammatory factors by initiating non-apoptotic mechanisms rather than apoptosis in macrophages. However, the impact of CD95L on M1/M2 polarization of macrophages is not known. To investigate the impact of CD95L on primary human macrophage polarization, human PBMC monocyte-derived macrophages were stimulated with soluble CD95L and simultaneously polarized into M1 with LPS+IFN-γ, M2 with IL-4 or M2c with IL-10. The expression levels of M1 and M2 markers in macrophages were analyzed by qPCR and flow cytometry. We demonstrated that CD95L stimulation did not change the non-polarized macrophage response. In M1 macrophages, while CD95L stimulation increased the expression of proinflammatory markers at mRNA level, it did not affect the expression at the protein level. In M2a macrophages, CD95L significantly reduced the surface expression of the M2a marker "CD206" at the protein level. However, M2c polarization was not affected by CD95L. Our findings suggest that CD95L has no functional effect on primary human macrophage polarization.

Keywords: Cell death, innate immunity, macrophage

P-0452

Anti-C1q-autoantibodies from patients with Lupus Nephritis recognize soluble C1q

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The serum molecule C1q is a typical autoantigen in many autoimmune disorders including Lupus Nephritis (LN). Two types of physiologically active domains characterise C1q structure – the collagen-like region (CLR) and the globular region (gC1q). Anti-CLR autoantibodies correlate with the clinical outcome of active LN and recognise neoepitope which is exposed on C1q upon immobilisation. Thus, clinical methods used to detect and to quantify anti-C1q antibodies include immobilized C1q. In contrast, anti-gC1q antibodies prevail before upcoming flare of LN. We aimed to analyse whether immobilisation of C1q is a prerequisite for auto-C1q antibodies to recognize the globular autoepitopes. The interaction of soluble C1q with immobilized IgG autoantibodies from sera of LN patients was analysed by: A) ELISA, where we used as autoantigens the native C1q and recombinant analogues of gC1q globular regions: ghA, ghB and ghC. B) Fluorescence spectroscopy with the same experimental design used for ELISA. Our data indicate dose-dependent interaction between LN autoantibodies and the soluble C1q. The IgG autoantibodies recognize all three globular regions of gC1q with the highest binding affinity to ghA. Soluble C1q expose conformational autoepitopes that are recognised from anti-C1q antibodies. These autoepitopes are formed by all three globular fragments - ghA, ghB and ghC. The recognition of these globular autoepitopes is not affected by conformational changes in the structure of C1q due to immobilisation.

Keywords: Antibody, autoimmunity, complement, engineering of antibodies and nanobodies

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POSTER PRESENTATIONS

P-0491

A role for IL-10 in cellular senescence and aging

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Ageing is a progressive, time-dependent loss of physiological functions, often associated with cancer, cardiovascular, metabolic and neurological diseases. Inflammatory responses are central to the aging process, with pro-inflammatory cytokines as a whole contributing to the "inflammaging" phenotype. In contrast, the role of anti-inflammatory cytokines, such as interleukin (IL)-10, has not been explored. Using an *in vivo* model of sustained high doses of IL-10, we show this cytokine mediates histological changes in the skin, as well as progressive hair greying, commonly found during aging. Sustained IL-10 expression led to reduction in the number of skin fibroblasts, and cellular damage, including mitochondrial damage, rough endoplasmic reticulum dilatation, vacuolization and dysregulation of chromatin organization, as revealed by electron microscopy. Furthermore, *ex vivo* live cell analysis of control or IL-10-exposed fibroblasts showed that IL-10 altered the cell cycle and triggered loss of mitotic fidelity. IL-10 impacted the whole skin transcriptome which reflected cellular damage. Surprisingly, we found that IL-10 induced cellular senescence in *in vitro* cultured mouse fibroblasts. We are investigating further the molecular network linking IL-10 to cellular senescence. We expect to elucidate novel IL-10 functions with potential therapeutic impact in inflammatory conditions and aged-associated diseases.

Keywords: Ageing, cytokines and mediators, immune senescence

P-0514

Dendritic cells derived IL-6 protects from epithelial damage during colitis and drives colitis-associated cancer by controlling RORγt+ cell accumulation

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IL-6 is known for its ability to drive tumor progression in the gut and to support intestinal barrier functions during acute colitis. IL-6-deficient mice develop severe colon inflammation in dextran sodium sulfate (DSS)-induced colitis model, but demonstrate reduced tumor burden in azoxymethane (AOM)–DSS colitis-associated cancer. Accumulating evidence suggests that dendritic cell-derived IL-6 may represent the major regulator of IL-6-dependent processes during gut inflammation. To test this hypothesis we compared IL-6 knockout (KO) mice, mice with cell-specific ablation of IL-6 in CD11c+ cells, and wild type littermate control mice in the models of acute DSS-induced colitis, and AOM-DSS-induced colorectalcancer. We found that CD11c-IL-6 KO mice developed more severe intestinal inflammation with elevated expression levels of TGFβ and RegIIIγ compared to wild type littermate control mice. In AOM-DSS model, CD11c-IL-6 KO, as well as IL-6 KO mice demonstrated lower tumor load than wild type littermate control mice. Interestingly, the frequency of both Th17 and Treg cells, dependent of the master transcriptional factor RORγt, were decreased in inflamed colon and in tumors of IL-6 deficient mice. RORγt+ cells are the key population controlling intestinal barrier function and homeostasis and their decrease with subsequent abrogation of IL-17A expression in the colon may represent the mechanism of enhancedtumor control. Taken together, our data suggest that IL-6 produced by dendritic cells may affect colon integrity during acute colitis and tumor progression in colitis-associated cancer model through activation of RORγt cells in the gut.

This work was supported by RSF grant № 19-75-30032

Keywords: Cytokines and mediators, dendritic cells, *in vivo* tumor models, inflammatory bowel disease, myeloid cells, tissue damage and repair

P-0578

Proteasome inhibition regulates inflammatory responses in a pDC cell line

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Protein aggregation is induced by a wide variety of cellular stresses, including amino acid starvation, virus infection, endoplasmic reticulum stress, lipopolysaccharide, and oxidative stress. It has been suggested that altered proteostasis impacts the inflammatory response, but the underlying mechanism between altered proteostasis and inflammation is still poorly understood. Here, we aim to analyse the impact of protein aggregation in the response of human Dendritic Cells, focusing on plasmacytoid dendritic cells (pDCs). We used CAL-1 cells, a plasmacytoid dendritic cell line, and inhibited either autophagy or proteasome, promoting accumulation of p62-based aggregates with different morphology and chemical composition. Upon autophagy inhibition the overall number of small aggregates increased and upon proteasome inhibition a more prominent and large type of aggregate was observed by laser scanning confocal microscopy. Interestingly, proteasome inhibition promoted an increase in IL-1β secretion, and a reduction on cell viability, suggesting inflammasome activation, in an irreversible and cell specific manner. Overall, we conclude that proteasome inhibition induces accumulation of protein aggregates associated with an inflammatory response in CAL-1 cells. Proteasome inhibitors are believed to have anti-inflammatory and immunosuppressive effects and, consequently, they could be used to alleviate inflammatory disorders. We propose that the inflammatory effect herein described must be considered when using proteasome inhibitors as potential drugs for the treatment of pDCs derived immune-mediated disorder.

This work was supported by FEDER through POCI (COMPETE2020) and by Fundação para a Ciência e a Tecnologia – references PTDC/BIA-CEL/28791/2017 and POCI-01-0145- FEDER-028791, POCI-01-0145-FEDER-030882 and PTDC/BIA-MOL/30882/2017, UIDB/04501/2020; and by Maratona da Saúde.

Keywords: Dendritic cells, immune regulation and therapy, innate immunity

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POSTER PRESENTATIONS

P-0583

The Elongator complex regulates TLR4 and type I interferon gene induction responses

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The evolutionarily conserved Elongator complex acts by modifying of transfer RNA (tRNA) molecules at the wobble base position, which is required to ensure the fidelity and efficiency of translation of mRNA codons ending in –AA. However, the specific role of Elongator in gene regulation during the innate immune response is poorly understood. USing CRISPR/Cas9 we generated immortalised bone-marrow derived macrophages (iBMDMs) lacking Elp3, the catalytic subunit of the complex, to investigate Elongators function in the innate immune response. Unbiased quantitative proteomic analysis of Elp3 -/- iBMDMs displayed an impairment of proteins required for type I interferon and antiviral signalling, and also impaired LPS-dependent IFNβ and IFN-stimulated gene (ISG) expression. IFNβ induced by LPS- stimulation amplifies type I IFN & ISG expression via the IFNAR receptor and the ISGF3 complex. IFNβ-mediated ISG expression was abolished in Elp3 KO iBMDMs similarly to LPS stimulation. Both LPS and IFNβ-mediated STAT1 phosphorylation and activation was abrogated in Elp3 KO iBMDMs. In contrast to LPS and IFNβ-stimulated cells, IFNγ stimulated STAT1 phosphorylation and IRF1 induction were unimpaired in Elp3 KO iBMDMs. LPS and IFNβ, but not IFNγ –mediated gene induction and STAT1 phosphorylation were abolished in the absence of Elp3. Thus the Elongator complex regulates TLR4 and type I IFN mediated STAT activation and antiviral innate immunity.
Keywords: Cell signalling, innate immunity, macrophage, molecular immunology

P-0708

Genome editing confirms functionality of autoimmunity-associated intergenic polymorphism rs12946510 in a T-helper cell line

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A non-coding polymorphism rs12946510 is associated with multiple sclerosis, inflammatory bowel disease and asthma. Several fine-mapping methods suggest its causality. It is also an eQTL for GSDMA, GSDMB, ORMDL3 and IKZF3 in primary immune cells. To test its functionality in the native genomic context and investigate its impact on transcription in isolation from the bunch of other tightly linked SNPs we obtained derivatives of the CEM cell line (Th-like) carrying alternative alleles of this SNP using CRISPR/Cas system. We detected higher expression of ORMDL3 and IKZF3 in the CEM subline bearing the CC genotype by real-time RT-PCR (Wilcoxon rank sum p-value 0.008). These results show that a single intergenic nucleotide substitution can indeed affect gene expression. Moreover, genome editing can help discover target genes for fine-mapped disease-associated non-coding polymorphisms and thus enrich our knowledge of molecular bases of diseases.
This work was supported by the Russian Science Foundation (http://rscf.ru/en), grants #18-75-00072 (editing and preliminary tests) and #19-14-00341 (additional tests and data analysis).

Keywords: Autoimmunity, molecular immunology, multiple sclerosis

P-0725

Lymphocyte-specific tyrosine-protein kinase Lck dimers boost T-cell antigen receptor signaling by *trans*-activatory auto-phosphorylation

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Engagement of the T-cell antigen receptor (TCR) initiates a signaling cascade resulting in T-cell activation and differentiation. Intracellular lymphocyte-specific kinase (Lck) plays a pivotal role in this process, transducing TCR/CD3 stimulation into tyrosine phosphorylation, calcium fluxing, synapse formation, and altered gene expression. Lck activity is regulated on multiple intercalated levels, including its subcellular localization, lipid anchorage, 2-D micro-domain distribution within the plasma membrane, and its phosphorylation status that is directly linked to its enzymatic activity. Another potential mechanism of Lck regulation conceptualized in reviews but not fully investigated is its homo-dimerization leading to trans-activatory auto-phosphorylation. Employing Blue Native gel electrophoresis, we found a fraction of endogenous Lck signals at dimeric and higher-order complex size. Co-immunoprecipitation experiments with differently tagged Lck proteins confirmed self-association of membrane-anchored Lck molecules. Via TOCCSL, a super-resolution imaging technique, we depicted a significant amount of Lck-mEGFP dimers in living T-cells. To further investigate the role of Lck homo-dimers during T-cell signaling, we established an inducible Lck-dimerization system in human Jurkat T-cells after CRISPR/Cas9 knock-out of endogenous Lck. Controlled and specific dimerization of Lck by a membrane-permeable X-linking agent significantly altered its phospho-status and enzymatic activity in a titratable fashion, modulating early TCR signaling events like calcium fluxing as well as late-stage CD69 surface expression. In conclusion, homo-dimerization of Lck represents a novel regulatory mechanism for controlling Lck kinase activity and thus thresholds for T-cell signaling.

Keywords: Adaptive immunity, cell signalling, molecular immunology

P-0736

Inhibition of RANK signaling in breast cancer induces an anti-tumor immune response orchestrated by CD8+ T cells

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Most breast cancers exhibit low immune infiltration and are unresponsive to immunotherapy. We hypothesized that inhibition of the receptor activator of nuclear factor-κB (RANK) signaling pathway may enhance immune activation. Here we report that loss of RANK signaling in mouse tumor cells increases leukocytes, lymphocytes, and CD8+ T cells, and reduces macrophage and neutrophil infiltration. CD8+ T cells mediate the attenuated tumor phenotype observed upon RANK loss, whereas neutrophils, supported by RANK-expressing tumor cells, induce immunosuppression. RANKL inhibition increases the anti-tumor effect of immunotherapies in breast cancer through a tumor cell mediated effect. Comparably, pre-operative single-agent denosumab in premenopausal early-stage breast cancer patients from the Phase-II D-BEYOND clinical trial (NCT01864798) is well tolerated, inhibits RANK pathway and increases tumor infiltrating lymphocytes and CD8+ T cells. Higher RANK signaling activation in tumors and serum RANKL levels at baseline predict these immune-modulatory effects. No changes in tumor cell proliferation (primary endpoint) or other secondary endpoints are observed. Overall, our preclinical and clinical findings reveal that tumor cells exploit RANK pathway as a mechanism to evade immune surveillance and support the use of RANK pathway inhibitors to prime luminal breast cancer for immunotherapy.

Keywords: Animal models, cancer immunology, immunotherapy, *in vivo* tumor models, molecular immunology

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POSTER PRESENTATIONS

P-0744

Dendritic cell–specific role for Pellino2 as a mediator of TLR9 signalling pathway

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Ubiquitination regulates immune signalling and multiple E3 ubiquitin ligases have been studied in the context of their role in immunity. Despite this progress, the physiological roles of the Pellino E3 ubiquitin ligases, especially Pellino2, in immune regulation remains largely unknown. Accordingly, this study aimed to elucidate the role of Pellino2 in dendritic cells (DC). We show critical role of Pellino2 in regulation of proinflammatory response following TLR9 stimulation. Pellino2-deficient DCs show impaired secretion of IL-6 and IL-12. Loss of Pellino2 does not effect TLR9-induced activation of NFκB or MAP kinases, pathways that drive expression of IL-6 and IL-12. Instead Pellino2-deficient DCs showed impaired production of type I interferon (IFN) following endosomal TLR9 activation. Thus, Pellino2 mediates feedforward loop of IFNβ that promotes IL-12 production in DCs. We also observe that Pellino2 is downregulated following TLR9 stimulation and its overexpression in DCs induces upregulation of both IFNβ and IL-12 demontrating the sufficiency of Pellino 2 in driving these responses. Our results suggests that Pellino2 is critical for excecuting TLR9 signalling and its expression is tightly regulated to prevent excessive inflammatory response. Overall, this study highlightes a novel role for Pellino2 in regulating DC functions and further supports important roles for Pellino proteins in mediating and controlling immunity.

Keywords: Cell signalling, cytokines and mediators, dendritic cells

P-0746

Complex interplay between MAZR and Runx3 regulates the generation of cytotoxic T lymphocyte and memory T cells

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The BTB zinc finger transcription factor MAZR (also known as PATZ1) controls, partially in synergy with the transcription factor Runx3, the development of CD8 lineage T cells. Here we explored the role of MAZR as well as combined activities of MAZR/Runx3 during cytotoxic T lymphocyte (CTL) and memory CD8+ T cell differentiation. In contrast to the essential role of Runx3 for CTL effector function, the deletion of MAZR had a mild effect on the generation of CTLs *in vitro*. However, a transcriptome analysis demonstrated that the combined deletion of MAZR and Runx3 resulted in much more widespread downregulation of CTL signature genes compared to single Runx3 deletion, indicating MAZR partially compensates for loss of Runx3 in CTLs. Moreover, in line with the findings made *in vitro*, the analysis of CTL responses to LCMV infection revealed that MAZR and Runx3 cooperatively regulate the expression of CD8α, Granzyme B and perforin *in vivo*. Interestingly, while memory T cell differentiation is severely impaired in Runx3-deficient mice, the deletion of MAZR leads to an enlargement of long-lived memory subset and also partially restored the differentiation defect caused by loss of Runx3. This indicates distinct functions of MAZR and Runx3 in the generation of memory T cell subsets, which is in contrast to their cooperative roles in CTLs. Together, our study demonstrates complex interplay between MAZR and Runx3 during CTL and memory T cell differentiation, and provides further insight into the molecular mechanisms underlying the establishment of CTL and memory T cell pools.

Keywords: Adaptive immunity, memory, molecular immunology, viral infections

P-0800

Regulation of thrombosis during Behçet disease through EBI3, IL-6 and IL-6R

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Behçet disease (BD) is multisystemic, multifactorial disorder; it's classified among the vasculitides which can affect all types and sizes of blood vessels. In this study we sought to explore the role of EBI3, IL-6 and IL-6R in the regulation of coagulation during Behçet disease. We enrolled 16 with BD (5 active and 11 inactive stage) and 14 healthy control (HC). Hemostasis parameters and factors were assessed in freshly collected plasma. EBI3, IL-6 and IL-6R were measured by ELISA. Statistical analyses were measured by Mann Witney U test for group comparison while Spearman test was used for correlation analyses. We observed a significant increase in EBI3 levels during vascular manifestations of BD in comparison to other manifestations and HC ($p<0.05$) while IL-6 levels were significantly increased in all manifestations of BD comparing to HC, in contrary, no significant difference was noticed for IL-6R. All Hemostasis parameters didn't show any differences between patients and control subjects ($p>0.05$). However, correlation studies showed significant relationships between cytokines and some hemostasis parameters in patients and not in control subjects. Interestingly, EBI3 was positively correlated with fibrinogen (r= 0.517, p=0.04) and FVIII (r=0.598, p=0.019). IL-6 was positively associated with FVII (r=0.650, p=0.022), while IL-6R was negatively correlated with fibrinogen (r= - 0.627, p=0.009) and Activated protein C resistance with (r= - 0.610, p=0.012). These findings highlight the role of inflammatory cytokines, especially IL-6 and EBI3, in the prothrombotic state while IL-6R could be a protective factor against thrombosis.

Keywords: Autoinflammation, cytokines and mediators, immune communication, inflammatory disease, inflammatory molecules, innate immunity

P-0837

Carbamylation affects the biological activity of an inflammatory mediator, bradykinin

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The inflammatory response following tissue injury plays important role in wound healing process. It leads to haemostasis and recruitment of the innate immune system, which defends us against the invading pathogens and helps remove dead tissues. It is characterized by the influx of immune cells, mainly neutrophils in early phase, which upon activation release among others the heme peroxidase: myeloperoxidase (MPO). One of the pitfalls of MPO activity is the generation of isocyanic acid - a highly reactive molecule responsible for a posttranslational modification known as carbamylation. This modification has been described as affecting the structure and activity of various proteins and peptides. One of the its possible substrates, bradykinin, is a 9-amino acid-long peptide mediating the inflammatory response. We have hypothesised that carbamylation will affect biological activity of bradykinin. Firstly, we confirmed that the peptide can undergo carbamylation at its N-terminus. This can directly affect the half-life of the peptide, as it lowers its affinity to the bradykinin-inactivating enzymes. Using a keratinocyte model we have shown that carbamylated bradykinin looses its potency in inducing B2 receptor-mediated calcium influx. Both native and carbamylated bradykinin induce the production of cytokines and, in case of carbamylated peptide, observed effect is abolished upon inhibition of B2 receptor. Lastly, we have shown that carbamylated bradykinin is less potent in promoting wound healing than its native counterpart. Taken together, our results indicate that, upon carbamylation, bradykinin loses much of its biological potency which may hinder the progression from inflammation to proliferation stage of wound healing.

Keywords: Cell signalling, cytokines and mediators, skin diseases, tissue damage and repair

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POSTER PRESENTATIONS

P-1007

DC subset–specific induction of T cell responses upon antigen uptake via Fcγ receptors *in vivo*

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Dendritic cells (DCs) are efficient antigen-presenting cells equipped with various cell surface receptors for the direct or indirect recognition of pathogenic microorganisms. Interestingly, not much is known about the specific expression pattern and function of the individual activating and inhibitory Fcγ receptors (FcγRs) on splenic DC subsets *in vivo* and how they contribute to the initiation of T cell responses. By targeting antigens to select activating and the inhibitory FcγR *in vivo*, we show that antigen uptake under steady-state conditions results in a short-term expansion of antigen-specific T cells, whereas under inflammatory conditions especially, the activating FcγRIV is able to induce superior CD4+ and CD8+ T cell responses. Of note, this effect was independent of FcγR intrinsic activating signaling pathways. Moreover, despite the expression of FcγRIV on both conventional splenic DC subsets, the induction of CD8+ T cell responses was largely dependent on CD11c+CD8+ DCs, whereas CD11c+CD8− DCs were critical for priming CD4+ T cell responses.

Keywords: Adaptive immunity, adjuvants and vaccines, dendritic cells

P-1094

Sitagliptin, dipeptidyl peptidase 4-inhibitor, modulate oxidative burst activity of human neutrophils *in vitro*

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Dipeptidyl peptidase (DPP)-4 inhibitors belong to a class of anti-hyperglycemic agents indicated for patients with type-2 diabetes. In addition to glucose-lowering effects, emerging data suggests that DPP4 inhibitors display anti-inflammatory effects which are insufficiently investigated. The aim of this study was to investigate the impact of Sitagliptin on apoptosis and functions of human neutrophils. Neutrophils were isolated from peripheral blood of five healthy donors by dextran sedimentation, density gradient centrifugation and lysis of erythrocytes. Neutrophils were pretreated with or without different concentrations of sitagliptin (125 μg/ml, 250 μg/ml and 500 μg/ml), and stimulated with phorbol myristate acetate (PMA), N-formyl-methionyl-leucyl-phenylalanine (fMLP), calcium ionophore (CaI) or opsonized zymosan (opZy). The production of reactive oxygen species (ROS) was determined by luminol-amplified chemiluminescence. We showed that none of the applied sitagliptin concentrations induced apoptosis and necrosis of human neutrophils as assessed by annexin-V/propidium iodide flow cytometry assay. Sitagliptin dose-dependently decreased the ROS production by human neutrophils activated with CaI and opZy compared to control. In contrast, sitagliptin increased PMA-induced ROS production at all tested concentrations, whereas had no effect on fMLP-activated neutrophils. Sitagliptin did not affect neutrophil extracellular traps formation induced by PMA or CaI, which was detected using the fluorescent method on the multi-mode microplate reader. Our results suggest, for the first time, that DPP4 inhibitor can modulate neutrophil functions, which could explain some of the anti-inflammatory actions observed for DPP4-inhibitors. The molecular mechanisms of these activities remain to be investigated

Keywords: Drugs for immune modulation, granulocytes, immunopharmacology, molecular immunology, neutrophils

P-1099

Molecular modeling provides motifs for identification of TCRs specific to SARS-CoV-2 epitope

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Multiple studies are focused on T-cell response to SARS-CoV-2 and epitopes recognized by T-cells. One of the immunodominant epitopes, YLQPRTFLL (YLQ), derived from the S-protein, is presented by HLA-A*02:01 (Shomuradova et al.,2020). Sequences of YLQ-specific T-cell receptors (TCRs) have high similarity and form several homology groups. Mutual similarity of epitope-specific TCRs can be used to predict the specificity of de novo sequenced TCRs (Dash et al.,2017, Glanville et al.,2017, Emerson et al.,2017). We used 8 alpha- and 2 beta-chain sequences from different homology groups to model TCR- peptide-MHC complex (Jensen et al.,2019) and demonstrated that TCR alpha-chains contacted with YLQ N-terminal region, while beta-chains interacted with its middle and C-terminal regions. Using this data, we identified amino acids important for recognition and formed CDR3-motifs, which were used to identify YLQ-specific TCRs in total TCR repertoires of COVID-19 convalescent patients (n=17) and healthy donors (n=12). The search for a YLQ-specific motif with fixed CDR3-length and motif start position revealed - convalescent patients had more motif-containing clonotypes in beta-chain TCR repertoire than donors, besides their total share in repertoire was also higher. (Median (clonotype frequency) - 0.0001588 vs 0.00001072, p=0.0022, median (total share) - 0.0001300 vs 0.00008384, p=0.0061, Mann-Whitney). In contrast, when motifs were constructed without consideration of the TCR-p-MHC structure, we did not find any significant difference between convalescents and healthy donors. That shows that identification of key amino acid residues by modeling TCR-p-MHC interaction may provide better motifs for epitope-specific TCRs identification.

Supported by the Russian Science Foundation grant 20-15-00395.

Keywords: Adaptive immunity, antigen processing and presentation, big data, modelling, RNAseq, viral infections

P-1111

Design, preparation and immune characterization of SARS-CoV-2 antigenic epitope chimeric protein

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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) can cause respiratory symptoms after infection, and even death can occur in severe cases. SARS-CoV-2 infection has no specific treatment drugs, mainly rely on vaccination to block its transmission. SARS-CoV-2 contains four structural proteins, Spike protein (S) and Nucleocapsid protein (N) are the main candidate proteins for developing SARS-CoV-2 vaccine and antibody detection reagents. METHODS: Antigenic epitopes of SARS-CoV-2 structural proteins were screened by molecular biology software, the selected antigenic epitopes were connected in tandem, and expressed high efficiently in *E.coli* as a chimeric protein by genetic engineering technology. The soluble chimeric protein of high purity was obtained after purification and renaturation. Mice were immunized with the purified chimeric protein together MF59 adjuvant, aluminum adjuvant or no adjuvant at different doses respectively, then the effect of humoral immunity and cellular immunity induced by them were evaluated. Results： The expressed chimeric protein was in the form of inclusion body, soluble chimeric protein was obtained after renaturation. The specific antibodies with high titer were produced in the immunized mice, and strong cellular immunity was induced also. More high concentration of chimeric protein, more better elicited immune effect. The immune effect induced by the chimeric protein with MF59 adjuvant was no different from that induced with aluminum adjuvant. This study provides novel ideas for the design and renaturation of SARS-CoV-2 chimeric protein, and the chimeric protein is expected to be used for the development of SARS-CoV-2 recombinant protein vaccine and diagnosis reagent.

Keywords: Adjuvants and vaccines, antibody, cytokines and mediators, immunological techniques

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POSTER PRESENTATIONS

P-1114

Cytoskeleton related proteins control complement receptor 3 expression during differentiation

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Activation of the integrin phagocytic receptors CR3/αMβ2 and CR4/αXβ2 requires Rap1 activation and RIAM function. RIAM was shown to control particle engulfment by recruiting VASP to phagocytic cups and inducing its phosphorylation, and VASP knockout results in abolished phagocytosis. Using a CRISPR-Cas9 knockout approach, we analyze the requirement for RIAM, VASP and Vinculin expression in neutrophil-differentiated HL-60 cells. Knockout of either RIAM or Vinculin recapitulated the abolishment of phagocytic function in VASP KOs. All knockouts showed a significant and specific reduction in integrin αM and αX surface expression. q-PCR revealed a profound reduction in ITGAM (αM) and ITGAX (αX) mRNA, which correlated with a reduction in cellular F-actin. When treated with jasplakinolide, which induces actin polymerization, normal surface expression of αM was reestablished. The Serum Response Factor (SRF) was selected as the potential transcription factor since its coactivator MRTF-A requires increased actin polymerization to induce transcription. Immunofluorescent MRTF-A localization in wild type cells was primarily nuclear, whilst knockouts exhibited a diffuse cytoplasmic pattern. Localization of FHL-2 (SRF corepressor), was membranous in wild type cells, but was in the nucleus in knockouts, suggesting these proteins sequester FHL-2 and inhibit its activity. Finally, reexpression of VASP in the VASP knockout resulted in a complete reversion of the phenotype, as knock-ins showed wild type levels of αM. Taken together, these results suggest that expression of proximal components of the integrin adhesome and integrin signaling are necessary for the correct expression of CR3 during myeloid cell differentiation, and the acquisition of a phagocytic phenotype.

Keywords: Cell signalling, neutrophils, cytoskeleton, molecular immunology, phagocytosis

P-1116

The role of protein kinase C isoforms in Neutrophil Extracellular Traps formation

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The aim of this research was to find out which isoforms of protein kinase C (PKC) are involved in Neutrophil Extracellular Trap (NET) formation activated by various stimuli, such as calcium ionophore A23187, PMA, and chemoattractant fMLP. Also, the role played by various PKC isoforms in reactive oxygen species (ROS) production was investigated. Neutrophils were isolated from the blood of healthy donors or patients with X-linked chronic granulomatous disease (CGD). ROS production was determined by luminol-amplified chemiluminescence assay. The release of NETs was detected using fluorescence microscopy. Using the inhibitory analysis on the model of oxidative burst, we showed that A23187 activates equally conventional (cPKCβ), novel (nPKCδ), and atypical (aPKCξ) PKC isoforms, while PMA and fMLP induce only PKCβ and PKCδ isoforms. NETosis of healthy donor neutrophils induced by A23187 and PMA depended on PKCβ and PKCξ isoforms. Using neutrophils isolated from patients with CGD activated A23187, we have shown that inhibitor of conventional PKC isoforms suppressed NET formation. Therefore, some additional unknown targets of PKCβ apart from NADPH oxidase subunits are involved in A23187-induced netotic pathway.

Keywords: Cell signalling, granulocytes, innate immunity, neutrophils

P-1120

Immune synapse reprograms DCs to increase migration to antigen-presenting hubs via Ccr7 upregulation and epigenetic modification

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Antigen presentation is a key event in the initiation of adaptive immunity. During antigen presentation, intimate contacts between antigen-presenting cells, mainly dendritic cells (DCs), occur via formation of specialized structures termed immune synapses (IS). The fate of T cell upon antigen presentation and immune synapse formation has been largely studied while the fate of dendritic cells (DCs) after productive immune synapses (postsynaptic DCs, psDCs) has been largely neglected. Here, we performed transcriptomic analysis of CD11c+ psDCs and demonstrated that psDCs upregulate an genes related to antiviral activities and other immune functions. Also, psDC upregulate genes related to motility such as chemokine receptor Ccr7. This data correlated with the epigenomic changes induced in psDCs that include changes in DNA accessibility and histone methylation in the Ccr7 region. In line with the transcriptomic and epigenomic changes observed, psDCs migrate more efficiently towards CCR7 ligands *in vitro* and showed enhanced homing to lymph nodes when administered *in vivo*. In summary, we describe that DC reprogram their transcriptomic and epigenomic signature correlating with an enhanced migratory capacity upon antigen presentation and IS formation.

Keywords: Antigen processing and presentation, cellular interactions, dendritic cells, epigenetic control and modulation of immunity, immune communication, omics technologies

P-1129

Key role of the IRE1-XBP1s pathway in metabolic reprogramming of human macrophages in response to saturated fatty acids and LPS

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Macrophages accumulate in obese adipose tissue and acquire a unique pro-inflammatory polarization, playing a key role in obesity-induced chronic-low grade inflammation and insulin resistance. Increased saturated fatty acids (SFAs) levels have been proposed to drive this specific polarization. Accordingly, we investigated the immunometabolic reprogramming in SFA-treated human macrophages in comparison with LPS-stimulated ones. The RNA sequencing of SFA-treated macrophages highlighted a strong pro-inflammatory profile and signatures like UPR, glycolysis, hypoxia. The activation of glycolysis was very rapid and transient with LPS, while late with SFA. Concomitantly, LPS and SFA also induced xbp-1s mRNA splicing via the endoribonuclease IRE1 and HIF-1α protein stabilization. Both transcription factors, XBP-1s and HIF-1α, simultaneously translocated to the nucleus. IRE1 pharmacological inhibition prevented both inducers-mediated glycolysis upregulation. Chromatin Immunoprecipitation assays are ongoing to demonstrate XBP-1s recruitment on HIF-1α-target glycolytic gene promoter. These data suggest for the first time a key role of XBP-1s in macrophages metabolic reprogramming.

Keywords: Cell signalling, inflammatory disease, macrophage, microenvironment

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POSTER PRESENTATIONS

P-1149

A secondary role for hypoxia and HIF1 in the regulation of (IFNgamma-induced) PD-L1 expression in melanoma

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Cancer cells are able to escape immune surveillance by upregulating programmed death ligand 1 (PD-L1). A key regulator of PD-L1 expression is transcriptional stimulation by the IFNγ/JAK/STAT pathway. Recent studies suggest that hypoxia can induce PD-L1 expression. As hypoxia presents a hallmark of solid tumor development, hypoxic control of PD-L1 expression may affect the efficacy of cancer immunotherapy. This study aims to explore the hypoxic regulation of PD-L1 expression in human melanoma, and its interaction with IFNγ-induced PD-L1 expression. Analysis of the cutaneous melanoma dataset from the cancer genome atlas revealed a significant correlation of the HIF1-signaling geneset signature with PD-L1 mRNA expression. However, this correlation is less pronounced than other key pathways known to control PD-L1 expression, including the IFNγ/JAK/STAT pathway. This secondary role of HIF1 in PD-L1 regulation was confirmed by analyzing single-cell RNA-sequencing data of 33 human melanoma tissues. Interestingly, PD-L1 expression in these melanoma tissues was primarily found in macrophages. However, also in these cells STAT1, and not HIF1, displayed the most pronounced correlation with PD-L1 expression. Moreover, we observed that hypoxia differentially affects PD-L1 expression in human melanoma cell lines. Knock-down of HIF1 expression, indicated a minor role for HIF1 in regulating PD-L1 expression. A more pronounced influence of hypoxia was found on IFNγ-induced PD-L1 mRNA expression, which is controlled at a 952 bp PD-L1 promoter fragment. These findings, showing the influence of hypoxia on IFNγ-induced PD-L1 expression, are relevant for immunotherapy, as both IFNγ and hypoxia are frequently present in the tumor microenvironment.

Keywords: Cancer immunology, checkpoint inhibition, macrophage

P-1162

BMP7 promotes the generation of DC2-like cells from CD34$^+$ hematopoietic progenitor cells

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Dendritic cells represent specialized antigen presenting cells in almost all tissues, which have essential immune system regulatory function. Hereby, cDC2 cells represent the most frequent subtype of conventional DCs. Single-cell RNA sequencing revealed that cDC2 comprise a very heterogeneous subset and shares features with the newly identified Axl$^+$DCs (ASDC). However, the developmental heterogeneity and its relevance in inflamed/cancerous tissue remains poorly understood. Bone morphogenetic protein-7 (BMP7) is aberrantly induced at high levels during skin inflammation, and its expression levels negatively correlates with clinical outcome in lung cancer. Here, we aimed to identify factors governing DC heterogeneity under inflammatory conditions. The effects of BMP7 and TGF-β1 was evaluated in a DC differentiation model of CD34$^+$ hematopoietic progenitor cells and in peripheral blood CD1c$^+$DCs. We observed that BMP7 promotes DC2-like cells in CD34$^+$ cells. Generated DC2-like cells express E-Cadherin, β-catenin and PD-L1. Upon further culture, DC2-like cells acquired LC characteristics (CD207$^+$, CD1a^{++}, Axl$^+$). Additionally, a fraction of TGF- β1-induced Axl$^+$ cells retained DC2 characteristics, but lacked LC markers, reminiscent of the recently described ASDCs. Studies on purified CD1c$^+$ DCs revealed that TGF- β1 also induced Axl by large percentages of generated cells. We observed that while BMP7 failed to induce Axl$^+$ cell generation from blood CD1c$^+$ DCs, it promoted TGF- β1-mediated Axl induction. Moreover, BMP7 promotes TGF-β-mediated Axl induction by CD1c$^+$ DCs. Inflammation and cancer-associated changes in epithelial cell-derived BMP vs. canonical TGF-β ligand synthesis might influence fate decisions of CD1c$^+$ blood DCs.

Keywords: Cell signalling, dendritic cells, inflammatory disease, regulatory cells

P-1167

Study of the regulation of haematopoietic lineage cell-specific protein 1 (HS1) by tyrosine phosphorylation

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HS1 was characterized as a substrate of kinases coupled to T- and B-cell receptor signaling. It is a multidomain protein that activates the Arp2/3 complex to promote the polymerization of branched filamentous (F-) actin. HS1 is required for stabilization of the immunological synapse and it participates in lymphocytic cell adhesion and migration. However how the protein contributes to the various actin-remodeling associated processes is barely understood, to the point that it is often merely described as an F-actin stabilizer. On the other side the tyrosine phosphorylation of the protein by various kinases seems to regulate the functions of the protein in hitherto unidentified ways. We are currently focusing on the regulation of the protein by tyrosine phosphorylation by Src kinase. To that extend, we have set-up a system of expression vectors that promotes the specific phosphorylation of HS1 in Jurkat T cells. We have found that, in our assay, Src phosphorylates HS1 specifically at tyrosine 378, as detected by using phosphospecific antibodies. Now we are analizing the effect of overexpressing phospho-Y378-HS1 over cell spreading and migration. Cell adhesion and migration of immune cells are very dynamic processes that require the action of "molecular switches" governing relevant nodes of signaling pathways. In view of our results, we propose that HS1 phosphorylation on tyrosine 378 might represent one important switch controlling cell adhesion and migration of T cells.

Keywords: Cell signalling, molecular immunology, cytoskeleton

P-1174

Characterization of GAD65 epitopes possibly presented by HLA-DR3 and/ or DQ2 molecules commonly associated with type 1 Diabetes (T1D) In north Indian population

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Studies have shown that HLA-DR3-DQ2 haplotypes not only associate with T1D but also with autoantibodies against GAD65, the major β-cell autoantigen. However, the exact mechanism of these associations is not known. Therefore, this study was planned to define GAD65 peptides possibly presented by HLA-DR3/DQ2 molecules to CD4 T cells. GAD65 peptide assay was performed using the PBMCs from T1D and healthy individuals. GAD65 peptides were pooled into 4 groups according to their amino acid positions and PBMCs were stimulated in a 16 hours culture with each group of peptides at conc.10μg/ml/peptide. CD4 T cells' stimulation in term of IFN-γ, TNF-α, IL-17 and IL-10 expression was analyzed using BD-FACSCantoII cell analyzer and DIVA software. All the four GAD65 peptide groups resulted in significant increase in IFN-γ and IL-17 expression but not TNF-α in T1D patients vs. controls. In contrast, IL-10 was significantly decreased in T1D vs. controls. Interpeptide group comparison for immunogenicity revealed significant increase in IFN-γ and IL-17 producing CD4+T-cells for peptide group 2 as compared to other groups (p=0.008 and p=0.017 respectively) in T1D patients only but not in controls. Further, group 2 GAD65 peptides yielded significant increase in IFN-γ (p=0.032) and IL-17 (p=0.026) and a decrease in IL-10 expressions by CD4+T-cellls in HLA-DR3/DQ2+ve compared to DR3/DQ2-ve patients. GAD65 peptides inducing IFN-γ and IL17 cytokines might have an important role in CD4+T-cell immune response in T1D. Group 2 GAD65 peptides possibly shift immune balance towards inflammatory phenotype in HLA-DR3/DQ2+ve T1D patients.

Keywords: Antibody, autoimmunity, diabetes, immune response tracing, MHC and polymorphic genes

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POSTER PRESENTATIONS

P-1180

Identification of glycosylation signatures in synovial fibroblasts culture systems

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A healthy joint microenvironment comprises immune cells and non-immune stromal synovial fibroblasts (SFs), which collaboratively mediate homeostasis through the release of soluble mediators. In rheumatoid arthritis (RA), impairment in the homeostasis create an autoimmune loop between SFs and immune cells that are leading to the perpetuation of inflammation and bone destruction. Glycans attached to cell surfaces regulate cellular interactions in the joint microenvironment, but the role of SFs glycome is not well-understood in RA. On the other hand, cell glycosylation is depended on the local 3D environments created by extracellular matrix proteins. Nevertheless, conventional 2D cultures might neglect physiological cell-matrix interactions and could generate artefactual results. Here, we increase our understanding of SF-mediated inflammation by investigating gene expression, pathway activation, glycosylation signature changes, and glycome in healthy, arthritic, and IL-1 stimulated SFs. We used (I) transcriptomics RNA-seq data in the SFs to show their inflammatory functions and alterations in the glycosylation signatures belonging to different culture models, and (II) experimental mass spectrometry data to show reduced sialylation levels in the glycome of arthritic SFs. We also demonstrate that 3D rigid scaffolds are a better model for studying RASFs *in vitro* than 2D and 3D Hydrogel culture systems since it allows cell-cell and cell-matrix interactions where most of the gene signatures are regained. Our findings emphasise the importance of cell glycosylation in SF-mediated inflammation during RA.

Keywords: Animal models, inflammatory disease, RNAseq, rheumatoid arthritis

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POSTER PRESENTATIONS

TRACK 3 - DISEASES AND IMMUNE RESPONSES

P-0059

Differences of immune cell subpopulations and anti-tumor antibodies in carcinoma tumor-bearing C57BL6/J mice treated with new high-frequency nanosecond electrochemotherapy

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Microsecond electrochemotherapy (µsECT) is already used in clinical trials for tumor ablation. We present a novel therapeutic approach for murine tumor ablation using nanosecond ECT (nsECT) that is still in research. However, further assessment of immune cell subpopulations and anti-tumor antibody levels is needed in ECT-treated mice. C57BL/6J mice carcinoma tumors were induced by injecting LLC1 cells subcutaneously. For tumor ablation, standard microsecond and four different nsECT were used with bleomycin. Tumor volumes were assessed with volumetric measuring every 2-3 days after the treatment. Mice blood sera were collected 10 days after the treatment for anti-tumor antibody determination. Afterward, mice survival was evaluated. Mice tumors, lymph nodes and spleens were collected for further investigation using flow cytometry. nsECT significantly reduced tumor volumes and promoted C57BL6/J mice survival, compared to the tumor-bearing untreated mice. Before electroporation, untreated mice sera had antibodies against tumor cells, but after electroporation antibody levels were significantly higher. ECT resulted in mice spleen enlargement relating to the increased numbers of lymphocytes and myeloid cells. Compared to untreated mice, the percentage of T and B cells was significantly diminished in spleens. ECT-treated mice had a significantly higher percentage of CD3-CD4-CD8- B200-Gr1-CD11c-CD11b- lymphocytes. A new lymphocyte population (CD3-CD4-CD8-B200-Gr1-CD11c-CD11b-) and anti-tumor antibodies may result in a better modulation of anti-carcinoma immune response. nsECT may be promising for future anticancer therapies, gradually replacing µsECT.

Keywords: Animal models, antibody, cancer immunology

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P-0074

Chemokine profiles differences in serum of patients with acute rejection after kidney transplantation

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Kidney allograft transplantation improved the prognosis and quality of life of patients with end-stage renal diseases but the acute rejection represents a limitation of the final outcome. Noninvasive biomarkers are needed as well as further advancements in the understanding of immune mechanisms of reaction to the allograft. Our study focused on one-year monitoring of serum concentrations of 12 chemokines regulating the recruitment of immune cells into transplanted allograft. 138 patients after renal transplantation were enrolled to the study. Concentration levels of 12 chemokines were measured in serum by Luminex technology in four time points, extended by the point of diagnostic acute rejection. Patients were divided into two groups (acute rejection and normal outcome). In a group of 44 patients with acute rejection, higher serum pretransplant levels of CXCL1, CXCL5, CXCL6, CCL2, CCL21, and particularly CXCL10, CX3CL1 were found suggesting their higher proinflammatory status as compared to subjects with uncomplicated outcome. In samples collected at the time of acute rejection, chemokines CXCL9, CXCL11 were found upregulated. Dynamic changes in blood concentrations of chemokines might be used as potential biomarker of the immune response against kidney allograft. From chemokines regulating neutrophils recruitment, CXCL1 seems to be the most relevant to mechanisms of acute rejection. Our study then demonstrated an association of increased serum levels of chemokines CXCL9, CXCL10, CXCL11 attracting preferentially Th1 lymphocytes with the acute rejection of kidney allograft.

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Keywords: Biomarkers, chemokines, transplantation

P-0107

Increased activity of follicular helper T and follicular cytotoxic T cells in Chronic Lymphocytic Leukemia

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in the elderly. CLL is characterized by the accumulation of small, mature-appearing CD5+ B cells in the blood, bone marrow, and lymphoid tissues and has a highly variable clinical course. T follicular helper cells (TFH) are a subgroup of CD4+ T lymphocytes with the chemokine receptor CXCR5, and follicular cytotoxic T (TFC) cells are a new subset of CD8+ T cells expressing CXCR5. The literature about the role of TFH and, especially TFC cells, in CLL is scarce. Herein, we aimed to investigate the ratio and surface molecules of TFC and TFH cells in CLL. Peripheral blood samples from thirty-four CLL patients and 19 healthy subjects were studied. CD3+CD4+CXCR5+ TFH and CD3+CD8+CXCR5+ TFC cell levels and ICOS, OX-40 and PD-L1 expression of these cells were analyzed by flow cytometry. Increased TFH and TFC cells were found in CLL patients compared to healthy controls. High ICOS and low PD-L1 expression were detected in TFH cells of patients. Similarly, high OX-40 and ICOS and low PD-L1 expression were detected in TFC cells of patients compared to healthy controls. To the best of our knowledge, this is the first study reporting the role of TFC cells in CLL. CLL patients present increased activity of TFH and TFC cells with high OX-40 and ICOS activator and lower PD-L1 inhibitor receptor expression. Activated TFH and TFC cells might have a role in the pathogenic augmentation of B cells in CLL.

Keywords: Cancer immunology, costimulatory pathways, follicular helper T cells

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POSTER PRESENTATIONS

P-0119

Exploring Type i interferon activity in ANCA-associated vasculitis

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ANCA-associated vasculitis (AAV) is an autoimmune disease characterised by inflammation of the small blood vessels. Type I interferons (IFNs) are cytokine mediators of innate immune responses. Dysregulation of type I IFNs can result in the development of autoimmune diseases termed type I interferonopathies and are thought to be drivers of chronic inflammation in these conditions. Despite this, type I IFNs have not been comprehensively studied in AAV. We hypothesised that type I IFN responses are systemically dysregulated in AAV, indicative of a type I interferonopathy. Peripheral blood and serum samples were collected from healthy individuals (n=72), disease controls (n=34), AAV patients (n=83) and patients with known type I interferonopathies (n=28) (peripheral blood samples only). The expression of seven type I IFN regulated genes (IRGs) (IFI27, IFI44L, IFIT1, ISG15, RSAD2, SIGLEC1 and STAT1) characteristic of type I interferonopathies were measured in peripheral blood samples using qPCR while serum type 1 IFN regulated proteins (CXCL10, MCP-1 and CCL19) were assessed by ELISA. Unlike the type I interferonopathy samples, IRG expression in AAV patients do not differ from disease and healthy control samples. No significant differences in MCP-1, CCL19 and CXCL10 expression were observed between each cohort. These results are independent of age, sex and disease activity. Additionally, markers of type I IFN responses did not correlate with clinical measures of AAV severity. Systemic type I IFN responses are not dysregulated in AAV and therefore AAV should not be considered as a type I interferonopathy.

Keywords: Autoimmunity, cytokines and mediators, innate immunity

P-0125

Sexual dimorphism in CX3CR1/Nrf2/HO-1 spinal cord axis affects therapeutic efficacy of β-adrenoceptor blockade in EAE rats

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Our previous studies showed more severe EAE in male compared with female adult rats, and moderating effect of propranolol-induced β-adrenoceptor blockade on EAE in female rats through stimulation of Nrf2/HO-1 signalling pathway in spinal cord microglia. This study was designed to examine putative sexual dimorphism in Nrf2/HO-1 signalling pathway and CX3CL1, as one of its activators. Propranolol treatment beginning from the appearance of the first clinical signs of EAE mitigated the disease severity in rats of both sexes, but its effect was more prominent in males. This correlated with more prominent effect of propranolol on the expression of CX3CL1 in spinal cord tissue, CX3CR1 on microglial surface, and Nrf2/HO-1 in spinal cord microglia in males. Consistently, the proportion of CX3CR1-expressing microglia and CX3CR1 density on their surface increased more prominently in males. Consistently, propranolol increased the proportion of IL-10/TGF-β-producing microglia and microglia expressing CD163, molecule highlighting ramified microglia with neuroprotective properties in damaged tissue, to a greater extent in males. Additionally, propranolol increased phagocyting capacity of microglia to a greater extent in males. Moreover, propranolol more prominently decreased the frequency of blood-borne myeloid cells and highly pathogenic IL-17+ T-cells, coexpressing GM-CSF/IFN-γ, in male rat spinal cord. This correlated with greater reducing effect of propranolol on the spinal cord tissue expression of CCL2/CCL19/CCL21 chemokines in males. The study as a whole indicates that sexual dimorphism in CX3CR1/Nrf2/HO-1 spinal cord axis could contribute to greater severity of EAE in male rats, and sexually dimorphic action of substances affecting its signalling capacity.

Keywords: Animal models, autoimmunity, chemokines, immunopharmacology, multiple sclerosis, myeloid cells

P-0185

Does Fc-glycosylation influence the pathogenicity of myelin-specific antibodies?

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Antibodies mediate their effector function via their Fc-domain. The affinity for Fcγ-receptors and complement is influenced by their isotype and the glycosylation of a highly-conserved asparagine residue. Here we explore the functional potential of Fc-glycosylation within the context of antibody-mediated autoimmune demyelination. To this end we cloned the myelin-oligodendrocyte glycoprotein (MOG) specific IgG1 mAb 8-18C5 and produced different Fc-glycovariants under polarising glycosylation conditions. All Glycovariants had an identical affinity for the target antigen MOG. The N174 glycan moieties on recombinant 8-18C5 produced by HEK cells, are fucosylated (G0F), while in YB2 cells it remained hypofucosylated (G0). The pathogenicity of Fc-variants was assessed in the mouse model of experimental autoimmune encephalomyelitis (EAE). A single 50 µg injection of the G0F variants aggravated MOG35-55 induced EAE in C57BL/6 mice. Strikingly, hypofucosylated variants caused a more aggressive EAE associated with increased mortality. To assess the underlying cellular mechanisms we analyzed tissue damage in the central nervous system. Increased inflammation and demyelination was demonstrated by immunohistochemistry for the G0F variant relative to the G0 8-18C5 recipients. This was correlated with a CD8 T cell infiltrate for all 8-18C5 treated mice and a significant increase in the magnitude of the CD4 T cell response for the G0 variant relative to the G0F 8-18C5. This study provides novel insight into the functional impact of Fc-glycosylation in antibody-mediated demyelinating diseases. Our results demonstrate the increased pathogenicity of hypofucosylated MOG antibodies which is of particular interest for the emerging demyelinating inflammatory disorders associated with MOG-IgG1 (MOGAD).

Keywords: Adaptive immunity, antibody, autoimmunity, autoinflammation, inflammatory disease, neuroimmunology

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POSTER PRESENTATIONS

P-0196

Immunoregulatory role of the IL-33/amphiregulin axis in acute and chronic liver disease

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In autoimmune liver diseases, IL-33 is released from necrotic hepatocytes and binds to the ST2 receptor thereby activating group 2 innate lymphoid cells (ILC2) and regulatory T cells (Tregs). Since activated ILC2 and Tregs express amphiregulin (AREG), which has been associated with tissue repair, immunosuppression but also fibrogenesis, we aimed at investigating AREG in the regulation of acute and chronic liver inflammation. C57BL/6 mice received ConA to induce acute immune-mediated hepatitis. The immuno-suppressive function of IL-33 was studied in mice that received IL-33 for three days before hepatitis induction. *Mdr2-/-* mice were used that develop chronic liver injury resembling PSC within 12 weeks. Liver injury in acute and chronic hepatitis was associated with elevated expression of hepatic *Il33* and *Areg*. *Areg* was the most up-regulated EGFR ligand in acute and chronic liver disease. While in acute hepatitis, ILC2 and ST2+ Tregs showed increased expression of AREG, chronic liver injury resulted in a reduced capacity of Tregs to express AREG. IL-33 pre-treatment suppressed acute hepatitis, which was associated with strong AREG expression by ILC2 and ST2+ Tregs. Interestingly, *Areg-/-* mice developed more severe acute hepatitis, which was associated with stronger activation of ILC2 and a reduced frequency of hepatic ST2+ Tregs. The immunoregulatory function of IL-33 in acute hepatitis might be driven by expansion and activation of ILC2 and ST2+ Tregs expressing AREG. In chronic liver disease, Tregs might lose their immunosuppressive function due to reduced AREG expression crucial for maintaining Treg function particularly under inflammatory condition.

Keywords: Autoimmunity, chronic inflammation and fibrosis, cytokines and mediators, innate lymphoid cells, regulatory cells, tissue damage and repair

P-0208

Regulatory potential of local Ly49$^+$CD8$^+$ memory T cell abundance in chronic kidney disease

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Upon inflammation, adaptive immune information is stored in specific cells to trigger future memory responses. Excessive or insufficient memory retrieval can have detrimental consequences. However, an abundance of cells promoting protective immunity could be harnessed for innovative therapeutic options. Our study focuses on the regulatory potential of Ly49$^+$CD8$^+$ memory T cells in chronic kidney disease. In male C57Bl/6J mice, low dose IL-15 treatment locally increased renal CD8$^+$CD44$^+$CD122$^+$Ly49$^+$ T cells in a setting of inflammation. Upon induction of nephrotoxic serum nephritis, a mouse model of chronic kidney disease, mice treated with IL-15 show higher expression of Ly49 on renal CD8$^+$ memory T cells and increased cell numbers in lymph nodes. No changes in numbers of tissue-resident CD8$^+$ T cells, iNKT cells, and infiltrating CD4$^+$ T cells were observed. Most importantly, the treatment group showed better disease outcomes reflected in lower albuminuria, lower glomerulosclerosis scores, and less renal myeloid cell infiltration. Beneficial effects of locally heightened Ly49$^+$CD8$^+$ memory T cell numbers in renal tissue were also confirmed in prolonged survival of mice pre-treated with IL-15 in an ischemia reperfusion-induced model of acute kidney injury. Highlighting the regulatory potential of Ly49$^+$CD8$^+$ memory T cells, we show that increasing the quantity of these cells mediates effective tissue protection in models of chronic kidney disease and injury. However, since IL-15 is *trans*-presented, understanding the contributions of cytokine presenting cell sources will be critical to explore the therapeutic potential of manipulating CD8$^+$ memory T cells.

Keywords: Autoimmunity, chronic inflammation and fibrosis, immune regulation and therapy, memory, regulatory cells, tissue damage and repair

P-0289

Immune suppressive pathways in wound healing - A role for CD200R and PD-1 in lymphocyte responses to wounding?

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Cutaneous wound healing requires a procedural transition from acute inflammation to a proliferative phase where keratinocytes proliferate and migrate to close the wound. However, in older individuals and those with diabetes, wounds often fail to progress and are arrested in chronic inflammation. This deranges healing and impairs wound closure resulting in chronic wounds. We propose that in chronic wounds, pathways which restrain immune responses are dysregulated. Over-activation of such pathways could suppress a productive inflammatory phase in healing, but conversely under-activation could prevent the resolution of inflammation. To test this hypothesis, we investigated the immune-suppressive receptor CD200R, and its ligand, CD200, which is expressed by epithelial stem cells involved in re-epithelialisation. We also investigated PD-1 since there is evidence for PD-L1 provision enhancing healing. Interestingly, in spite of significant upregulation of CD200R and PD-1 particularly by ILC2 as well as γδ and αβ T cells, modulating these pathways in young adults did not greatly alter normal skin healing. CD200R deficiency in young adults only subtly affected repair, enhancing wound lymphocyte numbers and reducing IL-17 production, which coincided with a slight enhancement in epithelialisation. However, in older CD200R deficient animals delayed healing was observed, which could suggest greater importance of CD200R in a 'stressed' system. Additionally, CD200R deficiency reduced PD-1 expression suggesting possible co-operativity between these pathways in wound inflammation which warrants further investigation.

Keywords: Gamma-delta T cells, inflammatory disease, innate lymphoid cells, skin diseases, tissue damage and repair

P-0294

An adenoviral vector vaccine against SARS-CoV2 elicits trained immunity in humans

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Trained immunity is a functional reprogramming of innate cells that bestows them with a form of memory; allowing a heightened response subsequent, unrelated insults. Adenoviral vectors have been shown to induce trained immunity in mice. Therefore, this study aimed to assess the capacity of the AstraZeneca COVID-19 vaccine to induce trained immunity *in vivo* in human monocytes. Monocytes were isolated from the blood of healthy volunteers before receiving the vaccine and at day 3, 14, 56 and 90 post vaccination. Using multiparameter flow cytometry, the expression of cell surface markers associated with trained immunity in monocytes was determined. Metabolic reprogramming in monocytes was analysed by examining changes in the gene expression of key metabolic enzymes over time. Monocytes were restimulated *in vitro* with unrelated stimuli such as LPS, Pam3Csk4 and *Mycobacterium tuberculosis*. Cytokine production in response to stimulation pre- and post- vaccination was determined by multiplex ELISA. Monocytes show marked features of trained immunity at day 14, 56 and 90 post vaccination compared with the pre-vaccination control. *Ex vivo* monocytes exhibit enhanced expression of HLA-DR, CD40 and CD80/86 up to 3 months post vaccination. Moreover, monocytes display signs of metabolic reprogramming and, when restimulated *in vitro*, have altered cytokine outputs, consistent with the induction of trained immunity. Vaccine strategies that combine the induction of trained immunity with the generation of traditional adaptive immune memory are postulated to have enhanced efficacy and may promote non-specific protection against a range of pathogens.

Keywords: Adjuvants and vaccines, infectious disease, innate immunity

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POSTER PRESENTATIONS

P-0299

Nasal *Staphylococcus aureus* colonization in mice induces pathogen-specific T cell response in cervical lymph nodes

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Staphylococcus aureus is an opportunistic pathogen that colonizes the anterior nares of approximately 30% of the human population. Although asymptomatic, colonization with *S. aureus* is the major risk factor for a subsequent life-threatening endogenous infection. Our knowledge on the pathogen-specific T cell response in *S. aureus* colonization is still scarce. Insights into these processes will help to identify facets of protective immunity against this pathogen and to develop an effective *S. aureus* vaccine. Using a mouse-adapted *S. aureus* strain, JSNZ, we investigated the T cell response induced by *S. aureus* colonization. C57BL/6N mice were inoculated *i.n*. with 1 x 10^8 *S. aureus* JSNZ. Three, six, and ten days after colonization, the bacterial load was quantified in the nares, cecum and feces. Cells from cervical lymph nodes (CLN) were labeled with CFSE and cultured for 4 days in the presence of *S. aureus* antigens. T cell proliferation and expression of transcription factors were measured by flow cytometry. Cell-free supernatants were analyzed for cytokine release by multiplex assay. JSNZ persistently colonized the nasopharynx and gastrointestinal tract of mice. Compared to naïve mice, $CD4^+$ T cells from mice colonized for 6 days showed high proliferation and increased proportion of $ROR\gamma t^+$ and T-bet^+ T cells when re-stimulated with *S. aureus* antigens *in vitro*. CLN cells produced pronounced levels of IL-17a, IL-17f, IL-22, TNF-α, and IL-10 at all tested time points after colonization. Persistent *S. aureus* colonization in mice leads to the formation of *S. aureus*-specific Th1/Th17 cells in the CLN 3-10 days after colonization.

Keywords: Adaptive immunity, animal models, cytokines and mediators, immune response tracing, infectious disease, memory

P-0300

The potential of immunoregulatory properties of mesenchymal stem cells in the treatment of retinal degenerative diseases

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Retinal degenerative diseases, such as age-related macular degeneration, diabetic retinopathy, retinitis pigmentosa or glaucoma, represent a group of diseases which cause a progressive damage of retinal cells, increased angiogenesis, scar formation and the development of local inflammatory reactions. There is currently no effective treatment for these pathological conditions. Promising therapeutic opportunities for patients could be the use of mesenchymal stem cells (MSCs). MSCs isolated from bone marrow constitutively produce TGF-β and in the presence of proinflammatory cytokines increase the expression of genes for immunomodulatory and neuroprotective factors. We have shown in an experimental mouse model that intraperitoneal administration of NaIO3 causes a rapid retinal degeneration. Using flow cytometry, we showed that one week after NaIO3 application the number of rhodopsin-positive cells in the retina is significantly decreased. In contrast, there was increased a local infiltration with F4/80 positive cells (macrophages) and increased expression of IL-1α and Iba-1 (a marker for activated microglia) at the gene level. *Ex vivo* cocultivation of damaged mouse retinal explants with bone marrow-derived MSCs significantly decreased expression of genes for IL-1α and Iba-1 in retinal cells. In addition, damaged retinas cultivated in the presence of MSCs showed higher expression of the gene for rhodopsin compared to the untreated control. These results indicate that the immunomodulatory and neuroprotective effects of MSCs could contribute to the treatment of retinal degenerative diseases.

Keywords: Immune regulation and therapy, stem cells, tissue damage and repair

P-0324

Driver mutations and single copy number abnormalities identify Binet stage A patients with chronic lymphocytic leukemia with aggressive progression

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Herein, we aim to study the impact of driver mutations and single copy number abnormalities (SCNAs) on progression of asymptomatic patients with chronic lymphocytic leukemia (CLL). The correlation between progression and the genetic characteristics of Binet stage A patients with CLL detected by whole exome sequencing was analyzed in 55 patients with a median follow-up of 102 months. During the follow-up, 24 patients (43%) progressed. Univariate Cox analysis showed that the presence of driver mutations, the accumulation of two or more mutations, the presence of adverse mutations, *IGHV* mutation status and unfavorable SCNAs were associated with a higher risk of progression. Nevertheless, only the occurrence of adverse mutations retained statistical significance in the multivariate analysis. All patients carrying an unfavorable mutation progressed with a median progression-free survival (PFS) of 29 months. The accumulation of two or more mutations also increased the risk of progression. The median PFS of patients with unfavorable SCNAs was 38 months. Combining mutations and SCNAs, patients may be stratified into three groups with different prognostic outcomes: adverse (17% probability of five-year PFS), protective (86% probability of five-year PFS) and neither (62% probability of five-year PFS, $p < 0.001$). The analysis of the mutational status of patients with CLL at an early stage of the disease provide a novel approach for the identification of patients with a high risk of progression. Nonetheless, the feasibility of an early therapeutic intervention in these particular patients requires further investigation.

Keywords: B lymphocytes, biomarkers, cancer immunology

P-0337

Enzymatic electrochemical biosensors of glucose: profibrotic and inflammatory effects of the glucose oxidase enzyme on host cells

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Nowadays, continuous blood glucose monitoring is becoming essential for management of diabetes, but the accurate long-term *in vivo* measurement of glucose with implanted biosensors still remains a challenge due to foreign body reaction (FBR). Electrochemical biosensors using glucose oxidase (GOx) as glucose sensing enzyme have gained importance due to their high glucose sensitivity. We hypothesized that GOx activity might also act on the physiology of cells of the microenvironment, such as modifications of macrophage polarization and fibroblast to myofibroblast differentiation. Indeed, GOx activity for glucose concentration measurement is associated with glucose consumption and release of hydrogen peroxide as a by-product. In the present study GOx was incubated 1) with human fibroblasts whose differentiation into myofibroblasts was analysed (a-SMA and collagen expression), and 2) with human macrophages whose polarization was evaluated. We showed that fibroblast viability was not affected by a GOx concentration lower than 8 mU/mL. In the next step, we are checking the effect of GOx on macrophages to assess H2O2 mediated macrophage polarization and pro-inflammatory changes via activation of the NLRP3 inflammasome. In conclusion, the aim of this work is to decipher the inflammation and fibrosis reaction due to GOx in order to validate its suitability for biosensor applications. This project has received funding from the state-operated "Agence Nationale de la Recherche" (ANR-16-CE19-0001-01) and from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement No 813006.

Keywords: Chronic inflammation and fibrosis, diabetes, macrophage, tissue damage and repair

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POSTER PRESENTATIONS

P-0338

Combined inhibition of IL-6 and TNF as a therapeutic option in acute allergic asthma

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Anti-cytokine therapy aimed at blocking pathogenic cytokines is promising in the treatment of disorders associated with chronic inflammation. TNF and IL-6 are the key mediators of inflammation that play an important role in the pathogenesis of asthma. Notwithstanding, studies using anti-IL-6 or anti-TNF therapeutic antibodies failed to provide positive outcomes for patients with poorly controlled asthma and the efficacy of combined inhibition of TNF and IL-6 in asthma were not studied. The aim of the study was to investigate whether the simultaneous administration of anti-TNF/IL-6 antibodies will be beneficial in the context of severe asthma. To address the efficacy of combined therapy we induced acute asthma in BALB/c mice by daily intranasal administration of 20 μg HDM extract for a week with sensitization of 5 μg HDM one week prior to the main course. Anti-IL-6 antibodies (MP5-20F3) and anti-TNF inhibitor (Etanercept) were administered intraperitoneally prior to each immunization. We found that combined anti-TNF/IL-6 inhibition decreased granulocyte infiltrate, as well as the production of IgE in BALF as compared to inhibition of these cytokines individually. In addition, the simultaneous administration of the inhibitors led to diminished expression of IL-17A and to reduction of Th17 cell recruitment in the lungs induced by anti-TNF monotherapy. Taken together, the data suggest that combined pharmacological inhibition of TNF и IL-6 in mixed granulocytic asthma may be more beneficial compared to inhibition of these cytokines individually.

The work was supported by the Russian Science Foundation, grant No. 19-75-30032.

Keywords: Adaptive immunity, allergen-induced immune responses, cytokines and mediators, granulocytes, immune regulation and therapy

P-0349

Leishmania major antigens combined with α-GalCer as promising vaccine candidates against cutaneous Leishmaniasis

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Leishmaniasis is is a group of diseases caused by protozoan parasites of Leishmania species that are transmitted by infected sand flies during blood-feeding. The disease can manifest as cutaneous, mucocutaneous or visceral Leishmaniasis. The lack of protective vaccines, treatment with pentavalent antimonials toxic to humans and the rise of drug resistance in Leishmania species has prompted us to explore preventative vaccine candidates against cutaneous Leishmaniasis. Parasite-derived exosomes, soluble leishmania antigens and lyophilized leishmania parasites were prepared from L. major as distinct antigen sources. These antigen preparations were combined with α-galactosylceramide (α-GalCer) as an adjuvant for subcutaneous immunization of Balb/c mice which were then challenged with transgenic EGFP/luciferase-expressing L. major using a footpad injection model. The development of cutaneous lesions was monitored by using a digital caliper and parasites loads in footpads were determined using an *in vivo* imaging systems (IVIS) based on luminescence readings for quantification. Leishmania antigen-specific IgG titers were quantified from sera by ELISA, and Th1, Th2, Th17 and Th10 responses of antigen-pulsed splenocytes were measured using cytometric bead array. Although tested vaccine candidates were not completely protective, the antigens tested in combination with α-GalCer had significantly reduced lesion size and reduced the parasite load in infected footpads. These results demonstrate that α-GalCer is a viable vaccine adjuvant choice for prevention of cutaneous Leishmaniasis.

Keywords: Adaptive immunity, adjuvants and vaccines, animal models, infectious disease, NKT cells, parasite infections

P-0354

Inflammasome induced caspase interplay in determine Sertoli cell fate with implications for men fertility

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Sertoli cells maintain immune privileged blood-testis barrier in testis, which is essential for male fertility. Recently, our research group have shown a functional Nlrp3 inflammasome, able to activate caspase-1 in Sertoli cells inflicting cell death. We aimed to investigate the signaling involved in Sertoli cell death incurred upon LPS and ATP danger stimuli challenge. We used inflammasome genes silencing, qPCR, ELISA and flow cytometry to evaluate the activation of caspase-1, caspase-3 and gasdermin D levels upon challenge. We found several subpopulations differentially responding to LPS and ATP challenge, based on caspase-1 and caspase-3 fluorescent reporters. Silencing Nlrp3, and Asc resulted in decreased caspase-1 and caspase-3 activity in some of high caspase-1 activity populations, but it has different effect in low caspase-1 activity population, suggesting only caspase-1 to be Nlrp3 dependent. The challenge surprisingly induced GSDMD transcripts and protein, in Nlrp3 and Asc dependent way, although this cell type did not undergo pyroptosis. Silencing GSDMD on its turn decreased caspase-1, but activated caspase-3, suggesting for GSDMD - caspases regulatory interactions, besides its effector role. The study suggests partial induction of caspase-3 by caspase-1 in Nlrp3/Asc dependent way, but also an independent caspase-3 activation and reciprocal gasdermin D regulation that have to be further investigated. Potential additional players are other caspases that could be directly induced by Asc or via alternative LPS mediated mechanisms independently of the Nlrp3.

This work was supported by Ministry of Education grant "INFRAACT, agr. DO1-275/16.12.2019 and agr. DO1-399/18.10.2020

Keywords: Cell death, immune networks, innate immunity

P-0397

Familial mediterranean fever attack during COVID-19 infection and after inactivated COVID-19 vaccination; a case report

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Coronavirus disease 2019 (COVID-19), the novel coronavirus pneumonia, was caused by Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 causes of severe pneumonia in human. The general symptoms observed in the infected individuals are fever, cough, dyspnoea and lesion in the lungs. In the advanced stage, the symptoms of this virus show pneumonia which progresses to severe pneumonia and acute respiratory distress syndrome. Familial Mediterranean Fever (FMF) and COVID-19 show a remarkable overlap of clinical symptoms and similar laboratory findings. Both are characterized by fever, abdominal/chest pain, elevation of C-reactive protein, and leukocytosis. Our case presents acute attacks of FMF during COVID-19 infection and after COVID-19 vaccination. This case is important because it is the first case of FMF attack after vaccination.

Keywords: Adjuvants and vaccines, inflammatory disease, viral infections

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POSTER PRESENTATIONS

P-0398

Terminal ileum tissue resident memory T cell vaccine-induced responsiveness is affected by the aging process in humans

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The immune system is affected during aging, a process known as immunosenescence. Human studies on immunosenescence have almost exclusively examined the systemic compartment. However, tissues contain most immune cells which exhibit differential cell characteristics and frequencies (e.g., memory T cells -TM). At the site of infection, tissue resident memory T cells (TRM) provide rapid effector immune responses. Very limited information is available in humans regarding TRM responses at the site of infection (e.g., terminal ileum -TI) upon exposure to pathogens during immunosenescence. Here, we investigated the effect of aging on TI S. Typhi-responsive TRM subsets using a human oral immunization model with the live attenuated typhoid vaccine Ty21a using flow cytometry. We found that aging influenced TI-lamina propria mononuclear cells (LPMC) TM and TRM cell numbers in both Ty21a-vaccinated and control groups. For example, LPMC CD103- CD4+ TRM frequencies displayed a positive correlation with age whilst LPMC CD4/CD8 ratio displayed a negative correlation with age in unvaccinated volunteers. Importantly, we observed that elderly volunteers have decreased S. Typhi-specific mucosal immune responses compared to adults. For example, CD103+ CD4+ TRM have reduced IL-17A production in the elderly compared to adults following Ty21a immunization. Likewise, LPMC CD8+ TRM and CD103- CD8+ T cell subsets displayed similar results. Further, comparisons of the multifunctional (MF) profiles of CD4+ and CD8+ TRM subsets between elderly and young adults revealed significant differences in elicited single (S) and MF responses. In conclusion, this study shows that aging impact the generation of TRM vaccine-induced responses in tissues.

Keywords: Adaptive immunity, adjuvants and vaccines, ageing, bacterial infections, immune senescence, infectious disease

P-0401

Characterization of the colitogenetic candidate gene Alpk1 during DSS induced colitis

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Inflammatory bowel disease (IBD) is a disorder that is dependent on host genetics, intestinal microbiota composition, and environmental factors. Knockout mice for IL-10 are a useful model of chronic colitis. Colitis severity in Il-10-deficient mice differs depending on the background strain with C3H/HeJBir-Il10tm1Cgn (C3Bir-Il10-/-) mice being more susceptible to colitis than C57BL/6J-Il10tm1Cgn (B6-Il10-/-) ones. We reported 10 quantitative trait loci (QTL) associated to colitis that were named Cytokine deficiency induced colitis susceptibility (Cdcs), each named from 1 to 10. The most remarkable locus is Cdcs1, located on chromosome 3, as several non –synonymous single nucleotide polymorphisms (SNPs) were found on candidate genes. One of them is alpha-kinase 1 (Alpk1), a protein for which different functions were described. We hypothesize that Alpk1 has a remarkable relevance in the orchestration of inflammation in the gut. Our *in vivo* studies include a Dextran sulfate sodium (DSS) model of acute colitis of specific-pathogen-free (SPF) B6N-Alpk1em2Wtsi (Alpk1-/-) and WT mice to determine gene expression of inflammatory cytokines, flow cytometry, body weight, and histopathological score. We firstly determined that 2% DSS is the needed concentration that made Alpk1-/- mice lose between 15-20% of body weight. We detected no differences in weight loss of Alpk1-/- and wild type mice. The histopathological score in DSS Alpk1-/- mice was slightly reduced than its parental wild-type strain. Flow cytometry and gene expression analysis determined no differences of pro-inflammatory cytokines in the mutant strain. These results show that ALPK1 is not involved in the process of acute chemically induced colitis.

Keywords: Adaptive immunity, autoimmunity, autoinflammation, biology of the immune system, chronic inflammation and fibrosis, immune networks

P-0402

Cancers related to primary immunodeficiency diseases in Turkish children: a single center experience from south Turkey

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Primary immunodeficiencies (PIDs) are genetic disorders that predispose to frequent and severe infections, autoimmunity, allergy and cancer. It is hypothesized that subjects with PIDD would have an increase incidence of cancer due to impaired immune function. The impact of PIDs-associated malignancy has increased significantly in recent years as the patients survival rates are improved due to significant advances in therapeutic strategies. Herein, medical records of 585 PID patients were analyzed retrospectively and 21 malignancies in patients (13 boys, 8 girls) with PIDs are presented dating between 1998 and 2018. 585 patients were diagnosed as having PIDs, and there were 21 cancer cases associated with PIDs. Lymphoid malignancies were observed in 10 of patients while 6 children with leukemia and 5 children with solid tumors were observed in our study.Solid cancers are observed as squamous cell carcinoma,neuroblastoma and malignant histiocytosis. Within our registry data, the PIDs more often associated with cancer were ataxia-telangiectasia (AT), common variable disease (CVID) and severe combined immunodeficiency. The remaining PIDs in 3 patients with cancer cases were observed as cartilage hair hypoplasia, DOCK8 deficiency and familial hemophagocytic lymphohistiocytosis. The children with PIDs, who developed cancer, had a very poor prognosis and mortality rate was 57.1% (12/21) in our study. The overall risk for children with PIDs of developing cancer in our study was 3.5%. Clinicians must be vigilant about this association, as the increased malignancy risk needs attention among physicians and patients for malignancy-associated signs in PID.

Keywords: Lymphoid organs, cancer immunology, immunodeficiency

P-0404

Does the HLA-C*06 Status (+/-), influence the response of Moroccan psoriatic patients to methotrexate: preliminary study

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Introduction Psoriasis is a chronic, systemic and multifactorial inflammatory skin disease. The association between HLA alleles and Psoriasis, especially HLA-C*0602 was reported. Methotrexate (MTX) is the first-choice therapy due to its high efficacy and affordable cost. Aims We aim to analyze the association between HLA-C polymorphism and the genetic predisposition to Psoriasis and evaluate HLA-C's impact on the response to MTX. Methods Thirty-six Moroccan Psoriatic patients who received MTX as a treatment and 77 healthy controls (HC) were genotyped for the HLA-C locus using the PCR-SSO-Luminex typing method. Patients were divided into two subgroups according to the age of the onset: G1: 12 patients ≤ 30 years old (33,33%) and G2: 24 patients > 30 years old (66,66%). They were clustered into two subgroups: responders and non-responders to MTX. Results Analysis of HLA-C allele frequencies distribution revealed an increase of three alleles in patients compared HC although not statistically significant: C*02(19,44%) C*06(50%) C*07(47,22) vs C*02(9,09%) C*06(41,56%) C*07(35,06%), respectively. Contrastingly, we identified a significant increase of HLA-C*07 allele frequency in G2 than G1 groups (84,62% vs 26,09%, p = 0.005, Pc = 0.03, OR = 4, 89, 95% CI:1.35-15.57). Similarly, only HLA-C*06 allele frequency was found significantly higher in responders than in non-responders, (p=0.028, OR=7,8, 95% CI: 0.96-63.59). Conclusions This is the first report of the possible involvement of the HLA-C (HLA-C*06 and HLA-C*07) gene polymorphism with Psoriasis susceptibility in Moroccan patients, which seems significantly related to the age of psoriasis onset, and a better response to MTX.

Keywords: Antigen processing and presentation, autoinflammation, inflammatory disease, MHC and polymorphic genes, skin diseases

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POSTER PRESENTATIONS

P-0405

Type of diet modifies the impact of hypotensive drugs on mouse contact hypersensitivity

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High sodium intake and dysregulation of inflammatory responses are associated with hypertension. Thus, one can speculate that hypotensive drugs may exert some of their therapeutic effects due to immunomodulation. However, little is known about the possible impact of hypotensives on mechanisms of immune responses. Therefore, our studies examined their influence on contact hypersensitivity (CHS) reaction in mice fed with standard or high-salt diet since weaning. Actively sensitized mice, macrophage donors, and effector cell recipients were intraperitoneally administered with either amlodipine, captopril, carvedilol, olmesartan, propranolol or verapamil for 8 days. Mice were sensitized by topical application of picryl chloride, and five days later were either challenged to elicit CHS ear swelling or sacrificed to collect effector cells that have been then transferred to drug-treated recipients. Oil-induced peritoneal macrophages were labelled with trinitrophenyl (TNP) hapten and then transferred to naive recipients. Captopril and carvedilol administration suppressed active CHS reaction in mice fed with high-salt diet, while amlodipine treatment induced the same effect regardless of the type of diet. In contrast, administration of all drugs increased CHS ear swelling in effector cell recipients on high-salt diet. Similarly, macrophages from donors fed with sodium-enriched fodder and treated with both beta-blockers induced stronger CHS reaction, while verapamil treatment exerted the opposite effect. Our results suggest that hypotensives influence not only the hypertension-related inflammation but also other immune reactions, including contact allergies, in medicated patients.

This study was supported by Polish Ministry of Science and Higher Education (grant No N41/DBS/000419).

Keywords: Allergen-induced immune responses, allergic disorders, cardiovascular diseases, immune regulation and therapy, modification allergic responses

P-0406

Predicting drug resistance and virulence of pathogenic transmembrane proteins in methicillin-resistant *Staphylococcus Aureus* with machine learning

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The mecA gene encodes the transmembrane protein penicillin binding protein 2a (PBP2a), which allows for antibiotic resistance to methicillin and penicillin-like antibiotics in methicillin-resistant Staphylococcus aureus (MRSA). 200 MRSA strains, including both hospital- and community-acquired MRSA, were isolated from patients with bacteremia. Whole-genome sequencing was performed to determine mecA gene sequence of each MRSA isolate. DNA sequence of each isolate was translated to derive PBP2a amino acid sequence. The amino acid sequences were subjected to K-means clustering. With the embedding layer of a model pre-trained with unbiased protein datasets, amino acid sequence of the mecA gene was automatically classified for all MRSA isolates into three groups. ANOVA and F-test were then used to validate the performance of the unsupervised clustering, through evaluating the differences in: (1) Source of infection, (2) vancomycin resistance, and (3) staphylococcal cassette chromosome mec (SCCmec) type, between the automatically classified groups. The density score of the protein classification model for vancomycin resistance was 0.89, with a specificity of over 0.9 and total accuracy of 0.65. Infection source was not significantly different among clustering groups ($P > 0.05$), suggesting that the model was not biased. Vancomycin resistance ($P = 0.01$) and SCCmec typing ($P < 0.001$) were significantly different among clustering groups, suggesting that classification of the K-means protein clustering model could be mapped to clinical outcomes including bacterial virulence of PBP2a encoded by the mecA gene in MRSA. K-means clustering automatically classified three groups of pathogenic MRSA isolates with a high specificity.

Keywords: Antibody, bacterial infections, infectious disease

P-0408

Utility of non-invasive biomarkers in diagnosis and monitoring of Celiac Disease in children

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Celiac disease (CD) is a chronic gluten-dependent autoimmune disorder that leads to small-bowel damage in genetically predisposed individuals. Mucosal villus atrophy in duodenal biopsies is the gold standard for CD diagnosis establishment. Aim of this study was to assess the utility of non-invasive biomarkers in CD diagnosis and monitoring. This study included 25 children with a broad spectrum of clinical symptoms finally diagnosed with CD. HLA-DQB1* molecular typing, was performed. In addition, anti-tissue transglutaminase (tTG) by ELISA and anti-endomysial (EmA) autoantibodies (Aabs) by indirect immunofluorescence were identified, at diagnosis and during a gluten-free diet 3 years monitoring. The most common manifestations in our patients (pts) were anemia (48%), poor weight gain (48%) and poor height gain (40%). All pts were positive in Aabs and carried a high risk HLA allele, specifically -DQB1*02 (DQ2) 72%, -DQB1*03:02 (DQ8) 8%, -DQ2/DQ8 20%. In 6 cases (24%) with low Aabs titre the diagnosis was confirmed by histological findings. In 19 cases (76%) the diagnosis was based in the typical clinical manifestations and the presence of genetic (HLA-DQ2 and/or DQ8) and serological (anti-tTGx10 normal, anti-EmA>1:320) markers, according to ESPGHAN criteria. During 3 years follow up, normalization of Aabs and clinical improvement were achieved in 24% of pts at the first, in 12% at the second and in 16% at the third year. In pediatric cases when the clinical suspicion of CD is strong, non-invasive biomarkers can be used as an alternative safe tool, instead of biopsy, for diagnosis and monitoring.

Keywords: Autoimmunity, biomarkers, MHC and polymorphic genes

P-0410

Analysis of γδ T cells in young patients with infectious mononucleosis (IM)

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Primary infection with Epstein-Barr virus can cause infectious mononucleosis (IM) which can lead to long-term complications. Previous studies have shown that γδ T cells can interact with EBV-infected cells, but the role of γδ T cells in IM remains unclear. Here we analysed the status of γδ T cells in IM and its aftermath. We acquired samples from 19 IM patients (median age 11 years, range 3–23) from the Munich Infectious Mononucleosis Study (IMMUC) at the day of the first visit and at about 1 and 6 months after onset of symptoms. Cryopreserved PBMCs were stained with one of two multicolour FACS panels, one including CD38/HLA-DR/CD16/CX3CR1, and the other CD45RA/CCR7/CD27/CD28. In the acute phase of the disease, a median of 40% of δ2 T cells and 32% of δ1 T cells were activated (CD38+HLA-DR+), compared to 82% of CD8+ T cells and 16% of CD4+ T cells. Effectors (CD45RA–CCR7–) and CD45RA–CD27+ cells were increased among δ1 and CD8 T cells, compared to healthy controls (median age 31 years) from the same geographic region. Over the course of six months after diagnosis, the proportion of both δ1 and δ2 T cells increased together with CD4+ T cells, while the proportion of CD8+ T cells decreased. After 6 months, when most patients were symptom-free, δ1 and δ2 T cells remained increased (each $p<0.01$) compared to controls. Our results suggest involvement of δ1 and δ2 T cells in IM, but the mechanisms remain to be clarified.

Keywords: Gamma-delta T cells, monitoring immunity, infectious disease, viral infections

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POSTER PRESENTATIONS

P-0412

IL-6 promotes colorectal tumor progression via modulation of gut microbiota

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IL-6 is known to promote gut carcinogenesis and to support epithelial barrier function during colitis. The gut microbiome is a crucial factor for shaping immune responses. Accumulating evidence suggests that IL-6 may regulate intestinal homeostasis via modulation of gut microbiota. To test this hypothesis, we studied susceptibility of IL-6 knockout (IL-6 KO) mice to tumor development in the model of AOM/DSS-induced colorectal cancer under separate housing and cohousing conditions. We found that housing conditions do not affect intestinal inflammation in the acute DSS-induced colitis model. However, in AOM/DSS-induced colorectal cancer model separately housed IL-6 KO mice showed a reduced number of tumors as compared to wild type controls, whereas no difference in tumor development was observed between cohoused IL-6-deficient and wild type mice. Similarly, IL-6 KO mice suffered from increased body weight loss during chronic intestinal inflammation induced by DSS under separate housing, but not cohousing conditions. Interestingly, the abundance of *Enterobacteriaceae* family was significantly higher in the feces of naïve IL-6-deficient mice cohoused with wild type mice, which did not occur during separate housing. Moreover, aged IL-6 KO mice demonstrated an increased level of commensal segmented filamentous bacteria in the ileum as compared to cohoused wild type controls. These results indicate that IL-6 plays an essential role in maintaining gut microbiota composition, and its disturbance may result in the altered susceptibility to intestinal carcinogenesis. Altogether, our data suggest that IL-6 can promote intestinal tumor progression by modulating gut microbiota.
This work was supported by RSF grant № 19-75-30032.

Keywords: Cytokines and mediators, animal models, cancer immunology, microbiome and environmental factors

P-0413

Bank1 signaling regulates the gut IgA response, microbiome, and inflammation in lupus

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The gene BANK1 is a susceptibility gene for SLE. The lupus-prone B6.Sle1.yaa mice Bank1 KO showed an improvement in the development of the disease, reduced IgG production and anti-dsDNA antibody production, type I interferon signaling, and improved kidney disease. Furthermore, BANK1 is implicated in the TLR signaling as BANK1 binds MyD88 and TRAF6 through its TIR domain and TRAF6-binding motifs, respectively. We thus hypothesized that an altered B cell response in the gut may be modulating the microbiome composition that may impact the lupus pathogenesis. We characterized by FACs the B cell populations in the gut and immunoglobulins in serum and fecal matter were quantified by ELISA. Microbiome composition was determined by sequencing the V4 region of 16sRNA. Gut permeability was measured with FITC-Dextran. In the TLR7tg lupus-prone mice, the absenceof Bank1 diminishes disease severity with a concomitant reduction in serum pathogenic IgG antibodies. Bank1 KO mice have reduced frequency of CD19+B220+ and IgA+B220- B cell populations in the gut. Fecal free IgA was also reduced. Bank1 KO mice have altered the baseline composition of their gut microbiome, and these changes were more pronounced after the development of lupus, causing increased gut permeability in the TLR7.Tg mice, but was reduced in TLR7Tg.Bank1 KO mice. Thus, having Bank1 a role in B cell function via Tlr7 signaling, our results link a susceptibility gene for lupus with the abnormalities in the microbiome composition of the gut known to occur in lupus and the production of IgA.

Keywords: Animal models, autoimmunity, B lymphocytes, microbiome and environmental factors

P-0415

MicroRNA-223 controls cardiovascular lesion formation in LCWE-induced Kawasaki disease vasculitis mouse model through regulation of Nlrp3 inflammasome

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Kawasaki disease(KD) is an acute systemic vasculitis and the primary cause of pediatric acquired heart disease. Although the etiology of KD are not yet fully understood, current knowledge revealed a key role of Interleukin-1 signaling thus clinical trials are being conducted to determine the efficacy of IL-1 blockade. Innate immunity signaling complexes known as inflammasomes are central producers of IL-1 family cytokines. Among these, Nlrp3 has been shown to be the main inflammasome complex involved in abdominal aortic aneurysm (AAA) formation in (LCWE)-induced KD vasculitis mouse model. Activation of this complex is tightly regulated by a group of short non-coding RNAs named as microRNAs. The elevated levels of microRNA-223, a negative regulator of Nlrp3, in serum, peripheral blood and coronary arteries of KD patients has been reported, however its specific role in disease progression remains undefined. Here, using the (LCWE)-induced KD vasculitis mouse model, we showed that the expression level of microRNA-223 was significantly increased in LCWE-induced cardiovascular lesions. Moreoever, microRNA-223(-/y) mice developed more severe coronary arteritis and aortitis, as well as more pronounced abdominal aorta aneurysms and dilations compared to WT controls. The severe phenotype was also accompanied by increased IL-1β levels demonstrating a direct role for microRNA-223 in IL-1 signaling. Overall, our data unveiled a previously unappreciated role for microRNA-223 in KD via Nlrp3 inflammasome regulation. Based on this data, it is clear that further studies are warranted to explore the use of microRNA-223 as a marker or as a therapeutic target in KD vasculitis.

Keywords: Animal models, cytokines and mediators, immune regulation and therapy, inflammatory disease, miRNA

P-0416

Inhibitory effect of Wharton Jelly derived mesenchymal stem cells on chronic myeloid cancer cell lines

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Mesenchymal stem cells (MSCs) have the capacity for self-renewal and pluripotency, making them a primary candidate for cell-based therapy. The purpose of this study was to evaluate inhibition effect of Wharton Jelly derived MSC (WJ-MSC) on the K562 cells as chronic myeloid leukemia (CML) cell lines. In this study, WJ-MSCs were isolated from an umbilical cord, and characterized by flow cytometry. WJ-MSCs (1×104/cm2) were obtained from Passage 1, and plated into 24-well plates in 1 ml DMEM complete culture medium solution. WJ-MSCs were incubated in a flask at 37˚C in a humidified air with 5% CO2. After 7 hours, K562 cells (1×105/ml) were added respectively K562: MSC; 1:2, 1:5, 1:10 ratio, low-glucose DMEM containing 10% FBS and 1% penicillin/streptomycin solution. The co-culture was incubated 72 hours at 37˚C in 5% CO2, and the co-cultured K562 cells were subsequently separated from adherent WJ-MSCs by slow and careful pipetting. The cell counts and viability were determined by trypan blue dye exclusion. The viable and non-viable cells were counted with a hemocytometer. The non-viable cell percentages were compared as 1:2 (42%), 1:5 (52,2%), 1:10 (33,3%) and control plates (%7,69) (untreated K562 cell line) after 72 hours incubation. The most effective dose was found 1:5 (52,2%). In conclusion, the results showed that WJ-MSCs can suppress K562 cell proliferation when using in proper ratio. This study will provide perspective on the next projects. In the future, WJ-MSCs may be an option for their clinic use for the inhibition of cancer cells.

Keywords: Cancer immunology, cellular interactions, stem cells

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POSTER PRESENTATIONS

P-0417

Inflammatory stratification in primary Sjögren's syndrome reveals novel tissue and immune cell alterations in patients' minor salivary glands

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There is a critical need to deconvolute the heterogeneity of minor salivary glands (MSG) from primary Sjögren's syndrome (pSS) patients. However, morphometric atrophy, adipose infiltration, and cell proportions inside the focal infiltrates (i.e., ≥50 mononuclear cells) of the MSG at distinct inflammatory phases have not been quantified in detail. To address this problem, we stratified 85 patients into early (S1), moderate (S2), and advanced (S3) stages using the Inflammatory severity index. Here, mild (<3%) and marked (≥3%) atrophy levels in the MSG parenchyma coincided with the respective levels of adipose infiltration in non-SS controls ($p<0.01$), but not in pSS patients. Adipose infiltration percentage correlated with the age of patients ($r=0.458$, $p<0.0001$). CD4+ T helper cells were reduced in the focal infiltrates in MSG of S2 patients compared to S1 ($p<0.01$), and in S2 compared to S1 and S3 combined ($p<0.05$). CD20+ B cells increased from S1 to S3 ($p<0.01$) and S2 to S3 ($p<0.01$), meanwhile CD138+ plasma cells diminished in S3 compared to both S1 and S2 groups combined ($p<0.01$). Positivity towards anti-Ro/SSA, anti-La/SSB, and rheumatoid factor increased over the course of inflammatory disease progression and were more common in the S3 group relative to S1 ($p<0.05$). Conversely, S2 measured a higher mean salivary flow relative to S1 and S3 patients combined ($p<0.05$). The proposed Inflammatory severity index stratification revealed pathological cell and tissue-associated aberrations in the MSG and their correlations to clinical outcomes, which could be directly transferred to the optimization of available diagnostic strategies applied for pSS patients.

Keywords: Antibody, autoimmunity, autoinflammation, inflammatory disease, tissue damage and repair

P-0418

Trained immunity increases lung antimicrobial activity of PMNs and protects from pneumococcal pneumonia

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Trained immunity characterizes innate immune memory in mammals. We reported that trained immunity confers broad-spectrum protection against bacterial infections (Ciarlo JID 2020). Yet, whether it protects from pneumococcal pneumoniae remains largely unknown. To test whether, and if so how trained immunity protects from lethal pneumococcal pneumonia. Control mice and mice trained with β-glucan were challenged i.n. with *S. pneumoniae*. Blood, lungs, spleen and bone-marrow were collected to quantify bacteria, hematopoietic stems cells, leukocytes, cytokines and lung injury, and to isolate PMNs used to measure metabolic activity, phagocytosis, and NETosis. The induction of trained immunity increased 2-4-fold PMNs and inflammatory monocytes in lungs, did not affect macrophages and innate lymphoid cells (ILCs), but increased the proportion of inflammatory ILC1. Trained mice survived pneumococcal infection and had reduced bacterial burden, lung injury and blood cytokines (P<10e-3-10e-4). The numbers of PMNs, monocytes, macrophages and ILCs remained stable in lungs of trained mice, while PMNs increased parallel to bacterial burden in lungs of control mice. Lung ILC2/ILC1 (i.e. tissue repair/inflammatory ILCs) ratio increased in trained mice but decreased in control mice. In response to *S. pneumoniae*, PMNs from trained mice showed increased metabolic activity, phagocytosis and NETosis. The accumulation of PMNs with enhanced antimicrobial activity, and the shift of ILCs toward ILC2 in the lungs suggest that the establishment of trained immunity promotes early antimicrobial defense mechanisms and later resolution/repair mechanisms associated with bacterial pneumonia.

Keywords: Bacterial infections, infectious disease, innate host defence, innate immunity, innate lymphoid cells, memory

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P-0419

The evaluation of aSTR mutations in disputed parenthood

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Autosomal Short Tandem Repeats (aSTRs) and Human Leukocyte Antigens (HLA) constitute genetic systems used in disputed paternity cases, but they both have limits due to mutation events and linkage disequilibrium respectively. The evaluation of HLA polymorphism contribution in deficient parentage cases previously analyzed by aSTRs. A total of 634 DNA samples (252 parentage testing cases, 34 of which were father-motherless ones) were investigated. Analysis was performed using 16 aSTRs typing by DNA sequencing, and HLA by PCR-SSP/SSOP. Power of Exclusion (PE), Random Man Not Excluded (RMNE), Combined Parentage Index (CPI), and Probability of Parentage (W) values were calculated using allele frequency of Caucasoid (aSTRs) and Greek (HLA) population databases. Fourteen aSTRs mutations [vWA(3), D12S391(3), SE33(3) and 1 from each D10S1248, D2S1338, D8S1179, FGA, D3S1358] and 1 null allele (SE33) were observed in 15(15/205, 7.32%) different parent/child allele transfers (1 of them was motherless case), without ruling out fatherhood. The ratio of paternal versus maternal mutations/null allele was statistically significant (14:1, p=0.001). Full HLA typing for paternity confirmation was performed. In these cases, the RMNE was ranged from 1.06x10^-9 to 1.76x10^-13, while PE was estimated over 0.9999999. The low CPI (6.938:1) and W (0.9988) as determined by aSTRs in one duo deficiency case was increased up to 9.44x10^5:1 and 0.9999989 respectively by both systems (practically proven parenthood). The combination of two systems (HLA-aSTRs) diminishes the possibility of false exclusion due to aSTRs mutations, and minimizes the risk of wrong inclusion due to the absence of mother's genotype.

Keywords: Biomarkers, immunological techniques, MHC and polymorphic genes

P-0420

Prevalence of anaphylaxis in emergency department in university clinical center of Kosovo

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Out of 15,131 persons presented at the ED in Prishtina in UCCK, for various emergency health problems, 74 allergic reactions were registered that required emergency medical assistance, gender ratio f / m = 53/21 ($p<0.001$), with a predominance of 15-30 years of age (39 of them). Dominant symptoms in our patients were skin changes in the form of urticarial changes (36 of them), erythematous changes (51of them) and angioedema manifested in 27 patients. Fortunately, during the research period 41 (55.4%) cases were with mild forms of generalized reaction, 23 (31.0%) moderate and only 10 (13.6%) cases with severe form, as after Brown's classification. Of these18 (24.3%) cases according to the criteria of the "Second symposium on the definition and management of anaphylaxis" were in anaphylaxis, this incidence would be 0.1% of all visits in the ED, even though no patient was diagnosed with anaphylaxis from the emergency doctors. The most common known causes were medications (44.6%). The most common causative drugs were the NSAIDs group with 48.3%, with ketoprofen lysine (brand name, OKI) leading the way with 25.8% of all cases of allergic reactions to the drugs. The second group of drugs were β-lactam antibiotics with 7 (22.6%) cases, led by cephalosporins and followed by penicillin. The second most common cause was food in 12.1% of cases.

Keywords: Allergen-induced immune responses, allergic disorders, skin diseases

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POSTER PRESENTATIONS

P-0422

Symptomatology and serology of COVID-19 patients in Kosovo

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In this paper we aimed to evidence general data and symptomatic characteristics of individuals tested for COVID-19 serology. We recruited 267 persons in our clinic between a one-week timeframe (14.07-20.07.2020). In order to diagnose SARS-CoV-2 seropositivity, we used 2019-nCoV IgG/IgM Rapid Test and to track the symptoms for these individuals, we used a survey containing basic questions about: chills, fever, muscle aches, sore throat, headaches, gastrointestinal discomfort (nausea, vomiting, diarrhea¬), shortness of breath, or lossof taste and smell. 41 (15.4) were seropositive for IgM and 43 (16.1%) for IgG. Only 19 (7.1%) were seropositive for both IgG and IgM.Respectively 84/267(31.46%) were positive to one of Ig or both of them. Results of chi square test showednostatistical significance differenceon frequency distributions among female and male seropositive and seronegative for IgM and IgG (p>0.05).63 out of 267 (23.59%) of the cases were seropositive with clinical symptoms, and only 4/267(1.50%) had symptoms while were seronegative. Most common symptoms among 67 seropositive patients were general inflammation symptoms:fever(90.8%), chills(80%), loss of smell and taste(64%), and muscle aches(80%).

Keywords: Infectious disease, innate immunity, viral infections

P-0425

Inflammasome activation drives inflammation in the skin disease hidradenitis suppurativa

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Hidradenitis suppurativa (HS) is a severe, chronic, inflammatory skin disease where current therapeutics are inadequate. We have previously implicated Th17 cells and therefore examined the role of the cells and factors that drive Th17 cells in HS skin. Flow cytometry and single cell RNA sequencing was performed on CD45+ cells within lesional skin from HS patients and healthy control skin. Skin explants were cultured in the presence or absence of the NLRP3 inflammasome inhibitor MCC950 and the release of inflammatory mediators was analysed. The data revealed a significant increase in frequency of T cells, B cells, neutrophils, and dendritic cells (DC), with striking differences in the transcriptomic profile of both innate and adaptive immune cells. The most upregulated genes in HS skin were IL-17A, S100A8/9 and those related to B cells/plasma cells, and here we focus on the upstream factors that might drive Th17 cells. The source of expression of S100A8/9 was dermal macrophages, with IL-23, IL-1β and inflammasome components localised to CD1c+ DC and Langerhans cells. In the presence of MCC950 we observed a significant reduction in the release of IL-1β, TNF, IFN-γ, IL-8 and CCL20 from HS skin explants. These data suggest that the NLRP3 inflammasome is an important driver of inflammation in HS and may represent a novel therapeutic target.

Keywords: Autoimmunity, autoinflammation, RNAseq, skin diseases

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P-0426

Single-cell transcriptomic profiling of hyperproliferative keratinocytes in hidradenitis suppurativa

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To elucidate the transcriptomic profile of hyperproliferative keratinocytes in the inflammatory skin disease hidradenitis suppurativa (HS) using single-cell RNA-sequencing. Single-cell RNA-sequencing was performed on sorted CD45- cells from 6 HS lesional and 3 healthy control skin samples. The data was interpreted using Seurat, Monocle3, enrichR and Nichenet packages. 23 distinct cellular populations were revealed, including keratinocytes, fibroblasts, melanocytes, endothelial cells, pericytes and smooth muscle cells. A keratinocyte population, termed hyperproliferative keratinocytes given its expression of multiple proliferation markers, was significantly enriched in HS lesions compared to healthy controls. These hyperproliferative keratinocytes were the source of a number of differentially expressed genes in HS, including the antimicrobial peptides S100A7, S100A8 and S100A9, and chemokines CXCL1 and CXCL8. Pseudotime trajectory analysis and ligand-receptor interaction analysis suggest that these hyperproliferative keratinocytes develop a unique transcriptome. Intriguingly, we have shown that this is likely driven by interactions with Th17 cells, which appear to play a major role in HS inflammation. These hyperproliferative keratinocytes also demonstrated a dysregulated metabolic profile with an increase in both glycolysis and oxidative phosphorylation pathways. These findings suggest that hyperproliferative keratinocytes play an important role in the pathogenesis of HS. To our knowledge, this is the first transcriptomic characterisation of hyperproliferative keratinocytes in HS, which may direct future studies.

This publication has emanated from research supported in part by a research grant from Science Foundation (SFI) under the SFI Strategic Partnership Programme Grant Number 15/SPP/3212 and research support from AbbVie Inc.

Keywords: Cytokines and mediators, immune communication, inflammatory disease, RNAseq, skin diseases

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POSTER PRESENTATIONS

P-0427

Antibodies to porphyromonas gingivalis associate with presence of specific autoantibodies and myocardial infarction in patients with periodontitis

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Rheumatoid arthritis (RA) is an autoimmune disease characterized by presence of anti-citrullinated protein antibodies (ACPA). ACPA precedes RA onset and it has been suggested that autoimmunity is triggered outside joints, at mucosal surfaces. Periodontitis (PD) is epidemiologically linked to RA, and the key pathogen driving PD, Porphyromonas gingivalis (Pg), has a unique capacity to citrullinate proteins. Hence, it was proposed that Pg is actively involved in triggering the ACPA response. We have previously shown elevated Pg antibody levels in ACPA+RA versus controls, and hypothesize that Pg antibodies could possibly be used as biomarkers for PD patients at increased risk of developing ACPA+RA. To determine if Pg antibodies could define a subset of PD, and if Pg antibodies associate with autoimmunity in general or the ACPA response in particular, we analyzed Pg antibodies in relation to different autoantibodies in a large PD cohort (ParoKRANK; n=1610, where 805/1610 have had a myocardial infarction (MI)), a small PD cohort (n=80), and an SLE cohort (N=200). Significantly increased Pg antibody levels were detected in PD versus controls, and associated with PD severity, MI and RA-related autoantibodies, but not SLE-related autoantibodies. When analyzing specific autoantibodies, we found an association with ACPA and dsDNA antibodies, but not with rheumatoid factor, anti-phospholipid, anti-Sm, anti-RNP, and/or anti-Ro/La antibodies. We conclude that Pg antibodies associate with specific autoimmunity and MI, and that Pg antibodies may be used as a biomarker for a subset of PD with severe disease. Future studies will determine whether they can predict ACPA+RA, and/or MI.

Keywords: Antibody, autoimmunity, bacterial infections, biomarkers, rheumatoid arthritis

P-0428

Functional properties of CD56+CD20 + natural killer cells and their relationship with demyelinating diseases

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Our objective in this project is to investigate the functional properties of CD56+CD20+ natural killer cells and their relationship with IDDs. PBMC isolation was performed from 30 patients with IDDs and 18 patients with NINDs. The presence of CD56+CD20+ cells was determined by flow cytometry. Groups were compared using Mann-Whitney U test. PBMCs were isolated from 12 healthy volunteers and CD56+ cells were separated by magnetic isolation. The functions of CD20+ and CD20- cells were performed via flow cytometry by examining the expressions of CD107a, Perforin, Granzyme B, NKP46, Trail, CD178/Fas Ligand and IFN-g. For this purpose, cells were inoculated in two sets in 96-well U bottom plates at 1x105 cells/100µl. The first set was stimulated with PMA/Ionomycin and the second set with IL-2 (10ng/ml) and IL-15 (10ng/ml). The percentage of CD56+CD20+ cells (7.2; 6.9-7.8) in IDD patients was found to be higher than in patients diagnosed with NINDs (4.8; 4.1-5.4) ($p<0.001$). As a result of PMA/Ionomycin and IL-2/IL-15 stimulation; CD107a, Granzyme B, Perforin, CD57, NKP46, Trail, CD178/Fas Ligand and IFN-g expressions were found to be significantly higher in CD20+ cells compared to CD20- cells. Cytokine levels of CD20+ cells were decreased after Retuximab treatment. With this study, we determined for the first time in the literature that a subgroup of NK cells express CD20 molecule, and these cells produce more cytotoxic molecules. CD56+CD20+ cells are higher in the blood of individuals with IDDs and may be associated with disease pathogenesis and response to treatment.

Keywords: Cytokines and mediators, multiple sclerosis, NK cells, neuroimmunology

P-0430

Empiric antibiotic treatment and use of moxifloxacin for hospitalized COVID-19 patients: a multi-country cohort study

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The use of antibiotics has been commonly adopted as a targeting therapy for COVID-19, however, there is a lack of consensus whether empiric use of antibiotics may reduce mortality and length of hospital stay. Thus, we aimed to compare the 30-day mortality rates and lengths of hospital stay in propensity score-matched cohorts of COVID-19 patients with versus without empiric antibiotics use. This retrospective cohort study used medical records of patients with COVID-19 from Italy and China in early 2020. Patients who received empiric antibiotic treatment and propensity score-matched patients without any antibiotic treatment were enrolled. The criteria of propensity score matching included demographic information, underlying comorbidities, medications, and lab data including hemoglobin, high sensitivity C- reactive protein, total bilirubin, white blood cell counts, platelet counts, and procalcitonin. Log-rank test was used to evaluate the effect of empiric antibiotic treatment. The effect of moxifloxacin in in-hospital mortality was also examined within the same cohort. There was a significant difference for in-hospital mortality between COVID-19 patients receiving standard treatment and those undergoing standard treatment with empiric use of antibiotics. After propensity score-matching, there was no statistical significance difference in 30-day mortality and length hospital stay greater than 14 days, no statistical significance in empiric use of moxifloxacin compared to those who never received antibiotics. Empiric use of antibiotics did not improve COVID-19 patients' survival outcome, clinicians should be aware of potential side effects and limited efficacy of empiric antibiotics while managing hospitalized patients with COVID-19.

Keywords: Drugs for immune modulation, infectious disease, viral infections

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POSTER PRESENTATIONS

P-0432

Effect of methylprednisolone treatment on COVID-19: An inverse probability of treatment weighting analysis of a binational cohort from Italy and China

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This study sought to investigate the outcomes of methylprednisone administration in a bi-national cohort of hospitalized patients with confirmed SARS-CoV-2 infection from China and Italy. Patients with confirmatory testing for SARS-CoV-2 were retrospectively enrolled, and segregated by administration of corticosteroids. In the Chinese cohort, 40mg of methylprednisolone was given every 12 hours for 5 days to patients with deteriorating oxygen saturation or rapid progression of radiologic imaging. In the Italian cohort, methylprednisolone was administered to patients not responding to pharmacological therapy and ventilatory support at 0.5-1mg/kg/day for 4 to 7 days. Inverse probability of treatment weighting (IPTW) was used to adjust for baseline differences between the steroid and non-steroid cohorts. Primary outcomes included acute respiratory failure (ARF), shock, and 30-day mortality among surviving patients. Among 560 patients enrolled, 169 patients received steroids and 391 did not receive steroids. Crude analysis prior to PS weighting revealed no statistically significant difference in 30-day mortality and ARF between the steroid and non-steroid groups. This persisted after IPTW: 30-day mortality, shock and ARF. Subgroup analysis revealed that 30-day mortality was associated with cardiovascular disease, chronic lung disease, and oxygen-dependent acute respiratory distress. No significant survival benefit was seen after steroid treatment. Use of methylprednisolone was not associated with significant reduction in 30-day mortality, shock, or ARF. A trend towards reduction in shock and ARF was observed and although a potential protective effect might be hypothesized in more severe patients, caution should be exercised and more studies are needed.

Keywords: Drugs for immune modulation, infectious disease, viral infections

P-0433

Familial co-aggregation of idiopathic inflammatory myopathies and cancers

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We aimed to examine if idiopathic inflammatory myopathies (IIM) share pathogenic factors with cancers by estimating the familial co-aggregation of these two disorders. This population-based family study included 8,640 first-degree relative pairs of patients with IIM versus 41,127 of matched individuals without IIM identified from the Swedish health-, and population registers. We ascertained lifetime cancer via linkage to the nationwide cancer and death registers. We estimated the adjusted odds ratios (aORs) of familial co-aggregation of IIM and cancers using conditional logistic regressions. We adjusted for sex and birth year of index individuals and their first-degree relatives. We did the analyses for cancer overall and for specific cancer types, and further stratified the analyses by IIM subtypes, kinships and sex-concordance of relative pairs. We found that patients with IIM were more likely to have full siblings (aOR=1.11, 95%CI 1.02-1.20) and offsprings (aOR=1.14 95%CI 1.00-1.31) affected by cancers overall compared to individuals without IIM. In the stratified analyses by sex concordance and IIM subtypes, we observed significant familial co-aggregation of cancer in male concordant relative pairs of patients with dermatomyositis in all types of kinship. A variety of cancers such as B-cell lymphoma, cervical cancer and small cell lung cancer showed familial associations with IIM, but the associated types differed between kinships and IIM subtypes. The observed familial co-aggregation of IIM and a variety of cancer types suggests shared pathogenic factors between IIM and cancers. These factors might have more profound effects in men than in women.

Keywords: Autoimmunity, autoinflammation, cancer immunology, inflammatory disease

P-0436

A novel mechanism linking mucosal bacteria with autoantibody response in RA: acetylated bacterial lysate as a model antigen

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Rheumatoid arthritis (RA) is characterized by autoantibodies recognizing post-translationally modified (PTM) proteins (AMPA) such as citrullinated, carbamylated and acetylated proteins. The antigens causing the breach of tolerance remain unknown, although microbial antigens are often suspected. Various bacteria are known to be capable of acetylation raising the question whether bacterial acetylated proteins can induce AMPA. To investigate whether acetylated proteins of bacterial origin can induce AMPA development in mice; and are recognized by human derived AMPA and AMPA-expressing B cells. Bacterial acetylated proteins were acquired by inducing *E. coli* auto-acetylation (intrinsically acetylated bacterial proteins, IABP) or by chemically acetylating *E. coli*-derived proteins (extrinsically acetylated BP, EABP), controlled by non-acetylated BP (NABP). The ability of these different acetylated bacterial proteins to induce AMPA in mice was assessed via immunization experiments. Furthermore, the recognition of these proteins by human AMPA and AMPA-expressing B cells was investigated. Intrinsic acetylation resulted in partial, while extrinsic/chemical acetylation resulted in complete acetylation of *E. coli* proteins. Repetitive immunization of mice with EABP resulted in high titers of AMPA recognizing acetylated, carbamylated and citrullinated proteins, which was not observed in mice immunized with NABP or IABP. Human-derived AMPA recognized EABP and IABP but not NABP; AMPA-expressing B cells recognized EABP and (to a lesser extent) IABP, but not NABP. Acetylated bacterial proteins can induce cross-reactive AMPA responses in mice and are also recognized by human AMPA, suggesting a role for acetylated bacterial proteins in the breach of tolerance in RA.

Keywords: Microbiome and environmental factors, adaptive immunity, antibody, autoimmunity, environmental factors in autoimmunity and allergy, rheumatoid arthritis

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POSTER PRESENTATIONS

P-0438

Association of IL-6 levels with morphometric changes of the brain in patients with schizophrenia

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The role of systemic inflammation and immune factors in the development of structural changes of the brain in schizophrenia has not been sufficiently studied. IL-6 is one of the key cytokines mediating systemic inflammation. Increased IL-6 levels promote neurodegeneration. Yet, the role of IL-6 as a possible marker of morphometric brain changes in schizophrenia remains unclear. The aim of this work was to assess the relationship of IL-6 level with systemic inflammation, immune profiles and structural changes detected by neuroimaging in schizophrenia. The study included 60 patients with schizophrenia, 25 healthy volunteers. All participants signed a voluntary informed consent. MRI was performed on a Siemens Magnetom Verio 3T tomograph. Determination of the key immune parameters, cytokines (IL-4, IL-6, IL-8, IL-10, IFNγ), inflammatory markers and immunoglobulins in blood serum was performed by ELISA. The level of IL-6 was associated with markers of systemic inflammation and immune activation in the patients. Significant negative correlations were detected between IL-6 levels and a number of structural MRI indicators. Patients with increased IL-6 levels (>15 pg/ml) had a reduced mean curvature in the left lingual gyrus and a reduced gyrification index in the left fusiform gyrus compared to the controls (p<0,005). According to the literature, morphometric changes is these regions of the brain are associated with the severity of symptoms in schizophrenia. The results indicate that excessive immune activation and systemic inflammation are associated with structural changes in certain regions of the brain in schizophrenia.

This work was supported by NRC "Kurchatov Institute" (№1059).

Keywords: Adaptive immunity, cytokines and mediators, inflammatory molecules, innate immunity, neuroimmunology

P-0441

Immunoprofiling of systemic juvenile idiopathic arthritis reveals distinct biomarkers characterizing disease activity

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Systemic Juvenile Idiopathic Arthritis (sJIA) is an autoinflammatory disease with unknown aetiology. To identify and clarify the underlying mechanisms of sJIA pathogenesis and disease activity, plasma samples from a clinically well-described cohort consisting of twenty-one sJIA patients and sixty age and gender matched healthy controls were analyzed by a highly sensitive proteomic immunoassay – Proximity Extension Assay (PEA). The significantly differentially expressed proteins were further studied by using Ingenuity Pathway Analysis (IPA). Our results confirmed the well-studied sJIA biomarkers, IL6, IL18 and S100A12, being expressed in higher levels in active sJIA than in healthy controls. Novel proteins, including CXCL1, CXCL11, CXCL5, CCL23, EIF4EBP1, KIT-ligand, MMP1, OSM, SIRT2, rarely described in sJIA, contributed to the clustering of active sJIA, inactive sJIA and healthy controls. IPA predicted these biomarkers to participate in cell migration, invasion and homeostasis, as well as inflammatory response. Pathway analysis showed that IL17 Signaling Pathway, HMGB1 Signaling Pathway, Inflammasome Pathway, Granulocyte Adhesion and Diapedesis Pathway, and Macrophage, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis Pathway tended to be enriched in sJIA; while Erythropoietin Signaling Pathway was potentially suppressed. This is the first study in which the PEA proteomics approach has been used to reveal immune mechanisms active during sJIA. We could both confirm previously reported biomarkers as well as report a set of previously unexplored biomarkers. Our findings enable a better understanding of the immunomechanisms driving sJIA independent of medical treatment and aid future diagnostic and therapeutic strategies.

Keywords: Autoinflammation, biomarkers, cytokines and mediators, inflammatory disease, inflammatory molecules, omics technologies

P-0442

Characterization of autoreactive T cells in Guillain-Barré syndrome

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Guillain-Barré syndrome (GBS) is considered an autoimmune disorder of the peripheral nervous system (PNS) in which the contribution of pathogenic autoreactive T lymphocytes targeting PNS antigens has been strongly supported by *in vivo* studies. However, the underlying immune-mediated mechanisms in humans are far from clear. The overall aim of this study is to gain insights into this issue by investigating the existence and providing an in-depth characterization of the autoreactive T cell response in GBS patients during the acute and recovery phases of the disease. Flow cytometry analysis of *ex vivo* PBMCs revealed increased frequencies in effector memory and TEMRA subsets among $CD4^+$ and $CD8^+$ T cells in GBS patients, thus pointing to an involvement of T cells in the disease. Notably, by using a recently established sensitive workflow based on *ex vivo* T cell screenings, generation of single T cell clones and TCR sequencing, here we reveal the existence of self-reactive T cells in GBS patients. Memory $CD4^+$ T cells targeting self-antigens of the PNS were detected in all GBS patients analyzed so far, whereas they resulted almost absent in healthy controls. Moreover, by analyzing more than 300 autoreactive T cell clones, we found that these cells show a polyclonal TCR repertoire, target multiple epitopes of the self-antigens with some immunodominant regions and are mostly HLA-DR restricted. Collectively, our data provide the first description of self-reactive T cells directed against PNS proteins in GBS patients, thus opening new perspective for biomedical application.

Keywords: Adaptive immunity, autoimmunity, neuroimmunology

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POSTER PRESENTATIONS

P-0443

T and B cell responses by Live attenuated oral rotavirus vaccine in human nasopharynx-associated lymphoid tissues

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Rotaviruses are the main cause of severe acute gastroenteritis worldwide. Despite a global reduction in acute gastroenteritis burden by rotavirus vaccination, significant differences in vaccine efficacy persist between developed and developing countries, although the immunological mechanisms remain undefined. AIMS: We evaluated the immunogenicity of a live-attenuated oral rotavirus vaccine using an *ex vivo* mucosal cell culture model with human tonsillar tissues and examined the effect of pre-existing immunity on vaccine-induced immune responses. Ninety immunocompetent children and adults referred to adenotonsillectomy were included in this study. Serum levels of rotavirus-specific antibodies were measured by ELISA. Mononuclear cells from tonsil tissues were isolated and stimulated with a monovalent rotavirus vaccine (RV1). T cell immunity and antibody responses were measured by flow cytometry and ELISA, respectively. Rotavirus-specific IgG and IgA antibodies were detected in serum samples from the recruited patients. A positive correlation was observed between serum rotavirus-specific antibody levels and patients' age. Rotavirus-specific antibodies were also detected in tonsillar cell culture supernatants following rotavirus vaccine stimulation. Mucosal B cell antibody response was positively correlated with patients' serum rotavirus-specific antibody titres and ages. Vaccine stimulation also elicited marked CD4+ and CD8+ T cell proliferative responses and increased frequencies of IFN-γ-producing T cells in NALT of children and adults. Interestingly, an increased expression of gut-homing receptor α4β7 was also detected in vaccine-stimulated human tonsillar T cells. Further studies are ongoing to analyse the effect of pre-existing immunity on vaccine-induced mucosal T and B cell responses.

Keywords: Adaptive immunity, adjuvants and vaccines, antibody, B lymphocytes, protection, viral infections

P-0444

Environmental, clinical, immunological and therapeutic relapse factors of pemphigus

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Pemphigus is a chronic autoimmune disease caused by humoral response with mediated auto-antibodies-directed against desmoglein-proteins. The aim of our study was to identify factors associated with clinical relapse in Moroccan pemphigus patients. A retrospective analysis was conducted of 92pemphigus patients seen between 1990-2020 in the Dermatology Department of Ibn-Sina Hospital. Patients were followed up every 3months. Clinical and environmental features considered relevant were collected at the first consultation. Immunological factors include the indirect immunofluorescence (IIF) at baseline and repeated at 6 months and in clinical relapse. Direct immunofluorescence (DIF) and skin biopsy was performed at study initiation and at the time of clinical switch-phenotype. The mean age was 50-years-old, female to male ratio was 1.48, chronic sun exposure was found in 64 patients, an excessive intake of allium group food in 38.7%, clinical forms were distributed as follow: 49%deep pemphigus, 45%superficial forms and 6% of pemphigus herpetiformis. Relapses were more frequent in patients with: cutaneous involvement at the onset of disease (43%), severe PDAI in91%, 50% experienced pruritus before the onset of blisters/erosions. 58 patients relapsed one time while 20cases relapsed 2times. Immunological factors found to be significantly associated with relapse was the IIF rate between 320-1280 in 39.1%. We present the first large sample study of relapse pemphigus with a follow up period of 30 years. The main limitation of our study is the absence of anti-Dsg enzyme at the onset and relapse of disease due to the lack of its dosage in our country

Keywords: Antibody, autoimmunity, skin diseases

P-0445

Anti-HLA antibody status in kidney transplant candidate

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We aimed to demonstrate the anti-HLA antibody status of the end-stage renal disease patients whose antibody screening and identification tests were performed. Two hundred fifty-six patients (female/male,152/104) who were tested for anti-HLA antibodies between 2017-2020 were included in the study. Anti-HLA antibody screening and identification tests were performed using an Luminex method. FCXM and CDCXM tests were performed with the donor derived fresh cells. PRA(+) group (MFI≥1000) consisted of 202 (78.9%) patients. Of the PRA(+) patients, 20.0% were only class I (+); 40.0% were only class II (+); 40.0% were both class I-II (+). The single antigen bead (SAB)(+) group consisted of 172 (67.2%) patients. Of the SAB(+) patients, 25.0% were only class I (+); 37.2% were only class II (+); 37.8% were both class I-II (+). FCXM(+) group consisted of 133 (52%) patients. Of the FCXM(+) patients, 63,2% were only FCXM-B(+), 36.8% were both FCXM-T-B(+). CDC positivity was found as 10.5% in 256 patients. PRA and SAB positivity was significantly associated with female gender (p=0.028) [MFI(median) Class I: 5500/Class II: 5604],pregnancy (p=0.012) [MFI(median) Class I: 8191 Class II: 7720], blood transfusions (p=0.06) [MFI(median) Class I: 4891 Class II: 8562], previous transplantation history (P<0.0001) [MFI(median) Class I: 4950/Class II:14198]. Anti-A24, anti-B51, anti-DR15, anti-DQ7 were the most frequent antibodies in this group. Our results showed that previous transplantation affect the antibody production of MFI levels of class II in sensitized patients and confirmed the significant correlation between female gender, pregnancy, blood transfusions and previous transplantation history with PRA and SAB positivity.

Keywords: Antibody, biology of the immune system, immunological techniques, transplantation

P-0446

ACPA status correlates with differential immune profile in patients with Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is a progressive erosive autoimmune disease that affects 1% of the world population. Anti-citrullinated protein autoantibodies (ACPA) are routinely used for the diagnosis of RA, however 20-30% of patients are ACPA negative. ACPA status is a delineator of RA disease endotypes with similar clinical manifestation but potentially different pathophysiology. Genomic and radiographic differences and importantly differential response to treatment highlight the need for a treat to target approach for ACPA- and ACPA+ RA patients. Profiling of key peripheral blood and synovial tissue immune populations including B cells, T follicular helper (Tfh) cells and CD4 T cell proinflammatory cytokine responses could elucidate the underlying immunological mechanisms involved and inform a treat to target approach for both ACPA+ and ACPA- RA. Detailed high dimensionality flow cytometric analysis with supervised and unsupervised algorithm analysis revealed unique RA patient peripheral blood B cell and Tfh cell profiles. Synovial tissue single cell analysis of B cell subpopulation distribution was similar between ACPA- and ACPA+ RA patients, highlighting a key role for specific B cell subsets in both disease endotypes. Interestingly, synovial tissue single cell analysis of CD4 T cell proinflammatory cytokine production was markedly different between ACPA- and APCA+ RA patients. RNAseq analysis of RA patient synovial tissue highlighted disease endotype specific gene signatures. Therefore, there is evidence of potentially distinct underlying immunological mechanisms involved, as well as, points of convergence in the pathogenesis of the two disease endo-types of RA, reinforcing the need for a treat to target approach.

Keywords: Adaptive immunity, cytokines and mediators, inflammatory joint diseases, rheumatoid arthritis, RNAseq

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POSTER PRESENTATIONS

P-0447

Impact of the C-type lectin receptor DCIR on T cell priming and antiviral immunity in Theiler's murine encephalomyelitis virus infection

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Theiler's murine encephalomyelitis virus (TMEV) infection represents an animal model for virus-induced neurodegeneration. Myeloid C-type lectin receptors (CLRs) are mainly expressed by antigen presenting cells (APCs), often act as pattern recognition receptors and thereby contribute to pathogen recognition. The inhibitory CLR Dendritic cell immunoreceptor (DCIR) is involved in immune homeostasis and regulation of APC functions. The aim of the present study was to investigate DCIR-mediated effects on T cell priming, viral load and neuropathology in Theiler's murine encephalomyelitis. C57BL/6 (WT) and DCIR-deficient mice were intracerebrally infected with TMEV. Histology and immunohistochemistry of the hippocampus were performed and peripheral T cell responses were analysed. To investigate DCIR-mediated effects mechanistically, bone marrow-derived dendritic cells (BMDC) from WT and DCIR-deficient mice were stimulated with ovalbumin (OVA) peptide-expressing TMEV and co-cultured with T cell receptor-transgenic $CD8^+$ T cells (OT-I) *in vitro*. Further, mixed bone marrow chimeras were generated using DCIR-deficient and CD11c-DTR bone marrow cells to assess the impact of DCIR expression on $CD11c^+$ dendritic cells on the course of TMEV infection. Hippocampal damage, neuronal loss and the number of TMEV-infected cells in DCIR-deficient mice were decreased compared to WT mice accompanied by an increased peripheral T cell activation *in vivo*. Our results also indicate an enhanced $CD8^+$ T cell priming during TMEV infection in the absence of DCIR. Our results indicate thatDCIR exerts inhibitory effects on antiviral responses following TMEV infection. In conclusion, DCIR deficiency ameliorates TMEV-induced neuropathology, thus rendering this CLR a promising target to modulate immune responses during neuroinfections.

Keywords: Animal models, antigen processing and presentation, dendritic cells, innate immunity, neuroimmunology

P-0448

CD101 is a type 1 diabetes susceptibility gene in NOD mice

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Type 1 diabetes (T1D) is a multi-factorial disorder characterized by an immune-mediated destruction of the insulin-producing pancreatic beta cells. The genetic analysis of NOD mice allowed the identification of many insulin-dependent diabetes (Idd) loci and candidate genes, one of them being Cd101. Thus, we elucidated the mechanism(s) by which CD101 mediates protection from T1D using congenic NOD.B6 Idd10 mice in which the susceptible NOD region had been replaced by T1D-resistant B6 genes including Cd101. The genotype-dependent expression of CD101 correlated with a decreased susceptibility to T1D in NOD.B6 Idd10 congenic mice compared to parental NOD controls. The knockout of CD101 within the introgressed B6-derived Idd10 region increased T1D frequency to that of the parental NOD strain. The loss of protection from T1D was paralleled by decreased Gr1-expressing myeloid cells and FoxP3+ regulatory T cells and an enhanced accumulation of CD4-positive over CD8-positive T lymphocytes. As compared to CD101+/+ NOD.B6 Idd10 donors, adoptive T cell transfers from CD101−/− NOD.B6 Idd10 mice increased T1D frequency in NOD scid and NOD.B6 Idd10 scid recipients. Increased T1D frequency correlated with a more rapid expansion of the transferred CD101−/− T cells and a lower proportion of recipient Gr1-expressing myeloid cells. Fewer of the Gr1+ cells in the recipients receiving CD101−/− T cells expressed also CD101. Thus, our results connect the Cd101 haplotype-dependent protection from T1D to an anti-diabetogenic function of CD101-expressing Tregs and Gr1-positive myeloid cells and confirm the identity of Cd101 as Idd10.

Keywords: Animal models, biomarkers, diabetes, immune communication

P-0450

Comparative analysis of Luminex-based Class I donor-specific antibody mean fluorescence intensity values with complement-dependent cytotoxicity and flow crossmatch results in live donor renal transplantation

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The objective of this study was to retrospectively compare donor spesific antibodies (DSA) results of solid phase assay and cell-based assay and explore the near accurate DSA mean fluorescence intensity(MFI)-cutoff value detected on solid phase assay above which the cell-based assay would show a positive result. The 335 patients and their donors who were scheduled for kidney transplantation occured in the year between 2018-2020 were included in the study. Serum samples from the cohort awaiting for transplantation were tested for the presence of DSA by CDC, FCXM methods with their donors besides for DSA detection, using the single antigen bead assay. Of the 335 patients 307 were positive for anti-HLA antibodies(MFI>1000). Of the 335 patients 107 were positive class I DSA. Three patients (DSA-MFI<1000), CDC-XM, T-B FCXM were negative. Thirty patients with DSA-MFI(1000-3000), have negative results for T-B FCXM or CDC-XM while 2 patients showed positivity for the B-FCXM. Seventeen patients with DSA-MFI(3000-5000) were negative for CDC-XM, T and B FCXM. Fifty-five patients (DSA-MFI >5000); 10 patients were showed positivity for the CDC-XM, T and B FCXM, 10 patients were showed positivity for the T-B FCXM, 2 patients showed positivity for the only B-FCXM. Thirty-three patients were negative for CDC-XM, T and B FCXM. Our results indicated that the DSA-MFI values of cut-off >5834 were significantly ($p<0.0001$) correlated with positive T -FCXM while DSA-MFI values of cut-off >6016 were significantly ($p=0.002$) correlated with positive CDC-XM. It was observed that MFI values above 5000 were associated with both CDC and FCXM.

Keywords: Antibody, immunological techniques, transplantation

P-0451

Specific antibody dynamics in healthcare workers during Covid-19 pandemics and vaccination

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During the major outbreak of viral infection in the modern history healthcare workers have encountered multiple challenges. One of the challenges was their constant contact with infected samples and patients. The purpose of our study was to monitor their antibody immune response during the pandemics and followed through vaccination protocol. In the present study, 103 healthcare workers with documented exposure to SARS-CoV-2-infected patients/samples were monitored for specific IgG and IgA throughout one year and in the vaccination period, namely after the first vaccination at 21 days and two weeks after the second vaccination with an mRNA-based vaccine. Using a validated ELISA method, we have monitored the specific antibodies level. Results show that infected subjects display sustained levels of antibodies compared to non-infected subjects. When subjected to vaccination a consistent increase in both IgG and IgA levels was obtained, even after the first dose in the majority of subjects from both prior infected and non-infected subjects. After the second dose, the circulatory levels of IgA and IgG were increased yet again. The associations between the IgG and IgA levels upon the first and second dose of vaccination was observed in the entire vaccination group, regardless of prior exposure to the infectious agent. The increment and the levels of IgG and IgA were similar. Thus, the levels upon vaccination were statistically similar irrespectively of the starting base-line before vaccination. In the present study, seroconversion was achieved in 100% of the group after the second dose with similar high antibodies levels.

Keywords: Antibody, infectious disease, viral infections

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POSTER PRESENTATIONS

P-0454

Elevated MMP-10 and MMP-13 in patients co-infected with pulmonary tuberculosis and soil-transmitted helminth infections

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Approximately 1/4 of the world's population are infected by soil-transmitted helminths (STHs) which cause malnutrition and anaemia. Tuberculosis (TB) causes around 1.5 million deaths per year. STHs, like TB, thrive where there is poverty, however, the impact of concurrent STH infection on the innate immune response to TB is poorly understood. STHs may drive increased tissue damage in TB secondary to augmented secretion of matrix-metalloproteinases (MMPs). We recruited 61 adults with confirmed active pulmonary TB and 51 healthy controls in Iquitos, a city in the remote Peruvian Amazon. Consecutive stool samples were obtained for parasitology, and plasma analysed for IgE by ELISA and MMPs by multiplex bead-assay. Plasma IgE concentrations were elevated in TB and STH co-infected individuals compared to STH-negative TB patients (median 5504 (IQR 3427-8467) vs 1267 (IQR 727-5104) p=0.0003). TB culture positivity was associated with at least one STH (OR 2.5, 95%CI 1.8-5.5) and the odds of being a TB patient increased with multiple helminth species per person (OR 3.9, 95%CI 1.5-11.6). Plasma MMP-10 concentrations were significantly elevated (median 1199pg/mL (IQR 998-1385) vs 896pg/mL (IQR 754-1105), p=0.001), as was MMP-13, a key collagenase, (median 861pg/mL (IQR 998-1385) vs 766pg/mL (IQR 754-1105), p=0.037) in co-infected subjects compared to TB-positive, STH-negative individuals. MMP-1 and -8 were also non-significantly elevated. We observed a significant association between TB and helminth infection with increased odds of TB in individuals with multiple helminth infections. STHs increased MMP-10/13 secretion in TB which potentially will increase the severity of immune-related lung damage in TB patients.

Keywords: Innate immunity, tissue damage and repair, bacterial infections, parasite infections

P-0455

Mesenchymal stromal cells reduce house dust mite induced allergic airway inflammation in a humanised MIF expressing mouse model

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Mesenchymal stromal cells (MSCs) are a subset of bone-marrow derived cells with cytoprotective and immunomodulatory capabilities. Clinical trials are currently examining MSCs as a potential novel therapeutic approach to protect against injury perpetrated by an over-zealous immune response in a range of inflammatory conditions. Our previous research has identified the need for MSCs to be activated by pro-inflammatory signals to facilitate or enhance their therapeutic efficacy. High expression levels of the pro-inflammatory cytokine macrophage migration inhibitory factor (MIF) are present in a range of inflammatory diseases including severe asthma. This study sought to elucidate the influence that MIF licensing has on human MSC cytoprotection and immune modulation *in vitro*. Moreover, the effect of MIF on MSC therapeutic efficacy *in vivo* was investigated using a clinically relevant acute house dust mite (HDM) (*Dermatophagoides pteronyssinus*) model of allergic airway inflammation in humanised MIF mice. MIF pre-stimulation enhanced MSC promotion of wound healing in airway epithelial cells in a VEGF dependent manner. Periodic acid-Schiff and Masson's Trichrome staining showed that intranasal HDM challenge induced severe mucus production and collagen deposition accompanied by inflammation in bronchial regions in the high expression MIF humanised mice. Importantly, human MSCs significantly reduced mucus production and collagen deposition along with reducing the expression of type 2 inflammatory genes. Differential cell counts from BALF illustrated that human MSCs have the ability to suppress the eosinophil population resulting from HDM challenge.

Keywords: Cell based therapies, cytokines and mediators, inflammatory disease, stem cells

P-0457

Clonal analysis of immunodominance and cross-reactivity of the CD4 T cell response to SARS-CoV-2

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The identification of CD4+ T cell epitopes is instrumental for the design of subunit vaccines for broad protection against coronaviruses. Here we demonstrate in COVID-19-recovered individuals a robust CD4+ T cell response to naturally processed SARS-CoV-2 spike (S) and nucleoprotein (N), including effector, helper, and memory T cells. By characterizing 2943 S-reactive T cell clones from 34 individuals, we found that RBD is highly immunogenic, and that 33% of RBD-reactive clones and 94% of individuals recognized a conserved immunodominant S346-365 region comprising nested HLA-DR- and HLA-DP-restricted epitopes. Using pre- and post-COVID-19 samples and S proteins from endemic coronaviruses, we identify cross-reactive T cells targeting multiple S protein sites. The immunodominant and cross-reactive epitopes identified can inform vaccination strategies to counteract emerging SARS-CoV-2 variants.

Keywords: Adaptive immunity, antigen processing and presentation, infectious disease, memory, viral infections

P-0458

Nasal polyps are characterized by a population of CD109+CRTH2- Th2 cells that secrete interleukin-4 and interleukin-10

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Chronic rhinosinusitis with nasal polyps (CRSwNP) is characterized by a chronic inflammatory process associated with comorbid asthma. In this study, we analysed the transcriptomes of single T helper (Th) cells from nasal polyps of patients with CRSwNP and validated these findings using multiparameter flow cytometry. Polyp tissue contained suppressive Treg and Th2 cells, type 2 innate lymphoid cells (ILC2) and 3 transcriptionally distinct subsets of cytotoxic CD4 T cells (CD4 CTL). GATA3 was a feature of polyp Treg while Th2 cells were the main population producing IL5 and IL13 mRNA, even in comparison to ILC2. Only a portion of polyp Th2 cells expressed the prostaglandin D2 receptor CRTH2, while a subpopulation of CD109+CRTH2- Th2 cells expressed mRNA for common inhibitor receptors and produced IL-10. Taken together, we resolve the complexity of T helper cells in CRSwNP patients and identify a population of CD109+CRTH2- Th2 cells with putative regulatory potential.

Keywords: Adaptive immunity, allergen-induced immune responses, allergic disorders

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POSTER PRESENTATIONS

P-0459

Pro-inflammatory changes in the brain of Cntnap2-/- and Shank3b-/- mice, animal models of autism spectrum disorders

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Autism spectrum disorders (ASDs) are a heterogeneous group of neurodevelopmental disorders associated to social communication deficits and repetitive sensory–motor behaviors. These symptoms affect children from the early childhood and produce clinically significant developmental impairments. Immune dysfunction has recently emerged as major contributor to the neurodevelopmental deficits observed in people with ASD. This condition is often linked with a strong inflammatory state, which contributes to neurodegeneration and impairments in synaptic plasticity. Cntnap2-/- and Shank3b-/- mice have widely been considered robust animal models of ASD. In the current study, we analyzed the expression of classical pro-inflammatory molecules in the cerebral cortex, hippocampus and cerebellum of mutant mice. mRNA and protein expression of IL-6, TNF, IFNγ and IL-1β were increased in the cerebellum of Cntnap2-/- and Shank3b-/- mice, in comparison with their WT littermates. In addition, increased levels of the same molecules were found in the blood of both knockout mice. Finally, a link could be identified between inflammation within cerebellum and impaired social and motor behaviors (common ASD-related features) in these mice. Taken together, these results suggest that cerebellar inflammation may support ASD-like behaviors in autism.

Keywords: Animal models, inflammatory disease, inflammatory molecules, neuroimmunology

P-0461

Respiratory syncytial virus regulates activation of the JAK-STAT pathway through its non-structural proteins

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Respiratory Syncytial Virus (RSV) is the leading cause of bronchiolitis and viral pneumonia in infants, causing significant morbidity and mortality. Part of RSV's impact on infants is its ability to limit IFN signalling though the JAK-STAT pathway in infected cells. The JAK-STAT pathway is crucial in the transduction of the Interferon (IFN) response, with tight regulation needed to prevent inappropriate signaling. Manipulation of the pathway by pathogens limits the anti-viral capabilities of the cell and makes them more permissive to infection. Previous work has linked the non-structural (NS) proteins, NS1 and NS2, to the subversion of IFN signalling. However, much of our understanding comes from immortalised kidney cell lines. In order to establish a physiologically relevant understanding of RSV's immune evasion strategies we have studied its effects in two immortalised epithelial cell lines and primary human cells. We expressed NS proteins in alveolar epithelial cells (A549) and bronchial epithelial cells (BEAS-2b) and analysed their effect upon the IFN-α pathway. We discovered that the NS proteins have varying effects in each cell line, though both cell lines had decreased ISG expression with NS1, and variable SOCS expression with both NS1 and NS2. Our work has shown that the activity of NS1 and NS2 is cooperative and targets the activity of the JAK-STAT signalling at multiple points to limit the antiviral response. These insights add to our understanding of RSV, and suggests that these proteins could be druggable targets in the future to treat RSV, which currently has no curative treatments.

Keywords: Cell signalling, infectious disease, innate immunity, molecular immunology, viral infections

P-0462

Interleukin 1 beta promotes the skin sensitization and the atopic march

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Atopic diseases, including atopic dermatitis (AD) and asthma, affect a large proportion of the population, with increasing prevalence worldwide. AD and asthma share common type 2 inflammatory response, with infiltration of Th2 cells, eosinophils as well as expression of Th2 cytokines and allergen-specific IgE. AD often precedes the development of asthma, which is known as the "atopic march". Allergen sensitization developed through the barrier-defective skin of AD skin has been recognized to be a critical step leading to asthma, however the mechanisms underlying cutaneous sensitization remain incompletely understood. Thymic stromal lymphopoietin (TSLP) has been previously shown to be a key player for allergen sensitization through the skin and the atopic march leading to asthma. In the present study, we obtained experimental evidence that TSLP is differentially required for house dust mites (HDM) sensitization-induced Th2/Tfh responses and the subsequent asthmatic inflammation, when allergen sensitization occurs at different depth of the barrier-defective skin in mice. We further identified that interleukin 1 beta (IL-1β) promotes skin sensitization and the atopic march in a TSLP-independent manner. Our data suggest that IL-1β and TSLP could represent context-dependent targets for preventing/stopping the atopic march.

This study is supported by funds from l'Agence Nationale de la Recherche (ANR) (ANR-17-CE14-0025; ANR-19-CE17-0017; ANR-19-CE17-0021) and Fondation Recherche Médicale (FRM; Equipes-FRM 2018).

Keywords: Interleukin 1 beta, TSLP, atopic march

P-0463

Enrichment of RORγt+ innate-like T cells in joint and gut samples from spondyloarthritis patients

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Dysregulated IL-23/IL-17 responses have been linked to development of spondyloarthritides (SpA), a cluster of inflammatory rheumatic diseases which is frequently marked by the presence of (subclinical) gut inflammation. IL-23/IL-17 inflammation is controlled by RORγt, the key Th17 cell transcriptional regulator, which is also expressed by innate-like T cell subsets such as iNKT, MAIT and γδ-T cells, but their role in SpA pathology is still unclear. Here we describe the presence of particular RORγt+T-betloPLZF− iNKT and γδ-hi T cell subsets in healthy peripheral blood. RORγt+ iNKT and γδ-hi T cells showed profound IL-23 mediated Th17-like immune responses and were clearly enriched within inflamed joints of SpA patients where they act as major IL-17 secretors. SpA derived innate-like T cells showed unique and Th17-skewed phenotype and gene expression profiles as determined by respectively FlowSOM and RNAseq analyses. Moreover, RORγt+ subsets were clearly enriched in intestinal biopsies of SpA-patients compared to healthy controls, and remarkably this was more pronounced in patients with subclinical gut inflammation. In conclusion, our findings highlight a unique diversity of human RORγt+ T cells and show that SpA innate-like T cells, both in gut and joint samples, are skewed towards a predominant pro-inflammatory Th17 profile. Overall, these data strengthen the existence of a gut–joint axis of inflammation in SpA.

Keywords: Autoinflammation, CD1-restricted T cells, gamma-delta T cells, NKT cells, rheumatoid arthritis

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POSTER PRESENTATIONS

P-0464

Effects of bacterial genotoxins on immune modulation, chronic inflammation and cancer development

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Genotoxins have recently been identified as a novel family of effectors in pathogenic and commensal bacteria. At present, three bacterial genotoxins have been identified: colibactin produced by some *Escherichia coli* strains, the cytolethal distending toxin (CDT) family produced by several Gram-negative pathogens and the typhoid toxin produced by *Salmonella enterica* serovar Typhi. Exposure to high toxin doses activates the classical DNA damage response, which will block proliferation and eventually will induce death in mammalian cells. However, exposure to low toxin doses have shown to promote classical sign of carcinogenesis *in vitro*, such as cell survival and acquisition of genomic instability. In spite of an extensive characterization of their mode of action *in vitro*, we have a poor understanding of their role in chronic infection and, considering the genotoxic potential, of their carcinogenic capacity. *Salmonella* Typhi is the only genotoxin-producing bacterium that is associated with increased risk of tumor development in humans. We demonstrated that upon infection with *S. enterica*, the typhoid toxin causes DNA fragmentation and promotes senescence *in vivo*, which were uncoupled from a pro-inflammatory response but, surprisingly, associated with an anti-inflammatory response in the intestine. Based on this evidence, we suggested the possibility that senescence may have multiple roles in immunomodulation, beside the well characterized pro-inflammatory effect. The results indicate that this unusual bacterial effector is not a classical toxin, but rather acts as immunomodulator, highlighting a complex and tissue specific crosstalk between two very conserved stress responses: the immune response and the DNA damage response.

Keywords: Cell death, cytokines and mediators, immune networks, inflammatory disease

P-0467

Invoking immune resistance in acute myeloid leukemia (AML) cells through ex-vivo allogeneic immune reactions

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This study aims at invoking immune resistance in acute myeloid leukemia (AML) cells through co-culturing with allogeneic activated PBMCs and elucidating the characteristics of viable immune-resistant AML cells. AML cell lines (THP-1, HL-60, U937) were co-cultured allogeneic PBMCs at 1:0.25 ratio in the presence of anti-CD3 stimulation for 48h. The viable AML cells based on propidium iodide staining and high CD13 expression were purified by FACS; hereafter, referred to as "immune-experienced" AML (ieAML) cells. Later, $CD4^+$and $CD8^+$ T cells isolated from healthy individuals were co-cultured at different ratios in two setups: one with freshly isolated ieAML cells and, another with rested ieAML in the presence of anti-CD3 stimulation for 72h to study changes in T cell proliferation and AML cells viability. RNA isolated from the viable ieAML cells was subjected to transcriptomics analysis. The ieAML cells were not negatively influenced even by the highest amount of T cells in terms of viability and proliferation, $CD4^+$ and $CD8^+$ T cells proliferation rate reduced in ieAML cells co-cultures, more significantly in THP-1. Viable ieAML found to be less adherent, less chemotactic and more polarized. May–Grünwald Giemsa staining showed ieAML cells more granular with less cytoplasmic to nuclear ratio. RNAseq data showed 77 genes upregulation and 7 genes downregulation in ieAML cells. Pathway analysis showed upregulation of major Inflammatory pathways, particularly of interferon signalling. As a malignancy with an immune (myeloid) cell origin, AML cells gain unique signatures to modulate the anti tumor immunity especially through interferon regulated pathways.

Keywords: Cell signalling, inflammatory molecules, myeloid cells, RNAseq

P-0468

CD206+CD163+ pathogenic macrophages enriched in Rheumatoid Arthritis synovial tissue with distinct transcriptional signatures

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Synovial-tissue macrophages play a key role in RA pathogenesis, yet the precise nature/function of macrophage subsets within the inflamed joint remains unexplored. RA, PsA, OA, Arthralgia and healthy-control synovial-tissue biopsies and synovial-fluid analysed via flow-cytometry: (CD40,-CD45,-CD64,-CD68,-CD163,-CD206,-CD253,-CCR4,-CCR7,-CXCR1,-CXCR3). CD206+CD163+ and CD206-CD163- macrophages sorted from RA synovial-tissue by FACSAria sorter; RNAseq, FLIM analysis and heathy-fibroblast experiments performed. A spectrum of macrophage activation states exists within the inflamed synovium. Within this spectrum, significant enrichment of dominant CD206+CD163+ macrophage subtype is present in synovial-tissue versus fluid($p<0.05$). CD206+CD163+ synovial tissue macrophages express significantly more CD40 ($p<0.0003$), positively correlating with disease activity($r=0.6$,$p<0.01$), with baseline levels predicting response to therapy ($p<0.05$). CD206+CD163+CD40+ macrophages are enriched in RA synovial tissue compared to PsA and OA ($p<0.05$). CD206+CD163+ subset is present in healthy synovial-tissue, however, expression of CD40 is completely absent ($p<0.05$). Protective barrier-like CX3CR1-expressing macrophages depleted in RA synovial-tissue and this occurs prior to clinical manifestations. RNA-seq analysis indicates that CD206+CD163+ macrophages are transcriptionally distinct from synovial-tissue CD206-CD163- and RA polarised-macrophages, with unique tissue-resident gene signatures. Differing metabolic demands between CD206+CD163+/CD206-CD163- subsets was demonstrated by RNAseq and FLIM analysis. Finally, CD206+CD163+ macrophages spontaneously secrete key pro-inflammatory mediators (reversed through inhibition of CD40 signalling) which in turn can activate healthy-synovial fibroblasts, thus further contributing to the local inflammatory response. This data identifies for the first-time, enrichment of a previously undescribed dysfunctional dominant and transcriptionally-distinct macrophage subtype in RA synovial-tissue, thus providing a greater understanding of the critical role tissue-resident macrophages play in perpetuating inflammation in RA.

Keywords: Autoimmunity, innate immunity, macrophage, metabolic control of immune responses, rheumatoid arthritis, RNAseq

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POSTER PRESENTATIONS

P-0469

Distinct inflammatory and anti-inflammatory profiles of water extracts from a cohort of medicinal plants from Norway

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With the re-emergence of infectious diseases and multidrug resistance, there is an increasing need for novel pharmaceuticals. We have in this project focused on medicinal plants with widespread use in Nordic folk medicine in the 16th-19th centuries, as potential novel sources of active pharmaceutical compounds. Twenty-three plants were selected based on reported usage interpreted to be related to immunological diseases or wound healing. Polysaccharides and polyphenols isolated from hot water extracts were screened for effects in immunological assays. Assessing NO-production from macrophages, PBMC proliferation, and TNFα- and IFNγ-secretion from PBMCs, we found very high activity of polysaccharide extracts from Norwegian angelica *Angelica archangelica*, February daphne *Daphne mezereum* and scurvygrass *Cochlearia officinali*. Further, we found the polyphenol extracts of bogbean *Menyanthes trifoliate*, Dooryard dock *Rumex longifolius* and Common agrimony *Agrimonia eupatoria* to have anti-inflammatory effects. The observed immune activating effect of the plant polysaccharides might be due to their ability to bind pattern recognition receptors on innate immune cells. The observed anti-inflammatory effects of the polyphenol extracts is consistent with other studies indicating anti-inflammatory effects of polyphenols through interaction with a variety of immune receptors. We also found the polyphenol-extract from grey alder *Alnus incana* to have potent antibacterial effect against four different strains of bacteria, most notably *Pseudomonas aeruginosa*. The results of this screening will serve as basis for further characterization of the natural compounds and their exact mechanism of action.

Keywords: Drugs for immune modulation, immune regulation and therapy, immunopharmacology, inflammatory disease, innate immunity, molecular immunology

P-0470

IL-6 is regulating physiological and metabolic adaptations to inflammatory conditions

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Tolerance represents an important part of adaptation to infection. Proinflammatory cytokines, such as IL-6, IL-1β, and TNF, are believed to take part in this process, driving the so-called "sickness behavior", but their relative contributions are not yet clarified. Recently, IL-6 was characterized as the exercise- and stress-induced factor that is regulating systemic metabolism, specifically, the blood glucose level. However, its role in physiological and metabolic adaptations in the context of inflammation remains elusive. In this study we aimed to investigate the contribution of IL-6 to "sickness metabolism state" that is characterized by the altered blood metabolome and reduced body physical endurance in the context of inflammation. We observed a drastic reduction in RotaRod exercise capacity of WT, but not IL-6 KO mice, following LPS challenge. Intriguingly, conditional inactivation of IL-6 in myeloid cells did not affect systemic IL-6 production and body endurance, while skeletal muscles were identified as the major source of inflammation-induced IL-6. Furthermore, inactivation of IL-6 resulted in elevated glucose and reduced triglycerides levels in the blood in response to LPS as compared to WT mice. Transcriptome analysis revealed deregulated glycogen synthesis and degradation pathways in the liver, as well as lipolysis pathway in white adipose tissue in LPS-challenged IL-6 KO as compared to WT mice. Altogether, we propose that physiological and metabolic adaptations to inflammatory conditions constitute a significant part of IL-6 functions, and they could be employed in the control of the adverse effects of infections, such as sepsis and cytokine storm.

Supported by RSCF (grant#19-75-30032)

Keywords: Animal models, biology of the immune system, cytokines and mediators, inflammatory molecules, innate immunity, metabolic control of immune responses

P-0471

Unraveling neglected tropical diseases: establishment of experimental models and investigation of mayaro fever pathogenesis

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Mayaro virus (MAYV) is an arthropod-borne virus of the Alphavirus genus circulating in Latin America that causes a febrile illness with arthralgia. To date, there are no vaccines or treatments against MAYV infection and risk of an epidemic indicates that development of treatments is urgent. In this study, we applied state-of-the-art technologies along with traditional methods in immunology and virology to investigate MAYV pathogenesis, aiming at the development of experimental models and at evaluation of therapeutic strategies against MAYV infection. Our results show that type I Interferon receptor deficient mice (ABR-/-) footpad infection with inocula as low as 100 PFU prompts disease development with inflammation that resemble arthritis in humans. Within 3 days post-infection, MAYV is detected in all organs tested, coinciding with peak of disease signs and weight loss. Using x-ray microtomography, we observed MAYV-affected mouse paws with high resolution (64 μm^3) in 3D, to assess volume and localization of edema, indicating increase in soft tissue volume due to mostly subdermal edema. In parallel, we tested over 1000 compounds from the NIH Clinical Collection (FDA-approved) using High-Throughput Screening, to select 20 candidates for hit confirmation and characterization *in vitro*. Three compounds progressed through our screening pipeline. We conclude our experimental models and methods will be helpful in a better understating of MAYV pathogenesis. As perspectives, best hit compounds will be tested in our mouse model of infection, hopefully contributing to host protection. Given the similarities between alphaviruses, our findings may also advance the understanding of other arboviral neglected diseases.

Keywords: Animal models, infectious disease, inflammatory disease, viral infections, visualizing immune responses

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POSTER PRESENTATIONS

P-0473

CNS-endogenous TLR7 and TLR9 induce different immune responses and effects on EAE

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Innate receptors, including Toll like receptors (TLRs), are implicated in pathogenesis of CNS inflammatory diseases such as multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE). TLR response to pathogens or endogenous signals includes production of immunoregulatory mediators. One of these, interferon (IFN)β, a Type I IFN, plays a protective role in MS and EAE. We have previously shown that intrathecal administration of selected TLR ligands induced IFNβ and infiltration of blood-derived myeloid cells into the CNS, and suppressed EAE in mice. We have now extended these studies to evaluate a potential therapeutic role for CNS-endogenous TLR7 and TLR9. Intrathecal application of Imiquimod (TLR7 ligand) or CpG oligonucleotide (TLR9 ligand) into CNS induced IFNβ expression, with greater magnitude in response to CpG. CNS extraparenchymal CD45+ cells were identified as source of IFNβ. Intrathecal CpG induced infiltration of monocytes, neutrophils, CD4+ T cells and NK cells whereas Imiquimod did not recruit blood- derived CD45+ cells. CpG, but not Imiquimod, had a beneficial effect on EAE, when given at time of disease onset. This therapeutic effect of CpG on EAE was not seen in mice lacking the Type I IFN receptor. Our findings show that TLR7 and TLR9 signaling induce distinct inflammatory responses in the CNS with different outcome in EAE and point to recruitment of blood-derived cells and IFNβ induction as possible mechanistic links between TLR9 stimulation and amelioration of EAE. The protective role of TLR9 signaling in the CNS may have application in treatment of diseases such as MS.

Keywords: Inflammatory disease, multiple sclerosis, innate immunity, neuroimmunology

P-0474

Dietary effect on host antimicrobial peptide expression and microbiota composition in the small intestine

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Secreted host antimicrobial peptides (AMPs) (e.g. α-defensins, RegIIIγ) are key effectors of the innate immunity that continuously protect the epithelium from the trillions of microbes residing in the intestine. Defects in AMP expression caused by a high-fat diet intake can cause an imbalance in the microbial communities in the gut and potentially contribute to a localized inflammatory response. Likewise, microbes are known to stimulate the production of AMPs, giving rise to a sustained bi-directional interaction between the microbiota and AMPs. The aim of this study was to assess the effect of a high-fat diet on AMP expression and investigate the changes in microbiota composition in the small intestine. We fed wild-type C57BL/6 mice from two cohorts a chow (control diet) or a Western style diet (WSD), a diet rich in carbohydrates and fat but low on dietary fiber content, for 8 weeks and determined the expression of epithelial and Paneth-cell produced AMPs in the small intestine. In addition, we investigated the changes in small-intestinal microbiota composition by 16S rRNA sequencing. We observed microbial alterations upon WSD feeding and that different AMPs in the two cohorts were differentially affected by the diet intervention. Previous reports have shown that the environment shapes the microbiota composition, thus highlighting the influence of steady-state microbial communities in the food-mediated antimicrobial response in the digestive tract.

Keywords: Effector molecules, innate host defence, microbiome and environmental factors

P-0475

The glucocorticoids receptor in intestinal epithelial cells alleviates intestinal inflammation in mice

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Glucocorticoids (GCs) are a major therapy in the treatment of ulcerative colitis (UC). An important relationship between intestinal epithelial cells (IECs) and UC is well established but the role of GCs and their receptor (GR) in this disease has not yet been explored. To understand the role of endogenous GCs in IECs in the context of UC, we induced an inflammation of the colon in mice carrying an inducible deletion of the GR in IECs (GRvillin mice). Colitis was induced by administration of dextran sulfate sodium (DSS) in mice and the disease activity was monitored during the experiment based on different criteria. Colon tissues, lamina propria cells (LPCs), and IECs were investigated by Evans Blue assays, flow cytometry and gene expression analysis. Absence of the GR in IECs aggravated disease progression and the damaged tissue resulted in a more pronounced loss of the IECs' barrier function, thereby increasing epithelial permeability in the colon. Gene expression of pro-inflammatory mediators was dysregulated in IECs and LPCs of GRvillin mice, leading to a reduced leukocyte recruitment into the colon and a hyperactivation of LPCs. Our results reveal an essential role of GCs in IECs of the colon and in the development of UC, which has consequences not only for the management of this disease but also for its progression to colitis-associated colon carcinoma.

Keywords: Animal models, immune communication, inflammatory bowel disease, molecular immunology

P-0476

Itaconate mediates control of *Coxiella burnetii* infection in murine macrophages

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The obligate intracellular gram-negative bacterium *Coxiella burnetii* is the causative agent of the worldwide zoonotic disease Q fever. It transmits to human by inhalation of infectious material from the reservoir animals (sheep, goats, cattle). Q fever is usually a self-limiting asymptomatic or mild flu-like respiratory infection, but can become chronic and cause endocarditis in some patients. *C. burnetii* is sensed by Toll-like receptors (TLR), and we recently found in a mouse model of infection with *C. burnetii* Nine Mile phase II that it induced IFNγ and its target genes in a Myd88-dependent manner. Here, we have investigated the role of MyD88-/IFNγ-induced genes (eg. *Irg1, iNOS, Gbp2, Ido*) in controlling bacterial replication and macrophage reprogramming during infection with *C. burnetii*. We noticed a massive increase in *C. burnetii* replication in *Irg1*$^{-/-}$ bone marrow-derived macrophages (BMM), while the bacterial burden was only modestly elevated in macrophages deficient in *iNOS*. In activated macrophages, the TCA cycle metabolite citrate is metabolized by *Irg1* to itaconate, which can have both anti-microbial and immune-regulatory effects. Treatment of *C. burnetii*-infected BMM from *Irg1*$^{-/-}$ mice with itaconate in non-toxic concentrations normalized bacterial burden as measured by qPCR and immunofluorescence microscopy. Mass spectrometry analysis of TCA cycle metabolites showed itaconate production and succinate accumulation in *C. burnetii*-infected wild-type, but not in *Irg1*$^{-/-}$ BMM; exogenous itaconate restored intracellular levels, indicating efficient uptake by macrophages. Our data establish that *Irg1* is required for the early control of *C. burnetii* and directly inhibits bacterial replication through the production of itaconate.

Keywords: Bacterial infections, innate host defence, innate immunity, macrophage, mass spectrometry, metabolic control of immune responses

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POSTER PRESENTATIONS

P-0477

Impaired priming of SARS-CoV-2-specific naïve CD8+ T cells in older subjects

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Advanced age is associated with severe symptoms and death upon SARS-CoV-2 infection. Virus-specific CD8+ T-cell responses have shown to be protective toward critical COVID-19 manifestations, suggesting that suboptimal cellular immunity may contribute to the age-pattern of the disease. The induction of a CD8+ T-cell response against an emerging pathogen like SARS-CoV-2 relies on the activation of naïve T cells. To investigate whether the primary CD8+ T-cell response against this virus is defective in advanced age, we used an *in vitro* approach to prime SARS-CoV-2-specific naïve CD8+ T cells from healthy, unexposed donors of different age groups. Compared to younger adults, older individuals display a poor SARS-CoV-2-specific T-cell priming capacity in terms of both magnitude and quality of the response. In addition, older subjects recognize a lower number of epitopes. Our results implicate that immune aging is associated with altered primary SARS-CoV-2-specific CD8+ T-cell responses.

Keywords: Adaptive immunity, ageing, immune senescence, infectious disease

P-0478

Exploring *Mycobacterium tuberculosis* immune evasion strategies to investigate the role of the host IL-1 axis

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Tuberculosis (TB) is an ancient disease that still remains a major public health. TB disease may present with mild to severe lung pathology, eventually resulting in death. The initial lung immune events critically define the outcome of TB, and interleukin (IL)-1 is critical for an early immune defense to TB. Our recent studies show that modulation of IL-1β induction in infected macrophages by the pathogen associates with different TB severities in humans. In particular, clinical isolates of *Mycobacterium tuberculosis* (Mtb) recovered from severe TB patients induced low IL-1β levels *in vitro*. However, the mechanisms linking differential IL-1β induction to disease severity remain unknown. Here, using a mild TB/ high IL-1β (4I2) and severe TB/ low IL-1β (6C4) inducing clinical isolates, we recapitulated in the mouse model the differential TB severity found in the patients. Indeed, we found that infections of wild-type mice with the high IL-1β inducer Mtb 4I2 resulted in both lower bacterial burdens and lung pathology, as compared to infections with the low IL-1β inducer Mtb 6C4. Furthermore, lack of IL-1 receptor in the myeloid compartment abrogates the lower virulence of Mtb 4I2. We are now investigating the underlying mechanisms, as well as the contribution of the IL-1R in non-hematopoietic compartments (endothelium or lung epithelial cells). With this study, we expect to offer novel insights on the mechanisms underlying the host-pathogen interplay during TB.

Keywords: Animal models, bacterial infections, infectious disease, innate immunity, myeloid cells

P-0479

SMAD-6 overexpression and CAV-1 underexpression as potential biomarkers of hepatitis C infection

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Hepatitis C virus (HCV) causes acute and chronic hepatitis C. Correlating with liver disease progression, hepatic fibrosis is a key factor for development of liver disease and risk of hepatocellular carcinoma (HCC). The aim of this study was to compare the transcriptomic profiles of 84 genes involved in the development of fibrosis genes in various stages of fibrosis associated with chronic hepatitis C. This study is a prospective analysis of genes associated with hepatic fibrosis in newly diagnosed hepatitis C infected patients receiving clinical care at the University Hospital for Infectious Diseases (UHID) in Zagreb, Croatia. Analysis was done using real-time quantitative RT PCR to study the expression of 84 fibrosis genes. Nine patients with HCV and 3 HCV- negative individuals were enrolled in the study. We compared expression of genes associated with hepatic fibrosis as a consequence of HCV infection. Of 84 analyzed genes, SMAD-6, IL-13 and MMP-8 were overexpressed in HCV patients when compared to the healthy controls. On the other hand, CAV-1 was downregulated and statistically significant in all stages of fibrosis compared to healthy controls. To conclude, overexpression of SMAD-6 is significant predictor of HCV infection. Also, overexpression of IL-13 and MMP-8 may play a role in the development of HCV chronicity. On the other hand, downregulation of CAV-1 may contribute to exacerbation of liver fibrosis and progression to HCC.

Keywords: Biomarkers, infectious disease, molecular immunology

P-0480

A distinct subset of YKL-40 expressing macrophages drive tissue destruction and neovascularisation in giant cell arteritis

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Giant cell arteritis (GCA) is a large-vessel vasculitis affecting temporal artery and aorta. Macrophages mediate inflammation, angiogenesis and vessel-wall destruction in GCA. Serum levels of the macrophage-associated protein YKL-40, previously linked to angiogenesis and tissue remodeling, remain elevated in GCA despite glucocorticoid treatment. Here, we aimed to investigate the contribution of YKL-40 to vascular damage in GCA. Immunohistochemistry was performed on GCA temporal artery biopsies (TABs; n=12) and aortas (n=10) for detection of YKL-40, its receptor IL-13Rα2, macrophage markers PU.1 and CD206, and the tissue-destructive protein MMP-9. Ten non-inflamed TABs served as controls. Dynamics of YKL-40 production was studied with GM-CSF- or M-CSF-skewed macrophages (GM-MØs or M-MØs), *in vitro*. Next, silencing RNA (siRNA)-mediated knock-down of YKL-40 in macrophages was performed to study its effect on MMP-9 production. Finally, the angiogenic potential of YKL-40 was investigated by tube formation experiments using human microvascular endothelial cells (HMVECs). A distinct CD206+MMP-9+ macrophage subset expressed abundant YKL-40 in inflamed GCA vessels. GM-MØs of GCA patients, but not of healthy controls, released higher levels of YKL-40, compared to M-MØs. In inflamed TABs, IL-13Rα2 was expressed by macrophages and endothelial cells. Functionally, knock-down of YKL-40 led to a 10-50% reduction in MMP-9 production by macrophages, whereas exposure of HMVECS to YKL-40 led to increased tube formation. In GCA, a GM-CSF-skewed, CD206+MMP9+ macrophage subset expresses high levels of YKL-40 which may stimulate tissue destruction and angiogenesis through IL-13Rα2 signaling. Targeting YKL-40 or GM-CSF may inhibit macrophages that are currently insufficiently suppressed by glucocorticoids.

Keywords: Autoinflammation, chronic inflammation and fibrosis, cytokines and mediators, inflammatory disease, myeloid cells

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POSTER PRESENTATIONS

P-0481

Stromal cells in the complex colorectal tumour microenvironment modulate the innate immune system in 2D and 3D culture systems

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Colorectal cancer is the 3rd most common cancer worldwide. Patients with a high density of mesenchymal stromal cells (MSCs) and a strong immune signature in the tumour microenvironment (TME) have the worst disease free survival rates. However, the mechanisms by which MSCs and innate immune cells interact to promote tumour growth is not fully understood. Inflammatory tumour conditioned media (iTCM) generated from colon cancer cell lines contains potent chemokines and cytokines, including MCP-1 and RANTES. MSCs treated with iTCM show increased enrichment scores for innate immune regulation gene sets from RNA sequencing data and increased cell surface expression of the immunomodulatory ligands PD-L1 and CD47 by flow cytometry. The iTCM treated MSC can alter macrophage migration and antigen up-take and processing. A Gelatin Methacryloyl hydrogel based model of colon cancer incorporating the 3 cell types of interest was developed. It combined cancer cell lines, MSCs and THP-1s a monocytic cell line. MSCs didn't increase cancer cell proliferation in this 3D model, but altered matrix deposition and increased the release of tumour promoting and immune modulating molecules including CXCL12 and IL-6 in the culture system. Colorectal cancer in the inflammatory TME alters MSCs to induce a potent immunomodulatory phenotype, capable of modifying innate immune cell function. The 3D triple culture model of colorectal further demonstrates the role MSCs play in modulating the TME by contributing to tumour promoting inflammation. This research highlights the potential of therapeutically targeting MSCs in colon cancer patients and provides a multicellular model to test novel therapeutic.

Keywords: Cancer immunology, macrophage, microenvironment

P-0482

IL-17A in human liver: an important source of liver inflammation and an old new friend of retinoic acid

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Il-17A is considered to guide liver inflammation and fibrosis. Using human liver slice culture, we aim to study whether IL-17A is capable of influencing the fibrogenesis, but also, to look into lymphatic immune cell composition, secreting IL-17A. Using human liver samples (F0-F4) and blood samples, collected after partial hepatectomy due to different pathologies, we analyzed IL-17A secretion and immune cell profile. Liver tissue was used for primary culture of human liver slices, followed by subsequent cytokine stimulation and analysis by Elisa of fibrotic markers. The Huh7.5.1 cell line was used for SeaHorse and WB analysis. IL-17A concentration in human liver tissue was significantly higher in the early fibrotic stage compared with the advanced stage. Th17 T cells and, to a lesser extent, MAIT cells are the main sources of IL-17A in both compartments, liver and blood. Moreover, the presence of liver Th17IL-17A+INFγ+ cells were detected. IL-17A stimulation of human liver slices increases the expression of profibrotic markers. Stimulation by IL-17A+TGF-β1 removes the reserve respiratory capacity in Huh7.5.1 cells, while RA addition is capable to restore it and to reverse the expression of LC3II/LC3I ratio. IL-17A, secreted by Th17 and MAIT cells, induce the expression of pro-fibrotic markers. However, the level of hepatic IL-17A secretion is probably more related to the underlying pathologies that cause fibrosis rather than to the mechanism of fibrosis itself. There is also a terrain of communication between IL-17A and retinoic acid in the liver, that should be investigated.

Keywords: Chronic inflammation and fibrosis, cytokines and mediators, immune communication, inflammatory molecules, MAIT cells, tissue damage and repair

P-0483

Strong viral infection causes γδT cell mediated relative hypoglycemia which promotes the innate anti-viral immune response

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Viral infection has a major impact on systemic metabolism. In humans, severe infection may lead to hypoglycemia and hyperglycemia, but how this is regulated on a molecular level, nor how it benefits the host is unknown. We recently showed that mild viral infection alters endocrine regulation of systemic blood glucose, without dysglycemia. Here, we investigated how severe infection impacts regulation of blood glucose. We showed that infection of mice with high, non-lethal titres of mCMV or LCMV caused transient, relative hypoglycemia. Low blood glucose levels were beneficial to the host as enforced hyperglycemia during infection resulted in a significant increase of viral titres in peripheral organs. This effect depended on IFNγ secreted by γδT cells, as TCRδ-/- mice and animals treated with IFNγ-neutralizing antibodies failed to develop hypoglycemia upon infection. Infection-induced IFNγ caused specific insulin resistance in muscle, but not in liver, leading to increased insulin secretion by the pancreas. As a result, hepatic glycogenolysis and liver glucose output were reduced, leading to lower systemic glucose levels. Limited glucose availability amplified cellular stress response of infected cells, resulting in higher production of type-I interferons which reduced viral replication. When glucose levels were increased, artificially or due to diabetes, viral loads were strongly increased because of an impaired type-I interferon response, both *in vitro* and *in vivo*. Our findings indicate that reduction of blood sugar levels during infection is a well-regulated part of the body's natural anti-viral response. This response is derailed in diabetes leading to increased susceptibility to infections.

Keywords: Diabetes, innate host defence, metabolic control of immune responses, viral infections

P-0484

Seroprevalence of viral hepatitis B and markers of seroprotection in a population living in Senegal

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Evaluate the immune status to hepatitis B virus (HBV) in a population living in Senegal by looking for markers of infection and seroprotection. This is a prospective, descriptive and analytical study that took place in 2019 at the Medical Biology Laboratory of the General Hospital Idrissa Pouye (LBM-HOGIP). The inclusion criterion was the presence at LBM-HOGIP for a blood test. HBsAg and anti-HBcAb were investigated in all participants and anti-HBsAb titrated in HBsAg negative individuals. A microparticular immunological technique was used as analytical method. Data have been analysed using SPSS Statistics. This study included 236 patients. The study population mainly consisted of women (52.54%). The median age was 38 years with extremes of 6 and 93 years. The most representated age group was 30 to 39 years (25.85%). The prevalence of HBsAg carriage was 19.91%. Anti-HBcAb were present in 72.46% of participants. No significant association was found between the risk factors studied and the carriage of HBsAg. Only 49.15% of the study population remained protected against HBV (anti-HBsAb >10 mIU/ml). HBV infection is a public health problem in limited resource settings such as Senegal because of its serious complications such as cirrhosis and primary liver cancer. Our data have shown a high prevalence of HBV infection in the study population as well as a large percentage of unprotected subjects. Therefore, vaccination remains the best means of prevention against HBV.

Keywords: Antibody, biomarkers, protection, viral infections

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POSTER PRESENTATIONS

P-0485

Cross-reactivity of antibodies against Spike protein of SARS-Cov-2 towards commensal microbiota

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Coronavirus Disease 2019 (COVID-19) is a systemic inflammatory disease initiated by a SARS-CoV-2 virus infection of the lung and gastrointestinal tract. SARS-CoV-2 virus infects cells via interaction of the Spike (S) protein with the receptor-binding domain (RBD) of the ACE2 receptor expressed by various cell types. Blocking of this crucial interaction by monoclonal anti-SARS-CoV-2-RBD antibodies confers protection of the host against infection of the target cells. Here we report the presence of anti-RBD IgA antibodies at mucosal surfaces in healthy individuals. Additionally we observed that neutralizing monoclonal anti-RBD antibodies bind distinct commensal bacterial species, suggesting a cross-reactivity of these anti-RBD antibodies towards the commensal microflora. Using 16S rRNA sequencing and bacterial cultures we have identified distinct microbiota strains recognized by these antibodies. Next, oral supplementation with isolated bacterial strains induced cross-reactive anti-RBD IgA antibodies *in vivo*. Finally, severe COVID-19 patients lack some of those bacteria in their oropharynx and feces. Taken together, distinct microbial species of the human oral and gut microbiota can induce secretory IgA antibodies, which are cross-reactive to the RBD of SARS-CoV-2.

Keywords: Antibody, microbiome and environmental factors, viral infections

P-0486

T cell activation, highly armed cytotoxic cells and a shift in monocytes CD300 receptors expression in severe COVID-19

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COVID-19 manifests with a wide diversity of clinical phenotypes characterized by dysfunctional and exaggerated host immune responses. Many results have been described on the status of the immune system of patients infected with SARS-CoV-2, but there are still aspects that have not been fully understood. The aim of this study was to analyze phenotypically monocytes, T cells, NK cells and B cells in COVID-19 patients with different disease severity. Peripheral blood mononuclear cells from 44 COVID-19 patients with mild, moderate and severe disease and 12 healthy donors were analyzed using multiparametric-flow cytometry. Conventional and unsupervised data analysis were performed. Patients with severe disease exhibited a higher state of activation in all T cell subsets (CD4+, CD8+, double negative and T follicular helper cells), high expression of perforin and granzyme B in cytotoxic cells, expansion of adaptive NK cells and the appearance of activated and immature dysfunctional monocytes. These monocytes are identified by a low expression of HLA-DR and an intriguing abrupt change in the expression pattern of CD300 receptors. More importantly, correlation analysis showed a strong association between these alterations and the clinical signs of severity. Patients with severe COVID-19 have altered immune cell phenotypes and these changes are associated with the severity of the disease.

Keywords: Myeloid cells, adaptive immunity, B lymphocytes, memory, NK cells, parasite infections

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P-0488

Vancomycin resistant bacteria exacerbate DSS colitis in mice by extraintestinal dissemination via TLR4-TNF axis

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The incidence of inflammatory bowel diseases (IBD) is growing worldwide in the last decades. Such increase is associated with reduced microbiota diversity caused by environmental factors and increased use of antibiotics. In particular, vancomycin is used as a broad-spectrum antibiotic in clinical practice as the last line of defense directed to bacterial infections. However, such treatment may also result in the appearance of vancomycin-resistant enterococci infection. The mechanism of this process is not completely understood. Here we report the mechanism of severe pathology induced by vancomycin modification of the microbiota followed by the acute injury of the gut. To study the effects of vancomycin-induced changes in microbiota on the development of inflammation, we have pretreated mice with antibiotic for 2 weeks followed by the induction of the acute dextran sodium sulfate (DSS) colitis. Such treatment in C57Bl/6 mice resulted in the increased weight loss and a higher level of mortality when compared to control counterparts. Increased mortality in vancomycin treated mice was associated with disseminating *Klebsiella sp*., and *E. coli* to extraintestinal tissues. Furthermore, vancomycin treatment significantly reduced the number of CD11b+CD11cint granulocytes during colitis. Moreover, this pathology was driven via TLR4, since TLR4KO mice were protected from high mortality and bacterial dissemination during the DSS colitis after vancomycin pretreatment. Vancomycin-induced modification of the microbiota predisposes mice towards opportunistic bacteria dissemination upon acute colonic injury via TRL4 mediated recognition. Material-Methods: murine DSS colitis model, FACS analysis, CFU's counting

Keywords: Inflammatory bowel disease, innate immunity, microbiome and environmental factors

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POSTER PRESENTATIONS

P-0489

Host defense antimicrobial peptides from pulmonary surfactant against multidrug-resistant respiratory pathogens

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The appearance of multidrug-resistant respiratory pathogens such as *Klebsiella pneumoniae* or *Pseudomonas aeruginosa* has increased the need to develop new antimicrobial therapies. Attention is focused on host defense antimicrobial peptides (AMPs), which play a critical role in warding off invading microbial pathogens and could improve the efficacy of conventional antibiotics. Two promising host defense factors are pulmonary surfactant protein (SP-A) and the antimicrobial peptide SP-BN, which exert synergistic action against *K. pneumoniae* K2. Therefore, the aim of this work was to evaluate and characterize the synergistic antimicrobial activity of SP-A and SP-BN, with and without conventional antibiotics, against respiratory pathogens. The effect of SP-A/SP-BN on bacterial membranes was studied by spectrophotometric techniques and transmission electron microscopy, while the effect on bacterial model membranes was analyzed by differential scanning calorimetry. Synergistic activity of SP-A and/or SP-BN with conventional antibiotics was studied through killing assay. The antimicrobial activity of SP-A/SP-BN is based on the capability to alter the integrity of bacterial membranes. SP-A/SP-BN alters the bacterial ultrastructure of *K. pneumoniae* due to the ability to bind to lipopolysaccharide molecules present in the outer membrane, forming pores that favor the translocation of both proteins to the periplasm and causing permeabilization and depolarization of the cytoplasmic membrane, perhaps through the induction of toroidal pores. On the other hand, SP-BN could act synergistically with conventional antibiotics against *K. pneumoniae* and *P. aeruginosa* and it did not show cytotoxicity in human alveolar epithelial cells. These results show the potential antimicrobial use of AMPs against respiratory pathogens.

Keywords: Infectious disease, bacterial infections, innate host defence

P-0490

Seroprevalence of viral hepatitis B and markers of seroprotection in a population living in Benin

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Evaluate the immune status to hepatitis B virus (HBV) of a population living in Benin by looking for markers of infection and seroprotection. This is a prospective, descriptive and analytical study that took place in 2020. The selection criterion of patients was the presence at the Medical Biology Laboratories of health centres of the Archdiocese of Cotonou for a biological examination. HBsAg and anti-HBc Ab were investigated in all patients and anti-HBs Abs titrated in HBsAg negative individuals. A microparticular immunological technique was used as analytical method. Data have been analysed using SPSS Statistics Software. This study included 234 patients, mostly women (61.97%). The median age was 29 years with extremes of 10 and 65 years. The most represented age group was 20 to 29 years (46.58%). The prevalence of HBsAg carriage was 12.39%. In our population, 51.28% of the participants were anti-HBc Ab positive. The association between piercing and HBsAg carriage was significant. Only 31.20% of the study population were protected against HBV (anti-HBs Ab >10 mIU/ml). Our population has a higher female predominance than the general population. Our HBsAg prevalence is consistent with data from similar studies but higher than the national prevalence. The low seroprotection rate in our population is in line with available data. It is important to strengthen the health policy against HBV.

Keywords: Antibody, biomarkers, protection, viral infections

P-0492

Malondialdehyde-acetaldehyde antibodies occur in systemic lupus erythematosus and associate specifically with neuropsychiatric involvement

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Systemic lupus erythematosus (SLE) is a heterogenous autoimmune disease characterized by a global loss of self-tolerance. Although autoantibodies are an important hallmark of SLE, many autoantibodies are not specific for SLE or for a specific SLE manifestation. Post-translational modifications (PTMs), including citrullination and carbamylation, have been studied extensively in rheumatoid arthritis and antibodies against these PTMs are associated with disease progression. While PTMs have also been detected in SLE patients, studies on the presence of anti-PTM antibodies and the relation to clinical aspects remain limited. IgG antibody responses against six PTMs (malondialdehyde-acetaldehyde adducts (MAA), advanced glycation end-products (AGE), carbamylation (CarP), citrullination (Cit), acetylation (AL) and nitration (NT)) were tested in sera of 349 SLE patients and compared to 108 healthy controls. Levels and positivity were correlated with clinical features and SLE manifestations. Anti-MAA, -AGE and -CarP antibodies showed significantly higher positivity in SLE compared to controls (MAA: 29 vs 3%, AGE: 18 vs 4%, CarP: 14 vs 5%, all $p \leq 0.0001$). Anti-MAA and anti-AGE antibodies both correlated with clinical and serological measures associated with SLE. Patients with major neuropsychiatric SLE (NPSLE) showed higher positivity of anti-MAA antibodies (39 vs 24%, $p = 0.007$) and anti-CarP antibodies (20 vs 11%, $p = 0.042$) compared to patients without NPSLE. In conclusion, SLE patients have anti-PTM antibodies against MAA, AGE and CarP modified proteins. Interestingly, anti-MAA antibodies clearly associate with NPSLE, a clinical condition for which virtually no biomarkers exist.

Keywords: Antibody, autoimmunity, biomarkers

P-0493

Multiple faces of IgG4-realted disease: case report

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IgG4-related disease (IgG4-RD) can be a diagnostic challenge for the physician. We present a case of a patient with IgG4-RD characterized with aortitis, periaortitis and involvement of different organs. A 59-years old male patient presented with a groin and abdominal pain and reduced urinary output that progressed over the period of 3 weeks. His medical history was positive for hypothyroidism, autoimmune thyroiditis and thyroid ophthalmopathy. Thorough evaluation with multidisciplinary approach was performed. MDCT of the abdomen and angiography of the aorta and its branches revealed the abdominal aortic aneurysmin the infrarenal segment, retroperitoneal fibrosis (RPF) and bilateral renal hydronephrosis. A JJ stent was placed. Laboratory analysis showed elevated inflammatory markers, total serum IgG (20.13g/l) and IgG4 (5.4g/l). Clinical course was complicated by the development of acute renal injury requiring temporary hemodialysis. PET CT scan showed an irregular soft-tissue mass retroperitoneally, which in the form of a muff wrapped the aorta and lower vena cava in their infrarenal segment, continuing caudally along the common iliac arteries on both sides. The retroperitoneal mass of 50x85x170 mm in diameter, with enhanced FDG uptake was detected. Diagnosis of IgG4-RD encompassing inflammatory abdominal aortitis, periaortitis, RPF, with elevated IgG4 level was made. The patient was treated with methylprednisolone with a good clinical and laboratory response. It was speculated that thyroid ophthalmopathy for which the patient was previously treated may be the first presentation of IgG4-RD. Multidisciplinary and comprehensive approach is necessary for timely recognition and treatment of patients with IgG4-RD.

Keywords: Antibody, autoimmunity, chronic inflammation and fibrosis, inflammatory disease

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POSTER PRESENTATIONS

P-0494

The role of obesity on allergic reactions and tolerance induction

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Along with allergic diseases, obesity is a severe health problem that is remarkably increasing in prevalence worldwide. Obesity has been recognized as an important risk factor for enhanced allergic reactions. However, the causal link between obesity and allergy is far from fully understood. C57BL/6 male mice were fed with high-fat diet (HFD) or standard chow diet (STD), and after nine weeks, mice were immunized and challenged with ovalbumin (OVA). To induce tolerance, mice were orally and intranasally treated with OVA prior to sensitization. Metabolic parameters and allergen-specific antibodies were measured in serum. The cell differential count was performed in bronchoalveolar lavage (BAL)-cytospins. Cytokine measurements were performed in lung tissue. HFD-fed animals exhibited increased body weight compared to STD-fed animals. In HFD-fed mice leptin levels were higher compared to STD-fed mice. Sensitization and challenge with OVA resulted in the infiltration of eosinophils and reduced macrophages numbers in BAL of HFD- and STD-fed mice. Allergen-specific antibody levels were induced in BAL and serum in OVA-treated groups. Th2-cytokine levels in the lung supernatants were significantly higher in the allergic group than in the PBS-treated mice. Furthermore, the HFD-fed group showed a significant increase of IL-5 and IL-13 compared to STD-fed animals. Oral and intranasal tolerance induction led to a shift towards the non-allergic phenotype in BAL, serum, and lung in sensitized animals. Further evaluation of the impact of HFD on immunological parameters is ongoing. Understanding a cross-talk: obesity – allergy – tolerance may identify novel approaches to combat morbidities associated with Westernized lifestyle.

Keywords: Allergen-induced immune responses, animal models, metabolic control of immune responses

P-0495

Evaluation of SARS-COV-2-specific T cell responses in COVID-19 patients and non-infected controls

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T-cell immunity plays a major role in viral infections: Th1 provide help to B Ly, CD4 and CD8 effectors eliminate virus-infected cells, limiting viral spread and replication. Unlike antibody response, viral-specific T cell immunity is less well studied. To assess SARS-CoV-2-specific CD4 and CD8 T in COVID-19 patients and non-infected exposed donors. Samples from hospitalized (A,n=7), convalescent (B,n=14) COVID-19 patients, and exposed controls (C,n=19, PCR(-), SARS-CoV IgG(-) were studied. Virus-specific CD4 and CD8 T were assessed according to CD69 and IFNγ expression after 16h *in vitro* stimulation with SARS-CoV-2 peptide megapools by flow cytometry. RBD-specific IgG and IgA were evaluated by ELISA (Euroimmun). SARS-CoV-2-specific T cells were detected in 95 % of patients, and 48% of controls. IFNγ+ CD4 and CD8 were significantly increased in active infection (mean% 0,70(A) vs.0,28(B) and 0,12(C); $p<0.05$), with CD8 responses dominating over CD4 (mean% IFNγ+ 0,44 vs.0,28;$p<0.05$). After recovery (B) CD69+CD4 T prevailed (mean% 0,35 vs. 0,23 CD69+CD8; $p<0.05$); IFNγ CD8 response dominated in a subset of donors. Levels of CD4 and CD8 SARS-CoV-2-specific T did not correlate with time past infection, or the presence and quantity of IgG and IgA virus-specific antibodies ($p>0.05$ for all). In group C, CD8 T cells specific for conservative non-structural peptides were mostly detected. Evaluation of virus-specific T cells aids monitoring of SARS-CoV-2 immune response. The levels of virus-specific CD4 and CD8 vary independently from the time past infection or exposure risk.

Keywords: Monitoring immunity, adaptive immunity, infectious disease, viral infections

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P-0496

PEGylation of SAAP-148 improves its immunomodulatory activities

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There is an urgent need for novel agents and strategies to combat bacterial infections not effectively treatable with current antibiotics. For this purpose we have developed the synthetic antibacterial and anti-biofilm peptide (SAAP)-148 which is highly effective against antimicrobial resistant (AMR) bacteria without inducing resistance. Challenges for further development of SAAP-148 include its cytotoxicity and short circulation half-life. To circumvent these drawbacks a library of SAAP-148 linked to polyethylene glycol (PEG) groups of various lengths was synthesized. This library was screened for *in vitro* antibacterial activity against (AMR) *Staphylococcus aureus* and *Escherichia coli*, anti-biofilm activity against *Acinetobacter baumannii* and haemolytic activity (as measure for cytotoxicity). Results indicated that PEGylated SAAP-148 variants combine antibacterial activities with reduced cytotoxicity compared to SAAP-148. Interestingly, several PEGylated variants showed enhanced immune modulatory activities. PEGylation of SAAP-148 enhanced peptide's capacity to chemoattract human neutrophils and monocytes and to redirect monocyte-macrophage differentiation towards type 1 macrophages. In addition, PEGylated SAAP-148 was found to lead to mature dendritic cells during the differentiation process of monocytes towards immature dendritic cells. It was found that the length of the PEG group, the position to which the PEG group is attached to peptide, and the sequence of the peptide are important for the conjugate's immune modulating activity. Together these results indicate thatPEGylated SAAP-148 is highly effective in redirecting monocyte-macrophage and promoting monocyte-mature dendritic cells development. Therefore, PEGylated SAAP-148 may be a promising agent to modulate inadequate immune responses in case of tumours and chronically infected wounds.

Keywords: Bacterial infections, dendritic cells, drugs for immune modulation, macrophage

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POSTER PRESENTATIONS

P-0497

Filaggrin-insufficient keratinocytes produce exosomes characterized by reduced capacity to promote CD1a-dependent responses

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Filaggrin (FLG) is a key structural protein expressed in epidermal keratinocytes. Mutations in the filaggrin gene (*FLG*) are strongly linked to atopic dermatitis (AD) and allergic inflammation occurring later in life at distant body locations in AD patients. Keratinocytes secrete exosomes, small, lipid-rich membrane vesicles, to communicate with distant cells, including with the immune system. Here, in a sh knock down model we investigated if filaggrin insufficiency impacts the ability of keratinocyte-derived exosomes (KCexo) to influence antigen-specific T cell responses. KCexo were isolated by ultracentrifugation. T cell responses to peptides, whole protein and lipid neoantigens were assessed by IFNγ ELISpot and ELISA. Lipidome and proteome analysis of keratinocytes and KCexo was performed by mass spectrometry. Reduced capacity of shFLGexo to mediate CD1a-dependent T cell responses was observed; T cell responses to peptides or whole protein were not affected. Decreased abundance of long chain polyunsaturated fatty acids (LC-PUFAs) and predominance of saturated fatty acids (SFAs) in shFLGexo was observed. ShFLG cells differed in their production of a number of proteins involved in the lipid metabolism, including downregulation of long-chain-fatty-acid--CoA ligase (ACSL3). FLG insufficiency in keratinocytes contributes to alterations in the lipid-enriched exosomal compartment. Decreased presence of LC-PUFA species in shFLGexo results in a reduced supply of lipids which act as sources of neoantigens for CD1a binding. This impairs CD1a-specific T cell response induction by shFLGexo, which could potentially contribute to chronic inflammation in tissues distant form the skin and promote additional allergic manifestations in AD patients.

Keywords: Adaptive immunity, allergic disorders, CD1-restricted T cells, skin diseases

P-0498

Functional validation of ZEB2 in antigen-specific CD8+ T cell responses

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ZEBs are known to regulate the transcriptional networks necessary for cell differentiation, maintenance, and function. Among ZEBs, ZEB2 plays a major role in promoting terminal differentiation, survival and function of effector/memory CD8+ T cells. Of note, the factual regulatory mechanism of ZEB2 in cytotoxic T cells is still unclear. Markedly, it would be intriguing to examine the functional and regulatory role of ZEB2 in antigen-specific T cell responses. Here, we performed whole-genome bisulfite sequencing on cytomegalovirus (CMV)-specific CD8+ T cells and identified four differentially methylated regions (DMRs) within the ZEB2 locus that were significantly hypomethylated in CMV-specific T cells when compared to memory and naïve CD8+ T cell controls. In concordance with these data, we found that the transcriptomic expression of ZEB2 is significantly upregulated in effector/memory CD8+ T cells when compared to central/memory and naïve CD8+ T cells isolated from the peripheral blood of CMV-seropositive donors. These data suggest that the expression of ZEB2 in antigen-specific effector/memory CD8+ T cells is firmly controlled through DNA demethylation. Additionally, the functional relevance of these DMRs is yet to be identified using luciferase reporter assays. Furthermore, the impact of ZEB2 on phenotypic/functional/cytotoxic properties of CD8+ T cells is currently studied by overexpressing ZEB2 in human CD8+ T cells. Collectively, our data will reveal the correlation between the expression of ZEB2 and the demethylation pattern of DMRs, and also improve the current understanding about the functional relevance of ZEB2 for antigen-specific T cell responses.

Keywords: Epigenetic control and modulation of immunity, infectious disease, memory, viral infections

P-0499

Besides strong inflammation, severe COVID-19 is determined by endothelial dysfunction and dysregulated cytokine networks

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To treat and manage life-threatening COVID-19 disease, it is required to understand processes involved in progression and recovery of severe cases. Despite intensive investigations, the mechanisms leading to severe COVID-19 progressions are still incompletely understood. We combined analyses of immune cells and cytokine/chemokine networks with endothelial activation and injury markers to define hallmarks of severe COVID-19 and to discriminate them from disease recovery in convalescent patients. Massive inflammation with prolonged loss of lymphocytes but expansion of granulocytes, monocytes and plasmablasts was observed in intensive care unit (ICU, N=28) patients. Immune hyperactivation was confirmed by the presence of highly activated HLA-DR+CD38+ late memory T and NK cells and high levels of SARS-CoV-2 specific antibodies. Plasma protein profiles of ICU patients revealed a highly inflammatory microenvironment and endothelial activation. ICU patients showed a core signature of seven plasma proteins including cytokines, chemokines, growth- and endothelial-factors, such as IL-6, CXCL-10 and HGF. Dynamic changes within this signature were associated with disease progression, starting with strong inflammation and followed by a disrupted endothelial barrier and multiple organ dysfunction. Recovery from COVID-19 correlated with increased levels of IL-13, IL-1β, GM-CSF and VEGF suggesting the induction of systemic repair mechanisms. Convalescent patients (N=17) regained regular numbers of all lymphocyte populations, although T and NK cells displayed a differentiated memory phenotype. Our data suggest that severe COVID-19 is not only driven by a strong inflammatory response but also by endothelial activation and barrier disruption, whereby recovery from COVID-19 is dependent on the regeneration of the endothelial integrity.

Keywords: Adaptive immunity, cytokines and mediators, tissue damage and repair, viral infections

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POSTER PRESENTATIONS

P-0501

High frequency of mature CD57+NKG2A- NK cells associate with poor disease progression after autologous hematopoietic stem cell transplantation in multiple myeloma patients

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Autologous hematopoietic stem cell transplantation (autoHSCT) is a worldwide-established treatment option for a diverse group of hematological malignancies, including multiple myeloma (MM). Natural killer (NK) cells are the first lymphocyte subset to recover during immune reconstitution after transplantation and day 15 post-autoHSCT NK cell counts associates with patient survival. The aim of this study was to phenotypically and functionally characterize NK cells during their reconstitution after autoHSCT. Peripheral blood mononuclear cells from 54 MM patients that underwent autoHSCT were studied by multiparametric flow cytometry. Six samples, collected before and after autoHSCT over a one-year period, were analyzed from each patient. Results showed that, shortly after leukocyte recovery, an extensive redistribution of NK cell subsets occurs in these patients. In addition, NK cells undergo a profound phenotypic change characterized, among other things, by their increased proliferative capacity and immature phenotype, while being fully functional. Importantly, MM patients who showed lower frequencies of the mature highly differentiated CD57+NKG2A- NK cell subset at +30 and +100 days after autoHSCT experienced superior progression-free survival and had a longer time to the next treatment than those who did not. The degree of NK cell maturation after autoHSCT affects the clinical outcome of MM patients treated with this therapeutic strategy.

Keywords: Biomarkers, cancer immunology, innate immunity, NK cells, transplantation

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P-0502

MiR-184 microRNA, overexpressed in Granulomatosis with Polyangiitis, regulates AKT2 in a granulocyte model

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MicroRNAs (miRNAs) regulate a wide range of biologic and pathologic processes mainly through translational repression. Previous data showed overexpression of miR-184 in nasal tissue of patients with Granulomatosis with Polyangiitis (GPA), an autoimmune vasculitis. Previous work suggests that proteinase 3 (PR3), the major autoantigen in GPA, may represent a direct target of miR-184. The aim of this study was to validate miR-184 as a putative regulator of GPA-relevant targets in a granulocyte model. NB4 cells were differentiated by all-trans retinoic acid for 24h. Granulocyte-like differentiation was analyzed by FACS, qPCR and western blot. Endogenous miR-184 expression was examined by miRNA-specific PCR. miR-184 and GPADH miRNA control was transfected using lipofection. Downregulation of target genes and proteins were analyzed by qPCR after 24h and by western blot after 48h. NB4 cells were differentiated into a CD11b+/CD14- granulocyte-like phenotype expressing PR3 mRNA and protein. Endogenous miR-184 expression was not found. Downregulation of GAPDH by control miRNA was validated on protein level. However, miR-184 did not downregulate PR3, but was able to downregulate a previously described target AKT2 on mRNA as well as on protein level. This study demonstrated that miR-184 regulates AKT2, but not PR3 in a granulocyte model. AKT2 is involved in cell death mechanisms such as apoptosis. Since miR-184 is highly overexpressed in nasal tissue of GPA patients, the miR-184/Akt2-axis might represent a potential new link to mechanisms of cell death regulation, driving autoimmunity in GPA.

Keywords: Autoimmunity, granulocytes, miRNA

P-0503

Investigating role of obesity, inflammation and immunity in cancer-related sarcopenia in oesophageal cancer

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Cachexia is the ongoing involuntary loss of muscle mass (sarcopenia), leading to poor quality of life, reduced treatment efficacy and increased mortality. This study aims to elucidate the soluble and cellular mediators underpinning the development of oesophageal cancer (OC) cachexia. Sarcopenic and non-sarcopenic patients within a cohort of 373 OC patients were identified by body composition measurements. Inflammatory mediators and muscle growth/breakdown markers were quantified by ELISA in serum and muscle conditioned media at time points pre- and post-chemoradiotherapy (CRT). Muscle biopsies were collagenase-digested and immune-phenotyped. The number of sarcopenic OC patients increased by 50% following CRT. Circulating levels of the muscle growth factor follistatin and the macrophage chemoattractant protein-1 (MCP-1) were significantly higher in patients following CRT ($p<0.001$). Interestingly, circulating interleukin-15 (IL-15) which has previously been shown to mitigate inflammatory skeletal muscle loss, was also increased following CRT ($p<0.05$). Intramuscular levels of follistatin, myoglobin, activin A and creatine kinase K together with MCP-1, IL-6, IL-33 and IL-1Beta, were significantly higher than circulating levels suggesting their involvement in muscle loss in this cohort ($p<0.05$). Moreover, marked differences were observed between the serum and muscle of nonsarcopenic and sarcopenic patients. Finally, immune-phenotyping data indicated higher intramuscular infiltrations of CD4+ and CD8+ T cells in sarcopenic OC patients. Our data indicate that CRT contributes to the progression of sarcopenia in OC. Further work will elucidate the role of follistatin, MCP-1 and IL-15 play and whether our panel of soluble mediators have prognostic and/or therapeutic utility in OC cachexia.

Keywords: Biomarkers, cancer immunology, inflammatory disease

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POSTER PRESENTATIONS

P-0504

Can IL-13 polymorphism act as a marker of disease activity in rheumatoid arthritis patients?

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Interleukin-13 (IL-13) is a key cytokine of Th2 cell-mediated immune responses. IL-13 has important immunomodulatory activities and exerts influence on broad range of immune cells. Recent studies have implicated role of IL-13 in pathogenesis of autoimmune diseases, including rheumatoid arthritis (RA). The purpose of this study was to assess a potential influence of IL-13 gene polymorphism on clinical parameters RA disease activity. In total 579 RA patients, including 113 of Greek and 466 of Polish origin, were enrolled to the study. Genotyping for IL-13 rs20541 was performed employing LightSNiP assays. Disease activity was evaluated according to Disease Activity Score-28 (DAS28). Both patient groups showed a similar distribution of rs20541 variants, but differed in the frequencies of the HLA-DRB1*04:01, *04:05, *10:01 shared epitope alleles ($p<0.001$, $p<0.001$, and $p=0.011$, respectively). The IL-13 rs20541 was significantly associated with disease activity in Polish patients diagnosed with RA. Patients bearing IL-13 rs20541-GG genotype had significantly lower DAS28 score than patients carrying other genotypes ($p=0.023$). On the other hand, significantly higher DAS28 score was more frequently observed among patients with IL-13 rs20541 heterozygous genotype as compared to other genotypes ($p=0.007$). No association was observed between IL-13 rs20541 polymorphism and DAS28 in Greek patients.

These results suggest that IL-13 polymorphism may influence disease activity in RA patients in Polish population. The lack of association in Greeks may be due to inter-population genetic differences between these patient groups and the number of patients studied.

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Keywords: MHC and polymorphic genes, cytokines and mediators, rheumatoid arthritis

P-0505

Epigenome-editing of CD8+ T cells to improve the efficacy of personalized CMV-specific T-cell immunotherapies for immunocompromised patients

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Infectious complications of persistent viruses like cytomegalovirus (CMV) remain clinically challenging in immunocompromised patients, including transplant recipients, despite antiviral therapy. Adoptive transfer of CMV-specific T cells offers an effective and non-toxic immunotherapeutic modality for the immediate and long-lasting control of CMV infections. However, the epigenetic signatures of adoptively transferred IFNγ+ T cells are not well defined. Our current study aims to identify the epigenetic signature of human CMV-specific CD8+ cytotoxic T cells (CTLs) with respect to global DNA methylation and to use this knowledge to improve the efficacy of personalized CMV-specific T cell immunotherapies for immunocompromised patients via CRISPR/dCas9-based epigenome-editing. Here, we performed whole-genome bisulfite sequencing from CMV-specific CTLs of five CMV-seropositive blood donors to identify the epigenetic signature of CMV-specific CTLs. Furthermore, the functional relevance of the newly identified differentially methylated regions (DMRs) of CMV-specific CTLs is determined using luciferase reporter assays. Additionally, correlation of the epigenetic signatures with cytotoxic activity of the T cells will be validated. Currently, two versions of epigenome-editing systems, a dCas9-TET1CD fusion molecule and the Casilio system, are established by targeting the well-studied methylation-sensitive FOXP3 locus. This tailored epigenome-editing shall be applied to improve the efficacy and cytotoxicity of CMV-specific CTLs by manipulating the methylation status of promising candidateregions. Prospectively, our data will broaden our knowledge of the epigenetic landscape of CMV-specific CTLs and advance adoptive T cell therapy beyond current state-of-the-art as a personalized and GMP-compliant T cell immunotherapy using epigenome-profiling and -editing.

Keywords: Epigenetic control and modulation of immunity, immune communication, immune regulation and therapy, memory

P-0507

Interleukin-10 and interleukin-23 in primary and metastatic brain tumors

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The aim of the study was to evaluate the serum levels of interleukin (IL)-10 and IL-23 in patients with primary and metastatic brain tumors. The serum samples were collected from 36 cases and 37 healthy subjects without current inflammatory or neoplastic diseases. The case included 20 persons with primary tumors (30% of cases with low-grade and 70% with high-grade gliomas) and 16 persons with brain metastases from primary lung, colorectal, or breast tumors. The cytokine serum levels were determined by ELISA assays. Data were analyzed by One-Way-Anova followed by Tukey test for multiple comparisons. The mean level of IL-10 in metastatic brain tumors (15.8±5.7pg/ml) was significantly higher in comparison with primary tumors (4.2±1.3pg/ml; $p=0.011$) and healthy controls (1.9±0.24pg/ml; $p=0.002$). In addition, cases with low-grade primary brain tumors showed lower IL-10 (3.41±1.22pg/ml) than cases with high-grade tumors (5.38±1.99pg/ml; $p>0.05$). These results confirm the role of IL-10 in brain tumors and point to IL-10 as a factor with metastatic potential. Contrary, serum IL-23 between cases with primary and metastatic brain tumors were very similar (26.09±8.18p/ml vs. 26.08±10.3pg/ml) and higher than controls (14.03±1.6pg/ml; $p>0.05$). The paired analysis of post- and pre-operative cytokine serum levels showed a tendency for the post-operative elevation of IL-23 in the majority of cases within a range of 0-112.53pg/ml, in contrast to IL-10 levels, among a limited group of patients with primary brain tumors. In conclusion, our data confirm the impact of IL-10 and suggest the potential of IL-23 in brain tumorigenesis.

Keywords: Cancer immunology, cytokines and mediators, immune networks

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POSTER PRESENTATIONS

P-0508

Effects of immunosuppressive drugs on characteristics and functional properties of bone marrow-derived stem cells isolated from patients with diabetes mellitus

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Patients with diabetes mellitus who suffer from diabetic foot ulcers receive autologous stem cell treatment (ASCT) as last therapeutic option. ASCT is indicated also to diabetic patients after solid organ transplantations who are using immunosuppressive drugs (ISs). However, there is still limited information about the impact of ISs on SCs. Therefore, the aim of this project was to investigate effects of ISs on characteristics and functional properties of bone marrow-derived SCs. SCs were isolated from bone marrow of diabetic patients, cultivated and phenotypically characterized by flow cytometry. These SCs were cultured in presence of different ISs (tacrolimus, mycophenolate mofetil, sirolimus) in various concentrations (0.05 – 500 µg/ml) and the impact of ISs on metabolic activity was measured by WST-1 assay, the expression of genes for immunoregulatory molecules was detected by RT-PCR, the apoptosis was studied by flow cytometry and the production of cytokines was determined by ELISA. The mononuclear fraction cultivated for 14 days contained 64% of mesenchymal stem cells (CD45-CD73+CD90+CD105+), 24% of myeloid angiogenic cells (CD45+CD146-), 7% of endothelial colony-forming cells (CD45-CD146+) and 5% of other cell populations. ISs significantly inhibited the metabolic activity, the expression of genes for immunoregulatory molecules, the production of cytokines and growth factors. On the contrary, the apoptosis was enhanced in the presence of ISs. The results showed that ISs in a dose-dependent manner have negative effects on characteristics and functional properties of SCs. Therefore, this study suggested that ISs could decrease the therapeutic potential of ASCT in diabetic patients.

Keywords: Cell based therapies, diabetes, drugs for immune modulation, stem cells, transplantation

P-0509

Age-associated changes to innate immune responses of resident lung cells

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The immune system undergoes age-associated alterations that render older adults at a higher risk of developing severe respiratory infections. However, little is known about how the lung innate immune system ages. Our work aims to identify potential age-associated functional differences in lung resident innate immune cells using young (8-14 weeks) and aged (17-18 months) male C57/Bl6 mice. *Ex vivo*: Young and aged alveolar macrophages (AMs) were stimulated with LPS for 16h. Cytokine and chemokine gene expression and production were analysed by RT-qPCR and ELISA. *In vivo*: Young and aged mice were intranasally challenged with LPS for 4h. Lung inflammation was investigated by flow cytometry. AMs and epithelial cells were FACS-sorted from whole lungs and their cytokine and chemokine gene expression evaluated by RT-qPCR. Aged AMs stimulated *ex vivo* with LPS exhibited significantly increased expression of *Cxcl2*, *Tnf* and *Il1ß* and a similar trend was seen for *Ccl2*. Significantly increased production of CXCL1, CXCL2 and TNF was detected in the supernatants from aged AMs. Following *in vivo* LPS challenge, neutrophil recruitment was significantly increased in the aged lung. In aged epithelial cells, *Ccl2*, *Cxcl2* and *Tnf* expression was significantly elevated. *Ccl2* was significantly increased in aged AMs and similar trends were observed for *Cxcl2* and *Tnf*. Combined, our results demonstrate increased inflammation following LPS challenge in aged mice and an overall more pro-inflammatory phenotype in aged lung innate cells, which could have major implications for the outcome of respiratory infections in older adults.

Keywords: Immune senescence, ageing, animal models, infectious disease, innate immunity, macrophage

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P-0511

Deep phenotypical characterization of human CD3+CD56+ T cells by mass cytometry

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CD56+ T cells are a group of pro-inflammatory CD3+ T lymphocytes with characteristics of natural killer cells, being involved in antimicrobial immune defense. Here, we performed a deep phenotypic profiling of CD56+ T cells of peripheral blood of normal human donors and individuals sensitized to birch-pollen or/and house dust mite by high-dimensional mass cytometry combined with manual and computational data analysis. A co-regulation between major conventional T cell subsets and their respective CD3+CD56+ cell counterparts which we herein demonstrate, appeared restricted to CD8+, MAIT, and TCRγδ+ T cell compartments. Interestingly, we find a co-regulation of several CD3+CD56+ cell subsets in allergic but not in healthy individuals. Moreover, using FlowSOM, we distinguished a variety of CD56+ T cell phenotypes demonstrating a hitherto underestimated heterogeneity among these cells. The novel CD3+CD56+ subset description comprises phenotypes superimposed with naive, memory, type 1, type 2, and type 17 differentiation stages, in part represented by a phenotypical continuum. Frequencies of 2 out of 19 CD3+CD56+ FlowSOM clusters were significantly diminished in allergic individuals, demonstrating less frequent presence of cells with cytolytic, presumably protective, capacity in these donors consistent with defective expansion or their recruitment to the affected tissue. Our results contribute to defining specific cell populations to be targeted during therapy for allergic conditions.

Keywords: Allergic disorders, MAIT cells, NKT cells

P-0512

Cytomegaloviruses induce massive reorganization of Arf proteins in the early phase of infection

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Cytomegaloviruses (CMVs) are herpesviruses that can cause life-threatening diseases in immunocompromised patients. During the early phase of the infection CMVs cause massive reorganization of the cellular membranes that finally results in formation of a cytoplasmic virion assembly compartment (cVAC) and impaired trafficking of many proteins, including molecules that modulate immune response. Arf proteins are small GTPases that organize cellular cytoskeleton and control membrane dynamics, thus regulating traffic within secretory and endocytic system. The aim of this study was to determine expression and intracellular localisation of Arf proteins in the early phase of CMV infection and to define how these changes reflect to endocytosis and recycling of MHC-I molecules. Murine embryonic fibroblasts (Balb/3T3) were infected with recombinant murine cytomegalovirus Δm138-MCMV and expression of Arfs was determined by confocal microscopy and WB. Arfs intracellular localisation was determined by co-localization with different markers of intracellular compartments. Kinetics of MHC-I endocytosis and recycling was determined by flow cytometry on uninfected and MCMV infected cells in which Arfs were normally expressed or silenced by siRNA. Arf proteins are overexpressed and/or overactivated during the early phase of MCMV infection and are accumulated in the juxtanuclear region in the area of cVAC formation. This leads to impaired endosomal trafficking of many proteins, including MHC-I molecules. Overexpression/overactivation of Arfs, especially Arf6, may represent the earliest alteration which leads to the reorganization of the endosomal recycling system and downregulation of MHC-I molecules during the CMV infection. Better understanding of these processes could result in development of new antiviral treatments.

Keywords: Endo- and exocytic vesicles in immunity, MHC and polymorphic genes, viral infections

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POSTER PRESENTATIONS

P-0513

Immune receptors modulate eosinophilic functions in viral immunity

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Eosinophils involvement in a broad spectrum of pathological conditions such as acute and chronic infections, cancer and thrombosis make them an interesting research topic. Despite their roles in parasitic infections, there is a growing body of evidence that eosinophils play roles in fungal, bacterial and viral infections. Studies on mouse and primary human eosinophils reveal the importance of eosinophils against viruses that cause airway inflammation such as influenza and respiratory syncytial viruses. Moreover, recent studies on SARS- Cov-2 infection show eosinopenia in Covid-19 patients. However, the question how eosinophilic PRRs and immune receptors (IRs) are affected by viral infections remains elusive. Thus, the aim of this study is to elucidate the regulation of eosinophilic functions in antiviral immunity by measuring the production of matrix metalloproteinases, eosinophil cationic protein, eosinophil-derived neurotoxin. Initially, human eosinophilic Eol-1 cells were stimulated with various viral stimuli such as Poly I:C, R848, ssRNA40. The mRNA levels of PRRs, IRs, ECP and EDN were determined by QPCR and protein levels of TLR3, TLR7, TLR8, CARD9 were measured by western blotting. Additionally, we determined the MMP2 and MMP9enzyme activities by gelatin zymography in response to viral stimulants. Of all the stimuli we tested, ssRNA40 was the most potent in inducing the mRNA expressions of TLR-3, TLR-7, TLR-8, CD147, CARD9, ECP, EDN, FcεR1a and FcεR2. Interestingly, CARD9 which is an key receptor for fungi infections was significantly elevated after treatment with TLR7/8 ligands, suggesting a primary role for TLR7 and 8 rather than TLR3 in viral defense by eosinophils.

Keywords: Molecular immunology, eosinophils, viral infections

P-0516

Children and adults with mild COVID-19 symptoms develop T cell immunity to SARS-CoV-2

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Severe acute respiratory syndrome coronavirus-2 virus (SARS-CoV-2) is causing a global pandemic that, even with current effective vaccines, is still not under control. The number of coronavirus disease-2019 (COVID-19) deaths worldwide exceeds 3 million, but most infections, especially among children, have a mild course. However, it remains largely unknown whether persistent immune memory is being developed after mild disease. To evaluate the development of SARS-CoV-2-specific memory T cell immunity, we performed IFN-γ ELISPOT and activation marker expression analyses on longitudinal samples collected from children and adults with mild to moderate COVID-19. After stimulation with a set of overlapping peptides of SARS-CoV-2 spike protein, high frequencies of interferon-gamma positive (IFN-γ+) T cells were found in infected children (83%) and adults (100%), whereas T cell responses were minimal or absent in samples from unexposed healthy children (0%) or adults (8.3%) taken prior to the pandemic. Frequencies of SARS-CoV-2-specific IFN-γ+ T cells were higher in infected adults, especially in those with moderate symptoms, compared to infected children. Predominantly CD4+ T cells of the effector memory (CD45RO+/CCR7-) subset were reactive against SARS-CoV-2-antigens as shown by co-expression of activation markers, CD25 and CD137. Low frequencies of SARS-CoV-2-reactive CD8+ T cells were detected. We found no evidence that the pre-existing T cell response against seasonal human coronavirus, HCoV-OC43, played a role in the mild course of SARS-CoV-2 infections. Importantly, our data indicate that T cell immune memory is developed in children and adults with mild symptomatic SARS-CoV-2 infection. These responses may provide protection against SARS-CoV-2 re-infection.

Keywords: Adaptive immunity, cytokines and mediators, follicular helper T cells, infectious disease, viral infections

P-0517

Evaluation of PD-1 and Tim-3 expression and their effect on cytokine production by T-cells in allergic asthma and rhinitis

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The aim of this study was to determine the expression of co-inhibitory receptors (PD-1 and Tim-3) on T-cells and to assess their effect on cytokine production by T cells in allergic asthma (AA) and rhinitis (AR) patients. Isolated PBMCs were cultivated in RPMI-1640/10% FCS, with PMA/Ionomycin and anti-CD3/anti-CD28 antibodies for 20 hours. Cells were stained with antibodies against the antigens CD3, CD4, PD-1, Tim-3, intracellular staining was for IL-4 and IFNγ. For PD-1 and Tim-3 assessment before cultivation, PBMCs were stained for surface markers only and then were analysed on the FACS CantoII cytometer. Data was analysed using non-parametric comparison methods. Patients with AR were characterised by a reduced number of $CD4^+Tim3^+$ cells in the blood compared to healthy controls, while there were no differences in the AA group. After cultivation, the percentage of T cells expressing PD-1 and/or Tim-3 was also lower in AR patients. However, there was increasing of $PD\text{-}1^+$ and $PD\text{-}1^+Tim\text{-}3^+$ T-cells in AA patients compared to AR patients. As for cytokine production, cells expressing PD-1 and/or Tim-3 produced more cytokines (IL-4, IFNγ) compared to the $PD\text{-}1^-Tim3^-$ populations within each group. In AA patients there were less cytokine-producing cells than in AR patients, despite the bigger percentage of cells with PD-1 and/or Tim-3 expression. Overall, regardless of the allergy presence, there was no decrease in the production of IL-4 and IFNγ by T-cells expressing PD-1 and/or Tim-3. On the contrary, individual populations, positive for PD-1/Tim-3 expression, showed an increase in cytokine production after stimulation.

Keywords: Adaptive immunity, allergic disorders, checkpoint inhibition, cytokines and mediators

P-0518

Influence of HCMV-variants expressing NKG2D ligands on immune cell activation

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HCMV-infection elicits protective NK/T cell responses. Patients with inherited or acquired NK/T cell impairments, e.g. recipients of organ transplants under immunosuppression, are at high risk of HCMV-infection. A HCMV-vaccine would not only greatly reduce the risk for reactivation/disease in those patients, but also lower the incidence of graft rejection. A HCMV-vaccine should be attenuated, but retain the ability to induce HCMV-specific T cells. Thus, we aimed at comparing HCMV-mutants that express the NKG2D ligand ULBP2 and retain or lack immune-evasins for HLA molecules. Such mutants are expected to differ in attenuation and control by NK cells and their ability to activate HCMV-specific CD8+ T.For this, ULBP2-expressing mutants based on the parental TB40-strain (ΔUS2-6) were constructed by replacing the UL16-gene with the ULBP2 transgene under control of different promotor strengths. Fibroblasts infected with TB40-WT or the mutants were phenotypically characterized with respect to HLA class I and NK-ligands. Furthermore, we co-cultured fibroblasts infected with these ULBP2-expressing variants with allogeneic PBMCs and measured T/NK cell responses. In such infected cells, we observed downregulation of HLA class I expression, owning to the presence of US11. Fine-tuned, increased surface expression of ULBP2 was observed for the ULBP2-expressing mutants, but not the WT-strain due to UL16-mediated retention. Activation of NK and CD8+ T by the HCMV variants was evidenced by upregulation of CD69 and CD25. Downregulation of NKG2D on NK/T cells correlated with the expression level of ULBP2. Therefore, expression of NKG2D ligands while simultaneously deleting HLA-I immune-evasins may have merit for HCMV-vaccine development.

Keywords: Adaptive immunity, adjuvants and vaccines, innate immunity, NK cells, transplantation, viral infections

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POSTER PRESENTATIONS

P-0519

Altered peri-implantation endometrial Treg in recurrent pregnancy loss

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For 50-70% of couples seeking medical advice for recurrent pregnancy loss (RPL), the consecutive loss of two or more pregnancies before 24 weeks' gestation, no cause can be found. A defect in the maternal endometrial immune system has been proposed as Treg depletion in the very early stages of murine pregnancy leads to pregnancy loss. This study analyses Treg and CD4T cells in the human peri-implantation endometrium, when an embryo first implants into this dynamic mucosal tissue and must trigger immune tolerance mechanisms for a successful pregnancy. We obtained peripheral blood and endometrial samples from women with RPL or fertile controls. We identified an enhanced regulatory transcriptomic signature in endometrial Treg compared to peripheral blood Treg, using RNA Sequencing. Validation studies using flow cytometry showed that whilst there was no difference in FOXP3 nor HELIOS expression levels, endometrial Treg expressed higher levels of key regulatory, residency and antigen presentation molecules: CXCR6, ICOS, CTLA4, TIGIT, PD1, IL18R, CD39 and TIM3 ($p<0.05$). Parous women had an altered endometrial Treg transcriptome compared to nulliparity, indicating acquired immune memory of pregnancy within the Treg population. We compared primary and secondary RPL to nulliparous or parous controls respectively. Both RPL subgroups displayed differentially expressed Treg gene transcriptomes compared to controls. We found increased cell surface S1PR1 (a tissue exit signal marker) and decreased TIGIT (an inhibitory checkpoint receptor) protein expression by Treg in primary RPL, confirming the presence of altered Treg in the peri-implantation RPL endometrium.

Keywords: Adaptive immunity, regulatory cells, reproductive immunology, RNAseq

P-0520

Circulating inflammatory protein and cellular profiles at time of diagnosis classify inflammatory bowel disease patients according to their underlying immune response and clinical disease course

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Chronicity of inflammatory bowel disease (IBD) is driven by inflammatory memory $CD4^+$ T helper (Th) cells which activate an inflammatory cascade. Because of disease heterogeneity, novel treatment strategies tailored to target the patient's immune defect are required. We hypothesize that combined analysis of circulating inflammatory protein abundance and Th cells allows to dissect underlying immune pathogenesis. We performed the analysis of 92 inflammatory plasma proteins in a cohort of pediatric Crohn's disease patients (CD), ulcerative colitis/IBD-unclassified patients (UC/IBD-U) and age-matched healthy controls (HC). Blood was collected at diagnosis and after induction treatment. Protein concentrations were assessed with Olink Proximity Extension Assay technology® and Th cells were analyzed with flow cytometry. Thirty-six plasma proteins discriminated IBD patients from HC. Increased abundance of interferon-γ was strictly associated with CD while interleukin-17A was more abundant in UC/IBD-U. In CD, three patient clusters were identified. CD#3 patients had lower clinical disease activity, lower C-reactive protein and higher blood albumin concentrations, while clusters CD#1 and CD#2 had comparable clinical parameters. CD#1 patients had higher abundance of 14 proteins associated with neutrophil function and interferon-γ signaling while CD#2 patients showed increase in frequencies of activated ($HLA\text{-}DR^+$) memory Th cells. The three CD clusters responded differently to therapy with CD#1 patients exhibiting more modulated proteins and greater fold changes, CD#2 patients showing intermediate modulation and CD#3 patients exhibiting only a few changes. In conclusion, combined profiling of plasma immune proteins and circulating Th cells discriminates subgroups of pediatric IBD patients which differ in their response to therapy.

Keywords: Cytokines and mediators, immune networks, monitoring immunity, inflammatory bowel disease

P-0521

Modulation of P2X7 receptor activity in experimental autoimmune encephalomyelitis models

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Multiple sclerosis (MS) is a chronic autoimmune and demyelinating disease of the central nervous system (CNS), characterized by axonal loss and neuronal degeneration. Current treatments remain ineffective during its progressive phase. Therefore, there is a great need to discover new therapeutical targets. P2X7 receptor (P2RX7) is a cell surface ion channel that senses ATP released from cells as endogenous danger signals during inflammation. The receptor is largely implicated during inflammatory events such as inflammasome, pro-inflammatory cytokine production, differentiation and survival of lymphocytes. The objective here will be to better understand the role of P2RX7 in the pathophysiology of experimental autoimmune encephalomyelitis models (EAE, mouse models of MS). To this end, we will use the AAVnano technology based on AAV vectors coding for single-chain antibodies named nanobodies (Nbs) specific of P2RX7. We demonstrated that the latter can efficiently target and modulate the activity of P2RX7 in the periphery as well as in the CNS (particularly microglia and T cells) during the course of EAE. Preliminary data suggested that AAVnano coding for P2RX7 Nb antagonists led to an exacerbation of the EAE clinical signs. The effect of the anti-P2RX7 Nb agonists is ongoing. This translational approach will evaluate the therapeutical potential of anti-P2RX7 Nbs during MS and potentially other inflammatory, autoimmune and/or neurodegenerative diseases.

Keywords: Animal models, autoimmunity, immunotherapy, multiple sclerosis, neuroimmunology

P-0522

Systemic and tissue-specific immune cell alterations in male with severe idiopathic infertility

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Male infertility affects 7% of all men with 15% of infertile men developing comorbidities. In the hypothesis that a deregulation of the immune system could be associated to idiopathic infertility and could also account for the development of linked comorbidities, we assessed the frequency and functionality of IL-10-related regulatory cell (DC-10, and Tr1 cells) and of effector cells in peripheral blood (PB) and seminal fluids (SF) of infertile men with one/two (INF) or three (OAT) seminal alterations and fertile men (FER). An extended immuno-phenotyping by multicolor flow cytometry and the analysis of pro-/anti-inflammatory mediators by bead arrays in PB and SF was performed. T cell proliferation and cytokine production profile was also analyzed. Compared to FER, OAT have more neutrophils and less T and B cells, and a higher proportion of exhausted-like (TIM-3+ and KLRG-1+PD-1+) CD8+ T cells in PB. Moreover, CD8+ T cells from OAT are less responsive than FER to TCR-mediated stimulation, in terms of proliferation and of expression of activation markers. The frequency of DC-10 is slightly decreased in OAT compared to FER. OAT compared to FER have a higher frequency of neutrophils and less DC-10 in SF. Finally, OAT and INF show a pro-inflammatory signature in the plasma. For the first time we showed that, despite being overall healthy, INF and OAT display alterations in the immune system. Further and more targeted investigations will define whether these alterations are linked infertility or to the development of co-morbidities.

Keywords: Adaptive immunity, monitoring immunity, myeloid cells, regulatory cells, reproductive immunology

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POSTER PRESENTATIONS

P-0523

Unique molecular signatures typify skin inflammation induced by chemical allergens and irritants

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Skin exposure to chemicals may induce an inflammatory disease known as contact dermatitis (CD). Distinguishing the allergic and irritant forms of CD often proves challenging in clinic.

To characterize the molecular signatures of chemical-induced skin inflammation, we conducted a comprehensive transcriptomic analysis on the skin lesions of 47 patients with positive patch tests to reference contact allergens and non-allergenic irritants. A clear segregation was observed between allergen- and irritant-induced gene profiles. Distinct modules pertaining to the epidermal compartment, metabolism and proliferation were induced by both contact allergens and irritants; whereas only contact allergens prompted strong activation of adaptive immunity, notably of cytotoxic T cell responses. Our results also confirmed that: (i) unique pathways characterize allergen- and irritant-induced dermatitis; (ii) the intensity of the clinical reaction correlates with the magnitude of immune activation. Finally, using machine-learning approach, we identified and validated several minimal combinations of biomarkers to distinguish contact allergy from irritation. These results highlight the value of molecular profiling of chemical-induced skin inflammation for improving the diagnosis of allergic versus irritant contact dermatitis.

Keywords: Allergen-induced immune responses, biomarkers, skin diseases

P-0524

Collagenase-induced mouse model of osteoarthritis – a transition of knowledge to humans

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Osteoarthritis (OA) is the most common form of degenerative joint disease, affecting millions of people worldwide. Pathophysiology of OA is a complex process in which inflammatory mediators are released from cartilage, bone, and synovium. The collagenase-induced mouse model of OA is based on the induction of joint instability by intra-articular injection of collagenase. The aim of the study was to analyze in-depth the phenotype of OA in the active (20 days) and chronic (30 days) stages of the disease development. Balb/c mice, intra-articular injection of collagenase, splenocytes, flow cytometry. The injection of collagenase resulted to a decrease of the total B cell number in the spleens of the CIOA mice, compared to the healthy controls. The percent of the CD3+CD4+ T cells was decreased during the active stage of the disease and on the 30th day, it moved to the levels of the control animals. The CD3+CD8+ T cells portion increased up to 5% towards the controls. We reported the shifting of the immune response from CD4 to CD8, and detected number of very high activated T cells. The NK cells portion was decreased on days 20 and 30 after disease induction and these NK cells were with high expression of CD107 activation marker compared to the healthy controls. Our assumption is that NK cells are redirected to the site of inflammation. The studied CIOA mouse model is very useful for translation of findings from basic science to practical applications that enhance human health.

Keywords: Animal models, chronic inflammation and fibrosis, inflammatory joint diseases

P-0525

Lamin A/C in T-cells protects against melanoma and colorectal cancer

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The immune system is an important defense mechanism against tumors, with IFNγ+ CD8+ T cells and Th1s as protective cells, opposed to Tregs. Lamin A/C in CD4+ T cells potentiates the activation of naïve T lymphocytes and promotes differentiation towards Th1 phenotype while inhibits Treg differentiation. Here we study the role of lamin A/C in T lymphocytes in the development of the solid tumors (melanoma and colorectal cancer) and in metastasis, to propose the modulation of lamin A/C protein levels in T cells as immunotherapy. To this end, we used a model of subcutaneous B16-F10 melanoma or MC38 colorectal cancer cell injection to generate solid tumor development in wild-type mouse and a mouse that lack of lamin A/C in T cells (Lmna-/- mice). Lamin A/C absence in T cells increases tumor progression and decreases the percentage of infiltrated leukocytes. In melanoma, the absence of lamin A/C in T cells also reduces the number of tumor infiltrating T cells (TILs), as well as the number of IFNγ-producing T cells as observed by flow cytometry and immunostaining. In colorectal cancer, the percentage of Tregs is increased in Lmna-/- mice. Lmna-/- mice also have reduced survival after intravenous injection of B16-F10 melanoma cells in metastasis studies. These results suggest that lamin A/C in T-cells might be a therapeutic target for the development of new treatments for tumor development and metastasis.

Keywords: Adaptive immunity, cancer immunology, immune regulation and therapy, immunotherapy, *in vivo* tumor models, regulatory cells

P-0526

Induction of TGF-β receptor signaling by Anaeroplasma, a member of the instestinal microbiota

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The cytokine tumor growth factor β (TGF-β) and immunoglobulin A (IgA) dominate mucosal immune responses. TGF-β suppresses unwanted immune responses in the gut while IgA is an important component of the intestinal barrier controlling the growth of intestinal bacteria and their attachment to mucosal surfaces. We have recently identified a distinct member of the microbiota, *Anaeroplasma*, which enhances mucosal IgA levels by inducing the expression of TGF-β in T follicular helper (Tfh) cells of Peyer's Patches (PP), the inducing cytokine for immunoglobulin class switch recombination to IgA. A strong inducer of TGF-β expression is TGF-β itself. We can show that bacterial extract containing *Anaeroplasma* is able to induce TGF-β receptor signaling directly. We have sequenced the genome of *Anaeroplasma* and are screening for putative proteins with the ability to activate the TGF-β receptor. To test the therapeutic potential of *Anaeroplasma*, I will test its anti-inflammatory properties in pre-clinical models of colitis.

Keywords: Follicular helper T cells, inflammatory bowel disease, microbiome and environmental factors

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POSTER PRESENTATIONS

P-0529

Fever, heat shock protein 70 and the regulation of macrophage IL-1β and IL-10 secretion following *mycobacterium tuberculosis* infection

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25% of the global population are infected with *Mycobacterium tuberculosis* (Mtb), which accounts for 1.4 million deaths per year. Patients infected with Mtb are classically febrile. However, the impact of fever on innate immune responses to Mtb are unknown. Heat shock protein 70 (HSP70) is a molecular chaperone activated by increased temperatures that may modulate inflammatory responses. We investigated the effect of fever on macrophage immune responses in Mtb infection and the role of HSP70. Primary human monocyte derived macrophages (MDM) were pre-treated with recombinant HSP70 or HSP70 antagonist VER155008, infected with Mtb and incubated at 37°C or 40°C. Samples were collected for Western blot analysis, RT-PCR, ELISA's and Mtb survival rates documented by colony counts. Lymph node tissue sections from Mtb culture positive patients were immunohistochemically stained for HSP70. The intracellular survival rates of Mtb decreased in febrile conditions (8.9±2.2 to 4.4±0.3 (x10^4 CFU/ml); P≤0.05). TNF-α, IL-1β, IL-6 and IL-10 secretion from Mtb-infected MDMs decreased at 40°C compared to 37°C, whereas extracellular HSP70 secretion increased (23.9±5.1 to 49.9±8.4 (ng/ml HSP70);P≤0.05). Fever had no effect on intracellular HSP70 accumulation or HSP70 mRNA expression. IL-1β and IL-10 secretion from MDMs decreased with VER155008 but increased with recombinant HSP70, whilst not affecting TNF-α or IL-6 secretion. HSP70 staining was detected around lymph node granulomas. Fever decreased macrophage proinflammatory cytokine secretion in Mtb and HSP70 is a regulator of fever-associated IL-1β and IL-10 secretion. Fever generation in Mtb may be key in modulating the human immune response to infection.

Keywords: Bacterial infections, cytokines and mediators, innate host defence, macrophage, infectious disease

P-0531

Mucosal Helicobacter spp. protect mice from *Citrobacter rodentium* infection by reducing virulence gene expression

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Lack of reproducibility and failure of transferring experimental findings into clinical therapies are the major drawbacks in biomedical research. Phenotype discrepancies are partly derived from differences in the microbiota of specific pathogen free (SPF) laboratory mice and wild mammals living in their natural habitat. *Citrobacter rodentium* (*C. rodentium*) is a natural mouse pathogen that mimics many aspects of EHEC and EPEC infection, which cause severe intestinal inflammation and diarrhea in humans, including attaching and effacing (A/E) lesion-formation. We evaluated the effect of gut bacteria that are widely distributed in the natural environment, especially Helicobacter species (Hb), on host resistance upon *C. rodentium* infection using two SPF isogenic mouse lines. Transfer of three Hb delayed the initiation of *C. rodentium* infection and attenuated *C. rodentium* induced hyperplasia and inflammatory cell infiltration in the colon. Strikingly, the protective effect was still detectable in *Rag 2*-/- mice, suggesting that Hb enhances host resistance independently of the adaptive immune system. Hb colonization triggered minor alteration in the luminal microbial composition and metabolites, which had no direct impact on *C. rodentium* growth *in vitro*. However, quantification of *C. rodentium* and the expression of virulence genes *Ler* and *Ler*-regulated T3SS component *Tir* unveiled that Hb limited *C. rodentium* tissue attachment and inhibited T3SS assembly in both WT and *Rag 2*-/- mice. Thus, the barrier constructed by mucosal associated microbes enhances host fitness against attaching enteropathogens especially when adaptive immunity is impaired. This may provide novel insights in developing therapeutic approaches to limit enteric pathogen induced inflammation.

Keywords: Adaptive immunity, animal models, bacterial infections, inflammatory disease, innate immunity, microbiome and environmental factors

P-0532

Anti-cancer efficacy and systemic toxicity of resveratrol nanocrystals in mice

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There has been significant research interest in nanocrystals as a promising technology for improving therapeutic efficacy of poorly water-soluble drugs such as resveratrol. Nevertheless, little is known about the interaction of nanocrystals with biological tissue, especially liver, kidney and tumor, since nanocrystals remain longer in tissue compared to the fluids. The need to understand potentially harmful side effects of resveratrol nanocrystals became clear, and our goal was to investigate the potential toxicity of resveratrol and its nanocrystals through biochemical, oxidative and histological changes of kidney, liver and tumor by monitoring apoptosis, necrosis and mitotic index. Tumor was caused by intraperitoneal injection of 2.5 x 10^6 EAT cells into the abdominal cavity of Swiss albino mice. Treatment of animals with EAT tumor in groups was started the following day by injecting resveratrol or resveratrol nanocrystals in a dose of 25 and 50 mg/kg every other day for 14 days straight. Results show that resveratrol nanocrystals significantly inhibit proliferation of tumor cells in the abdominal cavity and reduce number of blood vessels in the peritoneum, but also show systemic toxicity and reduce overall survival of animals. Hepatocellular necrosis and apoptosis, hepatic fibrosis around the central vein and degeneration with minor fatty change are shown. In addition, inflammation with high mitotic index in kidney and renal glomerulus swelling have also been observed with slight elevation of some biochemical parameters in groups on high-dose of resveratrol nanocrystals. With respect for human health and environmental safety, the mechanism of toxicity needs to be further clarified.

Keywords: Cancer immunology, *in vivo* tumor models, microenvironment

P-0535

Iron as an adjuvant of anti-tumor responses

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The idea that the immune system can control cancer has been initially proposed by P. Ehrlich in the early 20th century. Since then, numerous immunotherapy trials have been tested. Until recently, these treatments have been inconclusive, tarnished by the collateral toxicities observed and the failure of vaccine therapy, highlighting that the tolerogenic environment of tumors is dominant, preventing the immune system to mount efficient responses. At the beginning of the 21st century, J. Allison and T. Honjo described the existence of immune checkpoints corresponding, among other things, to the expression by T lymphocytes of inhibitory molecules blocking their effector functions. In the case of anti-tumor responses, this inhibition allows the tumor to escape the control of the immune system. Revolutionizing the management of many cancers such as melanoma, new immunotherapies like nivolumab (anti-PD1) increase patient survival. Unfortunately, only 20 to 30% of patients are responding well to these treatments by developing an effective anti-tumor immune response. Our first results show that iron significantly increases T lymphocyte responses, both *in vivo* and *in vitro*. This "adjuvant" effect results in a marked slowing down of tumor growth in models of transplanted tumor cell lines in mice. Therefore, combining iron supplementation with immune checkpoints inhibitors may offer healing prospects to a larger proportion of patients. Hence, our project aims to show the positive effect of iron supplementation on the reactivation of anti-tumor responses induced by immune checkpoints inhibitors and to confirm the benefit of combining immunotherapy and iron supplementation.

Keywords: Cancer immunology, checkpoint inhibition, immune regulation and therapy, *in vivo* tumor models

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POSTER PRESENTATIONS

P-0536

Serological markers of viral hepatitis B in two West African populations in Dakar (Senegal) and Cotonou (Benin)

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Determine the serological profile to hepatitis B infection in two West African populations in Senegal and Benin. This was a prospective, descriptive and analytical study. In HBsAg-positive individuals, HBeAg and anti-HBe antibodies (Ab) were tested. A microparticular immunological technique was used as analytical method. Data have been analysed using SPSS Statistics software. A total of 470 patients participated in the study, 234 in Cotonou (Benin) and 236 in Dakar (Senegal). The study population was predominantly female (61.97% for the Beninese population (BP) versus 52.54% for the Senegalese population (SP)). The median age was 29 years (BP) and 38 years (SP) respectively. The most represented age group was 20 to 29 years for the BP and 30 to 39 years for the SP. The prevalence of HBsAg carriage was 12.39% (n=29) (PB) and 19.91% (n=47) (SP). The association between piercing and HBsAg carriage was significant only in Benin. Five (5) out of twenty-nine (29) patients were HBeAg positive in Benin compared to six (6) out of forty-seven (47) in Senegal. Twenty-one (21) patients expressed HBeAb in Benin versus thirty-seven (37) in Senegal. Men were in the majority among patients. All HBeAg positive patients had quantitative HBsAg titres higher than 250IU/ml. The prevalence of HBsAg in Senegal is higher than in Benin. These results are in line with some previous studies in both countries. Our results also confirm the greater susceptibility of men to contract HBV compared to women as reported in previous studies.

Keywords: Antibody, biomarkers, viral infections

P-0537

Alteration in the immune response as a result of GnRH and hMG hormone stimulation in a MRL/lpr mouse model

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Systemic lupus erythematosus (SLE) is an example of autoimmune disease manifesting itself in immune cells activation against self-antigens. The devastating aftereffects of the disorder are especially evident among females where symptoms are frequently intensified during reproductive age. Furthermore, ovarian stimulation protocols during *in vitro* fertilization (IVF) procedures in female patients may represent direct risk for these individuals and more research is needed to establish better and safer approaches. MRL/lpr mice are suitable model to follow in details the molecular mechanisms behind SLE expression. In order to understand the connection between autoimmunity and the reproductive system, young (one month old) and old (four months old) mice were subjected to stimulation with different dosages of GnRH (80 μg/kg to 500 μg/kg) and hMG (5 IU/mouse) in accordance with IVF procedures in patients. The immune status was studied with FACS analyses, while organ damage in ovaries and kidneys was analyzed through histology. Compared to unstimulated control group, stimulated mice presented diverse set of autoimmunity aggravations, accompanied with slight changes in follicle formation. Notably negative effects were established in kidneys with significant increase in infiltration and organ damage. The results confirm that sex hormones affect immune cells and might lead to disease progression. Hence, more research is needed in order to provide possible adaptations of the protocols in consonance with case specificity.

Keywords: Animal models, autoimmunity, reproductive immunology

P-0538

Association of circulating adipokines and complement components with clinical and immunological parameters for treatment naïve lupus nephritis (LN) patients from western India

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To study the association of adipokines and complement components with clinical and immunological parameters for treatment naïve Lupus Nephritis (LN) patients. Treatment naïve renal biopsy proven (2003 ISN/RPS) lupus nephritis (LN) patients (n=30) and healthy volunteers (n=50) were enrolled (January 2017-December 2019). Serum levels of adiponectin, leptin, adipsin, resistin, progranulin and galectin-3 were tested by ELISA. C3 and C4 levels were measured by nephlometer, complement factor C1q, CFH, CFB, CFP were tested by ELISA. Anti-C1q and ANA specificities were tested by ELISA. Adiponectin, adipsin, resistin, progranulin and galectin-3 levels were statistically significantly elevated in LN patients when compared to healthy controls ($p<0.05$), while serum leptin levels were significantly lower in LN patients ($p<0.05$). Reduced levels of C1q (36.67%) and C3(96.67%) were noted, whereas C4 (80%), CFH (20%), CFB (13.33%) and CFP (46.67%). Adipsin levels were positively correlated with adipokine resistin and complement components C3, C4 and CFB levels ($p<0.05$). Serum leptin levels were negatively correlated with LN disease activity ($r = -0.499$, $p = 0.005$) and anti-dsDNA autoantibodies ($r = -0.494$, $p = 0.006$). Reduced C1q levels positively correlated with low levels of C3, C4, CFB, CFP and inversely correlated with presence of anti-C1q and dsDNA antibodies ($p<0.05$). Resistin levels were positively correlated with CFB levels ($p<0.05$) in our study. Association of these adipokines with disease activity and complement components suggested a regulatory effect on complement components indicating their possible role in disease pathogenesis of LN.

Keywords: Antibody, complement, cytokines and mediators

P-0539

Type 1 diabetes mellitus: prevalence of specific autoantibodies and concurrence with other organ-specific autoantibodies

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Type 1 Diabetes Mellitus (T1DM), an autoimmune disease with aberrant immune response to specific β-cell autoantigens, may also be a part of organ-specific multiple autoimmunity in the context of autoimmune polyendocrine syndrome (APS). The aim of this retrospective study was to investigate the prevalence of specific autoantibodies (Aabs) and to assess their coexistence with other organ specific Aabs, in T1DM patients. In 77 T1DM patients and 30 healthy individuals (blood donors-BD) the following Aabs were determined: anti-parietal cell (APCA), anti-adrenal (ADR), anti-endomysial (EmA), anti-islet cells (ICA), by indirect immunofluorescence (IIF), anti-glutamic acid decarboxylase (GAD65), anti-insulin (IAA), anti-tyrosine phosphatase (IA2), by radioimmunoassay (RIA), anti-thyroid antibodies (ATA), anti-intrinsic factor (IFA) and anti-tissue transglutaminase (tTG), by enzyme-linked immunoassay (ELISA). All patients had at least one of the specific T1DM Aabs. Fifty-five (71.4%) had anti-GAD65, forty-three (55.8%) anti-IAA and thirty-seven (48.1%) anti-IA2. ATA were detected in twenty-three (29.9%) and anti-tTg/EmA in eleven (14.3%), while in BD only one had anti-IAA, two (6.7%) ATA and none anti-tTg/EmA (p value=0.011 for ATA and 0.032 for anti-tTg/EmA). Eight patients (10.4%) had APCA whereas three (3.9%) anti-ADR, whilst in BD one (3.3%) had APCA and none ADR, without statistical significance. In our study the prevalence of T1DM specific antibodies appears significant while the anti-GAD65 display the highest specificity and sensitivity. Their concurrence with ATA and anti-tTg/EmA is statistically significant hence the necessity of their identification is suggested in the context of comorbidity with another autoimmune disease or the diagnostic approach of APS.

Keywords: Antibody, autoimmunity, biomarkers, diabetes

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POSTER PRESENTATIONS

P-0540

Rhamnogalacturonan-I from bell pepper mediates colonisation resistance to Salmonella infection

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Salmonellosis is one of the most common infectious diseases that can cause serious gastrointestinal illness in both animals and humans. In this study, we tested the capacity of pectin derived Rhamnogalacturonan-1 (RG-I) from bell pepper (bpRG-I) to protect mice from the effects of a gastrointestinal infection caused by Salmonella enterica serovar Enteritidis. Before infection, C57Bl/6 female mice were fed either a diet fortified with 1% w/w of bpRG-I or a standard control diet for 21 days. Infected mice were compared with uninfected control mice, and the course of the infection was followed for 4 days. Dietary supplementation with bpRG-I resulted in a fast reduction of faecal pathogen numbers following infection and prevented secondary pathogen outgrowth from days 2 to 4. The number of Salmonella recovered in the ileal tissue on day 4 was reduced to levels close to non-infected mice. Supplementation with bpRG-1 increased ileal tissue IgA, the number of activated CD8+ T cells and Treg cells in the Peyer's Patches but decreased numbers of pro-inflammatory M1 macrophages in Peyer's patches, and concentration of serum pro-inflammatory cytokines (TNF-α, IFN-γ, IL-6 and MCP-I) concurrent with an increased anti-inflammatory response (IL-10). Interestingly, feeding bpRG-I before infection modulated the gut microbiota composition, leading to increased levels of Bifidobacterium and Akkermansia muciniphila. Together, these data point to a dual mode of action of bpRG-I on the immune system and the gut microbiota that underly the preventive protective effect of this polysaccharide against colonization with Salmonella.

Keywords: Infectious disease, innate host defence, microbiome and environmental factors, nutrients, protection

P-0542

Serum / plasma diagnostic biomarkers for potential screening and prognosis evaluation of glioma patients

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Gliomas belong to the most frequent primary brain tumors that have the third highest mortality and morbidity rates among cancers in human population. More than half of patients present a diagnosis of glioblastoma. The majority of central nervous system tumors are diagnosed after clinical symptoms become apparent, though surgery, radiotherapy and chemotherapy may be less effective than in earlier stages of tumor. The markers of survival prognosis in patients with gliomas are still evaluated. We attempted to analyze multiple factors associated with anti-tumor immunity to identify profiles associated with grade II, grade III and grade IV gliomas and with their prognosis. The study group included 63 patients older than 18 years with partial or complete resection of glioma. The reference cohort in our case-control study comprised of 17-26 healthy volunteers. The serum levels of soluble sTREM-1, HMGB1, and plasma levels of IL-6, IL-10, IL-4, IL-13, sHLA-G, IFN-gamma, TGF-beta, VEGF, BDNF and CX3CL1 were analyzed by a sandwich ELISA test. The serum levels of 25(OH)D were evaluated by electrochemiluminescent binding test. In our study, these markers showed promising results in distinguishing patients with grade IV glioma from healthy controls: sTREM-1, sHLA-G, BDNF, VEGF, IL-10, IL-13 and CX3CL1. Soluble HLA-G levels above 40 U/ml and at 25(OH)D below 20 µg/L were associated with significantly worse overall survival in grade IV. In grade II and III, serum or plasma markers were not altered so much as in glioblastomas.

Keywords: Biomarkers, cancer immunology, Immune response tracing

P-0543

Anti-glutathione-S-transferase-theta 1 antibodies in a Menetrier´s disease case

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A 36-year-old female with no medical history of interest, consulted her doctor because of diarrhea with 5 normal-appearing stools a day, no abdominal pain or fever and edema in lower limbs. Complete blood analysis, image tests and biopsy were performed. Hypoproteinemia was evidenced in paraclinical studies. The gastroscopy revealed stomach thickening gastric folds and the gastric biopsy reported chronic atrophic gastritis with acute focal erosion and marked nonspecific foveolar hyperplasia. Menetrier's disease was diagnosed. Treatment was initiated with proton pump inhibitors, inhaled budesonide with formoterol and antihistamines. In addition, a high protein- based diet was prescribed. Since the patient condition improved, she was decided to be followed monthly. In subsequent controls, indirect immunofluorescence test on rat triple tissue revealed anti-smooth muscle antibodies at 1/160 titration and an abnormal particularly bright liver staining pattern surrounding cetrilobular vein together with renal tubule staining suggestive of anti-glutathione-S-transferase-theta 1 (GSTT1), with normal serum transaminase values and a classical allosensitization recorded event due to pregnancy. GSTT1 is an enzyme involved in the conjugation of reduced glutathione to a variety of compounds and may play a role in human carcinogenesis. GSTT1 gene is haplotype-specific and is absent from 38% of population.Menetrier's disease or hypoproteinemic hypertrophic gastropathy is a rare entity, clinically characterized by digestive symptoms and peripheral edema, associated with an increased risk of gastric cancer, even so its pathophysiology is not currently understood. More studies would be needed to elucidate the relationship between Menetrier's disease and anti-GSTT1 antibodies.

Keywords: Environmental factors in autoimmunity and allergy, antibody, autoimmunity

P-0544

Comparison of interferon-gamma release in response to SARS-CoV-2 antigens between vaccinated with BBIBP-CorV and BNT162b2 vaccines, convalescents and COVID-19 patients

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Cell-mediated immune response to different vaccines against COVID-19 is rarely compared. Additionally, apart from the phase 1/2 clinical trial, data on BBIBP-CorV vaccine against COVID-19 is scarce. The aim was to compare cell-mediated response of persons fully vaccinated with BBIBP-CorV and BTN162b2, the most frequently used vaccines against COVID-19 in Serbia, with the response of COVID-19 convalescents, hospitalized due to COVID-19 and naïve persons by interferon(IFN)-γ release assay. Heparinized venous blood was obtained from 56 healthy volunteers: at least three weeks after the second dose of BTN162b2 (24) or BBIBP-CorV (21) vaccine, convalescents (6) or naïve (5, without antibodies to receptor-binding domain of a SARS-Cov-2 spike protein or N-protein) persons; and from 10 hospitalized patients with PCR-confirmed COVID-19 pneumonia. IFN-γ release was measured by Qiagen QuantiFERON SARS-CoV-2 RUO (QFS), a test still in process of validation for diagnostic use. IFN-γ levels were statistically significantly higher in vaccinated, convalescents and persons with acute COVID-19 compared to naïve ones. Concentrations of released IFN-γ did not differ between BBIBP-CorV and BTN162b2, but trend towards lower values after BBIBP-CorV was observed in sera after both antigen simulations. Portions of positive QFS were 38% and 62% after BBIBP-CorV and BTN162b2, respectively ($p>0.05$). Although statistically significant differences were not detected, a tendency to lower cell-mediated immune response after BBIBP-CorV measured by QFS, in accordance with the lower efficiency of BBIBP-CorV in clinical trial compared to BTN162b2, warrants the need for further assessment in a greater number of participants.

Keywords: Adjuvants and vaccines, cytokines and mediators, effector molecules, immune response tracing, infectious disease, viral infections

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POSTER PRESENTATIONS

P-0545

The role of soluble HLA-G and cytokine levels on relapse and transplant related complications in children with leukemia undergoing allogeneic stem cell transplantation

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Relapse and transplant-related complications (TRCs) are significant barriers to the success of allogeneic stem cell transplantation (allo-SCT) in leukemia. As well, it has not been identified yet specific biomarkers to predict the relapse and TRCs in leukemia. Our aim was to investigate the role of cytokines and soluble HLA-G (sHLA-G) on relapse and TRCs in pediatric leukemia undergoing allo-SCT. A total of 41 patients with acute leukemia were recruited. They were examined at diagnosis (n=26), pre-transplant period (PreTx) (n=26), transplant day (Tx0) (n=41), posttransplant 14th (PostTx14) (n=41) and 28th (PostTx28) (n=41) days. Serum levels of cytokines (IL-1, IL-2, IL-4 IL-6, IL-10 and TNF-α) and sHLA-G were measured by ELISA (Bioassay Technology, China), and their cut-off levels to predict relapse (n=12) and TRCs (n=25) were determined by ROC curves. The median sHLA-G and cytokine levels at diagnosis and post-transplant periods were found to be significantly higher than the PreTx levels ($p<0.05$). The elevated IL-1, IL-4, IL-10, and sHLA-G levels at diagnosis were associated with relapse ($p<0.05$), and the elevated IL-2, IL-6, TNF-α and sHLA-G levels at Tx0 were associated with TRCs ($p<0.05$). The IL-1, IL-4, IL-10 and sHLA-G cut-off levels at diagnosis for predicting relapse in leukemia were 139ng/L, 521ng/L, 616ng/L and 19.4U/ml, respectively. The IL-2, IL-6, TNF-α and sHLA-G cut-off levels at Tx0 for predicting TRCs during allo-SCT were 1644 ng/L, 319 ng/L, 469 ng/L and 21U/ml, respectively. Cytokine and sHLA-G levels may be useful markers for predicting relapse and TRCs in pediatric leukemia undergoing allo-SCT.

Keywords: Cancer immunology, cytokines and mediators, transplantation

P-0546

The switching from neutrophil- to eosinophil-mediated immune response in *Aspergillus fumigatus*-induced allergic airway inflammation

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Neutrophils can suppress eosinophilia, but in allergic airway inflammation, the switching from neutrophil- to eosinophil-mediated response occurs. To investigate the mechanisms of such switching we analyzed the kinetics of eosinophil and neutrophil proportions in blood samples of mice during the course of allergen application. Mice received five oropharyngeal applications of *Aspergillus fumigatus* extract in a dose of 7 μg per mouse every 72 hours. Blood samples were collected before each allergen application. Neutrophils and eosinophils were detected by both flow cytometry and differential cell count of cytospins. By flow cytometry myeloid cells were identified as $CD45^+CD11b^+CD172^+$. Neutrophils among myeloid cells were identified as $Ly6G^+SiglecF^-$, eosinophils as $Ly6GlowSiglecF^+$. Significant elevation of neutrophil percentage was detected in blood 72 hours after the second allergen application. Later, neutrophils were replaced by eosinophils and the percentage of eosinophils was significantly elevated already 72 hours after the fourth application. Flow cytometry analysis of myeloid cells indicated together with neutrophil and eosinophil populations a population of Ly6G+SiglecF+ cells. The percentage of $Ly6G^+SiglecF^+$ cells steadily elevated after each allergen application. Such population was also found in the bone marrow of mice at 72 hours after the fifth allergen application. $Ly6G^+SiglecF^+$ cells were characterized with higher expression of CXCR2 compare to neutrophils, which indicated a higher potential of these cells to mobilize from the bone marrow to the periphery. Multiple application of *A. fumigatus* extract to mice induces elevation of the percentage of $Ly6G^+SiglecF^+$ cells that possessed high expression of CXCR2.

The work was supported by RSF 20-75-00111.

Keywords: Allergic disorders, animal models, eosinophils, neutrophils

P-0548

Association of dendritic cell frequencies and phenotypes with aging and frailty

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There are major differences in how individuals and their immune system respond to aging. The frailty phenotype describes older adults with declined health, leading to physical impairment, disease and impending death. It is currently unclear what causes frailty in older adults, although changes in the immune system have been implicated. As dendritic cells (DCs) are essential for initiating immune responses, we aimed to determine the phenotype of DCs in frail elderly blood donors. We selected frail elderly donors, age- and sex-matched non-frail controls and young controls from our Immunolines cohort (n=15 per group). Frail and non-frail donors were selected based on Tilburg Frailty Indicator scores. Conventional DCs (cDC1=CD11c+CD141+ and cDC2=CD11c+CD1c+) and plasmacytoid DCs (pDCs=CD303+) were identified by flow cytometry in PBMCs and simultaneously evaluated for TLR2, TLR4, PDL1, CD86, CCR7 and CD40 expression. Frequencies of circulating pDCs were significantly lower in healthy old donors compared to young donors. In frail and non-frail old donors, we found similar frequencies of pDC, cDC1 and cDC2. There was a trend towards reduced expression of CCR7 ($p=0.07$) and CD40 ($p=0.06$) in cDC1 of frail donors compared to non-frail donors. Whereas frequencies of pDCs decrease with age, they do not appear to be affected by frailty in older adults. Given the importance of pDCs in immunity against viral pathogens, their reduction with age may play a role in increasing susceptibility to severe infections. Frailty may affect the rare cDC1 subset, as a trend towards less CCR7 and CD40 expression suggests decreased activation of these cells.

Keywords: Ageing, antigen processing and presentation, dendritic cells

P-0549

Obesity alters adipose tissue energy metabolism profiles and inflammatory secretions: their influence on Dendritic Cell maturation

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Oesophageal Adenocarcinoma (OAC) is the most strongly associated cancer with obesity. Approximately 75% of OAC patients are obese which results in chronic systemic low-grade inflammation, which is believed to drive carcinogenesis as well as influencing radiation treatment response. Changes in metabolic oxygen consumption rate (OCR) has been associated with radio-resistance in OAC patients, however the role fat plays and how it responds to radiation is not understood making it the focus of this study. Following patient consent, ex -vivo Visceral Adipose Tissue (VAT) explants were exposed to increasing doses of radiation. Agilent Seahorse Xfe24 was used to measure oxygen consumption rate (OCR) and extracellular acidification rate (ECAR). ACM was analysed via MSD 54plex ELISA to assess changes in secretion on any of the angiogenesis, chemokine, cytokine, inflammatory, TH17 or Vascular injury panels. Levels of dendritic cell maturation following ACM exposure were analysed by flow cytometry to assess DC maturation. Fat explant energy metabolism showed significant increase for OCR and ECAR in obese patients compared with non-obese patients. Altered secretion of proinflammatory mediators were observed from the obese adipose secretome when compared to the non-obese. DC maturation showed decreased expression of HLA-DR and CD54 following exposure to adipose conditioned media from obese versus non-obese patients. We have demonstrated using fresh *ex-vivo* human fat samples from OAC patients, that obesity can significantly change real time metabolic profiling which could be directly linked with the inflammatory profile in VAT in these patients and hold downstream effects for DC maturation.

Keywords: Cancer immunology, chronic inflammation and fibrosis, cytokines and mediators, dendritic cells

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POSTER PRESENTATIONS

P-0550

Immunomodulatory effects of TGF-β in the patients with immune thrombocytopenia

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The novel concept of immune thrombocytopenia (ITP) pathogenesis is focused on *CD4 +* T cells, currently considered indispensable in stimulating B cells to produce anti-platelet antibodies. In this *in vitro* study, we examined the immune profile of *CD4 +* T cells from patients with ITP, as well as the possibility of its correction using *TGF-β*. The study included 6 subjects, both sexes, with an average of 56 (32-69) years, who were divided into two groups - control and ITP group. After collecting blood samples (10ml) using EDTA as an anticoagulant, blood was diluted in RPMI-1640 medium (1:1) and mononucleated cells (PBMCs) were isolated on a density gradient. The results of our study confirm the deviant polarization of the *CD4 +* T cell response in ITP patients and suggest that *TGF-β* may correct the imbalance in *Th1/Th2* and *Th17/Treg* cell ratios. This indicates that therapeutic strategies that are based on promoting *TGF-β* signaling may be a potentially novel approach in the treatment of this disease. The study also pointed out the shortcomings of an experimental model of prolonged *CD4 +* T cell cultivation. Patients with ITP show aberrant *Th1* and *Th17* polarization of the cellular immune response, which can be corrected by stimulated *TGF-β* signaling. However, it appears that long-term *CD4 +* T cell cultivation is not a suitable experimental model to study immunomodulatory properties in ITP due to dynamic fluctuations in the phenotype of these cells under *ex vivo* conditions.

Keywords: Cellular interactions, cytokines and mediators, immune regulation and therapy, immunotherapy

P-0552

ACKR2 limits skin fibrosis and hair loss through IFN-β

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The resolution of inflammation facilitates proper wound healing and limits tissue repair short of exaggerated fibrotic scarring. The atypical chemokine receptor (ACKR)2/D6 scavenges inflammatory chemokines, while IFN-β is a recently unveiled pro-resolving cytokine. Both effector molecules limit acute inflammatory episodes and promote their resolution in various organs. Here, we found fibrotic skin lesions from ACKR2-/- mice presented increased epidermal and dermal thickening, atrophy of the subcutaneous adipose tissue, augmented disorientation of collagen deposition, and loss of hair follicles compared to WT counterparts. In addition, affected skin sections from ACKR2-/- mice contained reduced levels of the pro-resolving mediators IFN-β and IL-10, but increased levels of the pro-inflammatory chemokines CCL2 and 3, the pro-fibrotic cytokine TGF-β, and the immune-stimulating cytokine IL-12. Notably, treatment with exogenous IFN-β rescued, at least in part, all the pro-fibrotic outcomes and lesion size in ACKR2-/- mice and promoted expression of the pro-resolving enzyme 12/15-lipoxygenase (LO) in both ACKR2-/- and WT mice. Moreover, Ifnb-/- mice displayed enhanced pro-fibrotic indices upon exposure to bleomycin. These findings suggest ACKR2 is an important mediator in limiting inflammatory skin fibrosis, and acts via IFN-β production to promote the resolution of inflammation and minimize tissue scaring.

Keywords: Chemokines, cytokines and mediators, macrophage, skin diseases, tissue damage and repair

P-0553

Mass cytometric analysis of the pulmonary immune profile upon MSC/placebo treatment of COPD patients with emphysema

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Chronic Obstructive Pulmonary Disease (COPD) is characterized by progressive inflammation causing irreversible alveolar tissue destruction (emphysema). Cellular therapy with Mesenchymal Stromal Cells (MSC) may present a new treatment for emphysema. We aim to delineate the immune cell profile in the lung tissue before and after treatment with MSC, and explore whether patients with emphysema develop anti-inflammatory and tissue repair responses upon MSC treatment. Therefore, we performed a comprehensive mass cytometry (CyTOF) single cell analysis to compare the immune compartment in lung tissue samples derived from emphysema patients (n=14) before and after treatment with MSC or placebo. Single cell suspensions were prepared from lung tissue and stained with a lymphoid and a myeloid antibody panel with altogether 76 markers to obtain a detailed overview of both the innate and adaptive immune cell profile in the lung tissue. The data sets were analyzed by a combination of Hierarchical-SNE and t-SNE based approaches. CyTOF analysis identified substantial heterogeneity in the CD4, CD8 and ILC lymphoid compartments based on differential expression of cell surface and functional markers (i.e. CD57, Granzyme B, T-bet). In addition several myeloid subsets could be distinguished due to distinct expression patterns of CD141, CD206 and CCR2. While our study is still blinded, the results already provide profound insight into the composition of the immune compartment in emphysema patients that may provide novel insight into disease pathogenesis. Moreover, upon unblinding, we expect to be able to determine local changes in the lung immune compartment due to the treatment with MSC.

Keywords: Big data, cell based therapies, immune networks, inflammatory disease, mass spectrometry

P-0554

Autoantibodies in patients with suspected post-treatment Lyme disease syndrome

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Lyme disease is a chronic multi-system infectious disorder caused by spirochetes belonging to the species complex Borrelia burgdorferi sensu lato. Although these bacteria are susceptible to many types of antibiotics and antibiotic treatment is effective in the most cases, in some patients the problems persist for a long time. This condition has been termed post-treatment Lyme disease syndrome (PTLDS.) It is still debated whether and to what extent the persistence of infection or the post-infectious immunopathological reactions are involved in the pathogenesis of this condition. We collected blood samples from 37 patients previously treated for Lyme disease and suspected of PTLDS due to persistent symptoms such as chronic fatigue, myalgia, arthralgia and cognitive deficit. None of the patients had known history of autoimmune disease. Wide range of autoantibodies was determined in sera of all patients by commercially available diagnostic immunoblot kits (Euroimmun, Germany). The manufacturer states a specificity of more than 95% for all antigens tested. Positive result for at least one autoantibody type was found in 16 samples (43,24 %). 4 samples were positive only for antinuclear antibodies, 10 samples for broad spectrum of myositis-associated/myositis-specific antibodies and 2 for combination of both groups. Although the causality between the previous Borrelia infection, presence of autoantibodies, and clinical signs cannot be clearly demonstrated from our data, the noticeably high frequency of autoantibodies suggests a likely involvement of autoimmune mechanisms in the pathogenesis of significant part of long lasting post-treatment Lyme disease complications cases.

Keywords: Chronic inflammation and fibrosis, autoimmunity, bacterial infections

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POSTER PRESENTATIONS

P-0555

Key roles of B cells and IL4I1 in melanoma

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Human cutaneous melanoma is the archetypal immunological cancer with a high density of tumor-infiltrating lymphocytes. In contrast to T cells, B cells remain poorly investigated in melanoma, whereas both conventional (B2) and innate-like (B1) B cells are present in skin at homeostasis and during inflammation. Our project aims to explore B1 and B2 roles in tumor progression and to understand the molecular mechanisms underlying. Here, we show that B cell depletion accelerates metastatic dissemination in RET mice, a spontaneous model of melanoma, through PMN-MDSC recruitment and impairment of CD4+ and CD8+ T cell functions. B2 cells massively infiltrate the primary tumor from the earliest stage of the disease, supporting their protective role, whereas B1 cells accumulate with the disease aggressiveness. In contrast, adoptive transfer of B2 in B-cell deficient mice (μMT) limits the progression of subcutaneous B16 melanoma. Expressed in B2 and myeloid cells, IL4-induced gene 1 (IL4I1) impairs B cell responses in physiological setting. Its genetic inactivation in RET mice delays tumor cell dissemination and increases the number of tumor-associated B cells. We thus expect that IL4I1 inactivation will potentiate the anti-tumor properties of B2 cells in melanoma. Accordingly, we are currently investigating how IL4I1 affects the functions and the recruitment of B cells in melanoma and modifies the tumor microenvironment to give a more comprehensive view of its pro-tumoral effects. Our data may strengthen the rationale for combining current immunotherapy targeting PD-1 in metastatic cutaneous melanoma with an IL4I1 antagonist, thus enhancing anti-tumoral B cells.

Keywords: B lymphocytes, cancer immunology, *in vivo* tumor models, regulatory cells

P-0556

Immunephenotyping of antiviral immune cells and in vitro investigation of cellular immune responses to SARS-CoV-2 spike proteins in coronary artery disease

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COVID-19 (coronavirus disease-19) is caused by a novel strain of SARS-CoV-2 virus. Mounting of antiviral immunity towards SARS-CoV-2 virus was greatly impaired among patients with coronary artery disease (CAD). Thus, the main aim of the study is to investigate the pre-existing antiviral innate and adaptive immune status among CAD patients. In addition, functional alterations with cellular immune responses under the influence of SARS-CoV-2 spike proteins are being investigated, underscoring the fact that CAD patients are more vulnerable to COVID-19 due to underlying chronic inflammation, which dysregulates the antiviral immune mechanisms. For this purpose, blood samples from 30 CAD patients and 15 older and 15 younger normal controls are examined for the immunophenotypic characterization of a) expression patterns of SARS-CoV-2 cognate receptors (ACE2 and CD147) on blood monocytes, lymphocytes and granulocytes; b) blood circulating frequencies of MAIT cells expressing migration markers and ACE2, b) blood circulating frequencies of CD8+ T cells and their activation (CD25, CD69) and exhaustion markers (PD-1, Tim-3). Furthermore, functional outcome of CD4+, CD8+ and MAIT cells in response to titrated SARS-CoV-2 spike proteins are being investigated in a Chandler loop system, simulating the extracorporeal blood circulation, in terms of a) T cell proliferation, b) apoptosis and c) cytokine and chemokine production. We have observed decreased MAIT cells and dysregulated antiviral immune subsets among CAD patients in comparison with normal controls. Of note, ACE2 expression is minimal in lymphocytes, followed by granulocytes and highly expressed in monocytes. Importantly, functional cellular immune responses to SARS-CoV-2 spike proteins are currently being investigated.

Keywords: Adaptive immunity, cardiovascular diseases, innate immunity, MAIT cells, viral infections

P-0557

IVIG and steroid pulses for overcoming critical status in cytokine storm associated with covid-19 and acute kidney allograft failure

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We present a case of a 54-year-old man, recipient of a kidney transplant, with a severe Covid-19 disease that evolved into a respiratory failure requiring attention in the intensive care unit (ICU) and mechanical ventilation on March 2019. Since first days at the ICU, the allograft function decreased to a situation of oliguric acute kidney failure that needed continuous renal replacement therapy (CRRT) and general condition worsened reaching a systemic inflammatory response with an increase of inflammatory serological parameters including ferritin, D-dimer, c-reactive protein, lactate dehydrogenase and cytokines such as IL-6, IL-8, TNF-α, IL-18, IL-10, MCP-1, MIF and MIP. The immunosuppression treatment of the patient was discontinued (except for 6-methylprednisolone -6MP- 40 mg IV/24h) and failed to respond to several therapeutical regimes recommended at that moment as antibiotic therapy, β-Interferon, hydroxychloroquine or lopinavir/ritonavir. At this point, we started a three-day consecutive course of high-dose IVIG and 6MP which was followed by a fast reduction of the pro-inflammatory serological markers and a clinical stabilization. The patient could stop CRRT within the 2 following weeks and was extubated in the third week after the treatment onset. He finally was discharged after 49 days with conserved allograft function and with no modification of his previous anti-HLA antibody panel, suggesting that IVIG and 6MP could be an alternative strategy to treat severe cases of Covid-19 pneumonia associated with an uncontrolled inflammatory response in transplanted populations.

Keywords: Biomarkers, cytokines and mediators, immune regulation and therapy, infectious disease, transplantation

P-0558

The effect of scFv antibody treatment targeted anti-C1q autoantibodies in MRL/lpr mouse model of systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a chronic inflammatory autoimmune disease characterized by tissue damage in multiple organs caused by autoantibodies and the resulting immune complexes. One possible way for complement system contribution to onset of autoimmune disorder could be realized by the impairment of C1q mediated apoptotic clearance as part of human homeostasis. The capacity of C1q to bind early apoptotic cells could be decreased or even lost in the presence of anti-C1q antibodies which are specific for epitopes within gC1q. A phage-displayed library expressing single-chain recombinant antibodies was screened to select scFv specific for anti-C1q autoantibodies from different groups of lupus sera. Two groups of MRL/lpr mice were used for *ex vivo* and *in vivo* experiments.: 7 weeks old mice that are still disease free and 16 weeks old with advanced disease manifestations. We have injected the mice with 20 μg/mouse weekly of the studied scFv antibody. Control groups were injected with PBS only. Blood samples were collected weekly and the sera were stored at -80 ºC for subsequent analyses. The data show that the scFv treatment modulates the percent of B and T cell subpopulations and splenocyte apoptosis. An increase of the proteinuria levels in the 7 weeks old MRL/lpr mice, splenocyte proliferation change and the number of plasmocytes producing anti-dsDNA antibodies in the treated group were observed also. The treatment with anti-idiotypic scFv antibody has modulatory effect on lupus symptoms in MRL/lpr murine model of SLE.

Keywords: Animal models, autoimmunity, complement

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POSTER PRESENTATIONS

P-0559

The systematic effect of mesenchymal stem cell therapy in critical COVID-19 patients: a prospective double controlled trial

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The aim of this clinical trial was to control the cytokine storm by administering mesenchymal stem cells (MSCs) to critically-ill COVID-19 patients, to evaluate the healing effect, and to systematically investigate how the treatment works. Patients with moderate and critical COVID-19 clinical manifestations were separated as Group 1 (moderate cases, n=10), Group 2 (critical cases, n=10), and Group 3 [(critical cases, n=10, treated conventionally plus MSCs transplantation therapy on days 0, 3, and 6, (as $3x10^6$ cells/kg, intravenously)]. The treatment mechanism was investigated with markers of the cytokine storm, via biochemical parameters, levels of proinflammatory and anti-inflammatory cytokines, analyses of tissue regeneration via the levels of growth factors, apoptosis markers, chemokines, matrix metalloproteinases, granzyme-B, and by the assessment of the immunomodulatory effects via total oxidant/antioxidant status markers and the levels of lymphocyte subsets. In the assessment of the overall mortality rates of all the cases, six patients in Group-2 and three patients in Group-3 died, and there was no loss in Group-1. Proinflammatory cytokines IFNγ, IL-6, IL-17A, IL-2, IL-12, anti-inflammatory cytokines IL-10, IL-13, IL-1ra, and growth factors TGF-β, VEGF, KGF and NGF levels were found to be significant in Group-3. When Group-2 and Group-3 were compared, serum ferritin, fibrinogen and CRP levels in Group-3 had significantly decreased. The results demonstrated the positive systematic and cellular effects of MSCs application on critically ill COVID-19 patients in a versatile way. This effect plays an important role in curing and reducing mortality in critically ill patients.

Keywords: Cell based therapies, chemokines, cytokines and mediators, immune regulation and therapy, stem cells, viral infections

P-0561

The effect of isoflurane and iron dextran on biochemical and inflammatory parameters of tissues and organs in rats

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Anesthetics are types of medicines in a gaseous, liquid or other form, whose function is the introduction and/or maintenance of anesthesia with the aim of facilitating surgical and other painful interventions. Isoflurane has rapid pharmacological activity and absorbance, and stimulates rapid recovery after anesthesia. Long-term exposure to anesthetic acts negatively on peripheral organs and tissues, stimulates inflammation, oxidative stress, necrosis, and apoptosis leading to neurodegenerative diseases such as Alzheimer's and Parkinson's disease. Iron is an indispensable part of the living organism and the disorder of its homeostasis produces redox active iron that affects the formation of ROS, tissue injury, cell growth disorder, and ultimately the appearance of oxidative stress. The aim of the study was to investigate the effect of isoflurane itself and/or the combination of iron dextran on the level of oxidative stress and consequent inflammation on liver, spleen and kidney tissues. The obtained results indicate that long-term use of isoflurane and iron-dextran alone or in combination increases the level of oxidative stress and inflammation in the liver, kidney and spleen cells and leads to their impairment, indicating an increase in biochemical parameters in serum and hemolysis of erythrocyte.

Keywords: Animal models, inflammatory disease, tissue damage and repair

P-0562

TME Analyzer: a novel multi-dimensional visualization and analysis tool to identify immune contexture-based predictors in tumors

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Spatial distributions of immune cell populations, and their changes in time, are critical in understanding tumor evolution. Here, we present TME-Analyzer, an in-house developed guided-user interface with option to gate according to cell characteristics and expression for quick and reproducible image and data analysis. We tested and verified TME-Analyzer with immune-fluorescently stained and multi-spectrally imaged triple-negative breast cancer (TNBC) whole tissue sections. Densities of immune effector cells and distances between these cell types in different tissue compartments were in agreement with benchmark software across patients and tissues. When using >400 TME-Analyzer-derived immune contextual parameters, we discovered a 10-parameter classifier that significantly predicted short versus long survivors. Validation was performed with multiplexed ion beam imaging by time of flight (MIBI-TOF) imaging data of a 2nd cohort of TNBC patients. This classifier outperformed single and combined parameters according to COX proportional hazard model, and revealed the importance of distances between CD4 and CD8 lymphocytes. In conclusion, TME-Analyzer is an efficient and diverse tool to analyze immune micro-environments, and its application to TNBC has led to the identification of immune contexture parameters that differentiate survival in TNBC.

Keywords: Cancer immunology, immunotherapy, microenvironment

P-0563

Global transcriptomics profiling of Coxsackievirus B infection in pancreatic ductal cells

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The incidence of type 1 diabetes (T1D), especially in young children has dramatically increased worldwide in recent years. Besides genetic factors, non-genetic factors including enteroviral infections, as well as epigenetic events are likely to play a major role in the development of T1D. A potential association between enteroviruses, particularly coxsackievirus B (CVB), and the development of T1D has been reported in few studies. The response to acute CVB infections *in vitro* has been studied extensively, however still little is known about enterovirus induced transcriptomics changes in persistently infected pancreatic cells and their impact on the development of T1D. In this study we have established persistent CVB infections in a human pancreatic duct cell line using two different CVB strains. In addition, by using the two CVB strains we induced acute infection in human pancreatic cells to identify characteristic molecular features for each infection model. Our results revealed extensive changes in the transcriptomes of coxsackievirus B infected human pancreatic cells. Based on our RNA sequencing results we identified up to more than 2500 differently expressed genes for persistently infected pancreatic cells while around 1000 genes were affected in acute infection. By comparing the gene expression results of persistently infected pancreatic cells to acute infection we identified common features as well as unique patterns for persistent infection that have not been reported previously. These results highlight the importance of global characterization of infection induced changes when looking for novel information on events that might trigger the development of T1D.

Keywords: Autoimmunity, diabetes, viral infections

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POSTER PRESENTATIONS

P-0565

Different isolates of Group B *Streptococcus* induce different macrophage phenotype

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Streptococcus agalactiae (Group B *Streptococcus*, GBS) is a Gram positive commensal of the vaginal and intestinal tract of healthy adults. It is also a major invasive pathogen responsible for sepsis, meningitis and pneumonia in neonates and elderly, immunocompromised people. Susceptibility to infection highly depends on the immune status of the individual. Macrophages are phagocytic cells and are, as part of innate immunity, the first line of defence against pathogens. They are extremely heterogeneous, capable to adopt different activation states, which result in different macrophage phenotype. Roughly, M1 macrophages are associated with inflammatory responses while M2 macrophages produce anti-inflammatory mediators. In our preliminary study we determined the phagocytic profile of 12 different clinical isolates of *S. agalactiae* using THP-1 cell line derived macrophages and flow cytometry. We observed that phagocytosis between isolates ranged from 10-70 %. With flow cytometry we also analysed infected macrophages for the expression of surface markers. Results showed that some isolates induce higher expression of co-stimulatory markers than others. To gain a better insight into the macrophage immune response, we used Seahorse Extracellular Flux analyser to measure glycolysis and oxidative phosphorylation of macrophages, infected with different isolates. Results revealed that all infected cells differ significantly compared to control, uninfected cells and that some isolates induce higher increase in glycolysis compared to others. Our preliminary results therefore show that different isolates of *S. agalactiae* elicit different macrophage immune response.

Keywords: Bacterial infections, infectious disease, innate host defence, innate immunity, macrophage

P-0566

Could myeloperoxidase be a biomarker or predictor for colorectal cancer?

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Myeloperoxidase (MPO) is intensely produced in the early maturation phase of neutrophilic granulocytes. Colorectal tumor tissue has a higher amount of MPO^+ cells than normal tissue. We aimed to compare the parameters of myeloid and lymphoid cells and MPO levels in the blood of patients diagnosed with CRC and healthy volunteers.Age and sex- matched volunteers and patients (n=56) who were diagnosed with colorectal carcinoma in the biopsy and who had not yet received any treatment were included. The mean MPO level of the patient was lower than the control (3.59±2.26, 5.2±2.1 pg/mL, p=0.007). White blood cell, neutrophil and neutrophil-lymphocyte ratio (NLR) values were higher in the patient group, while lymphocyte, hemoglobin and hematocrit values were lower. When we grouped the patients according to the cut-off value, there were significantly more CRC patients in the group with an MPO value of 3.66 and below (p=0.001; p<0.01). Having an MPO value of 3.66 or below increased the risk of CRC by 8.13 (CI 95%: 2.133-30.984) times. Increased neutrophils in blood and MPO^+cells in cancerous tissue are seen in CRC. The MPO level was lower in the CRC, suggesting that immune system cells in CRC tend to be havedifferently in cancerous tissue and the circulatory system. The effect of MPO on the induction of granulocyte apoptosis following activation may explain the association between low MPO and increased neutrophil and NLR in blood. Clarifying the relationship between immunotherapy response and MPO level can help predict the effectiveness of breakthrough immunotherapy agents in cancer treatment.

Keywords: Cancer immunology, granulocytes, immunotherapy, myeloid cells, neutrophils

P-0567

Features of T-cell immune response to tick-borne encephalitis vaccine

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For tick-borne encephalitis (TBE), as for many other viral diseases, vaccination is the best approach to reduce morbidity. Antibody titer is considered as the gold standard for vaccination efficiency evaluation, whereas T-cells response is equally important and informative. Therefore, study of T-cell response to immunization can give a new insight in antiviral defense and inform new vaccine development. For our research we took PBMC from five donors and isolated leukocyte fractions at six timepoints during two-step TBE-vaccination. At several timepoints IFNγ-producer and $CD137^+$T-cell small fractions were collected. RNA was isolated, TCRβ cDNA, RNA-seq and HLA-alleles libraries were prepared and sequenced using Illumina HiSeq. Deep TCR repertoire profiling was performed. By implementing two different mathematical approaches hundreds of significantly expanded T-clones were found. These clonotypes were detected in small fractions. The discovered T-clones had comparable peaks at different timepoints, that allows us to hypothesize about T-cell recruitment at various response stages. Response strength was connected with infection before vaccination. In this case response was moderate, but independently of donor status T-clones were at the same level on the 75th day. This is the additional evidence of successful immunity training despite light infection in two weeks before vaccination. T-clones of donors with the same HLA-allels formed amino acid sequence clusters reflecting similar specificity. That is an important observation in absence of TBE virus tetramers. Also we observed different immune associated genes expressions. These results help deepen the understanding of T-clones behavior during immunization.

The work was supported by RSF grant 20-15-00351.

Keywords: Adaptive immunity, adjuvants and vaccines, immune response tracing, molecular immunology

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POSTER PRESENTATIONS

P-0568

Adverse COVID outcomes in youngsters with immune deficiencies; inequality exists between subclasses

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Genetic deficiencies of immune system, referred to as inborn errors of immunity (IEI), serve as a valuable model to study human immune responses. In a multicenter prospective cohort, we evaluated the outcome of SARS-CoV-2 infection among IEI subjects and analyzed genetic and immune characteristics that determine adverse COVID-19 outcomes. We studied 34 IEI patients (19M/15F, 12 (min:0.6-max:43) years) from six centers. We diagnosed COVID-19 infection by finding a positive SARS-CoV-2 PCR test (n=25) and/or a lung tomography scoring (CORADS) ≥4 (n=9). We recorded clinical and laboratory findings prospectively, fitted survival curves and calculated fatality rates for the entire group and each IEI subclass. Nineteen patients had combined-immune-deficiency (CID), six with predominantly-antibody-deficiency (PAD), six immune-dysregulation (ID), two innate-immune-defects, and one in the auto-inflammatory class. Overall, 23.5% of cases died, with disproportionate fatality rates among different IEI categories. PAD group had a relatively favorable outcome at any age, but CIDs and IDs were particularly vulnerable. At admission, presence of dyspnea was an independent risk for COVID-related death (OR: 2.630, 95% CI; 1.198-5.776, p<0.001).Concerning predictive roles of laboratory markers at admission, deceased subjects compared to survived had significantly higher CRP, pro-calcitonin, Troponin-T, ferritin, and total-lung-score (p=0.020, p=0.003, p=0.014, p=0.013, p=0.020; respectively); and lower absolute-lymphocyte-count, albumin, and trough IgG (p=0.012, p= 0.022, p=0.011; respectively). Our data disclose a highly vulnerable IEI subgroup particularly disadvantaged for COVID-19 despite their youth. Future studies should address this vulnerability and consider giving priority to these subjects in SARS-Cov-2 therapy trials.

Keywords: Infectious disease, viral infections, immunodeficiency

P-0569

Serum cytokine levels induced in response to immunization with Chlamydia psittaci specific ornithosis antigen in tumor-bearing animal models

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Understanding of the interaction between antitumor and antigen-specific immune responses is necessary for development of strategies for vaccination in persons with cancer-related immune deficiencies. In this pilot work we investigated serum cytokine response and tumor growth dynamics to immunization with the specific ornithosis antigen contained the most perspective chlamydia vaccine candidate - major outer membrane protein (MOMP) of Chlamydia spp. possessing a certain inflammatory activity, in the model of tumor-bearing animals. A single injection of the specific ornithosis antigen derived from C. psittaci AMK-16 strain (0,025 -1,25 mg/kg in different groups) was performed, one group of animals served as a control (tumor-bearing without treatment). Serum Th1/Th2/Th17 cytokines were evaluated in the start and in the end of experiment, and tumor growth was monitored by routine measurement for 21 days. We found that high dose of specific ornithosis antigen stimulated a pronounced decrease in TNF-α and IFN-γ serum levels in tumor-bearing rats accompanied by a marked progress in tumor growth. In contrast, immunization with low doses of antigen did not influence on the tumor sizes and induced elevation of TNF-α and IFN-γ in sera of treated animals. We did not find any significant difference in IL-17A or IL-4 serum levels in antigen-treated and control rats. Our preliminary data indicates that inflammatory cytokines produced in tumor-bearing animal models in response to immunization with MOMP-based specific ornithosis antigen may influence on the tumor growth dynamics.

This study was supported by the Russian Science Foundation #17-16-01099.

Keywords: Cancer immunology, *in vivo* tumor models, cytokines and mediators

P-0570

NADPH oxidase modulates Ca2+-dependent release of neutrophil extracellular traps

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Chronic granulomatous disease (CGD) is a severe inherited immunodeficiency characterized by recurrent bacterial and fungal infections and aberrant inflammation. The CGD phenotype is due to deficiency of phagocytic NADPH oxidase, unable to generate reactive oxygen species (ROS). Such granulocytes are limited in phagocytosis and degranulation, as well as unique means of combating pathogens, neutrophil extracellular traps (NETs) formation, in response to many receptor and pharmacological stimuli. However, activation of NET formation by neutrophils isolated from the blood of CGD patients in response to calcium ionophores was described in our recent study. As was shown previously, neutrophils deficient in NADPH oxidase are not only unable to form ROS, but also have deficiency in the electrogenic activity of the enzyme and membrane depolarization upon activation. Therefore, these neutrophils have impaired extracellular Ca2+ influx and, as a result, multiple disorders in the synthesis of proinflammatory cytokines. In the present study, we showed that NET formation by CGD neutrophils in response to calcium ionophore A23187 is accompanied by excessive accumulation of intracellular Ca2+. We explain this disorder by the deficiency of the electrogenic function of mutant NADPH oxidase, which in healthy donor neutrophils causes membrane potential depolarization. The results obtained in our study indicate an important function of phagocytic NADPH oxidase as a modulator of Ca2+-dependent signaling pathways, and potentially can be used for treatment of CGD.

Keywords: Cell signalling, granulocytes, innate immunity, neutrophils

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POSTER PRESENTATIONS

P-0571

Glycan structures found in viral vaccines are largely dependent on the cell type in which viruses are cultivated

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The SARS-CoV-2 pandemic has imposed the question of immunogenicity upon mankind, especially of the immunogenicity of viruses in vaccines intended for human usage. Viruses are often glycosylated, meaning they have a certain amount of carbohydrates on their surface. In this work we set out to determine the glycosylation pattern of viruses in human vaccines, including influenza - TorVaxFlu, Hepatitis A and B - Twinrix, varicella-zoster - Varilix and SARS-CoV-2 Vaccine (Vero Cell), Inactivated (InCoV), using lectin molecules with various specificities, with a methodology similar to ELISA. Lectin molecules are carbohydrate binding proteins and, in a way, they can act like anti-carbohydrate antibodies, but with a lower affinity. Therefore, the reactivity of lectin molecules to viral vaccines indicates, to some extent, the reactivity of antibodies to these carbohydrate epitopes. Using this simple methodology, we found that the addition of aluminum adjuvants lowers lectin binding in this assay. More importantly, we show that glycosylation pattern in viral vaccines is heavily dependent on the cell type in which viruses are produced, hence influenza vaccines, which are produced in chicken eggs have markedly different glycosylation patterns compared to other vaccines analyzed, which show extensive similarities. We think this is an important finding which needs to be acknowledged, mostly because of high similarity of glycosylation in production cell lines with glycosylation found in humans. This could, in theory, have a major impact on the efficacy of such vaccines, especially whole virion particle based vaccines produced for heavily glycosylated viruses.

Keywords: Antibody, innate immunity, viral infections

P-0573

Investigation of the protein composition of extracellular vesicles in asthma and COPD

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Both asthma and COPD are a heterogeneous group of diseases. Currently, there are no biomarkers which can easily and reliably distinguish the two mentioned lung diseases from each other. Moreover, 5-10% of patients have severe refractory asthma, they are not able to keep their symptoms under control with the products available today. This shows that new drug targets are needed. Extracellular vesicles (EVs) have an important role in the intercellular communication. Our aim is to find EV-associated proteins to identify new therapeutic pathways or differential diagnostic markers. EVs were isolated from platelet-free plasma samples (PFPs) by differential centrifugation. PFPs were centrifuged two times on 12500 g. The pellet was exposed to liquid nitrogen. A mass spectrometry measurement was carried out with 12 adult asthmatic and 6 adult control samples to analyze the protein content of EVs. SPSS software was used for the evaluation of the results. Proteins with significant differences were validated by ELISA in an extended population. Ascending level of complement c9 (a,b) were measured in parallel with the severity of asthma and the comparison of moderately severe asthma vs. control group showed nominally significant association (p=0.014). In addition, c9(a,b) and talin-1 were also associated with controllability of asthma (p=0.02 and p=0.0005). Talin-1 and complement C9 has been selected for further examination. If further studies can confirm our results, complement C9 and Talin-1 could be used in differential diagnosis of asthma and COPD and in the exploration of new pathomechanisms.

Keywords: Complement, biomarkers, immune regulation and therapy

P-0574

Defective oogenesis in mice with pristane-induced model of systemic lupus

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Systemic Lupus Erythematosus (SLE) is an autoimmune systemic disease characterized by the appearance of autoantibodies directed against vast array of self-antigens. On account of the common reproductive complications in females during their active reproductive years as a result of autoimmunity, it is urgent to address the question how SLE can influence female fertility. Pristane-induced mouse model of SLE is a suitable tool for studying in details the present disease having healthy animals with the same background. Lupus-like symptoms were induced through intraperitoneal injection of hydrocarbon oil pristane in Balb/c mice. The immune status of the experimental animals was characterized using flow cytometry, ELISpot and ELISA. The oocytes of the corresponding groups were analyzed via fluorescent microscopy based on chromatin, tubulin and actin structures using Hoechst 33258, FITC-labeled alpha-tubulin antibody and rhodamine-labeled phalloidin, respectively. A single i.p. injection of pristane led to formation of SLE-like phenotype in mice including production of different autoantibodies accompanied by glomerular depositions of IgG-containing immune complexes in the kidneys. The total number of obtained metaphase I oocytes from lupus mice was lower compared to healthy controls. The maturation rate, i.e. the proportion of eggs reaching metaphase II, was also reduced for lupus mice. In addition, oocytes from lupus mice presented specific abnormalities, including long chromosomes, disorganized spindle and missing actin cap. Pristane-induced mouse model of lupus exhibited numerous impairments of the reproductive system which may be explained by disruption in the local microenvironment as a result of disease activity.

Keywords: Animal models, autoimmunity, reproductive immunology

P-0575

Work loss before and after diagnosis in patients with celiac disease

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Celiac disease (CD) is an immune-mediated disease triggered by gluten intake and affects around 1% of the population worldwide. Although patients with CD have an increased use of healthcare, data on work disability remains scarce. To estimate work loss in patients with CD before and after diagnosis. We identified 16,005 working-age patients with prevalent CD, and 4,936 incident working-age patients diagnosed in 2008-2015 through biopsy reports from Sweden's 28 pathology departments. CD was defined by presence of villus atrophy (Marsh 3) on biopsy (gold standard). Each patient was compared to up to 5 matched general-population comparators. Using nationwide social insurance registers, we retrieved prospectively-recorded data on compensation for sick leave and disability. In 2015, patients with prevalent CD had a mean of 42.5 (95%CI: 40.9-44.1) lost work days as compared with 28.6 (27.9-29.2) in the general-population comparators, corresponding to a relative difference of 49%. Among incident patients, the annual mean difference between patients and comparators was 8.0 (5.4-10.6) lost work days 5 years before CD diagnosis, which grew to 13.7 (9.1-18.3) days 5 years after diagnosis. In addition to the continuously increasing mean difference in lost work days over time, there was also a transient increase in work loss in patients with CD during the year of diagnosis (mean difference: 15.6 days, 95%CI: 13.1-18.0). Patients with CD miss more work days than comparators before their diagnosis, and this loss increases and persists after diagnosis despite presumed installation of treatment with gluten-free diet.

Keywords: Autoimmunity, inflammatory disease, inflammatory bowel disease

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POSTER PRESENTATIONS

P-0576

Elevated levels of the inflammatory marker C-reactive protein (CRP) are associated with multiple frailty measures in older adults

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As humans age, the immune system, particularly activation and regulation of inflammation, can be dramatically impacted. Translating the clinical significance of these changes can be difficult as ageing is physiologically heterogenous. Phenotypes which reflect the heterogeneity and systemic nature of biological ageing, such as frailty, enable assessment of age-related immunological changes. Inflammatory pathways have been implicated in frailty development and acceleration. We investigated associations between systemic inflammation and frailty measures in The Irish Longitudinal Study on Ageing (TILDA). Four major frailty measures – Fried's Phenotype (FP), Clinical Frailty Scale (CFS), FRAIL Scale (FS), and Frailty Index (FI) – were investigated cross-sectionally in TILDA (n= 8,174, mean age 63.8 years). Serum CRP levels were used as a marker for systemic inflammation, with multinomial logistic regression performed to investigate associations with frailty measurement types. Our results showed that prevalence of frailty varied by frailty measurement: 3.7% for FP, 8.6% for moderate – severe CFS, 3.0% for FS, and 13.7% for FI. Higher CRP levels were associated with all four frailty measures (relative risk: 1.03 – 1.13, $P < 0.001$) in unadjusted and adjusted models. Despite differences in frailty measure prevalence, frailty was consistently associated with higher levels of inflammatory marker CRP, agreeing with growing evidence that inflammageing may contribute to accelerated biological ageing. Expanding this study to include additional markers of inflammation, observations of frailty phenotypes over time, genetic and additional health and lifestyle markers will allow more patterns of ageing to be modelled – potentially furthering our understanding of biological ageing.

Keywords: Ageing, big data, innate immunity

P-0577

Consequences of an ageing alveolar macrophage response to bleomycin-induced lung fibrosis

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Ageing is associated with increased susceptibility to chronic inflammatory diseases. Impairments in immunity contribute to the persistent inflammation and dysfunctional repair that characterise age-related conditions, including lung fibrosis. While embryonically derived alveolar macrophages [AMs] maintain tissue homeostasis during early life, a lifetime of environmental perturbations constantly remodels the AM niche. Monocyte derived AMs [Mo-AMs] recruited from the bone marrow accumulate with age and are often detrimental in chronic lung diseases. Therefore, we aimed to understand how an ageing immune system affects the function of AMs and hypothesised that a lung niche reconstituted with AMs from an ageing bone marrow would exacerbate lung fibrosis. We, thus, irradiated young recipient mice and reconstituted them with either young or aged bone marrow, followed by bleomycin challenge to induce lung fibrosis. Mice harbouring aged bone marrow exhibited an increased fibrotic burden and AM numbers in comparison to control animals and this was associated with decreased levels of interleukin-10 [IL-10], a key anti-inflammatory cytokine and fibrogenic regulator, suggesting an impaired reparative response. To investigate if AM derived IL-10 could limit tissue damage, we pre-treated mice with CpG oligonucleotides [CpG-ODNs], which has been shown to induce the expansion of IL-10 producing lung macrophages. Systemic CpG-ODN pre-treatment conferred protection against bleomycin-induced lung fibrosis. This was associated with reduced Mo-AMs, decreased bone marrow myeloid progenitors and importantly, elevated lung IL-10 levels. Ultimately, we aim to test if priming the aged immune system can kickstart effective resolution and improve the outcome of lung fibrosis by modulating lung macrophage responses.

Keywords: Ageing, bone marrow transplantation, innate immunity, myeloid cells, tissue damage and repair

P-0579

Characterisation of SARS-CoV-2 specific T cell immunity in COVID-19 convalescent individuals varying in their anti-RBD antibody kinetics

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The in December 2019 emerged betacoronavirus SARS-CoV-2 causes the severe respiratory disease COVID-19. Cellular immune responses are directed against a range of viral proteins, including nucleocapsid (NCAP), spike (S) and its receptor-binding domain (RBD). Not much is known about the durability of the adaptive immune responses in relation to acquisition of long-term protection. SARS-CoV-2 antibody levels decline after clearance of the primary infection. We have observed that the kinetics of this process varies between patients, indicating a difference in short-lived and long-lived plasma cell formation. In this study, we analyse PBMCs of convalescent mild individuals with either short (n=13) or long (n=11) anti-RBD IgG half-lives to investigate if they also differ in their antigen-specific CD4+- and CD8+- T cells. The patient groups are matched for gender, age and days after symptom onset. The study focusses on CD4+ T cells because of their important role in B cell differentiation. In an activation-induced markers (AIM) assay, PBMCs are stimulated with S and NCAP peptide pools to detect S- and NCAP- specific T cell responses. By using multiparameter cell surface and intracellular flow cytometry, several T cell lineages (based on chemokine receptors) and activation markers are determined in addition to the Th1, Th2, Th17 and T follicular helper (Tfh) cells cytokine profile. We expect to find differences in the T follicular helper cell population between the two groups because of their involvement in B cell differentiation and their antibody production.

Keywords: Adaptive immunity, cytokines and mediators, follicular helper T cells, monitoring immunity, viral infections

P-0580

IgG4-mediated autoimmune diseases have a normal (IgG4) B cell compartment

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A range of autoimmune diseases is characterized by autoantibodies that are predominantly of the IgG4 subclass which are termed IgG4 autoimmune diseases (IgG4-AID). These include several neuropathies, skin blistering disorders, nephropathies and a haemolytic disorder. It is unknown why IgG4 predominates in these disorders. We hypothesized that dysregulated B cell maturation or aberrant isotype switching results in overrepresentation of IgG4 B cell and plasma cell responses. Thereto the B-cell landscape of 10 MuSK myasthenia gravis and 10 pemphigus vulgaris patients (two archetypical IgG4-AID) was examined using flow cytometry with validated EuroFlow B cell markers and compared to age-matched healthy donors. Most B cell subset counts at any maturation stage did not differ between groups. Furthermore, IgG4 B cell and IgG4 plasma cell counts were normal in patients. Immature and naïve CD5+ B cells were decreased in patients by 3-fold and 2.5-fold, respectively. In pemphigus patients, we found increased overall counts of CD21-CD27+ memory B cells. In conclusion, patients with IgG4-AID do not have altered B cell maturation or increased levels of IgG4 memory B cells or IgG4 plasma cells. This argues against aberrant B cell biology in these patients, but rather suggests antigen-driven IgG4 predominance.

Keywords: Autoimmunity, B lymphocytes, neuroimmunology, visualizing immune responses

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POSTER PRESENTATIONS

P-0581

Clinical phenotypic transition during pemphigus

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Deep pemphigus and superficial pemphigus are the two main types of auto-immune pemphigus,each having different clinical, histological and immunopathological features.The phenotypic transition during pemphigus is unusual. To describe clinical phenotypic shift of pemphigus relapse. We carried out a retrospective review of the clinical records of 302 cases of pemphigus at a single centre. Twelve patients experienced phenotypic switch during the first relapse.Two seborrheic has shifted to foliaceus,three vulgaris to vegetans,four vulgaris to seborrheic,one vegetans to vulgaris,one seborrheic to vegetans,one vulgaris to foliaceus.Mean age:43 years,sex ratio 8M:4F,mean duration of the disease was14.5months(2-38months).Mean peiod of the transition:48months(5 -156 months).Mean IIF 500.All the patients were treated before the transition with oralprednisone (1.5-2mg/kg/jr)and azathioprine; two received rituximab,one disulone and one bolus of methylprednisolone.Mean IIFduring relape 355.The reported complications were mostly infectious two herpes infection,two zona infection,one hydatit cyst of the liver,one septic shock and one cerebral abscess. The clinical phenotype of different forms of pemphigus is reportedly defined by the anti-desmoglein antiantibody.Our report is the largest case series allowed to hone-in on the analysis of the transition based on clinical and histological features.

Keywords: Epigenetic control and modulation of immunity, autoimmunity, skin diseases, visualizing immune responses

P-0582

Effects of *Cotinus coggygria* extract on human blood T and NK cells activity *in vitro*

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It is an important research area to define the bioactivities of plant fractions and to create appropriate utilization areas for them. Although Cotinus coggygria, which is widely used in folk medicine, which grows from Europe to the center of China, has a wide variety of bioactivities, there is limited information about its influence on the immune system. The anti-proliferative effects of *Cotinus coggygria* extract on stimulated peripheral blood mononuclear cells (PBMCs) were determined by CFSE dilution method. The effects of the extract on peripheral blood T and natural killer (NK) cells were followed by their proliferative responses. Annexin V / PI staining method was used to evaluate the apoptotic effects of the extract on PBMCs. The effects of the extracts on expression levels of IL-1β, IL-2, IL-4 and IL-10 genes were investigated. Effect of Cotinus coggygria on the proliferation of T cells and NK cell subsets were decreased in a dose dependent manner. Anti-proliferative effect of Cotinus coggygria extract on stimulated PBMCs was not directly related to its apoptotic properties, it also changes the cytokine genes expression on PBMCs. Our study suggests that *Cotinus coggygria* methanol extract, whose anti-inflammatory effects have not been investigated yet, may be a pioneer in the identification of new molecules with anti-inflammatory properties and the development of related therapies.

Keywords: Autoimmunity, autoinflammation, NK cells

P-0584

Immunological and clinical characteristics of systemic lupus erythematosus: study on 203 Tunisian patients

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Systemic Lupus Erythematosus (SLE) is a chronic inflammatory autoimmune disease with a wide range of clinical presentations resulting from abnormal immunological function. The aim of this study was to describe the clinical and immunological profile of Tunisian patients with SLE. This retrospective study describes the clinical and immunological featuresin a series of 203 SLE Tunisian patients in Fattouma Bourguiba University Hospital, Tunisia, between 2000 and 2020. The female to male ratio was 10:1. Mean age was 38 years. The main clinical manifestations were articular manifestations (84%), mucocutaneous manifestations (78%). Renal involvement was observed in 28% of patients. ANA were found in all patients, anti-DNA antibodies in 72%, anti-Sm antibodies in 34%, and anti-nucleosome antibodies in 18%. Anti-SSA, anti-SSB, and anti-RNP antibodies were detected in 44.8%, 28.1%, and 34.5%, respectively. Decreased C3 level was found in 21%. We found the following associations between autoantibodies and clinical manifestations to be statistically significant: anti-DNA with higher prevalence of lupus nephritis (p=0.05) and articular involvement (p=0,014), anti-Sm with higher prevalence of leucopenia (p=0.02), anti-nucleosome with higher prevalence of lupus nephritis (p=0.046) and neurological manifestations (p=0.042), anti-SSA with higher prevalence of mucocutaneous manifestations (p=0.02) and pulmonary arterialhypertension (0.006), anti-SSB with higher prevalence of pulmonary arterial hypertension (p=0.026) leucopenia (p=0.003) and thrombocytopenia (p=0.04), and anti-RNP with higher prevalence of Raynaud phenomenon (p=0.002), arthralgia (p=0.014) and interstitial lung disease (p=0.05). The clinical and immunological characteristics and correlations of our patients with SLE are largely comparable to other study populations.

Keywords: Antibody, autoimmunity, complement

P-0585

T cell repertoire sequencing to study the contribution of different donor T cell subsets to patient repertoire at the early stage after αβT/CD19-depleted allogeneic hematopoietic stem cell transplantation

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αβT/CD19-lymphocyte depletion allows decreasing the incidence of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation (HSCT). The additional infusion of donor CD45RA-depleted T cells (mDLI) can potentially compensate for the delayed immune reconstitution. Previously we've shown that during the first several months after αβT-depleted HSCT patients have extremely low T cell repertoire diversity, and T cell clones can be transferred with primary graft and/or mDLI. Here we used T cell repertoire sequencing to study the impact of different donor T cell subpopulations to the recipient repertoire early after αβT/CD19-depleted HSCT with and without mDLI (n=11,n=8). βTCR clonotypes from donor primary graft, mDLI and FACS-sorted CD4+, CD8+, Tscm(CD3+CCR7+CD95+CD127+), Tem(CD3+CCR7-RO+), Tcm(CD3+CCR7+RO+) cells were tracked in recipient blood at d60 and d120 after HSCT. A relatively small number of donor T cell clones expanded enough to be detected in patient blood at 2 or 4 months after HSCT. More donor clones with higher initial T cell proportion were detected in patients transplanted with mDLI. However, the infusion of mDLI not resulted in higher repertoire diversity. Both, CD4+ and CD8+ T cells contributed to the early patient repertoire. Surprisingly, clones from T cells with high proliferative potential (Tscm) didn't have a higher detection rate in patients. While the detection rate was higher for clonotypes from donor CD69+ T cells. Our results demonstrate that infusion of donor memory T cells enriches recipient repertoire after allogeneic HSCT, but other factors beyond initial clone size influence its survival and expansion.
The study supported by RFBR grant #19-015-00551.

Keywords: Adaptive immunity, bone marrow transplantation, monitoring immunity, omics technologies

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POSTER PRESENTATIONS

P-0586

Pathogen-specific γδ T cell response in pediatric sepsis

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Sepsis is a leading cause of childhood mortality worldwide, with newborns and young children being at the highest risk of developing severe or lethal infections. Extensive characterization of host-pathogen interactions in early life is required to develop novel preventive and therapeutic interventions. γδ T cells are an unconventional T cell population that possess characteristics of innate and adaptive immune system. Vγ9Vδ2 T cells are a main subset of γδ T lymphocytes in human adult blood that can react in a TCR-dependent way towards phosphoantigens, small metabolites that increase upon bacterial infections. Vγ9Vδ2 T cells are also abundant in human fetal peripheral blood, but compared to their adult counterparts they have a distinct developmental origin, and are hyporesponsive towards phosphoantigens *in vitro*. It is not known whether Vγ9Vδ2 T cells can react towards bacterial infections in early life. In order to address this question, we investigated the response of γδ T cells in young children with blood culture-proven sepsis. By analyzing γδ TCR repertoire using high throughput sequencing, we observed that infection of young children (< 2 years) with *S. aureus*, but not with other pathogens, was associated with the expansion of a highly public (shared between *S. aureus*-infected infants) fetal-like Vγ9Vδ2 TCR repertoire. This outcome was determined to be age dependent as we did not observe this kind of expansion in children older than two years. In conclusion, our data indicate that Vγ9Vδ2 T cells can respond towards bacterial infections in early life in a pathogen-specific way.

Keywords: Bacterial infections, fetal immunity, gamma-delta T cells, molecular immunology, omics technologies

P-0588

The clinical significance of anti-DFS70 antibodies

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Anti-dense fine speckled (DFS) 70 antibodies are known to have multiple biologic functions, but clear disease associations have not been confirmed. Isolated anti- DFS70 antibodies could facilitate the exclusion of antinuclear antibodies (ANA) associated rheumatic diseases (AARD).the aim of this study was to analyze the clinical significance of isolated anti-DFS70 antibodies. All the serum samples obtained in our hospital between December 2019 to December 2020, that were positive for ANA testing and anti-DFS70 antibodies were included. Demographic and clinical data were collected from anti-DFS70 positive patients. From 58 samples, 47 patients (81%) had isolated anti-DFS 70 antibodies; 85.1% were women and mean age was 41 years. The reasons for requesting ANA were mainly cutaneous manifestations in 10/47 (21.2%), followed by arthritis in 8/47 (17%), and pregnancy loss in 4/47 (8.5%). Only 6 patients (12.7%) had defined AARD: 3 patients had systemic lupus erythematosus, 2 patients had Hashimotos's thyroiditis and 1 patient had primary Sjögren syndrome. There were two patients with rheumatoid arthritis and one had sarcoidosis. Regarding patients without autoimmune disease, 19 patients (40%) had no complains. Patients with anti-DFS70 antibodies rarely present AARD, and only 13.7% of subjects showed autoimmune features (including AARD and rheumatoid arthritis). Patients with AARD had only mild clinical symptoms suggesting that anti-DFS70 antibodies can be considered as maker of benign autoimmunity.

Keywords: Antibody, autoimmunity, autoinflammation

P-0591

Retrospective analysis of pemphigoid gestationis in 19 moroccans patients

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Pemphigoid gestationis (PG)is a rare subepidermal bullous dermatosis occurring during the 2nd or 3rd quarter of gestation or in the postpartum period. The aim of this work is to draw a profile of the epidemiology, clinical aspects, treatment of PG.Methods retrospective study of 19cases of PG followed at a single center between 2004 -2020. mean age 31.5 years. 89.5% of cases occurred in multiparous women,8 patients were on oral contraceptives. PG appeared during the last 3 months of gestation in57,89%.52.6% of the patients were adressed by general practitioners,15.8 % by gynecologists,the other cases were either adressed by dermatologists or ER.In all cases, purities was the first symptom, followed by an erythematous maculopapular eruption. In the steady state of the disease, all patients had annular confluent erythematous papules with herpes like vesicles predominant in the umbilicus. The diagnosis of PG was confirmed by direct immunofluorescence. Peripheral eosinophilia was seen in 63.7%.we observed 5cases of preterm birth, 2cases of small-for-gestational age babies and one intrauterine fetal death. Systemic corticosteroids (0.5–1mg/kg/day) were used in 68,4%.31.6% of patients were treated with oral antihistamine and topical glucocorticoid. Exacerbation after delivery was observed in 5 patients and 7 patients had recurrence during the following pregnancies. Morocco is a country known as endemic for pemphigus, which suggests an environmental role in the pathogenesis of this cutaneous blistering autoimmune disease. Therefore, the behavior of other autoimmune blistering diseases, such as PG in this country is relevant. Our resultasts shared many similarities with other studies performed worldwide, indicating that environmental triggers are seldom related to the pathogenesis of PG.

Keywords: Autoimmunity, epigenetic control and modulation of immunity, fetal immunity, immune communication, immune development

P-0592

Iatrogenic Kaposi's sarcoma after immunosuppressive therapy: a retrospective study

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Kaposi's disease (KD) is a multifocal disease.It can occur in an endemic setting:it may be associated with human immunodeficiency virus or it may occur as a complication of immunosuppression, particularly of iatrogenic origin in transplant patients.This report aim to describe the epidemiological, clinical and therapeutic profile of iatrogenic KD in Morocco in a setting not involving organ transplantation. A retrospective analysis of 84 patients with KD covering 30 years period within this study 23 patients were presenting histologically confirmed iatrogenic KD. Fifteen men and eight women were included with a mean age of 61 years.All patients received corticosteroids, associated to cyclophosphamide in three cases, azathioprine in one case and methotrexate in three cases.The mean time of onset of lesions after starting immunosuppressive therapy was 31 months.All cases presented cutaneous lesions,the most common localization was the lower limbs. Impaired mucosal membrane was seen in 55.2% of patients and visceral involvement was seen in 11 patients (four patients:lymph nodes,two patients lung,five patients gastrointestinal tract).HIV serology tests were negative in all patients but HHV8 serology tests were positive in 78.5% of patients. Treatment consisted primarily of reduction or withdrawal of the immunosuppressant while 3 patients received bleomycine.The outcome was favourable in the majority of cases except one death from hypovolemic shock. This report emphasizes the value of regular follow-up and routine dermatological examination of patients on immunosuppressant therapy and suggesting the value of screening for HHV8 infection before initiating such therapy.

Keywords: Cancer immunology, immunodeficiency, microenvironment, viral infections

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POSTER PRESENTATIONS

P-0593

Vitiligo and scrotal calcinosis: a rare association

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A 57-year-old moroccan man, history of acro-facial vitiligo for 30 years,presented small calcified yellowish painless lesions covering his scrotum that have been progressively appearing for over 10 years with simultaneous depigmentation of the scrotum. He reports the recurrence of the lesions after surgical excision. Physical examination found amelanotic lesions affecting the scrotum.Laboratory tests including serum calcium and phosphorus were normal. Histological examination confirmed the diagnosis of scrotal calcinosis (SC). An excision of the affected scrotal skin was performed. SCis a very rare and benign condition; occurring in the absence of metabolic and phosphocalcic disorders.The association with vitiligo was previously described by Feinsteinetal in1984. SC usually affects young African men. It clinically manifests as firm yellowish usually multiple nodules that may spread on the whole penoscrotal area. It is generally asymptomatic; in some rare cases it can cause itching or pelvic/ perineal pain. The pathogenesis is mostly speculative. Some believed that the lesions are caused by dystrophic calcification of the dartos muscles of the scrotum. The role of repeated minor trauma has also been suggested.In our case, association with vitiligo, which is known to be triggered by Koebner phenomenon, on the same area may reinforce this hypothesis. However, most recent data suggest that the calcified lesions are probably the result of dystrophic calcification in the wall of epithelial cysts. In fact, in many patients with supposedly "idiopathic" SC, epithelial cysts with a variable amount of keratin and calcification or epithelial remnants were found along with the fully developed lesions of SC, suggesting that the calcification of these epithelial cysts is probably the main cause of scrotal calcinosis.

Keywords: Autoimmunity, immune networks, immune response tracing, memory, microenvironment, skin diseases

P-0594

Pemphigus foliaceus and Cerebrovascular aneurysm: a coincidence or a rare association

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A47-years-old moroccan man, history of smoking, chronic headache,admitted to dermatology for 7 months of erythroderma,flaccid vesicles,erosions,crusts and desquamation on scalp,face and trunk. The diagnosis of pemphigus foliaceus (PF) was confirmed through clinical, immunological and histopathological features.Coincidental discovery through CT-angiography of unruptured aneurysm of the posterior communicating artery. Surgery was performed while the patient was treated with oral prednisone and azathioprine. Cardiovascular and cerebrovascular morbidity occurs in multiple chronic inflammatory skin diseases due to chronic inflammation and immune cells. A retrospective study of 147 Brazilian patients with pemphigus vulgaris or foliaceus found they had higher blood pressure and higher rates of diabetes, obesity, hypertriglyceridemia and cardiovascular events. The circulating levels of vascular endothelial growth factor (VEGF) a potent endothelial activator, are increased in PF patients with erythroderma; tissue damage stimulates (VEGF) synthesis by keratinocytes, which interacts with VEGF receptor on both endothelial cells and keratinocytes. Angiogenic factors, particularly (VEGF) appear to play an important role in the pathogenesis and growth of cerebrovascular malformations via induction of endothelial cell proliferation. Several immunohistochemical studies demonstrated involvement of VEGF in the pathogenesis and enlargement of cerebral vascular malformations by locally enhanced expression. Through this case, we suggest the value of screening for cerebrovascular and cardiovascular diseases in pemphigus patients.

Keywords: Autoimmunity, autoinflammation, cardiovascular diseases, immune networks, inflammatory disease, skin diseases

P-0595

Psoriasis and glucose-6 phosphate dehydrogenase deficiency: coincidental case or a rare association?

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A45years old patient presented to our dermatology clinic with generalized psoriasis. Family/personal history revealed G6PDdeficiency. For our patient it was class 3 variant according to WHO classification. No history of recent hemolysis,infection/drug use. His skin examination: generalized erythematous, scaly plaques and lymphadenopathy PASI20. UVBphototherapy was proposed with good evolution. Deficiency of G6PD is the most common erythrocyte enzyme defect. It is genetically transmitted as X-linked recessive. Several papers has reported its association to other autoimmune diseases. Apaper on Sudanese vitiligo patients revealed an excess deficiency of G6PD compared to the controls. It has been shown Type 1 diabetic patients with G6PD deficiency suffer from accelerated microvascular complications such as retinopathy. Also a coincidental case of G6PD deficiency with celiac disease was reported.A research has shown decreased erythrocyte G6PD activities in patients with lichen planus in an area where an increased incidence of this skin disease coincides with higher frequency of favizm might suggest a biochemical overlap between these two conditions. It has been proven that in psoriatic skin lesion G6PD activity was important in malpighian layer, stratum granulosum and the overlying parakeratotic layer.Uninvolved skin of psoriatic patients and healthy control patients had confined G6PD activity only to pilosebaceous unit. Another paper on *in vitro* activity of anti-G6PD agents in psoriatic therapeutics stated that most active inhibitor was methotrexate followed by cGMP. Erythrocyte kinetics also change with psoriasis: membrane fluidity decreases and cytoskeleton is impaired. This caused reduced propensity to hemolysis. This is the second reported- case of psoriasis with G6PD deficiency. Even though it can't be concluded from this case, psoriasis and G6PD deficiency might overlap with more serious or more mild clinical tables.

Keywords: Autoimmunity, autoinflammation, immune communication, inflammatory disease, microenvironment, skin diseases

P-0596

The PD-1 / PD-L1 expression on lymphocytes subpopulations in patients with allergic rhinitis and bronchial asthma before and after the course of allergen-specific immunotherapy

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The PD-1 molecule and its ligands play an important role in allergen-induced immune responses. Currently, there are assumptions to use PD-1/PD-L1 as a markers of the effectiveness of allergen-specific immunotherapy (ASIT). The aim of this study was to analyze the PD-1/PD-L1 expression in patients with bronchial asthma (BA) and allergic rhinitis (AR) before and after the course of ASIT. The study of the PD-1/PD-L1 expression on subpopulations of blood T-cells and B-lymphocytes was performed by flow cytometry. Patients with BA (n=5, age 33.8±2.6), AR (n=7, age 31.5±2.8) and healthy donors (n=7, age 33.8±2.9) were included. All patients had sensitization to plant pollen allergens confirmed by skin prick tests. Statistical analysis was made by using Mann-Whitney criteria ($p<0,05$). The B-lymphocytes count decreased but PD-L1 expression by B-lymphocytes increased after ASIT in AR patients. The PD-1 expression by Treg cells decreased in patients with AR both before and after treatment comparing with donors. At the same time, these indicators were not changed in the group of BA patients. The expression of PD-L1 on Tregs increased in AR patients in comparison with donors before ASIT and decreased at the end of the course of treatment. Different situation was observed in BA: the level of PD-L1+T-regs increased after ASIT. Our results reflect differences in the pathogenesis of allergic disorders, which are associated with the imbalance of the processes of cell activation and suppression. Further studies are required to establish the role of PD-1/PD-L1 interactions in the process of ASIT-induced modification of allergic responses.

Keywords: Allergen-induced immune responses, allergic disorders, B lymphocytes, modification allergic responses, regulatory cells

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POSTER PRESENTATIONS

P-0597

Metabolic, transcriptional, and signaling disturbances in giant cell arteritis Tregs

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Giant cell arteritis (GCA) is an autoimmune disease which causes inflammation of large arteries. While Th1 and Th17 cells have been heavily implicated in disease pathogenesis, regulatory T cells (Tregs) failed to suppress conventional T cells proliferation, indicating dysfunction. However, transcriptional and signaling abnormalities in GCA Tregs have not been elucidated. This project aims to reveal molecular and cellular bases for the breakdown of tolerance in GCA Tregs. 34 GCA patients (active disease, n = 13; in remission, n = 21) were recruited for the study. RNA-Seq performed on sorted *ex vivo* Tregs (CD4+ CD25hi CD127lo) revealed lower expression of key Treg transcription factors (*FOXP3*, *IRF4*, *IKZF4*), glycolytic enzymes (*ENO1*, *PFKP*, *LDHA*), as well as molecules downstream to IL-2 signaling (*SOCS2*, *CISH*) in active GCA Tregs as compared to healthy Tregs. FOXP3 and IRF4 expression was validated at protein level by FACS. Furthermore, surface expression of induced CD25 (IL-2Rα) and GARP was also lower in GCA Tregs. On the other hand, the frequencies of FOXP3 exon 2-skipped Tregs (pathogenic Tregs) were higher in GCA patients than healthy individuals. Calcium flux assay revealed absent calcium signaling in Tregs from active patients which was normalized in patients in remission. Lastly, *in vitro* treatment of healthy Tregs with 2-deoxyglucose (2 mM, 48 hours) effectively abolished TCR-induced calcium flux, diminished CD25 and GARP upregulation, and increased the frequencies of pathogenic Tregs, which altogether recapitulated the phenotypes of GCA Tregs. These findings provide novel insights in the immunopathogenesis and potential clinical activity correlates of GCA.

Keywords: Autoimmunity, metabolic control of immune responses, regulatory cells

P-0598

Phenotypical and functional characterization of neutrophils in two pyrin-associated auto-inflammatory diseases

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Familial Mediterranean Fever (FMF) and Pyrin-Associated Autoinflammation with Neutrophilic Dermatosis (PAAND) are clinically distinct autoinflammatory disorders caused by mutations in the pyrin-encoding gene MEFV. We investigated the transcriptional, phenotypical, and functional characteristics of patient neutrophils to explore their potential role in FMF and PAAND pathophysiology. RNA sequencing was performed to discover transcriptional aberrancies. The phenotypical features, degranulation properties, and phagocytic capacity of neutrophils were assessed by flow cytometry. Production of reactive oxygen species (ROS), myeloperoxidase (MPO) release, and chemotactic responses were investigated via chemiluminescence, ELISA, and Boyden chamber assays, respectively. Neutrophils from PAAND and FMF patients showed a partially overlapping, activated gene expression profile with increased expression of S100A8, S100A9, S100A12, IL-4R, CD48, F5, MMP9, and NFKB. Increased MMP9 and S100A8/A9 expression levels were accompanied by high plasma concentrations of the encoded proteins. Phenotypical analysis revealed that neutrophils from FMF patients exhibited an immature character with downregulation of chemoattractant receptors CXCR2, C5aR, and BLTR1 and increased expression of Toll-like receptor 4 (TLR4) and TLR9. PAAND neutrophils displayed an increased random, but reduced CXCL8-induced migration. A tendency for enhanced random migration was observed for FMF neutrophils. PAAND neutrophils showed a moderately but significantly enhanced phagocytic activity as opposed to neutrophils from FMF patients. Neutrophils from both patient groups showed increased MPO release and ROS production. Neutrophils from patients with FMF and PAAND, carrying different mutations in the MEFV gene, share a proinflammatory phenotype yet demonstrate diverse features, underscoring the distinction between both diseases.

Keywords: Autoinflammation, innate immunity, neutrophils

P-0599

Umbilical cord blood DNA methylation in children who later develop type 1 diabetes

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Distinct DNA methylation patterns have recently been observed to precede Type 1 Diabetes in whole blood collected from young children. Our aim was to determine if such methylation patterns are present already at the time of birth. Reduced representation bisulfite sequencing (RRBS) analysis was performed on a unique collection of umbilical cord blood samples collected within the Type 1 Diabetes Prediction and Prevention (DIPP) study. Children later diagnosed with Type 1 Diabetes and/or testing positive for multiple islet autoantibodies (N=43) were compared to control individuals (N=79), who remained autoantibody-negative throughout the DIPP follow-up until 15 years of age. Altogether 24 clinical and technical covariates related to the pregnancy and the mother were included in a binomial mixed effects model, which was fit separately for each high-coverage CpG site, followed by spatial and multiple testing adjustment of P values. We discovered a strong inflation of P values, which was caused by a standard spatial adjustment method. Findings that were based on Benjamini-Hochberg corrected spatially adjusted P values, could not be validated by Pyrosequencing. We therefore used permutation-based significance analysis and showed that sex-associated differentially methylated cytosines could be reproducibly detected with this approach. After empirical type 1 error control, no differences in cord blood methylation patterns were observed between cases and controls. Differences between children who progress to Type 1 Diabetes and those who remain healthy throughout childhood, are not yet present in the perinatal DNA methylome.

Keywords: Autoimmunity, diabetes, molecular immunology

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POSTER PRESENTATIONS

P-0600

Targeting the immunomodulatory properties of stem-like colorectal cancer cell lines

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Consensus molecular subtyping of colorectal cancer (CRC) revealed the high level of intratumoral heterogeneity and highlighted that some tumors can be characterized by stemness properties such as chemoresistance, self-renewal, and EMT. Macrophages, the major population of tumor-infiltrating cells, have different roles in tumor progression, depending on their polarization. Cancer stem cells of breast and ovarian cancers were shown to induce the macrophage polarization towards the M2 type. However, the data on interactions between CRC cells and infiltrating macrophages is incomplete. In this study, we investigated the link between the stemness of CRC cells and their ability to induce macrophage polarization *in vitro*. We established the differences in the expression of stemness- and EMT-related genes and proteins in eight CRC cell lines. Furthermore, using an indirect co-culture system, we showed that CRC cell lines, belonging to stem-like molecular subtype, were able to induce the strongest macrophage polarization. In particular, the HCT116 cell line, characterized by the high expression of IL-4, demonstrated the trend to polarize macrophages toward M2 type, while the SW620 line, characterized by the high expression of TNF-α, was likely to induce M1 type polarization. In presence of various stemness inhibitors (salinomycin, SB-431542, JIB-04, napabucasin) and their combinations, CRC cell lines showed decreased ability to induce M2 polarization (a decrease of surface CD206), while favoring M1 polarization (an increase of surface MHCII, CD11c, CD80/86). Together, these data support the evidence for immunomodulatory properties of CRC cells and suggest targeting the stemness properties to disrupt the suppressory immune tumor microenvironment.

Keywords: Cancer immunology, immune communication, macrophage, microenvironment, molecular immunology, stem cells

P-0603

Anti SARS-CoV-2 antibodies: one virus, many questions

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One of the most questioned issues about SARS-CoV2 immunity is how long does it last. The answer to this question will determine key issues such as the reliability of individual and herd immunity or the need of social restrictions or periodical revaccination. The aim of this study was double: 1. to determine retrospective seroprevalence evolution among blood donors along and whether age, blood group or haematological parameters were related to recent past infection. 2. To know how long total anti SARS-CoV2 antibodies would persist in peripheral blood. A total of 14260 samples from 12062 donors (from July 2019 to October 2020) were tested for anti SARS-CoV-2 N protein total Immunoglobulins. Donors were 60.8% males and aged 46+/-13. Seropositive donation rate grew up from week 11 to week 21, reaching plateau at 8% donations. No differences by sex, age or blood group were found. Lymphocyte were significantly higher in positive women as compared to negative ones and haemoglobin were lower in positive men as compared to negative ones. 97.6% donors remain seropositve up to 46 weeks after first positive. Neither age nor blood group or sex were related to antibody waning. Seroprevalence due to asymptomatic cases would be equivalent to overall one. Sex and age would not affect COVID-19 susceptibility but its severity. Gender differences are present even in asymptomatic individuals: females might be protected from severe outcomes by their lymphocytes whereas males with decreased of haemoglobin would be weaker. Specific antibodies would last at least almost a year.

Keywords: Adaptive immunity, antibody, biomarkers, infectious disease, viral infections

P-0604

Our monoclonal antibodies show that eosinophils express high level of the purinergic receptor P2X4

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Extracellular nucleotides are important mediators of cell activation and trigger multiple responses via membrane receptors known as purinergic receptors (P2). P2X receptors are trimeric ligand-gated ion channels, activated by extracellular ATP. Their roles are partly coinciding but they do not have similar expression patterns across tissues and cell types. P2X4 is one of the most sensitive purinergic receptors, that is typically expressed by neurons, microglia, and some epithelial and endothelial cells. P2X4 mediates neuropathic pain via brain-derived neurotrophic factor and is also involved in inflammation in response to high ATP release. Therefore, it is involved in several inflammatory pathologies and neurodegenerative diseases. We have produced four monoclonal antibodies (mAb) directed against the human P2X4 receptor specifically. We have shown that these mAbs cross-react with mouse and rat P2X4 but with a lower avidity. We have demonstrated that our mAbs can be used in flow cytometry, immunoprecipitation and immunohistochemistry but not in Western blot assays, indicating that they target conformational epitopes. We used these mAbs to characterise the expression of P2X4 receptor on mouse and human peripheral blood lymphocytes (PBL). We showed P2X4 being expressed at the surface of several leukocyte cell types, with the highest expression level on eosinophils. P2X4 is expressed by leukocytes, in human and mice, with a significant gender difference, males having higher surface expression levels than females. Our findings reveal that PBL express significant levels of P2X4 receptor and suggest their important role in leukocyte activation, specially eosinophils, by ATP.

Keywords: Engineering of antibodies and nanobodies, inflammatory disease, neuroimmunology

P-0605

Centromeric KIR AA individuals harbor particular KIR alleles conferring beneficial NK cell features with implications in haplo-identical hematopoietic stem cell transplantation

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We have recently shown a broad disparity of NK cell responses against leukemia highlighting good and bad responders resting on the KIR and HLA genetic. In this study, we deeply studied KIR2D allele expression, HLA-C recognition and functional effect on NK cells in 108 blood donors in combining high-resolution KIR allele typing and multicolor flow cytometry. The KIR2DL1*003 allotype is associated with centromeric (cen) AA motif and confers highest NK cell frequency, expression level and strength KIR/HLA-C interactions compared to the KIR2DL1*002 and KIR2DL1*004 allotypes respectively associated with cenAB and BB motifs. KIR2DL2*001 and *003 allotypes affect negatively the frequency of KIR2DL1$^+$ and KIR2DL3$^+$ NK cells. Altogether, our data suggest that cenAA individuals display more efficient KIR2DL alleles (L1*003 and L3*001) to mount a consistent frequency of KIR2DL$^+$ NK cells and to confer an effective NK cell responsiveness. The transposition of our *in vitro* observations in T-replete haplo-identical HSCT context led us to observe that cenAA HSC grafts limit significantly the incidence of relapse in patients with myeloid diseases after T-replete haplo-identical HSCT. As NK cells are crucial in HSCT reconstitution, one could expect that the consideration of KIR2DL1/2/3 allelic polymorphism could help to refine scores used for HSC donor selection.

Keywords: MHC and polymorphic genes, NK cells, transplantation

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POSTER PRESENTATIONS

P-0606

Effect of interleukin – 6 on B cells responsible for Gd-IgA production in IgA nephropathy regarding to corticosteroid treatment

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IgA nephropathy (IgAN) is a frequent glomerulonephritis, characterized by deposition of IgA1 immune complexes in glomerular mesangium. The IgA1 molecule shows aberrant O-glycosylation consisting of reduced galactose content (Gd-IgA). B cells producing Gd-IgA haven't been properly characterized yet. IL-6 cytokine is one of the crucial factor in IgAN which affects Gd-IgA production. Corticosteroids (CS) are used for treatment of IgAN patients. PBMC of IgAN patients with or without Prednisone treatment were stimulated *in vitro* with different concentrations of IL-6 followed by the analysis of induced changes in populations of CD19+, IgA+ and Gd-IgA+ cells by flow cytometry. After stimulation with IL-6 concentration above 50 ng/ml, the reduction of CD19+ and IgA+ cell populations relative to total lymphocytes was detected. The percentage of Gd-IgA+ cell subpopulation relative to total IgA+ cells was enriched depending on increasing IL-6 concentration. The PBMCs from Prednisone-treated patients exhibited reduction in CD19+ and IgA+ populations irrespective to IL-6 stimulation at all tested concentrations indicating long-term phenotypic imprint of CS. Interestingly, analogical suppression of Gd-IgA+ cells was not detected after IL-6 stimulation indicating that corticosteroids could suppress the IgA producing but not Gd-IgA producing cells in IgA nephropathy. Our results could help to clarify the effect of IL-6 and also CS on the Gd-IgA production in IgAN patients.

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Keywords: Autoimmunity, B lymphocytes, immune response tracing

P-0607

Composition of the human pancreatic beta cell's peptidome bound to T1D-associated HLA alleles

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Presentation of autoantigen-derived peptides by MHC molecules is a key step in the autoimmune response resulting in human type 1 diabetes (T1D). Cellular autoantigens have been identified in T1D in addition to insulin, most residing in the insulin secretory granules of β-cells. β-cells are highly susceptible to ER stress, which could lead to altered protein processing and the possible generation of diabetogenic autoantigens. In order to identify β-cell-derived antigens presented by HLA-relevant alleles in T1D, peptides presented by HLA-DR3 and HLA-DR4 molecules were identified. Peptides were generated by pulsing moDCs with protein extracts of total cell lysates or insulin secretory granules and/or crinosomes enriched fractions from β-cells cultured under homeostatic or stress-inducing glucose concentrations. Peptides were obtained by acidic elution from HLA-DR-peptide complexes previously isolated by affinity chromatography. Mass spectrometry analysis led to the identification of more than 200 peptides derived from total lysates. Additionally, around 70 peptides from granule-derived fractions were obtained from each culture condition. Only around 30% of these peptides were found under both homeostatic and stress-inducing glucose conditions, which indicates alterations in antigen presentation. From the parental proteins identified, 51 were classified as potentially relevant to T1D. Noteworthy, a greater proportion of the potentially relevant proteins identified proceeded from granule fractions. These results show a predominance of HLA-bound peptides derived from proteins potentially relevant to human T1D in β-cell granules. Moreover, cellular stress modifies this HLA-DR peptide repertoire. Future experiments will further confirm if these variations give rise to new antigenic peptides.

Keywords: Antigen processing and presentation, autoimmunity, diabetes, mass spectrometry

P-0608

High dimensional analysis of intestinal immune cells during parasite infection

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Single cell isolation from helminth-infected murine intestines has been notoriously difficult, due to the strong anti-parasite type 2 immune responses that drive mucus production, tissue remodeling and immune cell infiltration. Through the systematic optimization of intestinal digestion protocols, we were able to be among the first to successfully isolate millions of immune cells from the intestinal duodenum after Heligmosomoides polygyrus bakeri infection. In parallel, we developed the first 23-parameter spectral flow cytometry panel to identify 46 different intestinal innate and adaptive immune cell populations and monitored their infiltration, proliferation and activation at different stages of infection. Our algorithm-assisted high-dimensional analysis confirmed many known hallmarks of anti-parasite immune responses throughout the entire course of helminth infection and identified transient immune cell phenotypes that we are currently investigating as potential drug targets.

Keywords: Big data, infectious disease, parasite infections

P-0609

SARS-CoV-2-associated ssRNAs activate inflammation and immunity via TLR7/8

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The inflammatory and IFN pathways of innate immunity play a key role in both resistance and pathogenesis of Coronavirus Disease 2019 (COVID-19). Innate sensors and SARS-CoV-2-Associated Molecular Patterns (SAMPs) remain to be completely defined. Here we identify single-stranded RNA (ssRNA) fragments from SARS-CoV-2 genome as direct activators of endosomal TLR7/8 and MyD88 pathway. The same sequences induced human DC activation in terms of phenotype and functions, such as IFN and cytokine production and Th1 polarization. A bioinformatic scan of the viral genome identified several hundreds of fragments potentially activating TLR7/8, suggesting that products of virus endosomal processing potently activate the IFN and inflammatory responses downstream these receptors. *In vivo*, SAMPs induced MyD88-dependent lung inflammation characterized by accumulation of proinflammatory and cytotoxic mediators and immune cell infiltration, as well as splenic DC phenotypical maturation. These results identify TLR7/8 as crucial cellular sensors of ssRNAs encoded by SARS-CoV-2 involved in host resistance and disease pathogenesis of COVID-19.

Keywords: Dendritic cells, innate immunity, viral infections

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POSTER PRESENTATIONS

P-0610

Circulating anti SARS-CoV-2 IgA in vaccinated individuals

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IgA is one of the key immunoglobulins for fighting against respiratory viruses, but a little number of studies focus on it. We aimed to estimate how many vaccinated individuals get their specific IgA and whether previous infection or completeness of vaccination can influence this fact. All currently in use vaccines against SARS-CoV-2 induce antibodies recognizing S protein, whereas immunization due to infection can elicit antibodies recognizing any of the virus antigens. 221 donations from 116 recently vaccinated donors (31.9% males) were tested for anti SARS-CoV-2 total Immunoglobulins recognizing N protein total antibodies together with a conventional ELISA for anti S IgA. Most included donors had received mRNA vaccines due to the dates of sample's collection and analysis. Most (89.54%) vaccinated donors developed IgA anti S antibodies, 37.73% presented as well anti N total antibodies, meaning that they have had a previous contact with SARS-CoV-2. Less than half of them (39.8%) had only received one dose of two. Anti N positive donors had more frequently developed IgA anti S (94.4 vs 80.8). Just one vaccine dose enhances specific anti SARS-CoV-2 IgA production, more efficiently when there has been a previous contact with SARS-CoV-2, but to a high degree as well, with no infection history.

Keywords: Viral infections, adjuvants and vaccines, antibody, biomarkers, infectious disease

P-0612

HLA allele and haplotype frequencies in a subset of 456 Turkish patients in the blood and bone marrow donor registry of Istanbul Medical Faculty

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Determining the HLA-matched donor is the primary goal in searching donors for successful allogeneic hematopoietic stem cell transplantation (HSCT). In this study, the aim was to evaluate the differences in HLA haplotype frequencies of patients who received HSCT from unrelated donor and who has not yet found a potential donor in one year. Four hundred fifty-six Turkish patients having been searched for unrelated donors between 2019-2020 were included in the study. While 123 of these patients are being transplanted, 333 of them are waiting for HLA matched unrelated donors. We identified 95 class-I, 45 class-II HLA alleles that are common in patients transplanted from an HLA-matched unrelated donor. A*02:01, B*51:01, C*04:01, DRB1*15:01, DQB1*03:01 were the most frequent alleles at each locus. Haplotype estimation of the 123 transplanted patients analyzed 3 different 5-locus-haplotypes are A*01:01, B*37:01, C*06:02, DRB1*04:01, DQB1*03:02 (0.8%); A*24:02, B*51:01, C*16:02, DRB1*15:01, DQB1*06:02 (0.8%); A*02:01, B*18:01, C*12:03, DRB1*11:04, DQB1*03:01 (0,6%).We identified 118 class-I, 57 class-II HLA alleles that are common in patients who are still waiting for potential donors. A*24:02, B*51:01, C*04:01, DRB1*11:04, DQB1*03:01 were the most frequent alleles at each locus. Haplotype estimation of the 333 patients analyzed 3 different 5-locus-haplotypes are A*11:01, B*51:06, C*14:02, DRB1*15:01, DQB1*06:01 (0.4%); A*01:01, B*13:02, C*06:02, DRB1*07:01, DQB1*02:02 (0.3%); A*02:01, B*18:01, C*07:01, DRB1*11:04, DQB1*03:01 (0.3%). The haplotype analyzes in the HSCT waiting list might be useful to predict the potential donor availability for Turkish patients. The Knowledge of the most frequent haplotypes should also be used to describe strategies to optimize donor searches.

Keywords: Bone marrow transplantation, MHC and polymorphic genes, molecular immunology

P-0613

Serum proteome analysis of innate antiviral mediators in patients with advanced liver disease awaiting transplantation

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Individuals with a chronic liver disease (CLD) have an elevated risk of severe SARS-CoV-2 infection with a 30-day mortality rate almost twice that of the general population. The biological basis for poor COVID-19 outcome in this population is not well understood. Patients with CLD display several physiological defects associated with reduced hepatocyte function including reduced levels of antiviral proteins and dysregulated inflammatory response. In this study we aim to explore the possible role that these defects play in COVID-19 progression by investigating whether changes in innate antiviral serum proteins from individuals with CLD influence SARS-CoV-2 infection. 53 serum samples from patients with CLD were analysed using mass spectrometry. MaxQuant and Persus software packages were used for bioinformatical analysis. The impact of specific serum proteins was assessed using SARS-CoV-2 pseudoparticle assays. Perseus analysis of MS/MS data identified 224 proteins after a >60% cut off for all samples was applied for valid m/z MS intensities. Clinical measures of mortality risk including Child-Pugh class and MELD-Na scores were assessed for all patients. PCA plots and heatmap analysis showed distinct clustering for samples with lower MELD-Na scores (5-15) versus higher MELD-Na scores (>15), as well as clustering based on underlying aetiology. Analysis of proteins differentially regulated in individuals with high MELD-Na scores suggests a dysregulation of systemic inflammation and coagulation. Patients with end-stage liver disease awaiting liver transplantation display a variety of changes in their serum proteome. These dysregulated proteins provide potential biological hypotheses for the poor COVID-19 outcomes in this patient population.

Keywords: Chronic inflammation and fibrosis, infectious disease, innate host defence, innate immunity, viral infections

P-0614

Cellular and humoral immune responses after SARS-COV2 mRNA vaccination. Preliminary results

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In January 2021, vaccination against SARS-COV2 was available for the health care workers in Greece. The aim of the study was to investigate the antibody and cellular response after vaccination in "Helena Venizelou" Hospital personnel. The study included 62 healthcare workers without evidence of previous COVID-19 infection, who received the BNT162b2 Pfizer-BioNTech COVID-19 mRNA Vaccine. The subjects were divided in two groups (males M and females F) separated in two age subgroups: M1 (20, 26-54 y), M2 (11, 56-67 y), F1 (18, 33-54 y) and F2 (13, 55-64 y). Samples were obtained 30 days after vaccine's second dose, for qualitative determination of IgG antibodies to SARS-CoV-2 in human serum on ARCHITECT i System. In 40 of them peripheral blood immunophenotyping was also performed. A second measurement is scheduled in 6 months after the second dose. In group M the antibody levels following booster vaccination were statistically significant lower than in group F (8007.8 AU/ml vs 12671.4AU/ml p=0.0296) and these difference was bigger in older individuals (M2:3711 vs F2:13141.1 p=0.0142). The same difference was observed between M1 and M2 (10.371.01vs 3711 p=0.0152) but not between subgroup M1 and F1 (10371.01vs 12332.3 p=0.4357) and F1 and F2 (12332.3vs13414.1 p=0.8080). No differences were observed in peripheral blood immunophenotyping between men and women or older and younger individuals. Antibody response following mRNA vaccine appears to be age and gender dependent. Our current extended study is expected to confirm these results and give information on the effect of immunogenetic and other factors.

Keywords: Antibody, immune response tracing, infectious disease

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POSTER PRESENTATIONS

P-0615

Effects of acute and persistent nervous necrosis virus infection of european sea bass (Dicentrarchus labrax, L.) on physiology parameters and immune-related genes expression

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Aim of the present study was to evaluate the long term effects of viral infection on host immune response and physiology parameters, to get insight into the mechanisms responsible for nervous necrosis virus (NNV) infection progress in European sea bass (Dicentrarchus labrax). NNV is a pathogen causing a disease resulting in mortalities up to 100% in sea bass which is economically important for European aquaculture. Fish were experimentally infected and studied for 28-days. Biometrical and haematological parameters, brain viral loads and specific anti-NNV antibodies were determined. Head-kidney tissues were subjected to qPCR to assess interferon pathway-, cytokines-, immunoglobulin-, T-cell markers- and antiviral peptide-related genes expression. Infected fish presented clinical signs 3-dpi and mortality reached 20%. Survivors had altered growth rates and blood count parameters. The highest brain viral load was observed 7-dpi. On 28-dpi viral load was increased, suggesting that the surviving fish are asymptomatic carriers. Specific anti-NNV Ab levels were increased from 7- up to 28-dpi. MxA expression increased 3-hpi and 7-dpi whereas the IRF7 expression decreased 6-hpi and 4-dpi. The ISG12, IL-1b and STAT3 expression was very high 14-dpi. IL-10 was increased 6-hpi and 4-dpi. TNFa expression was increased 3-hpi and lowered subsequently. IgHM increased 6-12-hpi and 14-dpi. CD4 and CD8a expression increased on 14-dpi. Hepcidin expression reached a maximum 24-hpi. Noteworthy, the infected fish appeared to be immunologically active at the early time points as expected but also 7- and 14-dpi providing information for fish immunological status on the carrier state

Keywords: Immune response tracing, infectious disease, molecular immunology, veterinary immunology, viral infections

P-0617

Itaconate attenuates anti-viral TLR3 responses in idiopathic pulmonary fibrosis patients: implications for disease progression during infection

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Idiopathic pulmonary fibrosis (IPF) is a fatal fibrotic lung disease of unknown aetiology, with a mean survival of less than 3 years. Currently, there is an unmet clinical need for the development of novel biomarkers and therapeutics in IPF. Bacterial and viral infections have been identified as important drivers of IPF disease progression. We established previously that TLR3-IRF3 induced anti-viral responses had a protective effect in IPF lung fibroblasts. Furthermore, defective TLR3-function accelerated disease progression and mortality in IPF patients. Itaconate is a metabolite generated during the Krebs cycle which has emerged as a potent immunomodulator with anti-bacterial and anti-viral properties. To date, the role of itaconate in viral infection in IPF is unknown. In this study, we investigated the effect of 4-OI, a synthetic analogue of itaconate, on TLR3 function in primary lung fibroblasts from IPF patients. Here, we demonstrated that 4-OI induced IRG1 mRNA expression for itaconate synthesis in IPF lung fibroblasts following poly(I:C) treatment. 4-OI treatment directly induced expression of the antioxidant proteins, NRF2 and HMOX-1 in IPF fibroblasts. In contrast, 4-OI treatment decreased TLR3-induced expression of anti-viral IFN-β, RANTES and RIG-I in IPF lung fibroblasts. These effects were associated with a concomitant reduction in TLR3 protein expression in IPF fibroblasts following poly(I:C) treatment. In this study, we demonstrate for the first time that itaconate (4-OI) can modulate TLR3 function in IPF lung fibroblasts. These findings warrant further investigation to elucidate the specific role of itaconate in IPF during viral infection and in disease progression.

Keywords: Bacterial infections, inflammatory disease, metabolic control of immune responses, tissue damage and repair, viral infections

P-0620

Ex vivo IFNγ T cell responses to a single dose of the AZD1222 vaccine 12 weeks following immunization

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To investigate the immunogenicity and kinetics of T cell responses induced by a single dose of the AZD1222 vaccine in Sri Lankan health care workers. Using IFNγ *ex vivo* ELISpot, we investigated T cell responses to the spike protein (253 overlapping peptides in two pools, S1 and S2), after 4 weeks (n=76) and 12 weeks (n=80) following immunization and compared these responses to those following natural infection (n=29). A positive response was defined as mean± 2SD from the background (spot forming unit/1,000,000 cells). 4 weeks post-vaccination, 46/76 (60.5%) responded to the S1, 24/76 (31.6%) to S2 and 50/76 (65.8%) for whole S. At 12 weeks post-vaccination, only 20/80 (25.0%) responded to S1, 6/80 (7.5%) to S2, and 33/80 (41.2%) to S. *Ex vivo* T cell responses to S1, S2 and S were significantly higher at 4-weeks ((p<0.0001) and also at 12-weeks than the pre-vaccination responses. However, responses to S1 and S2 significantly declined (p<0.0001) at 12 weeks post vaccination (p<0.0001) compared to 4-week responses. Although there was no difference in the 4-week post vaccination T cell responses (p=0.57) compared to the responses in naturally infected individuals, the 12-week post vaccination responses were significantly lower (p<0.0001), in those who had received a single dose of the vaccine compared to those who were naturally infected. A single dose of the AZD1222 vaccine induced *ex vivo* T cell responses comparable to natural infection but declined by 12 weeks of post-vaccination. It will be important to now test for central memory proliferative responses.

Keywords: Adaptive immunity, cytokines and mediators, viral infections

P-0623

Nrf2 regulates redox metabolism of CD4+T cells in chronic inflammatory conditions

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By entering inflamed tissues, T cells adapt to low levels of oxygen, lack of key nutrients and oxidative stress conditions. To study how this environment affects T cells, we analyzed T cell metabolism and function in synovial fluid cells from Juvenile Idiopathic Arthritis (JIA) patients. We aimed to investigate how oxidative stress regulates T cell responses within inflamed joints of JIA patients and analyzed Nrf2-the key regulator of the antioxidative stress response- and it's signaling pathways. Flow cytometry analyses were performed to determine oxidative status and metabolic characteristics in the mononuclear cells from arthritic joint and peripheral blood of JIA patients. Seahorse assay were performed to analyze their metabolic activity. qRT-PCR were performed to analyze expression of genes involved in glucose and fatty acid metabolism. We identified high ROS levels in CD4+ T cells from synovial fluid (SF). Nrf2 and its target gene Nqo1 were less expressed in SF compared to blood CD4+ T cells. SF CD4+ T cells expressed high levels of mitochondrial mass, high glucose uptake and ECAR levels and high fatty acid uptake. Vice versa, Nrf2 activation of SF T cells yielded in downregulation of ROS, ECAR and fatty acid uptake and also reduced secretion of IFN-g. These findings suggest that Nrf2 signaling regulates the metabolism of SF T cells and its dysregulation in T cells during chronic inflammation could contribute to disease progression.

Keywords: Autoimmunity, chronic inflammation and fibrosis, inflammatory disease, metabolic control of immune responses

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POSTER PRESENTATIONS

P-0625

(Bio)Chemical characterization of moss *Hypnum cupressiforme* extracts as potential immunomodulators

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In this study, we aimed to examine the chemical composition and biochemical activities (antioxidant, antidiabetic, and antineurodegenerative) of seasonal aspects (spring, summer, autumn) of moss *Hypnum cupressiforme* extracts. The extracts were prepared using Soxhlet extractor. Chemical characterization of extracts was performed via spectrophotometric assays and LC-MS. The antioxidant activity of the extracts was determined by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate), total reduction power, and β-carotene bleaching assays. Extracts were analyzed for their inhibitory activity on α-glucosidase, α-amylase, acetylcholinesterase, and tyrosinase. The chemical analysis of the moss extracts revealed the presence of flavonoids, phenolic acids, and triterpenoids. The highest concentration of these compounds was found in the moss from summer season, and was significantly higher in comparison to the spring and autumn aspects. Major compounds identified by LC-MS in *H. cupressiforme* extracts were kaempferol, *p*-hydroxybenzoic, protocatechuic, *p*-coumaric, gallic, and caffeic acid. According to biochemical assays investigated extracts exhibited significant antineurodegenerative potential, evaluated by the capacity to inhibit acetylcholinesterase and tyrosinase. Among the examined aspects, summer and autumn were found particularly efficient, especially at the lowest tested concentrations. Regarding β-carotene bleaching test, the summer aspect showed the best activity at the highest concentration, while the spring aspect was found more efficient at the lowest investigated concentration. The obtained data suggest that the most favorable season in terms of chemical composition and biochemical characteristics of moss *H. cupressiforme* is summer. This moss is a promising source of biologically active compounds that may be useful in the treatment of various pathological conditions.

Keywords: Immune regulation and therapy, immunopharmacology, inflammatory disease

P-0626

A nonsense mutation in DIAPH1 gene presents with major T cell defects

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Diaphanous related formin 1 (DIAPH1) is a member of formin family proteins and is an important regulator of actin polymerization and microtubule stability. DIAPH1 loss of function mutations are associated seizures, cortical blindness, and microcephaly syndrome (SCBMS). Very recently DIAPH1 mutations have been linked to a combined immunodeficiency. In this study we characterized a patients' lymphocytes presented with SCBMS and symptoms of immunodeficiency. Next generation sequencing of samples from a 10-year-old female patient, who was diagnosed with SCBMS revealed a nonsense mutation in DIAPH1 gene P.R31*. The patient has been followed up by pediatric neurology and pediatric immunology due to epilepsy, autism, cortical blindness and lymphopenia and had frequent infections (pneumonia and bronchiolitis), frequent ear drainage and otitis history. Peripheral blood mononuclear cells were studied with respect to cell proliferation, NK cell cytotoxicity, IL-2-mediated STAT5 phosphorylation, and induced Treg cell generation *ex vivo* and examined on FACSAria III. DIAPH1 deficient T cells showed proliferation defects in response to both CD3/28 and PHA stimulation. NK cells had cytotoxicity defects against K562 cells. As expected, DIAPH1 deficient PBMCs had migration in a trans-well plate. In addition, generation of Treg cells from naïve T cells was impaired. This impairment had more to do with cell expansion rather than conversion of naïve T cells into Foxp3+ T cells. DIAPH1 deficient PBMCs had impaired IL-2-mediated STAT5 phosphorylation. Lastly, DIAPH1 Mutant PBMCs have reduced CD4/CD8 ratio. Our data reveal that DIAPH deficiency results in major T cell defects in patients.

Keywords: Molecular immunology, NK cells, adaptive immunity, immunodeficiency

P-0627

Immunomodulatory properties of extracts of moss *Hypnum cupressiforme* from various seasons

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In this study, we aimed to examine the immunomodulatory potential (anti-neuroinflammatory/neuroprotective and antitumor activities) of various seasonal aspects (spring, summer, autumn) of moss *Hypnum cupressiforme* extracts. Moss extracts have been obtained using solvents of different polarities and their mixtures applying Soxhlet extractor. Activities of the extracts were tested on cell lines MRC-5, BV2, SH-SY5Y, HCT-116, and MDA-MB-231, for potential anti-neuroinflammatory and antitumor activities. The impact of extracts on cell viability/metabolic activity was measured by MTT assay, while the influence on the production of ROS and NO was determined using NBT and Griess assays, respectively. The biocompatibility of extracts was confirmed using MRC-5 cells. Extracts of moss from spring season have shown significant antiproliferative activity against MDA-MB-231 cell line (inhibition rate ~50%), as well as anti-neuroinflammatory activity. Furthermore, the effects of seasonal variation (spring, summer, autumn) on the immunomodulatory potential of ethyl-acetate extract were examined. Summer aspect of moss has led to the greatest reduction of ROS production, while autumn aspect had the greatest impact on the reduction of NO production by LPS-activated BV2 cells. Additionally, the supernatant-transfer model proved that treatment with moss extracts from summer and autumn seasons of LPS-activated BV2 cells exhibit neuroprotective activity towards SH-SY5Y neurons. The obtained data suggest that extracts from moss *H. cupressiforme* possess significant immunomodulatory potential evaluated *in vitro*, which may be useful in conditions such as Alzheimer's disease, Parkinson's disease, and breast cancer. In terms of anti-neuroinflammatory/neuroprotective activity, summer and autumn were characterized as the most favorable seasons.

Keywords: Cancer immunology, immunotherapy, inflammatory disease

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POSTER PRESENTATIONS

P-0629

Immunomodulatory potential of ethanolic extracts of selected Lamiaceae species

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The representatives of the Lamiaceae family are well-known medicinal plants and a number of them are used in traditional medicine since ancient times. Nowadays, people's health awareness is growing, so there is a need for scientific authentication of mechanisms of actions of these plants leading to different biological activities. Thereby, we aimed to analyze the anti-neuroinflammatory/neuroprotective and antitumor potentials of ethanolic extracts of the following Lamiaceae species: *Glechoma hederacea, Hyssopus officinalis, Lavandula angustifolia, Leonurus cardiaca, Marrubium vulgare, Melissa officinalis, Mentha × piperita, Ocimum basilicum, Origanum majorana, Origanum vulgare, Rosmarinus officinalis, Salvia officinalis, Satureja montana, Sideritis scardica, Teucrium chamaedrys, T. montanum, Thymus serpyllum* and *T. vulgaris*. The immunomodulatory activity of these extracts was determined on different cell lines: microglial (BV2), neuronal (SH-SY5Y), and colon cancer (HCT-116) cells by MTT, NBT, and Greiss assays. The results showed that these extracts normalize the viability of LPS-activated BV2 cells and reduce their production of reactive oxygen species (ROS) and nitric-oxide (NO). Moreover, the supernatant-transfer model proved that treatment with plant extracts of LPS-activated BV2 cells leads to neuroprotective activity towards SH-SY5Y neurons. Regarding the antitumor effects, only *L. angustifolia, O. basilicum*, and *R. officinalis* inhibited HCT-116 proliferation. The significant antiproliferative activity of *L. angustifolia* can be explained by increased ROS production, while *O. basilicum* and *R. officinalis* express their effects by increasing the NO production by tumor cells. Finally, this study provides experimental explanation for the application of the tested plants in traditional medicine and elucidates some of their effectory mechanisms.

Keywords: Cancer immunology, immunotherapy, inflammatory disease

P-0631

CTLA-4, LAG-3 and Tim-3 expressions are increased in NK cells of advanced stage ovarian cancer patients

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Ovarian cancer has the highest mortality rate among all gynecological cancers. Due to its limited response to conventional chemotherapy, immunotherapy is a promising target. The aim of this study was to investigate the expression of immune checkpoint receptors (ICRs) on tumor infiltrating lymphocytes (TIL) and peripheral blood natural killer (NK) cell subsets in patients with advanced ovarian cancer. Advanced stage ovarian cancer patients with stage III/IV (n=8) and healthy subjects (n=10) were included in the study. TIL were isolated from tumor tissue by using tumor dissociation kit. Expression of ICRs including PD-1, CTLA-4, LAG-3, Tim-3 and TIGIT on NK cell subsets were analyzed by flow cytometry. The ratio of cytotoxic (CD56neg/dimCD16br) and highly active (CD3-CD8+CD16+CD56+) NK cell subsets were significantly reduced in tumor tissue compared to blood of both patients and controls. CTLA-4, LAG-3 and Tim-3 expressions were significantly higher in patients compared to healthy blood and this increase was especially prominent in cytotoxic NK subset. Expression of LAG-3 and Tim-3 was significantly higher in tumor tissue compared to patient blood, as well. PD-1 expression tended to be higher in patients, but this difference was not statistically significant. Interestingly, TIGIT expression was reduced in tumor compared to both patient and control blood. Cytotoxic NK cells and highly active NK cell subsets were underrepresented in tumor tissue. Expression of CTLA-4, LAG-3 and Tim-3 was significantly higher in NK cells of patients and may serve as future targets for immunotherapy of ovarian cancer.

Keywords: Cancer immunology, checkpoint inhibition, microenvironment, NK cells

P-0633

Stimulation with mycobacterial glycolipids reveals different innate immune response profiles in active and latent tuberculosis

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Upon infection with *Mycobacterium tuberculosis* (Mtb) the immune response might clear the bacteria, control its growth leading to latent tuberculosis (LTB), or fail to control Mtb resulting in active tuberculosis (ATB). There is however no clear understanding of the features underlying an effective response. The immune response to Mtb is initiated mainly by the interaction of bacterial cell envelope components, mostly glycolipids, with cells of the innate immune system. Lipoarabinomannan (LAM) and its precursor phosphatidyl-inositol mannoside (PIM) are abundant in the bacterial cell envelope and interact with innate and adaptive immune cells. LAM and PIM have immunomodulatory properties, but the response patterns to glycolipids are still underexplored. We performed a detailed assessment and simultaneous comparison of the immune response to PIM/LAM, of peripheral blood mononuclear cells, in individuals with ATB or LTB. For that we dissected the immune profiling in response to PIM/LAM by mass cytometry measuring 37 cellular markers at the single-cell level allowing high-resolution of cellular composition. PIM induced a polyfunctional immune response, mainly in antigen-presenting cells, leading to the production of pro-inflammatory and anti-inflammatory cytokines, but not IFN-γ. LAM triggered weaker but similar responses. Expansion of myeloid subsets producing cytokines in response to PIM/ LAM was reduced in ATB/LTB compared to HC, suggesting a hyporesponsive/tolerance pattern. This effect on myeloid cells in ATB/LTB individuals suggests a pathogen-specific innate immune response that requires further exploration. By defining the mechanistic underlying the response to Mtb glycolipids, new vaccine strategies and correlates of protection may be developed.

Keywords: Bacterial infections, infectious disease, innate immunity, myeloid cells

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POSTER PRESENTATIONS

P-0634

Outcome of SARS-CoV-2 infection is linked to MAIT cell activation and cytotoxicity

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Immune system dysfunction is paramount in coronavirus disease 2019 (COVID-19) severity and fatality rate. Mucosal-associated invariant T (MAIT) cells are innate-like T cells involved in mucosal immunity and protection against viral infections. Here, we studied the immune cell landscape, with emphasis on MAIT cells, in cohorts totaling 208 patients at various stages of disease activity. MAIT cell frequency is strongly reduced in blood. They display a strong activated and cytotoxic phenotype that is more pronounced in lungs. Blood MAIT cell alterations positively correlate with other innate cell activation, proinflammatory cytokines, notably interleukin (IL)-18, and with the severity and mortality of severe acute respiratory syndrome coronavirus 2 infection. We also identified a monocyte/macrophage interferon (IFN)-α–IL-18 cytokine shift and the ability of infected macrophages to induce cytotoxicity of MAIT cells in an MR1-dependent manner. Together, our results suggest that altered MAIT cell functions due to IFN-α–IL-18 imbalance contribute to disease severity, and their therapeutic manipulation might prevent deleterious inflammation in COVID-19 aggravation.

Keywords: Biomarkers, infectious disease, inflammatory disease, MAIT cells, viral infections

P-0635

γδ T lymphocytes contribute in inflammation related to COVID-19: beneficial use of statins

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Statins have been considered an attractive class of drugs in the pharmacological setting of COVID-19 due to their anti-inflammatory and anti-thrombotic properties and their use showed a correlation with a decreased mortality in hospitalized COVID-19 patients. Furthermore, it is well known that statins, blocking the cellular mevalonate pathway, affect γδ T lymphocytes activation. As γδ T cells participate to the inflammatory process of COVID-19, we investigated on the therapeutical potential of statins as a tool to inhibit γδ T cell pro-inflammatory activities. We enrolled hospitalized COVID-19 patients with mild clinical manifestations and healthy control subjects. Peripheral blood mononuclear cells were isolated, treated overnight with different concentrations of Atorvastatin and subsequently stimulated with ionomycin/PMA or Zoledronate/IL-2. Intracellular expression of TNFα, IFNγ and IL-17 was evaluated on Vδ2$^+$ T cells by flow cytometric analysis. The frequency of circulating Vδ2$^+$ T cells of COVID-19 patients were lower compared to healthy donors, paralleling lymphopenia related to infection. Compared to healthy donors, Vδ2$^+$ T cells from COVID-19 patients produced slightly elevated levels of pro-inflammatory cytokines upon Zoledronate stimulation. Interestingly, Zoledronate activation was inhibited by Atorvastatin in a dose-dependent manner, causing a strong reduction of TNFα, IFNγ and IL-17 production. This effect specifically involved the contribution of the mevalonate pathway, as it was not observed if the cells were stimulated with Iono/PMA. Our data highlight a new therapeutic potential of statins in COVID-19 patients related to their inhibitory effect on γδ T cell activation and effector functions.

Keywords: Cytokines and mediators, drugs for immune modulation, gamma-delta T cells, infectious disease, viral infections

P-0636

Characterization of Th17 and Treg cells in leucocyte adhesion deficiency 1 patients

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Leucocyte adhesion deficiency (LAD) 1, which shows autosomal recessive inheritance, is one of the 3 types of LADs and is the most common. We investigated Th17 and Treg cells in 14 Turkish patients with LAD-1, with the aim of studying direct and indirect impact of ITGB2 mutations on Th17/Treg cell differentiation and functions. In the peripheral blood taken from LAD-1 patients and healthy donors; Treg, Th17 cells as well as ILCs were investigated by flow cytometry-based assays; trans-well migration, apoptosis were aso assessed. Selected naïve or memory CD4+ T cells were differentiated *ex vivo* to Treg or Th17 further. In addition, the amount of IL-17, IL-22, IL-23 and GM-CSF in the serum and supernatants of cultured PBMCs were measured by ELISA. The percentage of Treg cells in the peripheral blood of LAD1 patients and the percentage of induced Tregs differentiated from naive CD4+ T cells *in vitro* was decreased in the peripheral blood. Serum IL-23 level was elevated in LAD1 patients. Percentages of Th17 cells expanded from memory or naïve CD4+ T cell fraction obtained from LAD1 patients' peripheral blood were higher compared with those of healthy controls. ILC3 subset was marginally but significantly elevated in the LAD1patient peripheral blood. Finally, LAD1 PBMCs showed defects in trans-well migration, proliferation and were more resistant to apoptosis. In this study, we investigated the role of T helper (Th) cells and regulatory T (Treg) cells and related cytokines, and report Th17/Treg bias in favor of the former in LAD1 patients.

Keywords: Adaptive immunity, immunodeficiency, innate immunity, innate lymphoid cells, regulatory cells

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POSTER PRESENTATIONS

P-0637

Establishment of the cytomegalovirus assembly compartment initiated by the expansion of early endosome – endosomal recycling compartment interface

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In this study, we aimed to characterize the rearrangement of the cellular endosomal system during different phases of MCMV infection. We focused on the interface between early endosomes (EE) and endosomal recycling compartment (ERC) in the nascent viral assembly compartment (VAC). Balb3T3 fibroblasts were infected with recombinant MCMV, Δm138-MCMV. The samples were taken at different time points throughout the course of infection. Recruitment of endosomal regulatory proteins and distribution of viral proteins was analyzed by immunofluorescent confocal microscopy. VAC is established early in the MCMV infection by expanding the tubular membranous domains at the EE-ERC interface. The expansion is demonstrated as over recruitment of small GTPases that control tubulation of EEs and the ERC from the Rab (Rab8a, Rab10, Rab11, Rab22a) and Arf subfamilies (class I/III Arfs and their GEFs). Expanded membranes of the EE-ERC interface were surrounded by the compacted and fragmented Golgi stacks dislocated from its pericentriolar location, forming the VAC outer ring. Viral structural glycoproteins load the outer ring organelles, whereas viral tegument proteins loaded the inner AC area. Rearrangement of the membranous system is initiated in the early phase of MCMV infection as an expansion of the membranous organelles at the EE-ERC interface. The MCMV-induced expansion of this interface forms the pericentrosomal agglomerate of membranous organelles that may initiate Golgi unlinking, compacting, and displacement. Alternatively, the initial step of Golgi unlinking may dysregulate the Golgi linker compartments, including ERC, resulting in the expansion of tubular domains at the EE-ERC interface.

Keywords: Cell signalling, endo- and exocytic vesicles in immunity, viral infections

P-0638

TNF-a induces glycolytic shift in fibroblast like synoviocytes

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Tumor Necrosis Factor alpha (TNF-a) is a central cytokine in the pathogenesis of rheumatoid arthritis (RA) affecting immune cells and fibroblast like synoviocytes (FLS) in the joint. Nowadays, only 20-30% of patients experience remission after the standard of care therapy - antibodies against TNF-a. Interestingly, responders show reduced levels of GLUT1 and GAPDH, highlighting the importance of metabolic changes and especially glycolysis. We sought to study whether TNF-a directly affects the metabolic phenotype of FLS explaining the observed metabolic changes after successful anti-TNF-a therapies. We evaluated the impact of TNF-a on metabolism of FLS from healthy donors (H-FLS) using real-time respirometry (Seahorse), Taqman and Luminex assays. To study signal transduction, small molecule inhibitors of TAK1 and hexokinase were used. Real-time respirometry revealed that TNF-a upregulates glycolysis while modestly increasing oxidative phosphorylation in H-FLS. In addition, the expression of HIF1A and GLUT1 were significantly increased by TNF-a stimulation. Using a small molecule inhibitor against TAK1 we deciphered the TNF-a/TAK1/HIF1A/GLUT1 signaling axis. Further we confirmed that HIF1A and GLUT1 upregulation in H-FLS following TNF-a treatment reflects the enhanced level of these enzymes in RA-FLS. To prove that inhibition of glycolysis reduces the pathogenic phenotype of RA-FLS, we show that2-deoxyglucose, a competitive inhibitor of hexokinase, decreases secretion of RA biomarkers in RA-FLS. We identified a direct role of TNF-a on FLS glycolytic reprogramming and confirmed the potency of immunometabolism for RA. Further studies are needed to evaluate the therapeutic impact especially regarding non-responder data.

Keywords: Autoimmunity, biomarkers, cytokines and mediators, inflammatory joint diseases, metabolic control of immune responses, rheumatoid arthritis

P-0639

A novel mouse model for studying the function of neutral sphingomyelinase 2 in CD4+ T cells *in vitro* and *in vivo*

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T cells strongly depend on signaling of receptors within plasma membranes of which sphingolipids are major constituents. Previous work using pharmacological inhibitors and siRNA-mediated knock-down showed that the sphingolipid-metabolizing enzyme neutral sphingomyelinase 2 (Nsm2) plays a role in CD4+ T cell activation and migration. So far, a genetic mouse model to comprehensively study the role of Nsm2 in CD4+ T cells has, however, not been available. Therefore, we generated inducible Nsm2-knock-out (iNsm2-KO) mice to shed more light on the role of Nsm2 in different T cell subsets, in particular CD4+ Foxp3- conventional (Tconv) and CD4+ Foxp3+ regulatory T cells (Treg). We achieved Nsm2 deletion in CD4+ T cells *in vitro* leading to reduced neutral sphingomyelinase activity using either tamoxifen-inducible ERCre knocked into the Rosa26 locus or transgenic tamoxifen-inducible CD4-ERCre. After inducing Nsm2 gene (*Smpd3*) deletion *in vitro*, we cultured purified CD4+ T cells for 5 days to allow for loss of Nsm2 protein expression. The CD4+ T cells were then stimulated with anti-CD3/anti-CD28 mAb-coated Dynabeads for 24 and 48 h at different bead to cell ratios. In our preliminary experiments, we observed an elevated proportion of CD25- and CD69-expressing cells and stronger proliferation (marker: Ki-67) among iNsm2-KO versus wild-type (littermates) CD4+ Tconv. Nsm2 deficiency had less of an effect on Treg than on Tconv. Our data, thus, suggest that Nsm2 activity dampens CD4+ T cell activation which is of particular importance for CD4+ Tconv compared to Treg. This study was funded by the DFG (GRK2581-P3).

Keywords: Animal models, cell signalling, immune regulation and therapy, regulatory cells, viral infections

P-0640

The effect of local immune reactions in the development of atherosclerosis and vascular calcification in patients with chronic kidney disease

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Aim of the present study was to evaluate the role of immune mechanisms in the development of atherosclerosis and vascular calcification, in with Chronic Kidney Disease (CKD). Histological, immunohistochemical and morphometric analysis was performed on a radial artery specimen, obtained from CKD patients during the creation of a radiocephalic arteriovenous fistula (RC-AVF). Severity of vascular calcification was evaluated, (based on Verhoff's Elastic and von Kossa staining), as well as, expression of calcification regulators [Receptor Activator of Nuclear factor –kB ligand (RANKL), Matrix carboxyglutamic acid protein (MPG) and osteoprotegerine (OPG)]. Furthermore, severity of vascular inflammation [CD3(+), CD20(+), CD68(+) expression], and cellular activation [CD34(+), a-SMA(+) cells] were also assesed. Patients were divided in two groups, group A: CKD patients at pre- dialysis stage, and group B: CKD patients being on HD for at least 2 years. Correlations between Inflammatory activation and severity of vascular calcification and atherosclerosis were estimated. Significant differences were noticed between Group A, B and controls regarding the severity of MPG, RANKL and OPG expression, p=0.01, p=0.006 and p=0.005, respectively. The degree of MPG, RANKL and OPG showed positive correlation with CD3(+), CD20(+), CD68(+), CD34(+), a-SMA(+) expression; also severity of vascular calcification was correlated with CD3(+) p=0.001, CD20(+) p<0.0001, CD68(+) p=0.002, CD34(+) p<0.0001, a-SMA(+) p<0.0001, MPG p=0.01, RANKL p=0.04 and OPG p=0.03 expression. Atherosclerotic disease in CKD and its clinical effects appear to be directly related to inflammatory infiltration of blood vessels by CD3(+), CD20(+) cells, macrophages and myofibrolasts, as well as factors that affect calcification.

Keywords: Biomarkers, inflammatory disease, monitoring immunity

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POSTER PRESENTATIONS

P-0641

Memory T-lymphocyte response against SARS-CoV-2 reminds memory response against influenza virus in older patients

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Immunological memory against SARS-CoV-2 in older people is highly heterogeneous. To analyse if this variability is specific to the response against this virus or it depends on the previous immunocompetence, we studied the immunological response against influenza and SARS-CoV-2 in a group of 102 COVID-19 survival patients. Fifty-seven had been vaccinated in 2019-20 seasonal influenza vaccination campaign (VAC; age: 79.2±12.0) and 45 had not (NVAC; age: 65.7±5.3). Memory lymphocytes specific for influenza and SARS-CoV-2 were quantified using granzyme-B, IFN-γ and IgG1-ELISpot and antibodies were measured by ELISA. Cellular memory response against influenza was detected in all patients. However, only 96% of VAC and 71% of NVAC presented specific antibodies over the detection limit. As expected, we found a greater number of specific granzyme-B and IFN-γ-producing T-lymphocytes and higher anti-influenza antibodies in VAC (Student's T-test, $p<0.05$, $p<0.01$ and $p<0.001$, respectively). Moreover, positive correlation between memory T- and B-lymphocytes specific to influenza was found in both groups. When immunological memory to influenza and to SARS-CoV-2 was compared, positive correlation in IFN-γ-producing T-lymphocytes was found in both VAC and NVAC (Pearson test $r=0.450$, $p<0.001$ and $r=0.486$, $p<0.001$, respectively). Similarly, granzyme-B-producing T-lymphocytes and memory B-lymphocytes showed positive correlation in NVAC (Pearson test $r=0.601$, $p<0.001$) and in VAC (Pearson test $r=0.400$, $p<0.01$), respectively. No association was found in antibody titers against both viruses. Our results suggest that the cellular memory response against SARS-CoV-2 reminds the one developed against other viruses, such as influenza, in response to both seasonal vaccination and previous immunizations.

Keywords: Adaptive immunity, ageing, memory, viral infections

P-0642

Role of the endosomal phosphatidylinositol 3-phosphate in the biogenesis of the cytomegalovirus assembly compartment

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Cytomegalovirus rearranges membranous compartments to avoid immunological host response and develop an intracellular site for virion assembly and egress, known as cytoplasmic assembly compartment (AC). One of the earliest events identified in the AC development is the rearrangement of the early endosomal (EE) system where phosphatidylinositol 3-phosphate [PI(3)P] dynamics drive the membrane flow. Therefore, we aimed to determine PI(3)P role in the EE rearrangements in the course of CMV infection and AC biogenesis. Using retroviral vectors with cloned fluorescent PI(3)P-binding domains (2xFYVE, p40PX), we found high PI(3)P production and enrichment of PI(3)P membranous domains in the pre-AC and AC of CMV infected cells. The overexpression of PI3P-binding domains resulted in the inhibition of membrane flow from EEs to the endosomal recycling compartment (ERC). Similarly, short-term depletion of PI(3)P by inhibition of Vps34, the principal PI(3)P producer at EE membranes, abolished cargo sorting and membrane flow from EE towards the ERC. Despite these alterations, neither short-term depletion of Vps34-derived PI(3)P pool nor long-term saturation of PI(3)P by overexpression of PI(3)P-binding modules did not prevent the establishment of CMV infection, progression through the early phase of infection, and development of the pre-AC, as analyzed by confocal imaging and Western blot. Instead, inhibition of PI(3)P-associated functions affected CMV replication in the late phase of the infection, including the release of progeny virions. These results suggest that PI(3)P contributes to the biogenesis of the AC and final release of newly formed virions by a mechanism that affects entry into the late phase of infection.

Keywords: Cell signalling, endo- and exocytic vesicles in immunity, viral infections

P-0643

Modulation of human T cell responses by fungal enolase 1

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Candida albicans and Aspergillus fumigatus control the human immune response by secreting immune evasion proteins like Enolase 1 (Eno1) which is a moonlighting protein binding several human plasma proteins. Using bioinformatical methods, we predicted the interaction of Eno1 with CD4. This in silico prediction was confirmed in a Biacore assay where recombinant Eno1 from A. fumigatus bound to the extracellular domain of human CD4. To assess the relevance of this interaction for infection biology, we investigated the impact of Eno1 from either C. albicans or A. fumigatus (73% aa homology) on cytokine secretion by purified naïve human CD4+ T cells stimulated with anti-CD3/anti-CD28 mAb-coated Dynabeads. Here, we focused on secretion of Th1 versus Th2 cytokines promoting versus hampering, respectively, antifungal immunity. Concentrations of IL-17 were below the limit of detection. C. albicans Eno1 induced a Th2-biased response for the majority of healthy human blood donors (n=8). For Eno1 from A. fumigatus we noted a Th1 or Th2 bias in 50% of donors each. We speculate that differences in the aa sequences of both Enolases are responsible for their differential biological effects. Similar to anti-CD4 mAb RPA-T4 blocking TCR-HLA interactions, while by itself activating Lck, fungal Eno1 either blocked or enhanced human memory CD4+ T cell responses towards recall antigens. Therefore, our data suggest that Eno1 is capable of directly modulating human CD4+ T cell responses with the capacity to either enhance fungal pathogenesis or to strengthen anti-fungal immunity. This study was funded by the DFG (SFB-TR124-C6) and the DAAD.

Keywords: Fungal infections, adaptive immunity, biology of the immune system, cytokines and mediators

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POSTER PRESENTATIONS

P-0645

A tool for identification specific cellular immune response in nursing home residents

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To asses specific cellular and humoral responses in nursing home subjects after mRNA-based vaccine against SARS-CoV-2. A total of 624 nursing home residents in Cantabria (Spain), were included in the present study with a mean age of 80.56 years (SD 13.51). The clinical data of each subject were obtained from clinical records. The m-RNA based vaccine was administrated in each nursing center, and thirty days after the second dose, a sample for anti-S1 IgG antibody detection was obtained. Further T-specific assay, validated by Spanish Society of Immunology, was performed in the seronegative subjects. Pre-vaccine positive SARS-CoV-2 PCR result was observed in 132 subjects (21.15%), whereas in 6 residents (0.96%) a positive PCR result was detected after the second dose of the vaccine. One month after the second dose, 462 out of 486 PCR negative patients (95.1%) developed specific anti-S1 IgG antibodies. Twenty-two out of twenty-four subjects were further assessed for specific cellular T-cell vaccine response. A total of 10 subjects (45.45%) were identified as T-specific vaccine responders (T-RESP). Within 10 (T-RESP) subjects, 8 had no immunological relevant clinical records whereas 2 remaining subjects presented hematological disorder and dermatitis, respectively, treated with corticosteroids. SARS-CoV-2 T-specific response assay could be a valuable tool to identify vaccine responder subjects, especially in those cases without development of specific anti-S1 IgG antibodies against the vaccine.

Funding: This work was partially supported by grant from Cantabrian Government (2020UIC22-PUB-001) and from Instituto de Salud Carlos III (COV20/00170).

Keywords: Adaptive immunity, antibody, viral infections

P-0646

Dynamin-2 is important for production of infective virions in murine cytomegalovirus (MCMV) infection

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Murine cytomegalovirus (MCMV) as other members of the herpesvirus family, remodels cellular compartments of the endosomal system with the purpose of formation of viral assembly compartment (AC). In this study, we have investigated the role of dynamin in AC formation and virion production in MCMV infected murine fibroblasts by using dynasore – a short-term dynamin-2 inhibitor. In order to avoid influence of inhibitor on MCMV entrance and on imediate early phase of infection, dynasore has been added 4 hrs p.i. The influence of dynasore on AC formation has been followed by visualization of markers of inner AC (Rab10), and outer AC (viral late proteins like M55, M74, M25 (130 kDa)) 48 hrs p.i. We have found that inhibitor has prevented synthesis of late phase viral proteins and loading of Rab10 into inner AC. The inhibition of synthesis of late proteins has also been proved by flow cytometry and western blot analysis. Furthermore, viral DNA synthesis has also been inhbited (cells has been exposed to EdU Click 555 for labeling of newly syntesized DNA). In line with that, we have found that, after infection of fibroblasts with SCP-Cherry MCMV, dynasore treatment has also inhibited synthesis of small capsid protein (SCP). Finally, results of plaque assay have shown that applying of dynasore 4 hrs p.i. has significantly decreased release of progeny virions. These results suggest that dynasore inhibits production of infective MCMV particles, probably due to prevention of AC biogenesis, and synthesis of late phase viral proteins.

Keywords: Cell signalling, endo- and exocytic vesicles in immunity, viral infections

P-0647

Relationship between CD4+ cytotoxic T cell and stress protein responses in chronic conditions

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In some chronical conditions patients develop CD4+ cytotoxic T cells. Manuplating CD4+ T cell plasticity in this manner may be a therapeatic approch in some conditions. Relationship between stress protein expression levels and CD4+ cytotoxic T cell development is not clear. In this study we measured levels of CD4+ CD107a+ cytotoxic T cells by flow cytometry, and levels of HSP27, HSP72, and HSP90-alpha by ELISA in blood samples of patients suffering either of type-1 diabetes, hepatitis B infections, asthma associated with allergic rhinitis, and AIDS. According to the preliminary results, CD4+ cytotoxic T cell levels increased in last three groups and decreased in type-1 diabetes group. Change in mean HSP27, HSP72, and HSP90-alpha levels were correlated with change in mean CD4+ cytotoxic T cell level in all groups except for mean HSP27 level in asthma group, and mean HSP72 and HSP90-alpha levels in diabetes group. Two stress protein profiles were dominated. More anti-inflammatory HSP27 and less HSP90-alpha profile matched with diabetes and asthma groups; less HSP27 and more others profile matched with the infection groups. Comparison of the correlation values of mean HSP27 and CD4+ cytotoxic T cell levels in diabetes and asthma groups, and considering lack of correlation between relatively high HSP90-alpha levels and CD4+ cytotoxic T cell levels in diabetes group, suggested that there was no relationship between expression level of these stress proteins and CD4+ cytotoxic T cell development. Increase in HSP72 level showed clear correlation with CD4+ cytotoxic T cell level in all groups.

Keywords: Cell signalling, immune regulation and therapy, immune response tracing

P-0648

Probiotic bacteria differentially regulate IL-31 expression *in vitro*

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Certain probiotic strains of lactobacilli have been shown to dampen inflammation and proven efficacious in ameliorating atopic disease. In contrast, *Staphylococcus (S.) aureus* is a human opportunistic pathogen associated with immune mediated diseases such as atopic dermatitis, allergic asthma and sepsis. IL-31 is a Th2-like proinflammatory cytokine belonging to the IL-6 family of cytokines. IL-31 expression has been linked to several hypersensitivity disorders such as pruritis and atopic dermatitis, allergic asthma and inflammatory bowel disease, yet little is known regarding its regulation by probiotic bacteria. We have previously shown that cell free supernatants (CFS) or extracellular membrane vesicles (MV) from *Limosilactobacillus (L.) reuteri* DSM 17938 dampen *S. aureus* induced IFN-γ and IL-17A responses from PBMC. In the current project, we investigated the regulatory effect of two human-associated species of bacteria, *L. reuteri* DSM 17938 and *Lacticaseibacillus rhamnosus* GG (LGG) on inflammatory cytokine production. Interestingly, anti-CD3/CD28 stimulation of CD4+ T cells in the presence of conditioned medium from *L. reuteri*-primed monocyte-derived dendritic cells (MoDC) significantly increased IL-31 secretion. On the other hand, addition of *L. reuteri*-CFS during *S. aureus*-stimulated PBMC cultures had no effect on IL-31 secretion, suggesting a differential capacity of monocytes and MoDC to promote IL-31. Furthermore, stimulation of PBMC in the presence of LGG-CFS significantly reduced IL-31 secretion demonstrating a species-dependent regulation of IL-31.

Keywords: Adaptive immunity, cytokines and mediators, dendritic cells

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POSTER PRESENTATIONS

P-0649

Leptin modulates cytokine gene expression in childhood immune thrombocytopenia leading to a type-2 polarization

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High plasma leptin levels have been observed in patients with certain autoimmune diseases, implicating leptin in their pathogenesis. In this work, we studied the effect of leptin in childhood immune thrombocytopenia (cITP), a typical type-1 autoimmune disease. We measured plasma leptin levels in 39 children with ITP, before and after treatment with intravenous immunoglobulin and/or methylprednisolone, and 33 healthy age/body mass index-matched controls. We also cultured isolated peripheral blood T-cells and monocytes with recombinant leptin, to assess its effect on pro-inflammatory (type-1) and anti-inflammatory (type-2) cytokine gene expression. Plasma leptin levels were significantly increased in patients with active disease compared to controls, and negatively correlated with platelet count. Intravenous immunoglobulin treatment had no significant effect on leptin levels, whereas steroid treatment reduced leptin to below control levels. In remission, leptin levels were within control range. Culture of T-cells and monocytes with leptin resulted in a shift from type-1 to a type-2 cytokine polarization, mainly mediated through a significant increase of IL-10 and a significant decrease of TNF-α and IL-6 expression in patients' monocytes. Leptin had no significant effect on the expression of cytokine genes in T-cells and monocytes isolated from controls. In cITP leptin levels correlate with disease activity. Leptin acts as an active anti-inflammatory agent, mediating the secretion of IL-10, leading to an overall type-2 polarization.

Keywords: Autoimmunity, cytokines and mediators, immune regulation and therapy, innate immunity

P-0650

Expression of genes associated with pro-inflammatory but also anti-inflammatory/regenerative function by both Ly6Chi and Ly6Cint/low monocyte/ macrophage subsets in murine autoimmune liver disease

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Autoimmune liver diseases (AILD) like autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC) lead to chronic liver inflammation. To understand immunological pathways involved in hepatic immunity and tolerance, we focused on a phenotypic characterization of monocyte/macrophage subsets in murine models of AILD, which based on previous definition of inflammatory M1 (Ly6Chi/CCR2+) and anti-inflammatory/restorative M2 (Ly6Cint/low/CX3CR1+) subsets, to assess their contribution to liver disease pathology. C57BL/6 mice were treated with IL-33 on three days. C57BL/6 mice were treated with ConA to induce acute AIH. Mdr2-/- mice were used that develop PSC within 12 weeks. The PrimeFlow RNA assay was used for simultaneous analysis of mRNA and protein expression by flow cytometry. IL-33 pre-treatment suppressed acute AIH, which was associated with elevated frequencies of Ly6Cint monocytes/macrophages expressing genes associated with anti-inflammatory/restorative function (*Mmp9*, *Chil3*, *Tgfb1*). Interestingly, all subset showed increased expression of CCR2 that has been associated with a M1 phenotype. In acute AIH, Ly6Chi and Ly6Cint monocytes/macrophages were increased. These subsets were characterized by expression of genes associated with inflammatory (*Nos2*, *Tnf*) and anti-inflammatory/restorative function (*Il10*, *Arg1*, *Chil3*, *Tgfb1*). In PSC, Ly6Cint and Ly6Clow macrophages were increased while Ly6Chi monocytes were decreased. We detected a mixed gene expression profile with up-regulated expression of *Il12*, *Tnf*, *Il10*, *Chil3* and *Areg*. The analyses revealed that M1 and M2 characterization based on expression of Ly6Chi/CCR2+ and Ly6Cint/low/CX3CR1+ is not appropriate for hepatic monocyte/macrophage subsets, which are characterized by expression of genes associated with pro- and anti-inflammatory/restorative function in AILD.

Keywords: Animal models, autoimmunity, inflammatory disease, macrophage, myeloid cells, tissue damage and repair

P-0651

Head and Neck cancer can change neutrophil phenotype through secretion of tumor-derived exosomes

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Exosomes are heterogeneous, membrane-bound phospholipid vesicles secreted by different cell types, bearing various type of receptors and proteins from their host cells. Tumor-derived exosomes (TEX) serve as a communication system between tumor and immune system, and can be responsible for reprogramming of immune cells, such as neutrophils. Polymorphonuclear neutrophils (PMNs) were isolated from healthy humans and mice, and incubated with exosomes from head and neck cancer (HNC) cell lines. Exosome preparation was performed by using size exclusion chromatography. Nanoparticle tracking analysis and western blotting were applied for exosome characterisation. Transmission electron microscopy was used for evaluation of heterogeneity and purity of exosomes. The activity and survival of neutrophils were assessed by flow cytometry and functional assays. We have observed that neutrophils, after co-incubation with TEX, change their phenotype into pro-tumoral. Survival of such neutrophils is significantly up-regulated and expression of CD66b is elevated. At the same time, CD62L and CXCR4 are downregulated, suggesting their activation. Moreover, neutrophils co-incubated with TEX show significantly upregulated ROS-production, which can be *in vivo* responsible for creating a permissive growth environment for tumor cells. Similarly to the neutrophil phenotype that is observed during the progression of HNC, neutrophils after stimulation with TEX show increased life span and pro-tumoral status accompanied by elevated ROS production. Hence, TEX seem to be responsible for the activation of such pro-tumoral activity of neutrophils even in the absence of tumor microenvironment. Therefore, targeting TEX-mediated neutrophil modulation might provide a novel therapeutic approach for HNC.

Keywords: Neutrophils, cancer immunology, cell signalling, endo- and exocytic vesicles in immunity

P-0652

Immune assessment of BNT162b2 m-RNA-Spike based vaccine response in adults

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Currently, the assessment of the immune response after SARS-CoV-2 vaccination is scarce. Therefore, we aim to evaluate the specific humoral and cellular immune response after the first and the second dose of the BNT162b2 mRNA vaccine. A total of 53 health care workers were immunized with the same lot of BNT162b2 vaccine. The immunological response against the vaccine was performed using a T-specific assay based on the expression of CD25 and CD134 after stimulation with anti-N, -S, and –M specific peptides of SARS-CoV-2. Moreover IgG anti-S2 and -RBD antibodies were detected by ELISA. Furthermore, cell subsets involved in the secondary response to the vaccine were measured in peripheral blood by flow cytometry. Humoral specific response against vaccination was detected in 94% and 100% after the first and second dose, respectively. Therefore, anti-S T-specific response was observed in 57% and 90% of the subjects after the first and the second dose of the vaccine, respectively. Thirty days after two doses of BNT162b2-vaccine, a significant increase in T helper-1 memory cells ($p<0.001$), T follicular helper (TFH) cells ($p<0.001$), switched memory ($p=0.005$), and follicular B cell ($p<0.001$) was observed. This study describes the specific humoral and cellular immune response after vaccination with the new mRNA-based BNT162b2 vaccine. A mobilization of TFH and B follicular cells into the circulation occurs, reflecting a specific activation of the immune system.

Funding: This work was partially supported by grant from Cantabrian Government (2020UIC22-PUB-001) and from Instituto de Salud Carlos III (COV20/00170).

Keywords: Adaptive immunity, antibody, follicular helper T cells, viral infections

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POSTER PRESENTATIONS

P-0653

CVAC formation and Golgi apparatus reorganisation in the early phase of MCMV infection

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Murine cytomegalovirus (MCMV) extensively rearranges membranous compartments of the cell to form the virion assembly compartment (AC). The AC contains many membranous intermediates delimited at the periphery by the reorganized Golgi apparatus (GA). The loss of normal GA morphology and its relocalization from a juxtanuclear ribbonlike structure to a series of concentric rings on the AC periphery are the earliest landmarks of membranous system reorganization during MCMV infection. We aimed this study to identify whether GA transformation is the first step in AC biogenesis. Balb3T3 fibroblasts were infected with recombinant murine cytomegalovirus Δm138-MCMV for 6 and 16 hours. We analyzed a GA reorganization using confocal imaging and a panel of GA markers, including Rab6, GM130, Grasp55, Grasp65, GS15, Golgin 97, and Arf1. The requirement of GA in the AC biogenesis was monitored as over recruitment of Rab10 and Evectin-2 onto organelles of the inner AC after the dispersion of GA by Brefeldin A, and Golgicide A. GA analysis demonstrates that the outer ring of the AC is built by the C2-C7 Golgi cisternae, whereas the TGN and post-Golgi linker compartments are relocated to the inner area of the AC. The dispersion of the Golgi prevented the accumulation of Rab10- and Evectin-2-positive endosomal-derived intermediates within the inner AC area, indicating that intact GA is essential for the reorganization of the endosomal system in the early phase of MCMV infection. These data suggest that unlinking the Golgi ribbon may be the earliest event in the AC biogenesis during cytomegalovirus infection.

Keywords: Antibody, cell signalling, endo- and exocytic vesicles in immunity, viral infections

P-0654

Innate and adaptive immune assessment at admission to predict clinical outcome in COVID-19 patients

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Considering that many studies have been carried out to evaluate different immune system parameters against SARS-CoV-2 infection, we aim to predict clinical outcome through a broad study of cellular components of the innate and the adaptive immune response. A total of 155 SARS-CoV-2 patients were studied at admission in our hospital and categorized according to the requirement of oxygen therapy into mild (non-oxygen requirement) and severe (oxygen-requirement). Cellular components of the innate and the adaptive immune response were assessed using a broad multiparametric flow cytometry panel. At admission, patients with severe disease were older and presented higher levels of ferritin, D-dimer, C-reactive protein, troponin, interleukin-6, as well as neutrophilia and lymphopenia. Moreover, in comparison with severe disease patients, those with mild symptoms had significantly increased circulating non-classical monocytes (p=0.01), innate-lymphoid type-3 (p<0.001), and regulatory NK-cells (p=0.016). In contrast, severe patients had a significantly lower frequency of Th1 and regulatory T-cells with increased activated (p=0.028) and exhausted (p=0.019) CD8 phenotype (CD8+CD38+HLADR+ and CD8+CD27-CD28-, respectively). Finally, a logistic regression analysis was performed, with the following variables included in the final model: age, ferritin, D-dimer, lymph counts, C4, CD8+CD27-CD28-, and non-classical monocytes. Considering this result, severity could be predicted with an AUC of 78%. We proposed that the assessment of a composite of innate and adaptive immune parameters could be considered as potential predictive biomarkers of prognosis of COVID-19 disease.

This work was partially supported by grant from Cantabrian Government (2020UIC22-PUB-001) and Instituto Salud Carlos-III (COV20/00170).

Keywords: Adaptive immunity, innate lymphoid cells, viral infections

P-0655

HLA markers association with neuromyelitis optica spectrum disorders in a South Tunisian population

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Neuromyelitis optica spectrum disorders (NMOSD) are a group of autoimmune inflammatory demyelinating diseases of the central nervous system. A subgroup is characterized by seropositivity of anti-aquaporine 4 (anti-AQP4) antibodies which represent a specific marker of NMOSD. This spectrum of disorders seems to be rare in Tunisia. From a genetic point of view, human leukocyte antigens (HLA) associations with NMOSD, especially HLA class II associations, were investigated in different populations but not in our country. Our aim was to study HLA profile in South Tunisian NMOSD patients. Eleven anti-AQP4 seropositive NMOSD patients (recruited during 13 years) were enrolled. HLA class I (HLA-A and -B) typing was performed using micro lymphocytotoxicity complement dependent technique. HLA class II (HLA-DRB1 and -DQB1) alleles were determined by polymerase chain reaction with sequence specific primers. HLA markers polymorphism in our patients was compared to a control group (123 unrelated healthy controls from South Tunisia). Concerning HLA class I region, our study suggests an association, not previously reported, of HLA-B7 with NMOSD (45,45% versus 9,76%; p=0,003). Concerning HLA class II region, we didn't find any statistically significant association unlike studies in other populations. Further studies in our country may help to confirm our findings.

Keywords: Autoimmunity, MHC and polymorphic genes, neuroimmunology

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POSTER PRESENTATIONS

P-0656

Donor lymphocytes in peripheral blood of patients after lung transplantation comprise high frequencies of killer cell immunoglobulin-like receptor-positive T and NK cell subsets

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Acute and chronic rejections are major limitations following lung transplantation (LuTx) and better understanding of contributing immune responses early after LuTx is needed. Passenger leukocytes derived from donor lungs are primarily NK/T cells. We characterized the expression of killer cell immunoglobulin-like receptors (KIR), regulating NK and CD8+ T cell activity, on donor and recipient NK/T cells in recipient blood after LuTx. We investigated the functional capacity of donor vs recipient NK cells. Peripheral blood samples at pre, T0hr, T24hrs and 3wks post-Tx of n=51 LuTx recipients were analyzed using flow cytometry. Within the first 3wks after LuTx, donor NK/T cells were detected in n=51 patients. Donor NK cells showed significantly higher frequencies of KIR2DL1-positive cells 3wks post LuTx compared to recipient NK cells. This effect was also observed in donor T cells 3wks after LuTx. Higher activation levels of donor NK/T cells were detected as compared to recipient cells. Higher frequencies of donor NK and T cells expressing KIR compared to recipient NK/T cells argue for their origin in the lung as part of a highly specialized immunocompetent compartment. Despite KIR expression, the activation level of donor NK/T cells in the periphery of the recipient may be higher compared to recipient cells. Moreover, a positive correlation was detected for KIR surface expression on NK cells and cold-ischemic-times implying extended preservation times have an impact on the NK subset composition. Hence, donor NK/T cells might have a regulatory effect in balancing between tolerance, rejection and graft survival after LuTx.

Keywords: Monitoring immunity, NK cells, transplantation

P-0657

Persistence of skewed T regulatory cells in patients recovering from severe COVID-19

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Severe immune impairment, cytokine storm, inflammation and T regulatory cells dysfunctions exist in COVID-19 patients. Clinically, it is crucial to know whether and when the system recovers to the normal functionality and whether recovering patients should be considered immunologically healthy individuals. 41 COVID-19-recovered patients, divided based on WHO severity classification as severe (n=14), moderate (n=14) and mild (n=13), and matching healthy controls were analyzed at 1-3 and 6 months after the disease. For functional measurement of cytokine production by T cells, the CD3, CD4, CD8, intracellular Foxp3, TGF-β, IL-17, TNF-α, IFN-γ, IL-2, granzyme B and CD107a were detected by spectral cytometry. Plasma levels of IL-4, IL-2, CXCL10, IL-1β, TNF-α, CCL2, IL-17A, IL-6, IL-10, IFN-γ, IL 12p70, IL-8, TGF-β1 were quantified using LEGENDplex™ Panel. In severe post-COVID-19 patients we found higher percentage of CD4+ cells producing IL-2, TNF-α and CD107a, CD8+ subset showing higher production of TNFα, IFN-γ and CD107a and finally Treg producing more IL-2, IL-2+IL-17, CD107a and TNF-α. Such effector cytokine production indicated a potential Th1/Th17 state and aberrant plasticity of Treg. Kinetics analysis revealed the impaired state till 2-3 months after the recovery, however the cytokine storm was already attenuated. In conclusion, patients recovering from severe COVID-19 still show dysfunctional imbalanced immune system. Increased production of TNF-α, IFN-γ, IL-2, and IL-17 by CD4+ and Treg indicates Th1/Th17 phenotype promotion and skewed Treg activity. This might affect patients' health and should be considered before applying treatments to other disorders.

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Keywords: Cytokines and mediators, immune response tracing, monitoring immunity, regulatory cells, viral infections, visualizing immune responses

P-0660

Monoallelic R761H MEFV variant in two patients with Familial Mediterranean Fever

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Familial Mediterranean Fever (FMF) is an autosomal recessive disorder caused by pathogenic MEFV variants, characterized by recurrent attacks of fever, peritonitis, pleuritis, and painful, swollen joints, frequent in Mediterranean region. We present two Macedonian patients with FMF with a rare monoallelic R761H MEFV variant. Children with clinical features of periodic autoinflammatory syndrome were referred to the Institute for Immunobiology and Human Genetics, Medical faculty, Skopje for genetic testing. Genetic diagnosis was established using MEFV Reverse Line Strip (RLS) and Next-Generation Sequencing (autoinflammatory panel). Patient 1 is a 14-years old girl, whose symptoms started at the age 4, with recurrent fever (>38.5°C), transitory coxitis, atopic dermatitis and elevated inflammatory markers during the attacks. She is on therapy with colchicine and doing well. Patient 2 is a 6-year old boy, whose symptoms started at the age 5 with recurrent fever attacks, tonsillopharyngitis and mouth ulcers, with an average interval between attacks of 2 weeks. He was repeatedly treated with antibiotics, although his microbiology swabs were negative. These attacks stopped after 6 mounts and he is not on colchicine therapy. MEFV R761H was the only variant identified in both patients. The R761H variant has been reported in compound heterozygous state in <3% of FMF patients, with low allele frequency (not detected in healthy Macedonian population). This likely pathogenic MEFV variant should be considered in patients with FMF-like symptoms, even in the absence of a second variant.

Keywords: Autoinflammation, innate immunity, omics technologies

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POSTER PRESENTATIONS

P-0661

Study of exosomes derived from activated platelets in Multiple Sclerosis relapse and remission paired patients: potential plasma biomarkers

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Multiple Sclerosis (MS) is an autoimmune disease that affects the central nervous system (CNS), associated with a complex pathogenesis that shows high variability between patients. The 85% of patients present the clinical form known as relapsing remitting multiple sclerosis (RRMS) with relapses. The diagnosis of these relapses continues being clinical, which makes difficult their detection and early treatment. Exosomes are membranous vesicles from 40-150 nm released from a multitude of cell types, including nerve and immune cells. Exosomes have gained significant interest as potential diagnostic biomarkers because their number and origin change in pathological processes, including MS. Besides, they can be found in all biological fluids and can cross the blood-brain barrier. The study of platelet function in RRMS pathology has increased in recent years due to its involvement in both central and peripheral inflammation. However, how the level of platelets derived exosomes change in the same patient depending on its relapse or remission status and its possible role as inflammation prognostic biomarker has not been characterized yet. In our study, we analysed by flow cytometry exosomes released from activated platelets from patients with RRMS (in paired samples), comparing the number of active platelet exosomes between samples obtained in relapse and remission phases. Our results could help neurologists to identify and predict the development of a relapse in these patients, using plasma samples, taking steps towards personalized medicine.

Keywords: Autoimmunity, cell signalling, biomarkers

P-0662

Oral immunity induced by COVID-19 vaccine Tozinameran in comparison to natural infection

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SARS-CoV-2 causing COVID-19 challenges health care systems globally. Especially elderly people are at risk of fatal disease. The rapid development of vaccines, such as Tozinameran (BNT162b2, Comirnaty®) resulted in enormous vaccine campaigns. First studies indicate clear benefits through vaccination. However, an in-depth characterization of systemic and mucosal immune responses in the most vulnerable, i.e. elderly, and comparison to immunity through natural infection is still scarce. We thus investigated Spike glycoprotein-reactive T cells, IgG and IgA titers and neutralizing capacity in blood and saliva of elderlies (>70) and caregivers (<60) and in saliva of COVID-19 convalescents. We observed delayed and more heterogeneous cellular and humoral systemic and mucosal response in the elderly in comparison to younger individuals which mouned prompt and efficient responses to Tozinameran. In vaccinated individuals, neutralizing activity in serum was still higher at d49 as compared to COVID-19 convalescents. On the contrary, salivary neutralizing activity was still detecteable in COVID-19 convalescents at ~d49 and ~d94 after symptom onset. Our results demonstrate that Tozinameran evokes strong immune responses in middle-aged and elderly individuals. But, the elderly, exhibited delayed and more heterogeneous responses with some non-responders. The observed rapidly decreasing neutralizing activity in saliva after vaccination indicated that – in contrast to natural infection – vaccination cannot mount a long-lasting mucosal immunity.

Keywords: Adjuvants and vaccines, ageing, antibody, viral infections

P-0663

What is the role of Notch1 in liver fibrosis development?

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Hepatic fibrosis is a common feature of various liver diseases characterized by activation of hepatic stellate cells (HSC), a principal source of alpha smooth muscle actin (αSMA) liver myofibroblasts. The pathophysiological role of Notch activation has been well established, but the role of Notch1 in activated HSCs is still not sufficiently investigated. In the present research we first used two common murine models of liver fibrosis, carbon tetrachloride (CCL4) treatment for 6 weeks and 0.1% DDC-supplemented diet for 4 weeks to analyse the expression of Notch-related genes. In CCL4 model, qPCR analysis showed an upregulation of Notch2, Hey1, HeyL, and Jag2, while DDC-induced fibrosis was associated with increased expression of Notch2, Notch3, Hey1, Hes1, HeyL, Jag1 and Jag2. In the next set of experiments, we used double transgenic SMACreΔRbpjκΔ mice in which Notch1 signalling pathway was specifically inhibited in myofibroblasts by tamoxifen injections during the fibrosis development. Notch1 inhibition, however, did not change the degree of liver fibrosis, as evidenced by no significant difference in histological score on Sirius red liver staining and no difference in tissue expression of COL1A1 and ACTA2 between the control (SMACre-ΔRbpjκΔ) and Notch1 inhibited (SmaCre+ΔRbpjκΔ) mice. So far, our data show that canonical Notch1 signalling in myofibroblasts is not a crucial contributor to liver fibrosis development in CCL4 and DDC model.

Keywords: Animal models, biology of the immune system, chronic inflammation and fibrosis

P-0664

β-adrenoceptor blockade affects the germinal center B cell response to seasonal quadrivalent inactivated influenza vaccine (QIV) in mice

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The study was undertaken considering importance of antibody response for influenza vaccine efficacy and data indicating that β2-adrenoceptor stimulation may affect antibody generation. To elucidate influence of β-adrenoceptor-mediated signaling on antibody response to QIV, serum titers of QIV-specific IgG and draining lymph node and splenic germinal center (GC) reaction to QIV were examined in BALB/c mice treated with propranolol, non-selective β-adrenoceptor blocker, or vehicle (controls) daily, beginning two days before immunization. Four weeks after immunization IgG antibody titer was decreased in propranolol-treated mice. This correlated with lower frequency of GC B cells in lymphoid organs of propranolol-treated mice, and their diminished proliferation upon restimulation with QIV antigens in culture. Consistently, Tfh/Tfr cell ratio was shifted towards the latter in propranolol-treated mice. This correlated with lower frequency of QIV-specific CD4+ cells that produce IL-21, the key regulator of GC reaction, in lymphoid organs of propranolol-treated mice, as suggested by *in vitro* recall test. Additionally, propranolol treatment shifted IgG1/IgG2a antibody ratio towards IgG1 antibody (contributing mainly to the virus clearance). This was consistent with the shift in IFN-γ/IL-4 ratio to the site of IL-4 in QIV-stimulated splenocyte cultures from propranolol-treated mice compared with controls. Thus, the study suggests that chronic administration of propranolol, drug widely used for cardiovascular pathology, and recently repurposed for several other pathologies, including cancer, rheumatoid arthritis, and anxiety may impair efficacy of QIV by affecting CD4+ T-cell cytokine secretory profile and thereby overall efficacy of Tfh help to B cells, and its influence on IgG subclass switching pattern.

Keywords: Adjuvants and vaccines, antibody, follicular helper T cells, immune communication

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POSTER PRESENTATIONS

P-0666

High expression of PD1 in CD8 T-lymphocytes, PDL1 and blood-tissue correlation in patients with gastric adenocarcinoma

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Immunotherapy based on PD1 and PDL1 blocking antibodies has been a milestone for the treatment of cancer. These therapies require predictive biomarkers that allow the selection of patients potentially benefiting from these treatments. In this work we determined the presence of CD8 T-lymphocytes and PD1 expression in peripheral blood and in distal and tumor tissues of patients with gastric cancer, as well as PDL1 in tumors of these same patients, evaluating the correlation of the parameters in blood and tissues. Lymphocytes were obtained from peripheral blood and gastric tissue of patients with gastric adenocarcinoma and analyzed by flow cytometry. CD8, PD1 and PDL1 cells presence in tissue was determined by immunohistochemistry. We found a significant increase of PD1+ cells (24.9%±2.0 N=37) in peripheral blood lymphocytes of patients compared to controls (15.4%±1.9, N=21, p=0.003), with a higher increased in CD8 cells of patients (46.1%±4.3, N=26) as to controls (25.1%±4.2, N=15, p<0.002) and total T lymphocytes (p<0.0001) in patients. We confirmed PDL1 expression in 31% (N=29) of patients, an increase of CD8 cells in tumor tissue (6.4%±0.7 N=33) versus distal (4.1%±0.4 N=30, p=0.006) and an increase of PD1 cells in tumors (3.7%±0.6, N=27) versus distal tissue (1.5±0.3 N=28, p=0.002) of patients. The percentage of CD8 cells in peripheral blood correlated with the increase of tumor-infiltrating CD8 cells (R^2=0.2584, p=0.018), and the increase of tumor-infiltrating CD8 and PD1 cells correlated positively in patients (R^2=0.6248, p<0.0001). In conclusion, blood and tissue determination of these markers could identify patients likely to benefit from anti-PD1/PDL1 therapies.

Keywords: Biomarkers, cancer immunology, immunotherapy

P-0667

A single dose of BNT162b2 vaccine elicits strong humoral response in SARS-CoV-2 seropositive individuals

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The high demand for COVID-19 vaccines combined with a significant lack of supply prompts the question whether administering a single vaccine dose in SARS-CoV-2 seropositive individuals, could be a method for rationing available vaccine doses. The study included 41 seropoitive and 185 seronegative individuals. Testing was performed using the quantitative CLIA anti-SARS-CoV-2 RBD IgG kit on a Maglumi platform (Snibe, China). Anti-SARS-CoV-2 RBD IgG antibody levels after the first dose of BNT162b2 were significantly higher in seropositive individuals (922,9±948,6 AU/ml) compared to seronegative individuals (78,8±252,6 AU/ml, p<2e-16). The vast majority of the study participants reported at least one side effect (90,1%), mostly minor local pain (69,5%). Seropositive individuals had on average a higher count of side-effects (1,61±1,05) compared with seronegative individuals (1,31±0,95; p=0,045). Seropositive individulas had higher odds to experience fever/shivers (OR=2,98, p=0,048) and malaise (OR=3,15; p=0,005), and lower odds to experience minor local pain (OR=0,92; p=0,023) compared to seronegative individuals. Our findings are in line with previous reports of higher antibody levels in seropositive individuals after a single dose of BNT162b2 compared to seronegative individuals, and support the idea that a single dose of BNT162b2 in SARS-CoV-2 seropositive individuals might provide sufficient humoral immunity towards SARS-CoV-2. This idea should be validated in clinical trial setting as soon as possible, due to direct implications for public health policy in developing countries with limited access to vaccines. The more rational use of vaccines could accelerate the attainment of collective immunity at reduced costs.

Keywords: Adjuvants and vaccines, antibody, immune response tracing, protection

P-0670

Titre and avidity of peripheral blood antibodies reactive with gut microorganisms in local and systemic inflammatory diseases of bone tissues

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Chronic periodontal diseases (CPD) are suspected causal factors in the initiation and maintenance of the inflammatory response in rheumatoid arthritis (RA). However, the mechanisms of CPD and RA interconnection have not been clarified. Commensal microbiota is necessary for the establishment of an effective immune response but is also related to the onset of local and systemic inflammatory diseases of bone tissue. We analysed the titre and avidity of serum antibodies recognising *Lactobacillus plantarum*, *Lactobacillus reuteri*, *Escherichia coli* and *Candida albicans* in 25 CPD patients without any systemic disease and in 26 RA patients. Lower titre of IgM and IgG reactive to *E. coli*, higher IgM titre to *L. plantarum* and lower titre of IgG to *C. albicans* was detected in RA compared to CPD. Avidity of IgM reactive with the analysed bacteria was lower in RA then in CPD. Only the avidity of IgA to *L. reuteri* was higher in RA, while no difference was detected in the avidity of anti-*C. albicans* immunoglobulins. In CPD a strong correlation was found between the titres and avidity of anti-Candida IgM and anti-*E. coli* IgA antibodies, while in RA such correlation was found for all anti-Candida immunoglobulin isotypes and anti-*E. coli* IgG and IgA. The results showed that in RA patients the reactivity of serum antibodies towards the selected commensal bacteria differed from the reactivity observed in CPD. This might reflect a disturbed disease-related immunoglobulin reactivity and/or dysbiosis. The significance of the results remains to be elucidated.

Keywords: Adaptive immunity, inflammatory disease, inflammatory joint diseases, rheumatoid arthritis

P-0671

The association of PNPLA3, EGF and Notch3 gene polymorphisms with alcoholic liver disease and hepatocellular carcinoma

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We investigated SNPs of PNPLA3, EGF and Notch3 as genetic risk factors for alcoholic liver disease (ALD) and its progression to hepatocellular carcinoma (HCC). DNA was isolated from 245 ALD patients (124 without HCC and 121 with HCC) and 70 control patients. SNPs were determined by PCR using TaqMan assays. PNPLA3 rs738409 was associated with higher risk for ALD in dominant (OR95%CI = 3.36 (1.94-5.82) for GG/GC vs CC), recessive (OR95%CI = 3.71 (1.42-9.69) for GG vs GC/CC) and log-additive model (OR95%CI = 2.60 (1.69-3.99) for G allele). This SNP was also associated with a risk for HCC in recessive (OR 95%CI = 2.72 (1.43-5.17) for GG vs GC/CC) and log-additive model (OR95%CI = 1.63 (1.14-2.34) for G allele). EGF rs4444903 was not associated with a risk for fibrosis development. However, it was associated with a mildly increased risk for fibrosis progression to HCC in a dominant model (OR95%CI = 1.92 (1.11-3.32), for GA/GG vs. AA). Notch3 rs1043996 was associated with lower risk for ALD in recessive model (OR95%CI = 0.32 (0.14-0.72) for GG vs GA/AA). There was no association between the Notch3 genotype and risk for HCC (p>0.05). PNPLA3 rs738409 is a considerable risk factor for the ALD and its progression towards HCC, while EGF rs4444903 might be an additional risk factor for HCC development. Notch3 rs1043996 genotype is associated with a lower risk for the development of ALD.

Keywords: Biomarkers, cancer immunology, chronic inflammation and fibrosis

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POSTER PRESENTATIONS

P-0672

M-CSF-mediated macrophage development is dominantly inhibited by NOD2 signaling for replenishment of immunogenic dendritic cells

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Despite recent advances, it remains unclear whether monocyte-derived dendritic cells (moDCs) represent alternative context-dependent fate in the gut. We found here that Nod2-dependent sensing of bacteria lowers the ability of circulating monocytes to respond to M-CSF for generating moDCs. Such inhibitory effect on monocyte-to-macrophage transition was prevented upon blockade of TNF-α. Recognition of the gut microbiota by Nod2 was sufficient to promote the expansion of moDCs within the colonic mucosa. A competitive bone marrow transplant model further demonstrated that Nod2 promotes the conversion of monocytes into dendritic cells. Equally of importance, tumours with the highest transcript levels of NOD2 were associated with a favorable prognosis and characterized by an enrichment of a gene signature related to moDCs. This study implicates that Nod2-dependent sensing of the gut microbiota influences monocytic lineage commitment into dendritic cells, which sets the stage for future investigations to achieve accurate outcome prediction in colorectal cancer.

Keywords: Immune development, macrophage, dendritic cells, *in vivo* tumor models

P-0673

Ex situ heart perfusion and standard of care cold storage differentially affect the ischemic secretome of donor hearts in perfusates but not the reperfusion response in recipient plasma

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Allograft preservation procedures may influence the donor organ status and in turn affect heart transplant (HTx) recipient. Here we aimed to compare the secretomes in recipient plasma and perfusates in patients whose hearts were either preserved using *ex situ* heart perfusion (ESHP) or standard of care (SOC) cold static preservation in order to identify potential biomarker candidates for heart preservation. Using multiplex techniques, we measured 50 cytokines/chemokines in recipient plasma before (pre), after (T0), 24h and 3 weeks after HTx. Unsupervised cluster analyses identified top-10 plasma cytokines and chemokines clearly separating T0 from other time points after HTx and reflecting a reperfusion injury-specific pattern. Surprisingly, ESHP or SOC heart preservation did not have a significant impact on these inflammatory plasma profiles at T0, T24 or 3 weeks. The two strongest discriminators separating T0 from other time points i.e. IFN-γ, SCGF-β were detected in both ESHP and SOC recipients at comparable concentrations. In contrast, the preservation method clearly affected the cytokine/chemokine profile in perfusates highlighted by higher concentrations of pro- (IFN-γ, CXCL10) and anti-inflmmatory (IL-10, IL-1RA) mediators in ESHP compared to SOC samples. Although ESHP or SOC preservation did not affect the reperfusion response in plasma at T0 after HTx, normothermic oxygenated preservation of donor hearts was accompanied by secretion of pro- and anti-inflammatory cytokines, chemokines that may affect long-term functionality and longevity of the graft. With a better understanding of molecular changes during ESHP, we expect to identify biomarker candidates for improved organ function pre HTx.

Keywords: Biomarkers, chemokines, cytokines and mediators, transplantation

P-0676

The role of NK cells in the resolution of malaria-associated acute respiratory distress syndrome

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Malaria is a global health disease with >400 000 deaths each year, caused by complications such as malaria-associated acute respiratory distress syndrome (MA-ARDS). Despite efficient parasite killing with antimalarial drugs, 15% of patients with complications still die. This shows the need to study resolution and wound healing mechanisms involved in the recovery from these complications. Disease resolution is coordinated by several leukocyte subtypes. Here, we investigate the role of NK cells in the disease resolution of MA-ARDS. C57BL/6 mice were infected with *Plasmodium berghei* NK65 (*Pb*NK65), resulting in the development of MA-ARDS on day 8 post infection. On this day, antimalarial treatment with artesunate and chloroquine was started for 5 days. To study the role of NK cells, NK cells were depleted by injection of anti-NK1.1. Depletion of NK cells did not affect the development of MA-ARDS, but resulted in a decreased survival during resolution. In particular, only 50% of the anti-NK1.1-treated mice recovered from MA-ARDS upon anti-malarial treatment, compared to >80% without depletion. Interestingly, the resolution of alveolar edema occurred as efficiently in the NK cell-depleted mice that could be rescued compared to the non-depleted mice. The treatment of *Pb*NK65-infected C57BL/6 mice with antimalarial drugs serves as a good model to study the resolution of MA-ARDS. NK cells are not involved during the development of MA-ARDS, but are critical during the resolution process upon anti-malarial treatment.

Keywords: Innate host defence, NK cells, parasite infections, tissue damage and repair

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POSTER PRESENTATIONS

P-0678

Significant differences in the results of serologic HCV antibody detection assays approved for screening of donated blood

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Nowadays, a variety of serologic assays, approved for screening of donated blood for anti-HCV antibodies, are used. However, data on the level of agreement between the tests are rare. 76 serum samples of blood donors with anti-HCV antibodies detected by at least one of five automated, commercially available tests: chemiluminescent immunoassay (ICL) detecting IgM/IgG to C/NS3/NS4 HCV antigens; two ELISA detecting IgG (ELISA_1) or IgM/IgG (ELISA_2) to C/NS3/NS4; "lateral flow" immunochromatography (ICA) detecting IgG/IgM to C/NS3/NS4/NS5, and western blot (WB) detecting IgG to C1/C2/E2/NS3/NS4/NS5. A significant difference between the results of tests applied was observed ($p<0.001$; $\chi 2$ test). 86% positive samples for anti-HCV antibody were identified by ICL assay, while percentage of positive samples tested with ICA, and ELISAs did not exceed 40%. With WB, 33% positive and 21% indeterminated sera were detected. Kappa statistical analysis showed substantial degree of agreement ($\kappa>0.80$) between ICA and both ELISAs, moderate level of agreement ($0.55<k<0.75$) between ICA and WB, ELISA_1 and ELISA_2, WB and both ELISAs, and slight degree of agreement ($\kappa<0.2$) between ICL and all the other tests. Antibody reactivity to individual HCV antigens in ICA and WB showed moderate/substantial level of agreement between these tests. Whether the observed differences are related to applied technological platforms, HCV genotypes, and/or anti-HCV antibodies` structure, is not clear at this point. However, this study indicates the necessity for precise standardization and permanent development of more reliable anti-HCV tests with greater degree of concordance between the measurements made by different tests.

Keywords: Antibody, biomarkers, infectious disease, viral infections

P-0679

Targeting epigenetic chromatin modification enzymes upregulated by M2c macrophage polarization with a proteasome inhibitor ixazomib in lung cancer: a novel approach

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IIn the lung cancer microenvironment, tumor associated macrophages acquire M2a- and M2c-like phenotypes that promote epithelial mesenchymal transitition, cell invasion and tumor progression, thus leading to a poor prognosis. The ubiquitin-proteasome pathway is active in almost all cellular processes, including epigenetic modifications. In order to investigate epigenetic chromatin modification enzymes gene expression levels of A549 lung cancer cells within M2c macrophages interplay, THP-1 monocyte cells were polarized into M0 macrophages by 24 h incubation in 100 ng/ml phorbol 12-myristate 13-acetate (PMA) containing serum-free medium and rested for 48 h in the upper chamber of a 6-well co-culture plate. After 72 h treatment with 1 μM hydrocortisone and 24 h resting, they polarized into M2c macrophages. In parallel, A549 cells were seeded into the lower chamber of another 6-well plate. After 24 hours, the upper chambers were inserted into the six-well plates containing the A549 cells, and the two cell populations were co-cultured for 24 hours with or without the presence of 2.19 μM ixazomib (Ninlaro®) (24h IC50 concentration). After mRNA isolation and cDNA convertion, RT-PCR analysis was performed with a gene panel. According to the results, extremely high expression levels were found in 56 genes, up to 1848-fold. In the presence of the proteasome inhibitor ixazomib, overexpressed genes were downregulated. In conclusion, although proteasome inhibitors have been most successfully applied in the treatment of hematological malignancies, the combination of proteasome inhibitors with epigenetic drugs could be beneficial in the treatment of lung cancer in the future.

Keywords: Cancer immunology, epigenetic control and modulation of immunity, macrophage, microenvironment

P-0680

Temporal serum proteomics of mother-infant dyads with HLA-conferred T1D risk depicts gestation and maturation and reveals no formula related serum differences from dietary intervention with a hydrolyzed milk formula

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The incidence of autoimmune diseases has increased dramatically in recent years without clear accountability. Since priming of the immune system occurs both in-utero and in infancy, characterization of the biochemical profiles of these stages are important for distinguishing healthy and diseased states, and serve to monitor interventions. As part of a dietary invention study, serum samples from 22 mother-infant pairs with HLA-defined T1D risk were analyzed using LC-MS/MS-based label free quantitative proteomics. The temporal sample series included three samples from the mother (at the beginning of third trimester, the time of delivery and 3 months postpartum) and five samples from each child (cord blood, 3, 6, 9 and 12 months). To evaluate the influence of dietary intervention upon beta cell autoimmunity, the infants were weaned either to a hydrolyzed or regular milk formula during the period of sample collection. Analysis was made of cord blood and nine-month samples from an additional 39 children to distinguish early and dietary related differences, respectively. The proteomics data from the mothers provided distinct representation of changes in pregnancy specific glycoproteins and acute phase proteins pre and postpartum. Similarly, the data clearly defined changes associated with maturation of the infants. Comparison of the mothers postpartum serum profiles and the infants highlighted the differences between adult and child. With the analysis of the extended cohort for validation, there were no significant differences in the serum proteomes between the study formula groups and, as previously reported, there was no difference in the incidence of beta cell autoimmunity.

Keywords: Autoimmunity, diabetes, immune development, mass spectrometry

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POSTER PRESENTATIONS

P-0681

Immuno-structural changes in early tumor microenvironment

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The establishment of tumor microenvironment in its early phases is still under study. The colon structure with its immunological components is ideal model for modelling inflammation and cancer. We found the colon mucosa collagen matrix particularly reactive to induced immune activation (DSS-induced colitis in rat and mouse, AOM-induced carcinogenesis in rat and bacterial colonization of germ-free mice). Using second harmonic generation of 2-photon confocal microscopy, we evidenced changes of the collagen scaffold related to variations of the tissue immunological state. IL-6, IL-1, IL-10 and TGF-β differently interplay depending on the type of elicited inflammation, both at local and systemic level. In early period post-induction either with AOM a smouldering inflammation appear to be present but able to modify the mucosal collagen scaffold. The immune activation by modulation of inflammation appears confirmed in the comparison of the structure between germ-free and conventional animals and during the conventionalization of germ-free animals. Also in human colon cancer samples - normal mucosa, near tumor mucosa and cancer tissue - scaffold changes were found associated increased gene expression for collagen I, LOX2L, IL-1-β, IL-6, IL-13. Interestingly, the expression of PD-1 and PD-L1 expression appears possibly modulated also by these conditions. We hypothesize that deregulation of regulatory molecule expression (e.g. TGF-β, IL-10), may change the tissue inflammatory threshold ruling the homeostatic conditions of the mucosa allowing initial tumor microenvironment establishment... Acknowledgements: grants RVO 61388971 (CZ), UniCredit Bank s.r.o. (CZ), Generali CEE Holding B.V. (CZ

Keywords: Cancer immunology, *in vivo* tumor models, inflammatory molecules, microbiome and environmental factors, microenvironment

P-0682

Association between CMV infection and inflammatory status in chronic heart failure patients

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Chronic heart failure (CHF) patients show a high level of proinflammatory cytokines and CMV infection could exacerbate this status. The aim of this study is to analyze the association between CMV infection and serum proinflammatory cytokine levels in CHF patients. Forty patients were recruited and classified according to their CMV-serostatus, 27 were CMV+. Detection and semi-quantification of CMV-specific antibodies were determined by enzyme-linked immunosorbent assay (ELISA). The serum concentrations of pro- and anti-inflammatory cytokines (IFN-γ, IL-10, IL-12, IL-17, IL-1β, IL-2, IL-4, IL-6 and TNF) were measured by Luminex® xMap Technology and the basal level of mRNA expression of several immune molecules was quantified by TaqMan™ Array Human Immune Response plates in CD4+ T-lymphocytes from CMV+ and CMV- patients. High levels of IFN-γ, IL-17, IL-1β, IL-6 and TNF were found in CMV+ patients (Student t test, $p<0.05$). Additionally, a significant correlation between high levels of these cytokines and the antibody titer against CMV was found (Spearman Rho test, $p<0.05$). On the other hand, CMV+ patients presented under-expressed IL-8 and CCL2 genes, and over-expressed TBX21 and IFN-γ genes implicated in Th1 pathway. Moreover, molecules related to cytotoxicity characteristic of the CD4+CD28null such as perforin, granzyme B or granulysin, and other molecules present in activated CD4+ T lymphocytes or in antiapoptotic pathways were found overexpressed in CMV+ patients. Our results suggest that the dynamics CMV-infection exacerbate the inflammation characteristic of the patients with CHF. Furthermore, T-lymphocyte differentiation degree may be playing an important role in the inflammatory status of these patients.

Keywords: Cardiovascular diseases, adaptive immunity, ageing, inflammatory disease, viral infections

P-0683

Association of high total serum IgE and sensitization to peanut allergen components

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The sensitization to peanut allergen components among Lithuanian children has not been reported. We aimed to evaluate the molecular profiles of peanut sensitization and the association of extremely high (>500kUA/L) total serum IgE levels and the prevalence of sensitization to peanut allergen components in children. We performed a retrospective analysis of sensitization profiles in 171 children in Vilnius University Hospital Santaros Clinics. SIgE levels to peanut allergen components Ara h 1, 2, 3, 6, 8, 9, and 15 were measured using multiplex allergy test ALEX² Allergy Explorer. Categorical variables were compared using the Chi-square test. A total of 41 (24.0%) children had extremely high total serum IgE and 50 (29.2%) children were sensitized to at least one peanut component of those 31 (62.0%) children were sensitized to at least one non-pollen-related peanut component (Ara h 1, 2, 3, 6, 9, 15). High total serum IgE was associated to sensitization to Ara h 8 ($p = 0.007$) and Ara h 9 ($p = 0.036$), but there was no statistically significant association to seed storage proteins (Ara h 1, 2, 3, 6) and oleosin Ara h 15. Almost two-thirds of peanut sensitized children were sensitized to at least one non-pollen-related peanut allergen component. High total serum IgE is associated to sensitization to Ara h 8 and Ara h 9 ($p = 0.036$), but not to seed storage proteins or oleosin Ara h 15.

Keywords: Allergic disorders, antibody, molecular immunology

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POSTER PRESENTATIONS

P-0684

Role of the C-type lectin receptor MINCLE in Strongyloides ratti recognition and anti-helminth immune responses

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Approximately one third of the human population is infected with helminths, large multicellular parasites that are controlled by the mammalian immune system in the context of type 2 immune responses. To promote their survival and persistence, parasitic helminths have evolved sophisticated pathways to modulate and downregulate this immune response. Using Strongyloides ratti infection in mice as a model for intestinal helminth infection, we demonstrated several pathways of helminth induced immunosuppression that delayed expulsion of the parasites. Here, we aim to investigate a novel pathway of immune modulation during concurrent helminth infection via the engagement of the C-type lectin receptor (CLR) MINCLE by helminth-derived ligands. CLRs represent an ancient family of pattern recognition receptors (PRR)s, recognizing highly conserved pathogen-associated molecular patterns. Using a MINCLE reporter cell line and a MINCLE-Fc fusion protein library we could show binding of a S. ratti-derived ligands to MINCLE. Additionally, MINCLE deficient mice showed reduced parasite numbers in the small intestine in the context of increased Th2 cytokine production. Thus, we hypothesize that engagement of MINCLE by S. ratti-derived ligands antagonizes the protective anti-helminth immune response. This is of particular interest as the classic function of MINCLE as PRR would be expected to promote immunity. The underlying mechanisms are under current investigation.

Keywords: Cell signalling, infectious disease, innate host defence, innate immunity, parasite infections

P-0685

Dimethyl fumarate treatment induces changes in lymphocyte subpopulations in multiple sclerosis that could identify "NEDA" patients

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Optimal response to dimethyl-fumarate (DMF) in multiple sclerosis (MS) is mediated by a shift to an antiinflammatory and immunoregulatory profile. In a preliminary study of 22 MS patients followed 12 months, we observed that, at 3 months of treatment, patients with "No evidence of disease activity (NEDA)" had a decrease in the Th1-like Th17 effector memory (EM) subpopulation. To analyze long-term effect of DMF on lymphocyte subpopulations and its relationship with disease's activity. Longitudinal prospective study in MS patients undergoing DMF treatment. A panel of T and B lymphocytes in peripheral blood was analyzed by flow cytometry. Patients with a complete follow-up of more than 1 year are classified as: NEDA, MEDA (minimal clinical or radiological activity) or EDA. 48 patients have been analyzed. After a 2.66 (1-5) years of follow-up. Changes induced: increase of naïve subsets in T [CD4 and CD8] and B-cells, decrease of central memory (CM) and EM T-cells, and memory B-cells; remained stable in the long-term, being more prominent in NEDA patients. In these, we found lower percentages of Th1 CM and EM pre-treatment, and of Th1, Th17 and Th1-like Th17 CM and Th1 and Th17 EM during the first 12 months. MEDA patients appear to behave like EDA patients in changes in Th1/Th17/Th1-like Th17 subsets. Changes induced by DMF on the lymphocyte subpopulations remain stable over time. NEDA patients have an immunophenotype that seems to identify them. Immunomonitoring detects biological effect of the treatments.

Keywords: Autoimmunity, biomarkers, multiple sclerosis

P-0686

Immune and inflammatory genes possibly involved in the pathogenesis of severe COVID-19

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Severe COVID-19 is caused by a substantial immune reaction, together with cytokine storm. We aimed to determine putative immunological/inflammatory genes that may be involved in COVID-19 disease severity. Seven patients with severe COVID-19, seven with mild COVID-19, and two healthy controls were included. To uncover the molecular differences among severe and mild patient groups and healthy controls whole-exome sequencing was performed. The detected variants were classified according to the ACMG 2015 guideline, specifically for immune and inflammatory pathways. For each group, gene variants common in all seven patients and absent in the opposite patient group were picked for semiquantitative RT-PCR (run in triplicates). ITGB3, FERMT3, MX1, PRSS1, and DMBT1 gene variants were commonly detected in the severe disease group. MX2, ATG12, SHARPIN, INPPL1 gene variants were common for the mild group and missing in the severe group. TMPRSS2 and IKBKG (NEMO) were additionally analyzed with qRT-PCR. All genes for both patient groups exhibited higher expression than control group. In the severe group ITGB3 (β3 integrin), MX1 (activated via IFN pathway, involved in antiviral immune response) and PRSS1 (trypsin) showed higher expression while SHARPIN (involved in antiviral immune response through NFκB pathway), IKBKG (NEMO), FERMT3 (Kindlin-3, involved in leukocyte adhesion), TMPRSS2 (facilitates SARS-COV2 entry into host cells), DMBT1 (functions as an inhibitor of HIV and influenza A), and INPPL1 exhibited lower expression compared to the mild group. Our results suggest that multiple genes may be involved in the excessive inflammatory response in severe COVID-19.

Keywords: Inflammatory molecules, molecular immunology, viral infections

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POSTER PRESENTATIONS

P-0687

Fine analysis of lymphocyte subpopulations in SARS-CoV-2 infected patients: toward a differential profiling of patients with severe outcome

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Coronavirus disease 2019 (COVID-19) is caused by the human pathogen severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and has resulted in widespread morbidity and mortality. CD4+ T cells, CD8+ T cells and neutralizing antibodies all contribute to control SARS-CoV-2 infection. However, heterogeneity is a major factor in disease severity and in immune innate and adaptive responses to SARS-CoV-2. We performed a deep analysis of lymphocyte populations of 125 hospitalized SARS-CoV-2 infected patients on the day of hospital admission. Five clusters of patients were identified using hierarchical classification on the basis of their immunophenotypic profile, with specific lymphocyte subpopulation profiles and different mortality outcomes. Some characteristics were observed in all the clusters of patients, such as lymphopenia, an elevated level of effector CD8+CCR7- T cells (with extremely high levels in clusters 4 and cluster 5) and an elevated level of plasmablasts (with extremely high levels in cluster 3). Low levels of T cell activation are associated to a better disease outcome; on the other hand, profound CD8+ T-cell lymphopenia, a high level of CD4+ and CD8+ T-cell activation and a high level of CD8+ T-cell senescence are associated with a higher mortality outcome (clusters 2 and 5). Our study suggests that some lymphocyte parameters might be useful to physicians to better characterize patients at hospital admission, in order to treat them earlier and more appropriately.

Keywords: B lymphocytes, adaptive immunity, viral infections

P-0688

Tolerance to DNFB-induced contact hypersensitivity reactions is mediated by activated CD73$^+$ regulatory T cells

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Regulatory T cells(Tregs) which constitutively express CD73 play a crucial role in immune regulation. In a contact hypersensitivity model, we found that tolerance can be induced by Dinitrothiocyanobenzene(DNTB) against the hapten Dinitrofluorobenzene(DNFB) in WT mice but not in CD73$^{-/-}$ mice. To investigate the role of CD73 expressed by Tregs during DNTB-mediated tolerance. After tolerization with DNTB, we found more activated Tregs marked by Ki67$^+$CD69$^+$Helios$^+$CTLA4$^+$Lag3$^+$CD200R$^+$IL10$^+$ in draining lymph nodes during the sensitization phase, leading to decreased ear swelling after challenge. However, tolerance induction was abrogated by injection of PC61 (anti-CD25 antibodies), which blocks the suppressive function of Tregs. This indicates a role for Tregs in DNTB induced tolerance. Likewise, application of anti-CD73 antibodies abrogated tolerance in WT mice. In skin we observed reduced levels of IL1β, CXCL5, CXCL2 after tolerance induction which was nullified by anti-CD73 antibodies. As for the mechanism, anti-CD73 antibodies did not deplete Tregs, instead we observed downregulation of CD73 by Tregs. Moreover, CD73$^{-/-}$ mice were unable to develop tolerance at all, but transferred WT Tregs were able to reestablish tolerance. The expression of CD73 on regulatory T cells is mandatory to develop tolerance against haptens, which provides a novel approach to treatments of immune diseases.

Keywords: Animal models, immune regulation and therapy, skin diseases, regulatory cells

P-0689

IL-33 facilitates rapid expulsion of the parasitic nematode Strongyloides ratti from the intestine via ILC2- and IL-9-driven mast cell activation

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Parasitic worms leave a trail of destruction while migrating through their host's tissue. Thereby they trigger the release of tissue-derived alarmin cytokines such as IL-33 that promote the initiation of efficient anti-helminth immune responses. Here we use mice infected with the parasitic nematode Strongyloides ratti to unravel the chain of events leading from parasite sensing to parasite expulsion. S. ratti penetrates the skin of its mammalian host, migrates via skin and muscle tissue to the mouth, is swallowed and reproduces in the small intestine. The parasite is eventually expelled from the intestine by the action of mast cells that are activated via IL-9. Using inhibitors and enhancers for IL-33 we demonstrate that the release of IL-33 during S. ratti infection activates mast cells. Blockade of IL-33 elevated intestinal parasite burden and suppressed mast cell degranulation while stabilization of endogenous IL-33 or application of recombinant IL-33 reduced intestinal parasite burdens and increased mast cell degranulation. IL-33 mediated parasite expulsion independently of adaptive immunity, basophils or granulocytes but dependent on IL-9, innate lymphoid cells and mast cells. In summary we provide an example of how efficient sensing of a tissue-migrating parasite generates a hostile environment in the intestine that facilitates parasite expulsion.

Keywords: Infectious disease, innate lymphoid cells, mast cells, parasite infections

P-0690

Sex differences in the effects of early-life probiotic treatment on TNBS-induced colitis in rats

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We tested the effects of early-life probiotic treatment on the induction of colitis in female and male adult rats. Rat pups were fed an aqueous solution of *Lactobacillus rhamnosus* (from day 4 to day 30). Feces were collected for microbial analysis. Colitis was induced at day 85. Seven days later rats were graded for histological damage in colon, and samples of mesenteric lymph node (MLN) and peritoneal exudate cells were analyzed. Female rats developed slightly less severe symptoms of colitis than males, whereas early-life probiotic treatment had a more pronounced effect on males in nearly every analyzed parameter. Namely, it increased fecal bacterial diversity and ameliorated colon tissue damage, as well as increased percentage of resident peritoneal macrophages (CD163+), decreased peritoneal monocyte (HIS48+CD43+) influx, reduced production of IFNγ and IL10 by MLN cells, attenuated NO production in stimulated peritoneal macrophages and unstimulated MLN cells of male rats. Our findings reveal that effects of probiotic treatment are sex-specific to an extent. While microbial diversity was impacted by probiotic treatment in both sexes at an early age, the effect was more pronounced in young males, and it lasted to their adulthood. The change in microbial diversity correlated with improved outcome of TNBS-induced colitis, confirming the importance of microbiota for local inflammatory processes. It remains to be elucidated whether the sex differences in the effect of probiotic treatment on development of colitis may be a consequence of sex differences in early-life microbial diversity and severity of colitis symptoms in untreated rats.

Keywords: Animal models, inflammatory bowel disease, lymphoid organs, macrophage

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POSTER PRESENTATIONS

P-0691

Microbiota influence the proliferative capacity of IELs during small intestine infection

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Intestinal Intraepithelial lymphocytes (IELs) occupy top layers of epithelial barriers and are broadly composed of natural IELs (CD8ααTCRγδ) and induced IELs (CD8αβTCRαβ and CD4TCRαβ). IELs are kept in a heightened but a controlled state of activation, have reciprocal interactions with the intestinal epithelial cells (IECs) and microbiota. IELs have potent cytotoxic activity, express chemokines and cytokines (IFN-γ), but can also express inhibitory molecules (PD-1 and CTLA-4). Recently, there is more evidence to support that IELs are involved in the pathogenesis of gut disorders such as inflammatory bowel disease. Yet, the stimulus that leads to IELs activation and the molecular pathways involved are still unknown. In the context of a parasite infection, caused by "Eimeria vermiformis" that infect mice IECs, CD8αβ IELs, but not CD8aa IELs, are strongly activated. However, upon treated with broad-spectrum antibiotics, followed by "E.vermiformis" infection, the proliferation of CD8αα IELs is boosted. The bacterial depletion prior to "E.vermiformis" infection is time dependent with antibiotic treatment over a week resulting in the most robust proliferation. This proliferation boost is located within gram-positive bacteria, the major producers of short-chain fatty acids (SCFA) and lactate. Thus, I hypothesise that CD8αα IELs get activated upon disruption of microbial homeostasis, sensing the absence of microbial components or metabolites. Moreover, I will test different infection and chemical models to initiate intestinal inflammation, while being able to deplete the microbiota using antibiotics, to address the molecular stimulus that leads to IELs activation.

Keywords: Adaptive immunity, infectious disease, inflammatory bowel disease, parasite infections

P-0692

Follicular helper-like T cells in the lung highlight a novel role of B cells in sarcoidosis

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Pulmonary sarcoidosis is generally presumed to be a T helper-1- and macrophage-driven disease. However, mouse models have recently revealed that chronically inflamed lung tissue can also comprise T follicular helper- (Tfh-) like cells and represents a site of active T cell / B cell cooperation. We assessed the role of pulmonary Tfh- and germinal center-like B cells in pulmonary sarcoidosis. Bronchoalveolar lavage (BAL) fluid, lung tissue, and peripheral blood samples from sarcoidosis patients were analyzed by flow cytometry, immunohistology, RNA sequencing, and *in vitro* T cell / B cell cooperation assays for phenotypic and functional characterization of germinal center-like reactions in inflamed tissue. We identified a novel population of Tfh-like cells characterized by high expression of the B helper molecules CD40L and IL-21 in BAL of sarcoidosis patients. Transcriptome analysis further confirmed a phenotype that was both Tfh-like and tissue-resident. BAL T cells provided potent help for B cells to differentiate into antibody-producing cells. In lung tissue, we observed large peribronchial infiltrates with T and B cells in close contact, and many IgA+ plasma blasts. Most clusters were non-ectopic, i.e., they did not contain follicular dendritic cells. Sarcoidosis patients also showed elevated levels of PD-1high CXCR5- CD40Lhigh ICOShigh Tfh-like cells, but not classical CXCR5+ Tfh cells, in the blood. Active T cell / B cell cooperation and local production of potentially pathogenic antibodies in the inflamed lung represents a novel pathomechanism in sarcoidosis and should be considered from both diagnostic and therapeutic perspectives.

Keywords: Autoimmunity, B lymphocytes, chronic inflammation and fibrosis, follicular helper T cells

P-0693

Investigation of the mechanisms whereby the multiple sclerosis associated genetic variant CD226 Gly307Ser influences T cell functions

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The CD226 Gly307Ser (rs763361) single-nucleotide polymorphism (SNP) has been identified as a risk factor for several inflammatory diseases, including Multiple Sclerosis (MS). CD226 is a transmembrane receptor fundamental for T cell adhesion and activation, but its downstream signaling is still controversial and its integration in the context of autoimmunity mediated by T cells remains to be addressed. The aim of the project was to gain further insight on role of CD226 in T cells and the effect of the amino acid change Gly307Ser. Peripheral blood mononuclear cell (PBMCs) from 32 age- and sex-matched donors were genotyped for the rs763361 polymorphism and were analyzed by flow cytometry and mass cytometry for the distribution of different CD4 and CD8 T cell compartments (naive, effector, effector memory, central memory) and the expression of T cell-related markers. CD8 T cells were purified and stimulated *in vitro* with anti-CD3 and anti-CD28 mAbs and assessed by BCA and flow cytometry for survival, proliferation and cytokine production. In addition, signaling proteins were analyzed using phosphoflow. Analysis through flow and mass cytometry highlighted no major differences in the phenotype of PBMC between the two variants, although CD8 T cells isolated from donors carrying the risk allele displayed increased IFNγ production. Understanding the impact of this MS-associated genetic variant will provide essential pathophysiological knowledge for driving selection of novel therapies.

Keywords: Autoimmunity, cell signalling, inflammatory molecules, molecular immunology, multiple sclerosis, omics technologies

P-0694

Treatment by *Lactobacillus reuteri* influences the severity of chemicaly-induced colitis

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Inflammatory bowel diseases (IBDs) are associated with significant alterations in composition of commensal microbiota. The main characteristics of microbiota in IBDs is low abundance of beneficial bacteria belonging to *Lactobacillus sp.* and *Bifidobacterium sp.* The aims of our study were to explore whether daily treatment with *Lactobacillus reuteri* (LR; a strain isolated from the feces of C57BL/6 mice having confirmed probiotic characteristics) could alleviate severity of an experimental, chemically-induced, colitis, and to evaluate its impact on local immune response at the peak of disease. Experimental colitis was induced in outbred Intor Swiss:Albino mice by a single intrarectal administration of 2,4,6-trinitrobenzene sulfonic acid dissolved in 50% ethanol (day 0). LR/PBS ($5x10^6$ CFU/ml, p.o.) was given daily, 7 days prior and/or 7 days after day 0 (n=10 per treatment). Mice subjected to the induction of colitis without LR treatment were referent. Colon samples were collected on day 3 (peak of disease) for histological analyses (HE staining), evaluation of superoxide ions, IL-6 and TNFα productions, and MPO activity. A significant reduction in disease severity was noticed only in group treated by LR prior and upon colitis induction. The alleviation of disease severity correlated with lessening of local infiltration of leukocytes, a decrease in local inflammatory response (MPO activity, production of superoxide ions, IL-6, and TNFα), and an increase in local production of IL-10. Presented results show that LR triggers a regulatory mechanism which alleviates severity of experimental colitis, implying that it is worth further evaluation in prevention and treatment of IBDs.

Keywords: Animal models, cytokines and mediators, immune response tracing, protection

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POSTER PRESENTATIONS

P-0695

Comparative analysis and characterization of TCR repertoires in breast cancer TILs

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The characterization of T cell receptors (TCR) expressed by tumor-infiltrating lymphocytes (TIL) is gaining interest since TCR can be used as a tool of immunotherapy but also as a biomarker. Thus, it is necessary to understand the TCR features and their differences in the CD4+ and CD8+ infiltrating subsets. Biopsy fragments from 7 breast cancer patients were cultured as explants to analyze the TCR in the initial biopsy-derived T cells. Later on, expanded cells were sorted by their CD4 and CD8 expression. TCR HTS was performed to analyze the clonality, the sequence overlapping and different properties of the TRA and TRB CDR3, as well as to find motifs in the TRB sequences. Significant differences in the TRB CDR3 properties between CD4+ and CD8+ sets were found. Although the diversity was similar in both, a higher number of motifs shared between biopsies was observed in the CD4+ set. In most of these, an identical CDR3 sequence was found in several samples without a significant HLA relationship, suggesting a higher presence of public TCR in the CD4+ set. We observed a low sequence overlapping in the different fractions of the biopsies, indicating a certain tissular T cell compartmentalization. In overall, this data indicates that there are intrinsic characteristics in the TCR of TIL in each subset independently of the biopsy. However, the CDR3 sequences of the CD4+ cells show a higher similarity between biopsies than that of the CD8+ cells, suggesting that these are more restricted in the tumor environment.

Keywords: Adaptive immunity, cancer immunology, molecular immunology, monitoring immunity

P-0696

Skin infection induced eosinophil mobilization and its effect on subsequent allergic airway sensitization

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Infections are known risk factors for allergy development. Human studies indicate a correlation of *Staphylococcus aureus* (SA)–specific IgE with asthma severity. While reports demonstrate that bacterial infections drive type 2 immune responses at mucosal sites, detailed insight into the consequences of SA infection for asthma development is lacking. Given the frequent SA exposure of the skin, we hypothesized that SA epicutaneous infection transforms the skin into a type 2 immune environment that can drive sensitization against environmental antigens. We speculated that such atopic priming might mediate an increased systemic sensitivity state that potentiates allergy development in remote mucosal organs, such as the lung. To explore this idea, we used a mouse model combining SA infection and allergen skin sensitization, followed by intranasal allergen challenge. Our experiments showed that skin infection triggers a profound type 2 immune response, characterized by eosinophil tissue infiltration. Coupled with allergen exposure, it altered the course of allergic airway sensitization, resulting in enhanced type 2 antibody levels. This treatment shifted the lung cell composition upon allergen challenge, reflected by early recruitment of eosinophils into the lung. RNA-seq analysis of eosinophils revealed a distinct transcriptional profile compared to non-infected groups. Searching for a potential mechanism, we found that SA exposure induced expansion of the bone marrow eosinophil pool and increased expression of eosinophil chemoattractants upon airway challenge. We hope that our study will decrypt the mechanisms orchestrating systemic atopic immune responses upon local dermal priming, therefore opening new therapeutic avenues to treat asthma and bacterial skin infections.

Keywords: Allergen-induced immune responses, allergic disorders, bacterial infections, eosinophils, innate host defence, skin diseases

P-0697

Enhanced susceptibility of galectin-1 deficient mice to experimental colitis

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Galectin-1, a member of carbohydrate-binding proteins family with affinity for β-galactosides, is ubiquitously expressed in different tissues and immune cells. Several studies have demonstrated that administration of recombinant galectin-1 suppressed experimental colitis by modulating adaptive immune responses altering the fate and phenotype of effector T cells. However, the role of endogenous galectin-1 in intestinal inflammation is poorly defined. To this aim, experimental colitis induced by administration of dextran sulfate sodium (DSS) in drinking water was used to evaluate the inflammatory response of *Lgals1*$^{-/-}$ mice with respect to *wild type* (WT) mice. Colitic *Lgals1*$^{-/-}$ mice showed enhanced susceptibility to experimental colitis with respect to WT mice as showed by their increased in body weight losses and higher disease activity indexes (DAI). The mechanisms underlying the enhanced inflammatory response in colitic *Lgals1*$^{-/-}$ mice involved an altered Th17/Th1 profile of effector CD4$^+$ T cells. Strikingly, our results showed that in the absence of endogenous galectin-1 enhanced frequencies of T-Bet-expressing Foxp3$^+$CD4$^+$ T cells were observed in cLP in colitic *Lgals1*$^{-/-}$ mice. In addition, adoptive transfer of wild-type Foxp3$^+$CD4$^+$ regulatory T cells into *Lgals1*$^{-/-}$ mice during DSS-induced colitis reduces the severity of the disease, achieving a similar degree of intestinal inflammation as colitic WT mice. Altogether, these findings contribute to delineate the mechanisms underlying the immunosuppressive role of endogenous galectin-1 during intestinal inflammation.

Keywords: Animal models, autoimmunity, immune regulation and therapy, inflammatory bowel disease, regulatory cells

P-0698

Successful kidney allograft with repeatedly positive crossmatch result due to the presence of IgM autoantibodies

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The complement dependent lymphocytotoxicity (CDC) crossmatch is the standardized assay protocolized before transplantation, although the virtual crossmatch (VXM) have showed more accuracy, being able to predict CDC crossmatch result. The aim of this study was to elucidate the underlying cause of repeatedly positive CDC crossmatch result in a patient without anti-HLA antibodies and negative VXM. Anti-HLA antibodies were detected by Single Antigen Bead (SAB) in serum samples. The determination of anti-IgM and anti-IgG autoantibodies was achieved by extractable nuclear antigen (ENA) panel. CDC crossmatch was performed with neat and 5mM DTT (dithiothreitol) pre-treated serum. A 75-year-old woman with extracapillary glomerulonephritis lost kidney allograft after 19 years because of primary disease relapse. The immunosuppressant levels were at minimum dose (tacrolimus 0.5mg/24h + prednisone 5mg/24h) and anti-HLA antibodies were periodically monitored, which were always negative. At three different allocations with no DTT treated serum samples, the results of the CDC crossmatches were positive (40-60% of cell death), despite negatives VXM. Although no autoimmunity disease was diagnosed, an auto-crossmatch was performed given the suspicion of the presence of autoantibodies, with a positive result. The anti-ENA assay was positive for the presence of IgM antibodies against histones and nucleosomes. Eventually, the patient was successfully transplanted with positive neat-serum and negative DTT pre-treated serum CDC crossmatches and currently maintain a good allograft function (1.15 mg/mL serum creatinine). The presence of IgM autoantibodies against nuclear proteins can cause a positive CDC crossmatch, but they do not badly impact on allograft outcome.

Keywords: Antibody, autoimmunity, immunological techniques, monitoring immunity, transplantation

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POSTER PRESENTATIONS

P-0699

Prospective evaluation of immunological biomarkers during immunotherapy with honeybee venom

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Honeybee-venom allergy is a life-threatening disease, being immunotherapy (IT) the only curative available treatment. The aim of this study was to analyze the dynamics on immunological biomarkers underlying tolerance achieved by IT. A total of 20 allergic patients (AP) to *Apis mellifera* venom (AmV) eligible for IT were included. The basophil degranulation against AmV (Pharmalgen, ALK-Abelló) at 0,1 and 1 µg/mL, the amount of IL-4 and IL-10 (pg/mL), the level of specific-IgE (sIgE; UI/mL) and IgG4 (mg/L; sIgG4) to AmV, rApi m 1, rApi m 2, rApi m 3, Api m 4, rApi m 5 and rApi m 10 and the indoleamine 2,3-dioxygenase activity were prospectively studied throughout the build-up phase of IT and one year after its start. Honeybee sting challenge (SC) was performed to check the achievement of tolerance. sIgE to AmV, rApi m 1, rApi m3 and Api m 4 and sIgG4 to AmV and all its components were significantly higher when reaching the maximum dose at the build-up phase and decreased one year after starting IT. IL-10 was also significantly increased at the end of the build-up phase (p=0.0064), whereas IL-4 reached it maximum at the moment of SC (p<0.0001), like that of kynurenine (p<0.0001). In parallel, basophil degranulation progressively decreased, particularly when using 1 µg/mL of AmV (p=0.0002). All AP tolerated SC. The desensitization of basophils secondary to the increase of sIgG4, IL-10 and IDO activity could be one of the mainstays of tolerance to honeybee-venom achieved by IT.

Keywords: Allergen-induced immune responses, basophils, biomarkers, immunotherapy

P-0700

Synergistic effect of therapeutic combination of Sunitinib with a peptide based vaccine in cancer treatment after tumor microenvironment remodeling

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Immunotherapies and targeted treatments in cancer have shown remarkable advances in the past decades. Nevertheless, resistance and toxicity occur and combination approaches are emerging in the therapeutic arsenal to overcome these issues. We have previously demonstrated the therapeutic efficacy of a cancer vaccine (SVX) composed of long synthetic peptides containing CD8 and CD4 epitopes of the survivin protein, an inhibitor of apoptosis overexpressed in many cancers. To address the challenges of poor therapeutic efficacy of vaccines as monotherapy in advanced cancers, we are studying its optimization in combination with the antiangiogenic treatment Sunitinib, a tyrosine kinase inhibitor used in the treatment of mRCC. In this study, we have tested different combination approaches between Sunitinib and the therapeutic vaccine in CT26 tumor-bearing mice and have shown that associating SVX vaccine after two weeks of Sunitinib treatment results in therapeutic synergy between these two therapies compared to other scheduling approaches. In order to understand the microenvironment remodeling involved in this combination strategy, we studied the impact of each of these therapies alone on the vascular and immune microenvironment. We have highlighted different changes in the tumor microenvironment after Sunitinib treatment that could be involved in the permissiveness to the therapeutic vaccine in this timing as well as an impact of SVX therapeutic vaccine on tumor angiogenesis. These observations seem to explain the synergy observed in this precise timing and could be important parameters to study in a clinical setting.

Keywords: Anti-cancer vaccine, cancer immunology, drugs for immune modulation, immunotherapy, microenvironment

P-0701

Interaction between microbiome and immune system in allergy to lipid transfer proteins

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A significant proportion of patients allergic to Ole e 7 are co-sensitized to Pru p 3, non-specific-lipid-transfer proteins (nsLTPs) in olive-tree and peach, respectively. The aim of this study was to know the possible involvement of microbiome and immune system interaction in developing concomitant allergy to both nsLTPs. Oral and intestinal microbiome profile of 110 patients sensitized to Ole e 7, with or without sensitization to Pru p 3 (by specific-IgE >0.35 UI/mL) was explored, together with a thorough peripheral blood T-helper and T-regulatory phenotype, the cytokine production (IL-4 and IL-10) and the expression pattern of TLR pathway in oral mucosa. The relative abundance of oral and intestinal microbiome of bisensitized patients differed from that of monosensitized patients, with a predominant representation of Actinobacteria phylum and Gemellales order in oral mucosa and Bacteroidaceae family and Veillonella genre in intestinal mucosa. The proportion of CD39+ T-regulatory cells was significantly higher in Ole e 7-monosensitized patients (30.3 [19.0;37.2] vs. 24.4 [12.7;32.7]; p=0.017). Additionally, the T-regulatory proliferation ability through the expression of Ki67 was significantly higher in the monosensitized group (2.75 [1.50;4.65] vs. 1.95 [0.67;3.02]; p=0.023). Conversely, bisensitized patients had a higher proportion of CD4+Th2 cells when priming with Pru p 3 (0.90 [0.50;1.20] vs. 0.60 [0.40;0.98]; p = 0.051). In oral mucosa of these patients, TLR3 and TLR5 were significantly downregulated (p=0.03 and p=0.005, respectively). Microbiome and its interaction with the immune system could be a determinant factor in the development of concomitant allergy to nsLTPs.

Keywords: Allergen-induced immune responses, biomarkers, microbiome and environmental factors, regulatory cells

P-0703

Viral infection of the ovaries compromises pregnancy and reveals innate immune mechanisms protecting fertility

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Viral infections during pregnancy are a considerable cause of adverse outcomes and birth defects, while the underlying mechanisms are poorly understood. Among those, cytomegalovirus (CMV) infection stands out as the most common intrauterine infection in humans, putatively causing early pregnancy loss. We employed murine CMV (MCMV) as a model to study the impact of virus infection of the ovaries on pregnancy outcome and fertility maintenance. MCMV infection of different ovarian structures (follicles and corpus luteum - CL) was analysed by immunohistochemistry using antibody against MCMV IE1 protein, and virus titer was determined using standard plaque assay. Influx, phenotypic and functional characterization of various immune cells was analysed using multiparametric flow cytometry while the roles of individual cell types and cytokines in the immune response to CMV infection was analysed using a panel of mouse knock-out mutants and depletion with monoclonal antibodies. Cytokine secretion was analysed using cytometric bead array, qPCR and ELISA. Our data revealed highly selective MCMV infection in the ovaries, with the strong infection of CL and no signs of infection in the follicles. High CL infection resulted in progesterone insufficiency and pregnancy-loss. While corpora lutea infection might lead to miscarriage, infection of follicles may promote sterility. Yet, abundance of gap-junctions, absence of vasculature, strong type I interferon (IFN) responses and interaction of innate immune cells fully protect the ovarian follicles from viral infection. Our work provides fundamental insights into the impact of CMV viral infection on pregnancy loss and mechanisms protecting fertility.

Keywords: Innate immunity, reproductive immunology, viral infections

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POSTER PRESENTATIONS

P-0706

NK17 and NKreg responses in patients with active Behçet's uveitis

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Uveitis is the most common cause of morbidity in Behçet's disease (BD). The aim of this study was to characterize structural and functional changes in natural killer (NK) cells during active uveitis in BD compared to diseased (Ankylosing spondylitis, AS) and healthy controls (HC). The study included subjects with active BD uveitis (n=9), active AS (n=9) and HC (n=7). Peripheral bloods samples were taken from the patients during the active phase of the disease. NK cell subsets, KIR expressions (NKG2A, NKG2D, NKp46), cytotoxic activity (CD107a, perforin, granzyme A) and cytokine secretions (IL-4, IFN-γ, TNF-α, IL-10, IL-17, TGF-β) were analyzed by flow cytometry. There were no significant differences for the distribution of cytotoxic (CD56-/dimCD16br) and cytokine secreting (CD56br/dimCD16-) NK subsets between the groups. However, NKG2A expression was significantly higher in BD patients compared to AS and HC. NKG2D expression of BD patients was also significantly higher than HC and less than AS. IL-17 and TGF-β expressions were significantly higher in BD patients compared to HC and AS. A similar trend was observed in IL-10 expression and it was statistically significant in $CD8^+$ NK cell subset. CD107a and granzyme A expression of cytotoxic NK cells was significantly higher in BD patients compared to HC. Patients with active BD uveitis had increased expression of both activatory/inhibitory KIRs and inflammatory/regulatory cytokines. NK cell response in active BD uveitis was comparable to Th17/Treg axis of T helper cells. Interestingly, both arms of the NK17/NKreg axis were active during active uveitis.

Keywords: Autoimmunity, cytokines and mediators, inflammatory joint diseases, innate immunity, NK cells

P-0707

CLU rs11136000 T allele is associated with a reduced risk of late-onset Alzheimer's disease in APOE ε4 non-carriers

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The rs11136000:C>T polymorphism in the clusterin (CLU) gene has been identified as an important genetic risk modifier for late-onset Alzheimer's disease (LOAD). Clusterin is a multifunctional glycoprotein supposed to influence neurotoxic effects of amyloid beta deposits in the brain. In this study we aimed to validate the association between rs11136000 and LOAD in Slovaks and evaluate whether it is affected by the carrier status of the major LOAD risk allele apolipoprotein (APOE) ε4. CLU rs11136000 and APOE variants were genotyped in 292 LOAD patients and age-matched 489 controls using the PCR-RFLP method and direct sequencing. Association of rs11136000 with LOAD was evaluated by logistic regression analysis. Statistical analysis revealed a significant association of rs11136000 T allele with a reduced LOAD risk, which was observed only in subjects without APOE ε4 (log-additive model: P = 0.0063; OR = 0.66; 95% CI = 0.49–0.89) but not in APOE ε4 allele carriers (P = 0.65; OR = 0.91; 95% CI = 0.61–1.36). Moreover, interaction analysis was indicative of an antagonistic effect between the risk CLU rs111136000 CC genotype and APOE ε4 allele in LOAD risk (P = 0.052; synergy factor = 0.52). In conclusion, our results suggest that CLU rs11136000 T allele carriers have a decreased risk for late-onset Alzheimer's disease which is only evident in the absence of the major LOAD genetic risk factor APOE ε4. Interpreting the functional implication of CLU variants remains challenging given the plethora of its biological functions.

Keywords: Biomarkers, MHC and polymorphic genes, neuroimmunology

P-0709

Different migratory properties of CD4+CD28null and CD4+CD28+ T-cells in rheumatoid arthritis patients

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CD4+CD28null are highly differentiated cells with potent inflammatory activity. This T-cell subset is expanded in tissues and peripheral blood of patients with different autoimmune diseases such as rheumatoid arthritis (RA) being able to induce target tissue destruction. This work aims to study the migration ability of CD4+CD28null T-cells in RA patients as well as the effect that IL-15 could have in these processes. We analyzed the migration capacity, in transwell assays, and the transcriptional motility profile of both T-cell subsets with and without IL-15 stimulation. CD4+CD28null T-cells showed higher migratory capacity than CD4+CD28+ T-cells in transwell assays and this ability was enhanced by IL-15. The transcriptional analysis revealed that several genes have a significantly higher level expression in CD4+CD28null with respect to CD4+CD28+ T-cells. Among them, there are important genes involved in human cell migration pathways as *MMP9*, *SRC* and *PLAUR* or *PAK1*, *MYL9/MLC*, *VEGFA*, *BAIAP2*, *TIMP2*,*IGF1* and *HGF* with expression differences higher than 10 and 5 times, respectively. Oppositely, *PRKCA* and *DPP4* were downregulated in CD4+CD28null T-cells. However, there were almost no differences at gene expression level of the studied genes between basal conditions and IL-15 stimulation in neither of the two T cell subtypes studied. This study determined that the high migration capacity of CD4+CD28null is being importantly controlled at gene expression level, implicating several genes that are central in signalling pathways related to cell migration. These genes could be investigated as possible therapeutic targets for rheumatoid arthritis diseases.

Keywords: Cytokines and mediators, cytoskeleton, rheumatoid arthritis

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POSTER PRESENTATIONS

P-0711

Establishment of a mouse model of systemic autoimmunity to study the role of autoimmunity in post-myocardial infarction cardiac electrophysiological remodelling

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Systemic autoimmunity leads to accumulation of auto-antibodies resulting in long-term immune-mediated damage. The effect of autoimmunity on myocardial infarction (MI) and the post-MI remodelling process is unknown. We aimed to develop a model to investigate the effects of autoimmunity on electrophysiological remodelling post-MI. Induction of systemic autoimmunity using Resiquimod (R848) is an appropriate model for investigation of post-MI immune-mediated electrophysiological remodelling. 10-week old female BALB/c mice were repeatedly dosed with TLR-7 agonist R848. Induction of systemic autoimmunity was confirmed with anti-dsDNA auto-antibody ELISA. Autoimmune-mediated cardiac damage was investigated by anti-myosin auto-antibody ELISA and histological scoring of damage parameters. The electrophysiological phenotype associated with this model was investigated by ECG analysis and arrhythmia susceptibility tests. Systemic autoimmunity was confirmed by elevated anti-dsDNA auto-antibodies 4 weeks after treatment (control = 2.97 ± 1.36ng/ml, R848 = 116.5 ± 9.6ng/ml, $p < 0.0001$, mean±SEM). Increased anti-myosin auto-antibodies indicated cardiac specific damage (control = 0.12±0.03, R848 = 0.51±0.04, p=0.0002, mean±SEM). Histological scoring of myocardial infiltration revealed an R848-mediated increase (control = 0.17±0.16, R848 = 1.14±0.24, p=0.036). R848 treatment did not induce changes in heart rate, QRS duration, or QTc interval. Additionally, arrhythmia susceptibility tests did not show significant propensity for arrhythmia in R848 treated hearts. An inducible model of systemic autoimmunity without prior propensity for arrhythmia was investigated. BALB/c mice developed anti-dsDNA and anti-cardiac auto-antibodies indicating a systemic autoimmune phenotype with immune-mediated cardiac damage. No electrophysiological changes were recorded, indicating a suitable model for studying post-MI immune mediated electrophysiological remodelling.

Keywords: Antibody, animal models, autoimmunity, cardiovascular diseases

P-0712

Relationship of exosome profile with acute and chronic inflammation in brucellosis

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Brucellosis is a worldwide zoonotic infection caused by a type of bacteria from the genus Brucella. Exosomes are small lipid vesicles derived from multi-vesicular bodies released by many cell types. The exosomal cell cargo reflects the type of cell from which it originated. In this study, we aimed to contribute to the literature on the potential roles of exosomes in brucellosis immunopathogenesis. Our study included 10 acute and 10 chronic patients diagnosed with brucellosis and 10 healthy controls. Exosomes from serum samples were extracted using a commercial isolation kit. In the pre-designed panel, after staining with monoclonal antibodies (mAbs), the exosomes were immunophenotyped using Flow Cytometry. Confirmation of the presence of exosome was performed by staining of exosome-specific mAbs CD9, CD63 and CD81 and electron microscopy (SEM) evaluations. According to the results of the evaluation, granulocyte and G-MDSC (granulocyte-like myeloid derived suppressor cells) derived exosomes (%) were significantly higher in the acute group compared to the chronic and healthy groups, On the other hand, a significant difference was observed between the chronic group and the healthy control in exosome values(%) originating from G-MDSC. Taken together, we found that granulocyte and G-MDSC-derived exosomes may play a role in the acute phase responses of brucellosis. The significant differences between granulocyte and G-MDSC-derived exosomes at different phases of brucellosis may contribute to the immunopathology of the disease with new studies.

Keywords: Bacterial infections, endo- and exocytic vesicles in immunity, granulocytes, inflammatory disease

P-0713

A detailed analysis of regulatory T cell subpopulations in patients with autoimmune diseases

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Regulatory T cells (Tregs) are essential cells in peripheral immune tolerance. Their suppressor roles are pivotal in the development of autoimmune and allergic diseases. Reduced numbers of circulating Treg cells are associated with autoimmune diseases, such as systemic lupus erythematosus, autoimmune liver disease, juvenile idiopathic arthritis, and diabetes mellitus. In this study, we aimed to analyze CD4+ CD127lo- CD25hi- FOXP3+, CD4+ CD25+ CD127lo, CD4+ CD25+ FOXP3+ sub-populations of Tregs as well as CD127 expression in 30 patients with several autoimmune diseases and 19 healthy individuals. The autoimmune disorders in patients included dermatological (26%), hematological (22%), endocrinologic (21%), rheumatological (13%), gastrointestinal (9 %), neurological (9%) diseases. Four patients (13.3%) had multiple autoimmunities. Four patients (1.3%) had autoimmunity in addition to allergic manifestations. We found a statistically significant decrease in CD4+CD127lo-CD25hi-FOXP3+ Treg cells between patients and healthy controls (p=0,04). There wasn't any statistically significant change in CD4+ CD25+ CD127lo, CD4+CD25+FOXP3+ Treg sub-populations and CD127 expressions between the patients and the healthy controls. One of the patients had no FOXP3 expression, but he had a similar CD4+ CD25+ CD127lo Treg ratio compared to the healthy controls. This patient had a missense mutation in the *FOXP3* gene, and he had a severely decreased ratio of three Treg sub-populations. The analysis of different Treg subsets in distinct autoimmune disorders and syndromes aid molecular diagnosis of Tregopathies, such as IPEX and IPEX-like diseases (LRBA, CTLA-4 deficiencies). It also helps during the follow-up of the immunoregulatory treatment response of the patients.

Keywords: Adaptive immunity, autoimmunity, immunodeficiency, regulatory cells, visualizing immune responses

P-0714

Neuromyelitis optica spectrum disorders: a challenging diagnosis. case report

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We present a 26-year-old male who was admitted to hospital with an acute attack of headache, monocular vision loss, ophtalmoparesis and peripheral facial nerve paralysis. The analysis of the cerebrospinal fluid (CSF) showed lymphocytic pleocytosis and the serological study did not reveal any active infection. Evoked potential confirmed the lack of response in the right eye and injury in the centrum semiovale and corpus callosum were evidenced in the magnetic resonance imaging. Mitochondrial mutations related to Leber syndrome were discarded. The results of the autoimmunity studies, including antinuclear antibodies and antibodies against extractable nuclear antigens, anti-cardiolipin,anti-β2-glycoprotein, anti-neutrophil cytoplasmic antibodies (anti-myeloperoxidase and proteinase 3), anti-thyroglobulin, anti-thyroid peroxidase, anti-N-methyl-d-aspartate,anti-myelin oligodendrocyte glycoprotein (MOG) and anti-aquaporin-4 (AQP4), were negative in plasma and CSF. This study was extended by searching for antibodies against cornea, retina, choroid coat, recoverin and other antibodies related to paraneoplastic syndrome (CV2, PNMA2, Hu-ANNA1, Ri-ANNA2, Yo-PCA1, SOX1, titin, Zic4, GAD65 y DNER), but only a non-relevant anti-retina antibody was detected at a low titer. The clinical features and the evidence provided by diagnostic testing raised the likelihood of a neuromyelitis optica spectrum disorder. This entity is an inflammatory disorder usually involving the optical nerve and other structures of the central nervous system. Despite the correlation between this pathological condition and the presence of anti-AQP4 or anti-MOG autoantibodies, a seronegative onset is possible, which could imply a challenging diagnosis and a delayed treatment. An in-depth laboratory study and an appropriate differential diagnosis are essential for these patients.

Keywords: Biomarkers, autoimmunity, neuroimmunology

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POSTER PRESENTATIONS

P-0716

The age-dependent role of Th22, Tc22, and Tc17 cells in the severity of pneumonia in COVID-19 immunopathogenesis

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Lymphocytes are divided into different subtypes based on their cytokine production pattern. In this study, we investigated the role of cytokine expressions of CD4+ T helper, CD8+ T cell subsets as well as CD4-CD8- T cells in the pathogenesis of COVID-19. Peripheral blood mononuclear cells (PBMCs) were extracted by density gradient centrifugation from blood samples of 180 COVID-19 patients (children and adults) and 30 healthy controls. PBMCs were stimulated with PMA and ionomycin and treated with Brefeldin A in the 4 th hour, and a 10-colored Mo Ab panel was evaluated at the end of the 6th hour using flow cytometry. The numbers of Th22, Tc22 and IL-22 expressed DN-T cells increased in adult patients regardless of the level of pneumonia (mild, severe or asymptomatic) as compared to healthy controls. The number of Tc17 cells increased in mild and severe cases compared to the healthy controls. Both IL-22 and IL-17A production decreased after recovery. Our findings suggest that the increase in only IL-22 expressed Tc22 cells in the 0-12 age group who has a general symptom-free course and the presence of higher levels of Th22 and Tc22 in asymptomatic cases may indicate the protective effect of IL-22. The positive correlation between the severity of pneumonia and the elevation of Tc17 cells in adults may reveal the damaging effect of IL-22 when it is co-expressed with IL-17.

This work was supported by a grant from the BAP of the Bursa Uludag University of Turkey [Project no: OUAP(T)-2020/6].

Keywords: Adaptive immunity, biomarkers, cytokines and mediators, viral infections

P-0717

Association of cytotoxic T lymphocyte subsets with disease severity in COVID-19

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Lymphocytes and their subsets play a major role in the protection of immune system functions. It has been reported that notable differences in lymphocyte subsets are observed in immune system-related or infectious diseases. In our study, we aimed to investigate the role of CD8+ cytotoxic T lymphocyte subsets in COVID-19 infection and disease severity. 193 COVID-19 patients (juvenile and adults) and 31 volunteers as healthy controls were included in our study. Flow Cytometry analysis was performed with a 10-color monoclonal antibody panel from peripheral blood samples. In adult patients,

- Effector memory-1 (EM1) T cells (CD8+CD45RA-CCR7-CD27+CD28+) were found to be decreased in severe cases compared to asymptomatic, mild cases and healthy controls.

- Effector Memory-3 (EM3) T cells (CD8+CD45RA-CCR7-CD27+CD28-) were found to be increased in severe cases compared to asymptomatic and mild cases.

- Double-negative T cells (CD4-CD8-) were found to be decreased in all disease groups compared to healthy controls.

In juvenile patients,

-Effector memory (EM) CD8+ T cells (CD45RA-CCR7-) were higher in the 0-12 age range compared to the healthy controls.

In severe cases, the decrease of EM1 cells that produce low levels of effector mediators such as granzyme-B and perforin and the increase of EM3 cells which have effector properties and stronger cytolytic activity, suggesting that these cells play an important role in the immune response to the virüs.

This work was supported by a grant from the Scientific Research Projects Foundation (BAP) of the Bursa Uludag University of Turkey [Project no:OUAP(T)-2020/6].

Keywords: Adaptive immunity, cytokines and mediators, infectious disease, viral infections

P-0719

Evaluation of the roles of regulatory B (Breg) cells and B cell exhaustion in COVID-19

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Breg cells are immunosuppressive cells that support immune tolerance and suppress pathological immune responses. We aimed to investigate the role of Breg cells and B cell exhaustion in COVID-19. 102 COVID-19 patients (juvenile and adults) and 25 volunteers as healthy controls were included in our study. Flow Cytometry analysis was performed with a 10-color monoclonal antibody panel from peripheral blood samples. CD24highCD38high transitional B cell subset and CD24- CD38high plasmablast cells were found to increase in disease period and decrease in the recovery period in juvenile cases. In adults, transitional B cells increased in asymptomatic and mild cases compared to healthy controls and decreased in severe cases. The severity of the disease positively correlated with the percentages of the plasmablasts in adults. IL-10-expressing CD24high CD27+ B cells and CD21 + CD27 + high-proliferative memory B cells were found to be decreased in mild and severe cases compared to the healthy controls. CD24highCD38- memory-B cells were found to be lower in juvenile and adult patients compared to healthy controls and increased in the recovery period. It has been found that in both adult and juvenile patients, B-cell exhaustion increases with the disease and decreases after the recovery period. The results obtained may be instructive in understanding the mechanisms of Breg cells, B cell exhaustion by SARS-CoV-2, modulating their immunosuppressive activity, and increasing their immunoprotective effects on the host.

This work was supported by a grant from BAP of the Bursa Uludag University of Turkey [Project no: OUAP(T)-2020/6].

Keywords: Adaptive immunity, B lymphocytes, biomarkers, viral infections

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POSTER PRESENTATIONS

P-0720

Characterization of the immunological state of hospitalized patients with SARS-CoV-2 infection

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SARS-CoV-2 is being responsible for millions of contagions and deaths worldwide. The aim of this project was to explore possible immunological variables in SARS-CoV-2 infected patients which could be involved in their outcome. A total of 79 patients, 46 (58.2%) men and 33 (41.8%) women, with SARS-CoV-2 infection confirmed by PCR and requiring hospitalization were included. Serum inflammatory cytokines (IL-1β, IL-6, IL-8, IL-10, IL-12, IFN-γ, TNF-α, MCP-1 and IP-10), T-, B- and NK-cell subpopulations and acute phase reactants (c-reactive protein -CRP-, lactate dehydrogenase -LDH-, D-dimer and ferritin) were analyzed at time of admission. Patients were graded according to the need of Intensive Care Unit (ICU) care at any point throughout their hospital time. Among the 79 included patients, 35 (44.3%) were admitted to ICU and comprised ICU-group, while 44 (55.7%) comprised non-ICU group. The age was significantly higher in the ICU group (61.7±10.5 vs. 52.4±13.1; p=0,001). Similarly, ICU-patients showed significantly higher circulating levels of IL-6 (p=0.001) and MCP-1 (p=0.009) cytokines. Regarding lymphocyte populations, peripheral effector T-cells (CD4+ and CD8+) and CD16+CD56+ NK-cells also appeared significantly increased in ICU-patients (p<0.001, p=0.004, p=0.003), whereas transitional B-cells and central memory T-cells were significantly decreased (p=0.001 and p<0.05). The immunological state of SARS-CoV-2 infected patients at time of admission could identify those who would evolve towards a more severe form of the disease. This characterization would permit to implement early preventive therapies. Deeper studies are needed to evaluate the potential predictive value of the candidate biomarkers here described.

Keywords: Adaptive immunity, cytokines and mediators, innate host defence, innate immunity, monitoring immunity, viral infections

P-0722

Baseline iron status and presence of anemia determine the course of systemic Salmonella infection following oral iron supplementation in mice

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Iron deficiency anemia (IDA) is a major health concern. However, preventive and unbiased iron supplementation in regions with high burden of infectious diseases resulted in an increase of infection related morbidity and mortality. We fed C57BL/6N mice either an iron deficient or an iron adequate diet. Next, they received oral iron supplementation or placebo followed by intraperitoneal infection with Salmonella Typhimurium (S.Tm). We found that mice with IDA had a poorer outcome than mice on an iron adequate diet. Interestingly, iron supplementation of IDA mice resulted in higher bacterial burden in organs and shortened survival. Increased transferrin saturation and non-transferrin bound iron in the circulation together with low expression of ferroportin facilitated the access of the pathogen to iron and promoted bacterial growth. Anemia, independently of iron supplementation, went in hand with reduced neutrophil counts and cytotoxic T cells and increased splenic levels of the cytokine IL-10, which further negatively impacted on the control of S.Tm infection. Supplementing iron to anemic individuals worsens the clinical course of bacterial infections. This can be traced back to increased iron delivery to bacteria along with an impaired anti-microbial immune response. Our findings may have important implications for iron supplementation strategies in areas with high endemic burden of infections putting those individuals, who potentially profit most from iron supplementation for anemia, at the highest risk for infections.

Keywords: Bacterial infections, infectious disease, macrophage, neutrophils, nutrients

P-0724

Primary deletion immunodeficiency in homogogosis c.6155delA in the *LRBA* gene

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We present a 45-year-old male patient with multiple respiratory infections and chronic diarrhea since childhood. Family history of interest: brother with common variable immunodeficiency (CVID) died at 30 years of age and sister died in childhood from an unrelated cause. In 1984 he presented with immune thrombotic purpura (ITP), requiring splenectomy and monthly treatment with intravenous/subcutaneous immunoglobulin. The patient suffered from recurrent infections and in 1988 was diagnosed with CVID. He is subsequently diagnosed with chronic eczema in 1989, inflammatory enteropathy in 1991, pernicious anemia in 2004, and acute Campylobacter coli gastroenteritis in 2017 Following a genetic and functional study,a total of 323 genes related to primary immunodeficiencies were sequenced. Variant c.6155delA (p.Asn2052Thrfs*20) was detected in homozygosityin the LRBA gene, confirmed by Sanger sequencing. This mutation was not previously described in the databases. A functional cytometric study was performed which confirmed the LRBA deficiency. Despite the clinical manifestations compatible with various immunodeficiencies with autoimmunity, the analytical results ruled out ALPS and led to think more in LRBAD or ALPSV. The previously undescribed mutation in the LRBA gene and the confirmation by cytometry of the functional defect of LRBA led to the diagnosis of LRBAD. One of the highlights of this case is the genetic confirmation of a mutation not previously described in the databases and which is perfectly consistent with the diagnosis of LRBA deficiency, which highlights the importance of genetic testing when diagnosing this type of entity.

Keywords: Bacterial infections, immunodeficiency, infectious disease

P-0726

Evaluation of the potential roles of activin-A, activin-B, and follistatin molecules exhibiting immunomodulatory properties in brucellosis

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Brucellosis is a zoonotic disease caused by bacteria called Brucella. The mechanisms underlying the changes seen in the course of brucellosis are not adequately elucidated. It was aimed to reveal the potential roles of activin-A, activin-B, and follistatin molecules in sera taken from patients with acute and chronic brucellosis, healthy controls, and recovered individuals to overcome these deficiencies. Activins are members of the transforming growth factor-1 (TGF-β1) superfamily. Activin-A has been shown to play a role in both pro-inflammatory and anti-inflammatory processes. Follistatin is a protein that inhibits or neutralizes the functions of activins, and in this way, it can regulate the inflammatory process. 40 acute, 36 chronic brucellosis patients, 40 healthy, 8 healed donors were included in our study. In addition, analyzes have been associated with a predisposition to bone joint involvement (osteoarticular). All immunomodulatory molecules were studied by the ELISA method. The values for the activin-A, activin-B, and follistatin molecules in the acute and chronic patient groups were lower than the values in the healthy controls and the recovery group, but statistically significant differences were observed only in the activin-A and activin-B molecules. In our study, we observed that the serum levels of activin-A, activin-B, and follistatin decreased in the chronic and acute patient groups compared to the healthy control and the recovered group. According to this result, it is thought that the expression of these three molecules is suppressed in brucellosis infection, and in case of recovery, the suppression disappears and increases again.

Keywords: Immune response tracing, infectious disease, inflammatory molecules

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POSTER PRESENTATIONS

P-0727

Tolerogenic adjuvant Vitamin D3 synergizes with neutrophils: tipping the balance from Th17 to regulatory T cells

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Vitamin D3 (VD3) is well known for its tolerogenic capacities and is a promising adjuvant in tolerogenic vaccine formulations that target dendritic cells (DCs) in chronic inflammatory disorders e.g. allergic or autoimmune diseases, which are often associated with IL-17 producing T helper 17 (Th17) cells. We previously showed that presence of neutrophils is indispensable for Th17 cell development in humans. Here, we studied the effect of VD3 on DCs driving Candida albicans hyphae-specific Th cell differentiation, in the absence or presence of autologous neutrophils. Th1 (IFN-γ+) cells, Th17 (IL-17+) cells and regulatory T cells (Treg; FoxP3+CD25+CD127low) were measured by flow cytometry. Two hour priming of DCs with VD3 hampered neutrophil-induced Th17 cell formation and reduced neutrophil-independent Th1 cell development from naïve CD4+ T cells. Correspondingly, VD3 priming reduced IL-23 and IL-12 release by DCs. Moreover, VD3 priming significantly induced the polarization of FoxP3+CD25+CD127low cells. Strikingly, presence of neutrophils further enhanced this Treg development, especially when taking both FoxP3+ and IL-10 producing Tregs into account. Possibly correlated to the induction of Tregs, neutrophils increased the expression of tryptophan-metabolizing enzyme indoleamine 2,3-dioxygenase (IDO) in C albicans hyphae stimulated DCs. These data show that upon 2 hour priming of DCs with VD3, neutrophil-facilitated DC-driven Th17 cell polarization is impeded whereas tolerance-inducing Treg development is enhanced by neutrophils. This suggests that neutrophils can significantly influence the balance in generation of pro-inflammatory Th17 cells and tolerogenic Tregs. Development of DC-targeting vaccines for treatment of chronic inflammatory disorders could benefit from this new insight.

Keywords: Adaptive immunity, adjuvants and vaccines, dendritic cells, neutrophils, regulatory cells

P-0728

Potential effects of sCD40L, CD36, IL-23 and arginase-1 molecules on the pathogenesis of brucellosis

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Brucellosis is a systemic infectious disease that can be transmitted from animals to humans, ranging from mild to severe clinical pictures. In our study, we aimed to reveal the roles of sCD40L, CD36, IL-23 and arginase-1 (ARG1) molecules in the pathogenesis of brucellosis. sCD40L binds and activates CD40 on antigen-presenting cells, thereby promoting the secretion of pro-inflammatory cytokines and nitric oxide (NO) synthesis. CD36 promotes phagocytosis and apoptosis, can participate in pro-inflammatory responses. IL-23 is a pro-inflammatory cytokine and contributes to the maintenance and pathogenicity of the Th17 cells. ARG1 mediates down-regulation of NO synthesis and suppression of T cell immune responses. 30 acute, 30 chronic patients diagnosed with brucellosis and 20 healthy controls were included in our study. In addition, analyzes have been associated with a predisposition to bone joint involvement (osteoarticular). Standard ELISA procedures were performed for all molecules evaluated in our study. The values of sCD40L, CD36, IL-23 and ARG1 molecules were found to be lower in the acute and chronic patient groups compared to the healthy control group, but there was no statistically significant difference for ARG1. There was no significant difference between acute and chronic groups. In our study, we found that serum levels of sCD40L, CD36, IL-23 and ARG1 decreased in patient groups compared to healthy controls. According to this result, it can be thought that the expression of these molecules is suppressed in brucellosis infection. We believe that researching suppression mechanisms can contribute to the treatment of the disease.

Keywords: Bacterial infections, effector molecules, immune response tracing, infectious disease

P-0729

Prevalence of HLA-B*57 serotype associated with hypersensitivity reaction in HIV treatment in Turkish population

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Abacavir used in the treatment of HIV infection causes hypersensitivity reactions in some patients. These hypersensitivity reactions have been shown to be associated with the HLA-B*57: 01 allele. This study has the largest cohort investigating the prevalence of HLA-B* 57 in the Turkish population. In our study, 25318 person [14388 women (56.8%) and 10930 men (43.2%)] who applied to Bursa Uludag University Department of Immunology were included. DNA isolations were performed from the peripheral blood samples taken from the person using the commercial QIAamp® DNA Blood Mini Kit. HLA-B * 57 typing was performed using sequence-specific primers (SSP) or using sequence-specific oligonucleotides (SSO). A total of 25318 person were included in the study. 24491 person (96.7%) were found to be negative for HLA-B*57 and 827 person (3.3%) had serotype HLA-B*57. It was observed that 470 of these person were female and 357 of them were male. By evaluating women and men as separate groups, it was seen that the HLA-B*57 positivity rate in each group was 3.3%. As a result of the data obtained, the prevalence of HLA-B*57 in Turkey was found to be 3.3%. However, a hypersensitivity reaction to abacavir is usually observed in individuals carrying HLA-B*57:01. Therefore, the prevalence of HLA-B*57:01 in Turkey will be lower than the 3.3% rate obtained in our study. Our results will contribute to the determination of the cost-effectiveness analysis of the HLA-B*57:01 screening before abacavir-mediated therapy in HIV infection.

Keywords: Immunodeficiency, infectious disease, viral infections

P-0730

Antibody response after SARS-CoV-2 vaccination in a Healthcare workers cohort

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The aim of the study is to analyze the antibody response after receiving the complete vaccination schedule against SARS-CoV-2 in a healthcare workers (HCWs) cohort. Serum samples were obtained from 423 HCWs who received a complete vaccination schedule. In addition, 129 serum samples from HCWs infected by SARS-CoV-2 during March-May 2020 were analyzed. Antibody response to spike protein was measured using the Elecsys® Anti-SARS-CoV-2 S test while antibody response to nucleocapsid protein was measured using the Elecsys® Anti-SARS-CoV-2 IgM/IgA /IgG test (Roche Diagnostics International Ltd, Rotkreuz, Switzerland). HCWs were classified into 4 groups: Group 1, HCWs infected by SARS-CoV-2 not vaccinated; Group 2, HCWs infected by SARS-CoV-2 and vaccinated who did not lose the antibody response against nucleocapsid; Group 3, HCWs infected by SARS-CoV-2 who lost the antibody response against nucleocapsid and were vaccinated; Group 4, HCWs who were vaccinated, but they were not infected by SARS-CoV-2. The median of anti-spike protein antibodies for groups 1, 2, 3 and 4 was 30.0, 6465.5, 4878.5, 1142.0 U/mL, respectively. Groups 2 and 3 showed a statistically significant higher antibody titer than groups 1 and 4 ($p < 0.0001$), while no significant differences were observed between them. There were also significant differences in antibody titers between groups 1 and 4 ($p < 0.0001$). The antibody response to the spike protein induced by vaccination was greater than the one induced by natural infection, and was even greater in the group that had been previously infected.

Keywords: Antibody, infectious disease, viral infections

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POSTER PRESENTATIONS

P-0731

Biomarkers of bone metabolisms, gut barrier and immune response are associated with the form, activity and therapy of inflammatory bowel disease

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Crohn's disease (CD) and ulcerative colitis (UC) are main forms of inflammatory bowel disease (IBD). Moreover, primary sclerosing cholangitis (PSC) with concomitant IBD has recently emerged as another form of IBD. The pathogenetic mechanisms and molecular specifics of individual forms are, however, still not fully understood. The aim of this study was to test biomarkers of gut barrier and immune response to better understand the pathogenesis and to improve the IBD diagnostics. We enrolled 41 PSC-IBD, 52 UC, 20 CD patients and 28 healthy individuals. The sera were tested for Osteoprotegerin (OPG), Matrix metalloproteinase-9 and -14 (MMP-9, 14), Tissue inhibitor of metalloproteinase-1 (TIMP-1), Mannan-binding lectin (MBL), Lipopolysaccharide-binding protein (LBP), Trefoil factor-3 (TFF-3), Transforming growth factor- β1 (TGF-β1), Intestinal fatty acid-binding protein (I-FABP) and CD-14 using ELISA. We found, that MMP-9, -14 and TIMP-1 distinguished IBD patients from healthy controls (AUC>0.9). The decrease in OPG was typical for UC, where it distinguished UC from PSC-IBD patients (AUC>0.9). In combination with I-FABP and TIMP-1 it distinguished also UC from CD patients. The decrease in OPG was associated with anti-TNF-α therapy and increase in MMP-14 was associated with Mesalazine treatment. Low level of TGF-β1 was associated with disease activity and in combination with TFF-3, MMP-9 and LBP it distinguished patients according to the disease activity (AUC>0.9). Biomarkers of gut barrier and immune response reflecting specifics of IBD pathogenesis allowed distinguishing IBD by form, its activity and therapy. These findings are important in identifying of predictive biomarkers for disease progression and therapeutic success.

Keywords: Biomarkers, cytokines and mediators, inflammatory bowel disease, inflammatory molecules, tissue damage and repair

P-0732

Overexpression of endogenous retroviruses and immune checkpoint molecule CD200 under microenvironmental changes in neuroblastoma cell lines

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Neuroblastoma (NB) is the commonest solid tumor outside the central nervous system in infancy and childhood with unique biological heterogeneity. In patients with advanced, metastasizing NB, treatment failure is often marked by chemo- or immunotherapy resistance. Understanding of tumor microenvironment seems essential for developing effective therapy. We studied expression of immune checkpoint molecule CD200 and human endogenous retroviruses (HERV) as potential targets in NB cell lines during stem cell medium-induced microenvironmental change. To investigate microenvironmental changes, three NB cell lines (SH-SY5Y, IMR-32, SiMa) were cultured either in DMEM complete medium, stem cell medium supplemented with 10 % fetal bovine serum, or stem cell medium alone. Cells were harvested after 72 hours and prepared for flow cytometry analyses and total cellular RNA was isolated for gene expression analyses by quantitative PCR and RNAseq. A significant up-regulation of CD200 upon stem cell media incubation was detected both on transcriptional level by quantitative PCR and RNAseq, and on the protein level by flow cytometry indicating possible tumor escape mechanism in all NB cell lines. Interestingly, the overexpression of CD200 was associated with up-regulated transcription of HERV elements: HERV-K, HERV-W1 *ENV*, HERV-R *ENV* (ERV3-1), HERV-E1 and HERV-Fc2 *ENV* (ERVFC1-1). Our results suggest enhanced expression of immunomodulatory molecules like CD200 and HERVs by medium-induced microenvironmental changes in NB. Consequently, HERV-targeted drugs might be considered as novel therapeutic candidates.

Our study is supported by grant ZS/2018/12/96228 from the European Fund for Regional Development (EFRE) within the local program "Sachsen-Anhalt WISSENSCHAFT Schwerpunkte".

Keywords: Biomarkers, cancer immunology, drugs for immune modulation, microenvironment, RNAseq

P-0733

Th2-Tissue resident memory cells (TRMs) in a dexamethasone (Dex)-resistant model of relapsing allergic asthma in BALB/c mice

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Glucocorticosteroids are only partially effective at suppressing allergic lung inflammation in a relapsing-remitting mouse model of allergic asthma, and appears to resemble the 5-10% of asthmatics that are unresponsive to steroids. Mice recovered from acute onset allergic asthma have persistent allergen-specific lung Th2-TRMs, which we hypothesize might play a role in steroid-resistance. To address our hypothesis, we treated recovered mice with 3 mg/kg Dex intraperitoneally for 5 consecutive days and then either tracked circulating, resident, naïve, and memory lung CD4+ T cell subpopulations weekly for 4 weeks, or measured airway eosinophilia in response to ovalbumin-re-challenge at 72h and 4 weeks. Initially, Dex did not affect lung CD4+ T cell subpopulations, but by week 3, compared to untreated controls, Dex decreased CD4+ T cell subpopulations including: circulating naive (65%), resident naïve (65%), circulating memory (60%), and resident memory (48%). Surprisingly, at week 4, the Dex-treated mice had 10% more circulating and 30% more resident memory CD4+ T cells compared to untreated controls. To determine whether changes in cell numbers correlated with inflammatory responses, we evaluated eosinophilic airway inflammation as a marker of an ovalbumin-induced disease relapse. Dex reduced airway eosinophils by 25% compared to untreated controls in the first week following treatment, but by 4 weeks, Dex-treated mice had a rebound effect with 12% more airway eosinophils compared with untreated controls. These results suggest that TRMs are more resistant to systemic steroid therapy compared to other T cell subpopulations and they may even contribute to disease exacerbations occurring after therapy cessation.

Keywords: Adaptive immunity, allergen-induced immune responses, animal models

P-0735

Association of low-level cadmium exposure with alterations in lymphocyte cell subsets in cadmium exposed workers

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Occupational exposure to Cadmium (Cd) may have a profound health effect on workers. However, little is known about its effect on the immune system. Moreover, previous studies have been inconclusive in stating the effect of Cd on the immune system. AIM: Our study aimed to estimate immune cell subsets in workers occupationally exposed to Cd. 100 individuals occupationally exposed to Cd and 90 healthy non-exposed individuals were recruited for this study. Blood Cadmium levels were determined by Atomic Absorption Spectrophotometry (AAS). Lymphocyte subsets were analyzed using flow cytometry. The exposed group had significantly higher levels of B-Cd. The percentage (%) of CD8 cells were higher in the exposed while % of CD4 cells showed a decreasing trend in the exposed group. Among the CD3CD4 T cell subsets, Th1 (%) and Tregs (%) cells were lower while Th17 (%) were higher in the exposed group. Increased Th17/Tregs ratio in the exposed group also suggests an increased pro-inflammatory immune response in exposed groups. To conclude, even a low level of exposure to Cd in occupational settings is associated with alterations in Th17 cells, which may further predispose an individual to other systemic abnormalities.

Keywords: Biology of the immune system, biomarkers, visualizing immune responses

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POSTER PRESENTATIONS

P-0738

Quantitation of antibodies against SARS-CoV-2 spike protein after two doses of CoronaVac in health care workers

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Quantitation of antibodies to the spike protein of SARS-CoV-2 was performed for the detection of adaptive immune response in health care workers (HCWs) vaccinated with CorovaVac. We prospectively recruited HCWs from a university hospital in Turkey. Serum samples from 1072 HCWs were obtained following 28 days of the first, and 21 days of the second dose. Detection and quantitation of SARS-CoV-2 anti-spike antibodies was performed by the chemiluminescent microparticle immunoassay (CMIA) (SARS-CoV-2 IgG II Quant, Abbott, Ireland). Results greater than or equal to the cutoff value 50.0 AU/mL were reported as positive. After the first dose, anti-spike antibodies were detected in 834 of 1072 (77.8%) HCWs. Seropositivity was higher among females (84.6%) than males (70.6%) ($p<0.001$) and was found to be highest in both women and men between the ages of 18-34. After the second dose, antibodies were detected in 1008 of 1012 (99.6%) HCWs. Antibody titers were significantly higher in those who had COVID-19 before vaccination than those who did not ($p<0.001$). Antibody positivity and median antibody titers were significantly less in HCWs with chronic diseases compared to those without ($p<0.005$ and $p<0.001$, respectively). In conclusion, our findings indicated that a relatively high frequency (99.6%) of humoral immunity was produced in HCWs aged 18-59 after two doses of CoronaVac. Quantitation of antibodies may help facilitate longitudinal monitoring of the antibody response which will be especially useful in deciding the dose of the vaccine in vulnerable groups such as those over 60 years of age and those with chronic diseases.

Keywords: Antibody, monitoring immunity, viral infections

P-0739

Tumor cells and extracellular matrix dictate the pro-tumoral profile of macrophages in CRC

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Tumor associated macrophages (TAMs) are major components of the tumor microenvironment (TME). In colorectal cancer (CRC), a strong infiltration of TAMs is accompanied by a decrease of effector T cells and an increase of the metastatic potential of CRC. We investigated the functional profile of TAM infiltrating CRC tissue and their involvement in impairing the activation of effector T cells. In CRC biopsies we revealed a high percentage of macrophages with low expression of the antigen-presenting complex MHC-II and high CD206 expression. In accordance, monocytes co-cultured with tumor cells or decellularized tumor matrix differentiated towards a pro-tumoral macrophage phenotype characterized by decreased expression of MHC-II and CD86 and increased expression of CD206 and abundant release of pro-tumoral cytokines and chemokines. We demonstrated that the hampered expression of MHC-II in macrophages is due to the down-regulation of the MHC-II transactivator CIITA and that this effect relies on the increased expression of miR146b targeting CIITA. As result, macrophages become unable to present antigens to CD4 T lymphocytes. Our data suggest that tumor microenvironment contribute in defining a pro-tumoral profile of macrophages infiltrating CRC tissue, with impaired capacity to activate T cells effector functions. Furthermore, we recently highlighted the role for the immune receptor CD300e in hampering MHC-II expression in macrophages, resulting in an impaired antigen presentation capacity. Thus, we are currently investigating *in vivo*, adopting a conditional KO for CD300e in the myeloid lineage, the role of the immune receptor in a CRC mouse model.

Keywords: Cancer immunology, innate immunity, macrophage, microenvironment

P-0741

A new experimental allergic rhinitis model in mice

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Allergic rhinitis(AR) is an inflammatory disease of the nasal mucosa mediated by IgE after exposure to an allergen.The most well known related comorbidity of AR is asthma.This study was planned due to the need for an animal model for studies on AR-asthma coexistence.In this study,the frequency of AR accompanying in the asthma model created in mice,and the usability of the related model in AR studies will be investigated. In our study,6-8 week- old,18-20 g BALB /c mice were used.Chicken egg ovalbumin(OVA Grade V, Sigma)was administered through intraperitoneal(IP) route at doses of 10 µg on days 0 and 14.Mice were exposed to aerosolized 2.5% ovalbumin solution in sterile saline for 30 minutes 3 days a week for 8 weeks,starting 7 days after the last IP administration(21st day).After exposure to OVA,mice were observed for typical signs of AR including sneezing,runny nose, and nasal itching.The final diagnosis of AR was made by histopathological examination of the rhinotracheal tissues of mice. In our study,all mice exposed to ovalbumin received histopathologic diagnosis of AR.Increased number of capillaries lymphocytes, polymorphonuclear leukocytes and eosinophilsper square millimetre of rhinotracheal tissues were calculated in the murine model of AR compared to the the control group. This study introduced a new AR model, not cited in the literature, and induced with the longest-term ovalbumin exposure in the literature.It was concluded that this model, known as the asthma model, can also be used to induce an AR model and can be used in studies investigating coexistence of allergic rhinitis and asthma.

Keywords: Allergic disorders, animal models, modification allergic responses

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POSTER PRESENTATIONS

P-0742

CD4 T cells with identical TCR clonotypes show functional plasticity in a mouse model of relapsing-remitting colitis

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Chronic inflammatory diseases, like inflammatory bowel diseases are often characterized by a relapsing-remitting course. We established a mouse model of a reversible, CD4 T cell-induced colitis, where treatment with an anti-CD4 mAb leads to rapid remission, followed by a spontaneous relapse of colitis. We now compared by scRNASeq the transcriptome of distinct CD4 TCR clonotypes in this model of reversible colitis. Indeed, a large fraction of the immunodominant CD4 T cell TCR clonotypes detected in the blood during active colitis are also found in the colon during remission and relapsing disease. Our initial transcriptomic analysis reveals a unique gene expression profile of colonic CD4 T cells during remission with a distinct up-regulation of genes involved in the maintenance of a tissue residency, such as CD69 and Rgs1, and, intriguingly, also of genes encoding proteins that actively attenuate inflammatory responses, such as NR4A1, a potent repressor of AP-1 functions. During relapsing disease, the CD4 T cells regain their transcriptional signature seen during initial colitis induction. These findings thus demonstrate a high functional plasticity of CD4 T cells with identical TCR clonotypes during distinct phase of reversible colitis. Identifying the differentially expressed genes and their regulation may lead to more specific therapies to delay, or even prevent flares of active disease. Furthermore, the identification of the distinct signature of CD4 T cells that appear early in circulation mice may further allow to predict more accurately an imminent clinical relapse.

Keywords: Autoimmunity, adaptive immunity, animal models, inflammatory bowel disease, omics technologies

P-0745

The TBE-specific lymphocyte response after HSCT but before vaccination predicts the rapid establishment of humoral immunity after TBE vaccination

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Patients after allogeneic hematopoietic stem cell transplantation (HSCT) suffer from immunosuppression due to delayed immune reconstitution, sustained immunosuppressive medication, and underlying graft-versus-host disease and can therefore be considered as especially vulnerable to infectious diseases but also at risk for limited response to vaccination. The aim of this prospective study was to assess lymphocyte proliferative and cytokine response prior and following TBE-immunization among patients after HSCT and to correlate these findings with immunization success. Seventeen adult patients 11 to 13 months after HSCT and eight unvaccinated healthy adults received up to three TBE-vaccinations. Following *in vitro* stimulation with TBE antigen, lymphocyte proliferation and cytokine secretion were analyzed one week after the 2nd and 3rd vaccination. Ten patients (59%) showed significant baseline TBE-specific lymphocyte proliferation, but none of the unvaccinated controls ($p=0.002$). All patients with a TBE-specific antibody response after two vaccinations exhibited a strong lymphocyte proliferative response at baseline (stimulation index >10). Patients with sibling donors had a significantly stronger baseline lymphocyte proliferation and IL-13 cytokine response than patients with unrelated donors ($p<0.05$) at all three time points. In conclusion, a relevant proportion of patients showed TBE-specific lymphocyte proliferation and cytokine responses prior re-vaccination after HSCT. This specific cellular immune response to the vaccine antigens prior to actual vaccination seems to be a prerequisite for a humoral immune response in HSCT patients. Patients with (vaccinated) sibling donors were much more likely to elicit a cellular immune response than patients with unrelated donors of unknown vaccination status.

Keywords: Adaptive immunity, memory, transplantation

P-0747

Chicken cGAS is a key DNA sensor for antiviral immunity and regulation of macrophage effector functions

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The anti-viral innate immune response relies on pattern recognition receptors (PRRs) that are able to sense non-self DNA and initiate the innate immune response. Several cytoplasmic PRRs have been identified as DNA sensing PRRs that drive Type-I interferon production in mammals, but little is known about which of these PRRs function in birds, due to the lack of suitable tools and imperfectly annotated genome. In mammals, cyclic GMP-AMP synthase (cGAS) has been pinpointed as an essential component of the innate response in response to viral DNA. cGAS initiates a range of signaling outputs that are dependent on generation of the second messenger cGAMP that binds to the adaptor protein stimulator of interferon genes (STING). Using primary cells and CRISPR/Cas9 gene editing of the chicken macrophage HD11 cell line, we show that the cGAS/STING pathway is essential not only for the production of Type-I interferons in response to intracellular DNA stimulation, but also for regulation of macrophage effector functions including the expression of MHC-II and co-stimulatory molecules. After fowlpox infection, the cGAS/STING pathway was found to be responsible for Type-I interferon production and MHC-II transcription. The sensing of fowlpox virus is, therefore, essential for mounting an anti-viral response in chicken cells and for regulation of a specific set of macrophage effector functions. These results begin to define the pathways behind viral DNA sensing and the crosstalk between innate and adaptive immunity in poultry with further relevance for treatment and vaccine development.

Keywords: Infectious disease, innate immunity, macrophage, veterinary immunology, viral infections

P-0748

HLA loss detection: an application of HLA typing by next generation sequencing in the post-hematopoietic stem cell transplantation monitoring

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POSTER PRESENTATIONS

HLA loss relapse (HLA-LR) consists on genomic loss of HLA molecules incompatible between donor and recipient in re-emerging leukaemic cells. Until now, this intrinsic mechanism to evade graft versus leukaemia effect (GvL) by donor T-cells has been reported in retrospective studies and mostly in haplo-HSCT setting but use of next-generation sequencing (NGS) is scarce. Here, we present a case of HLA-LR in a 7 year-old boy suffering from atypical Chronic Myeloid Leukaemia. He achieved remission after a haplo-HSCT from his father but six months later a bone marrow (BM) relapse was detected. As various treatment lines were applied without response including one donor lymphocyte infusion (DLI), HLA loss assessment was requested to direct therapy options. BM and peripheral blood samples stored during post-HSCT chimerism monitoring were recovered and analysed retrospectively for NGS HLA typing in order to identify a possible HLA-LR. Shared, donor-specific and patient-specific haplotypes were detected in samples corresponding to early relapse. However, patient-specific haplotype was not detected anymore since DLI meaning that under-represented HLA loss leukemic blasts in early relapse escape from DLI GvL effect. Interestingly, donor-specific and patient specific alleles' deep coverage proportion correlated to chimerism results. Considering that NGS HLA typing is a useful tool for HLA loss detection (high sensitivity and possibility to analyse all relevant HLA loci), we currently initiate the first multicentric prospective study to evaluate the HLA loss detection capacity and prognostic value during post-HSCT monitoring in adult and paediatric patients with myeloid and lymphoid malignancies at high risk of relapse.

Keywords: Biomarkers, bone marrow transplantation, monitoring immunity, transplantation

P-0749

Activating Killer-cell Immunoglobuline-like receptors are associated with the severity of COVID-19

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One year after the first description of the coronavirus disease 2019 (COVID-19), clinical variability etiopathogenesis and the biological causes of its clinical variability remain unknown. Emerging studies in moderate and severe COVID-19 report low numbers of peripheral blood Natural Killer cells (NKcs) but increased numbers infiltrating the lung. NKcs are innate lymphocytes that represent the first line of defense against viral infections and cancer. However, a detailed role of NKcs in the landscape of clinical SARS-CoV-2 infection is still missing. The aim of this work was to investigate the role of Killer-cell Immunoglobuline-like receptors (KIR)/Human Leukocyte Antigen Class-I (HLA-I) interactions in the susceptibility and severity of COVID-19 by genotyping KIR and HLA-I and immunophenotyping NK cell receptor in 201 symptomatic patients (75 days post-infection) and 210 non-infected controls. NK cells with a distinctive immunophenotype suggestive of recent expanded in patients with severe COVID-19, which was associated with a higher frequency of the functional A-telomeric activating KIR2DS4 compared to mild/moderate patients and controls (83.7%, 55.7% and 36.2%, p<7.7x10-9). Besides, HLA-B*15:01 was associated with higher frequencies of activating B-telomeric KIR2DS1 and KIR3DS1 compared to patients with other HLA-B*15 subtypes and non-infected controls (90.9%, 42.9% and 47,3%, p<0.002). Although further investigations are needed, our results suggest that this atypical viriasis may be related to the appearance of neo-ligands that induce unusual anti-viral responses. Besides, they support a crucial role for NK cells in the clinical variability of COVID-19 and could be pointing to molecular targets for specific treatments.

Keywords: Infectious disease, Innate immunity, NK cells, Viral infections

P-0750

Differentiation of phenotypes in patients carrying the Q705K mutation in the cryopyrin-associated periodic syndrome

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We aim to describe NLRP3 gene mutations clinical presentation and relationship of phenotype and genotype. The data of 320 patients from our genetic database of autoinflammatory diseases were included in the study. In at least three months of follow-up, patients with autoinflammatory disease (AID) compatible recurrent episodes were included, in which other etiological reasons were excluded. 23 patients (16 male, 69.6%) were included in the study. The median age at disease onset was 29 months and the median disease duration (IQR) was 33 months (14-40 months). All patients were characterized by symptoms consistent with recurrent inflammatory syndrome. 52.2 % of patients (n= 12) presented with urticarial rash, 39% (n=9) with tonsillitis, 82.6% (n= 19) with arthralgia, 30.7 % (n=7) with conjunctivitis, 17.4 % (n=4) with headache. In 11/23 patients had worsening symptoms with cold contact.The phenotypes of PFAPA (n=5,21.7%), CAPS (FCAS and MWS) (n=11,47.8%) and undifferentiated AID (uAID) (n=7, 30.4%) were determined. There was no statistical significance between phenotypes in terms of median age at onset of attacks (>0.05). The most of patients (n=19, 82.6%) had Q705K variant in NRLP3 gene. Patients carrying the q705k variant had 26.3% PFAPA, 42% FCAS and 31.6% uAID phenotype.In 13 (59%) patients responded to colchicine treatment. Remained 9 (40.9%) patients had partial response or unresponsive to colchicine, were achieved to inactive disease with anti-IL 1 treatments. We suggest that the Q705K variant causes autoinflammatory syndromes in a variety of phenotypes.

Keywords: Inflammatory disease, inflammatory molecules, autoinflammation

P-0751

Naturalizing the microbiome by housing mice in a farm environment confers protection against colorectal carcinogenesis

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Mammals have co-evolved with the billions of microbes surrounding them and harboring their bodies. Thus, it is paradoxical that disease modelling studies using research mice take place under strictly hygienic conditions, far-away from the typical lifestyle of the end goal for such studies; humans. To close the gap between the preclinical mouse model and human lifestyles, we have established a system where laboratory mice are raised under a full set of environmental conditions present in a naturalistic, farmyard habitat. We call this process feralization, and the resulting mammal display a more mature states of immune cells and a diverse gut microbiota, likely surpassing conventional laboratory mice in resembling responses of free-living mice. We used the feralization approach with two different mouse models of colorectal cancer, showing that the mice feralized in a farm environment were protected against carcinogenesis. In contrast to conventionally reared laboratory mice, the feralized mouse gut microbiota structure remained stable and resistant to mutagen- and colitis induced neoplasia. Moreover, the feralized mice exhibited signs of a more mature immunophenotype, indicated by increased expression of NK and T cell maturation markers, and a more potent IFN-γ response to stimuli. In our study, conventionally born and raised mice subsequently feralized post-weaning were protected to a similar level as life-long exposed mice, downplaying the need for neonatal exposure. Collectively, we show protective implications of a farm environment on colorectal cancer development and demonstrate the utility of a novel animal modelling approach that recapitulates realistic disease responses in a naturalized mammal.

Keywords: Animal models, *in vivo* tumor models, microbiome and environmental factors

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POSTER PRESENTATIONS

P-0752

Increased serum levels of implantation and differentiation trophoblast factors in pregnant women with pre-eclampsia

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Preeclampsia (PE) is a multifactorial vascular disease characterised by newly onset hypertension with proteinuria during pregnancy. The multifactorial nature contributes to differences in its clinical presentation differentiating early from late PE depending upon the time of onset of symptoms. The adequate function of the immune system is paramount to achieve a successful pregnancy and its dysregulation leads to foetus-maternal boundary deficiencies giving rise to placental dysfunction. This work aimed to study the immune status of pregnant women diagnosed with PE. Forty pregnant women were included in this study from 2019 to 2020 at the University Hospital of Salamanca. A serum sample was obtained and tested for a panel of 25 cytokines (CCL3/MIP-1a, CXCL10, CXCL5, Galectin-1, Galectin-3, Galectin-9, GM-CSF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8/CXCL8, IL-10, IL-12p70, IL-13, Lymphotoxin-α/TNF-β, PIGF, TRAIL/TNFSF10, LIF, M-CSF, TNF-α, FasL/TNFSF6, IFN-γ, and CCL5/RANTES) by Luminex® 200™. 62,5% (n=25) of patients were grouped into the PE, and 37,5% (n=15) were grouped into the No-PE. Mean fluorescence for CXCL10 (p=0.031), Galectin-1 (p=0.05), IFN-γ (p=0.025), IL-6 (p=0.028) and PIGF (p=0.013) were significantly higher in PE than No-PE patients. Contrarily, M-CSF (p=0.007), TNF-α (p<0.001), and IL-8/CXCL8 (p=0.001) from PE showed significantly lower mean fluorescence than no-PE. Furthermore, LIF (p=0.004) was the only protein that shown higher levels in late PE vs No-PE. This preliminary result shows that in pregnant women with PE, there seems to exist an exacerbated response towards the implantation, differentiation, and proliferation of the trophoblast along with an altered pro-inflammatory innate immune response.

Keywords: Biomarkers, cytokines and mediators, fetal immunity, reproductive immunology

P-0753

The presence of Tks4 augments tumor progression

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Tks4's (tyrosine kinase substrate with 4 SH3 domains) key role in cell motility as well as in development of new metastases is well established. Little is known, however, about its function in earlier stages of tumor progression. Recently, we have found that B16-F10 melanoma cells injected into Tks4-deficient mice or lethally irradiated mice reconstituted with Tks4-deficient bone marrow develop significantly smaller tumors compared to wild-type animals, suggesting a fundamental role for Tks4 in immune surveillance controlling tumor growth. Here we examined the contribution of Tks4 to the immunosuppressive functions of tumor-associated myeloid-derived suppressor cells (MDSCs). Similar to the B16-F10 model, we found impaired tumor growth in Tks4-KO animals injected with Lewis lung carcinoma (LLC) cells. Differentiation of the major subsets of myeloid cells appears to be Tks4-independent as no major differences were detected in the number of tumor-associated myeloid or lymphoid subsets between Tks4-KO and wild-type mice. In functional studies we found that Tks4-KO MDSCs differentiated form bone marrow or sorted from LLC tumors showed decreased T-cells suppression, concomitant with impaired production of nitric-oxide and reactive oxygen-species. Tks4 appears to be a tumor-promoting factor in the tumor-associated immunosuppressive stroma. We propose that slower growth of LLC-tumors in the absence of Tks4 results -at least in part- from inefficient suppressor function of MDSCs allowing better control of the growing tumors.

Keywords: Cancer immunology, *in vivo* tumor models, myeloid derived suppressor cells

P-0754

Evaluation of the P2X7 functions in tumor context using a novel methodology combining rAAV vectors and anti-P2X7 nanobodies

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P2X7 is a receptor activated by extracellular Adenosine triphosphate, a danger signal that accumulates in inflammatory sites and in tumor microenvironment. P2X7 expressed by immune cells and by most malignant tumor cells was associated with release of pro-inflammatory cytokines, modulation of the activity and survival of immune cells, and proliferation and invasiveness of tumor cells. Hence, P2X7 plays an intricate role in the tumor microenvironment combining beneficial and detrimental effects that need to be further investigated. The aims of this work are to better understand the role of the P2X7 receptor in tumor microenvironment and to evaluate its therapeutic potential as a pharmacological target. We developed a novel methodology termed AAVnano based on the use of Adeno-associated viral vectors (AAV) encoding nanobodies capable to block or to potentiate P2X7. We demonstrate that a single intramuscular injection of AAVnano allow long-term expression of the selected nanobody resulting *in vivo* in potentiation or blockade of P2X7. We showed that blocking P2X7 in tumor model that express P2X7 leads to significant inhibition of tumor growth and better survival. We also gathered evidence indicating that P2X7 potentiation when combined with immunogenic chemotherapy can enhance anti-tumor immune responses. P2X7 plays an important role in inflammation, tumor progression and anti-tumor immune responses through distinct mechanisms acting on immune cells or on tumor cell. P2X7 represent an attractive therapeutic target in cancer in combination with immune checkpoint inhibitor or chemotherapy to inhibit tumor growth or to reactivate the anti-tumor immune responses.

Keywords: *In vivo* tumor models, cancer immunology, engineering of antibodies and nanobodies

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POSTER PRESENTATIONS

P-0755

Lack of cytomegalovirus (CMV)-specific cell-mediated immune response using QuantiFERON-CMV assay in CMV-seropositive healthy volunteers: fact not artifact

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The QuantiFERON-CMV assay (QF) measures cell-mediated immunity against cytomegalovirus (CMV-CMI), which is useful in individuals susceptible to CMV infection such as transplant patients. In CMV-seropositive individuals, a positive QF result identifies patients that are better protected against CMV infection. However, a negative QF result needs to be clarified. To evaluate whether CMV-seropositive healthy individuals with a negative QF result (CMV+QF-) show an impaired proliferative response against CMV lysate or this discordance is an artifact of the QF assay related to the type of stimulus. CMV-CMI was analyzed using the QF assay, and, in parallel, the Flow-cytometric Assay of Specific Cell-mediated Immune response in Activated whole blood (FASCIA). FASCIA assay measures T-cell proliferation using CMV lysate as stimulus whereas QF assay use CMV peptides. Anti-CMV IgG antibodies were determined by a semiquantitative (AU/mL) and a quantitative assays (IU/mL). A 18,3% (13/71) of CMV+ individuals showed discordance (CMV+QF-). Interestingly, with FASCIA assay CD4+ and CD8+ T-cell proliferations were lower in CMV+QF- than in CMV+QF+ individuals, 154.0 cells/μL vs. 301.5 cells/μL; p=0.038 for CD4+ and 4.0 cells/μL vs. 12.5 cells/μL; p=0.059 for CD8+. Furthermore, CMV+QF- volunteers had a lower level of anti-CMV IgG with both quantitative (1.7 IU/mL vs. 5.6 IU/mL; p=0.001) and semiquantitative assays (65.3 AU/ml vs. 96.7 AU/ml; p=0.008). Discordant CMV+QF- volunteers can be defined as "low responder" individuals since they show a lower CMV-specific humoral and cellular immune responses in comparison to CMV+QF+ individuals. Nevertheless, false-positive results for the semiquantitative method cannot be discarded.

Keywords: Immunological techniques, monitoring immunity, viral infections

P-0757

Examination of the value of Sars-Cov-2 class IgG antibodies in voluntary blood donors in the territory of central Serbia

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Blood collection from voluntary donors changed during the Covid 19 infection pandemic in the world as well as in Serbia. Collecting blood from people with a previous infection had the additional goal of plasma therapy depending on the presence of antibodies to the Sars Cov-2 virus. Therefore, in this paper, the values of IgG antibodies were analyzed, which were measured by ELISA test for the presence of Immunoglobulins of the IgG class and compared with the demographic characteristics of the voluntary donors. The concentration was determined in 823 voluntary blood donors aged 18 to 65 in the territory of central Serbia in the period from June 2020 to February 2021. Analysis of antibody values in all subjects showed that there were statistically significant differences in sex ratio, with men having higher IgG values, compared to women, and that there was a statistically significant difference in relation to the severity of the disease (p <0.5). Antibody values in relation to age showed that in voluntary blood donors the highest values of antibodies in plasma were in the age group from 55 to 59 years with a tendency that in the total population the value of antibodies was slightly positively correlated with aging (Spearman correlation coefficient). The data indicate that even in older people, after the previous infection, there is a significant production of antibodies and that aging does not lead to a decrease in the production of antibodies after the Sars-Cov2 infection in this population.

Keywords: Infectious disease, ageing, antibody

P-0758

İnitiation of antiretroviral therapy in early presentation contributes to normalization of CD8 T cell counts rather than late presentations in HIV infection

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Increased CD8 T-cell counts in human immunodeficiency virus infection (HIV) are an indicator of non-AIDS-related morbidity and morbidity. We evaluated the effect of active antiretroviral therapy (ART) initiation on the trajectories of CD8 cell counts within one year in early versus late presentations of the patients with HIV. Newly diagnosed 200 HIV/AIDS patients from 2007 to 2019 were enrolled in this study by our center in Izmir, Turkey. HIV-infected cases were divided into two groups according to the CD4 T-cell counts at the time of admission as early presentation (CD4 cell count> 350 cells/mm3) and late presentation (CD4 cell count <350 cells/mm3 or presentation with an AIDS-defining event, regardless of the CD4 cell count). Plasma HIV viral load (VL), CD4 and CD8 cell counts were evaluated at each study visit. CD8 counts was compared at 0 (baseline), 1, 3, 6, and 12 months from ART initiation. Early presenter had low pre-treatment HIV RNA levels, high CD4 T-cell count, CD8 T-cell count and CD4/CD8 ratios (p<0.001). The sixth month viral success rate of late presentations was low (p = 0.01). While a decrease in CD8 T-cell count was observed in early presentations during the treatment, an increase in CD8 T-cell count was observed in cases with late diagnosis, but no statistically significant difference was found. ART initiated in early presentation is associated with improved resolution of CD8 T-cell elevation compared with late presentation.Early ART may help reduce the risk of non–AIDS-related events by alleviating this elevation.

Keywords: Infectious disease, viral infections, immune response tracing, immunodeficiency

P-0759

Altered fatty acid oxidation in T and NK cells in myalgic encephalomyelitis/chronic fatigue syndrome

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There is overwhelming evidence that the immune system and metabolism play an important role in ME/CFS. We recently reported that circulating CD4+ and CD8+ T cells, and stimulated CD8+ T cells, from ME/CFS cases exhibit reduced basal glycolysis in comparison to cells from controls. Furthermore, multiple metabolomic studies have shown alterations in metabolites relevant to fatty acid metabolism. To further our understanding of metabolism within ME/CFS immune cells, we are carrying out metabolic assays in ME/CFS and healthy control CD56+ NK cells, CD4+ T cells, and CD8+ T cells to understand the contribution of fatty acid oxidation in these cells at rest and after stimulation using Seahorse extracellular flux analysis accompanied by a fatty acid oxidation drug panel. We have also used flow cytometry to analyze a fatty acid analog and two pertinent fatty acid transporters to quantify these energy dynamics. These experiments measure endogenous fatty acid oxidation, mitochondrial respiration, and spare respiratory capacity in ME/CFS and healthy control cells' mitochondria. Preliminary results from total T cells in 8 patients and 8 controls show an increased dependence of ME/CFS T cells on fatty acid oxidation. Furthermore, we see a trend of increased absorption of the fatty acid analog in ME/CFS CD8+ T cells and a higher proportion of CD8+ T cells with increased abundance of fatty acid transporters in ME/CFS cells, while we see opposing trends in ME/CFS NK cells. Our data on fatty acid oxidation in T and NK cells provides further evidence of immune metabolic dysfunction in ME/CFS.

Keywords: Chronic inflammation and fibrosis, inflammatory disease, metabolic control of immune responses, NK cells

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POSTER PRESENTATIONS

P-0760

High dimensional analysis of systemic immune signatures in post-transplant lymphoproliferative disorder

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Post-transplant lymphoproliferative disorder (PTLD) is a life-threatening complication of solid organ transplantation (SOT). Whilst the main risk factor of SOT PTLD is long term immunosuppression, the exact underlying immune deficiencies are not fully understood. The aim of this study is to conduct high dimensional analysis of systemic immunity in newly diagnosed SOT PTLD patients to uncover immune disturbances associated with disease. Peripheral blood samples from PTLD patients and age-matched healthy donors were assessed using a 35 marker CyTOF panel to simultaneously quantify and phenotype immune subpopulations. Preliminary analysis has shown significant differences in the frequency and phenotype of various immune subsets, including CD8+ and CD4+ T cells, myeloid subsets and B cells. PTLD patient CD8+ T cells had elevated expression of CD95 and co-expression of CD39 and PD-1, suggesting an exhausted phenotype. PTLD patient B cells had reduced expression of several homing receptors and increased expression of the regulatory markers CD39 and CD73. Consistently, patients had a significantly lower percentage of naïve B cells and a higher percentage of memory B cells compared to healthy donors. High dimensional analysis of PBMCs provides a far greater picture of overall immunity than can be achieved by analysis of individual cell subsets alone. Initial analyses have already revealed consistent and profound differences in various immune cell subsets and phenotype in PTLD patients compared to healthy donors. Ultimately, expanding this study may reveal a biomarker that can be used to predict PTLD and monitoring of at-risk patients.

Keywords: Cancer immunology, proliferative disorders, transplantation

P-0761

Crosstalk between stromal compartment and macrophages lead to CD169+ macrophages in pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is associated with an abundant stromal reaction, which accounts for up to 80-90% of the tumor mass. Macrophages represent one of the major immune cell populations in tumor microenvironment and have been previously shown to display a M2 phenotype and an immunosuppressive role. We sought to determine the respective roles of the tumoral and stromal compartment in impacting macrophages phenotype and function. We used two different primary cell lines isolated from stromal and tumoral compartment respectively from mice with neoplasia and PDAC and co-cultured them with bone marrow derived macrophages. We report here that, in contrast to the tumoral compartment, the stromal compartment induces i) macrophages polarization towards a M2-like phenotype by expressing PD-L1, CD206 and, ii) a significant increased production of the immunosuppressive extracellular protein βig-h3 and iii) suppression of $CD8^+$ T cell proliferation. Furthermore, upon stromal-macrophage crosstalk, we identified the induction of a newly described macrophage population $CD169^+Tim4^+$ and the production of CXCL12 chemokine by the stromal compartment. This work may lead to the identification of a new immunosuppressive population playing a role in PDAC progression.

Keywords: Cancer immunology, macrophage, microenvironment

P-0762

Microbiota analysis in allogeneic stem cell transplant patients with in AML and MDS

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In our study, microbiota analysis of patients and their donors who were applied allogeneic hematopoietic stem cell transplantation (allo-HSCT) was performed. The change of existing microbiota flora, engraftment and development of graft-versus host disease (GVHD) and the relationship of the microbiota with the HLA tissue group were investigated. Ten adult recipients/donors to be treated with Allo-HSCT were included in the study. Stool samples were taken from the donors and patients once before the conditioning regimen andin the 3rd week after Allo-HSCT from patients. Samples were sequenced by Oxford Nanopore sequencing method and analyzed using R software. In addition, OXA-48, KPC, NDM and CTXM gene screening in the stool samples of the patients after transplantation was performed by classical PCR method. The mean age was 49,14± 14,79 years in patients and 47,40±11,55 in donors. Gender distribution was M/F:6/4 in patients and donors. Stem cells were obtained pheripheral blood from 10/10 matched siblings. The overall frequency ofany degree of graft versus host disease was 10%. It was observed that the *Firmicutes* and *Proteobacteria* phyla changed significantly before and after transplantation. The number of *Entereccocus* species was found to be higher in patients who developed GVHD and died. Although KPC and NDM gene positivity were not found in the patients, 10% CTX-M geneand 20% CTX-M/OXA-48 association was found. Intestinal flora monitoring may provide guiding data for GVHD protection and/or treatment.

Keywords: Bacterial infections, bone marrow transplantation, microbiome and environmental factors, stem cells, tissue damage and repair, transplantation

P-0763

The serum complement in bone repair; between the norm and flaw

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The complement system is involved in repairing various tissues, particularly in response to the damage signals. The role of complement in bone repair is limitedly known and mainly based on the findings in other tissues or experimental studies. Here, the complement system was investigated in human sera to assess its involvement during normal bone repair. Additionally, the circulatory levels of the complement system was investigated in complicated bone repair. The serum samples from patients with normally healed long bone fractures were collected at three different time points covering the course of healing. Additionally, serum samples from patients diagnosed with non-union of long bone fractures and healthy controls were included in the analysis. The complement proteins, initiating factors, and products in serum samples were tested using mass spectrometry and ELISA. The data suggest an involvement/activation of the classic and lytic complement pathways during the early phase of normal bone repair. In contrast, the lectin pathway seemed disengaged in normal repair and highly expressed in the non-union cases. These data suggest distinctive roles for complement pathway during normal bone repair, and the lectin pathway might be linked to the complicated bone repair. Ongoing work is aiming to explain the complement-related molecular mechanisms, particularly those associated with abnormal bone healing. Such knowledge will help to understand better the immune response to bone damage in normal versus abnormal bone repair conditions and could potentially help to identify new targets for optimised regenerative therapies

Keywords: Complement, innate immunity, tissue damage and repair

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POSTER PRESENTATIONS

P-0764

A novel primary antibody deficiency due to FNIP1 mutation

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FNIP 1 deficiency is one of the recently identified primary antibody deficiencies. It's characterised by agammaglobulinemia, absence of B cells and hypertrophic cardiomyopathy in 6 patients in the literature. Here, we report a patient with a novel FNIP1 mutation and her clinical course during COVID19 infection. A fourteen month old girl was referred due to neutropenia and agammaglobulinemia. She was first and only child of consanguineous parents and had recurrent severe pneumonias, hypertrophic cardiomyopathy and rotavirus diarrhoea. Laboratory tests revealed severe neutropenia (ANC: 390/mm3), absence of B cells (CD19-20: %0.7) and a traction bronchiectasis was detected. IVIG, TMP-SMX and G-CSF treatments were started. Unfortunately, she didn't come to routine follow-up for 1.5 years. At 41 month old age, she was admitted to our emergency department with cough and fever (>38 C) lasting for two days. It was learned that her parents had COVID19 infection ten days ago, her nasal swab test for COVID19 PCR was found positive and she was followed without treatment. We hospitalized her with diagnosis of pneumonia. After reassessment, FNIP1 defect was considered and NGS analysis was performed. A novel homozygous mutation [NM_133372.3 C1630 A>G (p.R544G) (p.Arg544Gly)] was detected in FNIP1 gene that wasn't reported previously. Both parents were heterozygous for this mutation. She had complicated course requiring broad spectrum antibiotics, corticosteroid, IVIG, remdesivir (x2 courses), convalescent plasma(x3) therapy and non invasive mechanic ventilation. She was discharged with nasal oxygen support after 55 days. FNIP1 deficiency should be considered in patients with agammaglobulinemia and hypertrophic cardiomyopathy.

Keywords: Antibody, B lymphocytes, immunodeficiency, infectious disease

P-0765

Analysis of 14bp INS/DEL and +3142 C/G polymorphisms of the *HLA-G* gene in patients with gastric adenocarcinoma

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The *HLA-G* gene presents several polymorphisms related to the stability of the mRNA encoding HLA-G and, therefore, to its expression levels. Thus, those polymorphisms that increase the stability of the mRNA could be related to an enhanced ability of the tumor to evade immune system surveillance. We studied, in a group of 107 patients with gastric adenocarcinoma and 58 control individuals, the relationship of the polymorphisms 14bp-INS/DEL and +3142-C/G with the development and progression of this disease. We analyzed the presence of somatic mutations, the distribution of these polymorphisms and their haplotypes in patients, and the implication in survival. The aforementioned polymorphisms were analyzed by PCR-RFLP and sequencing techniques. No somatic mutations were observed when comparing DNA from tumor and distal tissue from the same patient. However, a significant increase in the frequency of the 14bp-DEL allele (70.0%) and of the +3142-C allele (55.0%) were found in patients compared to controls (57.0%; OR=1.65 (1.04-2.61) P=0.034 and 45.0% OR=2.68 (1.14-6.28) P=0.017, respectively). These variants confer greater stability to the HLA-G mRNA, leading to a potential increase in protein expression. Finally, 14bp DEL/DEL patients had lower survival rate (28.0%) than the rest of the patients (55.0%, P=0.041). Concomitantly, the DEL/C haplotype is more frequent in patients (54.6%) than in controls (44.4%; OR=1.74 (1.05-2.89) P=0.034). In conclusion, the 14bp-DEL and +3142-C variants are risk factors for the development and progression of gastric cancer; thus HLA-G may become a potential prognostic factor and target for the treatment of gastric adenocarcinoma.

Keywords: Biomarkers, immunotherapy, cancer immunology, miRNA

P-0766

Characterization of altered innate immune responses of RLTPR deficient patient

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RLTPR (CARMIL2) is a cytosolic scaffold protein which facilitates CD28 co-stimulation for T-cell activation. RLTPR facilitates recruitment of CARMA1, a cytosolic adaptor, along with the BCL10 and MALT1 which form CBM complex to CD28 site to activate NF-κB signaling pathway. The percentage of regulator T cells from the whole blood of 1 patient with RLTPR defect and 2 healthy controls were measured by cell flow cytometry. Peripheral mononuclear cells isolated from whole bloods of patients and healthy were stimulated with Pam3CSK4 (TLR1/2), Peptidoglycan (TLR2), Zymosan (TLR2) p(I:C) (TLR3), LPS (TLR4), Flagellin (TLR5), R848 (TLR7), Resiquimod (TLR7&8), D-ODN (TLR9), K-ODN (TLR9), transfected p(I:C) (RIG-I/MAVS), transfected LPS (Non-canonical Inflammasome), transfected flagellin (NLRC4), transfected Alum (NLRP3 Inflammasome), transfected p(dA:dT) (AIM2 Inflammasome), transfected cGAMP (STING) and transfected HSV (cGAS-STING) for 24 hours, then supernatants were collected to investigate IL-1β, TNF-α, IFN-α and IFN-γ secretion levels of patient and healthy controls with cytokine ELISA. Treg counts of the patient were reduced 6-fold as compared to healthy controls.TLR7 and TLR8 mediated IL-1β and TNF-α responses were impaired in patient. IFN-α response of the patient from all endosomal TLRs, STING and RIG-I/MAVS were reduced along with the impaired IFN-γ response upon TLR3, TLR7, TLR8, STING, RIG-I/MAVS and non-canonical inflammasome pathways. These results show that not only endosomal TLRs but also the cytosolic nucleic acid sensors were impaired in RLTPR patient which makes the patient vulnerable to viral infections.

This research was supported by TÜBİTAK 315S125 / 315S131 project and UMRAM Ministry of Development Vaccine Project.

Keywords: Cytokines and mediators, immunodeficiency, innate immunity

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POSTER PRESENTATIONS

P-0767

Cytomegalovirus infection is associated with an increase in aortic stiffness in older men which may be mediated in part by CD4 memory T-cells

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Cytomegalovirus (CMV) infection is associated with atherosclerosis, higher cardiovascular disease (CVD) risk, and an increase in memory T-cells (Tmem). T-cells are also implicated in CVD, independently of CMV infection. Thus, we examined the association between CMV (IgG) serostatus and central aortic (carotid-to-femoral) pulse wave velocity (cfPWV), an early predictor of CVD, and if this association might be reflected by the distribution of Tmem and/or other T-cell subsets. Healthy older volunteers (60-93 years) underwent routine clinical and laboratory evaluation, including cfPWV assessment, in eligible participants. Memory T-cells, CD28null T-cells, and CMV-specific T-cells were assessed by Flow-cytometry. The following associations were examined; CMV serostatus/cfPWV, CMV serostatus/proportion of Tmem, proportion of Tmem/cfPWV, CD28null T-cells/cfPWV, and CMV-specific T-cells/cfPWV. Linear regression models were used to adjust for age, sex, socioeconomic status, smoking, waist-to-hip ratio, cholesterol, and blood pressure as required. Statistically significant positive associations were found (p-values for the fully adjusted models are given); CMV serostatus/cfPWV in men ($p \leq 0.01$) but not in women, CMV serostatus/proportions of CD4 Tmem in men ($p \leq 0.05$) but not in women; proportions of CD4 Tmem/cfPWV among CMV-seropositive people ($p \leq 0.05$) but not CMV-seronegative. CMV infection increases the CVD risk of older men by increasing cfPWV. This may be partly mediated by increased proportions of CD4 Tmem that are increased in CMV+ older people, especially in men. Given the high prevalence of CMV worldwide, our findings reveal a significant global health issue. Novel strategies to mitigate CMV-associated CVD risk may be required.

Keywords: Adaptive immunity, ageing, immune senescence, inflammatory disease, memory, viral infections

P-0768

Study of 14 bp INS/DEL polymorphism of the 3'UTR region of the *HLA-G* gene in patients with uveitis

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HLA-G is a non-classical HLA class I gene encoding a molecule with immunomodulatory properties. The HLA-G protein has an important immunosuppressive and tolerogenic role in immunoprivileged organs, and variation in expression levels has been correlated with the risk of developing autoinflammatory pathologies such as uveitis, a disorder characterized by inflammation of the uvea. The *HLA-G* gene has different polymorphisms, some of them in the 3'UTR region, including the 14bp-INS/DEL polymorphism, which affects mRNA expression. In the present work, this polymorphism was studied by PCR in a group of patients with uveitis, with the aim of establishing a correlation between the 14bp-INS/DEL polymorphism and the risk of developing uveitis. For this purpose, the polymorphism was genotyped in DNA from 124 patients with anterior, posterior and intermediate uveitis, and from a group of 117 controls. The 14 additional base pairs insertion, makes the HLA-G mRNA more unstable, limiting its expression. No significant differences were observed in the frequency of INS (47.2% vs 40.6%) and DEL (52.8% vs 59.4%, Chi^2 p=0.15) alleles. Regarding genotypic frequencies, an overrepresentation, though not significant, of the INS allele was observed in the patient population compared to controls (21.0% vs 16.2%; p=0.14, OR 1.32 (95% CI 0.91-1.91)), under an additive inheritance model. The results obtained, thus far point to a possible association between HLA-G and uveitis. In addition, other polymorphisms of this region should be analyzed.

Keywords: Autoimmunity, autoinflammation, biomarkers, inflammatory disease

P-0769

Pregnant women with intrauterine growth restriction show differential profile expression of MicroRNA involved in immune response

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The pathophysiology of foetal growth restriction is defined by either an estimated foetal weight (EFW) by ultrasound for a given gestational age <10th or <3rd percentile for intrauterine growth restriction (IUGR) and small-for-gestational-age (SGA), respectively. Multiple causes have been associated with either entity such as genetics, malformation, or infection. This study aimed to isolate and characterised the extracellular vesicles (EVs) to identify surrogate microRNAs as biomarkers from peripheral blood of pregnant women with foetal growth restriction. 12 pregnant women were included in this study from 2019-2020 at the University Hospital of Salamanca, Spain. EVs were characterised using electron microscopy and nanoparticle tracking analysis (NTA). A TaqMan® array of 384 microRNAs was subsequently applied. 33.3% (n=4) of women were grouped as healthy control (HC), 33.3% (n=4) were grouped as IUGR, and the remaining as SGA for the stratification analysis of microRNA expression. The mir-146b, mir-450a, and mir-455 were significantly overexpressed in IUGR in comparison with HC and SGA. On the other hand, the expression of mir-454, mir-518d, and mir-548c decreased gradually as the severity of the pathology progressed. Neither the size nor the concentration of the EVs were significantly different amongst the study groups. Our preliminary results suggest that these MicroRNAs are implicated with antigen processing and presentation, TGF-β signaling pathway, oxidative stress, cell cycle, oocyte meiosis, and other pathways involved in viral and bacterial infections. These cellular processes could be deregulated due to differential expression on these MicroRNAs in women with IUGR.

Keywords: Biomarkers, cell signalling, endo- and exocytic vesicles in immunity, fetal immunity, miRNA

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POSTER PRESENTATIONS

P-0770

A novel frameshift mutation in *Malt1* gene resulted in defective TCR signaling and impaired immune response to fungal ligands

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A novel frameshift mutation in *Malt1* gene has been identified in a 3-year-old patient with diverse fungal infections in combination with severe diarrhea, vitiligo and failure to thrive. MALT1 is a crucial adaptor protein for NF-κB activation induced by TCR/BCR signaling and fungal recognition by certain CLRs. To understand the cellular level implication of MALT1 deficiency, we aimed to perform comprehensive analyses of T-cell response upon TCR stimulation. Additionally, we intended to investigate the immune response from MALT1-deficient monocytes induced by bacterial and fungal recognition. PBMCs from patient and healthy individuals were stimulated with anti-CD3/anti-CD28. CD4+ T-cell activation and proliferation was assessed through evaluation of CD25/ICOS upregulation and CSFE-dye dilution. Culture supernatants were used for cytokine measurement via ELISA. Phospho-p65 levels were determined in PMA/ionomycin stimulated PBMCs. TNF-α production in monocytes was analyzed from LPS, Zymosan and Depleted Zymosan-treated samples. TCR stimulation in patient's PBMCs resulted in defective T-cell proliferation, significantly diminished CD25 and ICOS upregulation, and reduced T-cell-specific cytokine production compared to healthy controls. Additionally, assessment of p65 phosphorylation upon TCR signaling revealed a complete loss of NF-κB activation in MALT1-deficient T-cells. Patient's monocytes showed normal TNF-α production in response to LPS (TLR4) stimulation. In contrast, Zymosan (TLR2/Dectin-1)-dependent TNF-α generation was significantly impaired compared to healthy donors. Furthermore, MALT1-deficient monocytes failed to constitute a TNF-α response upon stimulation with Depleted Zymosan (Dectin-1 ligand). Diminished pro-inflammatory cytokine response to fungal ligands concurrently with impaired T-cell activation may elucidate various fungal infections that the patient suffers from.

Keywords: Fungal infections, immunodeficiency, infectious disease, innate host defence, proliferative disorders

P-0771

Immune trombocytic purpura during the course of COVID-19 disease:three cases report

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Both the COVID-19 disease caused by SARS-CoV-2 itself and the drugs used in its treatment can cause immune thrombocytopenia (ITP). ITP may be primary and especially secondary to viral infections (HIV, EBV, CMV, HCV, SARS-CoV-2). Three cases of Covid-19 infection and immune thrombocytopenia are presented here. The first case; 69-year-old female patient with no comorbid disease platelet counts examined due to epistaxis on the 3rd day of favipiravir treatment with the diagnosis of Covid-19: 1000 / µL,The second case; 22-year-old male patient 7 days after receiving favipiravir treatment for Covid -19 disease, diffuse petechiae in the lower extremity the number of platelets examined upon occurrence: 1000 / Ml.Third case; A 55-year-old male patient with no comorbidity had ecchymosis and epistaxis in the lower extremity after suffering from Covid-19 disease, and the number of platelets checked was 1000 / µL, and the etiological reasons (autoimmune antibodies, hepatitis serology, TORCH panel, Brucella, AFAS) When thrombocytopenia was observed in the peripheral smear, 1mg /kg /day methylprednisolone was started in all three patients. IVIG treatment at a dose of 1g/kg was given to the second case because of steroid resistance. Platelet counts after treatment were as follows; It came as 211000 / µL, 230000 / µL, 187000 / µL. ITP should be suspected in case of thrombocytopenia that develops during the course of Covid-19 disease, SARS-CoV-2 should be accepted as a current viral agent that induces ITP and ITP treatment including corticosteroids should be initiated.

Keywords: Immunopharmacology, infectious disease, viral infections

P-0772

Tissue-restricted expression defines distinct and non-redundant functions of IL-1α and IL-1β in host defense against invasive bacterial infection

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IL-1α and IL-1β are potent pro-inflammatory cytokines, indispensable in restricting bacterial replication, limiting tissue damage and re-establishing homeostasis. Notwithstanding low sequence similarity and different regulation mechanism, both cytokines engage the same receptor. Stimulation of cells elicit comparable cellular responses; however, for poorly understood reasons, they are not redundant *in vivo*. In this study, we decoupled IL-1α and IL-1β functions that drive protective responses against invasive infection with *Streptococcus pyogenes*. IL-1β was central for efficient bacterial containment and recruitment of neutrophils to the infection site by inducing G-CSF and establishing emergency granulopoiesis. On the contrary, IL-1α seems to be utterly dispensable for pathogen elimination but governed reprogramming of liver metabolic pathways associated with adaptation to infection. The IL-1α-dominated hepatic regulation corresponded to high IL-1α induction in the liver during infection. Conversely, IL-1β was critical for transcriptome changes in the spleen which correlated with ample IL-1β and low IL-1α expression in this tissue. Our results uncover roles for IL-1α in resilience and IL-1β in resistance to bacterial infection. The data implicate a spatial restriction of both expression and action of IL-1α and IL-1β as a mechanism of their non-redundant functions.

Keywords: Biology of the immune system, cytokines and mediators, innate host defence, innate immunity, metabolic control of immune responses, neutrophils

P-0773

Patients suffering from long-term discomfort after initial mild COVID-19 disease exhibit dynamic changes in the lymphocyte compartment

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Despite mild disease during SARS-CoV-2 infection, many convalescent individuals continue to develop long-term symptoms clearly associated with former COVID-19 disease. Therefore, it is necessary to identify potential changes in immune cell composition that could contribute to the long-COVID syndrome, i.e. long-term discomfort persisting after acute COVID-19. We compared the peripheral leukocyte composition from n=60 convalescent long-COVID-19 patients two to twelve months after symptom onset with n=28 age matched unexposed donors by flow cytometry. Comparable frequencies and absolute cell numbers of lymphocytes, monocytes and granulocytes were observed between the two cohorts. However, CD4+ T cells were significantly increased in frequencies, absolute cell numbers and CD4/CD8 ratios in long-COVID-19 patients, showing a CD45RO-CCR7- terminally differentiated (TEMRA) phenotype with high CD57 expression as late memory marker. Moreover, long-COVID-19 patients displayed an unexpected B cell composition with increased frequencies of IgD+CD27-CD38+ naïve B cells and reduced proportions of CD27-IgD- effector memory B cells. The increase in the frequency and cell number of the CD4+ T cells indicates their sustained expansion and memory development. In contrast, naïve B cells seem to reconstitute during convalescence. In summary, our study reveals sustained changes in the immune cell composition of long-COVID, which may be associated with clinical long-term symptoms after recovery from COVID-19.

Keywords: Adaptive immunity, infectious disease, viral infections

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POSTER PRESENTATIONS

P-0774

Lower numbers of blood central memory T cells and altered phenotype of regulatory cells are present in newly diagnosed relapsing-remitting Multiple Sclerosis patients

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Failure at maintaining tolerance, particularly of T cells, which are generated in the thymus, is described to underlie multiple sclerosis (MS) pathogenesis. Patients with relapsing-remitting MS (RRMS), the most common clinical course of the disease, were shown to have reduced thymic function, an aged adaptive immune system and hampered activity by regulatory cells. However, the alterations present at clinical RRMS diagnosis are seldom explored. We aim to evaluate thymic function, and the phenotype of conventional and regulatory T cells (Treg), and of natural killer (NK) cells in newly diagnosed RRMS patients, naïve for disease modifying drugs (n=27), and in sex and age-matched healthy controls (HC; n=35). Newly diagnosed RRMS patients present equivalent thymic function to HC as observed by similar numbers of CD4+ recent thymic emigrants and sj/β T cell receptor excision circles levels. A higher naïve/memory CD4+ T cells' ratio was observed in RRMS patients, which was found to be mostly due to lower numbers of central memory cells. Similar but milder findings are observed for CD8+ T cells. Regarding regulatory T cells, patients have higher numbers of naïve and lower of activated HLA-DR+ cells. Percentages of immature NK cells expressing KLRG1 and of mature NK cells expressing NKp30 are higher in RRMS patients. Altogether, at diagnosis RRMS patients present reduced central memory T cells, which unbalances naïve and memory subsets, and altered regulatory cell populations, suggestive of a less suppressed immune system. Functional studies are essential unravel the mechanisms underlying these alterations and their impact on RRMS onset.

Keywords: Autoimmunity, immune senescence, memory, multiple sclerosis, NK cells, regulatory cells

P-0775

Macrophages and dendritic cells are not the major source of pro-inflammatory cytokines upon SARS-CoV-2 infection

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The exact role of innate immune cells upon infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and their contribution to the formation of the corona virus-induced disease (COVID)-19 associated cytokine storm is not yet fully understood. We show that human *in vitro* differentiated myeloid dendritic cells (mDC) as well as M1 and M2 macrophages are susceptible to infection with SARS-CoV-2 but are not productively infected. Furthermore, infected mDC, M1-, and M2 macrophages show only slight changes in their activation status. Surprisingly, none of the infected innate immune cells produced the pro-inflammatory cytokines interleukin (IL) 6, tumor necrosis factor (TNF)-α, or interferon (IFN)-α. Moreover, even in co-infection experiments using different stimuli, as well as non-influenza (non-flu) or influenza A (flu) viruses, only very minor IL-6 production was induced.

In summary, we conclude that mDC and macrophages are unlikely the source of the first wave of cytokines upon infection with SARS-CoV-2.

Keywords: Cytokines and mediators, dendritic cells, infectious disease, innate immunity, macrophage, viral infections

P-0777

New biological markers in the diagnosis and follow-up of brucellosis

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Brucellosis is the most common zoonotic disease Worldwide. Patients usually present with nonspecific symptoms, and physical examination findings. Specific tests are essential in the diagnosis, and follow-up of brucellosis and predicting of chronicity. This study was designated to find new markers that can be used in the diagnosis of acute and chronic brucellosis and predicting chronicity. Patients who were diagnosed with brucellosis with clinical and laboratory findings were included in the study. The patients were grouped as acute (0-2 months), subacute (2-12 months) and chronic (over 12 months). Levels of serum ABI3, PPP2R4, DDIT4L, WDR33, ZFP1, PIAS4, CLEC12B and IDO (SunRed Bio, Shanghai, China) were studied. One hundred and twenty nine patients who met the inclusion criteria were included in the study. There was no statistically significant difference between the groups in terms of age and gender distribution. Acute, chronic, recovering, and control groups, respectively, ABI3(ng/L);18.88(7.29), 16.57(4.47), 2.81(0.35), 3.15(2.91) (p<0.001), CLEC12B(ng/L);449.26(207.94), 207.94(219.52), 17.08(5.74), 23.31(5.00) (p<0.001), DDIT4L(pg/ml); 4.72(1.38), 4.67(0.97), 5.98(0.76), 5.87(5.53) (p<0.001), IDO(U/L);6.88(2.72), 6.23(2.10), 75.35(17.11), 79.26(140.64) (p<0.001), PIAS(pg/ml);8.01(3.04), 6.87(2.47), 9.98(1.65), 10.66(8392) (p<0.001), PPP2R4(ng/ml);42.85(16.72), 41.11(17.66), 3.61(0.69), 7.52(2.77) (p<0.001), WDR33(pg/ml);3.25(0.81), 3.13(0.60), 2.75(1.14), 2.89(3.67) (p=0,115), ZFP1(pg/ml);310.31(97.57) 219.69(83.98) 317.46(117.59) 395.71(408.30) (p<0.001). Our study has revealed that increased levels of ABI-3, CLEC12B and PPP2R4 can predict chronicity, and decreased levels can be used to monitor response to treatment. Decreased levels of DDIT4L, IDO, PIAS4 and ZFP-1, can be used to predict chronicity, and their increased levels can be used to evaluate response to treatment.

Keywords: Biology of the immune system, biomarkers, infectious disease

P-0778

Novel Role for complement 5a receptor 2 (C5aR2) in controlling the formation of pulmonary metastases

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The second receptor for the complement component and anaphylatoxin C5a was initially considered a decoy receptor. This view has been challenged by findings demonstrating that C5aR2 activation induces pro- and anti- inflammatory functions, depending on context and cell type. However, the functional properties of C5aR2 remain enigmatic. In the absence of C5aR2 we observed increased expression of the activating receptor NKp46 in NK cells, leading to the hypothesis that C5aR2 negatively regulates NKp46 expression and therefore is involved in modulating NK cell cytotoxic activity. Thus, C5aR2 might be relevant for influencing the outcome of metastatic cancer, where NK cell cytotoxicity is pivotal. To test this, we investigated the effects of C5aR2 deficiency in a NK cell-dependent murine pulmonary metastases model. B16-F10 murine melanoma cells were injected intravenously into wild type and C5aR2-deficient C57BL/6 mice. The disease progress was monitored daily. Mice were sacrificed 7, 14 or 21 days post injection. Lung surface metastatic foci were enumerated. Our investigations revealed that C5aR2-deficient mice developed fewer metastatic foci on the lung surface compared to their wild type counterparts. Consistently, C5aR2-deficient mice featured a lower disease score and a better overall survival over 21 days. Our findings demonstrate for the first time the importance of C5aR2 in the response to metastatic cancer. We hypothesize that C5aR2 negatively regulates NKp46 surface levels during NK cell development. In the absence of C5aR2 NKp46 expression is increased, leading to a more potent NK cell cytotoxicity. Therefore, C5aR2 could prove a useful target to control metastases formation.

Keywords: Animal models, cancer immunology, complement, *in vivo* tumor models, innate immunity, NK cells

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POSTER PRESENTATIONS

P-0780

Effect of probiotic on TLR-2, TLR-4 and NF-kB expression in the knee joint cartilage of rats with osteoarthritis

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The innate immune response is important in the pathogenesis of osteoarthritis (OA). TLR signaling, which activates nuclear factor NF-κB, is crucial for the control of catabolism in cartilage in OA. Several recent studies have reported the influence of probiotics (PB) on inflammatory processes during musculoskeletal pathologies, but these results are limited. The aim of this study was to investigate the effect of PB («Symbiter», Ukraine) on the expression of TLR-2/4, NF-κB and genes encoding them in the knee joint, and to determine the content of soluble forms sTLR-2/4 in the serum of rats with monoiodoacetate (MIA)-induced OA. The expression of TLR-2/4 and NF-κB in joint tissues were determined by the standard immunohistochemical procedures using primary antibodies. The location, intensity and number of immunopositive cells were considered. Expression of *Tlr2*, *Tlr4*, *Nfkb1* genes in cartilage were analyzed using one-step RT-PCR. The content of sTLR-2/4 was measured in the serum by enzyme-linked immunosorbent assay. MIA-induced OA was accompanied by degenerative joint disorders and stimulation of pro-inflammatory catabolic signaling pathways, increasing expression of TLR-2/4, NF-kB and *Tlr2*, *Tlr4*, *Nfkb1* genes in joint tissues and sTLR-2/4 content in the serum, that indicated possible involvement of chondrocytes in the adaptive immune response. PB administration had a positive action on the expression and content of above-mentioned parameters in the joint and serum during slowing the progression of OA. Thus, our data show that PB may affect the regulation of TLR-2/4-mediated NF-κB inflammatory pathway. Such an effect can be associated with its immunomodulatory, anti-inflammatory and antioxidant properties.

Keywords: Animal models, cell signalling, inflammatory joint diseases, microbiome and environmental factors, tissue damage and repair

P-0781

The main anti-inflammatory regulator IL-10 in the course of rabbit haemorrhagic disease virus (RHDV) infection

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IL-10 as a primary anti-inflammatory regulator is crucial to protect the host organism from organs damage during the acute phases of the immune response. IL-10 is produced by various leukocytes and is expressed in tissue epithelial cells. RHDV is an etiological factor of rabbit haemorrhagic disease. The disease usually leads to the death of animals due to multiple organ failure resulting from the development of disseminated intravascular coagulation. The present study aimed to quantify IL-10 expression patterns in liver tissue and lymphoid tissue-spleen of rabbits experimentally infected with RHDV. The specimen of liver and spleen tissue were collected from rabbits infected with RHDV, variant GI.1a (RHDVa) (n=10) and healthy controls (n=10). TaqMan®Real-Time PCR Assays has been used to analyze the expressions of IL-10. The mortality rate of the animals was 100%. Within the liver tissue of RHDV infected rabbits, IL-10 mRNA level was sharply increased (27-fold, p=0.01). Meanwhile, in spleen tissue has been observed 38-fold increase of IL-10 mRNA expression level compared to the control group (p≤0.0001). We found that RHDV infection induces rapid high-level production of IL-10 in the infected tissues as a natural inhibitor of inflammation. The increased expression of IL-10 in tissues of infected animals seems to represent an excessively intense local inflammatory response. It is considered that IL-10, despite its high expression, is an insufficient mechanism to block inflammatory reactions during RHDV infection.

This study was supported by the grant GSDUS 26/1/2021 from Doctoral School of the University of Szczecin.

Keywords: Biology of the immune system, cytokines and mediators, infectious disease, tissue damage and repair, viral infections

P-0782

Searching for lncRNAs stimulating the expression of the MYC proto-oncogene in B-lymphocytes and B-cell lymphomas

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The translocation of the Myc gene to the immunoglobulin heavy chain locus (IGH/MYC) is a characteristic chromosomal aberration of Burkitt lymphoma. This translocation leads to an increased expression of Myc in B-cells. This study is devoted to the search of enhancer RNAs (eRNAs) that can affect the proto-oncogene Myc expression in the case of its IGH/MYC rearrangement. In general non-coding RNAs play an important role in regulating cellular processes, blocking or activating transcription and translation, or interacting with the protein directly. In tumor cells, they can greatly affect the further development of the tumor via gene expression alteration. In our study, potential eRNAs from the IGH locus have been selected using an in-silico algorithm. Knockdown of a few of them has led to a reduced expression of the MYC gene in cells of the Namalwa B-lymphoblastoid cell line with IGH/MYC translocation. Moreover, overexpression of these eRNAs in the same cell line has increased the level of Myc mRNA. However, the overexpression of the eRNAs does not affect the level of MYC in cells of the MP1 B-lymphoblastoid cell line without IGH/MYC translocation or in B-lymphocytes.

Keywords: Cell signalling, B lymphocytes, lncRNA

P-0783

Immunological features beyond the CD4/CD8 T-cell ratio values in older people

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Since the CD4/CD8 ratio inversion was associated with mortality among aged populations, our aim was to explore immunological features beyond the CD4/CD8 values in older people. Sixty-five subjects (>65 years-old) were classified according to CD4/CD8 values in: lower (<1.4), intermediate (1.4-2) or higher (>2). PBMCs were immunophenotyped (CD3, CD4, CD8, CD25, CD45RA, CD31, PD1, CD98, CD57, HLA-DR, CD95, CD27, integrin-β7, CD28, Ki67, FoxP3 and CTLA4) by flow cytometry. Soluble biomarkers were quantified. The sj/β TRECs (T-cell Receptor Excision Circles) ratio was determined as thymic output. Kruskal-Wallis/Mann-Whitney tests were performed for group comparisons. Compared to others, the lower group showed lower thymic output, with depleted naïve T-cells and more mature T-cells. Their CD4 subset was enriched in CD95+ but depleted of CD98+ cells. Specifically, the regulatory T-cell compartment was enriched in CTLA-4+ cells. Their CD8 subset showed more CD95+ cells but less integrin-β7+ cells. Interestingly, the CD4 pool showed greater differences than the CD8 pool, mostly for cellular senescence in the intermediate group. Among inflammatory biomarkers, only hsCRP showed elevated in the lower group, but also negative correlations appeared between CD4/CD8 ratio and β2-microglobulin or sCD163. Subjects with lower ratios also showed trends to have more co-morbidities and less independence according to the Barthel index. In older people, CD4/CD8 ratio values reflect variable thymic output and immune profiles for CD4 and CD8 T-cells, which may affect immune capabilities, and hence their health status. CD4/CD8 ratio could be used as an integrative marker of biological age.

Keywords: Ageing, biology of the immune system, biomarkers, chronic inflammation and fibrosis, immune senescence, immunological techniques

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POSTER PRESENTATIONS

P-0784

Inflammatory profile of treated HIV-subjects with inverted CD4/CD8 despite normal CD4

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To explore the inflammatory profile of treated HIV-subjects achieving normal CD4 but failing to normalize the CD4/CD8 ratio. HIV-subjects from CoRIS with ≥500 CD4 after three years under antiretroviral treatment (ART), were classified into LOW (<0.8) and HIGH (>1.2) CD4/CD8 ratio groups. Pre- and post-ART plasma samples were analysed for inflammation-related markers: GM-CSF, TNFα, IFNα, IFNγ, ILs-1α, 1β, 4, 6, 8, 10, 12p70, 13, 17A, IP-10, MCP-1, MIP1α, MIP1β, sE-Selectin, sP-Selectin, ICAM-1 by ProcartaPlex™ Human Inflammation Panel 20plex. Biochemical parameters (D-dimers, lactateDH, hsCRP, ferritin, β2M, homocysteine) were also determined. Transversal and longitudinal comparisons were performed by univariate and bivariate analyses. CD4/CD8 ratio values were 0.63 [0.55-0.73] vs. 1.55 [1.33-1.72] in LOW and HIGH groups (n=18 each), respectively. Both groups were similar in all demographic and clinical/therapy characteristics, exempting for values of CD4, CD8 and CD4/CD8 ratios, both pre- and post-ART. None of the analysed parameters was different between groups in post-ART samples. However, at baseline, the LOW group showed higher levels of β2M, D-dimers, and E-selectin, but lower levels of MIP-1α. Nevertheless, such significant differences were lost after adjusting by baseline CD4/CD8 ratio. During ART, the LOW group greatly reduced several inflammatory markers, including β2M and D-dimers. The HIGH group only increased homocysteine and decreased IP-10 levels. Interestingly, we found correlations between post-ART CD4/CD8 ratio and baseline levels of IFNγ and MIP-1α in the HIGH group. Immunological alterations of treated HIV-subjects with normal CD4, but persistently inverted CD4/CD8 ratio, do not include an enhanced inflammatory profile.

Keywords: Biology of the immune system, biomarkers, cytokines and mediators, immunodeficiency, inflammatory molecules

P-0786

In vitro addition of adalimumab decreases the concentration of several cytokines produced by PBMCs favouring an anti-inflammatory environment in rheumatoid arthritis patients

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Rheumatoid arthritis (RA) is a systemic Th1 autoimmune disease characterised by synovitis leading to cartilage and bone destruction. The aim of this study is to analyse the Th1/Th2/Th17 cytokines profile found in serum and released by PBMCs at different stages of RA and to test the effect of adalimumab. Sera were obtained from 5 healthy volunteers (A), 5 recently diagnosed RA patients (B), 3 RA patients in outbreak (C) and 5 patients in remission (D). PBMCs were isolated by density gradient centrifugation using anticoagulated blood from 5 A, 2 B, 4 C and 6 D patients. PBMCs were cultured in RPMI supplemented with 10% FBS, 1% antibiotics and 1% glutamine and stimulated with 10 μg/ml PHA. Adalimumab (8 μg/ml) was also added. After 3 days of incubation at 37°C and 5% CO_2, the supernatant was collected. The cytokine profile was analysed with "BD™ CBA Human Th1/Th2/Th17 Cytokine kit", FACSCanto and FCAP array. Sera samples show a pro-inflammatory profile in B, while IL-10 increases in C. Cytokines released by PBMCs in unstimulated conditions showed in every RA patient an increase of pro- and anti-inflammatory cytokines. The addition of adalimumab decreased cytokine levels, but increased IL-10 in C. After stimulation, a general cytokine increase was found. The addition of adalimumab decreased all cytokine concentrations. In A and B, we found a pro-inflammatory environment, while C subjects have an anti-inflammatory profile. Adalimumab decreases pro-inflammatory cytokines, favouring the presence of an anti-inflammatory environment.

Keywords: Autoimmunity, cytokines and mediators, rheumatoid arthritis

P-0787

Generation and characterization of a broadly-reactive monoclonal antibody against fish parvalbumins

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Parvalbumins, knowing as main fish allergens, are small and highly stable calcium-binding proteins found in fish muscle. Common carp parvalbumin (Cyp c 1) is one of the commonly consumed fish species and is highly homologous to salmon and Atlantic cod parvalbumins. Monoclonal antibodies (MAbs) against fish allergens may provide useful reagents for fish allergy studies and diagnostics. The MAb against recombinant Cyp c 1-MBP was generated by hybridoma technology and characterized using ELISA. Recombinant salmon parvalbumin (Sal s 1), Baltic cod parvalbumin (Gad c 1), Atlantic cod parvalbumin (Gad m 1) and Atlantic herring parvalbumin (Clu h 1), fused with maltose-binding protein (MBP), were expressed in *E.coli* and purified. The pattern of reactivity of the MAb with different fish parvalbumins was analysed by ELISA and Western blot. The MAb (clone 3F6) against Cyp c 1-MBP was generated. It was shown to be of IgG1 isotype and demonstrated high affinity to the antigen. The cross-reactivity of the MAb with recombinant Sal s 1-MBP, Gad c 1-MBP, Gad m 1-MBP and Clu h 1-MBP was investigated. The MAb 3F6 reacted with all studied recombinant fish allergens and was shown to recognise linear epitopes of target allergens by Western blot. This study provides new data about the specificity of the developed MAb against Cyp c 1-MBP that may be used as a promising tool for antigenic characterization of fish parvalbumins. Moreover, these results indicate that all four fish parvalbumins share common epitopes that are recognised by the MAb generated against Cyp c 1-MBP.

Keywords: Allergen-induced immune responses, antibody, engineering of antibodies and nanobodies

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POSTER PRESENTATIONS

P-0788

Gut OX40+CD4+ T-cells strongly correlate with markers of progression in treated HIV infection

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OX40 (TNFRSF4) has a protector role in memory T-cells, particularly during clonal proliferation. OX40+CD4+T-cells are metabolically active, highly expressing Glut-1. In treated-subjects, circulating OX40+CD4+T-cells are enriched for clonally expanded HIV-1 sequences and Glut1+OX40+CD4+T-cells are permissive to *in vitro* HIV-infection. Thus, OX40+CD4+T-cells and HIV-reservoir are linked. To characterize this subset in gut mucosa, main site for HIV-reservoir, and explore relations with relevant progression markers. Biopsies of caecum and terminal ileum of treated-HIV-subjects (n=32) were obtained. Proliferating subsets, OX40+CD4+ and Ki67+CD4+T-cells were analyzed in MMCs and PBMCs. Several tissue T-cell subsets impacting mucosal integrity and homeostasis were also analyzed (Treg, Th17, Th22). Mucosal damage was estimated using a semi-quantitative scale by observation of histological sections. Soluble markers of inflammation (CRP, D-dimers, β2M) and thymic output (δ/β TREC ratio) were quantified. Spearman rank test was used to explore associations. Higher frequencies of OX40+CD4+T-cells were observed among mucosal, particularly at caecum, than circulating cells. Ki67+CD4+subsets slightly correlated with those expressing OX40 but were much less frequent in gut locations. The strongest associations were observed between caecum OX40+CD4+T-cells and nadir CD4 ($p<0.001$), CD4/CD8 ratio ($p<0.01$), thymic output ($p<0.001$), mucosal damage at caecum ($p<0.001$) and neutrophil-to-lymphocyte ratio ($p<0.05$). A strong inverse correlation was also observed between caecum OX40+CD4+T-cells and Th22 ($p=0.001$). Mucosal OX40+CD4+T-cells, particularly caecum, show notable associations with mucosal damage as well as relevant clinical, inflammatory and homeostasis-related parameters. Besides potentially representing a main cellular HIV reservoir, this cellular subset could be also involved in main homeostasis-related alterations subjacent in treated-subjects.

Keywords: Adaptive immunity, immunodeficiency, infectious disease, viral infections

P-0789

Synergistic interaction of estradiol with myeloid derived suppressor cells and T regulatory cells in pre-term birth

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Pre-term birth (PTB) is one of the major risk factors for neonatal mortality and morbidity worldwide. The aim of the study was to underpin the synergistic interaction of immunosuppressive T regulatory cells and Myeloid derived suppressor cells (MDSCs) with steroidal hormones in regulating maternal-fetal tolerance in PTB and their contribution towards compromised immunity of PTB babies. Peripheral blood from 20 pregnant mothers under spontaneous term and pre-term labor were collected along with their corresponding cord blood samples. Immunophenotyping of MDSCs, T regulatory cells and T cells were performed using flow cytometry. Serum samples were isolated from collected fresh blood samples in normal vial by centrifugation and were further processed for hormones estimation. Statistical analysis was done using GraphPad Prism software. PTB delivering mothers showed significant decrease in pooled MDSCs frequency specifically Granulocytic-MDSCs and T regulatory cells when compared to term controls. On contrary, PTB babies showed significant increase in pooled frequency of MDSCs, specifically Monocytic MDSCs and T regulatory cells when compared to term babies with subsequent decrease in total T cells (Th and Tc). Cord serum estradiol concentration in PTB babies positively correlated with the frequency of MDSCs and M-MDSCs. Alteration in immunological and hormonal milieu may set in early signals of parturition and initiate labor, resulting in pre-term delivery. Synergistic interaction of estradiol with MDSCs and T regulatory cells regulates the immune system in newborns.

Keywords: Fetal immunity, myeloid derived suppressor cells, regulatory cells, reproductive immunology

P-0790

Using flow cytometric cell distribution for predicting the severity of acute cholecystitis

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Acute cholecystitis develops in 20% of patients who have symptomatic gallstones. The aim of our study is to determine the changes in immune cells in patients with acute cholecystitis according to the severity, and predicting the prognosis. Patients who applied to Istanbul Training and Research Hospital with acute cholecystitis between 01.09.2019 and 01.06.2020 were included in the study. Patients were divided into three groups as mild, moderate and severe. Also healty volunteers were included as control group. Blood samples were obtained from all volunteers at the time of hospitalization, helper T lymphocytes, cytotoxic T lymphocytes, and HLA-DR expressions on CD 14+ monocytes were measured on by flow cytometry.

A total of 56 volunteers were included in the study. The mean age of all patients was 49.02 ± 16.34 years, and the female / male ratio was 11/17. In the study, there was no significant difference between the groups in terms of total lymphocyte count, CD3+ cell, CD4+ cell, CD8+ cell, CD4+/CD8+ cell ratios. A significant increase was observed in total leukocyte count and HLA-DR expression as the severity of cholecystitis increased. Although not significant, as the severity of the disease increases, the total lymphocyte count and the percentage of total CD3 decrease and the CD4 / CD8 ratio increases. The evaluation of lymphocyte count, total CD3+ cell ratio, CD4+/CD8+ ratio and HLA-DR expression on monocytes at the time of admission to the hospital may lead the clinician to have information about the prognosis of the disease.

Keywords: Adaptive immunity, infectious disease, inflammatory disease

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POSTER PRESENTATIONS

P-0791

Expression of the B-cell stimulatory factors BAFF and APRIL in gastric precancerous lesions and gastric cancer

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B cell stimulatory factors BAFF (B cell-activating factor) and APRIL (a proliferation inducing-ligand) have been previously shown to induce B cell maturation and survival. APRIL has been shown to over expressed in various cancers including gastrointestinal cancers. Additionally, BAFF was shown to accumulate in *Helicobacter pylori*-infected chronic gastritis patients. We investigated whether expression levels of BAFF and APRIL changes between controls with no gastric malignancies, gastric precancereous lesions and gastric cancer. We assessed the expression levels of BAFF and APRIL in total 74 patients (30 with gastritis, 19 with gastric ulcer, 10 with gastric cancer) and 15 controls by real-time quantitative PCR of gastric biopsy samples. In the selected cases immunohistochemical staining was performed of BAFF expression in CD68-positive macrophages and pan-cytokeratin-positive epithelial cells on paraffin-embedded gastritis and gastric ulcer samples. BAFF and APRIL were significantly up-regulated in gastric tissue of gastritis ($p< 0.05$), gastric ulcer ($p< 0.05$) and gastric cancer ($p<0.001$) compared with controls. No differences can be shown for the same genes between gastritis, gastric ulcer and gastric cancer groups. Immunohistochemical studies (n=2) suggested that expression of BAFF in macrophages and epithelial cells in gastritis and gastric ulcer patients. The higher expression of BAFF and APRIL in gastric lesions compared to control suggests an early involvement of BAFF and APRIL in gastric carcinogenesis. Additionally, the significance of the expression of BAFF in both macrophages and epithelial cells will be under investigation.

Keywords: B lymphocytes, cancer immunology, macrophage

P-0792

Influence of *H.pylori* density on PD-1:PD-L1 expression in gastric precancerous malignancies

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Chronic *Helicobacter pylori (H.pylori)* infection may lead to gastric cancer development. Tumor cells escape from immune response through expression of programmed death ligand 1 (PD-L1), which interacts with programmed cell death protein 1 (PD-1) on immune cells. In this study, we evaluated the influence of *H.pylori* density on the development of gastric immunopathology. A total of 86 gastric tissues of patients with gastritis (40), gastric ulcer (17), and gastric cancer (14) and controls without any gastric malignancies. were examined for the expression of immune-checkpoint inhibitors with real-time quantitative PCR. Moreover, *H.pylori* densities were assessed in Giemsa stained paraffin-embedded tissues. The intensity of PD-1 and PD-L1 mRNA expression was increased in gastric tissue of patients with gastric ulcer and gastric cancer compared with control subjects. Also, PD-1: PD-L1 expression was significantly higher in patients with gastritis who were infected with a marked density of *H. pylori* compared to its mildly infected counterparts. A statistically significant positive inter-correlation of PD1 and PD-L1 was determined in all disease groups. Our results suggests that the presence of PD1 and PD-L1 correlates with gastric cancer progression. Additionally, a positive association was present between the *H. pylori* density and expression of PD-1 and PD-L1 in gastric tissues of gastritis patients.

Keywords: Bacterial infections, cancer immunology, checkpoint inhibition

P-0793

Arginase 1 of myeloid cells accounts for disease chronicity in *Leishmania mexicana*-infected mice

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The enzymes arginase (Arg) 1 and Arg2 cleave L-arginine into urea and ornithine. Ornithine is a precursor of polyamines needed for cell proliferation. Arginase activity is antagonized by IFNγ-mediated induction of type 2 nitric oxide (NO) synthase (NOS2) which converts L-arginine into citrulline and antimicrobial NO. Here, we tested whether and how host cell arginases affect the course and the immune response of chronic cutaneous leishmaniasis (CL) caused by *Leishmania (L.) mexicana*. Following infection of C57BL/6 mice with *L. mexicana*, Arg1 mRNA was already present in the infected skin at around day 20 p.i. and massively increased thereafter until day 60 p.i. when pathology became prominent in WT mice. LC-MS metabolomics showed that the strong induction of Arg1 led to a depletion of L-arginine and significant rise in polyamines at the skin site of infection. Using IL-10ΔCd4 mice, $CD4^+$-T-cell-derived IL-10 was identified as Arg1 inducer. Unlike WT controls, mice with a cell-type-specific deletion of Arg1 (Arg1ΔTie2 or Arg1ΔCx3cr1) showed strongly reduced pathology and ultimately resolved their skin lesions despite parasite persistence. Interestingly, the clinical cure of Arg1-deficient mice did not result from increased NOS2 activity during the healing phase, because the NO levels detected in WT and Arg1ΔTie2 and Arg1ΔCx3cr1 tissues were comparable. Single cell RNAseq data from infected skin of WT and Arg1-deficient mice at day 35 p.i. rather revealed that alterations in the T cell compartment and myeloid cell recruitment are involved in Arg1-dependent induction of chronic pathology.

Keywords: Infectious disease, metabolic control of immune responses, myeloid cells, RNAseq

P-0795

Antiviral activity of ouabain against a Brazilian zika virus strain

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Zika virus (ZIKV) is an emerging flavivirus associated with neurological disorders, such as Guillain−Barre syndrome in adults and Congenital Zika Syndrome (CZS) in neonates. Currently, no specific vaccine or antiviral are available to treat this infection. Ouabain is a cardiotonic steroid able to inhibit the Na+/K+-ATPase pump. It has been described as an immunomodulatory substance by our group. In addition, several studies demonstrated the antiviral activity of ouabain using different models. Thus, this work aimed to evaluate the antiviral activity of this substance against a Brazilian ZIKV strain. Vero cells were ouabain treated before and after the infection. The antiviral effect was assessed by a fifty-percent tissue culture infection dose (TCID50) and reverse transcription quantitative PCR (RT-qPCR). Ouabain presented dose-dependent inhibitory effects against ZIKV. The reduction of the virus was accompanied by a decrease in ZIKV RNA levels, suggesting that the mechanism of ZIKV inhibition by ouabain occurred at the replication step. Moreover, time-of-addition experiments showed that ouabain did not interfere in the entry stage of the virus. Together, these data demonstrate the antiviral activity of ouabain against a Brazilian ZIKV strain and suggest that this substance interferes in the post-entry stage of the ZIKV life cycle. Additionally, these data evidence the potential of cardiotonic steroids as promising antiviral agents.

Keywords: Immune regulation and therapy, infectious disease, molecular immunology, viral infections

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POSTER PRESENTATIONS

P-0796

Meta-analysis of soluble HLA-G revealed an association with colorectal cancer onset

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Human leukocyte antigen-G (HLA-G) could be secreted as soluble HLA-G molecule (sHLA-G). This isoform has been linked to advanced cancer types. In this study, we meta-analyzed case-controls studies to evaluate sHLA-G levels linkage to colorectal cancer (CRC). A comprehensive systematic search was performed to investigate the association between sHLA-G in different fluidics (serum, plasma, ascite, saliva) and CRC onset. We identified five studies with a total of 380 patients and 243 healthy controls (HC). Association of sHLA-G levels with CRC was determined through the calculation of standardized mean differences (SMD) and its corresponding 95% confidence interval (CI). Soluble HLA-G levels, in all fluidics, were significantly higher in cases than in HC (N=5, SMD= 2.165, 95% CI=0.506-3.824, P=0.011). In serum/plasma samples, sHLA-G was near 3 fold times higher in CRC patients compared to HC (N=3, SMD= 2.686, 95% CI=0.159-5.214, P=0.037). In Asians, our meta-analysis revealed also a strong difference between sHLA-G in serum/plasma CRC compared to HC (N=2, SMD=2.266, 95% CI=2.016-2.516, P=0.000). The meta-analysis revealed a statistically significant increased level of sHLA-G patients with CRC in comparison to HC group for overall population and for Asians. Our findings, needing consolidation with further larger studies, suggested the potential use of sHLA-G to monitor CRC onset.

Keywords: Cancer immunology, biology of the immune system, biomarkers

P-0799

Neutrophil extracellular traps interaction with macrophages in wound healing

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In chronic wounds neutrophils set inadequate inflammation and sustain it together with macrophages. Factors that regulate timely neutrophils clearance and resolution of inflammation are not fully understood. To reveal spatiotemporal organization of neutrophils and neutrophil extracellular traps (NETs), we used the full thickness wound healing model in wildtype and diabetic mice. Ly6G gene expression and immunofluorescence (IF) analysis showed that in wildtype mice neutrophils infiltrate the wound bed at day 1, over time concentrate on the upper wound area and scab, before they are cleared from the tissue. Elastase activity peaks after initial injury and depletes with the disappearance of neutrophils as confirmed by IF analysis. Comparatively, Ly6G expression in diabetic wounds is lower, IF signals were found in wounds at day 5. Neutrophils persist in the wound bed, associated with increased elastase and gelatinase activity. NETs were identified by IF colocalisation of citrullinated histone, DNA and elastase. Consistent to mice, human diabetic wounds have more NETs in the wound bed, while acute wounds have NETs at open wound margins. *In vitro* experiments showed that NETs themselves release IL-1β. NETs co-culture with macrophages induce inflammasome-dependent IL-1β release in macrophages after TLR stimulation. Interestingly, the presence of saturated fatty acids together with NETs promoted IL-1β release independent of TLR signaling. In conclusion, our data suggest different pathways of neutrophil and NETs clearance in normal and diabetic wounds. Persistent NETs in diabetic wound may result in macrophage recognition and inflammasome activation, which contributes to increased inflammation in diabetic wounds.

Keywords: Neutrophils, macrophage, skin diseases, cytokines and mediators, immune communication, inflammatory disease

P-0801

Dysregulation of IL-17 and Calprotectin in the female reproductive tract affects pregnancy outcome following assisted reproductive therapy

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Embryo implantation is a critical stage of assisted reproductive technology (ART) and an appropriate endometrial immune environment is fundamental for a successful pregnancy. Here, we compared endometrial tissue from women who underwent successful (N=9) versus unsuccessful (N=11) ART cycles, to identify differences in their immune profile Mid-luteal phase endometrial biopsies were collected from 20 nulliparous women with unexplained infertility, who were undergoing ART; RNA-sequencing analysis was performed and endometrial and serum levels of IL-17A were measured by ELISA. Immune cell profiling was performed using Cibersortx. IL-17A and calprotectin, the antimicrobial peptide (AMP) dimer constituted by S100A8 and S100A9, were identified by immunohistochemistry in endometrial biopsies. AMP release upon IL-17A treatment in female reproductive tract (FRT) cells was measured by real-time qPCR. Data were analysed using GraphPad Prism. RNAseq revealed 204 differentially expressed genes (DEG) and particularly over-represented pathways analysis showed S100A9 and the 'IL-17 signalling pathway' to have decreased expression in the pregnant group. IL-17A protein levels were significantly increased in serum and endometrial tissue from 'non-pregnant' women, despite immune cell profiling showed similar proportions in the two groups. Immunohistochemistry on endometrial biopsies, displayed IL-17A and calprotectin in the endometrial stromal compartment to correlate with CD45 expression. *In vitro* stimulation of FRT epithelial cells by IL-17A stimulated AMPs expression. Differential expression of IL-17A and related genes may reflect functional changes in endometrial innate immunity, modifying receptivity for embryo implantation. Abnormal levels of circulating IL-17A may provide a screening tool for patients likely to have negative reproductive outcomes in ART.

Keywords: Biomarkers, cytokines and mediators, innate immunity, reproductive immunology

P-0802

Immunoprofiling of influenza vaccine responsiveness - transforming big data into knowledge for improved and adapted vaccination strategies

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Seasonal influenza infections represent a global health threat with up to 3 to 5 million cases of severe illness annually leading to a large economic burden. Children, elderly, individuals with comorbidities as well as immunocompromised individuals such as liver cirrhosis patients are at particularly high risk to develop severe disease. Although vaccination represents the most cost-efficient tool to prevent infectious diseases, vaccine-induced immune responsiveness varies quite a lot. Underlying immunological mechanisms responsible for non-responsiveness remain largely elusive. Computational analysis hold the capacity to process large amounts of information, and can thereby broadening the use of laboratory datasets. The presented project aims at identifying putative immunological and molecular mechanisms responsible for non-responsiveness, especially in high-risk groups. In this regard, in-depth immunological monitoring (diverse antibody titer assays, multiparametric flow cytometry of innate and adaptive immune cells) and molecular analysis (transcriptomics) of different cohorts will be performed. Preliminary results of an influenza vaccinated liver cirrhosis cohort suggest unexpectedly a higher responsiveness in the liver cirrhosis patients as compared to matched healthy donors. In an influenza vaccination study of elderly (≥ 65 years of age), immunoprofiling of triple responders and triple non-responders revealed deep insights in different immune cell distributions. Following, a knowledge graph will be applied to link lab-generated data with knowledge from diverse databases to identify putative mechanisms responsible for non-responsiveness to vaccination, and will contribute to the progression of improved vaccination strategies adapted to specific risk groups.

Keywords: Adjuvants and vaccines, big data, immune response tracing

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POSTER PRESENTATIONS

P-0803

Neutrophil Extracellular Traps during high fructose diet leads to low-grade inflammation and direct liver damage

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Fructose was reported to cause liver damage and none alcoholic fatty liver disease (NAFLD). Activated neutrophils were demonstrated to release Neutrophil Extracellular Traps (NETs) resulting in the initiation of gallstone formation. Neutrophils abundantly patrol surfaces of biliary ducts protecting liver from upcoming gut microbiota. Fructose tend to produce monourate sodium crystals, known inducers of NETs formation. Sugar content in some soft drinks can reach up to 150g/l with fructose being 2/3 of it. Here we tried to reproduce conditions of continuous fructose consumption (10% w/v in water) in the proposed model of mouse NAFLD or nonalcoholic steatohepatitis (NASH) and checked if fructose consumption lead to systemic inflammatory response. We collected blood every 2 week from the start of experiment with the aim to monitor serum neutrophil elastase activity, as well as evaluated NETs in gallbladder, and performed morphological analysis of liver and tested composition of circulating immune complexes and glycosylation (terminal sialylation, core fucosylation and branched N-glycans) of IgG, an indicators of systemic inflammatory response. Thus, NASH developed in mice after 6 weeks of fructose diet was accompanied by NETs formation in gallbladder, increased serum NE activity, and decreased sialylation of circulating IgG molecules. Continuous treatment of mice with s.c. injections of heparin-family glycosaminolycans mostly ameliorated pro-inflammatory influence of NETs during high-fructose load. We also present an animal model of diet-induce NASH, developed within 6 weeks, which is of great benefit over existing 12-16 week models of the disease.

Keywords: Cell death, granulocytes, neutrophils, nutrients, animal models, chronic inflammation and fibrosis

P-0804

CD4+CD25highCD127high cells are significantly increased in peripheral blood in renal grafts with subclinical inflammation

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Activated CD4 T cells (CD4+ CD25high) are characterized by high IL-7Rα (CD127) expression. These cells are increased in bothacute and chronic rejection in patients peripheral blood compared to healthy controls. In this study, we have measured the % of activated CD4 T cell population in 30 kidney transplant patients with low immunological risk who presented stable kidney function and underwent a protocol biopsy three months after transplantation. In 14 patients of them, the biopsy did not show signs of inflammation (NI), whereas in the remaining 16 it was founded inflammatory signs that were considered as subclinical inflammation (SCI) where IA rejection or higher was included (Banff19). The CD4+ CD25high CD127high population was studied in peripheral blood by flow cytometry before transplantation and three months after and the sample was obtained the same day of the biopsy. The results showed a significant increase of activated CD4 T cells in the group of SCI patients compared to the patients of the NI group (3.9% ± 1.9% NI vs 7.3% ± 2.7% SCI, p = 0.0001). The logistic regression showed activated CD4 cells as a risk factor associated with SCI: (OR 1,93 (IC 95% 1,2-3,1); p = 0.007). These preliminary results indicate that the measurement of the CD4+ CD25high CD127high population in patients with stable graft renal function might be used as a biomarker of subclinical inflammation.

Keywords: Biomarkers, monitoring immunity, transplantation

P-0806

Computational chemistry analysis of allelic variants of IL2, IL2RA and IL7R genes in the influence of mitochondria in pathogenesis of innate immunity and thymic control in multiple sclerosis

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The objective of this scientific work was to analyze the allelic variants of IL2, IL2RA and IL7R genes in the influence of mitochondria in pathogenesis of innate immunity and thymic control in multiple sclerosis. The simulation experiments were done on Matlab6.5 under the same condition as the physiological experiments. The molecular docking was conducted with the tool AutoDock Vina (version 1.1.2), as implemented in the MolAr (Molecular Architecture) software. ConSurf was used for the evolutionary conservation analysis. The key docking complexes were evaluated by molecular dynamics (MD) simulation using the GROMOSA7 all-atom force field and performed using GROMACS 5.1 software. The virtual cell based assay (VCBA) has been developed to simulate intracellular concentrations and to predict intra-mitochondrial concentrations in central nervous system (CNS). This scientific work suggests that the haplotype containing the IL7R rs1494558*T is associated with a higher solubility of the IL7R alpha chain than the haplotype containing rs1494558*C. The higher solubility of the IL7R alpha chain favors the appearance of failures in the phosphodiester binding regions, contributing to errors during the metabolic pathways that involve the deoxynucleotidyl transferase in V(D)J recombination during lymphocyte development. This work suggests that mtDNA deletions in the subunits of complex IV and disturbances in chemical bonds in complex I and III activities can induce active and chronic lesions in CNS. Changes in conformation of OXPHOS and proteins 4 and 5 can induce deficits in the functions of PGC-1α, stimulating the formation of a favorable environment for inflammation.

Keywords: Innate immunity, metabolic control of immune responses, modelling, multiple sclerosis, neuroimmunology

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POSTER PRESENTATIONS

P-0808

Germline STAT3-activating mutations from autoimmunity and lymphoid malignancy perturb mouse and human T lymphocytes

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Signal transducer and activator of transcription 3 (STAT3) is a latent transcription factor with pleiotropic roles in hematopoietic and non-hematopoietic cells, that regulates gene expression downstream of cell surface cytokine and hormone receptors. Heterozygous germline loss-of-function *STAT3* mutations lead to the primary immunodeficiency hyper-IgE syndrome (HIES) while somatic gain-of-function *STAT3* mutations recur in human solid organ malignancies and non-Hodgkin lymphoma. Recently, germline heterozygous gain- of-function mutations in *STAT3* were shown to result in early-onset and multi-organ autoimmunity with aspects of immunodeficiency. Affected individuals share characteristics with autoimmune lymphoproliferative syndrome (ALPS) and immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome, including reduced T regulatory cell numbers and suppressive activity. They present with variable early-onset autoimmune symptoms including type 1 diabetes, juvenile-onset rheumatoid arthritis, gut enteropathies and autoimmune cytopenias. Whilst many effects of STAT3 loss-of-function on immune cells have been described, the mechanisms behind autoimmunity and immunodeficiency in patients with STAT3-activating mutations remain unclear. Here, we present a detailed characterisation of T cell development and maturation in young and old mice on two different backgrounds with Crispr-engineered germline activating mutations in two domains of STAT3. We use mixed chimeras, flow cytometric analysis, T cell receptor deep-sequencing and high-throughput single-cell transcriptomics to reveal cell-extrinsic and -autonomous roles of STAT3 activation in T cells in autoimmunity versus immune malignancy. To our knowledge, this is the first report of mice with *Stat3* germline activating mutations identical to those in autoimmunity or malignancy. We validate our key findings in humans with gain-of-function germline *STAT3* mutations and childhood-onset autoimmune disease.

Keywords: Ageing, animal models, autoimmunity, cytokines and mediators, immunodeficiency

P-0809

Report on lower anti-pneumococcus IgG1 levels in men, especially over the age of fifty

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Streptococcus pneumoniae, the pneumococcus is an important human pathogen. It possesses a capsule which has been thoroughly studied, since it is an important factor in virulence. In this work *S. pneumoniae* ATCC 6301, belonging to Serogroup 1, was used as a model organism to determine the levels of different IgG isotypes specific to this bacterium in different sex and age groups. For this purpose an in-house ELISA was used. Although IgG2 is a major antibacterial antibody subclass, acting against polysaccharide antigens, the level of pneumococcus-specific IgG2 did not differ from the level of IgG2 specific to several tested lactic acid bacteria (LAB). On the other hand pneumococcus specific IgG1 level was significantly higher to the level of LAB specific IgG1. This is most likely a consequence of previous encounters with pathogenic pneumococcal microorganism and a distinguishing characteristic of this encounter. By analyzing different sex and age groups we found significantly lower anti-pneumococcus IgG1 levels in men, implying lower protection against invasion with pneumococcus, which possibly translates to other encapsulated bacteria. The Covid-19 pandemic has identified males as being at increased risk of hospital admissionand death and lower levels of anti-pneumococcus antibodies might be a contributing factor. Hence, we advocate the immunization with conjugate pneumococcal vaccines in male individuals, especially over the age of 50.

Keywords: Adaptive immunity, ageing, antibody

P-0810

A case of primary hemophagocytic syndrome seen in adult age

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Hemophagocytic lymphohistiocytosis (HLH) is an immune disorder with high mortality due to uncontrolled activation of T lymphocytes and macrophages and excessive proliferation of inflammatory cytokines. A 37-year-old female patient diagnosed with pimer HLH is presented here. A 37-year-old female patient, was hospitalized with complaints of fever, weakness, and joint pain. Laboratory tests; Hemoglobine: 6.8 gr/dl, Wbc: 1.06x103/uL, plt: 8x103/uL neutrophil: 0.73x103/uL, lymphocyte: 0.18x103/uL, fibrinogen: 160.18 mg/dl, ALT: 118 u/L, LDH: 302 u/L, total bilirubin: 1.72 mg /dl,albumine: 2.76 g/dl, BUN: 15 mg/dl, creatinine: 0,73 mg/dl, CRP: 32.81 mg/L, triglyceride: 405 mg/dl, ferritin: 2820 mg/dl. On abdominal USG, the liver was 167mm, spleen 200mm. The bone marrow pathology result was consistent with hemophagocytosis. The patient's NK cytotoxicity was low.As a result of the genetic whole exon sequencing performed upon the presence of a family history; PRF1 / NM-001083116 / EXZON 3, C.560C> G p (Pro187Arg) came as homozygous. HLH 2004 protocol was started with the diagnosis of familial hemophagocytic syndrome. Allogeneic bone marrow transplant was planned for the patient. Pulse steroid was initiated and plasma exchange was performed due to diffuse alveolar hemorrhage in thoracic tomography performed upon the development of dyspnea, tachypnea and tachycardia at the 7th week of the treatment. The patient died at the 8th week of the treatment. In adult patients, primary hemophagocytic syndrome should be considered in differential diagnosis, treatment should be initiated after diagnosis and bone marrow transplantation should be performed as soon as possible.

Keywords: Autoimmunity, cytokines and mediators, granulocytes, immunodeficiency, lymphoid organs, macrophage

P-0812

What about the cytokines for the etiology of idiopathic granulomatous mastitis?

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Few things are known about the etiology of Idiopathic Granulomatosis Mastitis. It was associated with autoimmunity, but no significant evidence was determined. In this study, we aimed to investigate the roles of cytokines in the etiopathogenesis of Idiopathic Granulomatosis Mastitis. Idiopathic Granulomatous Mastitis patients in active or remission who admitted to the breast diseases outpatient clinic and healthy volunteers were included prospectively in the present study. The IL-1β, IFN-α2, IFN-γ, TNF-α, MCP-1, IL-6, IL-8, IL-10, IL-12p (p70), IL-17A, IL-18, IL-23 and IL-33 values were measured with Flow Cytometry. The blood samples were taken before the treatment in active Idiopathic Granulomatosis Mastitis group. The ages, physical examination findings, menopausal conditions, smoking conditions, and treatment methods were also evaluated. A total of 32 patients, including 19 active and 13 remission patients, and 18 controls, were included in the present study, which made a total of 50 people. The mean age was 37.18±7.15. The IL-1β, TNF-α, IL-10 and IL-18 values were lower in patients with Idiopathic Granulomatosis Mastitis than the control group. Granulomatosis Mastitis patients smoked more than control. When the active patients, remission patients, and control group were evaluated together, no significant differences were detected. In our study, the low rate of cytokines which are important for autoimmune and granulomatous reactions, especially cytokines associated with Th1 and Th17, in patients with idiopathic granulomatous mastitis suggests that these cytokines may not play a role in the etiopathogenesis, and that different mechanisms may be effective.

Keywords: Adaptive immunity, autoimmunity, chronic inflammation and fibrosis, cytokines and mediators

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POSTER PRESENTATIONS

P-0813

Circulatory follicular helper T lymphocytes protect against CMV infection in kidney transplant recipients

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CMV-infection in kidney transplant patients (KTR) increases rejection risk. Circulatory T follicular helper cells (cTFH) may contribute to protection through neutralizing antibodies (Nabs) and/or IL-21, which has been shown to strengthen CD8+ cytotoxicity. We studied if cTFH could protect against CMV post-transplantation (Tx). PBMCs were collected pre- and post-Tx from 90 CMV seropositive KTR not receiving antithymocyte globulin or antiviral prophylaxis. cTFH were identified as CD4+CXCR5+ and activated cTFH as CD4+CXCR5+CCR7loPD1hi. CMV infection was defined as DNAemia > 1000 UI/ml. CMV-disease was considered when infection and symptomatology coexisted. Nabs titers were determined by microneutralization. CMV-specific CD8+ T cell were identified by a QuantiFERON-CMV modified test. KTR with CMV infection had significantly lower cTFH and activated cTFH pre-tx and early post-tx than KTR without CMV reactivation. Pre-tx and 14 days post-tx activated cTFH were lower within infected KTR who developed CMV disease (p=0.02, p=0.01). KTR with superior cTFH pre-Tx, 7 and 14 days post-Tx suffered the CMV infection later (p=0.02, p=0.01 and p=0.006). Pre- and 14 days post-Tx activated cTFH were an independent protective factor for CMV infection (HR 0.41, p=0.01; and 0.52, p=0.02, respectively). KTR with low cTFH 7 days post-tx (<11.9%) had lower CMV infection-free survival than KTR with high cTFH (28.2% vs 67.6%, p=0.002). cTFH associated with positivity for CMV-specific Nabs (p=0.008). IL-21 increased interferon-γ secretion by CMV-specific CD8+ T cells in healthy controls (p=0.007). cTFH may protectagainst CMV infection in KTR. Monitoring cTFH pre- and early post-tx could improve CMV risk stratification.

Keywords: Follicular helper T cells, infectious disease, transplantation, viral infections

P-0814

Long term follow-up of *in vivo* cellular immune response to SARS-CoV-2 using delayed-type hypersensitivity cutaneous test

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Our group has validated the use of delayed type hypersensitivity (DTH) cutaneous test using RBD antigen as a feasible (*in vivo*) method to assess cellular immune responses in natural (1) and vaccinated (2) SARS-CoV-2 exposed individuals. However, little is known about durability ot the positivity test in exposed and vaccinated individuals, and the correlation with specific anti-RBD IgG. Here we present the results obtained at 6 and 12 months of follow up of two infected individuals. IgG specific for the S1 protein of SARS-CoV-2 was measured using a commercial ELISA according to manufacturer´s instructions (Euroimmun, Lübeck, Germany). Results are semiquantitative. OD-ratios above 0,8 were considered positive. DHT-Skin Test Protocol was performed according to usual clinical practice consisting in the intradermal puncture of 25 microL (0.1 mg/mL final concentration) of the spike protein in the volar part of the arm. Skin test reaction was evaluated 12h, 24h and 48h after injection. A positive response was considered in the case of appearance in the intradermal injection site of a typical induration and erythema. 6 and 12 months after infection specific IgG values were 2,4-5,8 OD-ratio for patient A and 24,7-5,8 OD-ratio for patient B (calculated OD ratio after serial dilution of samples). DTH skin reaction diameters were 16 mm, 22 mm, 41 mm (after 6 months) and 8mm, 22mm, 35mm (after 12 months) for patient A and 6 mm,12 mm, 14 mm (after 6months) and 27 mm, 35 mm, 30 mm (after 12 months) for patient B. DTH test can be used as a routine method to follow up immune responses to SARS-CoV-2.

Keywords: Adaptive immunity, immune response tracing, visualizing immune responses

P-0815

Krakow air pollutants induce inflammatory response of the Th lymphocyte subsets

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In recent years, an increase in air pollution seriously impacts human health. However, the knowledge on the effects of particulate matter on the cells of immune system is still scarce. Here we investigated the effect of Krakow air pollutants on the activity of CD4+ T cell subsets from healthy donors and patients with rheumatoid arthritis or multiple sclerosis. Peripheral blood mononuclear cells (PBMC) were isolated and cultured with or without Krakow air pollutants *in vitro* and the expression of intracellular proteins, namely IFN-γ, IL-4, IL-17 and Foxp3, characteristic for Th1, Th2, Th17 and Treg respectively, was analyzed by flow cytometry. The results showed that treatment of PBMC with Krakow air pollutants increased expression of IFN-γ and IL-17A, specific for Th1 and Th17 cells, respectively. The expression of Foxp3 (Treg) and the frequency of cells positive for IL-4 (Th2) was negligible in all study groups. In conclusion, our results indicate that intensity of the response of CD4+ lymphocyte subsets vary depending on the concentration of pollutants in theair (seasonality). Moreover, *in vitro* treatment of human PBMC with Krakow air pollutants skews the balance of Th1/Th2 and Th17/Treg cells, promoting proinflammatory activity of the Th1 and Th17 subsets, confirming the role of Krakow air pollutants in the development and exacerbation of allergies, inflammatory and autoimmune disorders. This study was supported by grant from the National Science Centre (NCN) in Poland (grant APARIC no. 2015/16/W/ST5/00005) and by H2020-MSCA-RISE-2016 project "CHARMED" no. 734684.

Keywords: Autoimmunity, cytokines and mediators, environmental factors in autoimmunity and allergy

P-0817

SARS-CoV-2-specific memory CD4+ T cells in unexposed, post infected and vaccinated healthcare workers

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The aim of the study is to compare the AIM (activation induced markers) in CD4 memory T-lymphocytes by SARS-CoV-2 proteins between unexposed, post infected and vaccinated healthcare workers (HCW). Blood samples were collected from HCW (unexposed-unvaccinated, post infected-vaccinated, and unexposed-vaccinated). PBMCs (peripheral blood mononuclear cells) were extracted by ficoll density gradient (Ficoll-Paque, Cytiva), and stimulated during 24h at 37ºC and 5%CO2 in presence of DMSO (Sigma-Aldrich), PHA-M (phytohemagglutinin, Sigma-Aldrich), S M and N SARS-CoV-2 15-mer peptide pools (PepTivator SARS-CoV-2 Prot M, N and S. Miltenyi Biotec). The stimulated PBMCs were stained with 7-AAD, anti-CD3, anti-CD4, anti-CD8, anti-CD45RA, anti-CD137 (BD Pharmingen) and anti-CD134 (Cytognos) fluorescent monoclonal conjugated antibodies. The samples were acquired with BD FACSLyric cytometer (BD Biosciences), and analyzed with FlowJo v10.7.2 software. Statistical analyses were performed with Graphpad prism v5.0 software. Our preliminary results showed that the AIM CD4 T-specific response of infected-vaccinated against SARS-CoV-2 M and N proteins is time-independent from infection. Detection of AIM in CD4 + T lymphocytes stimulated with SARS-CoV-2 M, N and S differentiated infected individuals from non-exposed ones. The AIM in CD4 + T lymphocytes stimulated with SARS-CoV-2 S is not statistically different between post infected-vaccinated and unexposed-vaccinated. The T CD4-specific memory response to SARS-CoV-2 is maintained for at least one year and did not increase with post-infection vaccination.

Keywords: Adaptive immunity, memory, viral infections

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POSTER PRESENTATIONS

P-0818

Many faces of a protracted SARS-CoV-2 infection in a patient with autosomal-recessive agammaglobulinaemia and enteropathy

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Autosomal-recessive agammaglobulinemia (ARA) is a primary immunodeficiency characterized by the absence of mature CD19+B-cells and low serum immunoglobulins (Igs). Patients with ARA might develop more severe course or atypical form of COVID-19. We report a 35-year-old female with a history of ARA receiving intravenous immunoglobulin (IVIG) from the age of 16. A year later, at the age of 17, matched allogenic hematopoietic cell transplantation with an unfavourable outcome was performed. Enteropathy was diagnosed in 2018. Due to persistently low IgG (0.9g/l) despite of receiving IVIG every 3 weeks and escalating replacement dose to 900mg/kg, prednisone was started, with an improvement of IgG level (5.8 g/l), as measured in June 2020. The patient was diagnosed with COVID-19 pneumonia in August 2020, and treated with ABO-compatible donor convalescent plasma, favipiravir, hydroxychloroquine, and antibiotics. Despite of clinical/laboratory recovery, SARS-CoV-2 RT-PCR from nasopharyngeal swab (NFSw) remained positive until November 2020. During the period of prolonged SARS CoV-2 positivity, lower IgG levels were repeatedly measured (1.8g/l), suggesting possible reactivation of enteropathy. In April 2021, she presented with fever, cough, anorexia and elevated inflammatory markers. After negative RT-PCR for SARS-CoV-2 from NFSw, the test was positive from induced sputum. CT scan showed bilateral pneumonia. The patient was treated with remdesivir and antibiotics with a full clinical recovery within two weeks. Beside respiratory presentation, enteropathy worsening in ARA patients should be considered during COVID-19. Since NFSw can be negative, induced sputum analysis is recommended in suspected cases.

Keywords: Antibody, immunodeficiency, transplantation, viral infections

P-0819

A gut microbiota-based signature to characterize immune recovery in HIV-subjects

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To find an Operational Taxonomic Units (OTUs)-based signature from the gut mucosal microbiome of (CD4) non-recoverers HIV-subjects. Thirty-five treated-patients were classified as follows: early-treated (ET; CD4 >250 before ART) and late-treated (LT; CD4<250 before ART). LT were subdivided into high recovery (LT-HR) or low recovery (LT-LR), depending on CD4 recovery after two years of ART (threshold=250). Biopsies from terminal ileum and caecum mucosa were obtained. Microbial 16S rRNA V3-V4 region sequencing yielded OTUs whose relative abundances enabled classification of samples using a random forest (RF) algorithm. A multiple logistic regression analysis with 3-fold cross validation and Lasso regularization was performed to obtain a signature. RF analysis of OTUs' relative abundances from 58 biopsy samples showed a large overlap between ET and LT-HR samples, which were grouped together. A subset of 30 OTUs was selected as the best to classify samples as ET/LT-HR or LT-LR, according to their relative abundances, since 100% of ET/LT-HR and 61% of LT-LR were properly classified (ER=12%). To improve discrimination, we chose the 14 most relevant OTUs (mean-decrease accuracy>5) to build a logistic regression model that yielded 9 OTUs as the best candidates to predict belonging to ET/LT-HR or LT-LR groups: 5 Firmicutes (Blautia, Gemmiger, Clostridium, Ruminococcus, Oscillospira), 3 Bacteroidetes (Bacteroides, Parabacteroides, Barnesiellaceae) and 1 Proteobacteria (Escherichia). This predictive model gave an AUC of 0.97. Our abundance-based OTUs signature could discriminate (CD4) non-recoverers from recoverers/early-treated. Prospective studies are needed to explore whether this signature is already present before ART onset.

Keywords: Immunodeficiency, microbiome and environmental factors, viral infections

P-0820

The relationship between c-reactive protein, hdl and fibrinogen in overweight and obese patients wıth coronary artery disease

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Acute phase reactants, like fibrinogen and high sensitivity C-reactive protein, are well known cardiovascular risk factors. Low HDL-cholesterol concentrations are associated with increased cardiovascular risk and recent evidences suggest that HDL may aggravate the atherosclerotic process promoting inflammation. In this study we aimed the relationship CRP, as known inflammatory marker, HDL and fibrinogen in owerweight and obese patients with CAD. The study population consisted of 83 patients (40 men and 43 women) aged 58.96±10.18 years with obese and overweight with CAD. Patient age, fasting and postprandial glucose,uric aside, insulin, total cholesterol, LDL and HDL cholesterol, and triglycerides were measured, and body mass index (BMI) was calculated.CAD defined as have more than 50% stenosis in at least one coronary artery documented angiographically or a history of previous percutanous coronary intervention or coronary artery by-pass grefting. Patients age was 58.96±10.18 years and BMI 34.22±4.15. Serum CRP 3.43±7.67, fibrinogen 297.99±92.14, HDL 45.12±43.01, LDL 102.60±26.90, triygliseride 190.09±153.45. Negative correlation was found between CRP and fibrinogen and HDL in multivariate analysis (r=-761 p=0.002, r=-561 p=0.016 respectively). As CRP is a marker of inflammatory processes a negative correlation between serum CRP level and fibrinogen and HDL in obese and overweight coronary artery disease patients.

Keywords: Inflammatory disease, inflammatory molecules, metabolic control of immune responses

P-0821

Phenotypic characteristics of Natural Killer cells from melanoma-bearing mice

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Metastasis remain the major cause of death despite all therapeutic methods developed in recent years. Because of this, it is necessary to find new targets and therapeutic approaches for the treatment of metastasis. In this study we evaluated the phenotype of NK cells in two experimental tumour models. C57BL/6 mice, 8-10 weeks old, were subjected to subcutaneous and intravenous inoculation of B16F10 cells. Controls were healthy sex/age-matched mice. After 14 days spleens were harvested and immediately assessed using flow cytometry analyses for a large panel of NK cell surface markers (FACSCanto II flow cytometer with DIVA software). Evaluation of NK cell phenotype showed a significant increase in CD69, B220 and CD11c markers in both tumour models. Analysis of the B220+CD11c+NK1.1.+ cell population presented an important elevation in tumour-bearing mice, especially for those with pulmonary metastasis (intravenous inoculation). Regarding the activation and maturation markers of NK cells, such as CD335, CD122, CD49b, CD11b, CD43, CD27, KLRG1, there was a decrease in values for both tumour models compared to controls, the largest decrease being observed in subcutaneous tumours. NK cells are effectors involved in the immune response against tumours and a better understanding of their implication in the development and progression of melanoma is essential for establishing new effective NK cell-based therapies. Our data constitute additional findings about the NK cell phenotype changes in response to metastatic melanoma.

Keywords: Cancer immunology, *in vivo* tumor models, NK cells

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POSTER PRESENTATIONS

P-0823

Longitudinal dynamics of SARS-CoV-2-specific cellular and humoral immunity after BNT162b2 vaccination

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The Pfizer-BioNTech mRNA vaccine BNT162b2 is being administered within the vaccination plan against COVID-19. We longitudinally analysed the dynamics and duration of SARS-CoV-2 specific cellular (CIR) and humoral immune response (HIR). We determined S1-, M- and N-specific T-cell immunity by Fluorospot, anti-S1 antibodies by ELISA and neutralizing antibodies by pseudovirus neutralization assay, in a prospective cohort of 80 healthcare workers (19 previously infected and 61 non-infected) who received two 30 µg doses of BNT162b2, 21 days apart. Samples were obtained pre-vaccination, pre-boost, and 15 days, 1 and 3 months after completing vaccination. Pre-vaccination, 89.4% and 83.3% of previously infected subjects maintained S1-, M- and N-specific T-cells and anti-S1 antibodies, respectively. Anti-S1 response peaked 2 weeks after full vaccination in all subjects, and both CIR and HIR were significantly higher in previously infected than in naïve subjects at most time-points. In naïve individuals, both anti-S1-CIR and HIR decreased steadily from 2 weeks after completing vaccination (all, $p<0.0001$). These subjects developed neutralizing antibodies only after the boost dose, and their neutralization capacity started to decrease at one month ($p<0.01$). In previously infected subjects, anti-S1-HIR remained stable throughout the study and CIR started to decreased only 3 months after full vaccination ($p=0.0003$). Despite the decrease in anti-S1 response in some subjects, 97% and 100% had detectable anti-S1-CIR and HIR respectively, 3 months post-vaccination. In real-world conditions, the BNT162b2 vaccine develops a robust protective and coordinated anti-S1-CIR and HIR, up to three months post-vaccination.

Keywords: Adaptive immunity, adjuvants and vaccines, infectious disease, protection, viral infections

P-0824

Differential microRNA expression alters the immunometabolic response of human macrophages in tuberculosis and HIV infection

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Tuberculosis (TB) is the world's leading infectious disease, and TB/HIV co-infection represents a major barrier to effective disease management. Novel TB therapeutics research focuses on embellishing the host response, specifically on the alveolar macrophage's ability to clear infection, which is altered by HIV. Much of this work is on immunometabolism. Disrupted TCA cycle flux and metabolite accumulation is a hallmark of macrophage polarisation and the immunometabolic response. Key enzymes responsible for this metabolic reprogramming are under the influence of specific microRNA, and therefore represent potential targets as host-directed therapies. We hypothesised that Mtb infection or HIV stimulation of human monocyte-derived macrophages (MDM) would alter expression profiles of microRNA and their target mRNA transcripts involved in TCA cycling. MDM were infected with Mtb (H37Ra) or stimulated with HIV gp120. Cell lysates were harvested 24 hours following infection and RNA analysed by real time RT-PCR (microRNA) or multiplex mRNA analysis (NanoString nCounter Platform). Mtb infection and HIV gp120 stimulation resulted in differential expression of miR-378a-3p and appeared to alter the expression of several TCA enzymes, including aconitate decarboxylase (ACOD1) – the enzyme responsible for itaconate production. Human MDM have altered microRNA expression profiles in response to Mtb infection and HIV gp120 stimulation. miR-378a-3p specifically targets ACOD1 for degradation, and as such can restrict itaconate production during macrophage polarisation. Restricted itaconate synthesis following miR-378a-3p induction during HIV infection could hamper the anti-tuberculous response of the human macrophage. Targeting this microRNA may represent an avenue for host-directed immunotherapy.

Keywords: Infectious disease, innate immunity, macrophage, metabolic control of immune responses, miRNA, viral infections

P-0825

Frequencies and TCR repertoires of human 2,4,6-trinitrobenzensulfonic acid (TNBS)-specific CD154+CD4+ and CD137+CD8+ T cells

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Allergic contact dermatitis is a frequent chemical-specific T cell-mediated skin disease. We employed the extreme sensitizer 2,4,6-Trinitrobenzensulfonic acid (TNBS) as model allergen to determine human TNBS-specific T cell frequencies and receptors (TCR). This knowledge could provide insights into chemicals and T cells interaction. Human peripheral blood mononuclear cells (PBMC) were labeled, TNBS-modified and incubated in 1:1 ratio with unmodified *responder* PBMC to detect CD154+CD4+ and CD137+CD8+ T cells by flow cytometry. Activated cells were sorted for *in vitro* restimulation and TCR high-throughput sequencing. TNBS was non-toxic and suitable for modification at ~1–10mM. Stimulation with TNBS-modified cells induced 0.03%/0.04% CD154+CD4+ and 0.06%/0.24% CD137+CD8+ naïve(CCR7+CD45RA+)/memory(non-naïve) T cells (mean over control, n=12, 5h). After 16h, signals increased to 0.09%/0.30% (CD154) and 0.90% (CD137, memory) (n=8). CD154 and CD137 upregulation was TCR-mediated as confirmed by CD69 co-expression. In addition, 11/14 memory CD154+CD4+ T cell clones and 2/2 memory CD137+CD8+ T cell lines responded to TNBS-restimulation while anti-MHC blocking antibodies prevented activation. Among TNBS-specific TCR, we did not observe overrepresentation of gene segments. However, Tryptophans (α-/β-chain CD4, β-chain CD8) and lysines (β-chain CD8) were overrepresented in the CDR3 regions (n=3-6), indicating preferential interaction of this amino acids with TNBS. In conclusion, CD154/CD137 upregulation is suitable to detect human TNBS-specific T cells in a fast, comprehensive and quantitative manner. TCR repertoire analysis may reveal features underlying unusually frequent T cell activation by some chemical allergens. In the future, this approach may be adapted to improve *in vitro* diagnosis and chemical sensitizer prediction.

Keywords: Adaptive immunity, allergen-induced immune responses, modification allergic responses, RNAseq, skin diseases

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POSTER PRESENTATIONS

P-0827

Myc-driven lymphoma induces rapid metabolic re-programming of CD4+ T cells

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T cells are metabolically suppressed in tumors. This is marked by a loss of central metabolic pathways that drive normal T-cell effector functions. Reported mechanisms that impair T cell metabolism include: (i) metabolic exhaustion due to chronic T-cell receptor (TCR) signaling; and (ii) nutrient competition by tumor cells. The effects of tumors driven by MYC, an oncoprotein that drives cancer energetics and dampens immunogenicity, on immune cell metabolism has not been investigated. To test this we used a transplant model of *Myc*-driven B-cell lymphoma. Notably, increases in lymphoma burden have profound and selective effects on the numbers and fate of $CD4^+$ T cells, where there were increases in both Treg and Th1 phenotypes and markers of exhaustion. Interestingly, global metabolomic analyses indicated that most changes to intracellular metabolites of $CD4^+$ T cells occurred early during lymphoma progression when tumor burden is very low. These metabolite alterations include significant reductions in upstream substrates for central metabolic pathways that fuel the TCA cycle such as glucose, glutamine and branch chain amino acids. Notably, there were also significant reductions in mitochondrial phenotypes of lymphoma-derived $CD4^+$ T cells that occurred well before tumor-provoked polarization, and this was also manifest in populations of non-tumor-reactive T cells. Collectively, these data indicate that *Myc*-expressing B-cell lymphomas provoke rapid metabolic reprogramming of T cells that is independent of TCR engagement.

Keywords: Cancer immunology, cellular interactions, metabolic control of immune responses

P-0829

T-cell specific deficient mice for the PI3-kinase p110α catalytic subunit show attenuated experimental allergic encephalitis (EAE) in mature but not in young mice

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Phosphatidylinositol 3-kinases (PI3K) are essential to autoimmune responses, yet the role of the ubiquitously expressed class IA PI3K p110α catalytic subunits in these processes is not well established. Here, a mouse model with Cre/flox mediated T cell specific deletion of p110α catalytic chain (p110αΔT) was used to analyze p110α functions in MOG-induced EAE in young and mature WT or p110αΔT mice. EAE was chosen as a disease dependent or mediated by $CD4^+$ T cells and cytokines like IL-17A, IFN-γ, or TNF-α, and controlled by Treg cells. In the steady state, the percentage of naïve $CD4^+$ T cells in the spleen was significantly lower in six month-old (mature) than in two month-old (young) WT or p110αΔT mice, indicating participation of p110α in the homeostasis of these cells. In contrast, Treg cells were significantly enhanced in mature p110αΔT mice as compared to WT mice. EAE symptoms were similar in young WT and p110αΔT mice, or in WT mature mice. Strikingly, EAE symptoms and disease scores in mature p110αΔT mice were clearly lower than those of mature WT, or young WT and p110αΔT mice. Unlike young p110αΔT mice, cells from mature p110αΔT mice undergoing EAE secreted significantly lower levels of IFN-γ and IL-17A than WT mice. No significant differences in IL-10 or TNF-α were observed between p110αΔT and WT mature mice. These data indicate that the lower incidence of EAE in p110αΔT mature mice is linked to altered T cell homeostasis leading lower secretion of inflammatory cytokines.

Keywords: Autoimmunity, cytokines and mediators, immune regulation and therapy, immune senescence, multiple sclerosis

P-0830

In vitro effect of PI3 Kinase p110α and p110δ inhibitors in PBLs of rheumatoid arthritis patients

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Rheumatoid arthritis (RA) is an autoimmune disease involving T and B lymphocytes. Its high incidence (approx. 1% worldwide) makes RA an important target for drug discovery. Class IA phosphoinositide-3-kinases (PI3Ks) are heterodimers of different regulatory and catalytic subunits that are essential to function of different cells, including immune cells. The fact that p110δ PI3K contributes significantly to Ag-activation of B lymphocytes, but both the p110α and p110δ PI3K isoforms participate in B lymphocyte development in the bone marrow and in B cell survival in the periphery adds interest to the study of dual p110α/p110δ PI3K inhibitors in RA. T lymphocytes express high levels of the p110α and p110δ class IA PI3K catalytic isoforms. Whereas the functions of PI3K p110δ in immune and autoimmune reactions is well established, the role of p110α is less well understood. To evaluate the possible therapeutic application of PI3K inhibitors in humans, we have analyzed the effect of various new α-, δ- or dual p110 PI3K inhibitors on PBLs of RA human patients (before starting Methotrexate treatment) in a dose-response manner. *In vitro* viability, cytokine secretion and OKT3-mediated activation were evaluated. No significant effect on cell viability was found. OKT3-induced IFN-γ, TNFα and IL-10 secretion was strongly inhibited by p110α/p110δ and p110δ inhibitors, with a lower effect of p110α inhibitors. No significant effect on IL-6 secretion was found. These results highlight the relevance of p110α and p110δ in immune regulation, suggesting dual inhibition of these PI3K subunits as an effective therapeutic approach in immune-based pathologies.

Keywords: Autoimmunity, cytokines and mediators, immune regulation and therapy, immunopharmacology, rheumatoid arthritis

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POSTER PRESENTATIONS

P-0831

Longitudinal analysis of inflammatory response mild COVID-19 in the upper respiratory tract reveals an association with viral load, independent of symptoms

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SARS-CoV-2 infection leads to high viral loads in the upper respiratory tract that may be determinant in virus dissemination. The extent of intranasal antiviral response in relation to symptoms is unknown. Understanding how local innate responses control virus is key in the development of therapeutic approaches. SARS-CoV-2-infected patients were enrolled in an observational study conducted at the Geneva University Hospitals, Switzerland, investigating virological and immunological characteristics. Nasal-wash and serum specimens from a subset of patients were collected to measure viral load, S1-specific IgA and a cytokine panel at different time points after infection; cytokine levels were analyzed in relation to symptoms. Samples from 13 SARS-CoV-2-infected patients and six controls were analyzed. We found an increase in CXCL10 and IL-6, whose levels remained elevated for up to 3 weeks after symptom onset. SARS-CoV-2 infection also induced CCL2 and GM-CSF, suggesting local recruitment and activation of myeloid cells. Local cytokine levels correlated with viral load but not with serum cytokine levels, nor with specific symptoms, including anosmia. Some patients had S1-specific IgA in the nasal cavity while almost none had IgG. The nasal epithelium is an active site of cytokine response against SARS-CoV-2 that can last more than 2 weeks; in this mild COVID-19 cohort, anosmia was not associated with increases in any locally produced cytokines. S1-specific IgA local levels were heterogeneous.

Keywords: Cytokines and mediators, infectious disease, innate immunity

P-0832

NLRP3: a novel player in OPC proliferation and myelin regeneration

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Multiple Sclerosis (MS) is an immune-mediated demyelinating disease affecting about 2.5 million people worldwide. MS is characterised by a progressive loss of myelin and the death of oligodendrocytes in a process called demyelination, which can ultimately lead to neuronal degeneration. In early stages of MS, neurodegeneration can be prevented by regeneration of myelin (remyelination). Although the molecular mechanism underlying this process is poorly understood, it has been demonstrated that inflammation is required for efficient remyelination. Inflammasome associated proteins, such as the sensor protein NLRP3 and inflammasome product IL-1β, are abundant in plasma and CNS tissue of MS patients as well as in murine models of MS. Using a lysolecithin-induced focal demyelination model, we can study the different stages required for myelin restoration, in order to elucidate whether NLRP3 is involved in myelin regeneration of CNS lesions. Our preliminary data indicate that inflammasome components are present within demyelinated CNS lesions from early stages of myelin repair suggesting involvement in this regenerative process. Using mice deficient in the inflammasome sensor NLRP3, we analysed the glial cells involved in the repair processes. At early stages of the repair process, fewer proliferating oligodendrocyte progenitor cells (OPC), higher numbers of differentiated oligodendrocytes and microglia/macrophages were present within CNS lesions, suggesting that NLRP3 may have an active role in regulating OPC proliferation and/or differentiation after demyelination. Further analyses into the underlying mechanisms will help to define the role of NLRP3 and the inflammasome complex in remyelination and might uncover molecular targets to promote myelin repair.

Keywords: Inflammatory disease, innate immunity, neuroimmunology

P-0834

Peripheral blood lymphocyte subsets in arthritis in the elderly

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Lymphocyte subsets have been connected to joint infiltration and inflammation in rheumatoid arthritis (RA). Identification of leucocyte subsets dysregulated in arthritis development could provide insight into the aetiology of RA. Elderly-onset RA, EORA, is a RA starting at >60 years. Polymyalgia rheumatica (PMR) is another common rheumatic disease in the elderly. This study aimed to investigate the composition of the peripheral blood component. Newly diagnosed arthritis in patients >60 years, with blood samples collected at baseline and 12 months after treatment. Compared with control individuals of same age and gender. T and B cell subsets in whole blood were determined with flow cytometry.. 29 patients and 18 controls (HC) were analyzed (19 RA and 10 PMR). In patients with EORA, significant increase in percentage and numbers of CD4+ effector subset and significantly decrease in numbers of CD4+ central memory. In patients with PMR, a trend towards different B-cell subsets were observed with an increase in percentage of naïve B cells compared to HC and a significant increase in percentage and numbers of CD8+ central memory. Longitudinal analysis showed that several B and T cell subsets were significantly different at 12 months in both EORA and PMR patients suggesting an effect of therapy in the lymphocyte subsets. Patients with EORA and PMR demonstrate a change in cellular immune parameters apparent in the periphery. More studies are needed to show whether these subsets can be related with clinical remission and response to treatment.

Keywords: Adaptive immunity, biomarkers, rheumatoid arthritis

P-0835

Insights into otitis media: dissecting the interaction of C-reactive protein with non-typeable haemophilus influenzae

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Otitis Media (OM) is the inflammation of the middle ear (ME). Non-typeable Haemophilus influenzae (NTHi) is one of the leading otopathogens in causing OM. Phosphocholine (PCho) on the NTHi lipopolysaccharide influences host-pathogen interaction. C-Reactive Protein (CRP), an acute phase protein recognizes PCho, and can mediate bacterial killing. However, some strains of NTHi survive even in the presence of CRP. We aim to study the interaction of CRP with NTHi to understand its role in bacterial survival and OM. NTHi can efficiently infect the Junbo mouse, a characterised model of chronic and acute OM. CRP levels were highest 1 day post-intranasal inoculation in the ME fluid (MEF) and nasal passage (NP) washes. We show CRP is a localized response to NTHi as serum CRP levels were unaffected in NTHi inoculated and non-inoculated mice at 1, 3 and 7-day post intranasal inoculation. Further, we confirm the presence of NTHi influences CRP levels in the MEF and NP washes. We show CRP binding is influenced by the position and expression of PCho on the NTHi surface. Serum bactericidal assays indicate that the expression and position of PCho affects NTHi survival. The removal of CRP from the serum restores NTHi survival. The expression of PCho also influences opsonophagocytosis activity in macrophages, thereby confirming the importance of PCho in NTHi survival. The CRP-NTHi interaction is currently under investigation to advance our understanding of its role in the complex biological processes that influence bacterial killing and the onset, progression and resolution of OM caused by NTHi.

Keywords: Animal models, infectious disease, inflammatory disease, innate host defence, macrophage, phagocytosis

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POSTER PRESENTATIONS

P-0844

A homeostatic approach to malarial anemia

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Malaria is a severe disease that ranks among the most prevalent infections in tropical areas throughout the world. Anemia is a common complication in both acute and chronic malaria. Red blood cells (RBCs) are the main target of malaria parasites. However, malarial anemia cannot be explained simply by the direct destruction of infected RBCs alone, since both *Plasmodium falciparum* and *P. vivax* infections cause the abnormal removal of an important number of non-parasitized cells (npRBCs). Some patients of severe malaria-derived anemia have a negative blood smear for Plasmodium-parasites, showing that the destruction of npRBCs can continue long after the infection has been cleared. The abnormal removal of npRBCs during malaria has been attributed to multiple causes such as changes in the activity of macrophages leading to increased sequestration of RBCs in the spleen, reduced erythropoiesis, increased oxidative stress, or the presence of anti-RBC auto-antibodies. However, the relation of these observations with one another or with the presence of parasites remains poorly understood. We show that malarial anemia is ultimately caused by the interference of Plasmodium-parasites with the RBCs homeostatic mechanisms. In particular, we hypothesize that fluctuations in erythropoietin induced by massive destruction of RBCs in the early stages of malaria result in abnormal unbalance between RBC production and destruction in the later stages of the infection. We show that observations related to malarial anemia (such as changes in macrophage activity, decrease in RBC production, and lifespan or production of anti-RBC antibodies) can be explained as caused by such unbalance.

Keywords: Macrophage, autoimmunity, immune senescence, infectious disease, parasite infections, phagocytosis

P-0845

The follow up of hepatic serology, hepatic reactivation and lifespan of multiple myeloma transplant patients

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Multiple myeloma is a B cell malignancy and today cytotoxic agents, imides, protosome inhibitors, steroids, stem cell transplantation constitute the basic treatments. These treatments available will increase the risk of hepatitis reactivation. Between December 2008 and December 2019, 491 Multiple Myeloma patients who underwent haematopoietic stem cell transplantation at the Erciyes University were retrospectively analyzed in terms of hepatitis activation and its effect on patient survival. Median follow up time was 32 (0-146) months. 64.2% of the patients were male. IgG / kappa was the most Ig type (36.3%).Third month transplant responses were 67.6% (34.6% CR and 33%VGPR respectively). Most patients were stage 3(44.4%). Antiviral prophylactic treatment was given in 61 transplants because of Hbsag or antihbc positivity. Eight of them were given second prophylactic antiviral therapy. In 12 of these patients, hepatic enzyme increased despite prophylactic medication. Of these, eleven had Hbsag positivity prior and one was HCV. Six of the twelve patients were patients with a history of revlimid use after transplantation. Although the life expectancy of 61 patients was less than other patients at the beginning, then the overall survival did not change due to reasons such as recurrence, infections, etc. Patients with increased hepatic enzymes were hbsag + and half were taking lenalidomide after transplantation. So according to us, the prophylactic drug of Hbsag + and antihbc + should be different efficacy. Since the life expectancy of those who are positive for hepaticserology is low, these patients need early effective treatment.

Keywords: Cell based therapies, drugs for immune modulation, transplantation, viral infections

P-0846

Comparison of the results of PRA identification and single antijen bead assay in patients with kidney transplant candidate

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We aimed to compare the results of Single Antigen Bead (SAB) and Panel reactive antibody (PRA) class-I-II identification of the end-stage renal disease patients. We retrospectively analyzed 256 patients (female/male,152/104) who were tested for anti-HLA antibodies the years between 2017 and 2020. PRA identification and SAB tests were performed by using an Luminex method (immucor). Patients were classified as positive for PRA (MFI≥1000) (78%) and positive for SAB (37.2%) according to anti HLA antibodies. 39.6% of patients with PRA (+) and 33.3% of patients with SAB (+) had ''Donor Spesific Antibody''(DSA). Anti-HLA antibodies of the patients who had DSA with high MFI were as follows: A1 (16913), A2 (15715), B50 (9207), B51 (6000), DR15 (20000), DR16 (16350), DQ7 (22700), DQ2 (22400). Of the PRA (+) patients, 20% were only class-I (+); 40% were only class-II (+); 40% were both class-I-II (+). PRA (+) patients were divided into 4 groups according to their MFI values as follows: Group-1: MFI <1000, Group-2: MFI:1000-3000, Group-3: MFI:3000-5000, Group-4: MFI>5000. Class-I-SAB was performed in 106 of patients with PRA-class-I (+). Class-I-SAB was positive in 80%, 83%, 100% and 98% of patients in groups respectively. Class-II-SAB was performed in 160 of the PRA-class-II (+) patients. Class-II-SAB was positive in 57,1%, 54,5%, 77,7% and 89% of patients in groups respectively. Our results indicated that the PRA-class-I MFI values above 3000 were associated with Class-I-SAB positivity (p:0,049) and PRA-class-II MFI values above 5000 were associated with Class-II-SAB positivity (p:0,0001).

Keywords: Antibody, immunological techniques, transplantation

P-0847

DOCK8 deficiency in an adult patient with suspected diagnosis of hyper-IgE syndrome

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Male patient of 28 years since 2007 under investigation for clinical suspicion of Hyper-IgE syndrome (SHIE). Personal history includes egg allergy, severe atopic dermatitis, recurrent respiratory infections including severe SARS-CoV-2 pneumonia, elevated levels of IgE and eosinophilia. Family history reveals death of 2 brothers aged 5 and a 32 years due to anaphylaxis and septic shock, respectively. To determine the performance of a specific next generation sequencing (NGS) panel for SHIE. NGS was performed using AmpliSeq strategy (Ion Torrent PGM platform). The panel included five genes (STAT3, SPINK5, PGM3, DOCK8, TYK2). In this study, two mutations in compound heterozygosis in the DOCK8 gene not previously described were identified: a deletion of 6 nucleotides (c.6050_6055delTTAAGG), and a nucleotide change in intron region (c.1868 + 5C> G), outside the canonical region of RNA splicing. Alamut® Visual 2.4 predictor indicates that this variant could alter the donor site of the splicing region. Sanger sequencing of cDNA was performed confirming the splicing alteration, with the elimination of exon 16 of the mRNA. By using a specifically designed NGS panel we were able to identify a patient with DOCK8 deficiency. At the genetic level, an intronic genetic variant is described outside the canonical region of mRNA maturation and demonstrates the need to evaluate any intronic variant not previously described with computer tools of effect on mRNA maturation. The patient is planned to receive a matched sibling donor HSCT.

Keywords: Immune regulation and therapy, immunodeficiency, immunological techniques, transplantation

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POSTER PRESENTATIONS

P-0848

Hyperglycemia impairs the cell-protective effect of CD163-mediated scavenging of hemoglobin-haptoglobin complexes

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Hyperglycemia is a hallmark of diabetes and can induce inflammatory programming of monocytes and macrophages. Scavenger receptor CD163 internalizes and degrades hemoglobin-haptoglobin (Hb-Hp) complexes which are built due to intravascular hemolysis, particularly occurring in inflammatory conditions. Our aim was to identify the effect of hyperglycemia on CD163 expression and function in human primary macrophages. M(IFNγ) and M(IL-4) were differentiated out of primary human monocytes in normal (5 mM) and high (25 mM) glucose conditions. The effects of hyperglycemia on CD163 gene and surface expression were quantified by RT-qPCR and flow cytometry. CD163 mRNA expression was decreased 5.53 times in M(IFNγ) and 4.76 times in M(IL-4) compared to M0. Hyperglycemia elicited an additional suppression of CD163 mRNA in M(IFNγ) of 1.99 times. CD163 surface expression was significantly downregulated by hyperglycemia in M(IFNγ) (1.43 times); but not in M(IL-4). Flow cytometry demonstrated that hyperglycemia did not impair Hb-Hp complex uptake. However, hyperglycemia significantly enhanced pro-inflammatory response of M(IFNγ) to Hb-Hp complex uptake by stimulating production of TNFα, IL-1β, IL-6, IL-8 and IL-1RA. Hb-Hp1-1 complex uptake was the strongest stimulus for M(IFNγ) for acute (6h) cytokine release, however, secretion was diminished after 24h. Contrarily, Hb-Hp2-2 complex uptake resulted in the increased secretion of all read out cytokines after 24h, with the strongest effect on the release of IL-6 (3.06 times). These mechanisms are able to promote low-grade inflammation in diabetes mellitus and indicate the susceptibility of diabetic patients to vascular complications by a dysfunctional control of tissue damage.

Keywords: Diabetes, inflammatory disease, innate immunity, macrophage

P-0849

Trends in seroprevalence of SARS-CoV-2 in the Norwegian population through the first year of the COVID-19 pandemic

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COVID-19 is an infectious disease caused by the novel coronavirus SARS-CoV-2. Infection with SARS-CoV-2 induces antibodies that can be used as a proxy for infection.

We here present a repeated cross-sectional study with three sampling rounds to assess the seroprevalence of SARS-CoV-2 in Norway during the first year of the pandemic. Residual serum samples were solicited systematically from laboratories across Norway in April/May 2020 (round 1), in the late summer of 2020 (round 2) and in January 2021 (round 3), covering all age groups. Antibodies against SARS-CoV-2 were measured using a flow cytometer-based test. Samples with antibodies against both the receptor binding domain and the nucleocapsid protein of SARS-CoV-2 were considered positive. Antibodies against SARS-CoV-2 were measured in overall 4840 residual sera from nine to seventeen laboratories. Approximately 1% of the Norwegian population had been infected after the first wave of the pandemic based on the estimated seroprevalence of rounds 1 and 2. In January 2021, the estimated seroprevalence was found to be 3.2% (95% CrI 2.3-4.2). The seroprevalence estimates did not differ between the various age groups or males and females for either sampling round. The seroprevalence estimates shows a cumulative increase of SARS-CoV-2 infections over time in the Norwegian population. These estimates complement the reported number of confirmed cases of SARS-CoV-2 in Norway and suggests some under-recording of cases. The low seroprevalence estimate of January 2021 indicates that the great majority of the Norwegian population was still susceptible to SARS-CoV-2 infection after the first year of the pandemic.

Keywords: Antibody, infectious disease, viral infections

P-0850

Impact of carbamylation on the enzymatic activity of neprilysin (CD10) and its role in the modulation of lung immune response

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Neprilysin is a zinc-dependent metalloprotease present in the lungs as a membrane-bound protein and in its soluble form in the blood. It partakes in the regulation of the inflammatory responses through cleavage of hormone peptides, such as substance P, which stimulates vasodilation, chemotaxis and activation of immune cells, as well as expression of the proinflammatory cytokines. During inflammation, neprilysin may undergo modifications affecting its activity. In this study, we aimed to investigate whether neprilysins functions in the lungs can be affected by the carbamylation. This nonenzymatic modification, caused by the binding of the carbamyl group to the free α-amino and ε-amino moieties on the amino acids N-termini and lysine residues, is a result of increased concentration of the cyanate in the inflammatory milieu. Carbamylation of recombinant neprilysin was confirmed using the Western blot technique. 48h exposure of the enzyme to the cyanate resulted in the gradual loss of its activity, as assessed by the fluorogenic substrate degradation. Prolonged culture of lung fibroblasts (CCL210) in the presence of cyanate resulted also in decrease of the CD10 expression. Furthermore, differences in substance P cleavage by native and carbamylated recombinant neprilysin were evaluated via HPLC. Obtained results confirmed that carbamylation affects expression and activity of the neprilysin. This may lead to local increase of concentrations of the pro-inflammatory peptides, such as substance P, in the lung tissues.

Keywords: Inflammatory disease, inflammatory molecules, molecular immunology

P-0851

Plasma cytokeraton-20 is a promising novel biomarker of acute graft versus host disease (aGvHD)

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Acute graft versus host disease (aGvHD) is one of the most common complications of allogeneic hematopoietic stem cell transplantation (aHSCT). Early diagnosis is key to improve clinical care. We sought to perform a comparative analysis of select plasma proteins in adult patients undergoing aHSCT to identify novel diagnostic and predictive markers of aGvHD, and its gastrointestinal and cutaneous manifestations in particular. We selected the gut- and skin-restricted proteins FABP2, CK20, occludin, and CK15, respectively, to be tested for their diagnostic sensitivity, specificity, and predictive value in comparison with Reg3α and elafin, two established, tissue-specific diagnostic aGvHD markers as controls. Diagnostic value was analysed by ELISA on a sample set of 40-patients divided into four groups according to organ manifestations, i.e., aHSCT patients developing skin aGvHD, gut aGvHD, both, or no aGvHD (n = 10 each). Predictive value was assessed on an independent patient cohort analysed and on days 0, 7, 14, 21, 28, and 100 after aHSCT (n = 67 total). Our results suggest that plasma FABP2 may serve as a novel diagnostic marker of aGvHD in adult aHSCT patients (ANOVA p = 0.030) while plasma CK20 is a promising diagnostic biomarker candidate of severe aGvHD (ANOVA p = 0.025). The sensitivity and specificity of FABP2 (ROC = 0.754) and CK20 (ROC=0.759) was higher than that of elafin (ROC = 0.708) while it was lower than that of Reg3α (ROC = 0.868). However, while FABP2 has limited, if any predictive value, low plasma CK20 seems to predispose to severe GvHD.

Keywords: Biomarkers, bone marrow transplantation, tissue damage and repair

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POSTER PRESENTATIONS

P-0853

Pre-treatment serum markers of anti-tumour necrosis factor alpha therapy response in patients with inflammatory bowel disease

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Anti-tumour necrosis factor (anti-TNF-α) therapy are efficient treatment for large cohort of patient with inflammatory bowel disease (IBD). Unfortunately, there is no clinical predictor of responsiveness to therapy. The aim of this pilot study was to search for potential biomarkers that could predict the response to therapy. Serum samples were collected from IBD patients (n=21) receiving anti-TNF-α therapy (Adalimumab or Infliximab) at the beginning and at week 38 of the treatment. The detection of biomarkers of the gut barrier (intestinal and liver fatty acid-binding proteins (I-FABP, L-FABP) and trefoil factor 3 (TFF-3), tissue inhibitor of metalloproteinases 1 (TIMP-1)) or inflammation (mannan-binding lectin (MBL), CD14, lipopolysaccharide-binding protein (LBP)) were determined by ELISA. We found that IBD patients with full response to anti-TNF-α therapy have significantly elevated levels of MBL (p=0.042) or CD14 (p=0.006) and reduced levels of TFF-3 (p=0.008) before treatment compared to patients that did not respond. Subsequently during the anti-TNF-α therapy the levels of MBL (p=0.002), CD-14 (p=0.002) and TIMP-1 (p=0.049) are decreased. We did not detect any significant changes in the serum levels of I-FABP, L-FABP, or LBP in responders or non-responders receiving anti-TNF-α therapy at the beginning and at week 38 of treatment. Our results show several possible biomarkers of responsiveness to therapy. Nevertheless, larger cohorts of patients are needed to precisely describe their predictive value for efficiency of anti-TNF-α therapy in IBD patients.

This study was supported by Ministry of Health of the Czech Republic, grant no. NV18-09-00493.

Keywords: Immune regulation and therapy, biomarkers, immune response tracing, inflammatory bowel disease

P-0857

Decreased IFN-gamma levels are correlated with severe Covid-19

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Covid-19 caused by SARS-CoV2 virus is a pandemic disease which has affected and surrounded the world for more than 1 year. Although Covid-19 progresses seriously that it is fatal in some patients, it has been observed that some people experience the disease mildly or even without any symptoms. These differences among in clinical symptoms of patients exposed to SARS-CoV2 suggest variations in immune responses of these people. In this study, Covid-19 patients grouped as mild, moderate and severe according to clinical symptoms and age and gender-matched healthy controls were included. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood samples of donor groups and cultured for 4 hours with/without Cell Stimulation Cocktail. After culturing, PBMCs were stained for detecting intracellular levels of IFN-γ, TNF-α and IL-10 in NK cells and measured on flow cytometry. IFN-γ levels in NK cells of patient groups were shown to be decreased in comparison with healthy subjects and this decrease was correlated with the severity of the disease. TNF-α levels of NK cells in patient groups were also lower than healthy controls, though there were differences among patient groups. Conversely, IL-10 levels were demonstrated to be elevated especially in severe patients compared to healthy subjects. These findings indicate an imbalance between pro-inflammatory and anti-inflammatory immune responses of NK cells in Covid-19 patients. The decrease in levels of IFN-γ, which is an early defender of innate immunity against viruses might play a critical role in the course of Covid-19.

Keywords: Cytokines and mediators, NK cells, viral infections

P-0859

Titanium nanoparticles enhance production and suppress clearance of GDF15 in human primary macrophages

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Macrophages are key innate immune cells that mediate implant acceptance or rejection. Long term use of titanium implants generates wearing particles off. Titanium nanoparticles (TiNPs) favor a pro-inflammatory macrophage polarization (M1) and lower the tolerogenic activation (M2). GDF15 regulates energy expenditure and fibrosis, and is endocytosed by stabilin-1. How TiNPs affect the healing activities of macrophages and their release of circulating cytokines is a relevant open question in regenerative medicine. Our aim was to investigate the effect of TiNPs on GDF15 and stabilin-1 expression, on GDF15 secretion, and on the endocytosis in macrophages. Primary human macrophages were differentiated from CD14+ monocytes and incubated for 6 days under cytokine stimulation in the presence or absence of TiNPs. Affymetrix analysis confirmed by RT-PCR demonstrated that TiNPs stimulate GDF15 and suppress stabilin-1 expression. TiNPs also strongly stimulated GDF15 secretion in all subtypes of macrophages. Flow cytometry was used to quantify the effect of TiNPs on endocytosis of fluorescently-labelled stabilin-1 ligands acLDL and MS-1 (antibody directed against the extracellular domain of stabilin-1). Uptake of both ligands was significantly suppressed by TiNPs. Our data demonstrate that TiNPs have a dual effect on the GDF15/stabilin-1 interaction in macrophage system, by increasing production of GDF15 and suppressing stabilin-1-mediated clearance function. In summary, this mechanism can result in a significant increase of GDF15 in the extracellular space and in circulation leading the unbalanced pro-fibrotic and pro-inflammatory reactions and implant complications.

Keywords: Cytokines and mediators, endo- and exocytic vesicles in immunity, molecular immunology, innate immunity, macrophage, tissue damage and repair

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POSTER PRESENTATIONS

P-0860

Stemness acquisition in prostate cancer confers immune surveillance evasion through lncRNA SNHG16 and lncRNA CRNDE mediated alternative checkpoint B7-H3 (CD276) upregulation

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Identification of major signal transduction pathways associated with immune evasion in PC3 cancer stem-like cells, as *in-vitro* model of metastatic Castration-Resistant Prostate Cancer. We conducted *in-vitro* sphere formation assays to obtain stem-like enriched PC3 population, using serum-free and non-adherent conditions and subsequently only tumorspheres were subcultured. We validated the stem-like enriched phenotype of the PC3 line using flow-cytometry and stemness-related markers CD24 and CD44 staining. We enriched poly(A)+ and poly(A)- RNA out of rRNA-depleted total RNA, polyadenylated poly(A)- fraction and performed QC using QSEP-100 fragment analyzer. The mRNA was sequenced following Nanopore direct RNA sequencing (dRNA-seq) protocol on two MinION 9.4 flow-cells and real-time basecalling was performed with MinIT device. The Jupyter Notebook's EPI2ME Labs platform was used for primary bioinformatics analysis and transcriptome profile analysis was carried out with Cytoscape software. Pathway enrichment analysis of transcriptome profile of more than 6000 genes revealed PI3K/AKT/mTOR, MAPK6/MAPK4, WNT/beta-catenin, and the non-canonical NF-kB signaling pathways as significantly over-represented with Benjamini-Hochberg FDR score < 0.001. Furthermore, we found out full transcript coverage and expression above the median of the putative immune checkpoint molecule B7-H3(CD276), and of two lncRNAs - SNHG16 and CRNDE, implicated in RNA-RNA interactions with CD276 (www.rna-society.org). Cancer immune evasion is a major hindrance in designing effective anticancer therapies. Members of the B7 protein family are well-known source of co-inhibitory signals for T-cell mediated immune response. We identify the B7-family member – B7-H3(CD276) and two lncRNAs as potential targets for immune checkpoint blockade in prostatic cancer.

Keywords: Cell signalling, checkpoint inhibition, immune regulation and therapy, lncRNA, omics technologies, RNAseq

P-0861

COVID-19 and peripheral blood eosinophil counts: a retrospective study

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Coronavirus disease 2019 (COVID-19) is a current pandemic disease. Eosinopenia has previously been described in COVID-19. However, the role of eosinophils in the disease course remains under debate. With this study we aim to study the peripheral blood eosinophil counts in COVID-19 patients and investigate whether there is an association between the peripheral blood eosinophil counts and disease severity of COVID-19. We revised the electronical medical records of confirmed COVID-19 patients with polymerase chain reaction (PCR) assays in the Groene Hart Hospital, Gouda, the Netherlands. We divided patients in mild, moderate and severe groups based on clinical severity. COVID-19 severity was based on the therapy needed and clinical outcome of patients. We compared clinical characteristics, laboratory results and outcome between the three groups. Of the 230 patients included in this study, the mild, moderate and severe groups consisted of 16.5%, 46.5% and 37% patients, respectively. Mean age was 68 years (IQR 21). 63% of patients were male. A significant decrease in the peripheral eosinophil counts was found corresponding to COVID-19 severity. In the mild, moderate and severe groups the percentage of patients with eosinopenia was 73.7%, 86.9% and 94.1%, respectively. This study showed that eosinopenia is significantly more frequent present in patients with more severe COVID-19 disease course.

Keywords: Eosinophils, infectious disease, viral infections

P-0862

Cytokine profiling of FUS-transgenic mice with amyotrophic lateral sclerosis

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FUS-transgenic mice express aggregation-prone truncated human ΔFUS(1-359) protein resulting in the development of ALS symptoms by the age of three months. Gene expression analysis of the symptomatic transgenic mice exhibiting full-blown ALS showed activation of pro-inflammatory response of microglial cells. Transgenic animals exhibited vivid forelimb paresis and hindlimb paralysis which are indications of motor neuron lesions with the onset of progressive muscle atrophy. In this study, we aimed at comparing sera from transgenic and wild type animals for a number of cytokines and chemokines by using multiplex high throughput method in age-matched wild type CD-1 and symptomatic ΔFUS(1-359) mice. Symptomatic ΔFUS(1-359) mice showed increased levels of Th1-related cytokines and downregulated Th2 response. At the same time, cytokines involved in the recruitment of myeloid cells were reduced, while the rest of the analyzed cytokines were not different and were comparable to wild type animals. Interestingly, IL-6 might be elevated due to immune system activation acting as a myokine contributing to a generalized muscle dystrophy in FUS-proteinopathy. These results are the first indication of FUS-proteinopathy in transgenic animals, may help uncover molecular mechanisms of proteinopathy development and provide insight into the presence of inflammatory biomarkers for ALS.

The study was supported by #0090-2019-0005 and animals were housed at the Centre for Collective Use IPAC RAS.

Keywords: Animal models, chemokines, cytokines and mediators, molecular immunology, neuroimmunology

P-0863

To stress… to resist? The power of stress responses in Sepsis

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Restoration of organismal homeostasis after an insult is the result of a balanced response by the two main arms of host defence: disease tolerance and resistance. Inducing stress responses can trigger disease tolerance mechanisms, as demonstrated in several recent studies. There is growing evidence supporting the hypothesis that this could be an effective strategy, much needed for treating complex multifactorial conditions, such as Sepsis. However, the protective mechanisms initiated by stress responses have not been systematically studied. Thus, it is of key importance to understand the impact of stress responses on resistance mechanisms and the role of the immune system in these potential therapeutic approaches. Our laboratory has recently shown that inducing mild disruptions in the mitochondria could trigger disease tolerance programs. Herein, a murine model of Severe Sepsis showed increased survival and less tissue damage when treated with mitochondria-stress inducing drugs. Currently, we are working towards dissecting the impact of this stress response in resistance programs. So far, we have gathered compelling evidence that inducing mild disruption in the mitochondria can trigger pathogen clearance, a hallmark of resistance responses. Furthermore, we have supporting data showing that immune cell populations and their profile are impacted by such treatment. Understanding the mechanistic bases by which stress responses can induce both disease tolerance and resistance and dissecting the fine-tuned balanced cross talk that helps restore homeostasis could contribute to the development of synergistic therapeutic approaches to life threatening conditions, including sepsis.

Keywords: Drugs for immune modulation, immune communication, inflammatory disease, tissue damage and repair

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POSTER PRESENTATIONS

P-0864

Non-infectious immature dengue virus particles are sensed by peripheral blood monocytes through TLR2/TLR6/CD14 signalling axis

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Vascular pathologies stemmed from dysregulated inflammation triggered by infection with dengue virus (DENV) lead to an estimated 96 million hospitalizations each year. Due to incomplete understanding of the mechanisms governing inflammatory responses to the virus, to date, we have no means to predict or mitigate severe disease pathogenesis. We have recently uncovered that sensing of DENV particles by Toll-like receptor 2 (TLR2) on blood monocytes leads to endothelial activation. Moreover, DENV hijacks TLR2 to establish infection and increased TLR2 expression throughout acute phase of infection correlates with disease severity. Here, we show that also non-infectious immature DENV particles, which are released in large numbers by DENV-infected cells, drive endothelial activation via the TLR2 axis. We show that fully immature DENV particles induce a rapid, within 6 hours post-infection, inflammatory response in PBMCs. Furthermore, pharmacological blocking of TLR2/TLR6/CD14 and/or NF-kβ prior to exposure of peripheral blood mononuclear cells to immature DENV abrogates the initial production of inter alia TNFα and IL-1β by monocytes and prevents endothelial activation. However, prolonged TLR2 block leads to a second wave of TNFα production and exacerbated activation of endothelial cells, indicating that TLR2-mediated responses play an important role not only in the initiation but also the resolution of inflammation. Altogether, our study shows the maturation status of the virus has the potential to influence the kinetics and extent of inflammatory responses during DENV infection and thus adds yet another layer of complexity to the mechanisms underlying disease pathogenesis.

Keywords: Inflammatory disease, inflammatory molecules, innate immunity, myeloid cells, viral infections, cytokines and mediators

P-0865

PAD4 controls chemoattractant production and inflammatory cell trafficking in malaria

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Peptidyl arginine deiminase (PAD4) is an enzyme that catalyzes the post-translational modification of arginine residues to citrulline, thus altering the charge of a protein. Despite the accumulating in-vivo evidence suggesting that PAD4 regulates inflammation, its function in immune cells remains poorly understood. We investigate PAD4 in the context of malaria and demonstrate a role in regulation of immune cell trafficking and chemokine production. In a mouse malaria model, PAD4 regulates immunopathology by promoting neutrophil and CD8+ T cell trafficking into the diseased organs. CXCL1 chemokine, which binds to CXCR2 neutrophil receptors and guides them towards site of inflammation, was significantly reduced in the liver and the serum of the PAD4 knock-out mouse. We also found that PAD4 regulates the production of CXCL1 in human macrophages stimulated with Plasmodium falciparum (P. falciparum) and TLR7 ligands. Finally, using patient samples, we show that CXCL1 is a biomarker for severe malaria. In conclusion, PAD4 inhibition promotes disease tolerance and could therefore be a strategy for inducing disease tolerance in infections.

Keywords: Chemokines, granulocytes, infectious disease, innate immunity, neutrophils

P-0866

Melanoma-associated fibroblasts suppress CTL activation, impede cytotoxicity, and increase TIGIT and BTLA expression on CD8+ T cells by depletion of arginine

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Melanoma-associated fibroblasts (MAFs) secrete a large array of soluble mediators capable of suppressing NK cell activity. In this study we analyzed whether MAFs were able to influence CD8+ T cells in a similar manner and sought to clarify the underlying mechanism of action. MAFs and normal dermal fibroblasts (DFs) were isolated from surgically resected melanomas identified as Melan-A-/gp100-/FAP+ stromal cells. CD8+ T cells of healthy blood donors were activated in the presence of MAF- and DF-conditioned media (CM) and markers related to cytotoxic T-lymphocyte (CTL) functions, immune checkpoint regulation and *in vitro* killing activity were evaluated by flow cytometry and redirected killing assays, respectively. Soluble mediators responsible for MAF-mediated effects were identified by ELISA, flow cytometry and inhibitor assays. Activated CD8+ T cells exposed to MAF-CM showed impaired CD69 and granzyme B expression compared to DF-CM-treated cells. In addition, MAF-CM impeded CTL-mediated killing, and upregulated the inhibitory immune checkpoint receptors TIGIT and BTLA on activated non-naïve/memory CD45RO+ CD8+ T cells. Compared to DFs, MAFs displayed increased amounts of VISTA and HVEM, a known ligand of BTLA on T cells. MAF and DF did not differ in the production of most suspected immunosuppressive MAF mediators such as TGFβ, IL-10, PGE2, or kynurenine. However, MAFs showed higher L-arginase activity than DFs. Selective arginase inhibition decreased, while arginase-transfection increased MAF-induced ICR expression on CD8+ T cells. Our data indicate that MAF interfere with CTL activity via soluble mediators and modify ICR availability via L-arginine depletion.

Keywords: Checkpoint inhibition, adaptive immunity, cancer immunology

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POSTER PRESENTATIONS

P-0867

Impaired respiratory burst contributes to infections in PKCδ-deficient patients

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Patients with inherited autosomal recessive (AR) protein kinase C δ (PKCδ) deficiency suffer from childhood-onset autoimmunity. They also suffer from recurrent infections that overlap with those observed in patients with chronic granulomatous disease (CGD). Although their immunosuppressive treatment is a cofounding factor, we hypothesized that PKCδ-deficient patients may be intrinsically susceptible to infections. We studied the NADPH oxidase activity in EBV-B cells, primary and monocyte-derived phagocytes in a large international cohort of PKCδ-deficient patients. The patients' EBV-B cells produced little amounts of reactive oxygen species (ROS) and did not phosphorylate p40*phox* normally after PMA or opsonized *S. aureus* stimulation. Both phenotypes were restored by retrotransduction with wild-type *PRKCD* cDNA. The patients' circulating and monocyte-derived phagocytes displayed low levels of ROS production as well as a markedly reduced neutrophil extracellular trap formation and impaired phosphorylation of p40*phox*. Our results suggest a role for PKCδ in the activation of the NADPH oxidase complex through phosphorylation of p40*phox*. Patients with AR PKCδ deficiency have an impaired NADPH oxidase activity in various myeloid subsets, which may contributes to their CGD-like infectious phenotype.

Keywords: Immunodeficiency, infectious disease, molecular immunology

P-0868

Innate and adaptive immune activation contribute to radiotherapy-mediated tumour control in lung cancer

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Radiotherapy is widely used in lung cancer treatment but its role in regulating immune responses is only more recently appreciated. While activation of the adaptive immune system has been shown to be important in multiple mouse models, this has not been well characterised in the context of lung cancer. Furthermore, the innate immune response to lung radiotherapy remain unclear. Our work explored the activation and interplay of innate and adaptive immunity in response to targeted radiotherapy in the lung. We established a regiment of targeted lung irradiation using the Small Animal Radiation Research Platform (SARRP), which allowed specific targeting of the left lung lobe in mice, and used flow cytometry to quantify immune changes upon targeted radiotherapy. We demonstrated the effectiveness of this regiment in inducing significant reduction in tumour burden in the B16-OVA model of lung metastasis, and activation of dendritic cells (DCs) and adaptive CD4+ and CD8+ T-cells were observed by flow cytometry. Targeted radiotherapy in RAG-deficient mice led to reduced anti-tumour responses, confirming the importance of adaptive immunity. Additionally, we observed activation of innate natural killer (NK) cells, and antibody-mediated depletion of NK cells reduced the effectiveness of radiotherapy. We also identified activation of macrophages and Group 2 innate lymphoid cells (ILC2s). Our results highlighted the importance of both innate and adaptive immunity in the anti-tumour response induced by lung radiotherapy. These findings may inform therapeutic strategies to target these cell types alongside radiotherapy in lung cancer. We are now investigating potential crosstalk between these cell types.

Keywords: Adaptive immunity, cancer immunology, innate immunity

P-0870

Oral cadmium increases contact hypersensitivity reaction in rats

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Cadmium (Cd) in food and drinking water presents a health risk to the general population. We have shown previously that orally-acquired Cd affects basal immune homeostasis in the skin. In this study, we examined the effect of 30-days oral exposure of inflammatory disease-prone Dark Agouti (DA) rats to two environmentally relevant Cd doses (5 and 50 ppm) on contact hypersensitivity reaction (CHS) induced by topical 0,4% dinitrochlorobenzene (DNCB). Both Cd doses increased proinflammatory epidermal cell response (IL-1, TNF and IL-6 production) to DNCB sensitization, as well as epidermal cells' potential to stimulate naïve lymphocytes *ex vivo* (increased IFN-γ and IL-17 production in co-cultures). The proinflammatory milieu of epidermal cells induced by sensitization was accompanied by increased hapten-specific production of IFN-γ (at a lower Cd dose) and IL-17 (at both Cd doses) by draining lymph node (DLN) cells, compared to Cd non-treated animals. During the challenge phase of CHS, oral Cd increased ear swelling response and skin inflammation (edema, mononuclear and neutrophil cell infiltration) at both Cd doses, what correlated with increased innate (TNF) and hapten-specific effector (IFN-γ, IL-17) cytokine response by ear cells. Even in Albino Oxford (AO) rats generally less prone to inflammation, oral Cd increased the proinflammatory response of epidermal cells following sensitization, however, DLN cell responses were absent. Ear swelling response to hapten challenge was observed in AO individuals which acquired a higher Cd dose. Presented data imply the potential of food- and water-borne Cd to be risk factors for skin disease development and/or its exacerbation.

Keywords: Animal models, inflammatory disease, modification allergic responses, skin diseases

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POSTER PRESENTATIONS

P-0871

A mouse model of COVID-19 associated immunopathology

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While most infections with SARS-CoV-2 cause mild to moderate disease, 5% of COVID-19 patients develop critical illness and respiratory failure due to severe, inflammation associated immunopathology. To better understand the precise chain of events and molecular mechanisms that lead to severe COVID-19, preclinical models would be an important asset. However, mice are not susceptible to infections with SARS-CoV-2, due to inefficient binding between the viral spike protein and the mouse orthologue of the human receptor ACE2. To overcome this limitation, we have generated a mouse adapted SARS-CoV-2 strain (*maVie16*) by serial passaging of a human SARS-CoV-2 isolate through the respiratory tract of adult BALB/c mice. Sequencing of isolates from various passages revealed an accumulation of mutations in the receptor binding domain of the spike protein, resulting in two seemingly mouse specific mutations and one mutation (K417T) found in human SARS-CoV-2 variants potentially associated with increased infectivity and pathogenicity. Indeed, BALB/c mice infected with later passages showed progressive loss of bodyweight and significant upregulation of inflammatory genes in the lung despite having a similar pathogen load. Infection with mouse adapted *maVie16* (passage 16) led to severe disease in BALB/c mice with extensive lung inflammation indicated by immune cell infiltration and alveolar damage, resulting in mortality in 50% by day 4. This unique model of murine COVID-19 will enable us to identify novel cellular and molecular mechanisms of COVID-19 associated immunopathology and potentially contribute to an early identification of patients at risk and/or the discovery of novel therapeutic targets.

Keywords: Animal models, infectious disease, inflammatory disease, viral infections

P-0872

Sex differences in alveolar macrophages polarization during Streptococcus agalactiae-Induced pneumoniae

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Body of clinical and experimental investigations clearly indicate that sex is a contributing factor in the incidence and progression of several infectious diseases. This is particularly observed in respiratory diseases where men present higher morbidity and mortality than in women along the life course. We study the differences between the sexes in the inflammatory response in mice model of pulmonary group B streptococcus (GBS) infection. We found that the dynamics of pulmonary infiltration of alveolar macrophages differ between males and females after intranasal infection of GBS. This is associated with higher local production of the primary pro-inflammatory cytokines IL-1β and TNFα and higher expression of the chemokines CXCL2 and CCL3 (not CXCL1 and CCL2) in males compared to females. Alveolar macrophages represent a heterogeneous cell population and exhibit remarkable plasticity. In response to distinct micro-environmental stimuli, macrophages can polarize into different cell subtypes: classically activated macrophages (M1), involved in the pro-inflammatory process and in tissue damage, and alternatively activated macrophages (M2), involved in the damping of inflammation and tissue repair. We found that macrophages from GBS-infected male mice expressed higher levels of M1 macrophages markers (iNOS ans CD80). Taken together, our results indicate a differential sex polarization of alveolar macrophages after pulmonary GBS infection, hence suggesting the contribution of M1 macrophages in increased airway inflammation found in male mice.

Keywords: Bacterial infections, cytokines and mediators, infectious disease, innate immunity, macrophage

P-0878

Changes in monocyte population in response to Pfizer-BioNTech, Sputnik V and Sinopharm vaccine

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The aim of the study was to investigate the modulating effect of three vaccines being used in Serbia on the monocytes, as key immune effector cells. Blood samples were obtained from the subjects with no previous contact with the SARS-COV-2 virus (five participants per vaccine) before vaccination, two days after vaccination with the first dose (day 2.), and two days after receiving the second dose (day 23.). Peripheral blood mononuclear cells were stained with anti-CD14-FITC, anti-CD83-PE, and anti-CD11c-PCP antibodies and analyzed by flow cytometry. On day 2. in subjects receiving Pfizer (PfS) and Sinopharm vaccine (SinS) percent of circulating monocytes (Mo) decreased, whereas in subjects that received Sputnik vaccine (SpS) number of Mo doubled. In comparison to the starting level the percent of activated CD83+ Mo was lower in SinS, somewhat higher in SpS, and six times higher in PfS. The percent of CD11c+ Mo was about fifteen times higher in PfS and SpS, while in SinS the increase was less expressed. On day 23. the percent of circulating Mo and CD83+ Mo decreased below the initial level, while the percent of CD11c+ cells stayed higher in PfS and SinS, and, to a lesser extent, in SpS. The results of our study point to different dynamics of changes in the monocyte population in response to different vaccines.

Keywords: Adjuvants and vaccines, innate host defence, viral infections

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POSTER PRESENTATIONS

P-0879

Long-term immunological imprint of COVID-19 on human peripheral blood leukocyte populations

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SARS-CoV-2 has triggered a pandemic that is claiming many lives. We investigated the cellular immune responses of COVID-19 patients ten weeks and six months after the onset of disease and analyzed the impact of SARS-CoV-2 infection on the immune system. We used multiparametric flow cytometry to analyze whole peripheral blood samples, and determined SARS-CoV-2 specific antibody levels against S-protein, its RBD-subunit and nucleocapsid in a cohort of COVID-19 convalescent patients who had mild disease approximately 10 weeks and 6 months after infection (n=103) and healthy control subjects (n=98). Furthermore, we correlated immunological changes with clinical/demographic parameters. In a previous study we found that ten weeks after disease onset COVID-19 convalescent patients presented with significantly fewer numbers of neutrophils and CD25+Foxp3+CD127- Tregs, but elevated numbers of activated CD8+ T cells (HLA-DR+, CD38+), CD4+ and CD8+ effector memory T cells, transitional B cells and plasmablasts. Re-analysis of peripheral blood of this patient cohort 6 months after disease onset revealed persistently lower neutrophil numbers, and elevated CD8+ memory T cells. In contrast, other above mentioned cellular parameters returned to normal. Anti-RBD, S-protein and nucleocapsid specific antibody levels were reanalyzed and results will be discussed. In summary, acute SARS-CoV-2 infection leaves a significant imprint on the immune system early on, with significant changes sometimes lasting up to 6 months. Our findings may have implications for identifying patients suffering from "long COVID" and will help to determine optimal time-points for vaccination of convalescent patients to maintain SARS-CoV-2 immunity.

Keywords: Adaptive immunity, biomarkers, immune response tracing, innate immunity, memory, viral infections

P-0880

Opposing effects of *Bifidobacterium longum* and *Staphylococcus aureus* after recognition by paediatric T cells

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T cells in neonates, infants, and adults differ dramatically in the initiation, strength, and stability of their responses. In this study, we investigate cellular mechanisms of CD4+ T cells from neonates, infants and adults to show the antigen specific response to *Staphylococcus aureus*, *Staphylococcus epidermids* and *Bifidobacterium longum*. T cells from surgically excised adenoids of children in different ages, cord blood and peripheral blood from healthy donors were characterized by flow cytometry and functional assays. Therefore CD14+ monocytes were incubated with extracts of *S. aureus*, *S. epidermidis* and *B. longum* to stimulate enriched T cells. Analyses of T cells from the different age groups show that CD4+ T cell responses are initiated from birth with bacteria-specific characteristics. Surprisingly, an inverse correlation is observed between the percentages of proliferating T cells induced by *S. aureus* and the age of the children. In addition to a tremendous proliferative response, T cells activated with staphylococcus show increased plasticity and diversity, whereas *B. longum*-stimulated T cells are attenuated. This effect of *B. longum* is also reflected in the age-independent suppression of activation of *S. aureus*- and *S. epidermidis*-specific Thelper cells and suppresses the "cytokine storm" of severely ill COVID-19 patients, which may probably contributes to the resolution of harmful hyperreactions of the immune system.

Keywords: Bacterial infections, cytokines and mediators, immune development, innate host defence, regulatory cells

P-0881

Absolute quantification of epigenetic DNA modification products in patients with systemic sclerosis by 2D-UPLC-MS/MS system

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Systemic sclerosis (SSc) is a rare autoimmune disease characterized by progressive fibrosis of skin and internal organs. The underlying mechanisms of SSc are complex and remain unclear. Besides genetic mechanisms, epigenetic alterations including DNA methylation/demethylation processes may have crucial roles in SSc. This study aimed at the absolute quantification of the epigenetic DNA modification products in SSc patients using two-dimensional ultra-performance liquid chromatography-tandem mass spectrometry (2D-UPLC-MS/MS). Forty-one SSc patients monitored at DEU Research Hospital and 27 healthy individuals were included in this study. DNA samples were extracted from whole blood specimens. Waters Acquity 2D-UPLC and Xevo TQ-S tandem quadrupole mass spectrometer were used for detection of epigenetically modified DNA products which were 5-methyl-2'-deoxycytidine (5mdC), 5-hydroxymethyl-2'-deoxycytidine (5hmdC), 5-hydroxymethyl-2'-deoxyuridine (5hmdU), 5-formyl-2'-deoxycytidine (5fdC), and 5-carboxy-2'-deoxycytidine (5cadC). The 5hmdU concentration was higher in SSc patients while the 5hmdC level was lower compared to the healthy individuals ($p<0.001$, $p=0.012$, respectively). Besides, the 5-fdC and 5-cadC concentrations had an upward trend in SSc but the results could not reach a significant value ($p=0.066$, $p=0.062$, respectively). These findings demonstrate that 5hmdC may be efficiently converted to 5hmdU and SSc patients significantly tend to the alternative demethylation pathway at the systemic level. An upward trend in 5fdC and 5cadC levels also supports the increased hypomethylation pattern in SSc. We believe that absolute quantification of DNA methylation/demethylation products with novel technologies can provide a deep understanding to disease pathogenesis and has a potential to mark an era for developing new therapeutic strategies.

Keywords: Autoimmunity, epigenetic control and modulation of immunity, molecular immunology

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POSTER PRESENTATIONS

P-0882

Chimeric antigen receptor (CAR) NK cells for the treatment of allergy

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Allergen-specific CD4+ T cells play a central role in the pathogenesis and maintenance of allergic diseases. Currently, patients with allergic diseases are mainly treated with symptomatic therapy, while only few and suboptimal causal therapy options are available. Our aim is to develop a causal treatment of allergies based on the elimination of human allergen-specific CD4+ T cells using CAR cells. We created CARs with specificity for allergen-specific CD4+ T cells, consisting of HLA-DR heterodimers chimerized with different intracellular signalling tails (CD3 ζ, CD27, CD28, 4-1BB or Dap10). The different CARs were expressed in Jurkat E6-1 and NK-92 cell lines to investigate their functionality. The results showed that CARs are well expressed on Jurkat E6-1 cells, with 28–78% of the cells expressing the different CARs when introduced by lentiviral transduction. When the CARs were cross-linked by monoclonal HLA-DR antibodies, the CAR cells increased geometric mean of expression of CD69 up to 15-fold compared to controls. These results allowed us to select the CAR constructs with optimal signalling activity. To further confirm the functionality of the selected HLA-DR CAR, we transduced this construct into the NK-92 cell line. HLA-DR CAR NK-92 cells showed specific lysis of 78% of the target cells Jurkat TRAV17/BV18 at 10:1 effecter to target cell ratio. HLA-DR CAR NK-92 cells show strong cytotoxicity towards allergen specific T cells. Such treatment with CAR NK cells may help to reduce the systemic burden of allergen-specific CD4+ T cells in individuals suffering from severe forms of allergies.

Keywords: Allergic disorders, cell based therapies, immunotherapy, NK cells

P-0883

CUBIC visualization of the brain during experimental cerebral malaria

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Cerebral malaria (CM) is the most severe complication of malaria, a life-threatening disease caused by *Plasmodium falciparum* infection, responsible for over 400000 deaths annually. The molecular pathogenesis underlying CM is not completely understood, although sequestration of infected erythrocytes in cerebral microvessels is thought to play a role. Here, we visualize the whole-brain microvessels and the localization of *Plasmodium* parasites in three-dimensions (3D) to understand the spatial distribution and the potential sequestration of parasites during experimental cerebral malaria (ECM), a mouse model of CM. We utilized CUBIC (Clear, Unobstructed, Brain/Body Imaging Cocktails and Computational analysis) with light sheet fluorescent microscopy (LSFM), and reconstructed the images in 3D.

Keywords: Infectious disease, parasite infections, visualizing immune responses

P-0885

Antitumor potential of *Alchemilla vulgaris* L. in ortotopic mouse breast cancer model

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Alchemilla vulgaris L. has long history of usage in folk medicine especially against gynecological problems. Ethnomedicinal reports for the territory of Balkan are mentioning its well known biological properties against dysmenorrhea, menopausal complaints, infertility, cysts and endometriosis. Based on ethnomedicinal data on female illnesses, the objective of our study was to determine the effect of *Alchemilla vulgaris* L. ethanol extract against breast cancer cells *in vitro* and *in vivo*. Our results have showed remarkable viability decrease of mouse (4T1) breast cancer cells in dose-dependent manner after the treatment with *Alchemilla vulgaris* L. extract. Strong inhibition of cell prolifertion was observed in treated cells. In parallel with this, different types of cell death was found. Certain percentage of 4T1 cells was subjected to programmed cell death-apoptosis which was followed with caspase activation and confirmed by fluorescent microscopy observing tipical morphologicas features of apoptosis in treated culture. Estimation of the presence of autophagosomes shown that autophagy contributing to the cytotoxicity of the treatment. Also, enhanced production of ROS and intracellular NO after treatment with *Alchemilla vulgaris* L. was found. In parallel, metastatic potential of this cells is diminished. Apart from the direct effect of *A. Vulgaris* L. extract on tumor cells, strong potentiation of antitumor immune responce manifested dominantly through enhanced accumulation of activated dendritic cells and subsequently CD8+ T cells in spleen and tumor microenvironment. Above briefly described mode of action of *Alchemilla vulgaris* L. against breast cancer cells makes this plant worthwhile for further evaluation in the field of oncology.

Keywords: Cell death, cancer immunology, *in vivo* tumor models, microenvironment

P-0886

Could aortic stenosis have an infectious component?

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Recent studies suggest that aortic stenosis (AS) is an active inflammatory atherosclerosis-like process. Expansion of peripheral CD28null CD8+ and CD4+ T-cells has been observed in AS patients. These T-cell subsets expansion is associated with cytomegalovirus (CMV) infection. Additionally, expansion of CD8+CD57+ T-cells has been also observed in AS patients. We have demonstrated that CD57+ T cells are highly proinflammatory, cytotoxic and polyfunctional, and a hallmark of CMV infection. CD28null T-cells express CD57 and CX3CR1 chemokine receptor. Vascular endothelial cells produce fracktalkine (CX3CR1 ligand) in response to proinflammatory cytokines. Thus, here we studied the coexpression of CD57 and CX3CR1 on CD28null T-cells and their potential association with AVS and CMV. Expression of CD28, CD57 and CX3CR1 on peripheral blood T-cells from AVS patients (49-80 years), and sex/age matched healthy volunteers (HD) was analysed by flow cytometry. Proportion of CD28nullCD57+CX3CR1+ CD4+ and CD8 T-cells in AS patients were increased compared with HD (p=<0.001 for both). Additionally, we found a difference in CMV prevalence between patients (91%) and HD (66.7%). When considering CMV-serostatus, CD28nullCD57+CX3CR1+ CD8+ T cells percentage was increased in AVS patients only if they were CMV+, but not in CMV- individuals. CONCLUSIONS: Expansion of CD28nullCD57+CX3CR1+ CD8+ T cells associates with AS only in the context of CMV chronic infection, supporting a potential role of CMV infection in AS development.

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Keywords: Ageing, cardiovascular diseases, chronic inflammation and fibrosis, immune senescence, inflammatory disease, viral infections

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POSTER PRESENTATIONS

P-0887

Extracellular vesicles subtypes in sera of COVID-19 patients as indicators of immune dysregulation and disease severity

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COVID-19 is characterized by a wide spectrum of disease severity, ranging from asymptomatic to critical symptoms, hallmarked by immune dysregulation. Despite intensive investigations, the underlying mechanisms, and indicators of COVID-19 immunopathogenesis are largely missing. Extracellular vesicles (EVs) emerged as key mechanisms of cell-to-cell communication and excellent biomarkers in many infectious and immune-related diseases. However, their role COVID-19 is largely unknown. Here we analyzed a set of clinical, biochemical and immunological markers in 46 COVID-19 patients (20 with mild symptoms, and 26 with severe symptoms) and 16 sex/age-matched healthy individuals, along with the imaging flow cytometry EVs characterization from donors' sera. We found an increased number of CD13+ EVs/ml in COVID-19 patients, and their number was significantly higher in the group of severe patients, along with the number of CD82+ EVs. Additionally, the number of CD13+ EVs positively correlated with the number of inflammatory monocytes and IL-6-producing myeloid-derived suppressor cells (MDSCs) in severe COVID-19 patients. In contrast, the patients with mild COVID-19 symptoms displayed an increased number of HLA-ABC+ EVs compared to healthy donors, and significantly higher number of CD24+ EVs, compared to severe COVID-19 patients. HLA-DR-ABC+ EVs and CD24+ EVs predicted positive outcome of COVID-19, as they negatively correlated with disease severity and accumulation of IL-10-producing MDSCs, the mediators of immune paralysis in severe COVID-19 patients. These results indicate for the first time that EVs in sera are excellent indicators of COVID-19 pathogenesis and disease progression, but the exact mechanisms underlining EVs actions in COVID-19 require further investigations.

Keywords: Biomarkers, chronic inflammation and fibrosis, endo- and exocytic vesicles in immunity

P-0888

Impaired LAIR-1-mediated immune control due to collagen degradation in systemic sclerosis

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Fibrosis is characterized by the production and deposition of excessive extracellular matrix (ECM) products such as collagen. Systemic sclerosis (SSc), is a potentially fatal disease characterized by immune dysregulation and fibrosis of the skin and internal organs. LAIR-1 is an inhibitory collagen receptor highly expressed on immune cells in these tissues. We show that LAIR-1 deficient mice have increased bleomycin-induced skin fibrosis, supporting a protective role for LAIR-1 in controlling fibrosis. In SSc patients, LAIR-1 expression and function is intrinsically normal. However, the ECM produced by fibroblasts of SSc patients contains high levels of collagen degradation products that can act as decoy ligand and impair LAIR-1 mediated signalling. These collagen degradation products are dependent of matrix metalloproteinases and platelet-derived growth factor (PDGF) receptor signalling. We conclude that LAIR-1 represents a control mechanism in tissue remodelling and that the absence of LAIR-1 mediated control in SSc patients results in a perpetuating loop in which fibrosis continues. The presence of functional LAIR-1 in SSc patients provides a therapeutic opportunity that holds promise for disease control.

Keywords: Checkpoint inhibition, chronic inflammation and fibrosis, tissue damage and repair

P-0889

Multiple effects of *Alchemilla vulgaris* L. extract on melanoma cells and tumor microenvironment

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Several ethnobotanical reports on *Alchemilla vulgaris* L. pointed out diverse biological properties against problems such as dysmenorrhea, pruritus vulvae, menopausal complaints as well as related diseases in women. Also previous studies have shown that *Alchemilla vulgaris* L. extracts are exhibiting antiinflammatory, antioxidant, wound healing and neuroprotective activity. The aim of this study was to evaluate the direct effect of *Alchemilla vulgaris* L. ethanol extract against melanoma cells *in vitro* and *in vivo*, as well as its effect on tumor microenvironment *ex vivo*. This study was performed on two different mouse melanoma cell lines, B16 and B16F10, and on syngeneic mouse melanoma model *in vivo*. Obtained results revealed dose-dependent decrease of cell viability after 72 h- treatment with *Alchemilla vulgaris* L. extract. The observed effect was followed by loss of dividing potential in both tested cell lines. In parallel with this, certain percentage of B16F10 cells was subjected to programmed cell death in a caspase independent manner while in B16 cells estimation of the presence of autophagosomes by flow cytometry has shown that autophagy is occurring after the treatment and it is shown to be mechanism of death. Concerning *in vivo* studies *Alchemilla vulgaris* L. extract significantly reduced tumor growth in B16 melanoma model partly through stimulation of antitumor immune responce. It altered dendritic cells phenotype which activated cytotoxic and CD4+ T lymphocytes to successfully destroy tumor cells. In summary, these data indicate that *Alchemilla vulgaris* L. is valuable of further investigation in the field of experimental oncology.

Keywords: Cancer immunology, cell death, *in vivo* tumor models, microenvironment

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POSTER PRESENTATIONS

P-0890

Cold exposure reprograms monocytes and protects from neuroinflammation

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Obesity is linked to development of metabolic and inflammatory diseases. However, effects of a negative energy balance and a metabolically active phenotype on the immune system and immune-mediated diseases are poorly understood. Here we use cold exposure as an inducer of energy expenditure, which mainly acts by activating the UCP1-mediated brown adipose tissue thermogenesis. We show that cold exposure modulates monocytes and consequently T cell priming, resulting in decreased disease severity in a mouse modelof multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). Specifically, we found that cold exposure reduces monocytes in the bone marrow and changes their immunologic and metabolic phenotype in the circulation. Exposure to cold temperatures decreases the EAE severity independent of UCP1-mediated thermogenesis. Cold exposure reduces pathogenic T cell cytokine expression and MHCII expression of monocytes during EAE. Depleting the monocytes via genetic or pharmacological CCR2 blockade abolished T cell cytokine expression at EAE onset, implying that cold exposure may affect T cell priming via modulation of monocytes. Accordingly, EAE is unchanged when cold exposure is applied only during the effector phase of the disease. Our work provides systematic overview on the immune changes during exposure to cold and could have implications in prevention and treatment of immune-mediated diseases.

Keywords: Adaptive immunity, autoimmunity, metabolic control of immune responses, multiple sclerosis, myeloid cells, neuroimmunology

P-0891

Anti-melanoma effects of Hyper-harmonized hydroxylated fullerene water complex and hyperpolarized light *in vivo*

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In our recent study we have demonstrated antitumor effects of Hyper harmonized hydroxylated fullerene water complex (3HFWC) and hyperpolarized light (HPL) on melanoma cell lines *in vitro*. The aim of this study was to reveal their therapeutic effects *in vivo* in syngeneic model of melanoma in C57BL/6 mice. Treatment started when tumors became palpable. Mice were irradiated 2x20min daily with Bioptron® device equipped with HPL filter; with/without 3HFWC (0.145mg/ml) in drinking water ad libitum or 3HWC alone. Our results demonstrated the absence of 3HFWC and HPL side effects (no weight loss nor signs of nephro- and hepatotoxicity). Tumor growth was decreased in all treated groups with most profound effect in combined treatment. Histological examination revealed abolished proliferative capacity (significantly decreased mitotic index and nuclear PCNA immunopositivity of melanoma cells). Stimulation of melanin pigmentation and increased incidence of enlarged lipofuscin-filled melanoma cells suggest differentiation and pro-senescent effects of 3HFWC and HPL. Analysis of tumor infiltrating immune cells indicates strong potentiating of antitumor immune response through the increase of cytotoxic CD8+ and NK cells infiltration as well suppression of Treg, myeloid-derived suppressors and tumor-associated M2 macrophages. Taken together, our results reveal at least two mechanisms of anti-melanoma effects of 3HFWC and HPL: 1) directly, through the proliferation inhibition as well the stimulation of differentiation and senescence of melanoma cells; and 2) through the stimulation of antitumor immune response. In conclusion, the combination of 3HFWC and HPL presents potentially promising strategy in cancer therapy.

Keywords: Cancer immunology, *in vivo* tumor models, microenvironment

P-0892

Paucigranulocytic asthma: do sputum macrophages matter?

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Although paucigranulocytic asthma (PGA) is the most common phenotype of stable asthma, its features are not adequately studied. In this study, we aim to display the characteristics of PGA. 116 non-smoker adult asthma patients (80% female, mean age 39± 12.9) admitted to three tertiary centres were included. Their demographic and clinical features, allergy status, biochemical results, Asthma Control Test (ACT) scores, spirometry and exhaled nitric oxide (FeNO) measurements were obtained. Induced sputum cytometry was performed. According to induced sputum cell counts, four phenotypes were detected: eosinophilic (EA) (22.4%), mixed (MGA) (6.9%), neutrophilic (NA) (7.8%) and paucigranulocytic (62.9%). In sputum, macrophages were higher in the PGA group compared to other groups (PGA vs NA and PGA vs MGA: p<0.001, PGA vs EA: p=0.03). The atopy rate between phenotypes was the same. Although forced expiratory flow 1st second (FEV1) was similar in four groups, FEV1/FVC was higher (p=0.013) and FEV1 reversibility was lower in PGA patients than the corresponding values in other phenotypes (p=0.015). Low reversibility was comparable in both inhaled corticosteroid naive PGA patients and in those on ICS treatment. Although insignificant, FeNO and blood eosinophil counts were higher in MGA and EA groups while these were the lowest in the PGA group. Uncontrolled asthma ratio was low in PGA (16%) while it was 11% for NA, 25% for MG and 23% in EA. Macrophages are predominant in sputum of PGA patients. Besides lower uncontrolled asthma ratio, lower FEV1 reversibility is a prominent characteristic of this phenotype.

Keywords: Granulocytes, allergic disorders, eosinophils, macrophage

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POSTER PRESENTATIONS

P-0894

Salivary determination of SARS-CoV-2 anti-N IgG could be used as a diagnostic alternative

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Although SARS-CoV-2 antibody response in serum has been widely studied, we don´t know that much about this response in saliva. In this work, we used ELISA to test the salivary IgG response to SARS-CoV-2 N protein in individuals who were diagnosticated with COVID-19 between 3-6 months earlier, and we compared them with negative controls. Results inidicated that this method is meaningful with 81,82% of sensitivity, 83,34% of specificity, 81,82% of positive predictive value and 83,34% of negative predictive value. This study confirms that salivary IgG antibodies to N protein from SARS-CoV-2 remain at least until 6 months after diagnostic in most patients. Thus, the IgG response in saliva could be used as an alternative measure of the SARS-CoV-2 sistemic inmunity.

Keywords: Adaptive immunity, antibody, infectious disease, viral infections

P-0896

The HLA-G/ILT2 pathway modulates CD8 T cell effector function in gastric cancer

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Since not all cancer patients benefit from current immune check-point inhibitor therapies, new immune-checkpoint targets are required. HLA-G, a non-classical tolerogenic histocompatibility molecule is one of such potential targets. We studied the expression and function of HLA-G and ILT2, one of its ligands, in gastric cancer. Peripheral blood mononuclear cells (PBMCs) were isolated from 17 patients and 9 controls. Cells were stimulated with anti-CD3/CD28/CD2 beads. Both proliferation (CFSE assay) and phenotypic markers (CD8 CD3, PD1, ILT2) were assessed by flow cytometry. ILT2 expression is increased in PBMCs and CD8+ lymphocytes from gastric cancer patients (32.1% and 38.7%) compared to controls (16.1% and 15.1%, p=0.002 and 0.024, respectively), independently of PD1 expression. For a given patient, the percentage of cells able to produce IFNγ was higher in the CD8+ILT2+ population (21.9%) than in the CD8+ILT2- counterpart (7.9%, p<0.0001, paired T-test). Increased concentration of conditioned medium with soluble HLA-G (sHLA-G) results in decreased proliferation of PBMCs from healthy donors compared to cultures with no sHLA-G. Moreover, coculture of PBMCs with HLAG+ cells induced a decrease in IFNγ producing ILT2+ and CD8+ILT2+ cells, compared to HLA-G- cell line (paired t-test p=0.018 and 0.001, respectively). This implies that HLA-G, through ILT2, inhibits the activation of immune cells. ILT2+ and CD8+ILT2+ populations are abundant in peripheral blood from gastric cancer patients. In addition, ILT2+ cells have an increased capacity to produce IFNγ after TCR-mediated activation which can be blocked by HLA-G. The HLA-G/ILT2 pathway may become a new target in the treatment of gastric cancer.

Keywords: Biomarkers, cancer immunology, checkpoint inhibition, immune regulation and therapy

P-0897

Identification of dendritic cell populations and subsets in psoriasis patients on biological treatment

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Psoriasis is an immune-mediated inflammatory skin disease associated with systemic inflammation. Excessive production of innate cytokines IL-12 and IL-23 by dendritic cells (DC) drive differentiation of pathogenic T cell responses and the release of TNF and IL-17. Targeted therapies that inhibit TNF, IL-12/23, or IL-17 have revolutionized the treatment of psoriasis, yet there are some patients whose symptoms do not improve with applied treatment. The main goal of the study was to analyze the quantities of circulating DC subsets in psoriasis patients before and after 1 year of biological treatment of anti-TNF (etanercept and infliximab) and anti-IL12/23 (ustekinumab) and compare with treatment effect. Eleven patients with moderate-to-severe psoriasis vulgaris and eleven age-, gender- and BMI-matched healthy individuals were included. We used flow cytometry to define changes in the composition of peripheral DC subpopulations and subsets of psoriasis patients prior to and after 1 year of biological treatment. Data analysis showed a significant increase in the frequencies of conventional DC 1 (cDC1), cDC2, and most of cDC2 subsets during cytokine inhibition. For almost all subtypes and subsets of DC, we determined lower frequencies in psoriasis patients compared to healthy controls. We also observed stability in plasmacytoid DC subpopulation frequencies after comparing patients who responded or did not respond to treatment. These results suggest that the distribution of dendritic cells in peripheral blood could be a potential biomarker for disease severity in psoriasis. Further analysis should focus on assessing cell functionality to confirm these results.

Keywords: Dendritic cells, immunotherapy, skin diseases

P-0901

Tuning macrophage polarization to model myocardial infarction in the generation of functional cardiac organoids

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Myocardial infarction (MI) is an ischemic and inflammatory event majorly orchestrated by macrophages from infiltrating monocytes. In addition to the many functions played by macrophages in healthy heart, such as immunosurveillance, regeneration, electrical conduction and elimination of exophers, they also play a critical role in deciding the fate of the heart post-MI. Despite therapeutic interventions, there is a need to improve our understanding of the resident macrophage population in the heart *in vitro*, to better recapitulate the myocardium through tissue engineered models. Additionally, there is no cardiac disease model in existence that incorporates and immune response. Hence, the aim of this project is to develop a humanized model of MI, using induced pluripotent stem cell (iPSC) derived cardiomyocytes together with inflammatory cytokine stimulation, to model the disease environment. To obtain physiologically relevant inflammatory cytokines for cardiomyocyte stimulation, macrophages were derived from iPSCs. iPSC-derived macrophages (iMacs) were found to be positive for common macrophage markers such as CD14 and CD11b. They also displayed an increased expression of CX3CR1 and HLADR, as well as a decreased expression of CCR2, showing a more resident macrophage phenotype. Next, they displayed potential to polarise to classically and alternatively activated phenotypes, confirmed by flow cytometry and ELISA. Finally, iMacs also showed ability to perform phagocytosis. This preliminary data suggests that iMacs can be used as a potential candidate for modelling resident macrophage immune response *in vitro*.

Keywords: Cardiovascular diseases, macrophage, stem cells

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POSTER PRESENTATIONS

P-0902

Immunophenotypic analysis reveals differences in circulating immune cells in peripheral blood of segmental and non-segmental vitiligo patients

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Accumulating studies have indicated immune-based destruction of melanocytes in both segmental vitiligo (SV) and non-segmental vitiligo (NSV). Whereas SV often occurs unilaterally during childhood and stabilizes after an initial period of activity, the disease course of NSV is usually slowly progressive, with new lesions occurring bilaterally during life. This suggests involvement of distinct pathophysiology pathways, specifically increased systemic immune activation in NSV patients, but not in SV patients. This research aimed to identify differences in immune cells in blood of patients with SV and NSV, through immunophenotyping of circulating cells. Regulatory T cells (Tregs) were unaffected in patients with SV compared to healthy controls, but decreased in NSV patients. In NSV patients, the reduction in Tregs was associated with presence of other systemic autoimmune comorbidities, which were less present in SV. Likewise, absence of a melanocyte-specific antibody response in patients with SV, suggests less involvement of B cell immunity in SV. These data show that, in contrast to NSV, no increased systemic immunity is found in SV patients, indicating that SV pathogenesis is associated with a localized cytotoxic reaction targeting epidermal melanocytes.

Keywords: Antibody, autoimmunity, skin diseases

P-0903

Omalizumab in practice: ten-year experience of a tertiary referal allergy centre

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To evaluate the clinical outcome of omalizumab in different diseases. Patients receiving omalizumab for chronic spontaneous urticaria(CSU), severe allergic asthma(SAA), nasal polyposis with asthma(NPwA), idiopathic anaphylaxis(IA), mastocytosis, allergic bronchopulmonary aspergillosis(ABPA) were retrospectively included. The effectiveness of omalizumab with asthma control test(ACT) were assessed at baseline and 16th week of treatment in SAA and ABPA. Urticaria activity score-7(UAS7) and medication scores (MS) in CSU, SCORMA(SCORing MAstocytosis) in mastocytosis were determined at baseline, 3rd and 6th months of treatment. Nazal endoscopy for NP was performed at baseline, 6th month and first year of treatment. Clinical assessments at baseline and during treatment were performed for IA. 213 patients were included. In 59 patients with SAA, ACT scores increased at the 16th week($p<0.001$). Omalizumab was discontinued in median 15 months (min-max: 6-111) in 10 patients whose asthma was under control. In 4 of them, omalizumab had to be restarted in median 6 (min-max:3-12) months. In CSU (n=141), the baseline UAS7 and MS were higher than those at the 3rd and 6th months($p<0.001$,both). The treatment interval was increased to 45 days at the 6th month in 77 patients and 40 of them presented with relapse in mean 37.98±2.3 days. In ABPA(n=5), IA(n=4) and NPwA(n=2) were under control at assessment visits. In mastocytosis(n=2), SCORMA was lower at the 6th month($p:0.04$). Thrombocytopenia and anaphylaxis were the confirmed adverse reactions seen during treatment. This study demonstrates the effectiveness and safety of omalizumab in various diseases in long-term use in a real-life practice.

Keywords: Allergic disorders, mast cells, skin diseases

P-0904

Searching for long non-coding RNAs affecting the expression of the MYC proto-oncogene in B-lymphocytes and B-cell lymphomas

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The translocation of the Myc gene to the immunoglobulin heavy chain locus (IGH/MYC) is a characteristic chromosomal aberration of Burkitt lymphoma. This translocation leads to an increased expression of Myc in B-cells. This study is devoted to the search of enhancer RNAs (eRNAs) that can affect the proto-oncogene Myc expression in the case of its IGH/MYC rearrangement. In our study, potential eRNAs from the IGH locus have been selected using an in-silico algorithm. Knockdown of a few of them has led to a reduced expression of the MYC gene in cells of the Namalwa B-lymphoblastoid cell line with IGH/MYC translocation. Moreover, overexpression of these eRNAs in the same cell line has increased the level of Myc mRNA. However, the overexpression of the eRNAs does not affect the level of MYC in cells of the MP1 B-lymphoblastoid cell line without IGH/MYC translocation or in B-lymphocytes.

This work was supported by the Russian Science Foundation (project no. 19-74-10083)

Keywords: Adaptive immunity, B lymphocytes, lncRNA

P-0905

The C-X-C motif chemokine ligand 1 sustains breast cancer stem cell self-renewal and promotes tumor progression and immune escape programs

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Breast cancer (BC) mortality is mainly due to metastatic disease, which is primarily driven by cancer stem cells (CSC). The chemokine C-X-C motif ligand-1 (CXCL1) is involved in BC metastasis, but the question of whether it regulates breast cancer stem cell (BCSC) behavior is yet to be explored. Here, we demonstrate that BCSCs express CXCR2 and produce CXCL1, which stimulates their proliferation and self-renewal, and that CXCL1 blockade inhibits both BCSC proliferation and mammosphere formation efficiency. CXCL1 amplifies its own production and remarkably induces both tumorpromoting and immunosuppressive factors, including SPP1/OPN, ACKR3/CXCR7, TLR4, TNFSF10/TRAIL and CCL18 and, to a lesser extent, immunostimulatory cytokines, including IL15, while it downregulates CCL2, CCL28, and CXCR4. CXCL1 downregulates TWIST2 and SNAI2, while it boosts TWIST1 expression in association with the loss of E-Cadherin, ultimately promoting BCSC epithelial-mesenchymal transition. Bioinformatic analyses of transcriptional data obtained from BC samples of 1,084 patients, reveals that CXCL1 expressing BCs mostly belong to the Triple-Negative (TN) subtype, and that BC expression of CXCL1 strongly correlates with that of proangiogenic and cancer promoting genes, such as CXCL2-3-5-6, FGFBP1, BCL11A, PI3, B3GNT5, BBOX1, and PTX3, suggesting that the CXCL1 signaling cascade is part of a broader tumor-promoting signaling network. Our findings reveal that CXCL1 functions as an autocrine growth factor for BCSCs and elicits primarily tumor progression and immune escape programs. Targeting the CXCL1/CXCR2 axis could restrain the BCSC compartment and improve the treatment of aggressive BC.

Keywords: Cancer immunology, chemokines, stem cells

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POSTER PRESENTATIONS

P-0906

Induction of arthritis-like synovial inflammation in NOD-scid Il2rg null mice functionally engrafted with peripheral blood lymphocytes from rheumatoid arthritis patients

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Preclinical testing of novel therapeutic approaches for rheumatoid arthritis (RA) requires a mouse model that imitates the human disease. To circumvent limitations due to differences between murine and human immune systems, we aimed to establish a humanized mouse model of RA. Peripheral blood mononuclear cells (PBMC) of RA patients (or healthy subjects as control) were injected intravenously into 6-8-week old female NOD-scid IL2rg null (NSG) mice. Engraftment of human cells was monitored weekly and reactivity of human cells, recovered from spleens, was assessed by flow cytometry. Human cytokines and rheumatoid factor (RF) were quantified in mouse serum by CBA and ELISA, respectively. Joint sections embedded in paraffin were stained with H&E and analyzed by immunohistochemistry. Engraftment of human cells in NSG mice reached its maximum at six weeks post-injection. The human graft consisted predominantly of activated memory CD4+ T cells which preserved their capacity to express IFN-γ in response to recall antigen PPD, RA-synovial fluid (SF) or polyclonal stimulation. Engrafted B cells continued producing RF-IgM in NSG mice. Sequential intraperitoneal and intraarticular administration of RA-SF, or injection of autologous SF-pulsed dendritic cells, provoked leukocyte infiltrates and cartilage degradation in RA-PBMC-engrafted NSG mice. Engraftment of RA patient-derived lymphocytes in NSG mice was functional, leading to a chimeric human-mouse model which, after a specific inflammatory trigger, represented histological features of RA.

Funded by FONDECYT3150453, FONDECYT1140553, and PAI77180094 from ANID Chile.

Keywords: Animal models, autoimmunity, dendritic cells, inflammatory joint diseases, rheumatoid arthritis

P-0907

Influence of modified tumor-derived extracellular vesicles on the immunosuppressive activity of murine colon carcinoma-associated MDSCs

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Extracellular vesicles (EVs), profusely released by tumor cells, carry much information in various proteins, miRNA, or ligands. EVs may be absorbed by neighboring cells and affect the tumor microenvironment (TME), promoting tumor progression. For these reasons, scientists undertake researches focusing on limiting tumor EVs' pro-cancerous influence by their cargo modifications. Modified EVs may influence different cells in TME, including MDSCs, which possess high immunosuppressive activity. The purpose of the research was to determine the influence of modified EVs derived from murine colon carcinoma cells (MC38) with modified expression of IL-18 and/or TGF-β1 on the immunosuppressive activity of generated *in vitro* MDSC, including their ability to inhibition of lymphocytes proliferation and their differentiation towards Th1 cells. EVs were isolated from MC38 cells with the following modifications: overexpression of interleukin-18 (MC38/IL-18) and shRNA for TGFβ1 (MC38/shTGF-β1) and their combination (MC38/shTGF-β1/IL-18). EVs were isolated from hypoxia cell culture supernatant by centrifugation and size exclusion chromatography. Their quality was confirmed by dynamic light scattering (DLS). MDSCs were stimulated with EVs for 24 hours and then were co-cultured with CFSE-labeled splenocytes. After 72 hours, the proliferation intensity of splenocytes and differentiation towards Th1 lymphocytes were estimated using flow cytometry. EVs derived from MC38/shTGF-β1, and MC38/shTGF-β1/IL-18 lines diminished immunosuppressive activity of MDSCs, influencing the proliferation of splenocytes and their differentiation towards Th1 lymphocytes. EVs isolated from cancer cells modified to overexpression of IL-18 and shRNA for TGF-β1 may affect TME by limiting immunosuppression.

This study was funded by National Science Centre, Poland (project no 2018/30/E/NZ5/00711).

Keywords: Cancer immunology, cytokines and mediators, myeloid derived suppressor cells

P-0908

Effects of CARMIL2 deficiency on functional and behavioral characteristics of immune cells

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Focal adhesions are protein complexes that contain more than 200 proteins, including transmembrane integrins and cytoplasmic proteins, are organized by actin filaments inside the cell, formed by the extracellular network of cells, and they play a role in the regulation of the behavior of cells due to biomechanical and biochemical interactions with the extracellular matrix environment. The CARMIL (Capping protein, Arp2 / 3 and myosin-I binding protein) protein removes the protein bound to the blunt ends of the actin filaments, allowing monomeric actin proteins to bind to the filament end and regulates actin polarization through the cap protein. In our study, neutrophils isolated from patients with CARMIL2 deficiency; It was aimed to question the relationship between functional and behavioral responses with focal adhesion and actin polarization. Four patients with homozygous CARMIL2 gene mutation were included in the study. neutrophils were purified from peripheral blood samples, stimulated to ensure their activation and surface proteins associated with their activation were determined. In addition, cell adhesion and migration experiments and cellular imaging studies were conducted, in which functional experiments (proliferation, phagocytosis, ROS and NO production) and behavioral analyzes were conducted. It was observed that neutrophils could use their functional mechanisms in CARMIL2 deficiency. It was confirmed that neutrophils with CARMIL2 deficiency had very low migration and polarization capacities. Considering the low number of focal adhesions per cell in neutrophils compared to the control group and the weakness in migration capacity.

Keywords: Cytoskeleton, immunodeficiency, myeloid cells

P-0909

Lower functional and proportional characteristics of cord blood treg of male newborns compared with female newborns

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Allergic disorders are among the most common diseases, with incidence increasing worldwide. Understanding early events involved in induction of immune tolerance to harmless environmental antigens (allergens) and members of microbiota could reveal potential therapeutical targets, allowing us to limit allergy development in predisposed individuals. Regulatory T cells (Treg) are a key population in induction and maintenance of tolerance against allergens. Decrease in number and/or function of Treg could represent an early predictor of allergy development. In our study, we analysed proportional and functional properties of Treg in cord blood of children of allergic mothers (with high risk of allergy development) and healthy mothers (with relatively lower risk of allergy development). We have not observed significant difference in proportion of total Treg between children of healthy and allergic mothers, but there was a higher number of induced Treg in cord blood of females compared to males, suggesting impaired capacity of male immune system to set appropriate tolerance to allergens. This could conceivably contribute to higher incidence of allergic diseases observed in boys in early life. Generally decreased proportion of iTreg in cord blood compared with maternal peripheral blood further documents immaturity of neonatal immune system. We have also observed positive correlation in demethylation of Treg specific demethylated region (TSDR) and proportion of Treg in cord blood samples, although there was no difference between children of healthy and allergic mothers. Our data suggests that immaturity of neonatal immune system is more severe in males, predisposing them to increased risk of allergy development.

Keywords: Allergic disorders, epigenetic control and modulation of immunity, immune development, regulatory cells

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POSTER PRESENTATIONS

P-0913

Antinuclear autoantibodies as markers of autoimmunity in coronary artery ectasia

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Coronary artery ectasia (CAE) is defined as local or generalized aneurysmal dilatation of the coronary arteries probably due to excessive vascular wall remodelling in different clinical settings. The aim of the present study was to investigate the presence of autoimmune reactivity in patients with local or generalized CAE. In this case -control study, 39 patients suffered from CAE as a cohort study population, 10 patients with normal coronary arteries as a control group, and 10 patients with atherosclerotic coronary artery disease without an ectasia as a second atherosclerotic group were enrolled. All the patients were tested for antinuclear autoantibodies (ANA). The assessment of ANA was performed by conventional indirect immunofluorescence assay (IIF) (A. Menarini Diagnostics) (cut off for positivity: 1/160). The positive ANA titer was identified in 18 /39 patients (46%) with CAE and only 1/10 (10%) patient from the control group (chi-square 10.255; p = 0.001). Among CAE patients, 10 patients had ANA titre 1/160, six 1/320, and two 1/640. We observed a significant trend between number of ectatic vessels and prevalence of positive ANA (Cochran–Armitage test for trend, p < 0.001). According to our knowledge, this study is the first to report on the presence of ANA in patients with CAE with a prevalence of approximately 45%. This provides evidence for a role of autoimmunity in the pathogenesis of certain cases of CAE. More studies with larger sample sizes might be able to indicate the clinical usefulness of ANA in CAE.

Keywords: Antibody, autoimmunity, cardiovascular diseases

P-0914

Systemic lupus erythematosus and psychiatric disorders

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Systemic Lupus Erythematosus (SLE) may present severe mental disorders which could overshadow classical symptoms and obstruct the diagnosis.Clinical and laboratory findings of SLE were considered in patients with severe mental disorders without a history of autoimmune disease. Patients who admitted to the Psychiatric Department,June to August 2020 were studied upon written informed consent. A standard rheumatology questionnaire was completed, and physical examination and laboratory tests were performed. ANA performed by IIF (Menarini), anti-SSA by immunoblotting (Aescublotting-Aescu), C3 and/or C4 (nephelometry-Beckmann-Coulter), and antiphospholipid antibodies by Aesculisa. 43 patients (23 men,20 women) were enrolled mostly suffering from schizophrenia, schizoaffective disorder, depression, and bipolar disorder. Eight mentioned joint pain, 4 Raynaud's phenomenon and 14 photosensitivity. Three patients had pericardial or pleural effusion. Retinal detachment was observed in 2 patients and in 8 of them swelling ≥2 joints. ANA (+) (≥1/160) in 15 (34.9%) patients, of whom 2 had anti-SSA(Ro) and 7 (16.3%) had low C3 and / or C4. Two (4.7%) patients met EULAR / ACR criteria 2019 for SLE, while 3 (7%) SLICC criteria 2012. Seven patients had a history of vascular thrombosis and 2 pregnancy morbidity. aPL (+) was detected in 12 (28%) (10 had lupus anticoagulant, 3 and 1 anti-β2GP1). Criteria of antiphospholipid syndrome were met by 2 (4.7%) patients of whom one had triple positivity. In patients with severe mental disorder the prevalence of autoantibodies of SLE and APS was frequently detected underlining the challenge for an accurate diagnosis.

Keywords: Antibody, autoimmunity, immunological techniques

P-0916

Are hereditary angioedema patients satisfied with on-demand therapy with icatibant?

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The satisfaction level of hereditary angioedema (HAE) patients upon on-demand treatment with icatibant has not been addressed before. The aim of this study was to evaluate the opinions and satisfaction levels of the HAE patients about icatibant. 161 HAE patients were prospectively evaluated with a questionnaire including several questions about their icatibant-treated attacks. Details of demographic and clinical features were collected from their medical records and attack diaries. A total of 161 HAE-C1INH patients were included in the study. Patients reported a median of 2 (IQR:0.5-3) attacks per month and 16 (IQR:4.5-36) attacks per year. Median frequency of attacks treated with icatibant was 6 (IQR:0-20) per year. Mean duration of treatment with icatibant was 3±2.3 years. Self-administration rate was 91.3%. Mean time to administration and time to resolution were 1.6±1.1 and 1.7±1.3 hours, respectively. There was a correlation between the time to administration and time to resolution (r:0.566, p<0.0001). A total of 125 (77%) patients reported that they were very satisfied or satisfied with icatibant. A total of 52 patients reported 74 mild local reactions. No moderate or severe adverse events (AEs) related to icatibant treatment were reported. The current real-life study shows that icatibant is safe and effective. Moreover, patients' satisfaction level with icatibant in on-demand treatment, is high. We believe that availability of icatibant should be encouraged during the HAE attacks since patients are more involved in their disease management.

Keywords: Allergic disorders, immunodeficiency, skin diseases

P-0917

Modelling APDS2 syndrome for future gene and cell therapy

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APDS2 syndrome is a primary immunodeficiency caused by mutations in PIK3R1 gene, which results in immune dysfunction involving NK cells. To date, the only curative treatment against APDS2 disease consists in hematopoietic stem cell transplantation, bringing poor efficacy. For this reason, we aim to develop novel strategies to uncover effective specific-therapies directed to APDS2 patients by correcting the mutation in their own cells. Recently, we have generated a fibroblast-derived induced pluripotent stem (iPS) cell line from a young patient carrying a heterozygous single T deletion in intron 11 of the PIK3R1 gene using a non-integrative reprogramming technology. Then, we will correct the mutation in the PIK3R1 gene in APDS2-derived iPS by using CRISPR/Cas9 genetic engineering technology. Cas9 enzymes together with CRISPR sequences form the basis of a technology known as CRISPR/Cas9 that can be used to edit genes within organisms, providing a powerful tool for this purpose. Finally, corrected APDS2-derived iPS could be differentiated into hematopoietic stem cells expressing CD34+ marker, and these cells can be differentiated into the different types of blood cells, such as NK cells. In this way, we will be able to obtain healthy NK cells from this patient. Therefore, this cellular product will supply a personal source of healthy transfusable components. As a whole, our work represents an innovative therapy that will improve the current treatments, providing an exceptional tool to study APDS2 syndrome as well as personalized gene and cell therapy.

Keywords: Cell based therapies, immunodeficiency, NK cells, stem cells

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POSTER PRESENTATIONS

P-0918

A possible therapeutic agent for hyperinflammation in COVID-19: C-Vx directs T cells to display an anti-inflammatory immune response

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The Covid-19 pandemic, caused by the SARS-CoV 2 virus, has affected approximately 170 million people and caused the death of 3.5 million people worldwide as of today. In addition to the vaccines developed and applied to date, different treatment options are also being investigated. C-Vx, which was first developed by Hamida Pharma-USA in cooperation with Miracle Labs-Turkey for cancer treatment, has been changed in the formula with the emergence of the COVID-19 pandemic and the possibility of using it as a simultaneous treatment option for COVID-19 has been investigated. This study aimed to investigate the effects of C-Vx on various cytokine levels of $CD4^+$ and $CD8^+$ T cells of patients with COVID-19. For this purpose, heparinized-peripheral blood samples were obtained from COVID-19 patients (n=31; mild: 10, moderate: 11, severe: 10) and healthy subjects (n=10). PBMCs were isolated and cultured with/without C-Vx for 72 hours and then stimulated with Cell Stimulation Cocktail for 4 hours. Following culture, cells were labeled with anti-CD3, -CD4 and -CD8 mAbs prior to intracellular staining for IFN-γ, IL-4, IL-10, IL-17 and TNF-α cytokines. CD3, CD4 and CD8 expression of patients and healthy controls did not change with C-Vx. While IFN-γ and IL-17 levels of $CD4^+$ T cells as well as IFN-γ and TNF-α levels of $CD8^+$ T cells were reduced, IL-4 and IL-10 levels of both $CD4^+$ and $CD8^+$ T cells were increased by C-Vx stimulation. These findings suggest C-Vx driving $CD4^+$ and $CD8^+$ T cells to exhibit an anti-inflammatory rather than a pro-inflammatory immune response.

Keywords: Adaptive immunity, cytokines and mediators, immune regulation and therapy, infectious disease

P-0919

Cancer-associated fibroblasts isolation from tumor tissues of breast cancer patients and and investigation of Akt/FOXO and mTOR/eEF2K signalling pathway activations

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Fibroblasts are one of the most abundant cell types in the stroma and they become cancer-associated fibroblasts(CAFs) in the tumor microenvironment (TME). CAFs have different functions than normal fibroblasts due to the inflammatory factors they are exposed to. These functions may be responsible for the crucial roles of CAFs, such as sculpting of the TME. Our study aims to investigate the expression levels of proteins related to inflammation in CAFs for a better understanding of tumor microenvironment and its properties. We isolated primary fibroblasts from breast cancer tissues of patients who had total mastectomy surgery. The tissues were obtained from the pathology department in DMEM supplemented with %10 FBS. The tissues had sliced into 1mm3 pieces and were cultured in 12-well plates in DMEM medium in a 37oC incubator. Immunocytochemistry staining were performed on these fibroblasts to see if they are normal fibroblast or CAFs. Pancytoceratin antibody was used as negative and vimentin antibody was used as positive controls. The fibroblasts stained with α-SMA antibody were accepted as CAFs for the rest of the experiments. Further western blot analyses were conducted on these fibroblasts with varying selected antibodies to understand the CAFs' role in inflammation on the tumor site. mTOR, p-mTOR, eEF2, peEF2, eEF2K, p-eEEF2K, AKT, p-AKT, FOXO and p-FOXO, western blot analyzes were conducted. Our results show that cancer-associated fibroblasts with high α-SMA expression have more activated protein levels. These results will be further investigated with qPCR in our laboratory.

Keywords: Cancer immunology, cell signalling, microenvironment

P-0921

Comparison of peripheral blood chimerisms to tissue chimerisms in patients with allogeneic heamatopoietic stem cell transplantation

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In our study, we aimed to compare peripheral blood and tissue chimerisms on D+28 and to analyze its effect on disease outcome. Patients ≥18 years-old, who underwent allo-HSCT between were included. Pre-transplant DNA purified from WBCs, and DNA extracted from skin on D+28 were studied by STR-PCR. Forty-one patients were included(F/M:21/20; median age:40). Seventeen patients underwent related full-match, 13 unrelated full-match, 6 haploidentic, and 5 mis-matched unrelated(9/10 matched) HSCT. Fourteen AML, 8 MDS, 8 lymphoma, 4 ALL and 3 AA patients were analyzed. Recipients' DNA was found in 21 patients, and mixed chimerism was detected in 19 patients (DNA could not be extracted in one case). 9/21 patients who had self DNA in tissue lost peripheral blood chimerism at a median of 14 months. However, 3/19 patients with mixed chimerism in tissue lost blood chimerism(p=0.062). All 12 patients who lost blood chimerism had progressive diseases. 9/21 patients with self-DNA and 3/19 patients with mixed chimerism in tissue died(p=0.062). One-year PFS was %27,27 and %75, in self DNA and mixed chimerism in tissue, respectively(p=0.25). One-year OS was %61.54 and %87.84, in self DNA and mixed chimerism in tissue, respectively(p=0.095). In AML, progression was less prevalent(p=0.038) and OS was better(p=0.05) in mixed chimerism group. At a median 14 months of follow-up, we found that progression and death was less prevalent in AML with mixed chimerism in tissue and OS was better. We consider that some of the variables might affect survival and end-points with the extension of our follow-up time.

Keywords: Bone marrow transplantation, cancer immunology, transplantation

P-0924

Proliferative responses of COVID-19 patients infected with SARS-Cov2

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COVID-19 is a significant health problem with severe socio-economic consequences. The exploration of immune responses against SARS-CoV2 and the alterations in immune system revealed several abnormalities. Investigation of lymphocytes proliferative capacity is utilized both for immunological research and for diagnosis of immune deficiencies, allergic diseases, and response to various antigens. This study aimed to investigate proliferative responses of $CD4^+$ T and $CD19^+$ B lymphocytes in mild, moderate and severe COVID-19 patients, in comparison with healthy individuals. Patients infected with SARS-CoV2 and diagnosed with COVID-19 (n=28; mild: 11, moderate: 10, severe: 7), followed by Istanbul University, Istanbul Faculty of Medicine, and healthy individuals (n=11) were enrolled to this study. PBMCs purified with ficoll were stained with CFSE and cultured with the absence and existence of phytohemaglutinin (PHA) for 120 hours. Following cell culture, monoclonal antibodies against CD4 and CD19 were utilized for cell surface staining. PHA-induced proliferative capacity of total PBMCs and $CD4^+$ T cells was found to be diminished in harmony with increasing disease severity. Proliferation of PBMCs in severe patients was significantly reduced in comparison with both healthy individuals, mild and moderate patients. $CD4^+$ T cell proliferation was significantly diminished in comparison with that of healthy individuals and severe patients. Proliferation of $CD19^+$ B cells was observed to be diminished in concordance with increasing disease severity, but with no statistical significance. The decrease of proliferative responses in harmony with increased severity of disease might indicate a disrupted immune function in severe COVID-19 patients.

Keywords: Adaptive immunity, B lymphocytes, immune response tracing, visualizing immune responses

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POSTER PRESENTATIONS

P-0925

The immune landscape of human non-small cell lung cancer tumors is Th2 skewed

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The role of tumor-infiltrating CD4+ T helper (Th) cells is controversial. Th1 cells are considered important to help cytotoxic CD8+ T cells to fight cancer, whereas other Th subsets such as T regulatory (Treg) and Th2 cells may promote tumor development. We developed a multiplex chromogenic immunohistochemical method to analyze Th subsets in non-small cell lung cancer (NSCLC) tumors. Four locations were investigated: tumor epithelium, tumor stroma, tertiary lymphoid structures (TLS) and non-cancerous distal lung from 11 patients. In tumor epithelium and stroma, most CD4+ T cells identified had either a Th2 or Treg phenotype, whereas only low numbers of Th1, Th17, and T follicular helper (Tfh) cells were observed. Similarly, Th2 was the most abundant Th subset in TLS, followed by Treg cells. In contrast, Th1 was the most frequently detected Th subset in non-cancerous lung tissue from the same patients. There was a positive correlation between the Th1:Th2 ratios and the numbers of intratumoral CD8+ T cells. Thus, human primary NSCLC tumors are Th2-skewed and contain numerous Treg cells. A wrong type of ongoing antitumor immune response (Th2 instead of Th1) may potentially explain why many NSCLC patients do not respond to immunostimulatory treatment with immune checkpoint inhibitors.

Keywords: Adaptive immunity, cancer immunology, follicular helper T cells, immunological techniques, immunotherapy, microenvironment

P-0926

The impact of the presence and eradication of different subtypes of Blastocystis spp on the clinical course of chronic spontaneous urticaria

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To investigate the presence of Blastocystis spp. subtypes in chronic spontaneous urticaria (CSU) and the role of eradication in disease activity. Stool samples from 295 adult CSU patients and 38 healthy controls (HCs) were microscopically examined. The samples having parasites were cultured for the DNA isolation. Then, samples were evaluated by real-time (RT)-PCR. DNA sequences and subtypes were analyzed. Medication scores (MS), Urticaria Activity Score-7 (UAS-7) and laboratory values were compared at first and six months after anti-parasitic treatment between the patient groups (PGs) having various Blastocystis spp. subtypes. The parasite was detected in 45.8%(n=135) of the patients and %34.2(n=13) of HCs(p>0.05). 57(85.1%) of 67 samples in the PG and 12(92.3%) of 13 samples in HCs revealed a positive RT-PCR. Serum total IgE levels were higher in the PG(p<0.001). Subtype 3 was significantly higher in the PG(p<0.001) whereas subtype 1 was higher in HCs(p<0.001). The remission rate of the disease was higher(p=0.031) while UAS-7, MC and total IgE levels were lower in the patients having subtype 3 than those with other subtypes at the end of sixth month of anti-parasitic treatment(p=0.028,p=0.044,p=0.01). MS, UAS-7, total IgE levels, eosinophil counts decreased(p<0.001,p<0.001,p=0.001,p=0.009) and basophil counts increased in patients with subtype 3(p=0.045) whereas only UAS-7 decreased in those with subtype 2 after eradication(p=0.012). The presence and eradication of Blastocystis spp subtype 3 seem to be related to CSU course. Blastocystis spp. sequencing analysis seems helpful in determining the patients in need of Blastocystis eradication to control CSU activity.

Keywords: Eosinophils, parasite infections, skin diseases

P-0927

The mechanism of LRBA dependent CTLA-4 surface expression in regulatory T cell lymphocytes

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Primary immunodeficiencies (PID) are a very diverse group of disorders characterized by recurring infections, accompanied by autoimmunity, allergy, or malignancy. Novel PIDs caused by loss of function mutations in the genes encoding LRBA (lipopolysaccharide-responsive beige like anchor protein) and CTLA-4 (cytotoxic T lymphocyte antigen 4) demonstrate the importance of these molecules in regulatory T lymphocyte (Treg) function. Several studies have shown that the LRBA molecule regulates CTLA-4 cell surface expression. Because CTLA- 4 is a checkpoint inhibitor of T cell function, LRBA deficiency is accompanied by a loss of regulatory T cell (Treg) function and immune dysregulation syndromes. Besides CTLA-4, LRBA has an effect on the vesicular trafficking of epidermal growth factor receptor (EGFR) and cell surface expression of FasL. On the other hand, antibody responses have been interrupted in some LRBA deficiency cases, demonstrating that this protein modulates the expression and function of multiple receptors that are dependent on the vesicular transport. In this study, our main objective is to identify receptors that directly or indirectly interact with the LRBA protein. For this purpose, we use the CRISPR Cas9 genome editing technique to knock out this protein in the Jurkat T leukemia cell line. To induce CTLA-4 expression in Jurkat T cells, we will lentivirally express the FOXP3 transcription factor to make this cell line behave more like a Treg. To identify the differential expression of cell surface proteins in these Jurkat-Treg like cells, in the absence of LRBA, we are performing cell surface biotinylation coupled with mass spectrometry.

Keywords: Immunodeficiency, mass spectrometry, regulatory cells

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POSTER PRESENTATIONS

P-0928

T cell response towards tissue- and epidermal-transglutaminases in coeliac disease patients developing dermatitis herpetiformis

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The reason why only few coeliac patients develop the cutaneous manifestation of the disease, named dermatitis herpetiformis (DH), is still unknown. Epidermal transglutaminase (TG3) has been described as the main autoantigen of humoral immunity in DH but the mechanisms leading to this autoimmune response remain obscure. Here we characterized T cells from skin, gut and peripheral blood of DH and coeliac disease (CD) patients, evaluated the impact of the gluten-free diet on circulating T lymphocytes' phenotype and investigated antigen specific T cell response towards epidermal and tissue transglutaminase (TG2). DH patients showed an increased frequency of skin-derived T cells producing TNFα when compared to CD patients. Moreover, circulating T cells producing TNFα and IL-17A positively correlated with clinical score of skin disease activity and decreased after gluten-free diet. Finally, TG2 and TG3-specific T cells resulted more reactive to antigens stimulation in DH patients and showed cross reactivity towards the two autoantigens in both the groupof patients. Our data suggest a role of TNFα and IL-17A producing cells in the development of DH and, for the first time, show the existence of a crossed T cell response towards the two transglutaminases isoforms, thus suggesting new insights on T cells role in skin damage.

Keywords: Adaptive immunity, autoimmunity, cytokines and mediators, inflammatory disease

P-0929

Mapping the immunopeptidome of Philadelphia chromosome-negative myeloproliferative neoplasms identifies potential T-cell epitopes for immunotherapeutic approaches

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Philadelphia chromosome-negative myeloproliferative neoplasms (Ph^-MPNs) polycythemia vera (PV), essential thrombocytosis (ET), and myelofibrosis (MF) bear a risk for progression and leukemic transformation. Antigen-specific immunotherapy might enable to halt progression. Here, we characterized the immunopeptidome of Ph^-MPNs by mass spectrometry to identify HLA-presented tumor-associated antigens. HLA quantification on $CD13^+CD33^+$ myeloid cells and $CD34^+$ progenitors confirmed sufficient surface expression. HLA class I analysis of ET (n=23), PV (n=17), and MF (n=21) samples identified 21,935, 26,616, and 30,863 HLA ligands from 7,225, 8,239, and 8,517 source proteins. HLA-DR immunopeptidomes revealed 16,070, 17,140, and 17,989 peptides from 2,430, 2,469, and 2,602 proteins in ET, PV, and MF. Comparative HLA class I ligandome profiling using our dataset of benign tissues (n=351, 72,129 ligands, 13,179 proteins) revealed 21 ET-, 103 PV-, and 22 MF-associated ligands with representation frequencies of ≥17% in ET, ≥18% in PV, and ≥19% in MF. Comparative HLA-DR analysis (benign n=312, 110,255 peptides, 7,624 proteins) identified 28 ET-, 35 PV-, and 21 MF-associated peptides with frequencies of ≥33% in ET, ≥42% in PV, and ≥35% in MF. Moreover, we detected an overlap with AML-associated antigens and thus screened Ph^-MPN samples for AML-specific memory T-cell responses. Pre-existing polyfunctional ($IFN\text{-}\gamma^+TNF^+CD107a^+$) $CD4^+$ cells against 7/15 (47%) HLA-DR-restricted epitopes were detected in 5/11 (45%) MF and 2/12 (17%) ET samples. Polyfunctional $CD8^+$ cells recognized 3/3 HLA-A*01-restricted AML targets in 2/4 (50%) MF samples. Together, this work represents a step towards the identification and characterization of novel T-cell epitopes for the development of antigen-specific immunotherapy for Ph^-MPN patients.

Keywords: Anti-cancer vaccine, cancer immunology, cancer immunopeptidome, immunotherapy, mass spectrometry, proliferative disorders

P-0930

Cancer-associated fibroblasts and their relation to regnase-1

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Cancer-associated fibroblasts (CAFs) have different functions from normal fibroblasts. One of these functions is the effect of CAFs on monocyte recruitment and macrophage polarization. Unlike normal fibroblasts, CAFs express α-SMA and they lead to effective monocyte recruitment. Recruitment of monocytes by CAFs is mediated by monocyte chemotactic protein (MCP-1) and stromal cell-derived factor-1 (SDF-1). CAFs differentiate the recruited monocytes into M2-like macrophages which are capable of performing immunosuppressive roles. In our study, we aim to investigate macrophage polarization in CAFs for a better understanding of tumor microenvironment and its properties. We isolated primary fibroblasts from breast cancer tissues of patients who had total mastectomy surgery. The tissues were obtained from the pathology department in DMEM supplemented with %10 FBS. The tissue had sliced into 1mm3 pieces and were cultured in 12-well plates in DMEM medium in a 37oC incubator. Immunocytochemistry staining were performed to these fibroblasts to see if they are normal fibroblast or CAFs. Pancytoceratin antibody was used as negative and vimentin antibody was used as positive controls, and the fibroblasts stained with α-SMA antibody were accepted as CAFs for the rest of the experiments. Western blot analyses were conducted on these fibroblasts with varying selected antibodies to understand the CAFs' role in macrophage polarization. MCP-1 and Regnase-1 western blot analyzes were conducted and from our results, we suggest that cancer-associated fibroblasts with high α-SMA expression have a greater Regnase-1 and MCP-1 protein levels. These results will be further investigated with qPCR in our laboratory.

Keywords: Cell signalling, immune regulation and therapy, macrophage, microenvironment

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POSTER PRESENTATIONS

P-0931

ISG15-dependent activation of the sensor MDA5 is antagonized by the SARS-2 CoV-2 papain-like protease to evade host innate immunity

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Activation of the RIG-I-like receptors, RIG-I and MDA5, establishes an antiviral state by upregulating interferon (IFN)-stimulated genes (ISGs). Among these is ISG15 whose mechanistic roles in innate immunity still remain enigmatic. Here we report that ISG15 conjugation is essential for antiviral IFN responses mediated by the viral RNA sensor MDA5. ISGylation of the caspase activation and recruitment domains (CARD) of MDA5 promotes its oligomerization and thereby triggers activation of innate immunity against a range of viruses including coronaviruses, flaviviruses and picornaviruses. The ISG15-dependent activation of MDA5 is antagonized through direct de-ISGylation mediated by the papain-like protease (PLpro) of SARS-CoV-2, a recently emerged coronavirus that causes the COVID-19 pandemic. Our work demonstrates a crucial role for ISG15 in the MDA5-mediated antiviral response, and also identifies a key immune evasion mechanism of SARS-CoV-2, which may be targeted for the development of new antivirals and vaccines to combat COVID-19.

Keywords: Innate host defence, innate immunity, viral infections

P-0932

Molecular mimicry between peptides of SARS-CoV-2 and neutrophil extracellular traps related proteins

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Neutrophil extracellular traps (NETs) are observed in both COVID-19 pathology and autoimmune disorders. Molecular mimicry is a mechanism that can lead to autoimmune response. Homologous 15mer sequences with at least 50 bits value in alignments and 6 amino acid matches of SARS-CoV-2 proteins and NETs related plasminogen receptor KT (PLRKT), myeloperoxidase (MPO), proteinase 3, neutrophil elastase, matrix metalloproteinase 9 (MMP-9) are searched. Those human and SARS-CoV-2 sequence pairs are identified within top results of respective blastp searches. Identified sequence pairs with predicted strong-binding peptides or epitopes of the same HLA alleles among the supertype representatives are selected. Homologous regions of PLRKT and SARS-CoV-2 with high affinities to HLA-A*24:02, HLA-B*08:01, and HLA-B*15:01; of MPO and SARS-CoV-2 with high affinities to HLA-A*01:01, HLA-A*26:01 and HLA-B*15:01; and of MMP-9 and SARS-CoV-2 with high affinities to HLA-B*39:01, are detected. Results revealed potential involvement of molecular mimicry based autoimmunity in NETs-pathology within susceptible individuals, in case of being infected with SARS-CoV-2. Additional results of this ongoing study are planned to bepresented.

Keywords: Neutrophils, viral infections, autoimmunity

P-0933

Antibodies to spike protein of SARS-CoV-2 in convalescent COVID19 patients and subjects immunized with Sinopharm, Pfizer-BioNTech or Sputnik V vaccine

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The aim of this study was to measure the level of IgG antibodies specific to spike (S) glycoprotein of SARS-CoV-2. The study included 169 subject divided into the following groups: patients recovered from COVID19 and subjects who received Sinopharm, Pfizer-BioNTech, or Sputnik V vaccine. Also, the study included the subject completely immunized with Sinopharm vaccine before or after the onset of COVID19 disease. The level of antibodies was measured using Anti-SARS-CoV-2 QuantiVac ELISA (IgG) (Euroimmun). The level of antibodies was measured 3 weeks to 3 months after the infection with SARS-CoV-2 or after the application of second dose of vaccine. The mean level of antibody in convalescent patients was 139.34±111.68 BAU/ml, while in the subjects immunized with Sinopharm, Pfizer-BioNTech and Sputnik V vaccine were 79.98±60.84 BAU/ml, 333.78±52.44 BAU/ml and 158.08±57.47 BAU/ml, respectively. There were the significant differences in the levels of anti-S protein IgG antibodies between the tested groups (Independent samples Kruskal-Wallis test, $p<0.001$). The level of antibodies specific to S protein in subjects immunized with Sinopharm vaccine before or after SARS-CoV-2 infection (255.14±146.92 BAU/ml) was significantly higher than in convalescent patients ($p=0.002$) or subjects who were immunized with Sinopharm vaccine but did not get COVID19 ($p<0.001$). Our results indicate that the level of anti-S protein IgG antibodies in convalescent COVID19 patients and immunized subject differs significantly. The immunization with Sinopharm vaccine before or after the onset of the disease significantly increases the level of IgG antibodies to S protein of SARS-CoV-19.

Keywords: Adjuvants and vaccines, antibody, viral infections

P-0934

The immunomodulatory action of C-Vx substance on Cytotoxic T and Natural Killer cells in COVID-19

Nilgun Okumus Akdeniz[1], Ilhan Tahralı[1], Esin Aktas Cetin[1], Fatma Betul Oktelik[1], Yelda Ogutmen[2], Umut Can Kucuksezer[1], Hamida Heba[3], Mustafa Oral Oncul[2], Gunnur Deniz[1]

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COVID-19 is a global pandemic infectious disease caused by SARS-CoV-2 overactivated innate immunity and cytokine storms have been proposed as potential pathological mechanisms for rapid COVID-19 progression. Cytotoxic functions of natural killer (NK) and CD8+ T cells in patients with COVID-19 and their associations with disease severity were investigated. The study group consisted of mild (n=10), moderate (n=8) and severe (n=10) COVID-19 patients and healthy (n=10) individuals. The effects of C-Vx on the cytotoxic functions were determined using the CD107a degranulation, perforin and granzyme B expression by flow cytometry. When perforin and granyme expressions of CD3−CD16+CD56+ NK and also CD8+ T cells in response to C-Vx was investigated, no significant differences among any patient groups nor healthy individuals were observed. On the other hand, CD107a levels on NK cells was significantly up-regulated in mild COVID-19 cases as well as healthy individuals, in response to presence of C-Vx. While, in moderate and severe patients, C-Vx was not able to induce any further up-regulation of CD107a over K-562 stimulation. In all COVID-19 patients, CD107a expression of CD8+ T cells was increased with no statistical significance, in response to K-562 co-culture, regardless of C-Vx stimulation. These findings support the role of C-Vx in innate immunity by increasing the capacity of NK cytotoxicity. Prophylactic use of natural products like C-Vx may be a good approach to stop or at least slow down the transmission of COVID-19.

Keywords: Immune response tracing, NK cells, viral infections

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POSTER PRESENTATIONS

P-0935

Atopy and allergic diseases have no impact on the severity of COVID-19

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The clinical features of COVID-19 range from asymptomatic disease to severe pneumonia or even death. Therefore, many researchers have investigated the factors that could affect the severity of COVID-19. We aimed to assess the impact of aero-allergen sensitization and allergic diseases on the severity of COVID-19. We included 60 adult patients with symptomatic COVID-19 allocated into two groups equal in number as having severe and non-severe COVID-19. We evaluated the demographic features and allergic diseases in addition to clinical, laboratory and radiological findings of COVID-19. Skin prick tests (SPTs) with common aero-allergens, serum total IgE levels and blood eosinophil counts were evaluated 3 months after the patient's recovery of COVID-19. 73.3% of the patients were male and the mean age of the patients 52 ± 11 years. There was no significant difference between the two groups in terms of age, gender, smoking habits, obesity and comorbidities. Although the frequency of sensitization to aero-allergens and the allergic diseases were similar, the history of allergic diseases in the family was higher in the severe group($p<0.001$). The polysensitization in SPTs was associated with the presence of a cytokine storm during the infection($p=0.02$). Total IgE levels and blood eosinophil counts were not significantly different between the two groups. The presence of atopy or allergic diseases does not seem to be related to the severity of COVID-19. However, polysensitization and a family history of allergic diseases are more prominent in those having a cytokine storm and severe COVID-19, respectively.

Keywords: Allergic disorders, autoinflammation, viral infections

P-0936

Immunophenotyping of peripheral leukocyte subsets in children with acute SARS-CoV-2 infection and multisystem inflammatory syndrome reveals distinct patterns of immunological response

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Data demonstrating the nature of immune responses during acute SARS-CoV-2 infection and Multisystem Inflammatory Syndrome (MIS) in children are limited. The aim of this study was to determine whether different clinical phenotypes of SARS-CoV-2 infection in children are related to disparate distribution of peripheral leucocytes. The study cohort consisted of 35 children (M:F=20:15, aged:1-16 years) with COVID-19, confirmed by positive RT-PCR, or MIS temporally associated with SARS-CoV-2, which were admitted to Hippokrateion General Hospital of Thessaloniki during the pandemic. Patients were divided into three groups according to the clinical phenotype: Mild Disease (23), Severe Disease/Pneumonia (6) and MIS (6). Flow cytometry was performed to characterize peripheral leucocyte subpopulations during the acute phase of SARS-CoV-2 infection or the manifestation of MIS, respectively. Children with severe COVID-19 had significantly lower lymphocytes (p= 0.045), particularly CD4+T-helper cells (p=0.038), especially compared to the mild-disease-group. Furthermore, they had significantly higher percentage of: activated-CD3+HLA-DR+T-cells (p=0.019), CD4+CD45RO+memory-T-cells (p=0.053), CD8+CD27+CCR7+CD45RA-central-memory-T-cells (p=0.032) and CD8+CD27-CCR7-CD45RA-effector-memory-T-cells (p=0.031). The CD4/CD8 ratio did not vary between groups. Children with MIS had significantly increased neutrophil count (p=0.03) with elevated surface expression of FcγRI(CD64), including higher percentage of CD19+B-cells (p=0.025) and IgM-IgD-CD27+"switched"-memory-B-cells (p=0.057), when compared to both acute-COVID-19-groups. These results suggest distinct patterns of immunological response associated with clinical phenotypes of pediatric SARS-CoV-2 infection. In severe COVID-19 intense lymphocyte activation occurs, whereas immune dysfuntion underlying MIS-C reveals an inflammatory immunotype combined with indications of aberrant antibody production. These findings may have therapeutic implications in children with complicated SARS-CoV-2 infection.

Keywords: B lymphocytes, immune response tracing, memory, neutrophils, viral infections, visualizing immune responses

P-0937

Generation of sandwich ELISA by using in-house developed antibodies and antigen for detection of SARS-CoV-2

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COVID-19 first reported in 2019, caused the global outbreak. Early detection of the virus is important to slow the spread of the disease. In order to provide a detection system, we have chosen SARS-CoV-2 nucleocapsid protein (NP) because of its conservative structure. We developed anti-NP monoclonal antibodies(mAbs) and polyclonal antibody(pAb) for the generation of an antibody-based sandwich enzyme-linked immunosorbent assay (ELISA) system. By using hybridoma technology that includes steps like immunization of mice, checking appropriate antibody response, and fusion of the spleen and myeloma cells, 8 specific Anti-NP mAbs were developed. For the production of Anti-NP PAb, in-house developed and purified recombinant SARS-CoV-2 NP was used as an immunization agent. High volume serum was collected from immunized rabbit when the antibody response reached its maximum level. After completed of purification and characterization steps of all antibodies, it has been demonstrated that developed antibodies were a high affinity to NP antigen. It was also observed that some of these developed antibodies had approximately 2-fold higher affinity compared to commercial NP antibodies. The developed 8 mAbs and 1 pAb were labelled with biotin and horseradish peroxidase (HRP) from their Fc sites to check their usage potential in the sandwich ELISA test system on detection of the NP antigens. The appropriate antibody pairs were determined to detect NP quantitatively. To detect the NP in nasopharyngeal swabs taken from naturally infected patients, we carried out to develop the sandwich diagnosis systems such as ELISA and lateral flow test by using these in-house mAbs.

Keywords: Antibody, immunological techniques, infectious disease, viral infections, visualizing immune responses

P-0938

The frequency of HLA alleles and serological markers of infectious diseases in the North-Eastern population of Romania

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Allogeneic hematopoietic stem cell transplantation (HCT) is a potentially curative procedure for a variety of blood malignancies. The selection of suitable donors is crucial for a successful HCT. The present study aims to evaluate the frequency of HLA alleles and seroprevalence of Epstein-Barr-virus (EBV), Cytomegalovirus (CMV), Toxoplasma gondii (T.gondii) and Hepatitis-B core Antibody(HBcAb) among potential healthy registered in the Romanian National Registry of Voluntary Hematopoietic Stem Cell Donors (NRVHSCD). 1400 volunteers from NRVHSCD have been investigated between 2018-2019 at the Immunology Laboratory, "St. Spiridon" Clinical Hospital, Iași. The screening of anti-EBV, anti-CMV, anti-T. gondii (IgM and IgG) and total anti-HBc antibodies was performed by chemiluminescence and electrochemiluminescence using the Architect/Cobas/Immulite platforms. 57% and 43% were men and women, respectively, aged 18 to 52 years, with a 31 years median age. Among EBV IgG-positive individuals, 69% were also CMV IgG-positive and T. gondii IgG-negative, while 29% were both CMV and T. gondii IgG-positive. This triple association of IgG antibodies increased with age and correlated strongly with T. gondii IgG prevalence per age groups (R=0.98). The prevalences of the anti-EBV, anti-CMV and anti-T. gondii IgM antibodies was low: 0.4%, 0.5% and 0.7%, respectively. 11% of males and 14% of females were anti-HBc antibodies positive. Their serological profiles were ultimately correlated with their respective HLA alleles. The incidence and prevalence of the studied infections displayed an age-related increasing trend, a feature that needs to be considered when selecting potential donors.

Keywords: Antibody, transplantation, viral infections

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POSTER PRESENTATIONS

P-0939

Autosomal recessive agammaglobulinemia caused by a novel mutation in the *IGHM* gene

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Autosomal recessive agammaglobulinemia (ARA) is a rare primary immunodeficiency (PID) characterized by severe reduction of all immunoglobulin isotypes and lack of peripheral B cells, in the absence of *BTK* gene mutations. In ARA, mutations occur in genes encoding components of the pre-B-cell or B-cell receptor, or their signaling pathways. Molecular characterization is only confirmed in a minority of patients, suggesting that new mutations underlying the disease remain to be found. In this study we report the case of an ARA patient carrying a new mutation in exon 4 of *IGHM* gene. Patient: A male patient was referred to our laboratory for investigation of PID.

The exploration included: - Measurement of serum IgG, IgA and IgM levels by turbidimetry. - Lymphocyte sub populations immunophenotyping by flow cytometry. - Direct sequencing of *BTK* and *IGHM* genes by Sanger's method. The patient was born to consanguineous parents and presented at 4 years of age with a history of recurrent infections and petechiae. The results of immunological explorations allowed us to make the diagnosis of agammaglobulinemia according to the European society for immunodeficiencies (ESID) criteria. After confirming the absence of mutation in the *BTK* gene, we sequenced the *IGHM* gene, since it is the most frequently mutated gene in ARA.We identified a novel homozygous mutation in exon 4 (c.1165-1171delCCAGGCC); this 7-bp deletion results in a frameshift and a premature Stop codon (p.Pro389Glyfsx8) predicting the production of a truncated protein. Both parents of the patient were heterozygous for this mutation.

Keywords: Adaptive immunity, antibody, B lymphocytes, immunodeficiency

P-0940

House dust mite extract induces changes in metabolic and cytokine profiles of bone marrow-derived macrophages

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Phenotypically and functionally distinct macrophage populations can be protective or harmful in asthma. Despite the fact that polarization of macrophages is strongly associated with the development of allergic airway inflammation, the role of metabolic changes and their correlation with overall activation profile in allergic asthma is not fully understood. To define components of house dust mite (HDM) extract that may cause metabolic adaptations and induce cytokine production in myeloid cells, we investigated response to HDM, as well as to β-glucan, LPS or a mixture of β-glucan and LPS using gene expression and cytokine multiplex analysis, as well as measuring glycolysis and respiration in primary mouse bone marrow-derived macrophages (BMDM). We found that challenges with HDM, β-glucan or a mixture of β-glucan and LPS all resulted in similar altered profiles of extracellular acidification and oxygen consumption rates. At the same time stimulation with HDM or β-glucan resulted in higher basal and maximal respiration rates as compared to untreated and LPS-treated BMDM. Similarly, all of the treatments led to increased glycolysis in BMDM. Interestingly, cytokine production and metabolic changes in response to HDM were dependent on TLR4, while β-glucan induced metabolic changes but not cytokine production in the absence of TLR4-signalling. Taken together, our data suggest that macrophage metabolic reshaping in response to the first allergen encounter as well as molecular cascades underlying innate immune cell activation may be associated with asthma development and with its severity.

This work was supported by RFBR grant № 19-34-51030 and STU Sirius.

Keywords: Allergen-induced immune responses, macrophage, metabolic control of immune responses

P-0941

Cancer immunotherapy by NC410, a LAIR-2 Fc protein blocking human LAIR-collagen interaction

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Collagens are a primary component of the extracellular matrix and are functional ligands for the inhibitory immune receptor leukocyte associated immunoglobulin-like receptor (LAIR)-1. LAIR-2 is a secreted protein that can act as a decoy receptor by binding collagen with higher affinity than LAIR-1. We propose that collagens promote immune evasion by interacting with LAIR-1 expressed on immune cells, and that LAIR-2 releases LAIR-1 mediated immune suppression. Analysis of public datasets show that collagens, LAIR-1 and LAIR- 2 have unique and overlapping associations with survival in certain tumors. We designed a dimeric LAIR-2 with a functional IgG1 Fc tail, NC410, and showed that NC410 increases human T cell expansion and effector function *in vivo*. In humanized tumor models NC410 reduces tumor growth that is dependent on T cells. Immunohistochemical analysis of human tumors shows that NC410 binds to collagen-rich areas where LAIR-1+ immune cells are localized. Our findings show that NC410 might be a novel strategy for cancer immunotherapy for immune-excluded tumors.

Keywords: Cancer immunology, immunotherapy, *in vivo* tumor models

P-0942

Psoriasis and atopic dermatitis: co-existence or co-morbidity?

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Atopic dermatitis (AD) as well as psoriasis (PS) are inflammatory diseases as a result of skin barrier dysfunction. The goal of the study was to investigate the prevalence of comorbidity in patients with psoriasis of AD and the roleof proinflammatory cytokines in both diseases.To study the prevalence of comorbidity od PS and AD 1406 patient's histories of diseases were analyzed. PS patients with different lesion's localizations and severity as well as with different body mass index (BMI) were examined. The determination of the concentration of pro-inflammatory cytokines (IL-6, IL-8, IFNγ, IL-17, L-18 and TNFa) in sera and supernatants of 48h-cultivated peripheral blood mononuclear cell (PBMC) of psoriasis patients (n=85) and healthy volunteers (36 adults) were assayed by ELISA method. Analysis of the histories of diseases demonstrated that the number of combined forms of PS and different types of atopies was 42% and among them 87% of PS patients suffered from AD. It have been shown that serum levels of all studied cytokines (IL-6, IL-8, IFNγ, IL-17, IL-18 and TNFα) in most of studied patients were higher in psoriasis patients comparing to AD patients and healthy controls ($p<0.05$). Similar results have been demonstrated *in vitro* synthesis of IL-6 and IFNγ by PBMC. The study demonstrated low incidence of AD in patients with PS. Despite the pathogenic differences between AD and PS, they share some common features, such as high synthesis of proinflammatory cytokines. Atopic diseases of skin and PS co-exist as an overlapping syndrome.

Keywords: Cytokines and mediators, inflammatory disease, skin diseases

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POSTER PRESENTATIONS

P-0943

Effects of two pharmacological approaches on cytokine storm in severe COVID-19 patients

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The dynamics of immune responses to SARS-CoV-2 infection are currently under investigation. The cytokine storm is a major feature of the severe development of COVID-19. As already available treatments, Tocilizumab is used to target IL-6, and Ruxolitinib as Jak1/Jak2 inhibitor. Investigating the role of dysregulated cytokines could enforce the perspective of a combined treatment targeting more than one pathway. 8 ICU patients and 13 COVID-19 patients received, respectively, Tocilizumab and Ruxolitinib treatment. Phenotypic and functional properties of myeloid and lymphoid cell subsets were evaluated by flow cytometry and serum cytokine concentration levels were measured via Luminex Assay. At baseline,patients displayed reduction of circulating myeloid and plasmacytoid dendritic cells, activation markers on monocytes an increased terminal differentiation with impaired cytokine production by T cells, compared to healthy subjects. Ruxolitinib restored homeostasis of different immune cell subsets and induced a general decrease in levels of inflammatory cytokines (IL-1β, TNF-α, IL-8). Patients treated with Tocilizumab showed a significant increase of IL-6 and reduction of CXCL10 levels. The inhibition of the Jak signaling restores homeostasis with an anti-inflammatory effect, suggesting cytokine storm is strictly connected to immune response impairment. Higher levels of soluble IL-6 suggest efficiency of Tocilizumab binding with IL-6 receptor. A decrease in CXCL10 levels after anti-IL6 treatment offers a new direction for further investigation, since it increases with disease onset and possibly correlates with lymphopenia. Both IL-6 and CXCL10 could serve as biomarkers. Taken together, these results suggest cytokine storm is directly connected to illness degree in COVID-19.

Keywords: Biomarkers, cytokines and mediators, drugs for immune modulation, immune regulation and therapy, inflammatory molecules, viral infections

P-0945

Investigating neutrophil death in neuromyelitis optica and MOG-antibody disease

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Neutrophils accumulate in lesions of neuromyelitis optica spectrum disorders (NMOSD) but not in Multiple Sclerosis (MS) lesions. Our previous results demonstrated deficient functionalities of NMOSD neutrophils compared to MS. Thus, we hypothesized that NMOSD neutrophils may display impaired cell death mechanisms contributing with their accumulation in CNS lesions. To evaluate cell death of circulating neutrophils from AQP4-IgG seropositive NMOSD and MOGAD patients.

Neutrophils from 20 anti-AQP4 seropositive (AQP4+) 9 anti-MOG seropositive (MOG+) patients and corresponding age- and gender-matched healthy controls (HCs) were investigated. Cell death was induced *in vitro* with PMA for 30 min at 37ºC. Spontaneous and PMA-induced NETosis and apoptosis was analyzed by flow cytometry 2.5 hours after stimulation, using 7-AAD and Annexin V staining. Active Caspase-3 and MPO-DNA were additionally investigated by western blot and ELISA, respectively. Neutrophils from AQP4+ but not from MOG+ patients showed reduced percentage of Annexin V+ 7-AAD+ late apoptotic/NETotic neutrophils (29.6%), compared to HCs (44.7%, p=0.0006) in response to PMA. In contrast, AQP4+ NMOSD patients present a mild increase in Annexin V+ 7-AAD- early apoptotic cells (24.5%) compared to HCs (20.8%, p=0.048). Caspase-3 was increased only in neutrophils from AQP4+ patients compared to HCs. No difference in MPO-DNA levels after PMA stimulation in AQP4+ or MOG+ patients compared to HCs. The fraction of neutrophils undergoing apoptosis in response to PMA was reduced in AQP4+ patients after PMA, while NETosis markers were not altered. This impairment distinguishes AQP4+ NMOSD from MOGAD neutrophils.

Keywords: Autoimmunity, cell death, granulocytes, inflammatory disease, neuroimmunology, neutrophils

P-0946

Protein expression profiles in patients with primary immunodeficiencies

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Detection of protein expression levels and the functions of the proteins using various technics are fundamental to detect the pathogenicity of the variant following sequencing strategies. Nevertheless, in most cases protein expression analysis and functional studies are performed before the mutational analysis due to their quick results obtained by flow cytometry. Utilization of flow cytometry is a first priority in the designation of protein related evidence in primary immunodeficiencies. However, functions of proteins and the expression levels do not always reflect the genetic background and the detected variants do not always effect the protein expression and known function of the protein. In this study, our aim was to evaluate the level of protein expression and functions of the proteins in patients with primary immunodeficiencies with or without genetic variant followed up in our clinic. We investigated expression levels and molecular functions in 22 cell surface and intracellular proteins which are related to primary immunodeficiencies in 169 patients and 157 healthy control individuals. We detected decreased protein expressions in 28 patients. 26 of them had a mutation in the related genes. 10 of 14 patients were known to have mutations in the related gene had increased protein expression levels. Total loss of protein expression was observed in four patients who were confirmed to have mutation in the related genes. Four patients had normal expression level although they had mutations in the relevant genes. Two patients who have decreased CD40 protein expression had mutations in different genes.

Keywords: Immunological techniques, immunodeficiency, adaptive immunity

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POSTER PRESENTATIONS

P-0947

Genetic deletion of galectin-3 attenuates neuroinflammation and anxiety state in mice

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Galectin-3 (Gal-3), is involved in maturation and function of nervous system. It had been reported that Gal-3 regulates oligodendrocytes differentiation and that Gal-3TLR4 axis is involved in neuroinflammation. As both, CNS maturation and neuroinflammation may affect behavior, the aim of this study was to examine the effects of Gal-3 gene deletion on behavior. Male, 20-week-old wild-type (WT) C57BL/6 and Gal-3-/- (LGALS3-/-) C57BL/6 mice were used. Animals were intraperitoneally injected with a single dose of LPS (E. coli) 5 mg/kg, while the control groups recieved 0.9% saline. Gal-3 deficiency shows anxiogenic effect in mature untreated animals (basal conditions), accompanied with lower IL-6 and TNF-α gene expression and hippocampal content, with no effect on TLR4 expression. Gal-3 deficiency was also accompanied with lower BDNF gene expression and immunoreactivity in hippocampus (CA1 region). Besides, the Gal-3 gene deletion resulted in attenuation of the hippocampal gene expression of GABA-AR2S and GABA-AR5S, Elseways, Gal-3 deficiency attenuates LPS-induced neuroinflammation. The anxiogenic effect of neuroinflammation was accompanied with increased hippocampal IL-6, TNF-α and TLR4 gene expression, as well as decreased gene and BDNF expression in hippocampus, with significant decline in GABA-AR2S in WT mice in comparison to basal conditions. Gal-3 gene deletion prevented the increase in IL-6, the decline in BDNF gene expression and immunoreactivity, and reduction in hippocampal GABA-AR2S, and therefore attenuated the anxiogenic effect of neuroinflammation. Our data demonstrate that opposite effects of Gal-3 deficiency on anxiety levels seem to be related to the shift in IL-6, TNF-α and hippocampal BDNF.

Keywords: Animal models, molecular immunology, neuroimmunology

P-0948

Investigation of immune impairment status in primary immune deficiency

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Characterization of how immune system works in PIDs for better understanding of disease and treatment options is important issue.In this study, we investigated CD27 and STAT1 mutation and their effects on innate and adaptive arm of immune system.Immunological phenotypes of patients and their responses of adaptive and innate system were investigated in this study.To determine phosphorylation levels of STAT proteins, cells were stimulated with different ligands depending on type of STAT protein and analyzed.Innate immune responses against different PRR and inflammasome pathway ligands were investigated by cytokine ELISA.NETotic activities of neutrophils were analyzed by checking ROS production levels and production of NETosis formation.CD27 expression of the PBMCs, CD3+ T and CD19+ B cells were absent in CD27-/-.Increased phosphorylation level of STAT1 and decreased STAT5 levels were observed in CD27-/-. When PBMCs were stimulated with PMA/Ionomycin and analyzed, increased IFN-γ, IL-4 and IL-17a secretion were investigated in CD27-/-. Through activation of different TLR and inflammasome pathways, increased TNF-α and IL-10 was observed in CD27-/- as well.In STAT1 GOF patients, p-STAT1 levels were higher. Although the STAT1 phosphorylation level was high, IFN-γ released due to Th1 response was low.Th17 response were low in STAT1 GOF.Type II interferon responses of the patients to PRR and inflammatory pathway ligands were low while normal IL-12 secretion was observed.Some patients had increased NETotic activities at the basal levels supported by spontaneous NET formation, while some patients were not able to induce NETosis through PMA stimulation.Results suggest that impaired immune status was observed in CD27-/- and STAT1 GOF.

Keywords: Adaptive immunity, immunodeficiency, innate immunity

P-0950

Humoral immune response to the first dose of mRNA-1273 vaccine in a cohort of kidney transplant recipients

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To analyze the humoral response to the first dose of COVID-19 vaccine in a cohort of kidney transplant recipients (KTR) and to explore potential predictive factors of such response. Peripheral blood samples were collected before and three weeks after the first dose of mRNA-1273 Moderna vaccine. IgG antibodies against the trimeric SARS-CoV-2 S protein were quantified in sera by chemiluminescence assay. Soluble inflammatory and biochemical parameters, and immune cell populations were determined. Sixty-two KTR were included (34% females, 55[46-65] years-old). Time from transplantation was 46[16-118] months. Also 17 healthy-controls were analyzed three weeks after their first dose of the Pfizer-BioNTec vaccine (76% females, 38[31-44] years-old). Most of KTR patients were receiving corticosteroids (89%), tacrolimus (93 %) or mycophenolate-mofetil (73%) as immunosuppressive therapy. Three weeks after vaccination, we observed seroconversion (>33,8 BAU/ml) in 22% of patients whilst in 100% of healthy-controls ($p<0.001$), though Ab titers were not significantly different. Most non-responders KTR were older than responders (59 vs. 50 years, $p=0.019$), were under mycophenolate-mofetil (81% vs. 43%, $p=0.008$) and with higher dose of corticosteroids ($p=0.024$). Neither time since transplantation nor inflammatory or biochemical parameters showed any relation with the capability of seroconversion. Our results show a low rate of seroconversion after the first dose of vaccine in KTR, which was influenced by their age and immunosuppressive therapy. Soon, we will also have data about humoral and cellular responses to the second dose.

Keywords: Immune response tracing, immunodeficiency, transplantation

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POSTER PRESENTATIONS

P-0951

The populations of peripheral blood T-lymphocytes at different stages of differentiation in patients with Parkinson's disease

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Parkinson's disease (PD) is one of the most common neurodegenerative diseases. The mechanisms of PD development are largely associated with the processes of chronic inflammation in the brain tissue (neuroinflammation). The development of neuroinflammation involves the central nervous system's resident immune cells and the cells of the peripheral immune system migrating to the brain. We examined 31 PD patients, 33 old-aged healthy donors (OHD), 30 young healthy donors (YHD). Immunophenotyping was performed using flow cytometry. Was analyzed the populations of T-lymphocytes (CD3+) at different stages of differentiation: replicative senescence (CD56-CD57+), Naïve (CCR7+CD45RA+), Central memory (CM) (CCR7+ CD45RA-), terminally differentiated effector memory (TEMRA) (CCR7- CD45RA+), effector memory (EM) (CCR7- CD45RA-). The proportion of T-lymphocytes (CD3+CD56-) expressing the CD57 marker was lower in the PD group than in the OHD group (8.7 and 13.1, p = 0.02). The proportion of these cells in the group of the YHD was significantly lower than in the group of PD and OHD. There were no significant differences in the naïve, CM, EM, TEMRA populations between patients with PD and OHD in the population of T-lymphocytes (CD3+) at different stages of differentiation. This study demonstrates that the peripheral immune profile in PD is not typical for older donors. We found that there is no replicative senescence of T-cells (CD3+CD56-CD57+). However, in the group of PD and OHD in the population of T-lymphocytes (CD3+) at different stages of differentiation, no differences were found.

Keywords: Ageing, biomarkers, immune senescence, memory

Acknowledgments: The reported study was funded by RFBR, project number 20-315-90072.

P-0952

Severity of allergic rhinitis symptoms is associated with ceruloplasmin levels and a deficiency of microbial strains that sequester iron

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Since allergy is associated with iron deficiency, we sought to determine whether iron and microbial parameters correlate with the clinical response of allergic rhinitis subjects during nasal provocation. Female allergic subjects donated blood, and stool samples before they underwent a graded nasal provocation (n=38) with birch or grass pollen extract. Total nasal symptom scores (TNSS), visual analogue scale (VAS) and weight of nasal fluids were recorded. Complete blood cell counts and iron metabolism markers were determined. Stool samples were subjected to 16S rRNA amplicon sequencing. Serum hepcidin was assessed by ELISA. LegendPlex assay analysis was utilized to define levels of IgE, ceruloplasmin, lipocalin2 and cytokines in nasal fluids. Trace elements in serum and aqueous stool extracts were determined via inductively coupled plasma-mass spectrometry. Nasal and serum ceruloplasmin was the sole protein marker that positively correlated with all assessed symptom parameters (TNSS, VAS and nasal fluid weight), VAS scores in addition correlated with serum transferrin, haptoglobin and nasal IgE-levels. A positive correlation to symptoms was confirmed for serum copper, whereas gut iron and cobalt showed an inverse relationship to clinical symptoms. Particularly members of the order Bacteriodales and members of the genus Ruminococcus seems protective against TNSS and correlate well with the presence of gut iron. For the first time, we show that essential parameters in iron homeostasis, such as the copper-containing ferroxidase ceruloplasmin, correlate with the allergic outcome. Additionally, commensal bacteria sequestering iron seem to play a beneficial role against hay fever in adults.

Keywords: Allergic disorders, drugs for immune modulation, microbiome and environmental factors

P-0953

Overexpression of galectin 3 in pancreatic beta cells amplifies beta cell apoptosis and islet inflammation in type 2 diabetes in mice

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During obesity hematopoetic cells-derived galectin 3 induces insulin resistence. While the role of galectin 3 expressed in islet invading immune cells in both type of diabetes has been studied, the importance of expression of this molecule on the target pancreatic beta cells is not defined. We have used 10-12 weeks old C57/BL6 male mice (WT) and C57/ BL6 mice with transgenically enhanced Gal-3 expression in pancreatic β cells (TG). Obesity was induced with 16 weeks high fat diet regime. Pancreatic beta cells were tested for susceptibility to apoptosis induced by non-esterified fatty acids and cytokines as well as parameters of oxidative stress. The overexpression of galectin 3 increases beta cells apoptosis in HFD conditions and increases the percentage of proinflammatory F4/80+ macrophages in islets that express galectin 3 and TLR4. In isolated islets, we have shown that galectin 3 overexpression increases cytokine and palmitate-triggered beta cells apoptosis and also increases NO2- induced oxidative stress of beta cells. Also, in pancreatic lymph nodes, macrophages were shifted towards proinflammatory TNF-α producing phenotype. By complementary approach *in vivo* and *in vitro*, we have shown that galectin 3 overexpression facilitates beta cell damage, enhances cytokine and palmitate-triggered beta cells apoptosis and also increases NO2- induced oxidative stress in beta cells. Further, the results suggest that increased expression of galectin 3 in the pancreatic beta cells affects the metabolism of glucose and glycoregulation in mice on HFD, affecting the fasting glycemic values, as well as glycemia after glucose loading.

Keywords: Animal models, cytokines and mediators, diabetes, effector molecules

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POSTER PRESENTATIONS

P-0954

Humoral immune response to the BNT162b2 Pfizer vaccine in the elderly and predictive factors of its magnitude

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We aimed to compare the magnitude of humoral responses to the BNT162b2 vaccine between the elderly (>65 years-old) and younger (<65 years-old) vaccinees and to explore potential predictive factors. Volunteers (workers and residents) from the "Charity" home for the elderly were recruited. Blood samples were collected four weeks after the second dose of vaccine. IgG antibodies against the trimeric SARS-CoV-2 Spike protein were quantified by chemiluminescence assay. Soluble biochemical and inflammatory markers, as well as different immune subsets were determined. We also recorded the subjective perception of vaccine reactions in a medical questionnaire. 58 out of 101 volunteers were >65 years-old (77 [72-82], 100% men) and 43 were under that age (49 [36-62], 49% men). Four weeks after the second dose of vaccine, all participants achieved positive response (>33.8 BAU/mL), although antibody titers were significantly lower in the elderly (1150 [496-1625] vs. 1770 [1190-2070]); p<0.001), correlating antibody titers and age (r=-0.564, p<0.001). Antibody titers also correlated with total IgG (r=0.243, p=0.016), homocysteine levels (r=-0.295, p=0.003), absolute numbers of CD3 (r=0.471, p<0.001), CD4 (r=0.475, p=0.002), CD8 (r=0.311, p=0.005), B cells (r=0.346, p=0.002) and total-lymphocytes (r=0.470, p<0.001), and with the percentage of NK cells (r=-0.263, p=0.019). We found that subjects scoring ≥3 vaccine reactions showed higher antibody titers (1720 [1300-3180] vs. 1145 [1300-3180]; p<0.001). The magnitude of the response to the mRNA Pfizer vaccine in the elderly was lower to that of younger controls, being mostly influenced by the distribution of different immune subsets, including NK.

Keywords: Adaptive immunity, adjuvants and vaccines, ageing, antibody, memory, visualizing immune responses

P-0956

Aberrant B cell receptor signaling in naïve B cells from patients with idiopathic pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is a chronic and ultimately fatal disease in which an impaired healing response to recurrent micro-injuries is thought to lead to fibrosis. Recent findings hint at a role for B cells and autoimmunity in IPF pathogenesis. We previously reported that circulating B cells from a fraction of patients, compared with healthy controls, express increased levels of the signaling molecule Bruton's tyrosine kinase (BTK). However, it remains unclear whether B cell receptor (BCR) signaling is altered in IPF. Here, we show that the response to BCR stimulation is enhanced in peripheral blood B cells from treatment-naïve IPF patients. We observed increased anti-immunoglobulin-induced phosphorylation of BTK and its substrate phospholipase C2 (PLCγ2) in naïve but not in memory B cells of patients with IPF. In naïve B cells of IPF patients enhanced BCR signaling correlated with surface expression of transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI) but not B cell activating factor receptor (BAFFR), both of which provide pro-survival signals. Interestingly, treatment of IPF patients with nintedanib, a tyrosine kinase inhibitor with anti-fibrotic and anti-inflammatory activity, induced substantial changes in BCR signaling. These findings support the involvement of B cells in IPF pathogenesis and suggest that targeting BCR signaling has potential value as a treatment option.

Keywords: Autoimmunity, B lymphocytes, cell signalling, chronic inflammation and fibrosis

P-0957

Normal numbers of stem cell memory T cells despite strongly reduced naive T cells support intact memory T cell compartment in ataxia telangiectasia

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Ataxia Telangiectasia (AT) is a rare inherited disorder characterized by progressive cerebellar ataxia, chromosomal instability, cancer susceptibility and immunodeficiency. AT is caused by mutations in the ATM gene, which lead to hampered V(D)J recombination and consequently reduced numbers of naive B- and T-cells. Yet, AT patients in general have no clinical T-cell associated infections and the numbers of memory T cells are usually normal. In this study, we investigated the naive and memory T-cell compartments in five AT patients and five healthy controls using 24-color spectral flow cytometry. Using dimensionality reduction analyses, we report strongly reduced numbers of early naive T cells, i.e. CD4+CD31+ recent thymic emigrants and CD8+CCR7++CD45RA++ T cells. Interestingly, we found normal numbers of stem cell memory T (Tscm) cells expressing CD95 and CXCR3 within the naive compartment. The memory T cells of AT patients were normal in number and expressed chemokine receptors, activating and inhibitory receptors in comparable percentages as controls. Comparing memory T cell phenotypes by Boolean gating revealed similar diversity indices in AT compared to controls. We hypothesize that the presence of Tscm cells, with their longevity and self-renewal capability, supports the maintenance of the normal memory T cell compartment in AT, despite strongly reduced naive cells. The identification of Tscm cells via our spectral flow cytometric approach is highly relevant for better understanding of T cell immunity in AT. Moreover, it provides possibilities for further research on this recently identified T cell population in other inborn errors of immunity.

Keywords: Adaptive immunity, immunodeficiency, memory, stem cells

P-0958

Non-disease-originated leukocyte subset chimerism and the clinical outcome in hematopoetic stem cell transplantation

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We aimed to evaluate lineage chimerism for B and T cells and the relationship between total leukocyte chimerism and clinical outcomes. Patients with a hematologic malignancy, undergone allogeneic hematopoietic stem cell transplantation (HSCT) from HLA-matched donors enrolled in the study. Chimerism analysis was performed on +28th and +90th HSCT days. All patients' clinical outcomes as engraftment, graft versus host disease (GVHD), relapse were documented. A total of 21 patients were included in the study. The mean age was 38.1±13.2years (17-60). The HSCT indication was mainly myeloid neoplasm as being AML (n=6), MDS (n=5), CML (n=2) and PMF (n=1). Lymphoproliferative neoplasms the distribution was as Hodgkin lymphoma (n=3), ALL (n=2), CLL (n=1), ve follicular lymphoma (n=1). Three patients (14,2%) relapsed of whom two were during the first years. Acute GVHD developed in 52,3% and chronic GVHD in 61.9% of patients. During six-year-follow up, 11 (52,3%) of the patients died, one of them due to disease progression, 5 of them due to GVHD, 5 of them due to sepsis. Disease-free survival was found to be in a range between 4-78 months (mean 38.71 ± 28.2). The mean overall survival was found to be 44.62 ± 27.5 months (4-78 months). There was no significant relationship between total leukocyte chimerism, B and T chimerism, and clinical outcome. (P-value respectively 0.136 and 0.07). In our study, thenon-disease-originated leukocyte subset chimerism and the total leukocyte chimerism comparison did not point to significant different a clinical outcomes.

Keywords: B lymphocytes, bone marrow transplantation, stem cells

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POSTER PRESENTATIONS

P-0959

Development of a pseudovirus-based assay for analysis of neutralizing activity against SARS-CoV-2 in convalescent plasma

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For predicting treatment response to Convalescent Plasma (CP) therapy, the measurement of neutralizing activity against SARS-CoV-2 using wildtype virus is a reliable functional assay but its applicability is limited by the lack of suitable BSL3 facilities for virus culture. Instead, pseudovirus particles containing SARS-CoV-2 virus elements are widely used to test the activity of CP or other neutralizing agents such as monoclonal antibodies. In this study, we present our method for producing GFP-encoding lentiviral particles pseudotyped with the SARS-CoV-2 Spike (S), Membrane (M), Envelope (E) and Nucleocapsid (N) proteins for use in neutralization assays on 293FT cells overexpressing ACE2 and/or TMPRSS2. Our findings demonstrate the feasibility of producing pseudovirus particles using a C-terminally truncated Spike protein, which is greatly enhanced by the incorporation of the D614G mutation as well as the M, N and E proteins. Furthermore, we show that using Sodium Butyrate during pseudovirus production significantly increases titers. The analysis of neutralizing activity against particles pseudotyped with wildtype mutated version of Spike proteins on D614G and/or N501Y revealed a large range of activity in CP samples which did not necessarily correlate with the amount of anti-SARS-CoV-2 antibodies. Our findings show that the neutralizing activity in CP samples is determined by the quality rather than the quantity of humoral immune responses, and that it varies greatly between donors. If clinical efficacy is to be maximized, functional screening of neutralizing activity using pseudovirus-based neutralization assays can be accepted as a critical tool for selecting CP donors.

Keywords: Immunological techniques, infectious disease, monitoring immunity, viral infections

P-0960

Chemotherapeutic agent cisplatin impairs clearance function of tumor-associated macrophages by differential effect on the regulators of endocytosis

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Tumor-associated macrophages (TAMs) are a major component of innate immunity supporting primary tumor growth and metastasis. Scavenging function of TAMs is critical for the control of pro-angiogenic and immunosuppressive microenvironment. TAMs can accumulate in tumors after chemotherapy and contribute to chemoresistance. Our aim was to examine the transcriptional program and scavenging function of TAMs induced by cisplatin. Modeled human TAMs were differentiated from CD14+ monocytes stimulated by conditioned supernatants of breast cancer cells MCF-7. Transcriptional program induced in TAMs by cisplatin was identified by next-generation sequencing, and validated by RT-PCR. Endocytic activity of TAMs and stabilin-1+CHO cells was assessed by flow cytometry quantification of the uptake of acLDL and EGF. Intracellular transport of ligands was examined by confocal microscopy. We demonstrated that stabilin-1 ectopically expressed in CHO cells mediates endocytic uptake of EGF, key growth factor stimulating cancer progression. In TAMs cisplatin suppressed stabilin-1-mediated internalization and endocytic trafficking of EGF, without significant change in gene expression of stabilin-1. Molecular mechanisms of cisplatin effects on TAMs were analyzed by NGS. Cisplatin induced defects in endocytic machinery by suppression of the expression of genes that positively regulate endocytosis and vesicular transport, and inducing expression of factors, involved in negative regulation of endocytosis. Our data demonstrate that suppression of receptor-mediated clearance of tumor-supportive factors, such as EGF, by chemotherapeutic drugs may enhance tumor-supporting effect of TAMs creating the microenvironment supporting tumor chemoresistance or relapse after chemotherapy course.

The work is supported by the grant RSF №19-15-00151.

Keywords: Cancer immunology, immune networks, macrophage, microenvironment, molecular immunology, phagocytosis

P-0961

Ex vivo Th-1 biasing T cell responses in alum-adsorbed SARS-CoV-2 inactivated virus vaccine adjuvanted with K-type CpG ODN

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Emergence of the COVID-19 pandemic resulted in an urgent need of an effective vaccine against SARS-CoV-2. One effective vaccine strategy had been based on inactivated SARS-CoV-2/Alum combination, which has been widely distributed throughout the World. However, Herein, to enhance the immunogenicity and temper the potential Alum-induced immunopathology, we describe the immunogenicity of a Th1-biasing K-type CpG ODN vaccine adjuvant in combination with Alum and inactivated SARS-CoV-2 isolated from a Turkish patient. To assess the immunogenicity of the inactivated vaccine, BALB/c or C57/Bl6 mice were subcutaneously immunized with 3 different doses of beta-propiolactone inactivated SARS-CoV-2, separated by a 2-week interval. The vaccine was either administered with alum or in combination with alum plus K3 CpG ODN. SARS-CoV-2 antigen specific total IgG, IgG1 or IgG2a titers were evaluated with ELISA from sera of immunized mice. SARS-CoV-2-specific Th1/Th2/Th17 responses were measured via cytometric bead array from the *ex vivo* antigen restimulated splenocyte culture supernatants. Comparison of the antibody response elicited by inactivated vaccine/adjuvant combinations revealed that combining CpG ODN with Alum not only increased S-, N- or inactivated virus-specific total IgG, but greatly enhanced virus-specific IgG2a titers. Moreover, inactivated virus/Alum/CpG combination suppressed the secretion of Th2 dependent cytokines, demonstrating that combined adjuvantation supported a strong Th1 type of immune response. Preclinical data illustrates that in combination with CpG, inactivated virus/Alum formulation proved to be more effective as a vaccine against SARS-CoV-2, providing a rationale for its evaluation in human clinical trials.

Keywords: Adaptive immunity, antibody, adjuvants and vaccines

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POSTER PRESENTATIONS

P-0962

Virus like particle formation by using 4 structural proteins of SARS-Cov-2 and investigation of its effectiveness among wild type and prefusion stabilized spike variants

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SARS-Cov-2 has emerged in December 2019 and became a pandemic in a short term. Mainly, SARS-CoV-2 vaccine candidates target spike protein however, it has been shown that other structural proteins also plays a critical role in recognition and elimination of the virus. Herein, we developed a mammalian cell derived virus-like particle(VLP) which contains 4 structural proteins of SARS-Cov-2; Nucleocapsid(N), Envelope(E), Membrane(M) and Spike(S). Further, in order to improve the antigenicity, 2 different prefusion and thermo-stabilized spike proteins used beside wild-type form which are S-2P and S-6P(HexaPro) variants. VLP-protein encoding constructs designed and cloned in pVITRO1and pVITRO2 dual expression plasmids as in the combination of M,N and S,E. For S, M and E; CD33 signal sequence cloned at N-terminus while 6xHis-tag sequence cloned at C-terminus. After transfection and purification of VLPs, structural proteins observed via immunoblotting and silver staining, particle number and size distribution determined via tunable resistive pulse sensing. Ace-2 binding experiments performed with Ace-2 coated beads and CFSE labeled VLPs. VLPs morphological characterization performed via AFM and TEM. We observed that, VLPs carry 4 structural proteins and their size varies among 60-200 nm. Wild-type, S-2P and HexaPro spike associated VLPs showed that HexaPro spike expressed hence decorated more on VLPs also it is more stable compared to wild-type and S-2P. Ace-2 binding experiments showed that CFSE loaded VLPs are binding Ace-2 coated beads through spike protein. To conclude, this study suggested that prefusion stabilized spike decorated VLPs could be a good antigenic candidate for SARS-Cov-2 vaccines.

Keywords: Adjuvants and vaccines, immunological techniques, viral infections

P-0963

Helper T cell responses in SARS-CoV-2 virus like particle vaccinated mice

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Vaccine formulations that can generate neutralizing antibodies and strong Th1 cellular responses present a solution for the global SARS-CoV-2 pandemic. Most SARS-CoV-2 vaccines that have already been approved target spike protein. However, with the emergence of variants of concern, incorporating other potential viral antigens in vaccine design might prove advantageous especially with respect to broadening the coverage of cell mediated immunity. In this respect, herein, we compared the antigen-specific helper T cell responses in mice immunized with a Virus Like Particle (VLP) vaccine encoding the four main structural proteins (S, M, E, N) of SARS-CoV-2. The vaccine antigens were adjuvanted with either the Th1 polarizing CpG ODN, the Th2 stimulating alhydrogel or a combination of both. Splenocytes of immunized mice were harvested and restimulated with either recombinant spike, nucleocapsid or the inactivated virus, and antigen-specific cytokine production was determined from culture supernatants using a 12-plex mouse T helper cytokine panel. Results showed that interferon γ (IFNγ) production was increased when vaccines were formulated with Alum+CpG rather than Alum or CpG alone. Furthermore, Th2 associated cytokine levels (IL-4, IL-5, IL-13) were dramatically reduced in mice immunized with Alum+CpG. Our results demonstrate that Alum+CpG combination might be of interest in development of multi-antigen vaccines against SARS-CoV-2 infection.

Keywords: Adaptive immunity, adjuvants and vaccines, chemokines, cytokines and mediators, infectious disease, viral infections

P-0965

Regulatory B cell subsets are induced in patients with allergic rhinitis cured with allergen-specific immunotherapy

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Allergic rhinitis (AR) is an IgE-mediated disease which is triggered by inhalation of perennial or seasonal allergens. Allergen-specific immunotherapy (AIT) stands as the sole therapy option for induction of tolerance which in turn helps for long term cure of allergic diseases. Previous studies revealed that induced regulatory T (Treg) cells following AIT play important roles in maintenance of tolerance, but the role of regulatory B (Breg) cell populations is still unclear. This study aimed to investigate Breg subsets following a clinically successful long-term AIT. The patients enrolled to this study (25 new diagnosed patients with AR, 25 patients with long-term (32.52±5.25 months) house-dust mite specific sub-cutaneous AIT and 25 healthy subjects) were followed by Istanbul University, Istanbul Faculty of Medicine. Lymphocyte subsets were determined with a direct staining protocol in fresh blood samples. CD19+CD25+CD71+CD73- (Br1) and CD19+CD24hiCD27+ (B10) B regulatory (Breg) cell subsets were investigated. The initial results of this study revealed that CD19+CD25+CD71+CD73- Br1 subset was significantly increased following AIT. The lowest levels were observed in healthy subjects. CD19+CD24hiCD27+ B10 subset was also significantly increased following AIT, however the lowest content was observed in AR patients. The initial results of this study revealed induction of both Breg cell subsets in AR patients as a consequence of a successful AIT. The different expression patterns of these cell subsets may be due to differential regulatory roles in healthy individuals and also in patients, which should further be investigated.

Keywords: Allergic disorders, B lymphocytes, immunotherapy, regulatory cells

P-0966

Asthma-in which language should we speak on?

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After receiving passive consent from parents/ guardians, we elaborated a total of 1056 school children (adolescents) aged 13-14 years from randomly selected schools in the city of Prishtina. Out of 1056 school children 493 (46,64%) were male and 563 (53,26%) were female with gender ratio of 1:1,14. The percentage difference between the genders, was statistically significant Chi-square=9,26; df=1 p=0,0023) in favor of female. Social status of adolescents was analyzed through mother's level of education. We found that 102 (11,68%) of mothers had primary education (low status), 391 (44,79%) had secondary education (middle status) and 380 (43,53%) were with college/university (high status). We didn't find significant association between adolescents' gender and the social status (mother's level of education) for Pearson Chi-square=0,5918; df=2; p=0,7438. We found non normal frequency distributions of age for Shapiro-Wilk W=0,725; p=0,00001. The mean age of school children in the sample was 13,25±0,56 with min/max 12/16 and Median IQR=13 (13-14). The mean age of males was 13,27±0,56 with min/max 12/15 and for females 13,23±0,56 with min/max 12/16. About 50% of the children from both genders were older than 13 years for Median IQR)=13 There was no significant age differences between genders. Adolescents were analyzed related to weight, height, BMI and nutrition. The body mass index (BMI) was used to assess nutrition. The analysis indicated non normal distribution of weight (kg), height (cm) and BMI (kg/m²) for consequently Shapiro-Wilk W=0,8974; p=0,00001 vs. Shapiro-Wilk W=0,9683; p=0,0001 vs. Shapiro-Wilk W=0,8035; p=0,00001), so for the analysis we used appropriate statistical tests.

Keywords: Big data, inflammatory disease, allergic disorders

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POSTER PRESENTATIONS

P-0967

Renin-angiotensin system imbalance detected in simian immunodeficiency virus infected rhesus macaques

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The role of renin-angiotensin system (RAS) including ACE2, angiotensin (Ang) II, Ang type 1 receptor (AT1R) and Ang type 2 receptor (AT2R) in hypertention, cardiovascular diseases, renal diseases, and inflammation is well studied. Use of Ang receptor blockers and ACE2 inhibitors was associated with improved disease outcome in IBD patients. Therefore, understanding the role of RAS can reveal its importance in HIV mediated inflammation and comorbidities. TMPRSS2 and ADAM17 are two important protease enzymes that cleaves and process ACE2 expression. This study characterized the components of RAS and its importance in HIV pathogenesis and comorbidities in rhesus macaque (RM) model. Ten healthy adult Indian RMs were used and infected 100 SIVmac251 TCID50 intravenously. Frequency of ACE2, Ang II, AT1R, AT2R proteins in plasma were quantified by ELISA. Quantification of ACE2, AT1R, AT2R, TMPRSS2, and ADAM17 protein and/or mRNA expression were measured either by immunofluorescence, immunohistochemistry (IHC) or real-time QPCR. A significant downregulation of jejunum ACE2 protein (both in acute, $p < 0.0001$ and chronic, $p < 0.0001$), and TMPRSS2 protein (chronic, $p<0.0001$) expression detected SIV infected RMs. In contrast, a significant upregulation of jejunum AT1R and AT2R expression detected during chronic ($p<0.0001$) infection. No significant plasma ACE2 and AngII proteins and ADAM17 mRNA expression detected during SIV infection. Downregulation of ACE2 and TMPRSS2 by SIV/HIV invasion could be detrimental to the subsequent SARS-CoV-2 coinfection with baseline ACE2 and TMPRSS2 deficiency. Disparity in AT1R and AT2R expression may also accelerate the SIV/HIV mediate comorbidities.

Keywords: Animal models, immune networks, infectious disease, molecular immunology

P-0968

Autoinflammatory manifestations as a result of an elevated type I IFN signature in a case of NEMO deficiency

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NEMO (NF-κB essential modulator, IKKγ) deficiency, is a monogenic disorder caused by mutations in the IKBKG gene leading to EDA-ID (ectodermal dysplasia with immune deficiency) in males and IP (incontinentia pigmenti) in females. Patients rarely display autoinflammatory manifestations other than IBD (Inflammatory Bowel Disease) but present with developmental impairments in ectodermal tissues and experience recurrent infections. In a case of NEMO deficiency, we investigated the causal mechanisms of autoinflammation, evidenced by perivascular and interstitial neutrophilic infiltrations, nodular skin lesions, and subcutaneous swelling. Due to the immune deficiency, the patient could not generate IL-6 or IL-1β in response to TLR agonists or IFNγ upon TCR activation. Nevertheless, in the absence of infection, we detected high levels of IP-10 in the patient's plasma, free ISG15 (interferon stimulated gene 15) and ISGylation of PBMC proteins, suggesting an elevated type I IFN signature. Moreover, we identified LDGs (low-density granulocytes) in the PBMC fraction of the patient and observed spontaneous ROS production in neutrophils, indicative of a primed state. The patient later underwent HSCT (hematopoietic stem cell transplantation) which resulted in the normalization of type I IFN levels in the patient and disappearance of LDGs and pre-active neutrophils. Furthermore, gene expression analysis revealed that LDGs and neutrophils of the patient prior to transplantation, carry the potential to extravasate from the circulation into tissues and inflict inflammatory damage. Collectively, our results demonstrate that dysregulation of type I IFN due to NEMO deficiency might lead to LDG generation and neutrophil activation, contributing to disease pathogenesis.

Keywords: Autoinflammation, granulocytes, immunodeficiency, neutrophils

P-0969

Manufacturing of a SARS-CoV-2 virus-like particle vaccine adjuvanted with K3 CpG oligodeoxynucleotide and alhydrogel for phase 1/2 clinical trials

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Severe acute respiratory syndrome coronavirus 2(SARS-CoV-2) causing Coronavirus Disease 2019(Covid-19) have resulted in a global pandemic. Enveloped virus-like particles(VLP) used as vaccine antigens provide effective immunity against viruses. Upon production of four structural proteins; Spike(S), Nucleocapsid(N), Envelope(E) and Membrane(M) in producer cells, self-assembled SARS-CoV-2 VLPs are secreted to the cell supernatant. SARS-CoV-2 VLP production was achieved via transient transfection of HEK293 suspension cultures with two dual expression plasmids encoding S, N, M and E using PEIPro. VLPs were purified and concentrated from clarified cell supernatant by multimodal chromatography, followed by tangential flow filtration(TFF). Under good manufacturing process(GMP) regulations, scale up from 200ml shake flask to 5L bioreactor was achieved. 3.38-fold increase in total protein (from 39.2±2.3ug/ml to 132±0.85ug/ml) was achieved from shake flask to bioreactor process optimization. Host cell protein contamination was analyzed through HPLC-SEC and ELISA. Host cell DNA content for 5L batches were 4.8±0.3 ug/ml (<10ng/dose), in accordance with WHO ECBS. Accelerated(25°C, 1 week), long-term(2-8°C, 3 months) stability and stress(42°C, 48hour) tests indicate that bulk antigen is stable in tested conditions. Validation methods for determining the amount of adjuvants and antigen in the finished product were developed. Manufactured lots were tested for their *in vivo* immunopotency in BALB/C mice. Herein, we have reported the manufacturing of a SARS-CoV-2 VLP vaccine formulated with K3 CpG and Alhydrogel. Presence of humoral and cellular immune response to viral proteins other than S and effortlessness in adapting the vaccine for different variants makes this approach unique.

Keywords: Adaptive immunity, adjuvants and vaccines, infectious disease, innate immunity, viral infections

P-0970

Development of an antibody-based diagnostic system to detect S1 protein of SARS-CoV-2

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Coronavirus disease 2019 (COVID-19) is rapidly expanding worldwide until December 2019 and has been affecting human life dramatically since then. This makes the diagnosis of (Severe Acute Respiratory Syndrome Coronavirus 2) SARS-CoV-2 which causes COVID-19 very necessary. Monoclonal (mAbs) and polyclonal antibodies (pAbs) are very important in diagnostic systems due to their high affinity for specific antigens. Developed mAbs and pAb against the S1 subdomain of SARS-CoV-2 spike protein in our laboratory were used to integration of diagnostic systems such as enzyme-linked immunosorbent assay (ELISA) for detection of S1 protein in the sample. Also these antibodies were used in immunodot blot as a preliminary study before transition to membrane-based rapid assays. For this purpose, antibodies were first labeled with biotin or (Horseradish Peroxidase) HRP and the success of the procedure was demonstrated by direct ELISA and western blot. After optimization of the sandwich ELISA system, one of the mAbs was selected as the capture antibody and S1 pAb as the detection antibody. The results showed that this antibody pair will be able to used to detect the S1 protein of the virus at very low concentration and design the rapid diagnostic systems. On the other hand, the studies to evaluate the potential virus neutralization effect of developed mAbs on (Receptor Binding Domain) RBD - (Angiotensin Converting Enzyme-2) ACE2 binding are still ongoing.

Keywords: Visualizing immune responses, antibody, immunological techniques, infectious disease, viral infections

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POSTER PRESENTATIONS

P-0971

Determination of intracellular inhibitor candidates to abrogate pronounced interferon production profile in type I interferonopathies

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Intracellular pathogen invasion actives nucleic acid sensing mechanism, which in turn initiates production of specific subset of cytokines and chemokines. Among those, type I interferons are vital cytokines for recruitment of immune cells to limit, and clearance of an infection. The elevated type I interferon signature resolves once infection is cleared. The term "type I interferonopathies" was recently coined to describe a category of disorders characterized by perpetual type I IFN-mediated sterile inflammation. Here, we investigated the impact of dysregulated signalling in two interferonopathies, STING-associated vasculopathy with Onset in Infancy (SAVI) and Aicardi-Goutières syndrome (AGS) using *in vitro* cell line models. Type I IFN secretion from cold-stress induced vs uninduced STING M155V or N154S expressing cells was specifically analysed to determine the mechanism behind the cold-induced aggravation of inflammation seen in SAVI patients. Correspondingly, TREX1 knockout THP cells were investigated as an Aicardi-Goutières *in vitro* model. In both models, the suppressive efficacy of different inhibitors against type I IFN was tested. Our results showed that cold-induced type I IFN aggravation in the SAVI *in vitro* model triggered by upregulation of STING expression and subsequent STING phosphorylation. Experiments with particular inhibitors reveal that chronic type I IFN generation in the AGS model is more dependent on the STING/TBK1/IKK pathway than the autocrine/paracrine type I IFN-mediated JAK/STAT system. Furthermore, in this model, Amlexanox was the most potent inhibitor, capable of lowering type I IFN signature by 80%.

Keywords: Inflammatory disease, innate immunity, molecular immunology

P-0972

Genomic investigation into early onset protein-losing enteropathies: therapeutic implications and insights into clusters of shared pathomechanisms

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Protein-losing enteropathy (PLE) is a group of disorders characterized by pathological protein wasting into the gut; the pathomechanisms and treatment approaches are still unestablished. We carried a genomic approach to presumed PLEs (hypoalbuminemia and diarrhea) with early onset, hoping to identify distinct themes of presentations and discover putative shared pathomechanisms. We performed genome-sequencing on 46 PLE patients and evaluated the correlation between candidate gene variations with the clinical characteristics; and translated the findings into the management of patients whenever possible. The identified causative genetic mutations implicated diverse biological processes underlying PLE: i. innate and adaptive immune system: CD55(n=2); XIAP(n=1), and TTC37(n=1), ii. lipid metabolism: DGAT1(n=3), iii. protein glycosylation: MPI(n=1), DPAGT1(n=1), ALG6(n=1), and PGM3(n=1), iv. albumin production: analbuminemia(n=5), and v. lymphatic development: CCBE1(n=3). Interestingly, hypoalbuminemia was accompanied by hemostatic dysfunction and/or complement abnormalities besides immune deficiencies in multiple cases. The genetic diagnosis led to disease-specific therapies, or substantial therapeutic modifications: complement inhibitor, eculizumab, for CHAPLE; mannose supplementation for MPI deficiency; and bone marrow transplantation for the PGM3 and XIAP defects. We identified a common predisposition to subtle immunological, hematological or metabolic changes in youth with or without overt clinical symptoms, posing potential detrimental consequences. The overlap of presentations ties hypoalbuminemia with aberrant regulation of complement and hemostatic systems, and lipid- and protein- metabolism. The study of monogenic PLEs pointed to distinct clusters of molecular defects spanning across a broad range of biological pathways, shedding light into future PLE research.

Keywords: Complement, immunodeficiency, innate immunity, metabolic control of immune responses

P-0973

An unusual 3'UTR STAT3 mutation identified in a Hyper-IgE patient diagnosed with short stature and puberty delay

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Autosomal dominant hyper-IgE syndrome (AD-HIES) is a rare multisystem immunodeficiency typically caused by dominant negative mutations in Signal Transducer and Activator of Transcription 3 gene (STAT3). HIES patients are characterized by the triad of high serum IgE, eczema and recurrent skin and pulmonary infections. They may also present with non-immunological manifestations, such as connective tissue and bone abnormalities. We report a 17-year-old girl with a history of recurrent lung infection leading to bronchiectasis. She has also developed vaginal fungal infection, chronic onychomycosis and oral candidiasis. Her physical examination revealed short stature, puberty delay and retained primary teeth. Laboratory findings showed elevated IgE and eosinophilia. She had a score of 53 points according to the NIH clinical HIES scoring system. STAT3 phosphorylation was normal. However, we found reduced percentage of TH17 cells as assessed by flow cytometry. No STAT3 mutations were found in coding exons. Interestingly, we identified a rare mutation located in the 3'UTR region (c.*351delG). To assess the functional effect of this mutation, we evaluate the induction of SOCS3 by quantitative PCR, a STAT3 target gene, and found very high level of expression as compared to healthy controls. This result suggests that it could be a STAT3-GOF mutation. The absence of autoimmune disorders is intriguing since almost all reported STAT3-GOF mutations are associated with such manifestations. Regarding the presence of short stature and puberty delay, we plan to investigate the hypothesis that inhibition of STAT5 due to excess of SOCS3 subsequent to STAT3 GOF is responsible of GH defect.

Keywords: Cell signalling, immunodeficiency, molecular immunology

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POSTER PRESENTATIONS

P-0974

Subtyping for B cell- and antibody secreting cell-subsets in human melanoma reveals age-, metastasis- and tumor stage-associated changes

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The role of tumor-associated B cells in human cancer is only starting to emerge. B cells typically undergo a series of developmental changes in phenotype and function, however, data on the composition of the B cell population in human melanoma are largely absent including changes during tumor progression and their potential clinical significance. In this study, we subtyped B cells in the whole tumor sections of 154 human cutaneous primary or metastatic melanoma samples by seven color multiplex immunohistochemistry and revealed an enrichment of plasmablast-like, memory-like, and activated B cell subtypes in primary melanomas, while baring of the plasma cell-like cells, and not at all of the germinal center- and transitional/regulatory-like B cells. These B cell subpopulations showed a patchy distribution in the paratumoral and sometimes intratumoral stroma. Of the major clinicopathological prognostic factors for primary melanomas, metastasis was associated with decreased memory-like B cell numbers, whilst, a higher age and Breslow depth associated with high plasmablast-like cell numbers. Comparing the primary and metastatic melanomas, we observed a significantly higher proportion of plasma cell-like cells at distant metastatic sites, whereas, memory-like B cells majorly accumulated at locoregional sites. These data provide a first comprehensive and comparative morphological analysis of major B cell and antibody-secreting cell subpopulations in human melanoma and describe age-, metastasis- and tumor stage-associated changes, an important premise for B cell-related biomarker and therapy studies.

Keywords: Autoinflammation, B lymphocytes, cancer immunology, microenvironment

P-0975

Single cell sequencing of peripheral mononuclear cells reveals CHAPLE-specific distinct signaling pathways

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CHAPLE is a novel complement disease caused by genetic loss of CD55. However, limited data are available on the hallmarks of the disease. Here, we utilized high-throughput single cell RNA-sequencing (scRNA-seq) technology to assess the longitudinal transcriptional profile associated with CHAPLE disease with single-cell resolution. Peripheral-blood-mononuclear-cells (PBMCs) from two CHAPLE patients and three age-related healthy-controls (HC) were isolated, and frozen PBMCs were used for scRNA-seq. Clustering followed by visualization using uniform manifold approximation and projection (UMAP) for dimension reduction depicted 15 cell clusters, and immune cells such as T-cells, B-cells, monocytes and natural-killer (NK) cells were identified based on the expression of classic cell-type markers. By quantification of the cell type composition, we observed increase in B-cells (both naive and memory B), and decrease in memory CD4+ T-cells, CD8+ T-cells (both naive, central and effector memory) and NK-cells in CHAPLE patients versus HC. We detected 15 genes including *HLA-DPB1*, *HLA-DRB5*, *PELI1* to be up-regulated in CHAPLE versus HC. Further analysis revealed that "antigen processing and presentation", "inflammatory bowel disease" and "ras" signaling pathways activated in CHAPLE patients. When comparing HC, 18 genes including *CCL3*, *CCL4*, *HCST*, *PRF1*, *ACTB* were significantly down-regulated in CHAPLE patients. Other signaling pathways, such as "cytokine-mediated signaling pathway", "Toll-like receptor signaling pathway", "NK cell mediated cytotoxicity", "apoptosis" etc. in CHAPLE were down-regulated. Our results revealed CHAPLE-specific alteration of PBMCs and associated pathways. These results give a panoramic of PBMCs in composition, genes profile and pathway signatures that are driven by CHAPLE disease.

Keywords: B lymphocytes, complement, immunodeficiency, NK cells, RNAseq

P-0976

Development of monoclonal antibodies against SARS CoV-2 spike protein antigens to use in diagnosis and therapy

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Monoclonal antibodies (mAbs) are used clinically in the diagnosis and therapy of many diseases. There is a need for the use of high-affinity antibodies specific to SARS-CoV-2 in the rapid and accurate diagnosis of the global COVID-19 outbreak. In this study, mouse mAbs against the receptor-binding domain (RBD) and S1 subunit of the spike protein which are structural proteins of SARS-CoV-2, were developed using hybridoma technology. BALB/c mice were immunized with the recombinant S1 and RBD proteins. Spleen cells of the immunized mice which have high antibody response were fused with mouse myeloma cells (F0; ATTC CRL-1646) in the presence of polyethylene glycol to obtain hybrid cells. Selection and subcloning processes were performed for the hybridoma cells which have high-level antibody response, after that, the specificity of the developed mAbs to SARS-CoV-2 proteins was demonstrated by enzyme-linked immunosorbent assay (ELISA) and western blotting methods. Therewithal, the isotype of the developed antibodies was determined as IgG1 and the productivity of purification processes was compared by using Protein A and Protein G columns. As a conclusion 2 mAbs were obtained, one of them was only specific to S1 protein, but the other one could detect RBD protein at the same level as S1 protein even was immunized with S1 protein. Thanks to its high response to RBD, a neutralizing antibody candidate was obtained for potential drug development. At the same time, purified mAbs began to be used in the development of antibody-based detection systems for SARS-CoV-2 diagnosis.

Keywords: Engineering of antibodies and nanobodies, antibody, immunological techniques, viral infections, visualizing immune responses

P-0977

WHIM syndrome: a male patient and his father

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WHIM syndrome is a rare combined primary immunodeficiency disease named by acronym for the diagnostic tetrad of Warts, Hypogammaglobulinemia, Infections and Myelokathexis. WHIM syndrome is usually caused by autosomal dominant mutations in the G protein-coupled chemokine receptor CXCR4. We present a 4-year-old male patient diagnosed with WHIM syndrome and his father. Neutropenia was detected for the first time at the age of 4 months. No mutations were found in ELANE and HAX-1 genes that cause congenital neutropenia. When he was 2 years old, he was hospitalized for septic arthritis, hypogammaglobulinemia and neutropenia in the right knee. He refer to our pediatric allergy and immunology department when he was 3 years old. IVIG and antibacterial prophylaxis was initiated. A next generation sequencing was made for immunodeficiency. The genetic analysis revealed heterozygous mutation in CXCR4 gene, NM_ 003467.3: c.1000C>T (p. Arg334). The same mutation was detected in his father. The father had a history of neutropenia and was treated for bronchiectasis due to recurrent lung infection. The father was also initiated on IVIG and antibacterial prophylaxis treatment. WHIM syndrome should be considered especially in patients with neutropenia and hypogammaglobulinemia who also have a family history.

Keywords: Antibody, immunodeficiency, neutrophils

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POSTER PRESENTATIONS

P-0979

Preliminary results from peripheral blood immunophenotyping after SARS-COV2 mRNA vaccination

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The role of cellular immune responses in the development of immunity after vaccination against SARS-COV2 is under investigation since these vaccines have been authorized for use. In this ongoing study we evaluate the cellular response after vaccination with Pfizer/Biontech mRNA COVID-19 vaccine in Saint-Savvas Hospital healthcare workers. Peripheral blood samples were obtained from 29 healthcare workers who received the BNT162b2 Pfizer-BioNTech COVID-19 mRNA Vaccine, 7 of which were recruited following recovery from Covid-19 disease. CD3+, CD3+CD4+, CD3+CD8+, CD3-CD (16/56) +, and CD19+ cell populations were analysed by flow cytometry. Samples were collected previous to vaccination, before the second dose, 3 weeks and 2 months after the second dose. No statistically significant difference was observed in CD3+, CD3+CD4+, CD3+CD8+, CD3-CD (16/56) +, and CD19+ cell populations before first dose and 20 days after the first dose. Comparing the convalescent with the naïve group no significant change was observed in the levels of cell populations. The CD4/CD8 ratio 20 days after the second dose was decreased ($p<0.05$) in the naïve group compared to the ratio before vaccination. This was not observed in the convalescent group. Further investigation is necessary to reveal the role of cell immunity after SARS-COV2 vaccination.

Keywords: B lymphocytes, immune response tracing, infectious disease, NK cells, viral infections

P-0980

Relationship between antibodies to extractable nuclear antigens and clinical manifestations in systemic lupus erythematosus patients: results from a Gazi Univesity SLE cohort

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The aim was to investigate the role between antibodies to extractable nuclear antigens (ENA) and clinical manifestations in a cohort of SLE patients. The study included 187 adult patients with a diagnosis of SLE who followed at the outpatient rheumatology clinic.Patients fulfilled the 2012 SLICC classification criteria for SLE.Full medical history and general examination laboratory investigations. Analyses of the autoantibodies (ANAs, anti-dsDNA, anti-Ro/SSA, anti-La/SSB, anti-Sm and anti-RNP,) in the sera of the patients at diagnosis were also undertaken by the routine clinical immunology laboratory at the University Hospital. The median age was 38 years, and 187 (94.1%) patients were women.Median disease duration was 120 months. Median levels of serum anti-dsDNA, complement 3 and 4 were found 211,7 IU/ µL,78 mg /dl and 12,3 mg/dl respectively.The most frequent clinical manifestation of these patients was musculoskeletal disorders (60,4 %) the others were mucocutanous disorders(57,8%), nephritis (37,4%), haematological (18,7%),neurological disorders (12,3%). Anti-Sm, anti-RNP, anti-Ro and anti-La positivity were in 15%, 24%, 26,7% and 11,8% of SLE cases, respectively.Multivariable analysis revealed that discoid rash was independent predictors of anti-Sm positivity; Raynaud phenomenon and oral ulcer were independent predictors of anti-RNP positivity; alopecia, lupus pneumonia and pericarditis were independent predictors of anti-Ro positivity and pleuritis was independent predictors of anti-La positivity. Antibodies to ENAs are associated with clinical manifestations of in patients with SLE and may play a part in the assessment of disease activity. ENAs may lead to new and improved diagnostic test, exact evaluation of disease activity and lead to target approach for SLE.

Keywords: Antibody, autoimmunity, inflammatory disease

P-0982

Evaluation of in ovo antiviral activities of different plant extracts and bioactive molecules with immunomodulatory properties

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Viruses have increasingly caused severe economic losses, diseases, deaths and pandemics. The Covid-19 pandemic has once again revealed the importance of studies on potential antiviral agents. Therapeutic agent potentials of natural plant sources against different viruses have been evaluated *in vitro* and *in vivo* in previous studies. Plant extracts and bioactive molecules can stimulate the immune system against viruses by regulating the differentiation of immune cells, activating inflammatory signaling-pathways, or exerting immunomodulatory effects. This study aimed to investigate different plant extracts (*Camellia sinensis*, *Sambucus nigra*, *Echinacea purpurea*, *Melissa officinalis*, *Hypericum perforatum*, *Abies spp.*, *Laurus nobilis*), saponins and bioactive molecules (Oleuropein, Neohesperidin, Nobiletin, Gallic acid, Tangeretin, Catechin Hydrate) for virucidal antiviral activity against infectious bronchitis virus (IBV) by *in ovo*. Extracts/molecules incubated with IBV for 1-hour were inoculated into specific pathogen-free embryonated chicken eggs (SPF-ECE). After 48-hour incubation, allantoic fluid was collected for hemagglutination assay to detect IBV-titer. Cytotoxic activities of these samples were measured on different cell lines (MDA-MB-231, A549, PANC-1, PC-3, CCD-34Lu, HEK293, HepG2, HeLa, Caco-2) by MTT method. As a result, *H.perforatum* and *S.nigra* exhibited the most effective antiviral activity with 0.1 µg/g concentration by decreasing 5 and 3 HA $\log_2$ titers. Also, oleuropein molecule at 10 µg/g decreased HA as 5 HA $\log_2$ titers. Substances except for *H.perforatum*, *L. nobilis* and gallic acid did not showed significant cytotoxic activity against all cells. Results showed that these plant extracts and molecules having antiviral activity could be used as potential candidates and further mechanistic and *in vivo* studies are required.

Keywords: Infectious disease, modelling, viral infections

P-0983

Analyzing the effect of mannose-binding lectin on the prognosis of the SARS-CoV-2 infection

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Mannose-binding lectin (MBL) is an important player within innate immunity. MBL acts as a pattern-recognition receptor, interacting with different microorganisms. Its deficiency within the body is correlated with increased susceptibility to infections. The complement system has a role in the neutralization of infiltrating immune complexes from our body. It is aimed to measure the correlation between serum MBL levels and the severity of SARS-CoV-2 infection. To investigate the role of MBL in COVID-19, 96 healthy individuals and 96 COVID-19 patients divided into three groups as mildly ill, severely ill, and fatally infected will be used. Serum MBL levels will be measured with ELISA kits. T-test and ANOVA test will be used to analyze effect of MBL on healthy group-patients and severity of the infection, respectively. This study is an ongoing research and planned to be finalized in the August 2021. A negative correlation between serum MBL levels and the severity of COVID-19 infection is expected to be found. Previous research about MBL shows this negative correlation between SARS-CoV-1 and serum MBL level. It is anticipated that the results of this study will shed light on new treatment approaches that can be developed against SARS-CoV-2 and other viral infections.

Keywords: Immune networks, innate host defence, complement, innate immunity, viral infections

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POSTER PRESENTATIONS

P-0984

Dissecting immunomodulatory effects of liposomal methotrexate for treatment of rheumatoid arthritis

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Rheumatoid arthritis (RA) is a chronic inflammatory disease driven by activation of synovial macrophages that are known to express folate receptor (FRβ). Methotrexate (MTX) is a first treatment option and an anchor drug for RA. However, due to toxicity, significant proportion of RA patients is intolerant to MTX. In order to reduce side effects and improve MTX delivery to affected joints, we encapsulated MTX in liposomes functionalized with folate. We then compared an uptake of liposomal vs free MTX in human monocytic THP-1 cells and monocyte-derived macrophages with or without FRβ, as well as in primary human T cells. Consequences of the uptake were characterized using cytokine and activation marker profiling, cytotoxicity and proliferation assays. While free MTX exhibited considerable cytotoxicity in both FRβ$^+$ and FRβ$^-$ THP-1 cells, MTX-liposomes were predominantly taken up by FRβ$^+$ THP-1 cells, and this uptake and cytotoxicity were effectively mitigated by free folate. Neither MTX formulation induced cytotoxicity in monocyte-derived macrophages, but both modulated cytokine production. Similarly, both formulations inhibited activation of CD3+CD28 mAb-stimulated T cells, although different concentrations were required to achieve the effect. Finally, in a collagen-induced arthritis mouse model, MTX-liposomes alleviated inflammation to a higher degree than free MTX. In conclusion, our results indicate that MTX-liposomes effectively target FRβ$^+$ macrophages and could represent a promising treatment option for RA.

Supported by the European Union's Horizon 2020 research and innovation programme under grant agreement No 683356 (FOLSMART) and by the FP7 programme under grant agreement NMP4-LA-2009-228827-NANOFOL.

Keywords: Drugs for immune modulation, macrophage, rheumatoid arthritis

P-0985

Activation-Induced Cytidine Deaminase (AID) and Uracil N-glycosylase (UNG) novel homozygous gene variants in adult hyper-IgM syndrome

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Hyper IgM(HIGM) syndrome comprises a rare and heterogeneous group of primary immunodeficiency disorders presenting with recurrent infections and is characterized by impaired B and T-cell functions.In this study we report two novel variations in autosomal recessively inherited AID and UNG genes leading to HIGM phenotype in two adult Turkish patients. Two patients from consanguineous families were diagnosed with HIGM at the Haematology Outpatient Clinics of Istanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty based on IUIS criteria. Genomic DNA was isolated from the peripheral blood mononuclear cells by using QIAmp DNA Blood Mini Kit.A targeted next generation sequencing panel with known 22 genes (BTK, IGHM, IGLL1, CD79α, CD79β, BLNK, PIK3R1, TCF3, ICOS, CD19, CD81, MS4A1, CD21, TNFRSF13B, LRBA, TNFRSF13C, TWEAK, NFKB2, CD40LG, CD40, AID, UNG, TRNT1, TTC37) in primary immunodeficiency diseases were designed by SmartChip-TE technology. In this study, two novel variations associated with autosomal recessive(AR) type HIGM syndrome were reported for the first time.In Patient 1(P1#),a novel 11 bp deletion(c.278_288delATGTGGCCGAC) has been found in AID gene's coding sequence (Ref. ENST00000229335.6). In Patient2 (P2), two base pair insertion was identified in exon 7 of UNG gene (ENST00000242576.2) which cause a novel frameshift mutation(c.924_925insGG). Two novel variations associated with AR-HIGM syndrome.A novel 11 frameshift variant in AID gene was identified in a 34-years-old (P1#)male patient. Biallelic two base pair insertion in UNG gene was shown in HIGM patient for the first time in this study.

Keywords: Antibody, immunodeficiency, molecular immunology

P-0986

Evaluation of some blood parameters and percentage of CD4+ or CD8+ T cells from spleen and liver from the experimental autoimmune encephalomyelitis mouse model

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The experimental autoimmune encephalomyelitis (EAE) model in C57BL/6 mice is the most common animal model for Multiple sclerosis (MS) sharing many clinical and pathophysiological features. In this study, we focused the effect of EAE on hematologic, plasma total protein, albumin levels and CD4+ or CD8+ T cells in C57BL/6 mice. The hemogram and plasma total protein(TP) and albumin(ALB) levels were immediately measured by using Abbott CELL-DYN Ruby Hematology System and Abbott ARCHITECT c16000 Clinical Chemistry System (Illinois, USA) respectively, from the blood samples. In the hemogram, the red blood cell count (RBC,x106/mm3),the packed cell volume (PCV, %),the hemoglobin level(Hb,g/dl), the mean corpuscular volume(MCV,µm3),the mean corpuscular hemoglobin(MCH,pg), the mean corpuscular hemoglobin concentration(MCHC, g/dl), the red blood cell distribution width(RDW,%), the white blood cell count(WBC, x103/mm3),and the neutrophil-lymphocyte ratio(NLR,%) were measured. Percentage of CD4+ or CD8+ T cells were analyzed with flow cytometry in both spleen and liver. Our findings show that the EAE model in mice might not cause any significant change hematologically, except a slight increase in the white blood cell count, and might produce changes in the plasma protein level. The EAE-induced blood parameter effects, as the findings of the current study, could take consideration in terms of understanding the pathophysiology of the disease and developing a novel therapeutic approach for the disease.

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Keywords: Animal models, lymphoid organs, multiple sclerosis

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POSTER PRESENTATIONS

P-0987

Evaluation of monocytic/granulocytic cells from spleen and liver in the experimental autoimmune encephalomyelitis mouse model

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Multiple sclerosis(MS) is a chronic neuroinflammatory demyelinating disorder of the central nervous system with unclear exact etiology. The experimental autoimmune encephalomyelitis (EAE) model in C57BL/6 mice is the most common animal model for MS sharing many clinical and pathophysiological features to expand our knowledge on the pathophysiology of the disease and to develop novel treatment strategies. The soluble factors secreted during autoimmunity increase myelopoiesis; as a result, newly produced immature myeloid cells enter circulation and sometimes accumulate both spleen or liver. These cells regarded as myeloid-derived supressor cells (MDSC) that control over immune responses. Female C57BL/6 mice (8 weeks old, 16–20 g) were purchased from Animal House in the Department of Molecular Biology and Genetics at Bilkent University. The mice were housed as 4 mice per cage for one-week acclimation and quarantine period before starting the experiments in a room with a proper care environment for mice(20–220C;60–70% humidity; 12 h light/dark cycle). The experiment was terminated on the day6,12 and 18 after the first injection. Mycobacterium tuberculosis were injected to animals two different conditions (4mg/mL; 6mg/mL). Cell suspensions from the spleen and liver were obtained with mechanical agitation in phosphate buffered saline (1xPBS), and passed through 40 µm pore-sized filters. The leukocytes in the cell suspensions were separated by Ficoll-1.077g/mL density gradient centrifugation. The cells were labeled with monoclonal antibodies against CD11b, CD80, CD86, Ly6G and Ly6C. The immunophenotyping analyses were performed on flow cytometer. EAE model in mice might induce MDSC expansion but MS symptoms do not disappear as this effect regresses by D18.

Keywords: Inflammatory disease, multiple sclerosis, myeloid cells

P-0988

Intrathecal IgM synthesis as a biomarker of disease activity in algerian multiple sclerosis patients

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Intrathecal IgM synthesis appears to be associated with a poor prognosis in multiple sclerosis (MS). It identifies patients with unfavorable outcome, who are candidates at ontset for a more aggressive treatment. The aim of the present study was to determine the interest of the detection of an intrathecally synthesized IgM in multiple sclerosis (MS). 50 patients with MS (74% women, mean age 29,8± 9,4 years) presenting an intrathecally synthesized IgM (MS/IgM+), besides of 23 control patients (91% women, mean age 30,7± 11,5 years) not presenting an intrathecally synthesized IgM (MS/IgM-) were included in the study. IgM indexes higher than 20% were considered “increased”. IgM was measured in serum and in cerebrospinal fluid (CSF) by turbidimetry (SPAplus Binding site). Comparaison between MS/IgM+ patients and MS/IgM- patients showed a higher frequency (P=0,03) of relapses among MS/IgM+ patients (2,13 relapses/year) than MS/IgM- patients (1,57 relapses/year). Moreover, in our study, increased IgM index was associated with the presence of active lesions on brain and/or spinal cord MRI scan (P=0,048) with a frequency of (82%) in MS/IgM+ patients vs (53%) MS/IgM- patients. However, no significant difference was obsereved concerning age at onset, male predominance, type of clinical presentation at onset, a shorter interval between the first and the second relapse, EDSS score after treatment with steroids and the presence of spinal cord and infratentorial lesions. Intrathecal IgM production could be a biological marker of a more important inflammatory disease activity in MS.

Keywords: Antibody, biomarkers, multiple sclerosis, neuroimmunology

P-0989

The impact of the Rhesus factor on the immune response to a live virus in males and females using data from the Milieu Interieur cohort

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This study aims to investigate whether the Rhesus factor plays a role in immune phenotypes using data from healthy individuals from the Milieu Interieur cohort. As part of a comprehensive analysis of the variability on human immune responses, whole blood from 1000 healthy volunteers (500 males, 500 females) had been stimulated with a panel of ligands (Poly I:C,LPS) and a live virus (Influenza A) The expression of 560 immune genes was quantified using NanoString transcriptomics. Samples were genotyped for the Rhesus SNP (rs590787) using HumanOmniExpress and HumanExomeBeadChips. Rhesus positivity was determined based on the presence/absence of the SNP. Three phenotypes were determined, Rh-(Wild type), Rh+ (Heterozygous for SNP), and Rh+ (Homozygous for SNP). Data was analysed using R. The response to Poly I:C and LPS was similar in Rh + and Rh- males and females after FDR adjustment. 61 genes were differentially expressed among the Rh+ and Rh- males, when stimulated with Influenza A virus. In contrast Rhesus positive and negative females responded similarly to the influenza virus. Pathway analysis of these differentially expressed genes, identified the interferon gamma response. These findings may help to explain the higher protective response to infection of a viral origin, seen in Rhesus negative individuals.

Keywords: Innate host defence, innate immunity, viral infections

P-0990

HexaPro spike decorated virus like particle vaccine aganist SARS-Cov-2

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The global spread of SARS-Cov-2 necessitates the development of effective vaccines able to induce neutralizing antibodies and cellular immunity. Virus-like particle(VLP) vaccine formulations have been previously proven to be safe, effective and affordable. Therefore, we have developed a virus-like particle (VLP) vaccine platform, comprised of four structural proteins of SARS-CoV-2: spike, membrane glycoprotein, envelope, and nucleocapsid. For VLP production, we have cloned spike-envelope protein and membrane glycoprotein-nucleocapsid protein coding sequences in two different mammalian dual expression vectors. After transient transfection of suspension HEK cells, VLPs are collected from the culture supernatant, further clarified and concentrated. Antibody production against the spike protein is considered the most crucial step of the immune response against SARS-CoV-2 infection, as they are responsible of virus neutralization. As viral entry takes place during the prefusion configuration of the spike protein, its stability is essential for an effective immune response. In this context, we generated three forms of the spike protein; wild-type, 2-p, and HexaPro prefusion stabilized. Total IgG/IgG2a and IgG1 titers in BALBc mice following two doses of VLP vaccine adjuvanted with K3-ODN and alhydrogel demonstrated HexaPro spike protein decorated VLPs result in more superior immune responses compared to wild-type and 2-p. Moreover, our results show that HexaPro arrayed VLPs induced high neutralizing antibody titers in rats. Furthermore, challenge studies with Ace2 transgenic mice and ferrets demonstrated decreasing viral load in a dose dependent manner. Collectively, our results suggest that HexaPro-spike decorated VLPs are highly immunogenic and induce potent immune responses in mice and ferret models.

Keywords: Adaptive immunity, adjuvants and vaccines, antibody, viral infections

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POSTER PRESENTATIONS

P-0991

Evaluation of efficacy of CoronaVac vaccination in healthcare workers

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The COVID-19 pandemic is a huge public health problem over the world.In Turkey, from January 2021 administration of Coronovac vaccine to healthcare workers has started.In this study, we aimed to investigate the efficacy of CoronaVac vaccination and evaluate the preliminary data toward antibody responses. 849 healthcare workers were included in this study.COVID-19(+) status, COVID-19 vaccination status and COVID-19 vaccine-antibody response are evaluated. Of the 839 healthcare workers 630 of them (%75,1) were vaccinated. The ratio of participants who did not accept the vaccination was %20,74(n=174). 223(%26,6) healthcare workers were COVID-19(+) at any time. After vaccination period we have observed 61 new COVID-19(+) cases in 4 months. When the COVID-19 positivity is evaluated between vaccinated and unvaccinated groups, we have found a significant difference (p=0,000; 5,5% vs 14% respectively).SARS-Cov2 Spike antibody levels of 132 vaccinated healthcare workers were obtained.durin 2-4 month interval after vaccination.It is observed that 98.5% of them had antibody level above>50AU/ml; %62,9 of them had above 500 AU/mL and %39,4 above 1000 AU/ml. According to our findings, health care workers who were vaccinated with CoronaVac vaccine were less likely to get the infection. Antibody response was observed in 98.5% of the vaccinated population. Our data should be supported by a larger sample group and longer follow-up period.

Keywords: Adjuvants and vaccines, antibody, viral infections

P-0993

Anserin reduces MG-induced mortality in experimental Sepsis restoring endothelial integrity

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The current reactive carbonyl metabolite methylglyoxal (MG) has been associated to human early sepsis. When compared to proven inflammation markers, it did not only identify septic patients earlier, but was also able to depict a more precise survival prognosis. However, it is still not known whether MG has a causal influence on the course of sepsis. A loss of the endothelial barrier is a decisive factor contributing to serious disturbances of microcirculation, ultimately leading to septic shock. In mouse experimental sepsis (CLP), MG levels were increased and additional MG-derived carbonyl stress impaired survival of mice following Cecal Ligation Puncture (CLP). In Trans endothelial electrical resistance *in vitro* experiments, we could prove, that MG Methylglyoxal dose-dependently impairs endothelial barrier integrity by decreasing trans-endothelial resistance, increasing paracellular Dextran transport (4, 10, 70 kDa) in the same manner. We also found a distinct loss of the two key tight junction proteins ZO-1 and Claudine5 following MG exposure. The dipeptide anserine (β-alanyl-N-methylhistidine) was not only able to prevent the MG-induced resistance drop in a pre and co treatment setting, but could also reverse the MG-effect in a post treatment. *In vivo* application of anserine in a mouse experimental sepsis model (CLP) decreased mortality from approximately 65% to 40%. Finally, we investigated the serum carnosinase (anserine catalyzing enzyme), and found a decreased activity in septic patients making anserine a promising therapeutic option in human sepsis.

Keywords: Bacterial infections, immune regulation and therapy, infectious disease, inflammatory disease, inflammatory molecules, tissue damage and repair

P-0994

Evaluation of the immunomodulatory activity of murine colon carcinoma-derived exosomes - fresh vs frozen preparations

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Exosomes are circular vesicles of 30-150nm in diameter responsible for intracellular communication. Exosomes released by tumor cells (TEx) are a rich source of tumor antigens, genetic material and immunomodulatory molecules. TEx are defined as vesicles promoting tumor progression. Due to specific tumor antigen content, high stability in circulation, and biocompatibility, TEx are also considered as a tool for cancer therapy. This study aimed to answer whether the cryopreservation of MC38 murine colon carcinoma-derived exosomes affects their characteristics and immunomodulatory activity. Exosomes were isolated by size-exclusion chromatography (SEC) from supernatants harvested from MC38 cell culture maintained at hypoxia conditions. After isolation, exosome fractions were divided into two parts – the first was frozen at -80oC for 8 weeks, and the second was evaluated after isolation. To characterize the exosome preparations, the number, size, and protein concentration were determined. Then TEx were used as an antigen source for bone marrow-derived-dendritic cell (BMDC) stimulation. The phenotypic changes of BMDC under stimulation with TEx and their ability to primary activation of immune response were evaluated. Cryopreservation did not significantly affect the quality of obtained fractions of exosomes. Moreover, BMDCs stimulated with frozen fractions showed a high expression of costimulatory molecules and were more efficient in activating primary immune response than unstimulated control. On the other hand, differences observed between unstimulated BMDC and BMDC stimulated with fresh fractions were inconsiderable. In sum, cryopreserved TEx may be considered as a potential source of tumor antigens.

This study was funded by National Science Centre, Poland(project no 2018/30/E/NZ5/00711).

Keywords: Dendritic cells, endo- and exocytic vesicles in immunity, cancer immunology

P-0995

Rare pan-betacoronavirus monoclonal antibodies targeting stem-helix exhibit broad neutralizing capacity

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The emergence of novel SARS-CoV-2 variants highlights the need for the identification of conserved epitopes that can guide the design of future vaccines eliciting broad protection. Using a high-throughput method to screen the memory B cell repertoire of 42 convalescent and vaccinated individuals, we found that the majority of SARS-CoV-2-specific memory B cells are monospecific, and only few exhibit cross-reactivity to other coronaviruses. We isolated three clonally related monoclonal antibodies (mAbs) that cross-react with betacoronaviruses of the sarbeco-, merbeco- and embeco- subgenera from a SARS-CoV-2 convalescent donor. These mAbs bind to the stem-helix within the S2 subunit and are able to neutralize multiple human betacoronaviruses by targeting the fusion machinery. Reversion of mutations back to germline revealed that these antibodies were likely primed by HKU1 infection and acquired breadth through somatic hypermutations. Altogether, these data indicate that while the recall of pre-existing immunity comprises only a small subset of the whole antibody response, some of these antibodies, once recalled, can offer protective functions during an ongoing SARS-CoV-2 infection.

Keywords: Antibody, infectious disease, viral infections

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POSTER PRESENTATIONS

P-0996

Investigation of immunomodulation potential of dental pulp stem cells on infectious bronchitis virus infected lung alveolar epithelial cells

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Non-zoonotic chicken coronavirus (infectious bronchitis virus (IBV)) is responsible for a highly contagious acute viral respiratory disease in chickens. IBV is known to come from gamma coronavirus genus. Genome sequence analysis revealed that COVID-19 caused by SARS-CoV-2 belongs to the beta coronavirus genus. Whole genetic material and comparative genomic analysis also showed that IBV and SARS-CoV-2 specifically have the similar genomic structures and properties. Based on this information, alternative models using IBV can be developed instead of SARS-CoV-2 infection models in studies related to SARS-CoV-2 that require advanced laboratory safety levels. Recent mesenchymal stem cell (MSC) studies claimed MSCs are promising candidates for clinical treatment of inflammatory reactions as an immunological regulator during an acute viral infection. In this study, models including lung alveolar epithelial cell line (CRL-5807), THP-1-derived macrophage cells and dental pulp mesenchymal stem cells (DPMSCs) were used to create a model system for SARS-CoV-2. The lung tissue infection model created by IBV-infected CRL-5807 cells. THP-1 monocyte cells cultured with phorbol 12-myristate 13-acetate (PMA) to achieve differentiation into M0-THP-1 macrophages. IBV-infected CRL-5807 cells incubated for 24 hours were trypsinized and M0-THP-1 macrophage cells and DPMSCs added and triple co-culture incubated for an additional 24 hours. The IL-6, IL-1β, TGF-β and TNF-α cytokines level in the supernatants collected from tri-culture were measured by ELISA. As a result, a triple co-culture model system demonstrated that multicellular communication is important in investigating the viral microenvironment effect in regulating the *in vitro* immunomodulatory response in viral infection.

Keywords: Immune regulation and therapy, macrophage, stem cells, viral infections

P-0997

Cyclosporine a modulates antifungal responses in CD103+ DCs

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Cyclosporine A (CsA) is an immunosuppressant that protects against inflammatory diseases and graft rejection. Despite its strong efficacy, one side-effect of CsA is an increased risk of fungal infection. To minimise this, further research is required to determine the mechanisms by which CsA impacts on innate immune cell subsets that mediate antifungal immunity. Using both *in vivo* and *in vitro* models, these studies examined the impact of CsA on migratory CD103+ DCs and type I IFNs, both emerging mediators of antifungal immunity. During intranasal challenge with fungal Zymosan, a clinically available oral CsA formulation inhibited CD103+ DC trafficking to the lung-draining lymph nodes. In an *in vitro* CD103+ DC culture model, CsA inhibited Zymosan-induced migration and reduced the expression of the migratory molecule CCR7, as well as co-stimulatory molecules. Intriguingly, CsA also inhibited CD103+ DC secretion of type I IFNs and IFNAR signalling *in vitro*, which was essential for maturation and expression of CCR7, as well as migration to the lung-draining lymph nodes in the *in vivo* model. These data suggest that CsA inhibits type I IFN-dependent activation and migration of CD103+ DCs, providing further insight into how the drug increases susceptibility to fungal infection.

Keywords: Dendritic cells, drugs for immune modulation, fungal infections, innate immunity

P-0998

Kinetic of humoral response induced by SARS-CoV-2 infection in convalescent plasma receptors and donors

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During SARS-CoV-2 pandemic, use of convalescent plasma (CP) in high-risk patients was proposed as therapy. How CP impacted humoral response is still poorly understanding. AIM: Described kinetic of humoral response in COVID-19 hospitalized patients, receiving or not CP, and plasma donors. Subjects included, belongs to a previous RCT where they received or not convalescent plasma (CP) before 7 days of symptoms onset (hospitalized) and plasma donors (outpatients). Anti-SARS-CoV-2-S1 IgG was measured by ELISA (Euroimmune®) according to manufacture procedures. Samples were taken at day of enrolment, 3, 7, 14, 21 and 28 days, 3 and 6 months for hospitalized patients and the day of donation and after 3 and 6 months in plasma donors. 58 hospitalized patients, 32 received CP (median days since symptoms onset 5, range: 1-7days) and 15 did not, and 37 donors were included. Hospitalized patients were significantly older than donors (median age 62, 60 and 34 years respectively p<0,0001). Regarding humoral response of hospitalized patients, no differences were observed in term of seropositivity, seroconversion or IgG titer during the first month since symptoms onset between those who received or not CP. Anti-SARS-CoV-2 IgG titers were significantly lower at 3 and 6 months in donors compare to hospitalized patients receiving or not CP (GMT: 685, 1789 and 3005 at M3, p=<0,0001 and 182, 999 and 1600 at M6 respectively p=<0,0001). The humoral response was not affected by the infusion of CP. However, severity of illness seemed to impact the amplitude of humoral response.

Keywords: Antibody, biology of the immune system, infectious disease, monitoring immunity, viral infections

P-0999

Pentacyclic triterpenoid ursolic acid induced apoptosis with mitochondria dysfunction on adult T-cell leukemia cells

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In this study, we investigated the anti-tumor effects of oleanolic acid (OA) and ursolic acid (UA) on adult T-cell leukemia (ATL) cells. OA and UA inhibited cell proliferation of ATL cell lines in a dose-dependent manner. UA induced cell death of MT-4, which were rescued by caspase inhibitor Z-VAD-FMK. UA-treated cells had higher caspase 3/7 and caspase 9 activation than that of OA treated cells. PARP cleavage can be detected on UA-treated MT-4 cells. The activation of mTOR was inhibited by OA and UA. The expression of LC3-II can be detected on OA and UA treated MT-4 cells. Autophagosome can be observed in MT-4 cells after UA treatment by electron microscopy. The mitochondria membrane potential (MMP) in MT-4 cells was dramatically decreased by UA treatment. Furthermore, MT-1 and MT-4 cells were sorted depend on MMP to two regions. Both regions had high activation of caspase 3/7 which can be inhibited by Z-vad and high expression of cleavage PARP. UA treatment significantly reduced mitochondria respiration and aerobic glycolysis on MT-4 cells. Interestingly, after coculture with fresh MT-4 cells with sorted cells, MT-4 cells which coculture with UA-treated cells enhanced cell proliferation. These results suggest that UA-treated MT-4 cells occur caspase activation following mitochondria dysfunction and may produce some signals to surrounding cells for survival. UA induced cell death of PBMC and cleavage of PARP from ATL patient *ex vivo*. Taken together, UA induce mitochondria dysfunction on ATL cells, which will be useful in the development of clinical chemotherapy approaches.

Keywords: Cancer immunology, cell death, cell signalling

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POSTER PRESENTATIONS

P-1002

The possible cytotoxic function of natural killer cells of patients infected with SARS-CoV-2

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Natural Killer (NK) cells are critical components of the innate immune response against virus-infected cells. In this study, the possible cytotoxic role of NK cells in SARS-CoV-2 infection causing the ongoing COVID-19 pandemic was investigated. The patients diagnosed with COVID-19 (n=31; mild:11, moderate:11, severe:9) and healthy subjects (n=8) were enrolled in this study. CD56dimCD16+ and CD3-CD56brightCD16+/- NK cell subsets and their CD38 expression as well as perforin and Granzyme B levels were investigated by flow cytometry. Higher percentage of total NK cells and CD3-CD56dimCD16+ NK cell subset were detected in mild patients than those in other cases and healthy individuals. CD3-CD56dimCD16+CD38+ and CD3-CD56brightCD16+/-CD38+ NK cells were significantly increased in mild and moderate COVID-19 patients in comparison with healthy subjects. All forms of COVID-19 patients had significantly decreased in perforin expression in comparison with healthy subjects. In mild COVID-19 patients, Granzyme B expression of NK cells was significantly increased whereas CD107a expression was significantly decreased compared to healthy subjects. According to our findings, a general decrease in perforin expression of NK cells was observed in all patient groups while CD38 and Granzyme B expression levels were increased especially in mild COVID-19 patients. These findings confirm that CD38 is associated with NK cytotoxicity and further indicate that NK cytotoxic activity varies in the early and late phases of the disease.

Keywords: Innate immunity, NK cells, viral infections

P-1022

Transgenic overexpression of galectin-3 in pancreatic β cells attenuates type 1 diabetes in mice: synergistic antidiabetic effect with exogenous IL-33

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Galectin 3 (gal 3) has diverse roles in inflammatory and autoimmune diseases. There is evidence that galectin 3 plays a role in both, type 1 and type 2 diabetes. While the role of Gal-3 expression in immune cells in experimental type 1 diabetes has been already studied, the importance of the overexpression of Gal-3 in the target β cells is not defined. We used 10-12 weeks old C57/BL6 male mice (WT) and C57/BL6 mice with transgenically enhanced Gal-3 expression in pancreatic β cells (TG). Both groups, received STZ for 5 consecutive days at a dose of 40 mg/kg ip. Mice received exogenous mouse IL-33 (0.4 μg/injection) i.p., 12th, 14 th, 16 th, and 18 th day after the disease induction. Control animals were treated with intraperitoneally PBS + citrate buffer or IL-33 + citrate buffer. The overexpression of Gal-3 protected β cells from apoptosis and attenuated MLD-STZ induced hyperglycemia, glycosuria and ketonuria. The cellular analysis of pancreata and draining lymph nodes showed that Gal- 3 overexpression significantly decreased the number of proinflammatory cells without affecting T regulatory cells. The application of exogenous IL-33, attenuates the development of disease, by increasing the presence of regulatory FoxP3+ ST2+ cells, and competely abrogate diabetogenesis. We demonstrated the potential synergistic effect of exogenous IL-33 and TG overexpression of Gal-3 in β cells Not only enhanced expresion of Gal-3 in β cells reduced T cell mediated autoimmune inflammatory disease, but also exogenous IL-33 application had powerful terapeutic effect in TG mice.

Keywords: Animal models, autoimmunity, cytokines and mediators, diabetes, drugs for immune modulation

P-1110

Immunoregulatory properties of acridines derived from anti-malaria drug quinacrine

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FoxP3$^+$ regulatory T cells (Tregs) are a critical subset of CD4$^+$ T cells responsible for regulating immune homeostasis by suppressing excessive anti self-antigen immunity. The suppressive function of FoxP3$^+$ Treg cells also facilitates tumor cell proliferation due to inhibition of anti-tumor immunity. The key lineage-defining transcription factor FoxP3 in Tregs turns out to be a potential candidate target for anti-tumor treatment. Here, we identified the anti-malaria drug, quinacrine, as an inhibitor of FoxP3 expression in a cell-based high-throughput screening of a drug repurposing library using CD3$^+$ T cells from human healthy donors. Based on the structure of quinacrine, we established a small library containing quinacrine-like acridines by *in silico* prediction and functional verification. A subset of acridine analogues were found to have even more potent inhibitory effect on Treg suppression of effector T cells, by down-regulation of FoxP3 and critical Treg markers. This rescued proliferation and cytokine production of effector T cells. TCR signaling pathways analyzed by phospho-flow revealed that, these acridines could suppress FoxP3$^+$ Tregs more efficiently than other effector T cells. By SPR analysis, we found direct interactions between acridines and FoxP3, which could then interfere with FoxP3-DNA binding activity. The effect appeared selective for FoxP3 as other Forkhead proteins were not affected by these acridines, neither *in vitro* nor *in vivo*. Mice experiments further revealed anti-tumor properties of the several quinacrine-like acridines. We conclude that selected acridines could provide valuable toolsfor assessing Treg functions such as their role in tumor immune evasion.

Keywords: Cancer immunology, drugs for immune modulation, immune regulation and therapy, immunotherapy, regulatory cells

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POSTER PRESENTATIONS

P-1113

Hyper IgE syndrome phenotype associated with a new variant in CARD11: an update of the genetic spectrum

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CARD11 is a structural protein expressed mainly in T and B lymphocytes, which participates in the intracellular signaling of its main receptors. Germline mutations associated with different clinical phenotypes are described, such as: severe combined immunodeficiency, combined immunodeficiency and atopy; and B-cell lymphoproliferation. Likewise, since 2017, variants in CARD11 have been described in patients with the HyperIgE phenotype. 9-year-old male with egg allergy and atopic dermatitis. At 5 months of age, he suffered from acute otitis media due to *Pseudomonas aeruginosa*. A month later, he developed a gangrenous ecthyma caused by the same microorganism, complicated by sepsis. Due to this infectious susceptibility, the immunological study was expanded, ruling out chronic granulomatous disease, agammaglobulinemia, Toll-IL1R and combined immunodeficiencies. Low IgG was detected without specific antibody deficiency, normalizing levels at 2 years of age. At 4 years old, he was diagnosed with otomastoiditis and folliculitis due to *Staphylococcus*. At 6 years of age, as a result of a chronic functional impotence, he was diagnosed with chronic non-infectious osteomyelitis, requiring treatment with anti-TNFa. A persistent elevation of IgA and IgE was observed, with eosinophilia. Association between severe recurrent infections and autoinflammatory disease, led to extended genetic studies identifying an heterozygous variant *in-silico* pathogenic in CARD11. Family segregation was negative. Advances in genetic diagnosis have shown that variants in the same gene can give different clinical phenotypes. In our case, the variant detected in the patient could justify the Hyper IgE type phenotype, pending functional studies that validate its pathogenicity.

Keywords: Allergic disorders, immunodeficiency, inflammatory disease

P-1115

Evaluation of Bcl-2, Fas and FasL expression and their association with the extent of inflammation during primary Sjögren's syndrome

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Primary Sjögren syndrome (pSS) is a chronic autoimmune exocrinopathy characterized by epithelial atrophy with variable degree of lymphocytic infiltration of the affected organs and broad clinical manifestations. In pSS, atrophy of the epithelium is caused by an increased amount of apoptosis. The main aim of this study is to investigate the role of the apoptosis-related factors by studying Bcl-2, Fas and FasL expression in relation to the extent of inflammation. Fas, FasL and Bcl-2 plasma levels were assessed using enzyme-linked immunosorbent assays in 62 pSS patients, documented for their serological and clinical features. We also investigated Fas, FasL and Bcl-2 expression by immunohistochemistry analysis in the labial salivary glands samples in association with the extent of inflammation. Importantly, our results indicated that in pSS patients, the plasmatic Bcl-2, Fas and FasL levels were significantly elevated in comparison to the healthy controls and appear to be associated with the severity of inflammation. Moreover, we report a strong positive correlation between Bcl-2 and NO levels. The immunohistochemical study reveals a strong Bcl-2 expression in infiltrating mononuclear cells and a total absence in the acinar cells. The Bcl-2 level varies according to the severity of the pathology. However, the expression of Fas and FasL was less important and predominantly localized in infiltrating mononuclear cells. Our current study highlights the involvement of Bcl-2, Fas and FasL in pSS glands injury. These factors could represent a potential candidate for targeting inflammation during pSS.

Keywords: Autoimmunity, cytokines and mediators, inflammatory disease, inflammatory molecules

P-1117

Effective clearance of Chlamydia from the murine female reproductive tract in the absence of T-bet+ Th1 cells

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Protective immune responses to Chlamydia infection within the female reproductive tract (FRT) are incompletely understood. MHC class II-restricted CD4 Th1 responses are thought to be vital for bacterial clearance, due to their capacity to secrete IFN-γ, but Th1 responses as defined by the master transcription factor T-bet had not yet been fully interrogated in primary infection. Here, we investigated T-bet and IFN-γ involvement in primary infection clearance. We found that IFN-γ producing CD4+ T cells from the FRT exhibited surprisingly low levels of T-bet throughout primary infection, indicating low involvement of classical T-bet+ Th1 cells. Mice deficient for T-bet either globally or specifically in CD4+ cells both competently cleared infection along wild-type kinetics. T-bet-deficient mice also showed significant skewing towards Th17 responses, indicating potential compensation pathways through alternate Th fates. On the other hand, IFN-γ-, and IFN-γR-deficient mice resolved much of FRT infection, but experienced systemic dissemination and 100% mortality with an average median survival of approximately 4 weeks. Additionally, depletion of IFN-γ in T-bet-deficient mice illustrated that the Th17 shift observed in these mice was not sufficient to eliminate the requirement for IFN-γ to control systemic dissemination and prevent mortality. Together, this indicates that while IFN-γ is important to protect mice from death, classical T-bet+ Th1 cells are ultimately not required for primary clearance. Studies are underway to examine which alternative CD4 T cell mechanisms contribute to bacterial clearance within the FRT.

Keywords: Adaptive immunity, bacterial infections, infectious disease, reproductive immunology

P-1121

Identification of potential threshold to predict Ab persistence in subjects one year after SARS-CoV-2 infection

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A seroprevalence study in working adults (n=1600) showed that SARS-CoV-2-specific antibodies persist for at least six months. Twelve subjects of this cohort who had NT titers >1:10 after infection were followed-up after 1 year to assess long-term Ab persistence. SARS-CoV-2 Ab reactivity was evaluated in detail with semi-quantitative RBD-specific Ab ELISA, quantitative S1 IgG ELISA and in-vitro neutralization ELISA. Our results showed that S1-specific IgG (BAU/ml) correlated strongly with in-vitro neutralization ELISA results (% inhibition; r=0.939), and both S1 IgG and in-vitro neutralization correlated moderately with RBD-specific Ab ratios. One year after mild COVID infection, 7 of 12 individuals (58.3%) were still seropositive for S1-specific IgG and also showed >20% inhibition in the neutralization ELISA. Accordingly, S1-specific memory B-cells were present in respective frequencies in peripheral blood of these subjects. RBD-specific Abs remained above positive cut-off (ratio >1.1) for all subjects after 1 year, while NCP-specific IgG declined to levels below negative cut-off (ratio <0.7). Correlations of S1-specific IgG at different time-points indicated that subjects who had Abs >60 BAU/ml 3 months after infection retained detectable S1 IgG Abs (>35 BAU/ml) after one year (r=0.846). Similarly, subjects with in-vitro neutralization >20% 3 months post infection still showed inhibition >20% after 1 year (r=0.839). We report that S1-specific IgG levels >60 BAU/ml 3 months post-infection potentially persist at detectable levels for 1 year. Vaccine-induced immune responses to SARS-CoV2 apparently decline at similar rates as those following infection, thus our results have implications for the assessment of Ab persistence after vaccination.

Keywords: Memory, monitoring immunity, protection

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POSTER PRESENTATIONS

P-1123

Structure-based computational methods explain steroid sensitivity of a novel IL10 receptor deficiency

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IL-10 receptor mutations cause severe, very early-onset inflammatory bowel disease throughout the first year of life. Inflammation is typically unresponsive to immunosuppressive therapies such as corticosteroids, methotrexate, and anti-TNF-alpha. Here, we report a case presented with IL-10 receptor alpha (IL-10Rα) deficiency responding to steroid therapy. A 20-month-old Afgan girl with abdominal pain, recurrent diarrhea, fever, and vomiting complaints admitted to our hospital. After two weeks of birth, she had hospitalized due to several persistent diarrhea, fatigue, mouth ulcers, and sepsis. Furthermore, she suffered from perianal ulcers and hepatosplenomegaly. At six months of age, she underwent anal transposition and dilatation surgeries because of recurring perianal fissures and fistulae. Prolonged severe diarrhea required ileostomy. Despite broad-spectrum antibiotics and intravenous immunoglobulins, the patient's diarrhea did not improve, and high-dose corticosteroid treatment had initiated. After treatment, the frequency of diarrhea dramatically decreased in a week. Over the course of a year of therapy with lower dosages of methylprednisolone, she gained 4 kg of body weight. The gene sequence analysis showed the presence of IL-10Rα homozygous mutations (c.301 C>T, p.R101W). We used various bioinformatics techniques to predict stability and affinity. DynaMut tools computed that rigidity of IL-10 receptor alpha chain increased and flexibility significantly reduced. We have used the mCSM tools to analyze protein stability change and protein-protein complex affinity alterations. We predicted that receptor destabilized and affinity to IL-10 decreased mildly. In summary, we identified the new VEO-IBD subtype caused by homozygous IL-10RA mutation (c.301C>T), which is controllable with a low dose of corticosteroid.

Keywords: Immunodeficiency, chronic inflammation and fibrosis, immune regulation and therapy, inflammatory disease, inflammatory bowel disease

P-1124

Extracellular matrix modification in skin inflammation

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The extracellular matrix (ECM) builds a complex meshwork of fibres and associated glycoproteins. Previously it was thought that the ECM mainly has structural, supportive functions. However, recent studies showed that regulation of matrix modifications, its biochemical properties and architecture are important factors to maintain tissue homeostasis. Collagen is the major ECM component of the basement membrane and the dermal interstitial matrix. Enzymatic inter- and intramolecular crosslinking, which result in changes to the ECM geometry, structure and mechanical properties, can affect tissue-resident immune cell adhesion and migration. We hypothesized that inflammation-induced hypoxia can result in ECM modifications and thereby maintain chronic inflammatory skin diseases by influencing cell migration and tissue repair. Therefore, we would like to investigate whether the ECM is changed in inflammatory skin diseases, and if hypoxia may be a driving factor. We further want to explore molecules that modify the biochemical properties and architecture of the ECM, such as lysyl oxidase (LOX), one of the most prominent regulators of ECM topography. Here, we present a novel image analysis tool to investigate expression levels of HIF-1 and LOX in human healthy and psoriatic skin samples. We developed a semi-automated, customized analysis pipelines for cell and organelle segmentation and quantification in FiJi, machine learning-based programs and R to obtain localized intensity values. This pipeline will allow unbiased analysis of skin samples in batch mode and future findings will contribute to a better understanding of the role of hypoxia and matrix modifying molecules in skin tissue homeostasis and inflammation.

Keywords: Biomarkers, chronic inflammation and fibrosis, inflammatory disease, skin diseases

P-1125

Killer-cell immunoglobulin-like receptors (KIR) inhibitory genes and susceptibility to lung cancer

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Killer cell immunoglobulin-like receptors (KIR) are of crucial importance in education and regulating natural killer (NK) cell function. NK cells are considered as the first line of defense in eliminating tumors. Peripheral NK cells display attenuated cytotoxicity and reduced interferon-gamma (INF-γ) production in lung cancer patients. To determine the association of KIR polymorphisms with susceptibility to lung cancer, 16 KIR genes were genotyped utilizing sequence-specific primers-polymerase chain reaction in 186 confirmed cases of lung cancer (145 non–small cell (NSCLC), 42 small cell (SCLC)) and 271 healthy controls (CNs). A significantly increased frequency of inhibitory KIRs (iKIRs) 3DL1, 2DL2 and lower frequency of activating KIRs (aKIR) 2DS1, 3DS1, 2DS5 was observed in patients than CNs. We noted that carriers of genotypes with iKIR>aKIR and CXTX subset were more susceptible to lung cancer progression, while carrier frequency of genotypes with aKIR>iKIR, C4T4 subset, and T4 gene cluster was higher in CNs. These data suggest that possessing certain iKIRs, likewise the absence of T4 cluster associated genes increases the risk of lung cancer development. More investigations on NK cell role in lung cancer establishment may support the development of immunotherapies based on NK cells for lung cancer treatment.

Keywords: Innate immunity, cancer immunology, MHC and polymorphic genes, NK cells

P-1127

COVID-19 convalescent patients display distinct SARS-CoV-2-specific T-cell responses according to disease severity

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The aim of our study was to evaluate the presence of both CD4 and CD8 T-cell responses against SARS-CoV-2 virus in convalescent patients according to COVID-19 severity. To this end, we designed a prospective study (NCT04365322) that included 60 COVID-19 convalescent patients (1-month post infection) in two cohorts respectively entitled mild illness and severe pneumonia. Healthy donors (n=12) that were included prior SARS-CoV-2 epidemic were included in a control group. The monitoring of peripheral immune responses was performed using ELISPOT IFN_γ and flow cytometry. The serology index of each patient was investigated at the same time (IgG). Surprisingly we showed that T-cell responses in term of frequency and intensity (IFN_γ secretion) were clearly distinct between mild illness and severe pneumonia patients. We identified the presence of both CD4 and CD8 specific T-cells responses for S, M and N proteins whatever the disease severity. Furthermore, our results demonstrated that recent history of COVID-19 did not hamper viral memory T-cell pool against common viruses (Cytomegalo-, Epstein-Barr and Flu-virus). Finally, IL-10 production within the supernatants of PBMC stimulated 48 hours with SARS-CoV-2 peptides was lower in PBMC isolated from patients who underwent a severe COVID-19 infection. To our knowledge, this study reports the largest cohort of convalescent COVID-19 patients where both SARS-CoV-2 specific T-cells and serology were simultaneously investigated according to patient disease severity. At the time of this study, the presence of CD4 and CD8 SARS-CoV-2 specific T-cells sustain the rationale for the development of protective therapeutics against SARS-CoV-2 infection.

Keywords: Adaptive immunity, biology of the immune system, infectious disease

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POSTER PRESENTATIONS

P-1128

Anergy and exhaustion, rather than senescence, characterize T lymphocytes in patients with systemic lupus erythematosus

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Chronic inflammatory diseases, such as Systemic Lupus Erythematosus (SLE) are characterized by a shift of lymphocytes to exhausted and/or senescent phenotypes. Aim of the study was to evaluate the expression of exhausted and senescent lymphocyte subpopulations in SLE patients. Expression of Programmed Cell Death (PD1) as exhaustion, and CD45RA, CCR7 as senescent markers were estimated, by flow cytometry, on T and B lymphocytes of patients with SLE(n=20, Mean Age 43.4±17yrs). Compared to healthy controls, SLE patients had significantly reduced lymphocytes, 2100(1500-3000) vs. 1250(300-2000), p<0.0001, CD4+:1005(753-1591) vs. 607(0-1274), p<0.0001, CD8+:459(207-948) vs. 374(0-575), p=0.03, CD4+CD45RA+CCR7+(Naïve):453(231-1357) vs. 232(0-572), p=0.002, CD4+CD45RA-CCR7+(Central Memory):570(39-1001) vs. 328(0-972), p=0.01, CD4+CD45RA-CCR7-(Effector Memory):11.1(1.5-43) vs. 1.6(0-73), p<0.0001, CD4+CD45RA+CCR7-(Terminally EM):30(5.9-167) vs. 9.3(0-123), p=0.007. Similarly, CD8+CD45RA+CCR7+(Naïve) were reduced, 200(82-804) vs. 69(0-347), p<0.0001, but CD8+CD45RA-CCR7+(Central Memory) were increased: 15.6(0.1-55)% vs. 36(1.8-89)%, p=0.001. PD1(+) T lymphocytes were significantly increased in SLE patients compared to controls, CD4+CD45RA+PD1(%): 2.3(0.5-49) vs. 0.6(0-1.7), p<0.0001, and absolute numbers 11.9(0-291) vs. 6.7(0-21), p=0.02 and CD8+CD45RA+PD1+(%): 11.1(0.4-63) vs. 3.6(1.2-10.3), p=0.004 and CD8+CD45RA-PD1+(%): 18.6(7-88.6) vs. 9.1(0.7-27.4), p=0.006. CD19+ cells were reduced in SLE patients, compared to controls:64(0-514) vs. 214(84-501), p=0.003, CD19+IgD+CD27+:5(0-16) vs. 27.7(1.8-59), p<0.0001, CD19+IgD+CD27-:38.7(0-434) vs. 114(5.4-317), p=0.03, CD19+IgD-CD27+:14(0-45.8) vs. 50.4(15.6-147), p<0.0001 and CD19+IgD-CD27-:9.1(0-66) vs. 17(7.6-49), p=0.01, as were B cells expressing PD1:0.5(0-24.4) vs. 4.6(0.9-35), p=0.02. Both T and B lymphocytes were significantly reduced in SLE patients, equally affecting naïve and senescent subtypes, however, the process of T cell exhaustion seemed to predominate, as the expression of PD1 was increased.

Keywords: Adaptive immunity, B lymphocytes, immune senescence

P-1130

Effect of the elderly human lung mucosa on *Mycobacterium tuberculosis* infection of the alveolar epithelium

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As we age, there is an increased risk for developing tuberculosis (TB), a disease caused by the infectious agent *Mycobacterium tuberculosis* (*M.tb*). *M.tb* infection results in a bacterial deposition in the lung alveolus, where *M.tb* is bathed in alveolar lining fluid (ALF). We have published that age-associated changes in human ALF soluble components, such as increased levels of oxidized and dysfunctional innate proteins, accelerate *M.tb* growth within human alveolar macrophages. We are interested in studying the impact of human ALF on *M.tb* infection of non-professional phagocytes, such as alveolar epithelial cells (ATs). We hypothesized that *M.tb* exposure to elderly human ALF (E-ALF) drives increased *M. tb* replication and growth within ATs due to impairment of E-ALF innate soluble components. We observed that E-ALF exposed M.tb had significantly increased intracellular growth in ATs. Despite changes in intracellular bacterial growth, infected ATs with E-ALF-exposed M.tb did not show altered production of inflammatory mediators or cell activation. Interestingly, *M.tb* exposure to E- ALF drove bacterial translocation to both endosomal and cytosolic compartments in ATs. Overall, rapid bacterial replication and increased growthwithin ATs was observed in *M.tb* exposed to E-ALF, and together with inadequate AT activation, could lead *M.tb* to potentially exploit the AT cytosol as a favorable intracellular niche for replication. These results emphasize the effects of elderly lung mucosa on *M.tb* infection of non-professional phagocytes (ATs), which may conceivably explain why the elderly population is more vulnerable to developing active TB disease.

Keywords: Ageing, inflammatory molecules, innate immunity

P-1131

Longitudinal serological analysis following national seroprevalence study to investigate COVID19 infection in people living in Ireland

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Longitudinal assay evaluation, following initial assessment of sensitivity and specificity is necessary when temporally remote evidence of infection is sought. We report longitudinal serology results, paired with symptomatology and exposure history from 22 participants. We compare 4 SARS-CoV-2 serological assays, including 2 anti-(N) Nucleocapsid assays along with 2 anti-(S) Spike assays, up to 15 months following the 1 st peak of cases reported in Ireland (April 2020). Twenty-two participants with detectable anti-SARS -CoV-2 serology, identified in July 2020 (visit 1) in a national Irish seroprevalence study, had serology repeated at 3 months (visit 2, October 2020), 6 months (visit 3, January 2021) & 12 months (visit 4, July 2021- (results pending)). No significant change in intraindividual anti-(S) antibody titres were observed by 6 months follow up. Whereas all participants showed significant reductions in Abbott anti-(N) reactivity: at 3 months (mean 50% of visit 1) & 6 months (mean 42% of visit 1), with 81% of those initially having detectable Abott anti-(N) yielding negative results 6 months later. Yet, by 6 months, all participants remained positive on the Roche anti-(N) assay, despite declining titres at 3 and 6 months. Our data, provides vital information on assay performance, essential when selecting appropriate assays and interpreting results. Due to the significant under detection from as early as 3 months, the Abbott anti-(N) assay is unsuitable for seroprevalence studies. This is particularly relevant with investigators now seeking anti-(N) assays to differentiate natural infection from vaccine response, with anti-(S) assays alone, being insufficient.

Keywords: Adaptive immunity, antibody, immunological techniques, protection, viral infections

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POSTER PRESENTATIONS

P-1132

Biological testing of novel viral protease inhibitors against SARS-CoV-2 in mammalian model cell line VERO-E6

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The COVID-19 pandemic is still devastating the world causing significant social, economic, and political chaos. Corresponding to the absence of globally approved antiviral drugs for treatment and vaccines for controlling the pandemic, the number of cases and/or mortalities are still rising. Current patient management relies on supportive treatment and the use of repurposed drugs as an indispensable option. At early stages, Remdesivir drug and Pfizer vaccine have been only one approved by the FDA for emergency use, but as of today there are already several globally approved specific vaccines for official use. It is anticipated that vaccines and antibody-based drugs will be discovered before small molecules. However, vaccines might not be 100% effective and antibodies could have immunopathological consequences. Therefore, looking for putative drugs targeting SARS-CoV-2 main proteases is necessary. Of a crucial role in the viral life cycle, our ongoing studies are looking for potential inhibitors to main proteases (Mpro and PLpro). Here, we analysed effect of three newly designed compounds on replication of SARS-CoV-2 and cytopathology caused by this virus in VERO-E6 cell line. The goal is to reduce the amount of SARS-CoV-2 virus in infected cells after compound treatment to levels that are undetectable. The plague reductions assay was used to quantify the virus in infected cells. The cytotoxicity and the cytopathic effect were observed to varied degree; thus, the final antiviral effect was concluded in one case. The results were compared to other commercially available drugs to further highlight the strength of this newly synthesised inhibitors.

Keywords: Cellular interactions, infectious disease, viral infections

P-1133

The change of HLA DR expressions and monocyte subsets in non-small cell lung cancer

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Lung cancer is the leading cause of cancer-related deaths in the world. Monocytes are important innate immune cells with pro- or anti-tumor activity in cancer development and progression. The aim of this study was to investigate the subtypes of monocytes and their HLA-DR expressions in NSCLC progression. Thirty NSCLC patients with a mean age of 64.53±7.78 years and twenty-five healthy controls with a mean age of 62.28±11.77 years were included in the study. Monocyte subsets and their HLA-DR expressions were evaluated in patients with NSCLC and control. Mononuclear cells were collected using the ficol-histopaque method and monocyte subsets were determined by flow cytometry. CD14lowCD16+ non-classical monocytes were decreased in all patients and advanced stage compared to control (3.91±2.06% in all patients, 3.64±2.20% in advanced stage and 6.56±3.28% in control; p = 0.001, p = 0.002, respectively). There was no significant difference between groups in CD14+CD16- classical monocytes and CD14+CD16+ intermediate monocytes (p>0.05). The mean fluorescent intensity (MFI) value of HLA DR on classical monocyte were reduced in all patients and advanced stage (5207.66±2349.79 in all patients, 5379.06±2682.87 in advanced stage and 8763.76±5031.58 in control; p=0.007, p=0.024, respectively). Previous studies have reported that reduced HLA-DR expression of monocytes was associated with the immunosuppression on lymphocyte in cases of sepsis and COVID-19. This study suggested that decreased HLA-DR expression of monocytes may be a part of cancer-associated immunosuppression in patients with NSCLC, similar to sepsis and viral infections.

Keywords: Cancer immunology, innate host defence, innate immunity

P-1134

Hydroxyl radicals' production by human leukocytes under the influence of bacterial diamines

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It has been shown that nutrient deficiency, antimicrobial and other substances, presented in a large amount in the inflammation focus, increase the rate of synthesis of cadaverine and putrescine by microorganisms. The role of microbial polyamines (MP) as "scavengers" of free radicals has been described, but so far there is no data on their effect on the radical-producing activity of white blood cells (WBC). The aim of the investigation was to study the features of the hydroxyl radicals' generation by human WBC under the influence of MP. Peripheral blood samples were obtained from 20 healthy donors. To assess the production of hydroxyl radicals, a luminol-dependent chemiluminescence reaction was performed with leukocytes previously incubated with MP: cadaverine (0.01 M) and putrescine (0.01 M). The measurements were carried out on a Luminoskan Ascent® Thermo Labsystems luminometer (USA). For statistical analysis, an integral chemiluminescence index was used for the entire measurement period. It has been shown that the preincubation of WBC with cadaverine leads to an increase in the production of hydroxyl radicals (area under the curve was 6.7±0.7 units). Putrescine had small effect on the radical-producing activity (2.8±0.4 units, $p<0.05$). This may be due to the inactivation of MP with the formation of H_2O_2, which reacts with luminol. In this case, diamineoxidase inactivates other diamines, leading to the reduction of inflammation. Thus, the results of this study indicate the possibility of attributing MP to mediators that have a variable effect on the functional activity of human WBC

Keywords: Granulocytes, infectious disease, innate immunity, neutrophils

P-1135

Rapid determination of antibody-mediated neutralization against RBD from SARS-CoV-2 emerging variants using a flow-cytometry-based assay

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The rapid spreading of SARS-CoV-2 variants, raises concerns regarding their capacity to evade immune protection provided by natural infection or vaccination. The receptor-binding domain (RBD) of the viral spike protein is the major target of neutralizing antibodies, and viral variants accumulate mutations in this region. The aim of this study is to develop a simple method to rapidly determine antibody neutralization against SARS-CoV-2 RBD variants for the quick evaluation of the potential threat of the variants and update effective interventions. A non-adherent 300.19 stable cell line expressing the human ACE2 receptor was incubated with RBD-Fc-fusion proteins from several viral variants previously exposed to the plasma samples of 36 SARS-CoV-2 infected and 67 vaccinated individuals. Cells were stained with anti-mouse IgG-PE and plasma neutralizing capacity was determined by flow cytometry as RBD-ACE2 binding inhibition. We have established a simple and efficient flow cytometry assay to measure antibody-mediated neutralization against RBD of four variants: Alpha (B1.1.7), Gamma (P.1), Epsilon (B.1.427) and Kappa (B.1.617). These four RBD variants showed augmented binding to ACE2 compared to the original Wuhan strain. Variants containing mutations E484Q/K (Gamma, Kappa) with K417T (Gamma) or L452R (Kappa), and the variant encompassing only L452R mutation (Epsilon) showed increased resistance to antibody neutralization in comparison to the Wuhan strain or the Alpha variant encompassing N501Y. Our assay, which can be easily adapted to newly emerging variants, should be extremely valuable to assess the ability of these variants to escape immune protection from vaccination.

Keywords: Antibody, protection, viral infections

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POSTER PRESENTATIONS

P-1137

Modulation of the phospholipidome in circulating immune cells of obese patients are associated to insulin resistance or glycemic status

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Systemic metabolic disorders associated with obesity have been proposed to impact both intrinsic metabolism and function of circulating immune cells. To further investigate this question, we profiled the PBMCs phospholipidome in lean and obese with or without dysglycemia patients (OBNG or OBDysG). The overall phospholipidome of PBMCs is significantly downmodulated in OBDysG compared to lean patients. All classes of phospholipids, but mostly the PC (phosphatidylcholine), PE (phosphatidylethanolamine), SM (sphingomyelin), lysoPC/PE, are affected. A few PC, SM, lysoPC, lysoPE and lysoPI species are significantly downmodulated in OBDysG compared to OBNG individuals, making it possible to distinguish the two phenotypes. Multivariable linear regression models highlight interesting associations between membrane lipids and fasting insulin. Interestingly, membrane homeostasis is disrupted in PBMCs from obese patients with dysglycemia compared to normoglycemic individuals, as demonstrated by both an increased PC saturation and a reduced LysoPC/PC ratio. Moreover, the PC saturation is associated with HbA1c, which reflects glycemic status over past three months. Such modulation of the PBMCs phospholipidome could disrupt the cell membranes and the lipid mediators levels, driving an immune cell dysfunction.

Keywords: Big data, diabetes, inflammatory disease, metabolic control of immune responses

P-1138

Lack of antibody production after immunization with COVID19 mRNA vaccines in patients with certain immunosuppressive treatments

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COVID19 vaccines are the most effective prophylactic measures to combat the ongoing COVID19 pandemic. With the licensure of COVID19 vaccines, the question arose whether patients undergoing immunosuppressive treatments are able to mount a sufficient vaccine response. We therefore retrospectively analysed S1-specific antibody responses after intramuscular administration two mRNA vaccine doses in 242 patients with underlying chronic inflammatory or hematooncological diseases as well as patients that had received solid organ transplants or with metabolic diseases. Antibody testing was performed at an average of 31.7 days after the second dose. Within this group of patients, 15.9% were non-responders, where S1-specific antibodies were not detectable in sera. We found that non-responsiveness was associated with B-cell depleting therapies applied within the last11 months, ongoing therapies hindering lymphocyte trafficking (Fingolimod) or inhibiting proliferation (Tacrolimus). Therefore, it is important to discuss the possibility of vaccine non-responsiveness with patients on immunosuppressive therapies in order to ensure that they maintain non-pharmaceutical protection measures. The question, however, remains whether a booster dose would be successful to induce an antibody response in non-responders. Currently, we are evaluating prospectively whether patients that do not produce SARS-CoV-2 specific antibodies after COVID19 vaccination are able to mount a cellular response that would contribute to protection.

Keywords: Adjuvants and vaccines, immunodeficiency, infectious disease

P-1139

Gene editing of DRB1*04:01 at position 71 blocks collagen sensitization and avoids allorecognition

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Rheumatoid arthritis (RA) is a chronic autoimmune disease with strong genetic association with HLA-DRB1*04:01. Our previous work has demonstrated that a single amino acid change from lysine (K) to glutamic acid (E) at position 71 of DRB1*04:01 abrogated binding of the immunodominant collagen258-272 peptide. We investigated whether this novel mutation in DRB1*04:01 will prevent sensitization to collagen and will be accepted by the immune system in transgenic mice. DRB1*04:01 (DR4), DRB1*01:01 (DR1) and DRB1*04:01K71E (K71E) transgenic mice were created on the class II MHC knockout background. K71E mice contain a single amino acid change from K to E, at position 71 in DRB1*04:01. Transgenic DR4 and K71E mice were immunized with bovine type II collagen in complete Freund's adjuvant. CD4+ T cells from K71E did not respond when restimulated with collagen$^{258-272}$ peptide *in vitro* as compared to DR4-T cells (0.3% vs 2.4%). Additionally, Skin grafts were transplanted from DR4 (isograft), DR1 (allograft), or K71E (experimental) mice onto DR4 recipients to determine if DR4 -T cells would recognize the single amino acid change in K71E mice. The experimental skin graft from K71E was accepted by the recipient DR4 mice without any sign of rejection by day 70. The DR4 isograft was accepted while the DR1 allogenic skin graft was rejected by recipient DR4 mice. Thus, our results demonstrate for the first time that mutating DRB1*04:01 at position 71 blocks collagen sensitization and avoids allorecognition by the host suggesting that gene editing of DRB1*04:01 could be a potential therapy to halt RA.

Keywords: Autoimmunity, immune regulation and therapy, molecular immunology, rheumatoid arthritis, transplantation

P-1140

IL-18 and IL-10 act oppositely on endurance exercises in professional ice hockey players

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Introduction Interleukin 18 (IL-18) and interleukin 10 (IL-10) are pro- and anti- inflammatory immunoregulatory cytokines.. Down-regulation of free IL-18 as well as up-regulation of IL-10 may limit the magnitude and duration of excessive inflammatory responses to the exercise-induced tissue damage and contribute to elevated susceptibility to infection in athletes undergoing exhaustive exercise. Links between serum IL-18and IL-10 levels and physical activity, correlation between body mass index (BMI), muscle mass index (MMI), basal metabolic rate (BMR) as well as energy expenditure (EE) may exist. 34 male professional ice hockey players were examined. Sera concentrations of IL-18 and IL-10 were assayed by ELISA. Quantitative estimation of body composition was assessed by bioimpedance technology. Dietary energy consumption per person, in kcal per day, was estimated using an album of portions of different sizes of portions of most frequently consumed foods. An increase more than 3-4 fold occurred in the concentration of IL-18 in sera of athletes (327.86 + 45.67 pg/ml). The IL-18 concentration did bit correlate with BMI (p=0.040), but showed low correlation with MMI (p=0.234) and BMR (p=0,231). IL-10 levels had negative correlation with B MI (p= - 0.251), MMI (p= - 0.327) and BMR (p= - 0.301). Sera concentration of IL-18 and IL-10 highly correlated with physical activity and the diets of athletes. Sera concentrations of IL-18 and IL-10 nay be used study immune effects in high endurance athletes such as ice hockey players in whom opposite effects on the production of IL-18 and IL-10 were seen.

Keywords: Adaptive immunity, cytokines and mediators, effector molecules, metabolic control of immune responses

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POSTER PRESENTATIONS

P-1154

Persistent natural killer cell dysfunction in severe COVID-19

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Longitudinal analyses of the innate immune system including earliest time points are essential to understand the immunopathogenesis and clinical course of COVID-19. Here, we performed a detailed characterization of natural killer cells in 72 patients (163 samples, day 2-29 after symptom onset) from three independent cohorts using single-cell transcriptomics and proteomics together with functional studies. We found elevated IFN-α plasma levels in early severe COVD-19 alongside increased NK cell expression of ISGs and genes involved in IFN-α signaling, while upregulation of TNF-induced genes was observed in moderate disease. NK cells exert anti-SARS-CoV-2 activity but are functionally impaired in severe COVID-19. Further, NK cell dysfunction may be relevant for development of fibrotic lung disease in severe COVID-19, as NK cells here exhibited impaired anti-fibrotic activity. Our study indicates differential IFN-α versus TNF responses in severe versus moderate COVID-19 and associates prolonged IFN-α-induced NK cell response and persistent NK cell dysfunction.

Keywords: Biology of the immune system, NK cells, RNAseq, viral infections

P-1155

CD56+ monocytes and CD31+ endothelial cells as blood markers of COVID-19 disease severity

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Immune response dysregulation plays a key role in SARS-CoV-2 pathogenesis. Both too weak or too strong immune responses can lead to severe COVID-19 disease. In the present study, we evaluated immune and endothelial blood cell profiles of COVID-19 patients to determine critical differences in immune signatures between those with mild, moderate, or severe COVID-19 disease using spectral flow cytometry. We examined various immune phenotypes, including monocytes, T cells, NK cells, B cells, endothelial cells, and neutrophils, along with their activation status. Our results showed a significant increase in CD56+CD14+Ki67+IFNγ+ monocyte population in patients with moderate and severe COVID-19 disease. This population has not yet been described in terms of COVID-19. We also reported enhanced circulating endothelial cells (CD45-CD31+CD34+CD146+), circulating endothelial progenitors (CD45-CD31+CD34+/-CD146-) and neutrophils (CD11b+CD66b+) in blood of patients facing severe COVID-19 illness. However, there was significant progressive lymphopenia and depletion of T cell subsets (CD3+, CD4+, and CD8+) in patients with severe disease. Spearman correlation analysis revealed the synergistic effect of upregulated CD56+ monocytes, endothelial cells, decreased T cells, and higher age, obesity, and hypertension leading to severe outcomes of SARS-CoV-2 infection. With the presence of new variants of SARS-CoV-2 in progress and insufficient vaccinations in developing countries, it is essential that we continue to work toward a better understanding of productive immune responses against the SARS-CoV-2 virus and the immunopathological mechanisms underlying severe disease.

Keywords: Biomarkers, infectious disease, memory, viral infections, visualizing immune responses

P-1159

Impending cardiac tamponade as an atypical extrapulmonary tuberculosis manifestation in HIV patient with hepatitis B coinfection: diagnostic challenge in a resource-limited setting

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Immunocompromised patient has a higher risk of developing opportunistic infection, particularly tuberculosis (TB). An atypical presentation may be the clue towards emergency clinical situation which prompts early treatment. A twenty three year-old female came to the emergency department of Dr. Hasan Sadikin General Hospital, Bandung, Indonesia with a chief complaint of worsening dyspnea within three weeks prior to admission. The patient also felt palpitations concomitant with the dyspnea. There were no clinical features of heart failure and autoimmune observed in this patient. The patient had systemic manifestations of tuberculosis. Relevant sexual history as a major risk factor for HIV and Hepatitis B infection was present. Rheumatological and malignancy work-ups were negative. On presentation, the patient was hypotensive with elevated jugular venous pressure. No muffled heart sound was detected. Chest X-ray was significant for an enlarged cardiac silhouette. Transthoracic echocardiograpy demonstrated a large pericardial effusion with impending right ventricular collapse. One litre of serosanguinous pericardial fluid was drained during pericardiocentesis, of which it was positive for *Mycobacterium tuberculosis*. Oral tuberculosis therapy was started along with prednisone tapered dose. Her condition improved significantly and she was discharged several weeks later. Coinfection of HIV and Hepatitis B that the patient had rendered her more susceptible towards developing opportunistic infections, in this CASE: pericardial TB. It was accounted as a rare extrapulmonary TB manifestation, with an incidence rate of <1%. Extrapulmonary tuberculosis as an opportunistic infection should always be considered, notably in immunocompromised patients living in tuberculosis endemic area.

Keywords: Bacterial infections, cardiovascular diseases, infectious disease, viral infections

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POSTER PRESENTATIONS

P-1160

The interplay between SARS-CoV-2 infected airway epithelium and immune cells modulates the immunoregulatory/inflammatory signals

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infects primarily the airways tract, inducing the recruitment of inflammatory infiltrates in the alveolar space and a systemic inflammatory cytokine storm. To assess the cross-talk between immune cells and respiratory tract during SARS-CoV-2 dissemination, we analysed the relationships between the inflammatory response induced by SARS-CoV-2 replication and immune cells phenotype in a reconstituted human bronchial epithelium model. The SARS-CoV-2 infected organotypic human airway epithelium (HAE) was co-cultured with immune cells and the inflammation profile as well as the frequency of immune cell subsets was analyzed. The enriched network and signalling was finally evaluated. The results indicated that immune cells failed to inhibit SARS-CoV-2 replication in HAE model. In contrast, immune cells strongly affected the inflammatory profile induced by SARS-CoV-2 infection, dampening the production of several immunoregulatory/inflammatory signals (e.g., IL-35, IL-27 and IL-34). Moreover, these mediators were found inversely correlated with innate immune cell frequency (NK and ψ6 T cells) and directly with CD8 T cells. The enriched signals associated to NK and CD8 T cells highlighted the modulation of pathways induced by SARS-CoV-2 infected HAE. These findings are useful to depict the cell-cell communication mechanisms necessary to develop novel therapeutic strategies aimed in promoting an effective immune response.

Keywords: Cytokines and mediators, immune networks, infectious disease, viral infections

P-1166

Pre-clinical development of a vaccine against *Trypanosoma cruzi* to prevent Chagas disease

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Trypanosoma cruzi is an invading parasite causing Chagas disease, a neglected tropical disease with a devastating effect upon populations in rural areas of Latin America. As a result of globalization, Chagas disease has become a global concern. After an often-asymptomatic acute phase, it progresses to a chronic stage, causing lethal cardiomyopathy, gastro enteric manifestations or damage to the peripheral nervous system. Vaccines are not available, and treatment is difficult. The available drugs - beside their adverse effects - are only effective in the acute stage. The EC-funded CRUZIVAX consortium is developing a mucosal vaccine based on a structure-engineered trivalent chimeric antigen, Traspain, adjuvanted with c-di-AMP. Preclinical validation studies demonstrate the immunogenicity and efficacy of this candidate vaccine beyond gender and in different age groups. CRUZIVAXTM will be now advanced towards first-in-human studies.

Keywords: Adaptive immunity, adjuvants and vaccines, animal models, infectious disease, monitoring immunity, parasite infections

P-1170

Effect of HLA A, C, DQA1 and DQB1 alleles on the progression of HBV infection to liver cirrhosis and hepatocellular carcinoma (HCC)

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Decompensated cirrhosis and hepatocellular carcinoma are the worst complications of HBV infection and host genetic factors such as HLA polymorphisms that could lead to downregulation of antigen presentation – an immune escape mechanism in chronic infections and carcinogenesis are under investigation. Our aim was to study the effect of HLA polymorphism on the occurrence of HBV infection complications such as HCC/cirrhosis in North Greek HBV patients. The HLA frequencies of 151 HBV infected individuals (117 had a spontaneous clearance of HBsAg and 34 had complicated chronic HBV with cirrhosis/HCC) were compared to the HLA frequencies from the North Greece Bone Marrow Donor Registry (14506 samples – control group). HLA A, B, C, DRB1, DQA1, and DQB1 were genotyped by PCR SSP. Our statistical analysis was performed by IBM SPSS Statistics for Windows v.25. Our results showed: (i) a significantly increased frequency of HLA A*01, Cw*08, and DQA1*03 in HBV related cirrhosis/HCC versus control group (20,6% vs 9,7% p=0.031, 9,1% vs 2,2% p=0.017, and 14,3 % vs 3,8 % p=0,015, respectively), and (ii) an association of HLA DQB1*05 with the adverse HBV infection clinical outcome of cirrhosis when compared with HBsAg clearance group lower frequency of this allele (38,2% vs 22,2% p=0.038) and with control group (38,2% vs 22,3% p=0.05). Our results come in agreement with our previous research on this field and show that specific HLA alleles may contribute to the occurrence HBV infection complications in HBV patients while the recruitment of more patients would further reinforce them.

Keywords: Antigen processing and presentation, cancer immunology, infectious disease, molecular immunology, viral infections

P-1178

Mining cow's milk for novel immunomodulatory food ingredients for application in functional foods to promote muscle health and function

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The immune system is a key regulator of skeletal muscle (SM) function and health. The regulatory interactions between the immune system and SM during regeneration are intricate and orchestrated by a plethora of innate and adaptive immune cell phenotypes. Principal among these are macrophage. Briefly, damage to SM drives a pro-inflammatory immune response. This response is characterised by the presence and activity of M1 polarised macrophage. M1 macrophage clear the site of injury and drive the proliferation of quiescent muscle progenitor cells. As healing progresses the immune microenvironment shifts, characterised by the increased prevalence of anti-inflammatory M2 macrophage, which promote myoblast to myotube differentiation and quenches residual inflammation at the site of injury. Through ageing and lifestyle factors, inflammation can persist to become chronic, which detrimentally impacts the regenerative and metabolic functions of muscle tissue. Due to the role of macrophage in regeneration, and the extent at which their dysfunction can impair muscle function, modulating macrophage activity may offer a potential therapeutic avenue to treat chronic muscle disease. This research highlights the potential application of immunomodulatory cow's milk derived hydrolysates to promote muscle health through modulation of macrophage phenotypes. Additionally, this research highlights the impact that modulated immune function has on key aspects of muscle function including glucose metabolism, protein anabolism and wound healing. Novel, food derived immunomodulators may provide an alternative, natural route for the treatment of inflammation induced muscle damage and metabolic implications.

Keywords: Ageing, inflammatory disease, macrophage, tissue damage and repair

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POSTER PRESENTATIONS

TRACK 4 - INNOVATIVE TECHNOLOGIES AND IMMUNOTHERAPIES

P-0086

In silico analysis of FDA approved drugs against SARS-CoV-2 viral ORF3a protein

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SARS-CoV-2 (severe acute respiratory syndrome-coronavirus-2) is an enveloped, single-stranded RNA virus that belongs to the beta-coronavirus group. SARS-CoV-2 is a deadly infectious agent that can cause severe pneumonia, sepsis, uncontrolled coagulation, by interfering with the proper functioning of the immune system. SARS-CoV-2 encodes viral ion channel protein, open reading frame 3a (ORF3a), that stimulates NLRP3 inflammatory pathway at a high level and activates caspase enzymes. Activated NLRP3 complex leads to pyroptosis, inflammatory cell death and, maturation and release of proinflammatory cytokines (IL-1β and IL-18). Inflammasome activation can also trigger coagulation through pyroptosis by the release of F3 (tissue factor, CD147) from infected cells. We aimed to repurpose currently approved drugs to reduce dysregulated NLRP3 inflammasome activity which leads to severe tissue damage, cytokine storm, and immunocoagulation, by targeting ORF3a protein. In our study, we performed the virtual screening with FDA-approved 2515 small molecules that interact with ORF3a protein of the SARS-CoV-2. Screened molecules were evaluated according to their binding energy (ΔG). As a result, tannic acid (ΔG = -12.5), which is a phytotherapeutic, and grazoprevir (ΔG = -11.1), NS3 (NS3 / 4A, a serine protease enzyme) inhibitor used in the treatment of hepatitis C infection, are strongly bound to SARS-CoV-2 ORF3a protein.

Keywords: Cell signalling, drugs for immune modulation, immune networks, immunopharmacology, viral infections

P-0093

Ethyl pyruvate ameliorates experimental autoimmune myocarditis

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Ethyl pyruvate (EP) has profound anti-inflammatory and immunomodulatory properties. Here, its effects on experimental autoimmune myocarditis (EAM) induced in mice by heart-specific myosin-alpha heavy chain peptide immunization were determined. EP was applied intraperitoneally, daily, starting with the immunization. Severity of EAM was determined by histological assessment of immune cell infiltrates into the heart at day 21 post immunization. Cells were phenotypically characterized by flow cytometry. Concentration of cytokines in cell culture supernatants and sera was determined by ELISA. EP reduced infiltration of immune cells into the heart and lessened heart inflammation. Smaller number of total immune cells, as well as of $CD4^+$ T cells, $CD11b^+$ and $CD11c^+$ cells was isolated from the hearts of EP-treated mice. Reduced number of antigen-presenting cells, detected by CD11c, MHC class II, and CD86 antibodies, as well as of T helper (Th) 1 and Th17 cells, detected by CD4, IFN-γ and IL-17 antibodies, was determined in mediastinal lymph nodes draining the heart, in parallel. The number of $CD11c^+$, $CD11c^+$MHC class II^+, and $CD11c^+CD86^+$ cells was reduced in the spleen, as well. Lower production of IFN-γ and IL-17 by cells of the lymph nodes draining the site of immunization in response to the immunizing antigen was observed in EP-treated mice. Our results clearly imply that EP restrains autoimmunity in EAM. EP-based therapy for the treatment of myocarditis in humans should be investigated in the forthcoming studies.

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Keywords: Animal models, autoimmunity, immune regulation and therapy, inflammatory disease

P-0101

First exposure to rituximab is associated to high rate of anti-drug antibodies in systemic lupus erythematosus but not in ANCA-associated vasculitis

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Anti-drug antibodies (ADAs) to rituximab (RTX) can impair treatment safety and efficacy. RTX is approved for ANCA-associated vasculitis (AAV) and used off-label for systemic lupus erythematosus (SLE), but data regarding ADAs in these populations are limited. AIMS: To assess the frequency, and risk factors for ADAs to RTX in SLE and AAV. ADAs were detected using a bridging electrochemiluminescent immunoassay in sera from AAV (n=22) and SLE (n=66) patients taken 6 months after infusion. Clinical and laboratory data was retrieved from medical records. After the first RTX cycle, no AAV patient was ADA+ compared to 37.8% of SLE patients. The ADA+ SLE group were younger (34.0 (25.9-40.8) vs 44.3 (32.7-56.3) years, p=0.002) and with more active disease (SLEDAI-2K 14.0 (10.0-18.5) vs. 8.0 (6.0-14), p=0.0017) compared to ADA-. ADAs primarily occurred in nephritis patients, were associated with anti-dsDNA positivity but were not influenced by concomitant or previous treatments. Despite overall reduction of SLEDAI-2K (12.0 (7.0-16) to 4.0 (2.0-6.7), p<0.0001), ADA-positive patients had higher SLEDAI-2K (6.0 (4.0-9.0) vs 4.0 (2.0-6.0), p=0.006) and B-cell counts (%CD19+ 4.0 (0.5-10.0) vs 0.5 (0.4-0.95), p=0.002) at 6-months. Two of 16 (12.5%) ADA+ SLE patients developed serum sickness, and three had infusion reactions on retreatment (16%) in contrast to one ADA- patient with serum sickness (<3%). ADAs were highly prevalent among RTX treated SLE patients already after first cycle, mainly affecting younger patients with more active disease. These patients were more prone to adverse events, supporting ADA screening before retreatment in SLE.

Keywords: Antibody, B lymphocytes, immunopharmacology, immunotherapy

P-0115

Immune cells mediated cell death against gastrointestinal cancer cells via immunomodulators from Datura stramonium

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Datura stramonium is a miraculous Ayurvedic herb used in the treatment of innumerable ailments. This study deals with the three isolated compounds (Daturalactone (D1), 12 deoxywithastramonalide (D23) and Daturilin (D27) from D. stramonium leaves which were analyzed for their immunostimulatory activity. Human peripheral blood mononuclear cells (PBMC) model was used to study immunomodulatory activity of lactones invitro. The immunoenhancing potential of lactones was validated by analyzing cytokines release, cell surface markers (CD3, CD8, and CD56) and intracellular granulysin levels. Micro cytotoxicity assay was performed by co-culturing lactones activated lymphocytes and target cells (colon cancer cell lines; HCT-116 and SW620 and pancreatic cancer cells; Panc-1). ROS generation and mitochondrial perturbation were also analyzed in cocultured target cells to demonstrate cell death. The data revealed that the lactones significantly enhanced the proliferation of the human lymphocytes and increased the secretion of IL-2, IFN-γ and TNF-α. These lactones significantly up-regulated the expression of CD3, CD8, and CD56 while, an increased intracellular granulysin (immunomarker for activated CTLs and NK cells) expression was found in activated immune cells. The data revealed that lactones preactivated human lymphocytes exhibited enhanced target cells killing. D27 treated lymphocytes showed higher cancer killing potential as compared to D1 and D23 activated immune cells at 20:1 effector:target ratio. Additionally, mechanistic studies showed that lactones activated lymphocytes enhanced ROS generation and mitochondrial damage in target cancer cells. The data revealed an immunopotentiating effects of lactones on human lymphocytes via enhancing their killing capabilities of cancer cells.

Keywords: Cell death, drugs for immune modulation, immunotherapy, NK cells, cytokines and mediators

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POSTER PRESENTATIONS

P-0138

Immunotherapy combining allergen with low-dose IL-2 to generate antigen specific regulatory T cells

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Tregs play a central role in the control of allergic reaction. As a result, we developed a Treg-based immunotherapy and proved the therapeutic potential of low-dose IL-2 (ld-IL2) in a food allergy model. We are currently investigating the role of antigen-specific Tregs in the long-term protection and we aim to optimize our therapy by increasing selectively the allergen-specific Tregs after IL2 and allergen combination. To identify the best administration settings of ld-IL2 and antigen, we transferred OVA-specific TCR-transgenic CD4+ T lymphocytes, including both Tregs and non-Treg (Tconv) cells into non-TCR transgenic mice, and determined how the route, dose and timing of administration of OVA affect these two populations when combined with ld-IL2. Optimal identified strategy was then evaluated for its therapeutic efficacy in OVA-specific food allergy model. The kinetics of IL-2 and OVA administration influences directly the Treg and Tconv compartments. We show that administration of OVA before the initiation of ld-IL2 treatment favors the OVA-specific Treg expansion with almost no stimulation of the OVA-specific Tconv. This is not the case when we give OVA and ld-IL2 simultaneously. Importantly, this regimen of administration accelerates the immune protection against allergic reactions after per os OVA challenges in sensitized mice. The combination of allergen and ld IL-2 could be a significant improvement of allergen-specific immunotherapy efficacy. We are currently investigating the related mechanisms of this combo-therapy, including on the Treg biology.

Keywords: Animal models, immune regulation and therapy, immunotherapy

P-0221

BTLA/HVEM axis induces NK cell immunosuppression and poor outcome in chronic lymphocytic leukemia

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Immunosuppression is a hallmark of patients with chronic lymphocytic leukemia (CLL). Despite the encouraging results on immune checkpoint blockade-based therapies, no clinical benefits have been observed in CLL. Herein, we explore BTLA/HVEM axis, a novel immune checkpoint which has emerged as a powerful tool to reinvigorate anti-tumor responses. BTLA/HVEM expression was evaluated on leukemic and NK cells in samples from patients with CLL and healthy donors by flow cytometry. In silico analysis from publicly available data were employed to analyze *BTLA* and *HVEM* mRNA in CLL. Sera levels of soluble BTLA (sBTLA) were evaluated. Finally, blocking anti-BTLA monoclonal antibody (mAb) (Genentech) was employed to determine the relevance of BTLA/HVEM axis in leukemic cell count, cytokine production and NK cell-mediated anti-tumor responses by flow cytometry and calcein-AM assay. BTLA expression was upregulated on leukemic and NK cells from patients with CLL, whereas HVEM downregulation was observed on leukemic cells, correlating with diminished overall survival. Increased BTLA surface expression on NK cells was associated with poor outcome. sBTLA was increased in serum from patients with CLL and highly correlated with poor prognostic markers and shorter time to treatment. Finally, BTLA blockade restored, at least in part, NK cell-mediated anti-tumor responses, promoting leukemic cell depletion, IFN-γ production and cytotoxicity. Overall, this study brings to light the role of BTLA/HVEM axis in NK cell-mediated immune responses suppression in CLL and its impact on patient's prognosis, suggesting that BTLA/HVEM axis may be a potential therapeutic target in this disease.

Keywords: Antibody, cancer immunology, checkpoint inhibition

P-0251

Targeting intracellular inhibiting proteins DOK1 and DOK2 to improve CD8+ T Cell immunotherapy

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Cancer immunotherapy strategies targeting cytotoxic lymphocytes (CD8+/NK cells) show high potency. However, the majority of patients do not respond to available immunotherapies. New methods to improve T-cell based therapies of cancer are required. Our team previously showed that targeting intracellular inhibiting proteins is a promising strategy to improve CD8+ T cell anti-tumor efficacy. DOK-1 and DOK-2 proteins are two inhibiting proteins in the signaling cascade of T-cell receptor complex signaling. Therefore depletion of intracellular inhibition checkpoint DOK-1/2 could improve CD8+ T-cell based cancer therapies. To evaluate the role of DOK-1/2 depletion in physiology and effector function of lymphocyte T CD8+ and in cancer progression we have established a transgenic T cell receptor mouse model specific to melanoma antigen hgp100 in WT and DOK-1/2 KO mice. We have shown that DOK-1/2 depletion doesn't affect the development, proliferation, mortality, activation and cytotoxic function of naive CD8+ T cells. However after *in vitro* pre-stimulation DOK-1/2 KO CD8+ T cells had higher percentage of effector memory T cells as well as up-regulation of T cell receptor signaling cascade, including the increased levels of pAkt and pErk, two major phosphoproteins implicated in T cell cytotoxic activities and activation. However we didn't notice the improvement of CD8+ T-cell cytotoxic activities *in vitro*. Since *in vitro* tests don't represent the complexity of interaction between effector and target cells the tests in animal models are necessary. Further investigation in *in vivo* models would reveal if DOK-1/2 proteins could be used as a tool of T-cell immunotherapies potentiation.

Keywords: Cancer immunology, cell signalling, immunotherapy, molecular immunology

P-0253

Optimization of antibody-linked to immunoregulatory cytokines: *in vitro* and *in vivo* evaluation

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Immunoregulatory cytokines provide a significant therapeutical potential in many pathologies. However, their application is narrow because of their off-target actions leading to important side effects (systemic toxicity). To circumvent this matter, immunocytokines, resulting from the fusion of antibody and cytokine, allow a targeted delivery of the biomolecules and are being considered in the treatment of auto-immune disease. In this context, we aim to develop new biomolecules named Nb-cytokine by combining nanobodies (Nbs, single-chain antibodies) to a cytokine. Given its roles during inflammation acting on the inflammasome, T cell and myeloid cells, we chose P2X7 receptor as a working prototype using 7E2, an anti-P2RX7 Nb developed in the laboratory. We then conjugated 7E2-Nb to the immunoregulatory IFNβ with different linker types and lengths to generate several 7E2-IFNβ immunocytokines. The functionality and the specificity of each candidate are analyzed *in vitro* using cellular models expressing P2X7 receptors and/or IFN receptors. The selection is made on: (i) their ability to activate the IFNβ signaling pathway through flow cytometry and ii) their capability to inhibit cell proliferation. The selected 7E2-IFNβ immunocytokines will be further evaluated *in vivo* in models of multiple sclerosis. This work will provide a proof of concept that the optimization of such biomolecules will provide new therapeutical perspectives for a large spectrum of inflammatory and autoimmune pathologies.

Keywords: Animal models, autoimmunity, cytokines and mediators, engineering of antibodies and nanobodies, immunotherapy, multiple sclerosis

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POSTER PRESENTATIONS

P-0270

Relationship between genes and mirnas associated with the apoptotic pathway relevant to breast cancer

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The latest report by the World Health Organization (2020), confirms that breast cancer is the most prevalent in the worldwide female population; it also denotes the need for preventive measures such as molecular screening and identification of biomarkers. Points that can be explored in the analysis of genes and their respective microRNAs of the signaling pathways referring to *Hallmarks of cancer*. This study aims to identify new genetic targets, associated with the apoptotic pathway. Therefore, future *in vitro* studies may prove their importance as biomarkers. Molecular targets were selected through combined bioinformatics analysis based on the crossing of data from specific public banks: The Cancer Genome Atlas and mirWalk. The analysis was performed using algorithms written in the R language, executed through the IDE R Studio. The results were presented through the *GGCorPlot* graph, whose objective is to identify correlations between genes-miRNAs differentially expressed in the pathway. The genes chosen in a previous study were *BIRC5*, *LMNB1* and *TUBA1C*. Which are upregulated with high statistical significance and have positive correlations with each other. From them, the miRNAs of the current analysis were selected: *hsa-mir-133b*, *hsa-mir-665*, and *hsa-mir378c*. These were present in the graphs relative to the three genes and have important physiological action. The current analysis continues the previous one, so we are not only more certain of the role of the 3 genes as *gene signature*, but we also get closer to *in vitro* analysis aiming at validating the action of these and their miRNAs as biomarkers.

Keywords: Big data, biomarkers, cancer immunology, cell death, miRNA

P-0346

Interleukin-1 Receptor Associated Kinases (IRAK) as therapeutic target in chemo-resistant head and neck cancer

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Head and neck cancer is the 6th most common cancer worldwide. Induction chemotherapy with Docetaxel, Cisplatin and 5-Fluorouracil (5-FU), known as TPF-triplet-regimen is commonly used for the treatment of the cancer. However, acquired chemo-resistance leads to high mortality rate. Cytotoxicity upon different treatments lead to generation of various DAMPs from cancer cells which activates TLR signaling. TLR signaling engages Interleukin-1 receptor associated kinases –IRAKs and their phosphorylation which drives downstream signaling and activation of various transcription factors eventually inducing tumor survival and proliferation. Pro-oncogenic role of IRAK is reported in various solid tumors and also confer resistance to paclitaxel in breast cancer. Based on these reports, we evaluate the status of IRAK in chemoresistant head and neck cancer. We developed a TPF resistant cell line using human laryngeal carcinoma cell line HEp-2 by subjecting it to low concentrations of combination of the three chemodrugs. We observed overexpression of IRAK-1 and IRAK-4 and various markers for cancer stem cells (CSCs), proliferation, pro-survival, EMT and inflammation in the chemoresistant cell line. Pharmacological inhibition of IRAK-1 and 4 using small molecule based IRAK inhibitor in combination with the individual chemodrugs at sub-optimal concentrations increased the cytotoxicity of the chemoresistant cell line and downregulated the expression of these markers significantly. Results suggest IRAK overexpression as a biomarker and potential therapeutic target of chemoresistant head and neck cancer. Combination therapy of chemodrugs and IRAK inhibitor is active against resistant head and neck cancer and could replace molecularly targeted inhibitor treatments toxic to normal cells.

Keywords: Biomarkers, cancer immunology, cell death, immunotherapy

P-0431

Development of engineered transgenic TCR targeting SALL4 for cancer immunotherapy

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Splat like transcription factor 4 (SALL4) is a stem cell transcription factor frequently expressed in human cancers and plays an important role in the development, progression and drug resistance of cancer cells. To develop an engineered transgenic TCR directed against SALL4 positive cancer cells for adoptive T cell-based cancer immunotherapy. T cell epitope prediction algorithms were used to design SALL4-derived peptides binding to HLA-A2. SALL4 specific T cell responses were studied in cancer patient's peripheral blood using IFNγ ELISpot assay. After *in vitro* repetitive stimulation of HLA-A2$^+$ PBMCs with SALL4-derived peptides, SALL4 specific CD8$^+$ T cell clones and SALL4-TCR transduced peripheral blood lymphocytes were evaluated for functional specificities. The natural presentation of SALL4-derived peptides was validated using SALL4$^+$ HLA-A2$^+$ cancer cell lines. SALL4 specific responses against MHC-I restricted peptides were detected in long standing cancer patient's peripheral blood but not in healthy donors. These specific responses were directed against SALL4-derived S9V peptide. CD8$^+$ T cell clone specific to S9V peptide cytotoxicity was linked to IFNγ and Granzyme B secretion. S9V-specific T cell clones and genetically modified T cells expressing transgenic TCR were able to kill SALL4$^+$ tumor cell lines naturally processing S9V peptide. SALL4-derived S9V peptide is immunogenic and naturally processed by tumor cells. SALL4-specific engineered T cells showed reactivity against HLA-A2$^+$ tumor cell lines expressing SALL4. Altogether, these results suggest that adoptive cell therapy using engineered SALL4-specific transgenic TCR holds considerable promise for future cancer immunotherapy.

Keywords: Cancer immunology, cell based therapies, immunotherapy

P-0465

Effect of anti-Notch 1 neutralizing antibody on the activity of human and mouse osteoclast progenitors stimulated by Notch-ligands DLL1 and JAG1

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Bone loss associated with rheumatoid arthritis (RA) is caused by enhanced osteoclast differentiation and function. We aimed to determine the Notch receptor profile on human and mouse osteoclast progenitors (OCP) and the effect of Notch receptor signaling inhibition on OCP activity in murine collagen-induced arthritis (CIA) and human RA samples. Peripheral blood was collected from RA patients. Periarticular bone marrow (PBM) and spleen (SPL) were harvested from mice with CIA, additionally treated by i.p. injections of anti-Notch 1 neutralizing antibodies (1 mg/kg). FACS sorted OCPs were stimulated by osteoclastogenic factors (M-CSF/RANKL), in Jagged (JAG)1 or Delta-like (DLL)1 coated wells, with or without anti-Notch 1 neutralizing antibodies. The research was approved by the Ethics Committee. Both mouse (CD45+CD11blo/+CD115+CCR2+ or CCR2lo) and human (CD45+CD11b+CD14+CCR2+) OCPs express Notch receptors, with Notch 1 and 2 being the most abundantly detected. *In vitro* stimulation with DLL1 significantly increased, while stimulation with JAG1 significantly decreased osteoclastogenesis. The addition of anti-Notch 1 to ligand-stimulated OCPs resulted in an increased number of TRAP+ osteoclasts, partially reversing JAG1 inhibition. Both PBM and SPL OCPs from mice *in vivo* treated with anti-Notch 1 produced a higher number of TRAP+ osteoclasts, with increased expression of osteoclast differentiation genes. Our results confirm that Notch signaling regulates osteoclast differentiation, with DLL1 exhibiting a stimulatory, and JAG1 a suppressive role. Anti-Notch 1 neutralizing antibodies partially reversed suppression by JAG1, proposing Notch 1/JAG1 pathway as a possible therapeutic target for the regulation of osteoclast activity in arthritis.

Funding: IP-2018-01-2414, UIP-2017-05-1965, DOK-2018-09-4276

Keywords: Animal models, autoimmunity, cell signalling, myeloid cells, rheumatoid arthritis

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POSTER PRESENTATIONS

P-0487

The antitumor activity of therapy composed of methotrexate nanoconjugate and dendritic cells with downregulated IL-10R and its influence on local antitumor immune response

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To achieve satisfactory therapeutic effects, a comprehensive approach in anti-cancer therapy needs to be applied. For this reason prior the beginning of the immunotherapy, in order to modulate the tumor microenvironment (TME) methotrexate nanoconjugate (HES-MTX) were administered. Moreover, to reduce the unfavorable effect of IL-10 present in TME, the DCs were genetically modified for downregulation of IL-10 receptor expression (DC/shIL-10R) on their surface. These combined therapy was applied in murine colon carcinoma (MC38) tumor model. For this purpose, mice with subcutaneously growing MC38 tumor intravenously received HES-MTX and three days later, immunotherapy started (multiple peritumoral injections of mature DCs with downregulated IL-10R). Genetically modified DCs were obtained by transduction with lentiviral vectors encoding shRNA silencing IL-10R expression and next DCs were stimulated with tumor antigen-lysate (TAg). To determine the therapeutic effect of applied therapy, the tumor growth were monitored and after seven days form the last DC/shIL-10R/TAg vaccine, the tumor nodules from mice were collected for further *ex vivo* analyzes. With the use of multiparametric flow cytometry analyses the alterations in percentage of tumor infiltrating lymphocytes and myeloid cells were evaluated. The results demonstrated that therapy with HES-MTX and DC/shIL-10R/TAg, contributed to significant tumor growth delay. Moreover, only in HES-MTX+DC/shIL-10R/IL-10R group the considerable increase in the percentage of effector immune cells (CD4, CD8, NK and NKT cells) infiltrating in tumor tissue were observed. This was accompanied by reduced infiltration of immune cells with suppressor activity (Treg, Mfs TAM, MDSC).

The study was funded by National Science Centre (project no. 2015/19/N/NZ6/02908).

Keywords: Anti-cancer vaccine, dendritic cells, drugs for immune modulation, immunotherapy, *in vivo* tumor models

P-0500

DeadEnds: a protein contaminant repository preventing you from chasing down blind alleys in your proximity-biotinylation interactomics

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Proximity-biotinylation techniques (PBTs) are becoming an increasingly popular approach for the identification of protein-protein interactions *in vivo*. Compared to other, non-proximity-based methods, PBTs coupled to mass spectrometry allow a more inclusive approach for the detection of interactions, yielding extensive sets of potential protein hits. However, differentiating between the *bona fide* protein interactors and background proteins, which are obtained due to e.g. their inherent biotinylation status, has proven to be challenging. Fortunately, the experimental setups of PBTs are largely similar across experiments and hence offer a possibility to generate databases of background proteins, which aid in future data analysis. Here, we present the generation of DeadEnds, a repository, which incorporates the data from multiple proximity-biotinylation experiments and scores protein hits based on their likelihood of being background contaminants. The scoring matrices can be adapted to the biological system used and offer an improved way of differentiating between genuine hits and protein contaminants.

Keywords: Big data, cell signalling, immunological techniques

P-0530

Photon correlation spectroscopy analysis of circulating immune complexes in rheumatoid arthritis

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Immune complexes (IC) functions are dependent on their physicochemical characteristics, such as colloidal particle size, and stability. Nevertheless, IC particles' size has not been widely studied, primarily due to methodological limitations. Rheumatoid arthritis (RA) is IC-mediated disease, and in this study, we analyzed circulating IC (CIC) particles' size in RA using photon correlation spectroscopy, the method used for protein aggregates detection in the preparations of therapeutic antibodies. CIC were isolated by PEG precipitation from blood serum of 32 RA patients and 32 (age and sex matched) healthy individuals. The CIC level (PEG precipitates OD350nm) for RA and control group was 0.600±0.450 and 0.450±0.380. Hydrodynamic diameter of CIC colloidal particles was analyzed with Zetasizer Nano ZS (Malvern Instruments, UK, software version 7.03). The measurement of each CIC sample was repeated ten times and mean particle diameter/light scattering dependency curves for RA and control groups were constructed. In CIC of control group, we identified 10 particle types (1/10/18/44/79/122/295/459/825/1990 nm), where the strongest light scattering signals were obtained from 79 and 825 nm particles. Multimodal size distribution of CIC in RA revealed 12 particle categories (2/4/7/12/18/33/51/122/342/615/955/1484 nm), where the dominant components had a diameter of 33, 51, 122, and 615 nm. The only mutual with CIC of healthy donors were particle fractions of 18 and 122 nm. Whether observed RA CIC particle size distribution is of significance for the onset and development of the disease and whether it can be used as a disease biomarker remains to be analyzed.

Keywords: Adaptive immunity, antibody, inflammatory joint diseases, rheumatoid arthritis

P-0541

IL-21R blockade reduces atherosclerosis development in young female LDLr-/- mice

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Aging is the most prominent driver of cardiovascular disease (CVD) and has a major impact on the immune system. Several pro-inflammatory cytokines increase with age, such as IL-21. IL-21 binds to the IL-21 receptor, which is expressed on most lymphoid and myeloid cells. Downstream effects of IL-21 signaling are target-cell-specific. IL-21 has stimulatory effects on B cells and most CD4+ T cells and inhibitory effects on regulatory T cells (Tregs). Whether IL-21/IL-21R signaling contributes to atherosclerosis, the main underlying cause of CVD, remains to be elucidated. In this study, we therefore aim to provide further insight into the role of IL-21 on atherosclerosis development by blocking the IL-21/IL-21R axis. Young (~12 weeks) female LDLr-/- mice were fed an atherosclerosis-inducing western-type diet for five weeks, during which mice received 200µg IL-21R blocking antibody (n=15) or 200µg isotype control (rat IgG2a, n=15) three times a week intraperitoneally. IL-21R blockade significantly reduced atherosclerosis development by 38%. This coincided with increased atheroprotective CD4+FoxP3+ Tregs in aortic plaques (MFI αIL-21R: 931.9±35.4 vs ctr: 826.6±22.5, p<0.05), spleens (αIL-21R: 14.0±0.5% vs. ctr: 12.2±0.3%, p<0.01) and HLN (αIL-21R: 22.0±1.1% vs. ctr: 18.8±1.1%, p<0,05). Similarly, increased anti-inflammatory IL-10 was observed in serum (αIL-21R: 617.6±156.9 vs ctr: 36.0±29.9 pg/mL, p<0.001) and culture supernatant from splenocytes (αIL-21R: 64.9±10.2 vs ctr: 38.6±5.4 pg/mL, p<0.05) of αIL-21R-treated mice. Collectively, we show that IL-21R blockade reduces atherosclerosis by promoting atheroprotective regulatory T cell immunity and elevating anti-inflammatory IL-10 production, thereby representing a promising novel therapeutic strategy to extend health span and to combat CVD.

Keywords: Ageing, cardiovascular diseases, cytokines and mediators, immunotherapy

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POSTER PRESENTATIONS

P-0587

Enhancing the immunomodulatory potential of MSC-derived extracellular vesicles for therapeutic applications through cytokine licensing

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The immunosuppressive potential of mesenchymal stromal cells (MSCs) is thought to be mediated in part through their secretion of extracellular vesicles (MSC-EVs). MSC therapeutic efficacy can be enhanced with pre-activation or 'licensing' with stimuli, which alter their secretion and surface expression of immunosuppressive factors. The aim of this work was to assess changes in MSC-EV cargo and functionality following licensing with IFNγ or TGF-β1, compared to control MSC-EVs. Murine BALB/c MSCs were licensed with 50ng/ml IFNγ or 25ng/ml TGF-β1, or were cultured in growth medium alone. Conditioned medium was collected after 72h and EVs were isolated by size exclusion chromatography. Changes in MSC-EV surface profile was analysed by flow cytometry, and compared to that of the parent cells. Nanoparticle size and concentration was determined by nanoparticle tracking analysis. MSC-EVs were then cultured with LPS-stimulated macrophages to evaluate their uptake and potential to suppress expression of pro-inflammatory markers. TGF-β1, but not IFNγ, licensed MSCs exhibited higher levels of EV secretion compared to controls. Licensing induced expression of the immunoinhibitory protein PD-L1 on IFNγ-MSC-EVs, and increased CD73 expression on TGFβ-MSC-EVs, showing a similar profile to the parent cells. MSC-EVs were efficiently internalised by macrophages, as demonstrated through fluorescent-labelling of the nanoparticles, and suppressed the expression of TNFα in LPS-stimulated macrophages, with TGFβ-EVs being more potent compared to control EVs. MSC-EVs represent a novel cell-free therapeutic strategy for the treatment of inflammatory conditions, and different licensing strategies may be more suitable for the treatment of specific conditions depending on their mechanism of action.

Keywords: Cell based therapies, cell signalling, cytokines and mediators, immune regulation and therapy

P-0590

The atlas of inflammation resolution: a platform for data-driven analyses of acute inflammatory phenotypes

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Acute inflammation and its resolution is a non-linear spatial-temporal process involving several cell types and numerous regulatory molecules interacting in a coordinated fashion. The non-linear interactions among components pose a big challenge to investigate underlying mechanisms for the identification of diagnostic/therapeutic markers. We present the Atlas of Inflammation Resolution (AIR), which is a web-based comprehensive collection of information about cells, molecules and their interactions in acute inflammation (https://air.bio.informatik.uni-rostock.de). As of now, the AIR contains 20 manually curated submaps with >20000 interactions of signaling pathways associated with acute inflammation initiation, transition, resolution and homeostasis. Additionally, the AIR is enriched with regulatory layers of microRNAs, lncRNAs and transcription factors building a large molecular interaction map (MIM). The directed structure of the MIM enables the networks for in silico perturbation experiments directly on the web interface. In order to support the identification of diagnostic/therapeutic markers, we recently developed a suite of plugins for the AIR. The plugins provide functionalities including (i) mapping experimental and clinical data onto the MIM to identify potential regulators; (ii) enrichment analysis; (iii) mapping of human phenotype ontologies; (iv) in silico perturbation experiments; (v) deciphering the causal relationship of molecules to phenotypes; (vi) integration and visualization of gene variant information. With the newly developed plugins, the AIR becomes more than a knowledge base, enabling users to perform bioinformatics and systems biology based analyses. Our goal is to support the research community to formulate and validate hypotheses, combining data-driven modeling and model-driven experiments.

Keywords: Immune networks, immune response tracing, inflammatory disease, innate immunity, modelling, visualizing immune responses

P-0601

Galectin-1 expression by regulatory T cells is required for sustained modulation of chronic colitis in regulatory T cell-based therapy

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Cell-based therapy with FOXP3$^+$CD4$^+$CD25$^+$ regulatory T cells (Tregs) have been proposed in the recent years thanks to their potential to maintain homeostasis and capacity to control unwanted immune responses. Galectin-1, a β-galactoside-binding protein, exhibits broad anti-inflammatory and pro-resolving activities by targeting multiple immune cell types. Previous data from our group found that galectin-1 is expressed by human and mouse regulatory T cells and contributes to their immunosuppressive function *in vitro*. The aim of the study was to further investigate the role that galectin-1 plays in Treg cell function. We tested the ability of *Lgals1*$^{-/-}$ CD4$^+$CD25$^+$Foxp3$^+$ regulatory T cells (*Lgals1*$^{-/-}$ Tregs) to suppress gastrointestinal immune responses using a T-cell transfer model of experimental colitis. *Lgals1*$^{-/-}$ Tregs showed reduced immunomodulatory capacity compared to *wild type* Tregs, as suggested by the increase number of *Lgals1*$^{-/-}$ Tregs required to modulate intestinal inflammation induced by CD45Rb$^+$CD4$^+$ naïve T cells into *Rag-1*$^{-/-}$ mice. Strikingly, long term survival of those mice treated with *Lgals1*$^{-/-}$ Tregs was compromised with respect to mice treated with *wild type* Tregs mainly due to a systemic loss of *Lgals1*$^{-/-}$ Tregs. This was accompanied by an increase of IL-17$^+$ expressing CD4$^+$ T cells in colon lamina propria and spleen. These evidences highlight that a correct expression of galectin-1 is relevant in Tregs mediated regulation of chronic colitis in order to achieve sustained protection in the long term. An adequate expression of galectin-1 should be taken into consideration in future cell-based therapy protocols with regulatory T cells.

Keywords: Adaptive immunity, autoimmunity, cell based therapies, immune regulation and therapy, inflammatory bowel disease, regulatory cells

P-0611

The choice of stimulation method affects the lifespan and the number of NK cells modified by the catalytic telomerase subunit gene

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Catalytic telomerase subunit (hTERT) restores telomeric repeats. Insertion of additional copies of hTERT can help to expand NK cells *in vitro* for immunotherapy. However, we have shown earlier that simple transduction of this gene does not induce the long expansion of NK cells. In this work, we explored the possibilities for activating hTERT-overexpressing NK cells with IL-2 or with a combination IL-2 and IL-21-expressing K562 feeder cells to induce prolonged proliferation. Preliminary activated NK cells were transduced with GFP-P2A-hTERT-containing retroviral vector with RD114 envelope protein. Next, transduced NK cells and non-transduced controls were cultivated with 100 U/ml IL-2 added every 3 days. The dynamics of cell number were similar in both cultures during the first two weeks, but with further cultivation hTERT+ NK cells began to outnumber non-transduced controls. The lifespan of hTERT-NK cells comprised 3 months compared to 2 months for the non-transduced cells. Alternatively, transduced NK cells were sorted by GFP reporter expression into hTERT+ and hTERT– 4.5 weeks after the transduction, then added with feeder cells and cultivated as described above. Both hTERT+ and hTERT– NK cells expanded within the first two weeks, compared to the cells cultured with IL-2 alone, but the expansion of hTERT+ NK cells was greater. hTERT– NK cells died after 3.5 months, while hTERT+ NK cells proliferated up to 10 months. Thus, the addition of K562-mbIL21 feeder cells after transduction significantly increased the intensity and duration of hTERT+ NK cells proliferation.

This work was supported by the Russian Science Foundation # 21-74-30016.

Keywords: Cancer immunology, immune regulation and therapy, immunotherapy, innate lymphoid cells, molecular immunology, NK cells

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POSTER PRESENTATIONS

P-0630

A billion synthetic 3D-antibody-antigen complexes enable unconstrained machine-learning formalized investigation of antibody specificity prediction

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Machine learning (ML) is a key technology to enable accurate prediction of antibody-antigen binding, a prerequisite for *in silico* vaccine and antibody design. Two orthogonal problems hinder the current application of ML to antibody-specificity prediction. (i) The lack of a formalized mapping of antibody specificity prediction problems from immunological to ML notation and (ii) the unavailability of large-scale antibody-antigen binding datasets. To address the missing-data problem, we developed the *Absolut!* software suite that allows the parameter-based unconstrained generation of synthetic lattice-based 3D-antibody-antigen binding structures with ground-truth access to conformational paratope, epitope, and affinity. We have created an online database of 1 billion antibody-antigen structures, the extension of which is only constrained by moderate computational resources. We translated immunological antibody specificity prediction problems into ML tasks and used our database to investigate paratope-epitope binding prediction accuracy as a function of structural information encoding, dataset size, and ML method, which is unfeasible with existing experimental data. In summary, the Absolut! framework enables the development and benchmarking of ML strategies for biotherapeutics discovery and design.

Keywords: Antibody, modelling, omics technologies

P-0632

Design of a CD19-specific chimeric antigen receptor based on a monoclonal antibody LT19

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CAR T-cell therapy is a powerful approach for treating patients with B cell malignancies including r/rALL, CLL, and NHLs. Not all patients are known to respond to CAR-T therapy, and many responding patients may still relapse due to antigen escape and/or poor CAR-T cell persistence. To address these issues and expand the repertoire of targetable epitopes, we set out to design a CAR based on a novel CD19-specific monoclonal antibody LT19. We designed lentiviral constructs encoding two LT19-based second-generation CARs with antigen-recognition modules composed of either Vh-Vk or Vk-Vh sequences. Surface expression of CAR-encoding constructs was confirmed by FACS, as well as by the ligand-specific activation assay based on the up-regulation of CD69. *In vitro* cytotoxic activity of LT19-CAR-T against CD19-expressing HEK293T target cells was measured by FACS and iCelligence RTCA system We show that both variants of LT19 CAR-T cells obtained demonstrate pronounced *in vitro* cytotoxicity against CD19+ targets. Additional experiments and LT19 epitope mapping are in progress. Preliminary data indicate that LT19 and FMC63, which is used in the four FDA-approved CAR-T cell products, have distinct epitopes. Thus, LT19-based CARs may be considered for applications where the performance of FMC63-based CARs will likely be suboptimal. We also believe that the information obtained in our project will guide the design of bi-specific CARs.
This study was supported by the RFBR grants #20-415-540024, #18-29-07025

Keywords: Cancer immunology, cell based therapies, immunotherapy

P-0822

In situ anti-tumor immunization using the tumor microenvironment reprograming with a TLR4-agonist induces strong CD4 and CD8 T cells responses, long-living T cell memory, and protection against 4T1 metastatic breast cancer in mice

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To estimate tumor immunotherapy efficacy with the combination of a tumor surgical resection and the microenvironmental macrophage/dendritic cells reprogramming into their anti-tumor state using TLR4-activating signaling. BALB/c mice were subcutaneously inoculated with 15,000 4T1 cancerous cells. Surgical resection of the primary tumor was performed on day 11. Pharmaceutical TLR4-agonist Immunomax (14 μg) was injected every 4 days. Tumor-reactive IFNγ-secretory T cells were counted using ELISPOT. Sorted CD8 effector T cell cytotoxicity was measured in co-culture with 4T1 cells. 20-30% of mice with the deadly metastatic disease recovered upon a combination of the primary 4T1 tumor resection and immunotherapy with TLR4-agonist. Following the treatment, the complete responder mice developed CD4 T cells and CD8 T cells responding to 4T1 tumor antigens by secretion of IFNγ and capable of killing 4T1 tumor cells. The repeated inoculation of the 4T1 tumor cells into complete responders did not induce any tumor growth in 50%. Significant numbers of CD4 T cells that respond to 4T1 tumor antigens, as well as CD8 T cells that kill 4T1 tumor cells were found in the tertiary tumor and draining lymph node. T cell-mediated memory in complete responder mice persisted for 260 days post-treatment (day of observation). Macrophage/dendritic cell reprogramming with the TLR4-agonist for the post-resectional immunotherapy of 4T1 cancer metastatic disease induces tumor-specific T cell responses and T cell-mediated long-living immune memory.
This research was supported by the Russian Science Foundation (project no. 20-15-00391).

Keywords: Microenvironment, cancer immunology, immunotherapy, *in vivo* tumor models, memory, protection

P-0840

Engineering of anti-hPD-1 and anti-hPD-L1 into caninized mAbs with specific IgG1 and IgG4 canine constant regions by MOE-PCR Ligation-Independent Cloning (LIC)

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Anti-cancer immunotherapy in dog using mAbs check-point inhibitors against PD-1 and PD-L1 is far behind the equivalent application in humans. To address this limitation, we generate of caninized anti-PD-1 and anti-PD-L1 mAbs with specific canine IgG1 and IgG4 constant regions. We used EMBOSS Needle webtool to evaluate the identity of human and dog PD-1 and PD-L1 protein sequences. Moreover, we evaluated the binding efficiency of human APC-labelled therapeutic mAbs targeting PD-1 (Pembrolizumab, Nivolumab and Cemiplimab) or PD-L1 (Atezolizumab, Avelumab, and Durvalumab) was evaluated on the canine macrophage-like cell line DH82, and on the human monocytic cell lines THP1 and U937 as controls by flow cytometry. Using the multiple overlap extension PCR (MOE-PCR) LIC strategy we generated canine versions of the human anti-PD-1 Pembrolizumab and anti-PD-L1 Atezolizumab mAbs. Pairwise sequence alignment analyses showed: 1) 66.2% identity between human-PD-1 and canine-PD-1 (Uniprot Q15116 vs. A0A024FCJ9, respectively), 2) 75.7% identity between human-PD-L1 and canine-PD-L1 (Uniprot Q9NZQ7 vs. E2RKZ5 respectively). The APC-labelled anti-hPD-1 Pembrolizumab and anti-hPD-L1 Atezolizumab showed the highest binding efficiency in FACS experiments on canine macrophage cell line DH82. The MOE-PCR LIC strategy created a pVitro1 plasmid with human variable heavy and light chain sequences and canine constant IgG1 or IgG4 heavy and κ light chain (by IMGT). We show a new strategy to produce caninized anti-PD-1 and anti-PD-L1 mAbs that promise to fill the gap of available checkpoint inhibitors for dog anti-cancer therapies.

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Keywords: Antibody, cancer immunology, checkpoint inhibition, engineering of antibodies and nanobodies, immunotherapy, veterinary immunology

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POSTER PRESENTATIONS

P-0842

Small polymeric nanoparticles drive potent anti-tumour immunity

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It is estimated that 1 in 2 people will develop cancer in their lifetime, highlighting the urgent need for improved preventative and therapeutic strategies. Cancer immunotherapy has emerged as a key pillar of cancer treatment and in this context, cancer vaccines have shown significant promise. Cancer vaccines have the potential to induce long-lasting anti-tumour immunity through the cross-priming of cytotoxic T lymphocytes (CTLs) and T helper 1 (Th1) cells. However, the development of such vaccines has been hindered by a lack of adjuvants that effectively drive cell mediated immunity. We have found that polymeric nanoparticles are powerful adjuvants for inclusion in anticancer vaccines owing to their capacity to drive potent antigen-specific CTL and Th1 responses and the magnitude of these responses is critically dictated by adjuvant particle size. Here we aimed to demonstrate the efficacy of the adjuvant nanoparticles in anticancer vaccines, and identify immune correlates of vaccine induced tumour protection. Utilising a murine preclinical tumour model, involving prime boost vaccination prior to B16 melanoma challenge, we have demonstrated that nanoparticles induced potent anti-tumour immunity, diminishing tumour establishment and growth, leading to complete survival from tumour challenge. $CD8^+$ T cells and to a lesser extent IFNγ were shown to be essential mediators of this protection, demonstrated through cell depletion studies and the use of KO models respectively. These results demonstrate the potent anti-tumour functionality of immune responses induced by polymer nanoparticles, highlighting their potential use as adjuvants in cancer vaccination strategies.

Keywords: Adaptive immunity, adjuvants and vaccines, anti-cancer vaccine, cancer immunology, immunotherapy, *in vivo* tumor models

P-0854

Optimization of the method for generating human monoclonal antibodies from peripheral blood of vaccinated donors on the model of Hepatitis B Virus

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The monoclonal antibodies are rapidly growing class of therapeutic molecules for treating a broad specter of human diseases. The human antibody repertoire represents a large source of potential therapeutic antibodies. Human antibodies against specific antigens are produced by affinity-matured B cells, found in fresh blood of immunized individuals at the peak of their humoral immune response, due to disease or vaccination. The goal of our project was to develop an efficient and simplified strategy with available laboratory equipment to generate antibodies from isolated single B cells (plasma cells and memory B cells from peripheral blood mononuclear cells (PBMCs)) from blood of vaccinated donorsagainst hepatitis B virus surface antigen (HBsAg) by recombinant technology. The main focus was on isolation of plasmablasts and memory B cells that express surface B cell receptors (BCRs) for our chosen antigen with flow cytometry. Due to low frequencies of antigen specific B cells among PBMCs we used magnetic negative selection kit to enrich B cells before performing flow cytometry. We established flow cytometry protocol based on selecting B cells with surface markers CD19, CD27, IgG and BCR specific for HBsAg and sorted cells into single cell plates. On sorted cells we performed single cell RT-PCR and nested PCR on sorted to amplify variable regions of antibody genes, to clone them into expression vectors and to transfect them into human cell lines. This approach can be used to rapidly generate human monoclonal antibodies against various antigens in short time with reasonable budget.

Keywords: Antibody, B lymphocytes, Memory

P-0869

Liposomal vaccines targeting to human and mouse CD169+ antigen presenting cells activate strong T and B cell responses

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CD169-expressing macrophages are present in lymphoid organs at the site of antigen entrance to capture pathogens. Previously, we have shown that these CD169+ macrophages efficiently transfer antigen (Ag) to cross-presenting dendritic cells and B cells and thereby stimulate strong CD8+ T cell and antibody responses. CD169, also known as siglec-1, is a sialic-acid binding lectin which binds to several gangliosides. We have analyzed ganglioside-containing liposomes for their binding and uptake by human and murine CD169+ cells and their capacity to induce immune responses. Both human and murine CD169+ antigen presenting cells specifically bound and took up ganglioside-containing liposomes. *In vitro* studies with human CD169+ monocyte-derived and *ex vivo* Axl+ Siglec-6+ dendritic cells demonstrated Ag presentation to CD8+ T cells after uptake of ganglioside/Ag-containing liposomes. Intravenous immunization of mice with ganglioside/ovalbumin-containing liposomes showed clear binding to CD169+ macrophages and the induction of ovalbumin-specific CD8+ and CD4+ T cell and B cell responses. T cell, but not B cell, responses were dependent on CD169+ macrophages as well as Batf3-dependent cross-presenting dendritic cells pointing to an efficient collaboration between these two cell types. In conclusion, ganglioside-containing liposomes target Ag to human and mouse CD169+ antigen presenting cells, demonstrate a strong capacity to stimulate immune responses and should be further explored as a vaccination strategy.

Keywords: Adaptive immunity, antigen processing and presentation, cancer immunology, dendritic cells, macrophage

P-0873

Incorporation of α-galactosylceramide and ganglioside GM3 into liposomal nanovaccine results in uptake by CD169+ macrophages, iNKT cell activation and induction of CD8+ T cells

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To be successful, anti-cancer vaccines should achieve optimal antigen targeting to and activation of the correct antigen presenting cell (APC) to enable priming of naïve T cells. In our previous studies we have shown that vaccines that specifically target to splenic CD169+ macrophages via antigen-antibody conjugates or liposomes containing the CD169 ligand ganglioside GM3, result in strong induction of CD8+ T cells. In this study we analyzed GM3-α-galactosylceramide (αGC)-ovalbumin (OVA) liposomes for antigen delivery to splenic CD169+ macrophages and for their capacity to induce adaptive immunity. αGC is known to act as a strong adjuvant by stimulation of invariant natural killer T (iNKT) cells. GM3-aGC-OVA liposomes were efficiently taken up by CD169+ macrophages and exhibited excellent iNKT and NK cell-activating capacity, at 2h post injection. On day 7 after immunization, GM3-αGC-OVA liposomes induced higher frequency of cytotoxic CD8+ T cells than GM3 liposomes co-injected with a strong systemic adjuvant (anti-CD40/poy I:C). Observed robust activation of CD8+ T cell responses was highly dependent on CD169+ macrophages and cross-presenting dendritic cells (cDC1). In conclusion, our results demonstrate the potential of αGC-GM3 liposomes as an efficient nanoparticle platform for a simultaneous co-delivery of antigen and adjuvant to the correct APC, that engages different immune cells (CD169+ macrophages, cDC1, iNKT cells, NK cells, B cells and T cells) and results in robust activation of adaptive immunity. Incorporation of αGC into GM3 liposomes is expected further improve the anti-tumor efficacy observed following therapeutic GM3 liposomes co-administered with the systemic adjuvant.

Keywords: Adaptive immunity, adjuvants and vaccines, anti-cancer vaccine, dendritic cells, macrophage, NKT cells

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POSTER PRESENTATIONS

P-0899

Therapeutic potential of mir-15b/16-2 targeting in T-cell acute lymphoblastic leukemia

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Prognosis of T-cell acute lymphoblastic leukemia (T-ALL) has greatly improved in recent decades; however, relapsed/refractory patients still present a dismal outcome. Consequently, current efforts focus on developing novel and more effective targeted therapies against relapses. In 2018, the FDA approved the first small RNA to be used in the clinics and, subsequently, several microRNAs-based clinical trials against different types of cancer were initiated. In this work, we investigate the therapeutic potential against T-ALL of mir-15b and mir-16-2, two microRNAs that are tightly regulated during human T-cell development. Primary human T-ALLs and T-ALL cell lines were transduced with lentiviral vectors encoding mir-15b/mir-16-2 genomic cluster and IRES-GFP, or IRES-GFP alone as control. Cell cycle was analyzed both in non-synchronized and Nocodazole- or Thymidine-synchronized cells by DAPI staining and flow cytometry. Apoptosis was analyzed by Annexin-V/7'AAD staining. BCL-2/CyclinD3/CyclinE1 expression was determined by Western Blot and flow cytometry. T-ALL patient-derived xenografts (PDX) established in NSG mice were used to assess mir-15b/16-2 impact on T-ALL *in vivo*. We found that mir-15b/16-2 block T-ALL cell cycle progression, through negative regulation of Cyclin D3 and E1 expression, and also induce apoptosis through inhibition of BCL-2 protein expression. Supporting their role in T-ALL pathogenesis, overexpression of mir-15b/16-2 markedly impaired T-ALL expansion and tumor progression in PDX models. Based on their dual role as regulators of cell cycle progression and apoptosis leading to impaired tumor progression, we propose that mir-15b/16-2 could be potent therapeutic agents against T-ALL relapse.

Keywords: *In vivo* tumor models, miRNA, proliferative disorders

P-0900

In vivo experimental validation of the novel online tool NAP-CNB, a platform to predict MHC-I restricted T cell tumor epitopes in mice

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During the malignization process, tumors accumulate mutations which may result in neoantigens (TNA). TNA can be recognized by tumor-infiltrating lymphocytes, triggering T cell cytotoxic responses. Consequently, recent trend in cancer therapy has been shifted to tumor-specific epitope-based immunotherapies. However, the lack of an integrated bioinformatic pipeline for TNA discovery in mice hinders preclinical research. Hence, we have developed a web server tool (NAP-CNB) to predict putative binding epitopes to H-2Kb MHC-I molecules from tumor RNA sequencing reads. Since *in vivo* validation of *in silico* results has been already recognized as a key need, here we validate the NAP-CNB TNA prediction using the murine B16 melanoma model. Top-scored (ADAR, PNP, LRRC28 and WIZ), one bottom-scored (HERC6) and the positive control TRP2 epitopes were synthesized. Then, antitumor immunogenicity was validated in immunocompetent mice vaccinated with each peptide or with a combination of the top-scored ones, before melanoma implantation. ADAR alone did not induce any antitumor response, whereas PNP and LRRC28 elicited mild antitumor responses and slightly incremented survival rates. However, vaccination with the peptide combination (ADAR, PNP and LRRC28) prompted a strong antitumor reaction with augmented survival, even more efficient than TRP2, indicating that targeting different TNA may be a better approach to prevent immune escape. WIZ vaccination alone also elicited a more powerful antitumor response than TRP2, with considerably improved survival. HERC6 immunization did not result in any positive outcome. Thus, we show that our TNA prediction tool supports the development of epitope-based immunotherapy in the context of personalized medicine.

Keywords: Anti-cancer vaccine, cancer immunology, cell based therapies, immunotherapy, *in vivo* tumor models, RNAseq

P-0949

In vitro and *in vivo* toxicity/immunotoxicity of γ-Fe2O3-based nanoparticles in theranostic perspective

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The progress of biotechnologies allowed us to study various types of nanoparticles (NPs) for nanomedical applications. Based on their unique properties, many different types of NPs have taken the spotlight in theranostic approaches. Their growing use as carriers in targeted treatments, MRI and/or thermotherapy requires careful evaluation of their possible short- and long-term toxicity and immunotoxicity. In our study, tumour and non-tumour cell lines (murine CT26 colorectal carcinoma and 3T3 fibroblasts) were challenged with 4 types of γ-Fe_2O_3-based NPs, with or without nickel incorporation (either polymer-coated or naked). These NPs were tested *in vitro* for effects on cellular viability (MTT, crystal violet assay) and on apoptosis and ROS production (FACS). Their effect on immune cells after systemic administration *in vivo* was evaluated on murine splenocytes by FACS. All NPs were also tested for visualization in nuclear magnetic resonance (NMR). Our data showed very small to no changes in viability and/or ROS production in almost all *in vitro* conditions in all methods. Preliminary *in vivo* data showed almost no significant changes 24h after systemic administration, with the exception of slightly reduced percentage of NK/NKT cells, mild activation of B-lymphocytes and small recruitment of naïve macrophages into spleen in some conditions. All NPs tested in NMR *in vitro* produced signal similar to the standard contrast agent for MRI. In conclusion, γ-Fe_2O_3-based NPs showed small to no toxicity/immunotoxicity *in vitro* and *in vivo*, and they generated signal in NMR *in vitro*, which makes them appropriate candidates for further application in theranostic approaches.

Keywords: *In vivo* tumor models, cancer immunology, immunotherapy

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POSTER PRESENTATIONS

P-0955

A novel trispecific T cell engager antibody for dual-targeting in colorectal cancer inmunotherapy

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The Trispecific T cell Engager (TriTE) is a novel antibody format for dual-targeting of cancer cells, comprising two anti-tumor-associated antigen (TAA) VHH binding domains linked to an anti-CD3 scFv. As a proof of concept, the anti-EpCAM x anti-CD3 x anti-EGFR TriTE has been designed to redirect specifically the T cell cytotoxic response toward single- or double-positive colorrectal cancer (CRC) cells. Anti-EpCAM and anti-EGFR VHHs were linked genetically to the N-/C ends of the anti-CD3 scFv. Control anti-EpCAM x anti-CD3 and anti-CD3 x anti-EGFR bispecific antibodies (Light T cell Engagers, LiTEs) were also produced. Equimolar quantities of the purified proteins were tested by ELISA and flow cytometry to establish their ability to recognize their cognate antigens. T cell specific activation and cytotoxicity against a panel of single- or double-positive CRC cells were determined by CD69 upregulation and bioluminescence assay, respectively. Additionally, IFN-γ secretion was assessed in the supernatants of activated T cells. TriTE enabled strong dose-dependent T cell activation and specific cytolysis of EGFR- and/or EpCAM-expressing CRC cells, without inducing nonspecific T cell response against double-negative cells. Of interest, TriTE EC50 value was significantly lower than those of LiTEs on double-positive CRC cells. In addition, IFN-γ secretion was promoted more efficiently by TriTE. At first, this strategy could display a safer profile than bispecific antibodies because of increased tumor specificity consequently of the dual-targeting ability. Furthemore, we hypothesize that TriTE may reduce the risk of tumor escape due to antigen loss upon single-targeted antibody pressure.

Keywords: Antibody, cancer immunology, engineering of antibodies and nanobodies, immunotherapy, molecular immunology

P-1000

Naked-Eye assessment of polymorphonuclear leukocytes activation status with plasmonic nanosensors

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Polymorphonuclear leukocytes (PMNs) are hyperactivated in septic patients (SP) and undergo massive degranulation. Neutrophil granule proteins (NGPs) display basic isoelectric points (pI>9), an infrequent particularity by which they show greater positive-charge at physiological pH. NGPs could interact with the negative-charge of gold nanoparticles (AuNPs) in colloidal suspensions, causing clusters formation (color change) under pH conditions in which most of proteins show lower positive-charge. We propose a method to detect basic NGPs using AuNPs as a sensor. We evaluated the aggregation pattern of AuNPs in the presence of the neutrophil granule myeloperoxidase (MPO) at a wide pH range (from pH-6 to pH-11). We also studied the behavior of Au-NPs using supernatants (SNs) enriched with NGPs from PMNs cultures. Purified PMNs from healthy donors (HD) and SP were cultured and activated with ionomycin. We used flow cytometry to confirm activation of neutrophils (CD11bHighCD16+CD13+) by CD63 expression. AuNPs-MPO aggregation occurred at very low protein concentration (3nM), preferentially at pH-6 (when MPO shows higher positive-charge). Similarly, AuNPs aggregated more at pH-6 with SNs from activated PMNs cultures (enriched with NPGs). We evaluated the higher SNs dilution allowed to obtain a positive sensor response. AuNPs still aggregated up to 1:1000 dilution with SNs from HD. Since PMNs are degranulated *in vivo* during sepsis, AuNPs remain dispersed (negative response) with diluted SNs from SP. These results point to NGPs as main contributors in colloidal AuNPs aggregation. Our sensor could be useful as a cytometry-independent method to monitoring PMNs activation in sepsis.

Keywords: Granulocytes, infectious disease, neutrophils, visualizing immune responses

P-1004

Network-based repurposing identifies anti-alarmins as drug candidates to control severe lung inflammation in COVID-19

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COVID-19 remains a major health issue with dramatic ensuing economic consequences. Repurposing existing drugs remains the fastest cost-effective approach to alleviate the burden on health services by reducing the incidence of the acute respiratory distress syndrome associated with severe COVID-19. We undertook a computational repurposing approach to identify drugs to control progression towards severe airways inflammation during COVID-19. Molecular profiling data were obtained from public sources regarding SARS-CoV-2 infected epithelial or endothelial cells, immune dysregulations associated with severe COVID-19 and lung inflammation induced by other respiratory viruses. From these data, we generated a protein-protein interactome modeling the evolution of lung inflammation during COVID-19 from inception to an established cytokine release syndrome. This predictive model supports a role for known contributors to the cytokine storm such as IL1β, IL6, TNFα, JAK2, but also less prominent actors such as IL17, IL23 and C5a. Our study also points out to alarmins such as TSLP, IL33, members of the S100 family and their receptors [ST2, RAGE] as targets of therapeutic interest. By evaluating the network-based distancesbetween severe COVID-19-related proteins and known drug targets, we identified drugs which could be repurposed to prevent or slow down progression towards severe airways inflammation. This analysis confirmed the interest of dexamethasone, anti IL6-R, JAK2 inhibitors and further identified various drugs either available or in development interacting with the aforementioned targets. We most particularly recommend considering various inhibitors of alarmins or their receptors, currently receiving little attention in this indication, as candidate treatments for severe COVID-19.

Keywords: Drugs for immune modulation, inflammatory molecules, modelling, tissue damage and repair, viral infections

P-1005

Early innate immune responses triggered upon infusion of mesenchymal stem/stromal cells

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Mesenchymal stem/stromal cells (MSCs) have emerged as a novel cell-based therapy for treatment of immune-mediated disorders thanks to their immunomodulatory properties. Although a significant number of preclinical studies and clinical trials have been conducted with very promising outcomes, the precise mechanism of action of the MSCs is incompletely understood. Hence, the aim of this study is to dissect the early immune responses that follow the intraperitoneal (IP) infusion of adipose-derived MSCs (ASCs) in mice. To this aim, ASCs were infused IP in healthy and dextran sulphate sodium salt (DSS)-induced colitic mice and, 24 hours later, immune populations were analyzed in peritoneal cavity, peripheral blood (PB) and colon lamina propria. In ASC-infused healthy mice an increase in $Ly6G^+Ly6CdimCD11b^+$ granulocytic and $Ly6C^+Ly6GdimCD11b^+$ monocytic populations were measured in the peritoneal cavity with a concomitant decrease of these populations in PB with respect to non ASC-infused healthy mice. Similar results were observed in DSS-induced colitic mice infused with ASCs. Although in this instance, a decrease of both populations was also noticed in colon lamina propria when compared to untreated colitic mice. These changes were accompanied by a decrease of $Ly6GdimLy6CdimF4/80^+CD11b^+$ macrophage population in the peritoneal cavity in ASC-treated healthy and colitic mice with respect to untreated healthy and colitic mice. These results suggest that IP-infused MSC therapy induces early changes in the *in vivo* distribution of granulocytic, monocytic and macrophage populations in different compartments that are partially modified according to the inflammatory status of the recipient mice.

Keywords: Animal models, cell based therapies, immune regulation and therapy, inflammatory bowel disease, myeloid cells

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POSTER PRESENTATIONS

P-1006

Development of COVID-19 vaccines through augmenting antiviral immune responses: a systematic review and meta-analysis

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We aim to evaluate strategies on augmentation of host immunity against Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection. We searched clinical trials registered at the National Institutes of Health through December, 2020, and conducted analyses on inoculated population, involved immunological processes, source of injected components, and trial phases. We then searched PubMed, Embase, Scopus, and the Cochrane Central Register of Controlled Trials for their corresponding reports. A bivariate random-effects meta-analysis was used to derive the pooled estimate of seroconversion and adverse events (AEs). A total of 540,269 participants were enrolled in 225 identified trials. The working mechanisms included heterologous immunity, active immunity, passive immunity, and immunotherapy. A total of 2,565 healthy adults from 10 clinical trials were included for meta-analyses. The odd ratio (OR) was 90.82 for kinetics of serologic responses to anti-SARS-CoV-2 antibody IgG titer (95% CI =36.1 – 228.49; p< 0.00001). The pooled ORs were 2.57 for solicited systemic AEs (95%CI =1.57 – 4.21; p = 0.0002), 5.72 for solicited local AEs (95% CI=2.59 – 12.67; p < 0.0001), and 2.08 for unsolicited systemic events (95% CI=1.42 – 3.05; p = 0.0002), compared to placebo or conservative treatment. Among all immune-augmentative interventions, a paradigm shift to vaccines providing active immunity was observed. The efficacy of these interventions was promising although systemic adverse events were noted.

Keywords: Immune development, adjuvants and vaccines, immune response tracing

P-1008

Methyl- rich diet ameliorates lupus-like disease in MRL/lpr mice by altering DNA methylation profile

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Systemic lupus erythematosus is a chronic autoimmune disease with various clinical manifestations and impairments both in innate and adaptive immune systems. Previous studies have reported that aberrant DNA methylation profile in mouse T- and B- cells correlates with the development of lupus-like disease in MRL/lpr mice. In addition, the presence or the absence of dietary micronutrients that participate in the methylation process affects the DNA methylation state. We hypothesized that the deliberate methylation of DNA from lupus-prone mice by specific micronutrient diet could modulate the immune response and thus could alter the disease course. Groups of 10 female MRL/lpr mice were treated starting from 6th week of age as follows: one group of animals was fed with a standard diet; the second group of animals was fed with methyl-rich diet and the third group was fed with a standard diet from the 6th week of age and after the appearance of proteinuria the diet was switched to the methyl-rich diet. The levels of anti-dsDNA antibodies, proteinuria, skin lesions, survival rate, kidney pathology and DNA methylation were measured from mice of all experimental groups. The results confirmed that the administration of methyl-rich diet decreased the levels of anti-dsDNA antibodies, of proteinuria and of skin lesions and kidney pathology. The observed amelioration is a result of increased DNA methylation level of B lymphocytes which suggests DMA methylation as novel technique for manipulation of immune response.

Keywords: Autoimmunity, B lymphocytes, epigenetic control and modulation of immunity, immune regulation and therapy

P-1009

Transfection of vitamin D3-induced tolerogenic dendritic cells for the silencing of potential tolerogenic genes. Role of CSF1R

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Reestablishment of the immune tolerance by using tolerogenic dendritic cells (tolDC) is a promising strategy under investigation in several clinical trials. Identification of surface cell markers specific of tolDC is essential for their translation to the clinic. To set up an easy and fast transfection methodology of monocytes during their differentiation to tolDC using vitamin D3 (VitD3-tolDC). To analyze the relevance of CSF1R (CD115) and CD209 genes in their tolerogenic function. mDC and VitD3-tolDC were differentiated from monocytes cultured for 6 days in presence of IL-4, GM-CSF (and vitD3 for tolDC). At day 1, Viromer blue reagent combined with specific or control siRNA were added to VitD3-tolDC. Maturation was induced on day 4 with a pro-inflammatory cytokine cocktail (IL1β+TNFα+prostaglandin E2). On day 6, phenotype, functionality, and gene and protein expression were determined. Optimization of transfection using 0.75µL of Viromer blue + 2.75µM of siRNA/ 10^6 cells resulted in >80% of transfected cells without affecting phenotypical or functional characteristics of VitD3-tolDC. The transfection with specific siRNA for CD115 and CD209 genes allowed 80% gene silencing compared to non-transfected VitD3-tolDC, although only CD209 protein significantly reduced its expression. A partial reduction in the proliferation induced by VitD3-tolDC to allogeneic cells was observed in siCD115-treated VitD3-tolDC (p= 0.018, n=5). Additionally, reduction of glycolysis and lactate secretion in siCD115-VitD3-tolDC was observed compared to untreated VitD3-tolDC (p= 0.010, n=3). Viromer blue is an adequate transfection technology for VitD3-tolDC. CSF1R gene is related (but not essential) to the tolerogenicity of VitD3-tolDC.

Keywords: Autoimmunity, biomarkers, dendritic cells, immunotherapy, regulatory cells

P-1010

Developing vaccines to augment the anti-tumor effect of immune checkpoint blockade

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In the last decades immune checkpoint blockade (ICB) therapies have been developed as a new and promising strategy for cancer treatment. However their efficacy remains low, being around 30% among all the different solid tumors. The effectiveness of ICB depends on the frequency of tumor mutations, the neoantigens generated and the T-cell response against them. Therefore, it is expected that a vaccine targeting neoantigens would improve ICB therapy efficacy. The aim of this study was to develop a highly immunogenic vaccine containing novel combinations of pattern recognition receptors (PRR) agonists together with long peptides in order to induce a robust peptide-specific CTL response. We tested the immunogenicity of different vaccine formulations containing long OVA peptides and different combinations of adjuvants. We found a high frequency of OVA(257-264)-specific T cells compared to the frequencies found in mice immunized only with peptide in Addavax or saline. Alternatively, mice were immunized with neoantigens predicted by WES from3 different tumor models, B16-F10, AE17 and 4662. We found high antigen-specific responses in all cases. Finally, we tested the vaccines in B16-F10-OVA tumor bearing mice, in combination or not with ICB therapy. We found a reduction in the size of tumors compared to unvaccinated mice and when the vaccination was combined with ICB therapy we found a synergetic effect leading to a slower tumor growth and enhancing the efficacy of ICB therapy alone. In summary, we present a novel vaccine strategy that induces robust T cell immunity against neoepitopes and can enhance ICB therapy.

Keywords: Adjuvants and vaccines, anti-cancer vaccine, cancer immunology, checkpoint inhibition

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POSTER PRESENTATIONS

P-1012

Ex vivo expanded Natural Killer cells and INTASYL self-delivering RNAi targeting TIGIT as novel immunotherapeutic strategy in breast cancer

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Natural Killer (NK) cell-based immunotherapy shows promise as treatment option for cancer patients. Tumor cells upregulate inhibitory ligands leading to functional exhaustion of NK cells. Blockade of inhibitory receptors can synergize with cell-based approaches to overcome exhaustion of NK cells. We hypothesize that RNAi silencing of corresponding receptors is an effective strategy to enhance NK anti-tumor function. Since NK cells are notoriously difficult to genetically modify, we evaluated INTASYL self-delivering RNAi targeting the inhibitory receptor TIGIT for this purpose. NK line KHYG-1 or K562/mIL21/4-BBL feeder cell-expanded primary NK cells (pNKs) were incubated with anti-TIGIT INTASYL followed by flow cytometric analysis of TIGIT expression levels. To determine the effect on NK effector function, silenced or control NK cells were used in cytoxicity and degranulation assays with K562 CML or MCF-7 and SKBR3 breast cancer cell lines as targets. TIGIT was expressed at relatively high levels on KHYG-1 and pNKs. INTASYL silenced TIGIT in a concentration dependent manner, efficiently reducing TIGIT cell surface expression of KHYG-1 and pNKs as compared to the non-targeting siRNA control (42% in KHYG1, 25% in pNK) without impairing cell viability. TIGIT silencing enhanced degranulation (CD107a) of NKs against K562 and MCF7 compared to the non-targeting control and enhanced trastuzumab (anti-HER2) induced antibody dependent cellular cytotoxicity against SKBR3 illustrating effectivity for multiple NK effector functions. These results demonstrate the potential of INTASYL RNAi silencing of immune checkpoint molecules such as TIGIT to enhance the anti-tumor efficacy of expanded NK cells for adoptive cell therapy.

Keywords: Cell based therapies, checkpoint inhibition, immune regulation and therapy, NK cells

P-1013

A novel polymer-based STING activator with potent anti-tumour activity

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Since the discovery that the cGAS-STING pathway occupies a central role in the activation of anti-tumour immune responses, there has been a surge in research aimed at identifying natural and synthetic cyclic dinucleotides and non-nucleotidyl STING agonists for use in cancer settings. Despite pre-clinical evidence of efficacy, no widely applicable, clinically effective and safe agonist has completed phase III trials. The primary barriers to clinical translation are low cellular uptake and intracellular accessibility, poor pharmacokinetics, and STING variability, necessitating personalised STING agonists. This work has identified a chitin-derived polymer with no acetyl groups, as an attractive alternative to conventional STING agonists. The adjuvant promotes potent STING and IFNAR-dependent cellular immunity and tumour growth suppression upon intratumoral injection in B16 melanoma models. Unlike most licensed adjuvants, we have delineated the mechanism and physicochemical properties required for C100-induced immune activation. In a cellular uptake-independent and calcium-dependent manner, the adjuvant triggers mitochondrial stress that is pivotal for both cGAS-STING and NLRP3 inflammasome activation and subsequent Th1 immunity. Polymer-induced mitochondrial stress damages nuclear DNA, triggering its accumulation in the dendritic cell (DC) cytosol and activation of STING-dependent type I IFNs. Complete deacetylation of the polymer backbone is critical for adjuvanticity, as addition of acetyl groups reduces the degree of mitochondrial stress, nuclear damage, IFNAR-dependent DC maturation, NLRP3 activation, Th1 responses and protective anti-tumour immunity. Altogether, these results reveal an effective STING-dependent cancer vaccine adjuvant with unique properties that sidestep common limitations of existing STING therapeutics.

Keywords: Adjuvants and vaccines, cancer immunology, dendritic cells, *in vivo* tumor models

P-1014

Regulatory T cells in oral cancer pateints and impact of their inihibition on growth and establishment of cancer cells

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Oral squamous cell carcinoma (OSCC) is one of the major cancers affecting Asian countries. The main causative factor has been the tobacco habit. We assessed the phenotypic and functional characteristics of Regulatory T (Treg) CD4+CD25+FoxP3+subsets in patients with OSCC by multicoloured flow cytometry. Subsequently we investigated the effects their inhibition via TDG on growth of OSCC cell lines *in vitro*. An increased ($p<0.05$) prevalence of Treg phenotypes (CD4+CD25+, CD4+FoxP3+, CD8+FoxP3+, CD4+CD25+FoxP3+) was observed in the peripheral circulation of OSCC patients that positively correlated with clinico-pathological features. The increased frequency of CD4+CD8+CD25+FoxP3+, a unique T cell subset, CTLA4+, GITR+, NrP1+ and granzyme B+ (GzmB) Tregs also showed a significantly higher prevalence in OSCC patients. Functionally CD4+FoxP3+ Tregs showed skewed expression of IL2, IL10 and IL35 in patients as compared with the normal controls. Higher expression of TGFβ in tumor tissues suggests their dominant role in the up regulation of differentiation of Tregs from naive T cells in the tumor bearing host. Further, enhanced expression of CCR5 and CCR7 on Tregs with up regulation of their ligands (CCL5, CCL19 and CCL21) in tumor cells indicates efficient recruitment to the tumor site. The treatment with its inhibitor TDG resulted in inhibition of Treg subsets and also decreased the frequency of IL10+ and IL35+ Tregs indicating its immunomodulatory effects. It seems reasonable to assume that modulation of functional dynamics of selective Treg subsets may be useful in enhancing anti-tumor immunity and developing immunotherapeutic strategies for patients with oral squamous cell carcinoma.

Keywords: Biomarkers, cancer immunology, cytokines and mediators, immune regulation and therapy

P-1016

Antitumor effect of proton beam irradiation and anti-PD-L1 antibody combination therapy in subcutaneous murine models of pancreatic cancer

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Most pancreatic cancers (PC) are diagnosed in advanced stages, and chemotherapy efficacy is limited. A new non-surgical treatment by combining proton irradiation and immunomodulatory therapy was investigated. We analysed *in vitro* the effect of proton irradiation (0, 2 and 8 Gy) on two distinct murine PC cell lines: PAN02 (NCI-DTP) and mT3-2D (CSHL); colony formation assay and gene expression analyses were performed. Subcutaneous PAN02 PC and mT3-2D PC murine models were established by inoculating cells into the lower leg and dorsal area of C57BL/6J mice. The tumor in the leg was irradiated by proton (8 Gy), combined to the intraperitoneal administration of anti-PD-L1 antibody (combination therapy); tumor growth was monitored, and inflammatory cells within tumors were assessed by immunohistochemistry. For both cell lines, proton irradiation decreased the colony forming ability in a dose-dependent manner, and cytokines/chemokines were clearly induced by 8 Gy irradiation. Combination therapy inhibited tumor growth in the leg ($p<0.01$; versus no treatment) in both tumor models; in mT3-2D model, combination therapy also inhibited tumor growth on the dorsal tumor ($p<0.05$). Immunohistochemistry results, in both models, confirmed higher infiltration of CD8+ T cells in leg and dorsal tumors, meanwhile no apparent difference in the number of infiltrating CD4+ T cells was observed. In two distinct PC models, the antitumor effect at the irradiation site was promoted by proton beam irradiation plus anti-PD-L1 antibody. Abscopal effect was observed in mT3-2D model but not in PAN02 model; further analysis is ongoing to elucidate this difference.

Keywords: Animal models, checkpoint inhibition, immune regulation and therapy, immunotherapy, *in vivo* tumor models

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POSTER PRESENTATIONS

P-1017

A rapid CRISPR competitive assay for *in vitro* and *in vivo* discovery of potential drug targets affecting the hematopoietic system

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CRISPR/Cas9 can be used as an experimental tool to inactivate genes in cells. However, a CRISPR-targeted cell population will not show a uniform genotype of the targeted gene. Instead, a mix of genotypes is generated - from wild type to different forms of insertions and deletions. Such mixed genotypes complicate analyzing the role of the targeted gene in the studied cell population. Here, we present a rapid and universal experimental approach to functionally analyze a CRISPR-targeted cell population that does not involve generating clonal lines. As a simple readout, we leverage the CRISPR-induced genetic heterogeneity and use sequencing to identify how different genotypes are enriched or depleted related to the studied cellular behavior or phenotype. The approach uses standard PCR, Sanger sequencing, and a simple sequence deconvoluting software, enabling laboratories without specific in-depth knowledge to also perform these experiments. As proof of principle, we present examples studying the role of different genes for various aspects related to hematopoietic cells (T cell development *in vivo* and activation *in vitro*, differentiation of macrophages and dendritic cells, as well as a leukemia-like phenotype induced by overexpressing a proto-oncogene). In conclusion, we present a rapid experimental approach to identify potential drug targets related to mature immune cells, as well as normal and malignant hematopoiesis.

Keywords: Bone marrow transplantation, immune development, immunological techniques, stem cells

P-1018

Evaluation of new dye technology: Using quantitative criteria to improve biological resolution and reduce spectral spread in flow cytometry panel design

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Fluorescent dye performance heavily impacts both the quality and the dimensionality of flow cytometry experiments. For example, PE and PE-based tandems, although bright, are notoriously cross-excited by multiple laser lines, introducing substantial fluorescence into off-target channels, compromising the full potential of blue and yellow-green lasers. The development of fluorescent labels engineered with targeted excitation and emission spectra using the Invitrogen™ Phiton™ DNA platform has allowed us to expand the number of biological questions that can be asked in a single panel. Using an iterative process of panel design, we are able to evaluate existing panels and determine how to reduce spread and improve biological data resolution. In this presentation, we will show methods to evaluate both low and high dimensional flow cytometry panels and how underlying dye performance affects the cellular immunology that can be measured. For research use only. Not for use in diagnostic procedures.

Keywords: Biology of the immune system, biomarkers, visualizing immune responses

P-1019

Immunomodulatory effects of targeted delivery of heat shock proteins 70 kDa to the surface of tumor cells

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One of the promising directions in antitumor immunotherapyis associated with the ability of cytotoxic effectors of immune system, in particular NK cells, to recognize heat shock proteins 70 kDa (HSP70) present on the surface of tumor cellsas a marker forcytolytic reaction of cytotoxic lymphocytes.Therefore, this phenomenon can be used to develop new approaches to antitumor immunotherapy.At the same time, it is known that the presence of HSP70 on the cell surface is not an universal feature of cancer cells. Many types of tumor tissues do not express membrane-associated HSP70, which limits the potential clinical possibilities of these approaches. In this regard, targeted delivery of exogenous HSP70 to the surface of cancer cells can be considered as one of the promising means for antitumor immunotherapy. In order to assess the possibility of this approach we created two-module recombinant constructs containing in the first module mini-antibodies or darpins directed to the HER2/neu antigen, and in the second module - the HSP70 molecule or its fragment. The selectiveinteraction of the first and second modules was ensured by a docking link formed by a pair of barnase:barstar. The results obtained demonstrated the selective binding of the developed constructs to tumor cells expressing the HER2/neu antigen, and a significant stimulating effect of these preparation on the cytotoxic activity of effector cells of the immune system in relation to the corresponding target cells.

This work was supported by the Russian Science Foundation, grant number 19-75-10120.

Keywords: Cancer immunology, cellular interactions, NK cells

P-1020

Hidden activators of immunity: How house dust mites induce immune activation via dendritic cells

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Dendritic cells (DCs) are potent antigen-presenting cells and play a central role in orchestrating adaptive immunity, which makes them an important target for immunomodulatory therapies. Aiming to improve allergen immunotherapy we are focusing on the development of next-generation allergen-specific vaccines specifically targeting DCs. Therefore, we investigated the influence of the major house dust mite (HDM) allergens Der p 1 and Der p 2 on immune activation. Both natural purified allergens did not induce potent immune activation by themselves, as assessed by the expression of surface maturation markers (CD83 and CD86) and the cytokines IL-6, IL-10 and IL-12. In contrast, HDM extract as well as endotoxin-reduced HDM extract triggered a strong immune response, strongly suggesting that the HDM-induced DC activation is not restricted to endotoxins. To further confirm that major HDM allergens like Der p 1 play no significant role in DC activation, we showed that depletion of Der p 1 from HDM extract did not impair immune activation. To asses which structures other than its major allergens or endotoxin are driving DC maturation, identification of the involved receptors on DCs may provide some clues. We observed that blocking the tyrosine kinase Syk reduced the HDM-induced cytokine responses, narrowing down the spectrum of involved receptors. Identification of the main receptor and its ligand in the HDM extract will provide valuable insights that will allow selection of adjuvants which can more effectively override pro-allergic immune modulation by HDM than currently achieved by e.g. alum.

Keywords: Dendritic cells, immune response tracing, immunotherapy, modification allergic responses

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POSTER PRESENTATIONS

P-1021

A novel cationic liposome for validation of a multi-epitope chimeric protein for vaccine development against visceral leishmaniasis

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Subunit proteins provide a safe source of antigens for vaccine development, but they are often limited by their low immunogenicity. Thus, to achieve strong and long-lasting immune responses, they should be encapsulated into a stable antigen delivery system combined with an appropriate adjuvant. Toward this goal, we encapsulated a multi-epitope chimeric protein into novel cationic liposomes formulated or not with the Toll-like receptor 7/8 (TLR7/8)-agonist Imiquimod (IMQ), serving as adjuvant, for vaccine development against visceral leishmaniasis. The multi-epitope peptide vaccine consisted of several Helper and Cytotoxic T lymphocyte epitopes obtained from different *Leishmania infantum* proteins through reverse vaccinology approaches as well as the N-terminal domain of HBHA from *Mycobacterium tuberculosis* indicated as TLR4 agonist. We showed that one intramuscular injection of these formulations in BALB/c mice led to their enhanced uptake by antigen-presenting cells in draining lymph nodes with subsequent production of antigen-specific IgG antibodies irrespective of IMQ encapsulation. Moreover, administration of two vaccine doses promoted a high proportion of memory T cell subsets versus control group towards a Th1 profile and enhanced humoral immunity. Surprisingly, absence of IMQ adjuvant improved vaccine's protective efficacy against VL, as assessed by significantly reduced parasite loads in spleen and liver of vaccinated BALB/c mice compared to control groups. Reduction of parasite load was inversely correlated with the frequency of memory CD4+ T cells populations in spleen. Taken together, these findings suggest that this vaccine formulation that is capable of eliciting memory T cells could be considered as a promising vaccine candidate against VL.

Keywords: Adjuvants and vaccines, infectious disease, memory, parasite infections

P-1023

LAG-3 blockade with relatlimab (BMS-986016) restores anti-leukemic responses in chronic lymphocytic leukemia

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Diminished immunosurveillance and immunosuppression associated to chronic lymphocytic leukemia (CLL) along with disappointing clinical results of PD-1 and CTLA-4 blockade reinforce the need to evaluate emerging checkpoints in this malignancy. Herein, we study the anti-tumor potential of relatlimab, an anti-LAG3 monoclonal blocking antibody currently under clinical trial in CLL, and its underlying mechanisms. LAG3 expression was evaluated on leukemic, T and NK cells from patients with CLL and healthy donors by flow cytometry. Sera levels of soluble LAG3 (sLAG3) were also determined by ELISA. Peripheral blood mononuclear cells from patients were treated with relatlimab alone or in combination with lenalidomide and leukemic cell count, proliferation, cytokine production and NK cell-mediated cytotoxicity were evaluated by flow cytometry and calcein-AM-based assay. We report the profound dysregulation of LAG-3 expression on leukemic, NK and T cells, hence suggesting a role for this checkpoint in CLL-associated immunosuppression. High LAG-3 expression, as well as increased sLAG-3 levels, correlated with adverse cytogenetics and poor outcome in CLL, reinforcing its clinical relevance. Treatment with relatlimab induced leukemic cell depletion and restored NK cell- and T cell-mediated responses. Importantly, combination of LAG-3 with the immunomodulatory drug (IMiD) lenalidomide significantly increased IL-2 production by T cells as well as antibody-dependent cytotoxicity. Herein, our data provide new insights into the anti-leukemic activity exerted by relatlimab, currently under clinical trial in CLL, providing the rationale to further investigate its combination with IMiDs for the management of hematological malignancies.

Keywords: Cancer immunology, checkpoint inhibition, drugs for immune modulation, immunotherapy, NK cells

P-1024

Identification of peptides presented by MHC: exploring new vaccine antigen candidates against tuberculosis

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Intracellular pathogens are not efficiently recognized by T cells, but rather by peptides presented on the surface of infected cells, via MHC molecules. The immune response against intracellular pathogens relies, at least in part, on CD4+ and CD8+ T cells. Therefore, protective vaccines require the induction of antigen-specific T cells through peptides presented by MHC-II and MHC-I, respectively. Multiple approaches have been used to identify peptides recognized by T cells, with various degrees of success. These identification methods include biochemical fractionation and purification of antigens, cloning and expression of antigens, T cell screening of antigen libraries and bioinformatics predictions. Recent advances in mass spectrometry instrumentation combined with bioinformatics led to an unprecedented improvement in sensitivity. This unbiased technology, known as immunopeptidomics, allows for the direct identification of peptides bound the MHC molecules. Recently, antigens presented by MHC-I and MHC-II in macrophages infected with *Mycobacterium bovis* BCG, were identified by immunopeptidomics. This resulted in the pre-clinical development of three new vaccine candidate antigens, designed to boost BCG, with improved efficacy against tuberculosis. To further explore and identify additional vaccine antigen candidates, I will present an extensive immunoinformatics characterization and selection of antigens, to be used in the design of new vaccines against tuberculosis.

Keywords: Adjuvants and vaccines, antigen processing and presentation, bacterial infections, infectious disease, MHC and polymorphic genes

P-1025

Near-infrared irradiation as a potential immunomodulator – *in vitro* study

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Light energy is the most preferred in photobiomodulation due to its ability to safely penetrate through the skin and affect directly the cellular functions. Near-infrared (NIR) irradiation improves cell survival, modulates cytokine secretion, and reduces inflammation on the cellular and organismal level. These effects tightly correlate with mitochondrial activity. Peripheral blood mononuclear cells (PBMC) from healthy donors (n=10), stimulated with lipopolysaccharide (LPS), were irradiated with 15 and 30 J/cm2 by diode laser (810 nm). After 24h the MTT-test was performed to detect the cytotoxic effect. The supernatants were collected and the levels of cytokines IL-1β, IL-6, and IL-10 were measured by ELISA. Seahorse-assay for detection of mitochondrial activities of irradiated and non-irradiated cells was used. The results from the MTT-test showed that after NIR irradiation with both energies PBMC kept their viability similar to the control cells. In non-irradiated cells the secretion of IL-1β, IL-6, and IL-10 was 2,2 pg/mL, 82,8 pg/mL and 10 pg/mL, respectively, while LPS stimulation strongly increased the levels to 110 pg/mL, 249 pg/mL and 31 pg/mL. After 15 and 30 J/cm2 irradiation, the levels of IL-1β decreased to 17 pg/mL, and 2,5 pg/mL; IL-6 to 131,8 pg/mL and 21 pg/mL; and IL-10 to 9,9 pg/mL and 8,2 pg/mL. The results of Seahorse-assay showed that NIR-energy affects the mitochondrial activity of PBMC by increasing ATP levels and the spare respiratory capacity. NIR irradiation is a safe and effective anti-inflammatory and immunomodulatory therapy in healing processes, pain relief, neurodegenerative disorders, cancer, etc.

Keywords: Cellular interactions, cytokines and mediators, immune regulation and therapy

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POSTER PRESENTATIONS

P-1026

A methodological approach using rAAV vectors encoding nanobody-based biologics to evaluate ARTC2.2 and P2X7 *in vivo*

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On murine T cells, mono-ADP ribosyltransferase ARTC2.2 catalyzes posttranslational modification, ADP-ribosylation at arginine residues of various surface proteins when nicotinamide adenine dinucleotide (NAD+) is released into the extracellular compartment. Covalent ADP-ribosylation of the P2X7 receptor by ARTC2.2 thereby represents an additional mechanism of activation, complementary to its triggering by extracellular ATP. P2X7 is a multifaceted receptor that may represents a potential target in inflammatory, and neurodegenerative diseases, as well as in cancer. We present herein an experimental approach using intramuscular injection of recombinant AAV vectors (rAAV) encoding nanobody-based biologics targeting ARTC2.2 or P2X7. We demonstrate the ability of these *in vivo* generated biologics to potently and durably block P2X7 or ARTC2.2 activities *in vivo*, or in contrast, to potentiate NAD+- or ATP-induced activation of P2X7. We additionally demonstrate the ability of rAAV-encoded functional heavy chain antibodies (hcAb) to elicit long-term depletion of T cells expressing high levels of ARTC2.2 or P2X7. Our approach of using rAAV to generate functional nanobody-based biologics *in vivo* appears promising to evaluate the role of ARTC2.2 and P2X7 in murine acute as well as chronic disease models.

Keywords: Engineering of antibodies and nanobodies, immune regulation and therapy, immunological techniques

P-1027

Characterization of conducting airway phagocytic cells that internalize SARS-CoV-2 receptor binding domain-coated 100-nanometer particles in conducting airways of mice

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SARS-CoV-2 penetrates the epithelial barrier of the respiratory tract and invading the airways immune cells can either provide the defense or facilitate virus dissemination. In this study, we compared the phagocytic potential of conducting airway immune cells in response to 100-nanometer fluorescent particles in the presence or absence of peptide – receptor-binding domain (RBD) of SARS-CoV-2. Mice received 100 nm carboxylate-modified fluorescent particles dissolved in phosphate buffer (PBS) or 0,1 % solution of RBD of SARS-CoV-2. Control mice received particles in 0,1% solution of bovine serum albumin (BSA) and 0,1 % solution of RBD of SARS-CoV-2 without particles. Particles were applied to mice oropharyngealy in a dose of 10 million particles per mouse. Conducting airway was dissected and subjected to immunohistochemistry as a whole mount. Specimens were analyzed using confocal laser-scanning microscopy. 24 hours after the particle application slight infiltration of $CD11b^+$ phagocytes was detected in conducting airways of mice that received particles both in protein or peptide solution or alone. Infiltrated airways $CD11b^+$ phagocytes did not participate in the uptake of particles dissolved in PBS or BSA. These particles were mostly ingested by $CD11c^+CD169^+$ cells in the luminal side of the airway epithelium. RBD of SARS-CoV-2 solution alone stimulates $CD11b^+$ phagocytes infiltration to the conducting airway. Dissolved in RBD of SARS-CoV-2 solution particles were ingested by both $CD11c^+CD169^+$ cells and $CD11b^+$ phagocytes. Thus, RBD of SARS-CoV-2 stimulates $CD11b^+$ phagocytes infiltration and 100 nm particle uptake in conducting airways.

The work was supported by RFBR, project 20-04-60311.

Keywords: Inflammatory disease, innate host defence, viral infections, visualizing immune responses

P-1028

The mithralog EC-7072 induces chronic lymphocytic leukemia cell death by targeting tonic B-cell receptor signaling

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In this study, we evaluate the therapeutic potential of the mithralog EC-7072 and its impact on leukemia B-cell homeostasis in chronic lymphocytic leukemia (CLL). Peripheral blood mononuclear cells (PBMCs) isolated from untreated patients with CLL fulfilling the diagnosis criteria for this malignancy and healthy donors were analyzed. RNA-sequencing analyses of CLL cells treated with EC-7072 were performed. The effect of EC-7072 on cell viability, apoptosis or BCR-signaling was analyzed by flow cytometry and western blotting. Herein, we demonstrate that the mithralog EC-7072 displays high *ex vivo* cytotoxic activity against leukemia cells from CLL patients independently from high-risk prognostic markers and *IGHV* mutational status. EC-7072 is significantly less toxic against T cells and NK cells. EC-7072 directly triggered caspase-3-dependent leukemia cell apoptosis, which was not abrogated by microenvironment-derived factors that sustain leukemia cell survival. RNA-sequencing analyses revealed a dramatic EC-7072-driven reprograming of the transcriptome of CLL cells, including a wide downregulation of multiple components and targets of the BCR signaling pathway. EC-7072 exerted similar or higher antileukemic activity than that of several available CLL therapies and displayed additive or synergistic interaction with these drugs in killing CLL cells. Our findings provide evidence that EC-7072 induces leukemia cell death by hampering BCR signaling in CLL cells. Further, the compound enhances the antileukemic activity of approved therapeutic agents, hence opening the question of whether EC-7072 may be a potential novel standalone or combination therapeutic option for patients with CLL and other B-cell malignancies.

Keywords: B lymphocytes, cancer immunology, cell death, RNAseq

P-1029

Preclinical evaluation of a novel rosmarinic acid derivative on the pathogenesis of type 1 diabetes in a mouse model

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Rosmarinic acid (RA) is a polyphenol compound that naturally occurs in plants of the *Lamiaceae* family. A novel rosmarinic acid derivative (RAd) has been developed and tested in the animal model of type 1 diabetes (T1D) and the animal model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). T1D was induced in male C57BL/6 mice using streptozotocin that was applied intraperitoneally for five consecutive days. EAE was induced in Dark Agouti (DA) rats by subcutaneous injection of autologous spinal cord homogenate. For T1D, intraperitoneal administration of RAd (10 mg/kg bw) began from the first streptozotocin injection and continued for 20 days, while for EAE, subcutaneous administration of RAd (28 mg/kg bw) started with the first clinical signs of the disease and continued for 15 days. RAd-treated mice exhibited lower incidence of T1D (monitored up to 45 days from the disease induction), and fluorescent histochemical analysis showed that their pancreatic islets expressed more insulin. Additionally, RAd ameliorated EAE in DA rats. In T1D, RAd treatment significantly down-regulated the proportions of $CD11b^+$ and $CD11c^+$ myeloid cells in the immune cell infiltrates in the pancreas, detected on day 10 after T1D induction. However, the proportions of cells of adaptive immunity ($CD4^+$, $CD8^+$, Th1, Th17) were comparable between the groups. These results suggest that chemically modified RA shows great promise for anti-inflammatory approaches in autoimmune and inflammatory diseases, while our previous research illustrated that unmodified RA exerted no effect on T1D pathogenesis.

Keywords: Animal models, autoimmunity, diabetes, drugs for immune modulation, immune regulation and therapy, multiple sclerosis

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POSTER PRESENTATIONS

P-1030

Modulation of the immune response by protein-engineered chimeric molecules in a spontaneous mouse model of type 1 diabetes

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Type 1 Diabetes (T1D) is characterized by B and T cells attack on the insulin-producing beta cells of the pancreas. Diabetogenic B lymphocytes are able to activate T cells and can direct the immune response against many autoantigens, like the membrane bound enzyme Glutamic Acid Decarboxylase 65 (GAD65). NOD mice are famous for the spontaneous development of T1D, caused by severe insulitis and hyperglycemia, that are initially induced by autoantibodies against insulin and GAD65. The inhibitory Fc-gammaRIIB receptor is found on B cells and, if activated, delivers a powerful blocking signal to the particular cell. We hypothesized that the elimination of GAD65 specific B lymphocytes by chimeric molecules, containing a specific antibody for the Fc-gammaRIIB receptor, coupled to peptide epitopes derived from the GAD65 protein, may delay the onset of T1D. The peptides and the Fc-gammaRIIB specific antibodies were linked by EDC coupling and the modulating ability of the newly constructed chimeric molecules was tested in a NOD model of T1D. The overall effect of the treatment was analyzed by flow cytometry, ELISA, ELISpot and histological assays. The so constructed chimeric molecules were able to bind specific B lymphocytes and to modulate the autoimmune response, by co-crosslinking of the inhibitory Fc-gammaRIIB and the BCR. Based on these results, we can assume that the constructed chimeric molecules are able to selectively modulate the activity of GAD65 – specific B lymphocytes. This treatment presents a possible way to alter the autoimmune nature of the diabetogenic autoimmune cells.

Keywords: Autoimmunity, diabetes, immune regulation and therapy

P-1031

Targeting of the cGAS-STING pathway on autoinflammatory and autoimmune disease

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The cGAS-STING pathway is crucial for antiviral immune response. cGAS-STING pathway activated in cellular stress, tissue damage, immune response to microbes. cGAS is located in the cytoplasm, nucleus membrane, and senses host-derivated and microbial DNA molecules. In autoinflammatory conditions, the cGAS pathway is exaggerated and causes dysregulated interferon response. DNA viruses sensed by the cGAS-STING pathway viral DNA particles bind the cGAS and enzyme produce cyclicGAMP (Cyclic guanosine monophosphate–adenosine monophosphate) cGAMP binds ER-located STING protein and STING protein goes via ERGIC pathway to Golgi. STING is palmitoylation in Golgi and released from Golgi. Then activated IRF-3 transcription factor goes nucleus and causes the expression of type-1 interferon genes. In host-derivated DNA molecules are sensed by the same mechanism. Some pathogens such as Mycobacterium tuberculosis produce cyclic diAMP molecules these molecules activate the STING pathway. In autoinflammatory or autoimmune conditions such as STING-associated vasculopathy with onset in infancy (SAVI), Aicardi–Goutières syndrome (AGS), COPA syndrome, Systemic lupus erythematosus, and Rheumatoid arthritis if can inhibit this pathway on this disease maybe taken good response on clinical progression. In our study, we aimed the immunological drugs to repositioning on cGAS enzyme. We created a library for virtual screening this library contains 2515 drugs. These drugs were docked by the virtual screening tool PyRx module. We targeted cGAS whole protein structure. (PDB code:5VDO). According to our results show the BT-11 (ΔG=-12.1), Picfeltarrenin (ΔG=-11.3), ammonium glycyrrhizinate (ΔG=-11.3), SC75741 (ΔG=-11.0), and RN486 (ΔG=-11.0) have a high affinity to the cGAS enzyme.

Keywords: Autoimmunity, autoinflammation, cell signalling, drugs for immune modulation, inflammatory disease

P-1032

Liver-resident gamma delta T cells are long-lived and can be induced to target hepatocellular carcinoma

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Gamma delta (γδ) T-cells are promising candidates for cancer immunotherapy, with potential for HLA-unrestricted tumour reactivity. We characterised the long-lived tissue-residency profile of γδ T-cells in the human liver and hepatocellular carcinoma (HCC), and explored novel strategies to recapitulate and exploit tissue-resident Vγ9Vδ2 T-cells for efficient targeting of HCC. Lymphocytes isolated from paired blood, liver, and tumoural tissue in HCC were analysed by flow cytometry. γδ T-cell counts were determined by immunostaining. Long-lived persistence of liver-resident γδ T-cells was examined using donor/recipient HLA-mismatched liver allografts. Aminobisphosphonate (Zoledronate) and IL-2 expanded blood Vγ9Vδ2 T-cells, intrahepatic lymphocytes, and tumour-infiltrating lymphocytes, were co-cultured with human hepatoma cell-lines treated with Zoledronate to promote tumour-cell phospho-antigen accumulation for Vγ9Vδ2 T-cell receptor activation. Intrahepatic γδ T-cells exhibited a tissue-resident memory (TRM) phenotype (CD69+CD49a+), absent from blood, with a distinct functional profile (higher IFNγ, IL-2), and long-lived persistence in the liver (>10years). Higher intra-tumoural γδ T-cell counts associated with greater patient survival. The Vγ9Vδ2 T-cell subset was depleted within HCC, but displayed the highest intra-tumoural γδTRM phenotype. Zoledronate-based expansion of blood Vγ9Vδ2 T-cells induced a de novo TRM phenotype with improved cytotoxicity. Direct treatment of hepatoma cell-lines with Zoledronate further enhanced the anti-tumour function (IFNγ,TNFα) of co-cultured induced, intrahepatic and intratumoural Vγ9Vδ2 TRM cells, with increased tumour-cell lysis. γδTRM cells demonstrate long-lived immunotherapeutic properties for HCC. Aminobisphosphonates can be used to induce Vγ9Vδ2 TRM cells for potential adoptive cell-transfer, and directly sensitise HCC tumour cells to enhance Vγ9Vδ2 TRM cell targeting.

Keywords: Cancer immunology, cell based therapies, gamma-delta T cells, immunotherapy

P-1033

Characterization of extracellular vesicles in plasma during acute graft-versus-host disease

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Graft versus host disease (GvHD) is the most common lethal complication for patients undergoing allogeneic hematopoietic stem cell transplantation. Recently, extracellular vesicles (EVs) has emerged as novel diagnostic and prognostic biomarkers for both acute and chronic GvHD. EVs are nanosized particles released from different cellular sources, and contain mRNAs, microRNAs, and proteins that may modulate target cells. We have investigated small EVs in a rat model of allogeneic bone marrow transplantation, and found an increased EV load in plasma of rats suffering from acute GvHD compared to controls receiving a syngeneic bone marrow transplant. EVs were isolated and characterized after precipitation and differential ultracentrifugation, and shown to have EV morphology and express canonical markers. Preliminary results indicate higher expressions of microRNA for miR-20a, miR-29c, miR-155 and miR-191 in EVs from rats with acute GvHD compared to healthy controls. Further work include proteomic analysis of the EV isolates, and tracking of the cellular sources of plasma-derived EVs. The exact contribution of these EVs to the development of and/or progression of aGvHD is currently unknown, as is the cellular sources of the vesicles. While several questions still remain, these data so far suggests that EVs may serve as potential biomarkers for the prediction, diagnosis, and prognosis of GvHD. A full understanding of the role of exosomes in GvHD might offer new therapeutic approaches.

Keywords: Animal models, biomarkers, bone marrow transplantation, cell based therapies, endo- and exocytic vesicles in immunity, immune regulation and therapy

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POSTER PRESENTATIONS

P-1034

Two-pronged Sézary syndrome immunotherapy

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Sézary Syndrome (SS) is an aggressive leukemic form of cutaneous T-cell lymphoma characterized by circulating malignant CD4 T lymphocytes (Sézary cells). Patients with SS have poor prognosis and current treatments show high rates of relapse. Thus, there is an unmet need for an efficient treatment. Sézary cells have unique clonal potentially targetable epitopes, including its TCR, and TCR-, neoantigen- and/or overexpressed proteins-derived HLA-restricted peptides. Our aim is to design a patient-tailored two-pronged strategy against SS. The specific aims are 1) to target SS clonal TCR B cell epitopes using mAb and/or CAR T cells, 2) to target SS HLA-restricted T-cell epitopes using TCR peptide-, neoantigen- and/or overexpressed protein-specific human T cells, and 3) to validate efficacy *in vitro* and *in vivo*. For generation of mAb, SS TCR CDR3beta peptides were used for immunizations, and screening was done on SS vs non-SS CD4 cells as defined by flow cytometry using CD26 and/or PD-1. mAb screening is currently under study. For *in vitro* expansion of SS peptide-specific T cells, SS patient-derived non-SS PBMC were stimulated with pooled HLA class I+II SS peptides defined by SS WGS, WES and RNAseq-based predictions or peptidome studies. After one week, cytokine production was analyzed by flow cytometry. We have obtained preliminary data studying two SS patients, including T-cell hits likely specific of a SS TCR HLA class-I-restricted CDR3beta sequence and/or overexpressed HLA class-II-restricted MAGE-1. In conclusion, normal HLA-restricted peptides expressed by SS cells can induce T immune responses that could be used as potential immunotherapy tools.

Keywords: Anti-cancer vaccine, cancer immunology, immunotherapy

P-1035

Selective silencing of disease-associated B and T-lymphocytes by chimeric molecules in humanized NSG murine model of hashimoto's thyroiditis

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Hashimoto's thyroiditis is one of the most common endocrine disorders affecting up to 20% of the adult population. We hypothesize that it may be possible to suppress selectively the production of anti-Tg IgG antibodies from pathological B cells in humanized NOD-SCID gamma (NSG) mice by the administration to the animals of a chimeric protein molecule, containing a monoclonal antibody specific for the human inhibitory receptor CR1, coupled to peptides derived from Tg. We expect that this treatment will down-regulate B and T cell auto-reactivity and will prevent the development of thyroid lymphocite infiltration and destruction. Methods: Protein engineering, FACS, NSG animal model, ELISpot. Two EpiDOCK-predicted synthetic peptides derived from Tg and another irrelevant peptide were coupled with monoclonal anti-human CR1 antibody to construct two chimeric antibodies. Their binding to CD35 on human B cells and the effects of the chimeric constructs on PBMC from patients with HT were tested using flow cytometry, ELISpot assay and ELISA. Humanized NSG model of HT was developed by reconstitution with PBMCs from HT patients to immunodeficient NSG mice. The chimeric molecules reduced the number of anti-Tg antibody-producing plasma cells from humanized mice and suppressed B and T cells proliferation. The degree of thyroid gland destruction and cell types were determined by Immunohistochemical and FACS analyses. The constructed chimeric molecules are able to modulate selectively the activity of Tg-specific B-lymphocytes and the production of anti-Tg auto-antibodies by co-crosslinking of the inhibitory CR1 and the BCR, thus silencing the autoimmune response.

Keywords: Animal models, autoimmunity, cell based therapies

P-1036

CD73-Mediated adenosinergic immunosuppression by human dental pulp cells

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Human dental pulp contains cells with properties that are characteristic of mesenchymal stem cells (MSC). MSC are commonly isolated from bone marrow and are known for their regenerative and immunomodulatory capabilities. One essential mechanism of immune regulation is the conversion of pro-inflammatory ATP to immune suppressive adenosine (ADO) through the sequential actions of ectonucleotidases CD39 and CD73. This study explores the immunoregulatory potential of dental pulp cells (DPC) and investigates the role of ADO signaling in this context. DPC homogeneously expressed high levels of CD73 but almost no CD39 on their surface, while CD8+ T cells strongly upregulated CD39 and lost surface-bound CD73 upon activation. When co-cultured *in vitro* with DPC (0.5:1, DPC:CD8+ T), stimulated human CD8+ T cells reduced proliferation by 72% and activation (CD25/CD38 surface expression) by 38%. Furthermore, addition of ATP resulted in high ADO concentrations in co-culture supernatants in comparison to supernatants of CD8+ T cells cultured alone or with human dermal fibroblasts as a negative control. Preliminary findings demonstrate that ADO production is inhibited extensively when CD73 is blocked, pointing towards a critical role of CD73 in hydrolyzing AMP. We conclude that ADO signaling is an essential pathway underlying DPC mediated immunosuppression. The accessibility and abundance of DPC as well as their regenerative and immunomodulatory capability make DPC a reasonable alternative to bone marrow MSC and a valuable source of cell-based clinical treatments. One such example are COVID-19 patients with a severe manifestation of the disease and a hyperactivated immune response.

Keywords: Infectious disease, autoimmunity, drugs for immune modulation, immune regulation and therapy, stem cells

P-1037

Influence of IL-12 and IL-18 transduced dendritic cells on the induction of local anti-tumor response

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Dendritic cell-based vaccines are an example of currently conducted immunotherapy with the use of immunostimulants that affect tumor microenvironment (TME) changes. They play a special role in activating antigen-specific CTL lymphocytes, in the TME. The main purpose of our study was to determine the effect of administration of IL-12 and IL-18 transduced DCs (dendritic cells) on tumor growth and on the creation of local antitumor response. On the 15th, 22nd and 29th days, IL-12, and IL-18-transduced DCs stimulated with MC38 tumor cell lysate were administered peritumorally (p.t.) to C57BL/6 female mice with established MC38 tumors. Then, the effect of cell vaccine administration on tumor growth rate was monitored and the TGI (tumor growth inhibition) parameter was assessed. On the 29th and 36th day tumor nodules and sentinel lymph nodes were collected from mice for further analysis. The multiparameter flow cytometry method was applied to determine changes in the percentage of immunocompetent cells infiltrating tumors and lymph nodes. Statistically significant reduction in tumor volume on the 36th day of experiment was demonstrated after administration of DC/IL-12/TAg compared to the untreated group of mice. Moreover, we observed changes in the percentage of the myeloid cell (DC, TAM, MDSC, macrophages) and lymphoid cell ($CD4^+$ and $CD8^+$ T cells, $CD19^+$, NK) in lymph nodes and tumor tissue. In summary, the use of IL-12 and/or IL-18 transduced DCs for MC38 cancer therapy affects the tumor growth and the formation of local antitumor response.

This study was funded by National Science Centre, Poland (project no 2015/17/N/NZ4/02834).

Keywords: Cytokines and mediators, dendritic cells, immunotherapy, *in vivo* tumor models

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POSTER PRESENTATIONS

P-1038

Effects of the STAT3 inhibitors on proliferating and senescent tumor cells

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Aberrant and constitutive activation of the STAT3 pathway is a frequent event in tumor cells that promotes malignancy. STAT3 thus represents an attractive target for novel anti-tumor therapeutic approaches. Cellular senescence is a process of cell proliferation arrest that can be induced by chemotherapy, irradiation, and other stimuli. Senescent cells produce a number of secreted cytokines, chemokines, and growth factors that may either stimulate or inhibit cell proliferation. One of the major cytokines that play role in regulation of cellular senescence is IL-6. IL-6/STAT3 signaling pathway is considered to represent one of the decisive regulatory factors in cellular senescence. We have previously demonstrated that docetaxel-induced senescence in the TC-1 and TRAMP-C2 murine tumor cell lines. The objective of this study was to compare the effects of several novel and existing small molecule STAT3 inhibitors on senescent and proliferating tumor cells. *In vitro* experiments were performed using TC-1 (HPV16-associated) and TRAMP-C2 (prostate cancer) cell lines. Both TC-1 and TRAMP-C2 cells displayed elevated IL-6 secretion and have activated STAT3 signaling pathway. Therefore, STAT3 inhibitors and their novel analogues were tested for their effects, such as cytotoxicity, ability to inhibit STAT3 phosphorylation, cell proliferation on mentioned above tumor cell lines. Our data showed that STAT3 phosphorylation inhibitory and cytotoxic effects of the STAT3 inhibitors were observed in both proliferating and senescent cells. However, we observed significant differences between the cell lines and our data also suggest that senescent cells are *in vitro* less sensitive to the treatments, as compared to their proliferating counterparts.

Keywords: Cell signalling, cytokines and mediators, immunotherapy

P-1039

An MCMV Based Vaccine Vector against COVID-19 elicits strong, long-lasting humoral and cellular immunity

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Global pandemics by SARS-CoV-2 has caused severe disruptions to the public health and severe morbidity and mortality in the elderly, leading to unprecedented speed in vaccine development. However, the length of immune protection conferred by approved vaccines remains unclear. Cytomegalovirus (CMV) induces uniquely robust immune responses that are maintained for life. Hence, the potential of CMV as a vaccine vector is investigated in preclinical models and early clinical trials. We tested the immune responses induced by a recombinant mouse CMV (MCMV) vaccine vector expressing the full-length spike (S) protein of SARS-CoV-2 (MCMV-S). Single administration of MCMV-S induced antigen-specific humoral responses against the S protein up to 90 days upon mice infection. A neutralization assay with pseudotyped VSV expressing S variants (WH01, B1.1.7, B1.351) demonstrated high neutralization titers against all tested variants at 8 weeks post vaccination, albeit the neutralization against the B1.351 variant was 4-fold lower than against the other variants. Neutralization assays with SARS-CoV-2 showed an increasing neutralization titer up to 8 weeks post immunization in the IgG serum fraction and no signs of decrease upto 90 days. Similarly, CD8 T cells specific for an immunodominant MHC-I S peptide expanded over time. Therefore, MCMV induces lasting functional immune responses against SARS-CoV-2, indicating that CMV based vaccine vectors may protect against COVID-19 disease in the long-term. *In vivo* challenge experiments are underway, and results will be presented.

Keywords: Adaptive immunity, adjuvants and vaccines, animal models, antibody, infectious disease

P-1040

Role for G-CSF in neutrophilic extramedullary myelopoiesis in systemic juvenile idiopathic arthritis

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Systemic juvenile idiopathic arthritis (sJIA) is an auto-inflammatory disease of childhood-onset. sJIA is associated with neutrophilia, including immature granulocytes, which might be driven by the growth factor granulocyte-colony stimulating factor (G-CSF). This study aimed to unravel the role of G-CSF in the pathology of sJIA. Injection of complete Freund's adjuvant (CFA) in BALB/c mice induces mild inflammation and neutrophilia in wild-type (WT) mice and a more pronounced disease, reminiscent to sJIA patients, in interferon-γ (IFN-γ) knock-out (KO) mice. Extramedullary myelopoiesis was studied in CFA-immunised mice by single cell RNA-sequencing and the effect of G-CSFR-blockage on neutrophil development and sJIA pathology was evaluated. Additionally, in sJIA patients, plasma G-CSF levels were measured. Both in sJIA patients and in the corresponding mouse model plasma levels of G-CSF are increased. Using the mouse model, we demonstrate that G-CSF is responsible for the observed neutrophilia and extramedullary myelopoiesis and the induction of immature neutrophils and myeloid-derived suppressor cells. Administration of a G-CSF-receptor antagonising antibody blocked the maturation and differentiation of neutrophils in CFA-immunised mice. In IFN-γ KO mice, anti-G-CSF-receptor antibody treatment was associated with almost complete inhibition of arthritis development. We describe for the first time the role of G-CSF in a mouse model of sJIA and point towards an important G-CSF-induced myelopoiesis and neutrophilia, regulating the development of arthritis.

Keywords: Autoinflammation, cytokines and mediators, neutrophils

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POSTER PRESENTATIONS

P-1041

Molecular dynamics analysis of ERAP1 and ERAP2 monomer, homodimer and heterodimers as relevant players in ankylosing spondylitis

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Ankylosing Spondylitis (AS) is a chronic inflammatory autoimmune disease manifesting as arthritis and can lead to bone fusion in severe cases. Endoplasmic Reticulum associated Aminopeptidase-I (ERAP1) and -II (ERAP2) were implicated in AS pathogenesis as risk factors along with HLA-B27. ERAP1 and ERAP2 are homologous proteins involved in antigen processing and the MHC-I assembly pathway. Although the whole landscape of AS pathogenesis is currently unclear, it is already known that different ERAP1 haplotypes can cause a change in enzyme function which can lead to abnormal peptide trimming, eventually triggering inflammation and the beginning of AS. Moreover, it was recently discovered that, *in vitro*, ERAP1 and ERAP2 proteins were able to form a heterodimer structure, increasing their trimming efficiency. In the framework of this study, monomers of ERAP1 and ERAP2, homodimer and heterodimer complexes of ERAP1 and ERAP2 structures were analyzed via Molecular Dynamics (MD) simulations in an attempt to understand the effect of homo- and hetero-dimerization on the protein structure and function. To this end, we have performed MD simulations using the NAMD program. Each system was solvated with 10 A° thick TIP3P water and neutralized with 0.15 M KCl. CHARMM36m force-field was adopted under PBC. After successfully minimizing the structures and gradually increasing their temperature to 310.15 °K, equilibration and production simulations were run using NVT (2 ns) and NPT (100 ns) ensembles, respectively. The impact of dimerization on the enzymes' structure and function were analyzed via RMSD, RMSF, PCA, the radius of gyration calculations.

Keywords: Antigen processing and presentation, autoimmunity, autoinflammation, inflammatory disease, modelling

P-1042

Macrophages as potential carriers of boron-rich compounds in boron neutron capture therapy

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Boron neutron capture therapy (BNCT) is classified as a targeted anti-cancer radiotherapy based on boron delivery to tumor cells followed by irradiation of the affected area with a neutron beam. One of the most important challenges for the development of this therapy is the search for new carriers capable of carrying an appropriate amount of boron to cancer cells. Due to playing an important role by macrophages in the tumor environment, we intended to focus on the harnessing the phagocytic cells as carriers to deliver boron-rich compounds to treated tissue. In our research we used newly synthesized two fractions of boron carbide nanoparticles with a defined surface and shape by dynamic light scattering and atomic force microscopy. To evaluate the toxic effects of tested compounds on RAW 264.7 macrophage cells, the Annexin V Assay, Propidium Iodide protocol and MTT cell viability assay were performed. Additionally, the transmission electron and fluorescence microscopy techniques were used to demonstrate the uptake of the tested compounds by RAW 264.7 cells. Fraction I of boron carbide included nanoparticles with a size not exceeding 100 nm and fraction II included nanoparticles with a size between 100 and 200 nm. Our results demonstrated that RAW 264.7 cells tolerated boron carbide in a wide range of concentrations and are able to uptake the tested compounds. We postulate that designed by us cellular carriers of boron compounds possess a potential for the application in BNCT.

Keywords: Cancer immunology, cell based therapies, macrophage, microenvironment, phagocytosis

P-1043

Computational analysis of two arthritogenic peptides in complex with HLA-B*27:05 and HLA-B*27:09 associated with ankylosing spondylitis

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Ankylosing spondylitis (AS) is an autoimmune disease affecting the axial skeleton. Several studies have found out the association between AS and HLA-B27. As an MHC-I, HLA-B27 is responsible for antigen presentation and involved in immune responses. Although HLA-B*27:05 and HLA-B*27:09 differ in only one amino acid, HLA-B*27:05 is implicated in the pathogenesis of AS, and not HLA-B*27:09. This strong association between HLA-B*27:05 and AS could be explained by the arthritogenic peptide theory focusing on the similarity between the self and arthritogenic peptides. In this study, the underlying mechanism of interactions between two arthritogenic peptides (ARGQPGVMG-DRASFIKNL) previously identified by Atagündüz et. al. (2005) and aforementioned subtypes have been studied through molecular docking and molecular dynamics (MD) methods to understand their role in AS pathogenesis. Firstly, a flexible docking approach for HLA-B27 subtypes including beta-microglobulin with two self-peptides was employed using HADDOCK. Representative complexes were selected based on HADDOCK scores, clusters, and known HLA-B27 and peptide complexes. For each protein-peptide complex, 50-100 ns MD simulations have been carried out using NAMD with CHARMM36m force field. The complexes were solvated in TIP3P water (~ minimum 10 Å) and neutralized with 0.15 M NaCl, the temperature of the system was increased gradually to 310 K°. The systems were minimized 50,000 steps. Equilibration and production steps were performed in NVT (2 ns) and NPT (50-100 ns) ensembles, respectively. Lastly, RMSD, RMSF, PCA, H-bonding analyses were performed to compare the structural effects of these peptides on antigen presentation by MHC-I.

Keywords: Antigen processing and presentation, autoimmunity, autoinflammation, inflammatory disease, modelling

P-1044

Molecular biomakers of mouse tumor immunogenicity and prediction of response to dendritic cell vaccines and anti-PD-1 treatment

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Less than half of oncology patients are sensitive to immunotherapy and recent studies show that this may be due to different tumor ability to process and present Ag with MHC I molecule. The aim of our study was to evaluate whether disorders of Ag processing and presentation mechanism (APM) could affect tumor response to treatment with dendritic cell vaccine DCV or anti-PD-1 in murine model. For this reason the expression of genes involved in APM was evaluated in LLC1 and GL261 tumors (RT-PCR). Efficacy of DCV and anti-PD-1 treatment was assessed by LLC1 and GL261 tumor size, immune cell infiltration (FC) and expression of immune-related genes (RT-PCR). DCV were generated from murine bone marrow cells by differentiating with GM-CSF+IL-4 for 7 days and maturing with LLC1/GL261 cell lysate+E.coli lipopolysaccharide. Impaired APM in LLC1 cells resulted in low tumor immunogenicity leading to the absence of anti-cancer immune response. The formation of effective antitumor immune reaction was seen in GL261 tumors where APM was not altered. Differences were also seen then treating tumors with immunotherapy where it effectively slowed GL261 development and almost had no effect for LLC1 tumors We show that changes in Ag processing and presentation mechanism might result in tumor insensitivity to treatment with dendritic cell vaccines and anti-PD-1. Therefore, evaluation of APM involved genes expression (Psmb8, Psmb9, Psmb10, Tap1, Tap2, Erap1, B2m) might help to stratify primary untreated murine tumors and predict their response to DCV and anti-PD-1 treatment.

Keywords: Animal models, antigen processing and presentation, biomarkers, checkpoint inhibition, dendritic cells

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POSTER PRESENTATIONS

P-1045

NK-cell derived extracellular vesicles target and kill resistant cancer cell-derived spheroids

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NK cell-based therapies has shown promising results for hematological cancers. Their therapeutic use against solid cancers is hampered by their poor tumor-infiltrating ability, as well as a suppressive tumor microenvironment. Extracellular vesicles (EVs) secreted from NK cells (NK cell-derived EVs; NK-EVs) are shown to contain the cytolytic material necessary for targeting and killing cancer cells, and may even circumvent the suppressive tumor microenvironment. We have in this study compared output, proteomic profiles, and functional characteristics of EVs from primary NK cells and the NK cell lines NK92 and KHYG-1 cultured in IL-15 alone or in combination with IL-12 and IL-18. EV isolation was verified by electron microscopy and nanoparticle tracking analysis, as well as expression of canonical markers by western blotting. We found similar production output between the two culture conditions, and that NK92 cells produced significantly more EVs per cell then primary NK cells. EVs from primary NK cells and NK92 expressed more cytolytic proteins compared to EVs from KHYG-1 cells, and this translated to higher killing rate of cancer cell targets. We further demonstrate that NK-EVs from primary NK cells or NK92 cells induced apoptosis ofspheroids derived from a wide range of cancers including breast, colon, prostate, brain, skin, and ovarian cancer. Infiltration of EVs into the spheroid core was demonstrated. NK-EVs showed killing of tumor spheroids even in situations where NK cell killing of the cancer cell line is low, indicating that NK-EVs may be suitable therapeutic agents.

Keywords: Cancer immunology, endo- and exocytic vesicles in immunity, immunotherapy, innate lymphoid cells, NK cells

P-1046

Mutant RUNX1 may encode relevant neoantigens for immunotherapy in acute myeloid leukemia

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About 10% of patients with acute myeloid leukemia (AML) present with mutations in Runt-related transcription factor 1 (RUNX1). Mutations occur throughout the entire gene, but insertions and deletions often result in translation of the same alternative reading frame creating RUNX1 proteins sharing C-terminal amino acids. Here, we investigated whether this alternative reading frame is a relevant target for immunotherapy. A construct with the c.883dupT-mutated RUNX1 gene encoding p.S295FfsTer305 was introduced into 4 different EBV-B cells expressing common HLA class I alleles. Peptides presented in HLA class I were eluted and identified by mass spectrometry. A total of 13 mutant RUNX1 peptides were identified including 9 peptides in HLA-B*07:02. PBMCs from healthy HLA-B*07:02+ individuals were isolated and screened for specific T cells recognizing the 4 most C-terminal RUNX1 peptides. Multiple peptide-MHC tetramer-positive CD8 T cell clones were isolated. Clones specific for 2 out of 4 RUNX1 peptides show specific recognition of both HLA-B*07:02 expressing AML cell lines transduced with mutant RUNX1, and cell lines expressing an endogenous RUNX1 mutation induced by CRISPR/Cas9 resulting in the desired alternative reading frame. In conclusion, we show that various neopeptides translated from RUNX1 in an alternative reading frame are presented on the cell surface in HLA class I and can also be recognized by specific T cells.

Keywords: Antigen processing and presentation, cancer immunology, immunotherapy

P-1047

Meta-analysis of gene popularity: a focus on gene-specific biological features

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The reasons for selecting a gene for further study might vary from historical momentum to funding availability, thus leading to unequal attention distribution among all genes. However, certain biological features tend to be overlooked in evaluating a gene's popularity. Here we present a meta-analysis of the reasons why different genes have been studied and to what extent, with a focus on the gene-specific biological features. From unbiased datasets we can define biological properties of genes that reasonably may affect their perceived importance. We make use of both linear and nonlinear computational approaches for estimating gene popularity to then compare their relative importance. We find that roughly 25% of the studies are the result of a historical positive feedback,which we may think of as social reinforcement. Of the remaining features, gene family membership is the most indicative followed by disease relevance and finally regulatory pathway association. Disease relevance has been an important driver until the 1990s, after which the focus shifted to exploring every single gene. We also present a resource that allows one to study the impact of reinforcement,which may guide our research toward genes that have not yet received proportional attention.

Keywords: Big data, omics technologies, RNAseq

P-1048

Small recombinant non-cognate ligands of HIV-1 broadly-neutralizing antibodies as antigens eliciting broadly neutralizing sera in mice

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The development of HIV-1 vaccine has been the main goal of HIV research since dicovery of HIV. Important obstacle in vaccine development is an enormous HIV-1 antigenic variability of HIV-1 envelope (Env) glycoprotein - neutralizing antibody target. Rare population of elite neutralizers provided source of strong and exceptionally broad neutralizing antibodies. Our novel strategy is based on the identification of proteins binding to a paratope of selected broadly neutralizing antibodies (bNAb), that could mimic cognate HIV-1 Env epitope, acting in similar fashion to antiidiotype Ab, and could be used as potent immunogens for induction of protective virus-neutralizing antibodies. We tested this "non-cognate ligand" concept using two highly complex combinatorial libraries derived from scaffold of streptococcal protein G and from human myomesin-1 protein domain. We identified binders of several HIV-1 bNAbs confirmed by molecular modeling as structures biochemically similar their respective Env epitopes. After immunization of experimental mice several binders elicited serum antibodies neutralizing panel of Clade A, B, C, and AE pseudotyped HIV-1 viruses. We confirmed that this strategy is effective to elicit antibodies neutralizing HIV-1 due to recognition of dificult to target Env epitopes such Membrane Proximal External Region, CD4 binding site, and V3-loop. This strategy proved to be a new promising approach in HIV-1 and other infection preventing vaccine development.

Keywords: Animal models, B lymphocytes, infectious disease, viral infections

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POSTER PRESENTATIONS

P-1049

Exosome: an emerging participant in liver disease progression

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Exosomes are involved in many cellular processes including antigen presentation, immune escape and tumour progression. However, their involvement in liver diseases pathogenesis and immunotherapeutic applications of exosomes are still not clear. In this study, the role of exosomes in liver disease progression in the course of HBV infection, Cirrhosis and HCC was investigated. Furthermore, we also sought to test the anti-tumour activity of ligand-loaded tumour derived exosomes as potential anti-tumour therapeutics in HCC xenografts. Exosomes were purified from healthy controls' and HBV carrier, Cirrhosis and HCC patients' peripheral blood plasma. Then, exosomes were characterized by flow cytometry and western blot. After, the levels of circulating pro-inflammatory and Th1 as well as anti-inflammatory and Th2 cytokines/chemokines in control, HBV Carrier, Cirrhosis, HCC groups were determined. The immunomodulatory role of exosomes were assessed by stimulating healthy PBMCs with patient exosomes for 24hrs. The similar stimulation assay was performed by incubating murine splenocytes with patient exosomes. Finally, exosomes were utilized as cargo-delivery systems to encapsulate different TLR and NKT agonists and tested *in vivo*. The data demonstrated that PD-L1 expression was gradually upregulated on patients' exosomes from HBV carrier to HCC state. Cytokines that play a major role in inflammation showed a slight increase especially in Cirrhosis and HCC group when compared to controls. Similarly, murine splenocyte stimulation showed the similar pattern regardless of immunologic background of the mice. Remarkably, ligand-loaded exosome administration enhanced the survival of HCC bearing mice by suppressing the tumour growth showing a promising pattern as potential immunotherapeutics.

Keywords: Immune regulation and therapy, immunotherapy, *in vivo* tumor models

P-1050

Hypoxic and pro-inflammatory priming of mesenchymal stem cell-derived extracellular vesicles to reduce disease severity and immune responses in inflammatory arthritis

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Novel biological therapies have revolutionised the management of Rheumatoid Arthritis (RA) but no cure currently exists. Mesenchymal stem cells (MSCs) immunomodulate inflammatory responses through paracrine signalling, including via secretion of extracellular vesicles (EVs) in the cell secretome. We evaluated the therapeutic potential of hypoxic and pro-inflammatory primed MSCs-derived small EVs in an antigen-induced model of arthritis (AIA). EVs isolated from MSCs cultured normoxically (21% O2, 5% CO2), hypoxically (2% O2, 5% CO2) or with a pro-inflammatory cytokine cocktail were applied into the AIA model. Disease pathology was assessed post-arthritis induction through swelling and histopathological analysis of synovial joint structure. Activated CD4+ T cells from healthy mice were cultured with EVs or MSCs to assess deactivation capabilities prior to application of standard EVs *in vivo* to assess T cell polarisation within the immune response to AIA. All EVs treatments reduced knee-joint swelling whilst only normoxic and pro-inflammatory primed EVs improved histopathological outcomes. *In vitro* culture with EVs did not achieve T cell deactivation. Polarisation towards CD4+ helper cells expressing IL17a (Th17) was reduced when normoxic and hypoxic EV treatments were applied. Normoxic EVs applied into the AIA model reduced Th17 polarisation and restored Th17:Treg homeostatic balance. Priming of MSCs in EV production can be applied to alter the therapeutic efficacy however normoxic EVs present the optimal strategy for broad therapeutic benefit. The varied outcomes observed in MSCs priming may promote EVs optimised for therapies targeted for specific therapeutic priorities. EVs present an effective novel technology with potential for cell-free therapeutic translation.

Keywords: Animal models, inflammatory disease, rheumatoid arthritis, stem cells

P-1052

Neutrophil PD-L1 expression as a novel predictive biomarker for IV stage melanoma patients

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To evaluate the roles of peripheral blood normal density (NDNs) and low density neutrophils (LDNs) as predictive biomarkers in anti-PD-1 therapy in advanced melanoma patients. 65 stage IV melanoma patients were prospectively recruited. NDNs and LDNs were isolated from peripheral blood, before and during anti-PD-1 therapy, to evaluate their activation status and PD-L1 expression. Melanoma patient NDNs displayed an activated phenotype (increased percentages CD16+Cd62L− cells) and increased PD-L1 levels compared to healthy controls (HCs). Melanoma patients had increased percentages of LDNs, which displayed higher levels of PDL-1 compared to autologous NDNs. PD-L1 expression levels on NDNs and LDNs did not change during immunotherapy. Patients with low NDN PD-L1 expression displayed better progression free survival (PFS) and overall survival (OS) compared with patients with high NDN PD-L1 expression. Our results show that the activation status and PDL-1 expression of stage IV melanoma patient NDNs were modified compared to HCs and significantly predicted anti-PD-1 immunotherapy response. NDN PD-L1 expression emerges as a novel predictive biomarker in stage IV melanoma patients undergoing anti-PD-1 immunotherapy. Our findings call for a rigorous assessment of neutrophil subsets role in melanoma immunotherapy.

Keywords: Biomarkers, cancer immunology, checkpoint inhibition, immunotherapy, innate immunity, neutrophils

P-1053

Genetic modification of NK cells with a chimeric antigen receptor specific to the prostate stem cell antigen (PSCA)

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Modification of cytotoxic immune cells with chimeric antigen receptors (CARs) for cancer treatment is becoming increasingly popular. Along with CAR-T technologies, CAR-NK technologies are also developing for both hematological and solid tumors. In this work, we obtained primary NK cells specific to the prostate stem cell antigen (PSCA) by transducing a chimeric PSCA-specific receptor (PSCA-CAR) gene into the NK cells isolated from human peripheral blood. The envelope protein RD114 was used to assemble retroviral particles with PSCA-CAR. The efficiency of NK cell transduction reached up to 25%. Next, we developed a system for testing functional activity of the transduced NK cells: HEK293T cell line was modified using lentiviral transduction to express surface PSCA, and cytotoxic potential of PSCA-CAR NK cells was verified in a degranulation assay by measuring CD107a surface expression after incubation with HEK293T-PSCA target cells. 71.1 ± 0.45% of PSCA-CAR-NK cells expressed CD107a degranulation marker after co-incubation, while for the unmodified NK cells the value was 40.8 ± 0.5% of cells. Thus, successful modification of NK cells with the PSCA-CAR gene allowed us to obtain cytotoxically active NK cells which specifically recognize the PSCA ligand.

The work was supported by Russian Science Foundation, grant # 20-75-00129.

Keywords: Immune regulation and therapy, immunotherapy, innate lymphoid cells, cancer immunology, NK cells

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POSTER PRESENTATIONS

P-1054

From affinity maturation to kinetics maturation in GC modeling

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Germinal centres (GC) are microanatomical structures, in which B lymphocytes proliferate, undergo somatic hypermutations (SHM) and positive selection to produce high-affinity antibodies (Ab). Ab affinity (KA) is defined as the ratio of kinetic constants kon and koff which determine the kinetics of bond between a B-cell receptor (BcR) and antigen (Ag). Earlier research suggest that GC may not only select based on affinity but may preferentially select clones with specific kinetic properties (kinetic maturation [1-3]). We, therefore, hypothesized that under specific modes of selection the GC will preferentially select clones with specific kinetic properties. Mechanisms underlying such selection process are unclear but we propose two putative mechanisms that explicitly include both kinetic constants and compare this to the mechanism in which B-cell selection is solely based on affinity. We use a pre-existing agent-based model of the GC that comprises primary cellular mechanisms of the GC reaction, and which implements affinity-based selection [4]. We extended this model to allow for two modes of kinetic maturation. We show that if binding and unbinding of the BcR to the FDC-presented Ag is made dependent on kon and koff respectively, there is no competitive advantage for specific clones. However, considering the extraction of the Ag from the FDC also depends on koff and, consequently, can be interrupted before completion, a selective advantage for B cells with low association/dissociation rates is the result. Although this finding needs experimental validation, it shows that selection mechanisms for binding kinetics might operate as part of the GC reaction.

Keywords: Adaptive immunity, B lymphocytes, modelling

P-1055

Effect of different maturation stimuli on phenotype and function of clinical grade dendritic cells from prostate cancer patients

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Immunotherapy using monocyte-derived dendritic cells (moDC) has long been a promising approach for the treatment of cancer. However, the clinical effect has been rather disappointing, most likely due to a combination of suboptimal DC populations used and presence of inhibiting mechanisms in the patients. In this study we tested two maturation cocktails previously shown to be able to induce immunogenic moDC from healthy individuals utilizing cells from patients with metastatic prostate cancer. The cocktails consisted of i) TNF-α, IFNγ, Toll-like receptor (TLR) 7/8 agonist R848, TLR3 agonist polyI:C, and ii) OK432 (Picibanil), TLR7 agonist CL097, and PGE2. Both cocktails resulted in mature cells able to induce antigen-specific T cells analyzed by IFN-γ secretion with tuberculin as recall antigen, even though the moDC showed high expression of inhibitory markers PD-L1 (CD174) and PD-L2 (CD173). Phenotypic analyses of the induced T cells revealed activation of mainly CD45RO+ CD4+ T cells with high expression of CD25, especially in those cells stimulated with OK432-DC. More functional assays will have to be performed in order to evaluate potential immunosuppressive capacities of these cells.

Keywords: Anti-cancer vaccine, cell based therapies, dendritic cells, immunotherapy

P-1056

A metastatic MSI-H colorectal cancer: long-term benefit from nivolumab

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Colorectal cancers (CRC) and colorectal cancers with metastasis (mCRC) that are associated with deficient DNA mismatch repair mechanism systems and High Levels of Microsatellite Instability (MSI-H) benefit less from traditional chemotherapy regimens. Nivolumab, a PD-1 checkpoint inhibitor, was shown to be efficient in patients with chemotherapy-resistant MSI-H mCRC in phase 2 studies. In this report, we present a case of MSI-H mCRC who is long-term benefiting second-line Nivolumab monotherapy. A 62-year-old woman presented with lower gastrointestinal bleeding. Colonoscopy revealed a mass located in the left colon and pathology demonstrated adenocarcinoma. The patient then underwent a laparoscopic left hemicolectomy and started chemotherapy with Xelox. The disease relapsed, metastasizing to the spleen, liver, abdominal lymph nodes, and soft tissue. Molecular tests showed K-RAS, N-RAS, BRAF wildtype, and MSI-H. The patient was commenced on FOLFOX (oxaliplatin and 5-fluorouracil) and Panitumumab. After six cycles, PET/CT scan showed disease progression. In June 2019, she was started on Nivolumab monotherapy. The patient could not attend the therapy due to the Covid-19 pandemic between January and July 2020. However, an abdominal MRI screening in July 2020 demonstrated no new lesions. Prominent regression was observed in the peritoneal involvement in the subdiaphragmatic space. The patient has been receiving Nivolumab for two years and doing well. Although the patient paused Nivolumab therapy for six months, her disease did not show any progression. Further investigations on the durability of Nivolumab therapy response and the timing of discontinuation of anti-PD-1-therapy in responders are needed.

Keywords: Cancer immunology, checkpoint inhibition, immunotherapy

P-1057

Increased T cell infiltration and T cell receptor diversity in homologous recombination repair-deficient prostate cancer

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Homologous recombination repair deficiency (HRD) is observed in approximately 10% of castrate-resistant prostate cancer (CRPC) patients and is mainly a consequence of *BRCA* inactivation. HRD has been suggested to increase tumor immunogenicity, partly explained by a higher tumor mutational burden and STING pathway activation. Primary and/or metastatic prostate cancer samples were used of 15 patients with *BRCA* inactivation and/or an HRD signature (HRD/BRCA; 19 samples) and 47 patients without biallelic DNA damage repair alterations (WT; 61 samples). CD3+, CD8+ and FoxP3+ tumor-infiltrating lymphocytes (TIL) were quantified by multiplex immunohistochemistry; PD-L1 expression was assessed using the SP263 assay. TIL density was dichotomized by median split using a tissue site-specific cut-off and PD-L1 expression by a cut-off value of 1%. Additionally, T cell receptor (TCR) sequencing of peripheral blood mononuclear cells was performed in 49 patients, of which 16 HRD/BRCA and 32 WT. HRD/BRCA patients more frequently had intratumoral CD3+ or Foxp3+ TIL above median compared to WT patients (both: 77% vs 35%, chi-square p-value: 0.013), but there was no significant difference in CD8+ TIL (63% vs 39%, p=0.17) or PD-L1 expression on tumor or immune cells. Additionally, HRD/BRCA patients showed a more diverse (Wilcoxon test, p=0.0045) and evenly distributed (p=0.050) peripheral TCR repertoire compared to WT patients. HRD/BRCA patients have increased CD3+ and FoxP3+ TIL and an altered peripheral TCR repertoire compared to WT patients. These data warrant further research into the efficacy of checkpoint inhibitors in CRPC patients with HRD/BRCA tumors.

Keywords: Cancer immunology, checkpoint inhibition, immunotherapy

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POSTER PRESENTATIONS

P-1058

The effect of immunotherapy based on a combination of modified dendritic cells overproducing IL-12, IL-15 or IL-18 and anti-IL-10R antibodies on the inhibition of tumor growth in MC38 colon carcinoma

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Dendritic cells (DCs) are the most potent antigen-presenting cells and are the key initiator of tumor-specific immune responses. Their activity can be enhanced by IL-12, IL-15, and IL-18, while IL-10 can block their interaction with immune cells. Therefore, it is suitable to harness anti-IL-10R antibodies to abolish the negative IL-10 effects. On the 16th, 23th and 30th days, C57BL/6 female mice with established MC38 tumors were administered intraperitoneally (i.p.) with an anti-IL-10R antibody. The next day, overproducing IL-12, IL-15, or IL-18 DCs stimulated with MC38 tumor cell lysate (TAg) were administrated peritumorally (p.t.). Besides, mixtures of transduced DCs were also applied. The efficacy of anti-tumor therapy was estimated based on tumor growth inhibition, on the 38th day. On the same day, tumor nodules were collected from mice to identify myeloid and lymphoid cells infiltrating the tumor tissue. An increase in the percentage of CD4+ and CD8+ lymphocytes among infiltrating cells was found after administration of each type of dendritic cell-based vaccines which was accompanied by statistically significant decrease in Treg compared to the untreated group. We also observed an increase in M1/M2 macrophage ratio in the mice groups which obtained mixture of transduced DCs, meanwhile, the highest inhibition of tumor growth was observed after administration of DC/IL-12 /TAg+DC/IL-15/TAg. In conclusion, therapy based on the administration of the anti-IL-10R antibody, and the dendritic cell-based vaccines effectively inhibits tumor growth and promotes influx of the immune cells into tumor tissue.

This study was funded by National Science Centre, Poland (project no 2017/27/B/NZ6/02702).

Keywords: Cytokines and mediators, dendritic cells, immunotherapy

P-1059

Modular assembly of immune-event-labeled synthetic AIRR-datasets for the development and benchmarking of AIRR-based machine learning

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Machine learning on adaptive immune receptor repertoire (AIRR) data enables diagnostics and therapeutics design. Experimental immune receptor data is usually only sparsely annotated in terms of immune event information such as immune state or antigen specificity. The lack of large-scale immune-event-annotated data renders the development and benchmarking of robust, explainable, and interpretable machine learning approaches unfeasible. We are currently developing the LIgO software suite that enables the modular ("lego"-like) assembly of immune-event-labeled synthetic AIRR-datasets for the development and benchmarking of AIRR-based machine learning. LIgO will contain different methods for simulating immune events and confounding factors in both repertoire-based (diagnostics-related) and sequence-based (therapeutics-related) settings. In a case study, we will show how the implantation of immune signals (k-mers) into immune receptor sequences changes immune receptor generation probability instigating a data-driven discussion of the nativeness of the simulated immune repertoires.

Keywords: Adaptive immunity, big data, modelling

P-1060

Extracellular vesicles from dendritic cells are more potent than PD-1/PD-L1 blockade in a mouse melanoma model and their combination further enhances the anti-tumour response

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Extracellular vesicles (EVs) are important mediators of intercellular communication and immune responses. Dendritic-cell (DC) derived EVs loaded with tumour antigens have been shown to induce antigen-specific immune responses and reduce tumour growth in mice. Immune checkpoint blockade, mostly on the PD-1/PD-L1 axis, is highly effective in a subset of patients. However, most patients do not respond, due to mechanisms including reduced T cell priming to cancer antigens and T cell infiltration into the tumour, and decreased antigen presentation by tumour cells. Therefore, a combination of EVs, which induce antigen-specific T cell responses, and anti-PD-1/PD-L1, which prevent T cell exhaustion, could be beneficial. To test this, we first isolated EVs loaded with ovalbumin (OVA) and α-galactosylceramide from DCs. To determine the effect of these EVs on antigen-presenting cells, we incubated them with DCs *in vitro* and observed an increase in MHC II and CD86, but also an increase of PD-L1, indicating a possible benefit of the EV and checkpoint combination. When administered to mice inoculated with OVA-expressing melanoma, treatment with EVs alone, but not anti-PD-1/PD-L1 alone, induced strong anti-cancer responses. EVs also increased MHC I and PD-L1 expression on cancer cells. Furthermore, there was a trend towards increased OVA-specific CD8+ T cell induction, but this did not translate into reduced tumour growth. However, EV administration before tumour inoculation and subsequent PD-1/PD-L1 treatment, clearly prolonged survival compared to the monotherapies. These data indicate that combining EVs and PD-1/PD-L1 improves anti-tumour responses, making it a promising treatment strategy for the clinic.

Keywords: Anti-cancer vaccine, cancer immunology, endo- and exocytic vesicles in immunity, immunotherapy

P-1061

Polymer therapeutics for treatment of rheumatoid arthritis

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Rheumatoid arthritis is a chronic inflammatory autoimmune disease caused by alteration of both the innate and adaptive immune system. Current therapy includes glucocorticoids, nonsteroidal anti-inflammatory drugs. Unfortunately, it is often connected with various side effects. Generally, the conjugation of the drug to polymeric delivery systems improves therapeutic properties of the carried drugs. In some cases, the drug must be derivatized prior the attachment to polymer with the aim to introduce suitable functional groups for the attachment via stimuli sensitive spacers. Therefore we aimed to evaluate the biological properties of dexamethasone modification intended for synthesis of drug delivery systems targeting the affected joints. The derivatives of dexamethasone (DEX) were prepared by reaction of hydroxyl group of DEX with different oxo-acids. These derivatives were afterward attached to water-soluble linear N-(2-hydroxypropyl) methacrylamide copolymer platform. The biological effects of dexamethasone derivatives were evaluated using murine peritoneal macrophages RAW 264.7 activated by lipopolysaccharide (LPS). The toxicity was evaluated by measuring of lactate dehydrogenase release. The anti-inflammatory effects were studied by measurement of nitric oxide and cytokine production. The modifications of dexamethasone did not cause significant acute toxicity. The derivatives of the DEX showed at least similar inhibitory effects on inflammatory response of RAW 264.7 macrophages activated by LPS effect as free DEX. The data demonstrate that anti-inflammatory activity of dexamethasone was retained after its modification. The data suggest that the modification of DEX did not affect its biological activity. Therefore, these derivatives are suitable for preparation of drug delivery system.

Keywords: Drugs for immune modulation, autoimmunity, inflammatory joint diseases, macrophage

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POSTER PRESENTATIONS

P-1062

Development of a delayed burst delivery system for single dose vaccination

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Vaccination represents one of the most important healthcare advances but vaccine compliance remains a major issue in disease eradication. Single dose vaccines could provide an effective solution to improve global vaccination coverage while easing the logistical burden during outbreaks. Our aim is to develop a vaccine delivery system based on injectable core-shell polymer particles that enable a delayed burst release *in vivo*, removing the need for booster vaccination. Core-shell particles were produced using a high precision emulsification technique on a custom-designed scaleable microfluidic platform. The release mechanism and its tuneability were investigated using fluorescent model payloads (dextran and proteins) from particles incubated *in vitro*, using spectroscopy, fluorescence and confocal microscopy. Safety and release studies (IVIS imaging) were performed *in vivo* in mice and experiments are ongoing to evaluate the stimulation of a delayed immune response. Uniformly sized core-shell particles were successfully produced with a diameter that could be varied between 40-100µm to facilitate needle injection while retaining the ability to persist at the injection site. *In vitro* release studies demonstrated that a delayed burst release is achievable by this architecture and that the delay can be tuned to by varying the polymer shell composition, in the range 4 to 9-weeks. Release was found to be the result of pore formation in the particle shell, and hence also influenced by the shell thickness and media pH. The immunogenicity results will be presented at the meeting.

Keywords: Adjuvants and vaccines, animal models, protection

P-1063

Nematode-derived galectin inhibits colitis via modification of mast cells activity

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Galectins are group of proteins that bind β-galactosides and play key roles in inflammation and autoimmunity. Galectins produced by parasites show similarity in molecular structure to human galectins. *Teladorsagia circumcincta*, a parasite of the sheep, produces galectin (TcGal) that has a similar structure to galectin-3. We hypothesised that nematode-derived galectin inhibits mast cell degranulation and decreased activity of mast cells can reduce the severity of colon inflammation. The aim of this study was to evaluate the influence of galectin derived from *T. circumcincta* on rat mast cell line RBL-2H3 activity *in vitro* and the course of dextran sulphate sodium (DSS) - induced colitis in mice. RBL-2H3 cells were cultured in the presence of TcGal. BALB/c mice with induced colitis received TcGal subcutaneously. This study has shown that TcGal can bind to and/or be bound by IgE, This binding influenced the degranulation of RBL-2H3 cells and the release of eicosanoids. Treatment of mice with TcGal resulted in reduced severity of DSS-induced colitis. Our results indicate that the production of large quantities of galectin by nematodes is likely to enhance their establishment and survival. In addition, nematode galectins may alleviate autoimmune diseases.

Keywords: Autoimmunity, drugs for immune modulation, inflammatory bowel disease, mast cells, parasite infections

P-1064

Cellular platform for studying antigenic peptide binding to SLA-I molecules in pigs

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Rationally design of vaccines is required for pathogens where empirical vaccines have not been successful. However, the development of new recombinant vaccine relies on the ability to identify antigenic epitopes or antigens that the immune system detects to subsequently mounting a protective immune response. Recognition of epitopes by lymphocytes from different species and individuals is restricted by the major histocompatibility complex (MHC) molecules – named swine leukocyte antigen (SLA) in pigs- which is responsible for foreign antigen presentation. Our group has already identified new antigenic epitopes from swine influenza virus (SwIV) presented by three MHC-I in pigs (SLA-I) -SLA-1*0702, SLA-1*14:02 and SLA-2*11:04-. Here, we have set up a cellular platform based on MHC reconstitution assay which implements a novel cell platform to characterize the binding proprieties of SLA-I proteins. C1R, a lymphoblastoid cell line selected for loss of HLA class I antigen expression was chosen for SLA-1*14:02 transfection. Stable transfectants were used for analysis of their peptide biding properties by 1) acid stripping and 2) reconstitution of SLA-I molecule with each peptide. Antigenic peptides from different pathogens and variations of those were tested. Their differential binding abilities to SLA-I were observed by flow cytometry. Our results have successfully generated a cellular platform showing specific binding to this SLA in low concentration of antigenic peptides. This is the first time that a cellular platform was used to define and refine peptide binding abilities for SLA-I molecules. Our results will have implications in the rational design of vaccines.

Keywords: Molecular immunology, viral infections, adjuvants and vaccines, protection

P-1066

Omics biomarkers as translational medicine tools in skin malignancies

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The immune setting holds a key role in tumorigenesis, from which potential biomarkers for therapy response prediction or disease monitoring are continuously discovered. Omics methodologies represent a mandatory robust tool heading for data consistency, accuracy and reproducibility, and providing vigorous biomarkers for disease management. Within omics platforms, protein microarray plunges in a specific and important niche for biomarkers discovery, both for research and diagnostic purposes. In skin cancer, especially in cutaneous melanoma, high tumor heterogeneity and complex immune interactions claims a combined analysis to decode the cellular phenotype. As proteins are those "pointers" that delineate the cellular phenotype, proteomic approaches would accurately measure the magnitude of biological effects caused by different genomic alterations. Serum from patients diagnosed with cutaneous melanoma and squamous cell carcinoma were assessed for simultaneously 42 analytes detection (RayBio® G-Series Human Cytokine Antibody Array). Among these biomolecules, some factors (e.g., MIF, IL-6, IL-10 etc.) present an altered level, knowing that they could modulate the inflammatory milieu in malignancy. Some alternate markers in the tumor framework, such as leptin, could correlate immune and metabolic axis helping in tumor therapy assessment and/or disease monitoring. Our data indicated an increased level of at least 5 times for leptin especially for melanoma. Leptin is a hormone increased in obesity that could directly modulated T cells functionality; this connection could further impacts the PD-1 immune axis blockade response, therefore proteomic approaches detect protein alterations and expand knowledge to new therapeutic targets characterization.

Keywords: Biomarkers, cancer immunology, cytokines and mediators, omics technologies, skin diseases

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POSTER PRESENTATIONS

P-1067

Humanized MISTRG mice as a preclinical model to study neutrophil-mediated immunotherapy in neuroblastoma

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The MISTRG mice are considered an improved humanized mouse model as compared to other conventional humanized mice. They have been genetically modified to develop a proper human myeloid compartment, making them a unique and very suitable model to study the innate immune system *in vivo*. Despite being nicely represented, the neutrophil population in the humanized MISTRG mice seems to be retained in the bone marrow and does not mobilize into circulation. Here, we aim at characterizing the human neutrophil population further so as to establish a model in which human neutrophil biology and their contribution in neuroblastoma tumors can be studied *in vivo*. We found a method to isolate and enrich for human bone marrow neutrophils in humanized MISTRG mice. All neutrophil maturation stages were found in the bone marrow and their capacity to perform some of the basic neutrophil functions (i.e. degranulation, ROS production, adhesion and ADCC towards neuroblastoma cells) were tested. In addition, we managed to mobilize mostly mature human neutrophils into the bloodstream of humanized MISTRG mice by means of two well-established neutrophil-mobilizing agents (i.e. G-CSF and Plerixafor). Last, under an inflammatory state we also detected human neutrophils in the blood of melanoma tumor-bearing humanized MISTRG mice.

Keywords: Cancer immunology, granulocytes, immunotherapy, *in vivo* tumor models, neutrophils

P-1068

CD169 defines activated CD14+ monocytes with enhanced CD8+ T cell activation capacity

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Monocytes are antigen-presenting cells (APCs) that play diverse roles in promoting or regulating inflammatory responses, but their role in T cell stimulation is not well defined. In inflammatory conditions, monocytes frequently show increased expression of CD169/Siglec-1, a type I interferon-regulated protein. However, little is known about the phenotype and function of these CD169$^+$ monocytes. Here, we investigated the phenotype of human CD169$^+$ monocytes in different diseases, their capacity to activate CD8$^+$ T cells, and the potential for a targeted-vaccination approach. Using spectral-flow cytometry, we detected CD169 expression by CD14$^+$ CD16$^-$ classical and CD14$^+$ CD16$^+$ intermediate monocytes, and an unbiased high-dimensional analysis showed that they were distinct from dendritic cells, including the recently described CD14-expressing DC3. CD169$^+$ monocytes expressed higher expression of co-stimulatory and HLA-molecules, suggesting an increased activation state. IFNα treatment highly upregulated CD169 expression on CD14$^+$ monocytes and boosted their capacity to cross-present antigen to CD8$^+$ T cells. Furthermore, scRNA-seq and flow cytometry analyses showed that CD169$^+$ monocytes were present in the blood and bronchoalveolar lavage fluid of COVID-19 patients, and in the blood of patients with five different types of cancers. Finally, we evaluated two CD169-targeting nanovaccine platforms, antibody- or liposome-based, and we showed that CD169$^+$ monocytes efficiently presented tumor-associated peptides gp100 and WT1 to antigen-specific CD8$^+$ T cells. In conclusion, our data indicate that CD169$^+$ monocytes are activated monocytes with enhanced CD8$^+$ T cell stimulatory capacity and that they emerge as an interesting target in nanovaccine strategies, because of their presence in health and different diseases.

Keywords: Antigen processing and presentation, dendritic cells, innate immunity, myeloid cells, viral infections, anti-cancer vaccine

P-1069

Use of monoclonal antibodies to various epitopes of HSP70 to analyze the expression of this protein in lymphoid cells

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Previously, we elaborated a panel of six monoclonal antibodies interacting with different epitopes localized in C- or N-terminal domain of the heat shock protein 70 kDa (HSP70) (Boyko et al. Russ J Bioorg Chem, 2014, 40(5): 488-498. doi: 10.1134/S1068162014050045). Using these antibodies and flow cytometry, we analyzed the expression of HSP70 in mononuclear cells and neutrophils isolated from human peripheral blood. It was revealed that all tested types of antibodies differ in the pattern of recognition of intracellular HSP70. Especially, significant differences in detection of HSP70 contained in human peripheral blood leukocytes were observed between groups of antibodies specific to the C- or N-domain of this protein. These features of the detection of intracellular HSP70 using tested panel of antibodies were confirmed in experiments with tumor lymphoid cells. Obviously, the registered differences provide an opportunity to obtain additional information on the state of the pool of intracellular chaperone HSP70, since the exposure of certain sites on the surface of a molecule of this protein is closely related to its conformational changes in the processes of aggregation and interaction with other intracellular molecules. The results of our experiments also indicated significant differences between the tested antibodies in their ability to bind to HSP70 present on the surface of tumor cells. Our data suggest the possibility of practical use of the obtained antibodies in biomedical research.

This work was supported by the Russian Science Foundation, grant number 19-75-10120.

Keywords: Antibody, engineering of antibodies and nanobodies, immunological techniques

P-1070

Improvement of Virus-like nanoparticle (VNP)-based hypoallergenic allergy vaccines by membrane-bound cytokines

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A prime candidate for allergy vaccination should not only avoid the release of atopic mediators but also alleviate allergen-specific Th2 dominance. In the context of allergen- specific immunotherapy (AIT), we harnessed a virus-like nanoparticle platform to encapsulate full-length Art v 1, the major mugwort pollen allergen, armed with surface-decorated immunomodulatory cytokines aiming to direct a Th1/Treg response through decoration-dependent VNP uptake. HEK293T cells were co-transfected with expression constructs coding for MAp15 (viral matrix protein) fused to Art v 1 and various cytokines fused to the CD16b GPI anchor attachment sequence for VNP generation. The presence of the cytokines tethered to VNPs shielding MAp15::Art v 1 was validated with flow cytometry of the producer cell line and immunoblotting of the SDS-PAGE-resolved VNPs under reducing conditions. Preliminary results with MA::Art v 1-IL10::GPI, MA::Art v 1-IL-7::GPI, MA::Art v 1-IL-12::GPI and MA::Art v 1-IFN-γ::GPI decorated VNP co-incubated with splenocytes derived from Art v 1-specific TCR/DR1 humanized allergy mice showed modulated T lymphocyte responses compared to the unchaperoned MA::Art v 1 expressing VNP or soluble recombinant cytokines supplementing the proliferation medium. Likewise, the ratio of Th1(Treg)/Th2 cytokine production determined through multiplexing (Luminex) was influenced by the cytokine-decorated versions of VNP. Cytokine-decorated VNP delivering a shielded version of Art v 1 in a hypoallergenic form may prove instrumental in optimizing VNP-based prophylactic and/or therapeutic allergy vaccines.

Keywords: Allergen-induced immune responses, allergic disorders, cytokines and mediators, immune regulation and therapy

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POSTER PRESENTATIONS

P-1072

The EIF4 translational inhibitor pateamine a improves immunological and neurological functions in BXSB.yaa lupus mice

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Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by loss of tolerance and activation of the immune response. Clinical manifestations are heterogeneous and several organs can be affected including skin, joints, central nervous system and kidney. Traditional treatments include the use of hydroxychloroquine, glucocorticosteroids, immunosuppressive and more recently, biological drugs such as belimumab or rituximab. In the last decade new alternatives have been proposed based on targeting interferon and cytokines. Mouse models have been extremely helpful to test the efficacy of new SLE therapies. In this work we analyze the therapeutic potential of a natural compound, Patemina A (PatA) to treat SLE. Pat A is an inhibitor of the translation initiation process with immunosuppressive properties that has been tested successfully in cancer mouse models. To test Pat A efficiency in SLE we used the BXSB.Yaa lupus model. Animals were treated for 8 weeks starting at the initial stages of disease (12 weeks). Our results show that Pat A treatment increases survival rate and is able to reduce circulating levels of proinflammatory cytokines and autoantibodies, with no side effects. We also observed improvement of cognitive functions (learning/memory, and depression behavioral tests) together with a reduction of proinflammatory cytokines locally in the hippocampus. These data suggest that translation inhibition improves the disease at the immunological and neurological level opening a new line of research based on translation inhibition to treat lupus and other autoimmune diseases.

Keywords: Autoimmunity, drugs for immune modulation, immune regulation and therapy, neuroimmunology

P-1073

Lyophilization based exosome loading is an efficient antigen delivery for cancer vaccines

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Lack of adequate methods of on demand multiple ligand loading within exosome lumen and off the shelf administration of lyophilized cancer vaccine formulations against tumors hampered translational advances of exosome-based therapies in the clinic. Herein, we describe a fast, efficient and high-yield biological cargo loading method that warrants co-entrapment of a protein antigen, single stranded nucleic acid-based immunoadjuvants and lipidic antigen to exosomes from biological fluids. The lyophilization method retained the exosome membrane integrity and protected labile ligands from premature digestion, enhanced internalization by target cells, and amplified *in vivo* immunostimulatory. The immunotherapeutic utility of multiple ligand encapsulated exosome vaccines were examined either in preventive or therapeutic tumor models in mice. Administration of exosomes incorporating model tumor antigen ovalbumin (OVA), elicited strong Th1-biased immunity and antigen-specific cytotoxic T-cell responses that protected mice from T-cell thymoma challenge six months after vaccination. Similarly, OVA/CpG-ODN/α-galactosylceramide incorporating exosomes regressed established murine melanoma and hepatocellular carcinoma progression, respectively. Our findings suggest a clinically adaptable approach that promises to expand the therapeutic outcome of exosome-based technologies.

Keywords: Adjuvants and vaccines, anti-cancer vaccine, antigen processing and presentation

P-1074

Possibility of functional protein kinases detection in primary immunodeficiencies by biosensors

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Protein kinases act as a trigger for regulating a wide range of cellular processes including development, survival, apoptosis, control of metabolism, transcription signals, and beyond. These molecules play a vital role in the appropriate regulation of immune responses. Of note, different primary immunodeficiency disorders (PIDs) have been linked to the protein kinases defects. Bruton's tyrosine kinase, Janus Kinases family, Phosphoinositide 3-kinases and a lot of other protein kinases related to PIDs. As recently the significant advances have been made in biomolecular detection methods, particularly in biosensors technology, the protein kinases related to the PIDs seem to be an attractive area for detection by biosensors. This review offers suggestions based on which the fast and real-time identification of immunodeficiency disorders related to the protein kinases will be possible in the future. We briefly refer to the important protein kinases involved in the PID conditions to emphasize their pivotal roles, while sensors are designed for them have not been regarded compared to the other protein kinases already detected by the different biosensors until now. It is anticipated that a promising future will happen for the detection of the related functional anomalies by biosensors.

Keywords: Immunodeficiency, immunological techniques, omics technologies

P-1075

Priming and boosting immunity in pancreatic cancer: strong anti-tumour effects by combining a CD40 agonist with IL-15 treatment

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Improving treatment outcome for pancreatic cancer is a big challenge in cancer research. We sought to explore the therapeutic potential of combining priming and activation of the immune system by combining a CD40 agonist with interleukin-15. Two different mouse models of pancreatic cancer were used. We demonstrated profound reduction in tumour growth and increased survival of mice with the majority of mice being cured when both agents were combined. RNA sequencing analysis demonstrated involvement of natural killer cell and T cell mediated anti-tumour responses and the importance of antigen-presenting cell pathways. This immunotherapeutic drug combination resulted in enhanced infiltration of tumours by both cytotoxic T cells and natural killer cells, as well as in a striking increase in the ratio of CD8+ T cells over regulatory T cells. We also observed a significant increase in dendritic cells in tumour draining lymph nodes, particularly CD103+ dendritic cells with cross-presentation potential. Depletion experiments showed a critical role for CD8+ T cells and involvement of natural killer cells in the anti-tumour effect. Importantly, strong immune memory was established as detected in rechallenge experiments, with an increase in memory CD8+ T cells only when both the CD40 agonist and interleukin-15 were combined. In conclusion, we demonstrated profound synergistic anti-tumour effects upon combination of a CD40 agonist and interleukin-15 treatment in mouse models of pancreatic cancer. These preclinical data support initiation of a first-in-human clinical trial with this combination immunotherapy strategy in pancreatic cancer.

Keywords: Cancer immunology, cytokines and mediators, immunotherapy, innate immunity, memory

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POSTER PRESENTATIONS

P-1076

How do leukemic cells escape natural killer cell-mediated surveillance? Uncovering novel immune evasion mechanism(s)

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Immune cells are well known to execute cancer immunosurveillance, a term that describes the orchestrated elimination of malignant cells from the body. Immunoediting however, illustrates the dual function of the immune system in the course of tumor development that can be tumor-suppressive and/or -promoting. The importance of the adaptive immune system, especially of T cells, in immunoediting is well established, but the role of innate lymphocytes, such as natural killer (NK) cells, remains elusive. NK cells are important effector cells against leukemia. We hypothesize that besides direct killing, NK cells put selective pressure on leukemic cells causing tumor editing and the outgrowth of highly aggressive tumor cell clones characterized by recurrent changes in key immune surveillance-related pathways. To challenge our hypothesis, we transduced leukemic tumor cell lines with DNA barcodes, which allow the tracking and quantification of distinct tumor cell clones *in vivo*. Barcoded tumor cells were transplanted into immunodeficient mice in presence or absence of adoptively transferred NK cells. NK cell-reconstituted mice showed a significantly prolonged disease latency, indicating that NK cells mediate strong anti-tumor activities. The barcode diversity present in the diseased animals will give a first quantitative and qualitative measure of NK cell-mediated surveillance of leukemic cells *in vivo*. Additionally, whole transcriptome analysis of the disease-causing cells will enable us to identify the underlying mechanisms of tumor escape. This project will provide deeper insight into tumor evasion strategies from NK cells and aims to discover novel targets for future cancer immunotherapies.

Keywords: Cancer immunology, immunotherapy, innate immunity, NK cells

P-1077

Pre-T cell receptor as a therapeutic target in T-cell acute lymphoblastic leukemia

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T-cell acute lymphoblastic leukemia (T-ALL) is an aggressive hematological malignancy originated by dysregulation of signaling pathways involved in thymic T-cell development. Aggressive chemotherapy has greatly improved T-ALL prognosis, but disease relapse is still a major problem and a main cause of decease. Therefore, developing therapies that target leukemia stem cells (LSCs), the ultimate responsible of relapses, is a critical challenge that has been approached in this work. To identify molecular pathways suitable for T-ALL LSC targeting, we developed a novel model of de novo generation of human T-ALL in immunodeficient mice transplanted with haematopoietic progenitors expressing oncogenic NOTCH1. The model allowed tracing human LSC appearance and T-ALL progression *in vivo*, revealing the expression of pre-TCR along T-ALL pathogenesis. Given that pre-TCR, a complex expressed transitionally during T-cell development, is expressed in >65% of human T-ALL cases, several *in vivo* assays were performed to assess the role of pre-TCR in T-ALL pathogenesis and progression, including limiting dilution patient-derived xenotransplantation (PDX), Notch1-dependent leukemogenesis, and therapeutic targeting PDX assays. We show that pre-TCR is a biomarker of LSCs, with a key role in leukemia-initiating cell (LIC) activity and *in vivo* progression of T-ALL. Accordingly, Notch1-dependent leukemogenesis in mice required a functional pre-TCR. Moreover, treatment of T-ALL PDX with an anti-pre-TCR mAb resulted in a marked decrease of leukemia burden and an increase in overall mice survival. Collectively, these results have important therapeutic implications, pointing to pre-TCR targeted immunotherapy as a promising strategy against T-ALL relapse.

Keywords: T-ALL, pre-TCR, immunotherapy

P-1078

Identification of bacterial-derived HLA-bound peptides in melanoma

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While different mechanisms by which bacteria can affect the immune response were reported, the role of bacterial antigen presentation as the mediator of immune recognition and response has remained unclear. In this study, combination of human leucocyte antigen (HLA) peptidomics with 16S rRNA sequencing of 19 melanoma metastasis derived from 9 different patients, lead us to the unbiased identification of hundreds of HLA-I- and tens of HLA-II-bacterial peptides. We were able to validate these results by controlled cell culture work, from the step of bacteria invasion, by co-culturing the bacterial species identified by 16S sequencing with the patient derived melanoma cells, throughout validating the peptide's presentation by preforming HLA peptidomics on the infected cells. Importantly, we were able to identify common bacterial peptides from different metastases of the same patient as well as from different patients. Some of the common bacterial peptides, as well as others, were able to elicit an immune response by the autologous tumor infiltrating lymphocytes. By identifying immunogenic microbial-derived antigens presented on tumor HLA molecules, we demonstrate that tumor bacteria may not only shape the immune tumor microenvironment but also directly affect T cell immune-reactivity. Antigen presentation of bacterial antigens provides insights into a new mechanism by which bacteria influence immune system activation and response to immunotherapy. Introducing bacterial derived antigens to the repertoire of tumor associated antigens, potentially extends the variety of targets for cancer immunotherapies.

Keywords: Cancer immunology, cancer immunopeptidome, immunotherapy, mass spectrometry, microbiome and environmental factors, omics technologies

P-1079

Microfluidic on-demand control of cell cultures for integrated analysis of temporal homocellular and heterocellular immune signaling dynamics

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Microfluidics offers precise and dynamic control of inputs for the study of temporal cellular responses. Despite the possibility in studying either homocellular (single-cell, population) or heterocellular response on specialized microfluidic platforms, investigations from one perspective may produce insufficient output information, which possibly leads to the partial comprehension about the underlying mechanisms of immune signaling events. An integrated approach allowing for temporal homocellular (single-cell, population) and heterocellular responses remains inaccessible, pertaining to limitations such as special structure designs for each condition. In this study, we present a microfluidic system for integrated confinement, (co)culture, and analysis of temporal dynamics of immune signaling in homotypic (single-cell or population) and heterotypic cells on demand. Variable inputs such as 'one-pulse' and 'continuous' signals can be delivered to cells in a precisely controlled fashion. This platform enabled exploring the temporal dynamics of signal transducer and activator of transcription 1 (STAT1) in single fibroblasts and a population upon treatment with different modes of interferon γ (IFNγ) stimulation. Using the same platform, we werealso able to analyze STAT1 and STAT2 dynamics of fibroblasts cocultured with macrophages subjected to one-pulse lipopolysaccharide (LPS) exposure. Our findings demonstrate that fibroblasts have varied STAT1 dynamics with a similar overall trend in respective single-cell and population-based analyses, while LPS indirectly induced diverse activities of STAT1 and STAT2 in co-cultured fibroblasts. By applying more self-defined input types, this system allows the exploration of complex cellular responses and enables a multi-perspective understanding of temporal immune signaling dynamics in the future.

Keywords: Cell signalling, cellular interactions, immune communication, immune networks

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POSTER PRESENTATIONS

P-1080

Single cell multi-omics identifies evolving phenotypes of CAR CD19 and endogenous T cell clones

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Chimeric antigen receptor (CAR) T cells have shown significant promise in B cell malignancies. Successful treatment relies in part on a better understanding of CAR T and immune cell phenotypes, and their evolution over different treatment stages. To this end, we compiled a single-cell multi-omic atlas of circulating endogenous immune cells and CAR T cells from longitudinal blood samples from pre- and up to one month post-infusion of patients with blood malignancies who were treated with anti-CD19 CAR T cells. We revealed heterogeneity in endogenous and CAR CD8+ T cells, defined by cytotoxic, cell-cycle and memory signatures. A combination of clonal, proteomic, and transcriptomic data identified the evolutionary trajectory of endogenous CD8+ T cells from an active cell cycling state to a cytotoxic phenotype. We also discovered clonally expanded endogenous Tγδ cells which evolved from a cytotoxic to a memory-like phenotype. Finally, we identified subsets of cytotoxic CD8+ T subsets undergoing rapid proliferation that were significantly correlated with CAR T cell count and specific cytokines circulating in blood. This analysis forms a comprehensive description of the evolution of CAR T cells, revealing a rapid immune-remodelling of the patients' immune cells which explain in part the rapid clonal expansion of cytotoxic T cells and inflammatory response observed in patients.

Keywords: Cancer immunology, immunotherapy, omics technologies

P-1081

Microarray analysis of alternative rheumatoid arthritis markers

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Despite the fact rheumatoid factor (RF) is strongly associated with rheumatoid arthritis (RA) development, its diagnostic efficiency is fair. To analyze alternative RA markers, that could help to close the serological gap in RA, we manufactured hydrogel microarray that contained immobilized protein arginine deiminase 4, mutated citrullinated vimentin, carbamylated fibrinogen. For the analysis, the samples were diluted and incubated on the microarray. For the detection of the complexes, microarray was incubated with the solution of secondary anti-human IgG-Cy3 and and anti-IgM-Cy5 with prior visualization with laser biochip analyzer. Exceeding the fluorescence from empty gel in 2 times for the pads with immobilized marker was considered as the presence of autoantibodies to this marker. The serum samples from 81 RA patients and 30 healthy donors were analyzed. The occurrence of autoantibodies to the analyzed markers was expectedly higher ($p<0,05$) for RA patients. However, for RA patients the signals from the pads were strongly affected by the sample diluent: the fluorescent levels were lower in case the diluent contained mixture of animal (rabbit, mouse and goat) sera. Except for additional blocking, animal sera could probably prevent interference from RF that acts like heterophilic antibodies and confound results of the analysis. Thus, the developed microarray can be used for the detection of alternative RA markers, however, the interpretation of the results and comparison with results obtained by the other methods should be carried out taking into account possible RF interference.

This work was supported by the Russian Science Foundation (http://rscf.ru/), Grant No. 19-15-00283.

Keywords: Antibody, biomarkers, inflammatory joint diseases, rheumatoid arthritis

P-1082

Accelerating development and improving access to CAR- and TCR-engineered T cell therapy in Europe

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T2EVOLVE is a breakthrough alliance of academic and industry leaders in cancer immunotherapy that started in January 2021, under the European Union's Innovative Medicines Initiative (IMI). The key objective of T2EVOLVE is to accelerate development and to increase access of cancer patients to immunotherapy with reprogrammed immune cells. Reprogramming is accomplished by genetic engineering with a T cell receptor (TCR) or synthetic chimeric antigen receptor (CAR). Combined expertise of leading researchers in the field aims for identification of gaps and methods to improve efficacy, toxicity and engineering of modified T cells. Granting EU patients access to the best available medical care, while providing guidance on the implementation of this novel treatment into the EU healthcare system in a sustainable way, is the strategic objective of T2EVOLVE. Moreover, patient involvement will ensure that the perspectives of cancer patients are at the center, in the research setting as well as along the cancer care continuum.

Keywords: Bone marrow transplantation, cancer immunology, cell based therapies, drugs for immune modulation, immunopharmacology, immunotherapy

P-1083

Isolation and characterization of nanobodies that bind the BTB domain of the PATZ1 transcription factor

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PATZ1 is a transcription factor that belongs to the ZBTB protein family. It was found to be critical for CD8 gene expression and regulatory T cell (Treg) function. As for other ZBTB family members, the transcriptional activity of PATZ1 is mediated by oligomerization of its BTB domain and protein-protein interaction; however, these interactions remain poorly understood at a molecular and structural level. The aim of this study is to generate potent and selective nanobodies against the PATZ1 BTB domain as a tool to assist the characterization of the structure and function of this protein. A phage display nanobody library was screened for PATZ1 BTB domain binders in two selective rounds of bio-panning, then high-affinity binders were selected by ELISA. Nanobody clones were expressed in E.coli and purified using affinity and size exclusion chromatography. Binding affinities were determined using surface plasmon resonance (SPR), and further characterization for binding specificity and affinity were done using size-exclusion chromatography (SEC) and a fluorescent two-hybrid (F2H) assay. Three nanobody clones were produced as soluble proteins of high concentration with nanomolar range affinities. SEC Co-Elution showed complex formation specifically with the PATZ1 BTB domain. Additionally, in the fluorescent two-hybrid (F2H) assay, these nanobodies showed colocalization with their target protein in live cells. Our results demonstrate specifically binding nanobodies to the BTB domain of PATZ1. Further evaluation of these nanobodies is required to assess their potential usage as modulators of PATZ1 function.

Keywords: Engineering of antibodies and nanobodies, antibody, molecular immunology

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POSTER PRESENTATIONS

P-1084

Acute phase proteins and rheumatoid factor microarray analysis in rheumatoid arthritis

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One of the defining biomarkers of rheumatoid arthritis (RA) is the rheumatoid factor (RF). However, RF detection has insufficient diagnostic sensitivity and specificity. It is reasonable to add acute phase proteins (APPs) (C-reactive protein, CRP, and serum amyloid A, SAA) as the companion biomarkers to RF since APPs involved in immunological processes will be increased in RA patients. For efficient simultaneous determination of IgM RF, CRP, SAA, a hydrogel microarray was developed. In the individual pads of microarray IgM RF, anti-CRP, and anti-SAA antibodies were immobilized. After incubation with samples and developing antibodies (anti-human IgM antibodies with a fluorescent label and monoclonal anti-protein antibodies conjugated with biotin), fluorescent images were obtained from microarrays using an analyzer developed in EIMB RAS. After processing such images, the concentrations of the proteins were determined using calibration curves and calibration samples. Diagnostic accuracy of IgM RF, CRP, and SAA in RA was assessed on a sample of 36 RA patients, 8 patients with ankylosing spondylitis, and 8 healthy donors. In RA each marker individually had the following diagnostic sensitivity/specificity: RF 86.1%/81.2%, CRP 77.8%/75%, SAA 83.3%/81.2%. According to logistic regression results based on three markers, the sensitivity of the analysis increased to 88.89%, while the specificity decreased to 68.75%. Thus, the microarray was developed that allowed the determination of RF IgM and APPs and allowed assessment of the contribution of APPs as biomarkers.

This work was supported by the Russian Science Foundation (http://rscf.ru/en), Grant No. 19-15-00283.

Keywords: Autoimmunity, biomarkers, rheumatoid arthritis

P-1085

Role of tumor-derived extracellular vesicles with cytokine modified cargo in the induction of antitumor response

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In the tumor microenvironment (TME), immune cells are unable to function properly. Tumor-derived extracellular vesicles, being part of the TME, induce immunosuppression as well as angiogenesis and metastasis. Given that the cytokine balance in the TME serves a crucial role both in the tumor progression and in the proper activation of immunological antitumor response, the change in the cytokine cargo of exosomes may affect tumor growth inhibition. The study aimed to assess the ability of extracellular vesicles isolated from murine colon carcinoma cells (MC38) with overexpression of IL-18 and/or shRNA for TGF-β1 to activate dendritic cells (DCs) and to induce a cellular antitumor response. Multivesicular vesicles (MVs) and exosomes (TEx) produced by genetically modified MC38 cells were isolated from culture supernatants using sequential centrifugation and size exclusion chromatography. Vesicles were then evaluated and used for the 24-hour stimulation of DCs. Phenotypic changes and the ability of DCs to activate specific antitumor response were further determined using flow cytometry methods and functional assays on spleen cells. MVs isolated from MC38/shTGF β1/IL-18 cells, opposite to TEx, were characterized by high immunostimulatory activity. DCs stimulated with the MVs showed the highest expression of costimulatory molecules and MHC II and were able to induce cytotoxicity toward MC38 cells by activating CTLs and NKT cells. MVs isolated from cells with increased production of IL-18 and/or shRNA for TGF-β1, appear to be better stimulators of the antitumor response than vesicles from unmodified cells.

This study was funded by National Science Centre, Poland (project no 2018/30/E/NZ5/00711).

Keywords: Cancer immunology, cytokines and mediators, dendritic cells, immune communication, immune regulation and therapy

P-1086

New approaches for biomarker discovery in oligoarticular juvenile idiopathic arthritis

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New biomarkers are demanded for the management of Oligoarticular Juvenile Idiopathic Arthritis (OJIA), the most common chronic pediatric rheumatic arthritis in Western countries. This study was aimed at identifying new biomarkers able to predict disease progression and response to treatment. We developed an integrated strategy that combines classical approaches for the study of inflammatory cells in liquid biopsies and system biology–driven omics methods (miRNomic, proteomic) for the analysis of extracellular vesicles (EV) released by these cells EV miRNA (EV-miR) and EV-protein (EV-Prot) expression profiling were carried out in paired plasma (PL) and synovial fluids (SF) from 30 new-onset OJIA patients using TaqMan Array RT-PCR and mass spectrometry.PL from 25 age-matched healthy children was used as control. Macrophages and T cells were isolated from SF aspirates and characterized by cytofluorimetry. Clustering analysis identified 16 and 34 EV-miRs significantly up- and down-regulated, respectively, in SF vs both paired and control PL. A few differentially expressed EV-miRs were able to discriminate subgroups of patients within the OJIA cohort, suggesting their potential predictive value. EV-Prot analysis showed mean expression of about 1000 protein in both SF and PL samples.The potential correlation between EV-miR and EV-Prot expression levels and patient clinical data are under study. Different ratio of M1/M2 macrophages and CD4/CD8 T cells were observed among outcome groups. We provide the first database containing EV-miR, EV-Prot, and cell phenotypic data of new-onset OJIA patients, identifying potential candidate disease biomarkers for the development of personalized therapeutic strategy.

Keywords: Autoinflammation, biomarkers, immune networks, omics technologies

P-1087

Proliferative responses of patients infected with SARS-Cov2 and the possible modulatory effects of C-Vx as a candidate novel therapeutic against COVID-19

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COVID 19 as a serious global health problem has led to investigation of immune responses against SARS-Cov2, both for better understanding of the pathology and also for development of therapy options. C-Vx was originally developed by Hamida Pharma-USA in collaboration with Miracle Labs-Turkey for cancer treatment. With COVID-19 outbreak, alterations in C-Vx formula were claimed to form a possible concomitant therapy option against COVID-19. This study aimed to investigate the effects of C-Vx on proliferative responses of T cell subsets and NK cells in COVID-19 patients. Patients diagnosed with COVID-19 (n=28; mild: 10, moderate: 8, severe: 10) and followed by Istanbul Faculty of Medicine, and healthy subjects (n=10) were enrolled. Ficoll-purified PBMCs stained with CFSE were cultured with PHA, C-Vx and their combination for 120 hours. Following cell culture, monoclonal antibodies against CD3, CD4, CD8, CD16 and CD56 were used for identification of cell subsets. C-Vx induced proliferation of total PBMCs, $CD3^+$, $CD4^+$ and $CD8^+$ T cells, the strongest in healthy subjects, the weakest in severe COVID-19 patients. NK cells significantly responded to C-Vx only in healthy controls. PHA-induced proliferation of PBMCs, $CD3^+$ and $CD8^+$ T cells, and also NK cells were diminished with increased disease severity, which was regained to some extent with the presence of C-Vx, in all cell populations but not in $CD4^+$ T cells. The decreased proliferative responses with the severity of disease might indicate a disrupted immune function in severe COVID forms which could be re-gained by C-Vx.

Keywords: Immune response tracing, Immunopharmacology, viral infections

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POSTER PRESENTATIONS

P-1088

Tungsten disulfide nanoparticles potentiate suppressive properties of human myeloid-derived suppressor cells *in vitro*

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Myeloid derived suppressor cells (MDSCs) play a pivotal role in the regulation of immune response. Their targeted modulation by multifunctional nanoparticles opens new perspectives in theranostics of many diseases, including cancer and autoimmunity. Tungsten disulfide nanoparticles (WS2-NPs) were shown to possess excellent optical properties and wide surface available for bioconjugation, making them ideal platform for novel theranostics. However, their biocompatibility and immunomodulatory properties, especially in interaction with MDSCs, are still unknown. To investigate this, here we used a model of human monocyte-derived MDSCs-like cells differentiated with GM-CSF and IL-6, which enables the differentiation of CD14+CD11b+HLA-DRlow cells that are suppressive in co-cultures with PHA-stimulated PBMCs. According to side-scatter parameter and microscope analyses, MDSCs displayed a high capacity to internalize WS2 nanoparticles in a dose-dependent manner. Thereby, WS2-NPs were not cytotoxic for MDSCs up to 100 μg/ml. However, WS2 (50 μg/ml) up-regulated the expression of CD73, CD44 and Arginase-1, and down-regulated CD86 and CXCR4 expression by MDSCs, especially after additional challenge of the cells with LPS/IFN-γ. WS2-treated MDSCs, also produced less IL-6 and TGF-β compared to control MDSCs, irrespective of LPS/IFN-γ stimulation. In co-culture with PHA-stimulated PBMCs, WS2-NPs potentiated the suppressive properties of MDSCs, which remained even after LPS/IFN-γ challenge. Moreover, WS2-treated MDSCs reduced IL-17 and IFN-γ levels in co-cultures with PHA-PBMCs, and increased the levels of IL-10. These results suggest that WS2-NPs are highly biocompatible and suitable for targeting MDSCs, particularly for their potentiating tolerogenic mechanisms, which could be exploited in designing new therapies in autoimmune and chronic inflammatory conditions.

Keywords: Autoinflammation, drugs for immune modulation, myeloid derived suppressor cells

P-1090

Functionalized nanoparticles of noble metals as stimulators of immune response in HSV-1/2 infection

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Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) infections are the most common cause of oral and genital herpes, respectively. There are no vaccines and treatment strategies for herpes infections are limited to antiviral agents blocking viral replication. In this project, we tested the antiviral and adjuvant properties of functionalised nanoparticles (NPs) of noble metals (silver and gold) sized 5 and 30 nm by employing the intranasal HSV-1 infection and genital HSV-2 infection models in C57BL6 mice. Our results showed that intranasal treatment of HSV-1 infection with tannic acid (TA) modified Au/AgNPs of both sizes resulted in better responses of NK cells and HSV-1-specific CD8+ T cells, followed by lower viral titers in trigeminal ganglia and brains. Furthermore, sera from NPs-treated mice showed better neutralisation titers. In HSV-2 genital infection, only treatment of primary infection with 10 and 30 nm TA-modified NPs led to better antiviral responses. NPs treated HSV-2 infected mice showed lower HSV-2 titers in vaginal lavages and spines and significant infiltration of monocytes, cytotoxic lymphocytes and NK cells into the mucosal tissues. Microarray analysis showed that application of TA-modified Au/AgNPs upon nasal mucosal tissue and AgNPs sized 30 nm upon the vaginal mucosal tissue helped to induce local type I IFN response and chemotactic response activating local infiltration of monocytes and dendritic cells. We conclude that local application of TA-modified Au/AgNPs of different sizes may play a role of adjuvant mounting antiviral response in viral infections of mucosal tissues.

Keywords: Innate immunity, viral infections, adjuvants and vaccines

P-1092

Characterization of B cell responses during checkpoint immunotherapy in metastatic melanoma

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Immune checkpoint inhibitor (ICIs) therapies have been approved for treatment of malignant melanoma. Although a survival benefit and better overall response rate is observed in many patients, not all patients respond to these ICIs treatments. Thus, identifying novel biomarkers is necessary to predict the response of patients and eligible patient selection for ICIs treatment. Characterization of B cell phenotypes by their surface markers will improve the understanding of B cell immunity in melanoma and how ICIs affects B cells. Therefore, we analyzed circulating B cells from healthy controls and in a cohort of patients (n=25) with metastatic melanoma before and after the administration of anti-PD1 and/or anti-CTLA4 mAbs from 2 different visits, representing early and late response. We detected statistically significant alterations of naïve B cell, switched B cell and IgA+ B cell frequencies in non-responding (n=12) patients during therapy. We observed BAFF (B cell activating factor) receptor expression was higher on all subsets of B cells in responders compared to non-responders at baseline and at early response. TACI expression was higher on switched B cells in all timepoints in responders while BCMA+ switched B cell frequency didn't significantly change. We also detected BAFF protein levels in the serum, which were significantly higher in non-responders than responders at baseline. Consequently, our results suggest that ICIs treatment alters the B cell characteristics during ICIs therapy through the BAFF related receptor expression. Furthermore, soluble protein BAFF may represent a predictive biomarker for the response of metastatic melanoma patients to ICIs therapy.

Keywords: B lymphocytes, biomarkers, cancer immunology, checkpoint inhibition, immune response tracing, immunotherapy

P-1093

Immunoprotective effects of Echinococcus granulosus laminated layer's crude extracts on experimental autoimmune uveitis

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In order to investigate the possible protective effect of the crude extract of the laminated layers from Echinococcus granulosus (LL) on an experimental model of autoimmune uveitis (EAU). The EAU was induced by sub-cutaneous injection of retinal crude extracts in Wistar rats (positive control). The extract (LL) was injected intraperitoneally before (pretreated group) or seven days after the disease induction (treated group) or two days before for the disease induction. Control groups received either PBS (negative group) or CPA (vehicle group) solutions alone. The disease was assessed macroscopically and histologically. The oxidative/ nitrosative markers (nitric oxide, MPO, MDA), antioxidant markers (SOD, Catalase) and TNF-α concentrations were measured in plasma of the different groups. Statistical comparison and correlation studies were performed using GraphPad Prism 8. Significance was reached when $p<0.05$. Our results showed that LL animals' pre-treatment improved the clinical symptoms and attenuated significantly the histological alterations of the retinal tissue. Concomitantly, pre-treatment with LL induces a significant reduction of TNF-α, MDA and NO concentrations as well as the activity of MPO, while it increased significantly the activity of the antioxidant enzymes ($p<0.05$). The protective effect of LL was less efficient in the treated group. Collectively, our data suggest a preventive effect of the LL during EAU. This effect appears to be mediated by the inhibitory effect of LL on inflammation and restoration of the oxidant/antioxidant balance. Therefore, we suggest that the laminated layer has a potential value in new preventive strategies against inflammatory autoimmune uveitis.

Keywords: Autoimmunity, immune regulation and therapy, inflammatory disease, inflammatory molecules, protection

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POSTER PRESENTATIONS

P-1095

Virus Like Particle (VLP) – based SARS- CoV-2 vaccine induces a potent immune response in rats

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Several SARS-CoV-2 vaccines are currently approved for emergency use in many countries, but the global need greatly exceeds the supply, and different formulations might be required to immunize specific populations. Virus-like particle (VLP) is a self-assembled nanostructure incorporating key viral structural proteins. SARS-CoV-2 VLPs that are constructed in mammalian expression system are efficient antigen for vaccines as a safe and relevant substitute of naturally pathogenic viruses. In this study, we aimed to show the immunogenic effects of our vaccine candidate, which is developed by VLP technology, in rats. We combined two adjuvants which are CpG and ALUM with VLPs displaying envelope (E), membrane (M), nucleocapsid (N) and the full-length transmembrane S glycoprotein, locked in its prefusion conformation by the substitution of six residues with proline (S6p). For this study, 5 rats were immunized with two doses of our vaccine formulation including 10 µg VLP, 600 µg ALUM and 300 µg CpG, 14 days apart. Serum samples were collected at day 14, 21 and 28 for the ELISA experiment. Inactivated virus, trimeric S, S1 and RBD recombinant proteins were used to detect total IgG responses. In rats, one subcutaneous dose of the vaccine elicits a high IgG response at day 14 and antibody levels had increased further 7 days after dose 2. Furthermore, live virus neutralization assay was performed, and fifty percent neutralizing dilution (ND50) titers positively correlated with the serum concentration of IgG against spike and RBD. In conclusion, our VLP vaccine has produced neutralising antibodies against SARS-CoV-2 virus.

Keywords: Adjuvants and vaccines, animal models, viral infections

P-1096

Production of recombinant proteins used in ELISA experiments

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused the outbreak of Coronavirus disease 2019 and became pandemic. Virus-like particles, which contain four structural proteins of SARS-CoV-2 virus, is absorbed with Alum and formulated with CpG ODN and used as a vaccine that produce efficient immune response against viruses. Since the vaccine is recombinant protein-based, different constructs of Spike, Nucleocapsid and RBD proteins were designed and they are fused with His-tag at the C-terminus. Recombinant proteins were produced in HEK suspension and adherent cells. Cell supernatants was clarified by micro-filtration and recombinant proteins were purified by tangential flow filtration (TFF) followed by His-tag affinity chromatography and desalting chromatography. Proteins were optimized by coating ELISA plates with different concentrations and IgG responses were detected. Purification and specificity of proteins were detected with Silver staining, Western Blotting and analytical SEC. Primary and secondary antibody responses against recombinant proteins of immunized 6-8 weeks old BALB/c mice with combination of different doses of adjuvants and VLP were studied. IgG responses of homemade and commercial recombinant proteins at different concentrations were compared and studied with patients. Based on optimization results, Spike, Nucleocapsid and RBD recombinant proteins were coated between 1 to 4 µg/ml. Here we reported, the successful production and optimization of recombinant proteins which are necessary for studying immune responses by ELISA experiments.

Keywords: Adjuvants and vaccines, antibody, immune response tracing, viral infections

P-1097

Lunasin suppressed breast cancer cells MCF-7 and MDA-MB-231 growth via regulating inflammatory mediators, estrogen receptor expression, and aromatase activity

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Breast cancer is one of the highest prevalence cancer in women. The pathology of breast cancer is comprised of tumor cells and nearby stromal cells, accompanied with cytokines, extracellular matrix, and stimulated molecules, resulting a favorable microenvironment for tumor progression. Lunasin is a seed peptide with multiple bioactivities derived from legumes and cereals. The study aims to explore the molecular mechanisms of chemoprevention by lunasin involved in inflammatory mediators, estrogen signaling, and cell growth and apoptosis impacted to MCF-7 and MDA-MB-231 breast cancer cells. The results showed that lunasin did not affect normal MCF-10A cells growth, but inhibited both breast cancer cells growth. Lunasin increased IL-6 gene expression and protein secretion at 24 h, and then decreased IL-6 production at 48 h in both cells, while lunasin increased COX-2 mRNA and PGE2 secretion in MCF-7 cells, but decreased COX-2 mRNA and PGE2 secretion in MDA-MB-231 cells. In both breast cancer cells, ERα and aromatase genes expression, and aromatase activity were decreased by lunasin treatment. Additionally, lunasin increased the populations of low vitality and total apoptosis, and decreased healthy cells. The estradiol presence in medium was significantly increased MCF-7 cells proliferation, but not MDA-MB-231 and MCF-10A cells. Lunasin still inhibited cell growth and vitality in MCF-7 cells under estradiol condition. In summary, lunasin inhibited breast cancer cells proliferation through regulating pro-inflammatory mediators, reducing estrogen signaling related molecules, decreasing cell vitality, and inducing apoptosis, suggesting lunasin executed a promising chemopreventive agent in both MCF-7 and MDA-MB-231 cells.

Keywords: Cancer immunology, cytokines and mediators, immune regulation and therapy, nutrients

P-1098

Adjuvant based *in vivo* work for novel VLP vaccine technology

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Vaccination studies started quickly to control SARS-CoV-2. In protein vaccines, antigenic particles are obtained from biotechnological reactors that mimic them. Because there are no molecular motifs associated with the disease agent that will strongly trigger immune cells in VLP technology, adjuvants are important to activate the immune system. In this study, we aimed to show the adjuvant effect combined with VLP vaccine technology *in vivo*. Several mice were used for each formulation and two different adjuvants, CpG and nanoring, were used. Two different concentrations (low and high) of VLP have been formulated with CpG and nanoring (NR). After injections, serum samples run for the ELISA experiment. Inactivated virus, trimeric S and RBD recombinant proteins were used to detect total IgG, IgG1 and IgG2a responses. It is shown that high dose VLP in combination with CpG and NR have almost twice the effect than only high dose VLP for total IgG response against inactive virus. Secondly, recall assay has been planned and splenocytes from each mouse were stimulated with ConA, VLP, recN and recS1 for 48h. IL-17A, IFN-γ and IL-4 levels were checked with cytokine ELISA. In stimulation of VLP, the splenocytes from mice which were injected with CpG and nanoring formulated high dose vaccines have shown increased type II interferon production compared with control. In conclusion, this study showed the importance of using a strong adjuvant to trigger the immune system for novel protein based VLP vaccine technology in order to produce an effective antibody and cytokines response against SARS-CoV-2.

Keywords: Adjuvants and vaccines, biology of the immune system, molecular immunology

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POSTER PRESENTATIONS

P-1100

TLR7 and TLR9 agonists as efficient adjuvants in Salmonella vaccine formulations

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Salmonella is a zoonotic pathogen causes infections in humans such as gastroenteritidis and thyphoid. Development of multi-antibiotic resistance in Salmonella strains limits their use. Inactive or live attenuated vaccines are important tools for fighting against Salmonella infections. However there are still serious concerns about risks of using live vaccines. Therefore, novel adjuvants, increasing vaccine potency is of considerable interest. TLR7 and TLR9 ligands (R848 and CpG-ODNs, respectively) are promising vaccine adjuvants for different infectious disease agents. In this study, *in vivo* efficiency of vaccines containing CpG-ODN or R848 together with extracts of heat-killed prevalent Salmonella strains, such as S. enteretidis, S. Typhimurium, S. infantis and S. Virchow were investigated in mice. BALB/c mice were i.p injected twice with three weeks intervals with heat-killed bacterial extracts (10μg/mice) alone or with either R848 (5μg/mice) or K3 (15μg/mice). Sera were collected from tails of immunized mice every 4 weeks after first injection. Anti-Salmonella IgG subtypes (total IgG, IgG1 and IgG2a) and IFNγ were measured via ELISA method. Anti-Salmonella total IgG, IgG2a, and IgG2a: IgG1 ratios levels were significantly higher in mice immunized with formulations containing CpG ODN or R848 in all Salmonella strains. In addition to antibody response, serum IFNγ levels of immunized mice were higher compared to naive group. Vaccines formulated with nucleic acid based adjuvants demonstrated stronger and longer Th1-based humoral and cellular immune response against Salmonella strains compared to only bacterial extract injected groups implicating potential of these molecules considered as adjuvants in vaccines against Salmonella.

Keywords: Adjuvants and vaccines, bacterial infections, veterinary immunology

P-1101

CD4+ Th-memory associated gene networks are rewired during mite specific allergen immunotherapy

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Multiple regulatory mechanisms have been identified that contribute to outcomes of allergen-specific immunotherapy. Allergen-induced immune responses are thought to involve thousands of genes functioning within complex networks, and how these operate/integrate to maintain immunological homeostasis is incompletely understood. To identify allergen-specific immunotherapy induced changes to Th-memory responses employing systems-level gene co-expression network analysis. Genome-wide allergen-induced Th-cell responses were profiled prospectively during 24 months subcutaneous immunotherapy (SCIT) in 25 rhinitis. Molecular profiles of Th-cells were interrogated employing upstream regulator, pathways and co-expression network analysis. Prior to immunotherapy, mite-induced Th-cell response networks consisted of multiple discrete co-expression networks; Type1-IFN-, Th2-, Inflammation-, and FOXP3/IL2-associated signalling. A 109 gene signature correlated with symptom scores relating to cytokine signalling/T-cell activation-associated pathways. Upstream drivers demonstrated activation of key Th1/Th2- and Inflammation-associated genes. At 3.5mths SCIT updosing, symptoms reduced by 32.5% and minimal changes to pathway/upstream regulator profiles were detected. In contrast, network changes were evident, displaying merging of FOXP3/IL2- with Inflammation- and Th2-associated modules. By 12mths SCIT, symptoms had reduced by 41%, whilst pathway/upstream regulator/network profiles remained unchanged. At 24mths of SCIT, symptoms stabilised at 47% of baseline, and the Type1-IFN-associated network module merged into the Th2/FOXP3/IL2/Inflammation module. Stabilisation of the clinical effectiveness of SCIT underlies progressive rewiring of mite-induced Th-cell-associated Th2-, FOXP3/IL2-, Inflammation- networks and Type1-IFN-signalling subnetwork. Importantly, integration only occurs following 24mths of SCIT treatment. The recruitment of Th2-antagonist Type1-IFN signalling into a coordinated expression network may be central to stabilising clinical effects of SCIT.

Keywords: Adaptive immunity, allergen-induced immune responses, immune networks, immunotherapy, molecular immunology, omics technologies

P-1104

Exosomes co-encapsulating antigen and immunoadjuvants act as an effective therapeutic cancer vaccine

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Exosomes are excellent vehicles for protein/peptide, gene or short sequences of RNA/DNA delivery. Dendritic cell derived exosomes although used in the immunotherapy of cancers, multiple ligand loading is not possible via cell feeding. Herein, we describe a simple method to externally load ligands within cell-line derived exosomes. Exosomes were first purified from the cell supernatant and loaded with cancer antigen ovalbumin (OVA), CpG ODN,α-GalCer. Therapeutic effect of these exosomes was tested on B16 F10-OVA model. After palpable tumor formation, animals were treated twice either with mixture of OVA, ODN and αGC or their loaded counterparts within exosomes. Tumor infiltrating lymphocytes were analyzed by flow cytometry from tumor cell suspensions. Therapeutic effect of the exosome formulation was evaluated by OVA-specific ELISA. Also, splenocytes were treated with MHC-I peptide specific epitope and CD8+ T-cell specific IFNγ secretion was detected. Triple ligand loaded exosome injected group showed significant regression in tumor development compared to PBS or free mixture treated groups. OVA-specific antibody titers showed development of a Th1-biased anti-OVA immunity after a single injection of exosome. Tumor infiltrating T cells, CD8+ T-cell CD4+ T-cells, NK cells, NKT-cells, pan and M1-like macrophages were significantly high in lyophilized exosome treated animals. When MHC-I peptide epitope was incubated with splenocytes, CD8+ T-cells had significantly higher levels of IFNγ secretion compared to PBS and free mixture treated groups. In conclusion, exosomes could be externally loaded with multiple ligands via lyophilization method and these exosomes provide sufficient immune activation and antigen-specific immunity capable of reducing established melanoma in mice.

Keywords: Adjuvants and vaccines, anti-cancer vaccine, immunotherapy, *in vivo* tumor models

P-1105

Preclinical immunogenicity of a B + W meningococcal outer membrane vesicle (OMV) vaccine adjuvanted with nucleic acid based TLR ligands and comparisons with existing meningococcal conjugate- and polysaccharide vaccines: preliminary report

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Invasive meningococcal disease (IMD) is caused by Neisseria meningitidis, and the main serogroups that cause the disease are A, B, C, W and Y. Vaccines targeting ACWY and B have not been included in the same formulation up to date because of the poor immunogenic property of B-type meningococci (MenB). Since the most common cause of IMD in Turkey are MenW and MenB, in this study, we prepared outer membrane vesicle (OMV) vaccine from MenB and MenW strains, and compared the immunogenicity of this vaccine with existing meningococcal conjugate and polysaccharide (PS) vaccines in mice. Balb/c mice were immunized with preclinical batches of the B + W OMV vaccine adjuvanted with Al (OH)3 and CpG or with commercially available vaccines; a MenACYW conjugate vaccine (NimenrixTM, Pfizer) or a MenB OMV-based vaccine (Bexsero®, GlaxoSmithKline) with a two-week interval. Immune responses were tested via ELISA and high levels of IgG antibodies against both B and W OMV were detected in mice receiving the B + W OMV vaccine adjuvanted with Al (OH)3. Furthermore, the B OMV vaccine was shown to induce IgG antibodies against MenW of the same magnitude as the titers induced by the W OMV vaccine. In conclusion, the B + W OMV vaccine induced high levels of antibodies that were shown to be higher or equal to the levels induced by licensed meningococcal vaccines. Thus, a B + W OMV vaccine could potentially serve as an alternative or a supplement to existing conjugate and PS vaccines in Turkey.

Keywords: Adjuvants and vaccines, antibody, bacterial infections

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POSTER PRESENTATIONS

P-1106

Antitumor immunity boosted by GSDMD-induced necrosis

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Pyroptosis is a programmed mechanism of cellular self-destruction, stimulated by divergent pathogens and endogenous stimuli. Also known as caspase-1 dependent cell death due to gasdermin D (GSDMD) pore formation, it accommodates immunogenic properties, whose outcome results in release of activated inflammatory cytokines IL-1β and IL-18. GSDMD contains two defined domains, N-terminal pore-forming and C-terminal auto-inhibitory domain, separated by a linker region. Upon caspase-1 cleavage, N-terminal GSDMD initiates pyroptotic cell death and release of IL-1β through the GSDMD pores. The mechanism of GSDMD induced pyroptosis could efficiently be used for modulating immune response against tumor. Properly controlled formation of GSDMD pores would not only evoke cancer cell destruction but also promote T-cell infiltration into the tumor microenvironment and stimulate response against tumor neo-antigens. Additionally, GSDMD treatment can be further improved when combined with clinically established cancer remedies such as checkpoint inhibitors, or mediators of the inflammatory response. We designed various GSDMD variants and tested their pore forming ability *in vitro*. The foremost was further used for release of cytokines and tumor antigens *in vivo* in murine melanoma model.

Keywords: Cell death, cytokines and mediators, immunotherapy

P-1107

Development of an *in vitro* B cell assay for Tetanus and Diphtheria containing vaccine batch testing using antigen specific B cells from healthy donors

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Protection against bacterial toxin-mediated diseases such as Tetanus, Diphtheria and Pertussis is mediated by antibodies. Induction of antibody production by toxoid vaccines containing inactivated toxins confers protection from these diseases. The aim of our project is, therefore, to develop a cell-based assay that mimics the induction of toxoid-specific antibodies *in vitro* in replacement for the currently required *in vivo* challenge and immunogenicity studies. We are developing an assay based on the stimulation of the B cell response to the vaccine antigen in tetanus/diphtheria toxoid-specific memory B cells isolated from buffy coats. Exposure of TT/DT-specific memory B cells to vaccine antigen induces differentiation into antibody-secreting cells and their specificity is confirmed by the detection of specific IgG antibodies in ELISpot. The results obtained indicate that the established protocol with TT/DT specific B cells from buffy coats delivers a specific response to the antigen and can be used to evaluate the immunogenicity of TT and DT vaccine compounds. Quantification of TT/DT specific IgG memory B cells in this assay allow reduced variability of a cell based assay and improves standardization for routine use in a future. Our test system will serve to prove the functional integrity of the vaccine bulk antigens, by confirming the induction of the expected specific human antibody response. It might offer a potential alternative to time-consuming animal experiments currently used for batch testing of vaccines containing TT and DT components.

Keywords: Antibody, B lymphocytes, immune response tracing, visualizing immune responses

P-1109

ExoPred: The first method for predicting vertebrata secreted proteins via exosome using random forest algorithm

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Protein secretion is an essential process for intercellular communication in eukaryotes. Protein secretion occurs mostly through a conventional pathway involving the endoplasmic reticulum and the Golgi apparatus. Proteins secreted through this pathway bear a signal peptide at the N-terminus known as 'leader sequence'. However, a significant number of leaderless proteins are secreted through unconventional routes, including that mediated by exosome vesicles. Currently, no method is available to predict protein secretion via exosomes and here we approached the subject using machine learning. First, we first obtained 2992 of exosome-secreted proteins from UNIPROT and/or EXOCARTA. Only highly curated proteins were considered. This dataset did not include membrane bound proteins or proteins with leader sequences. We also generated a negative, non-exosome dataset, mirroring the exosome dataset, and assembled a final training dataset consisting 2961 proteins. Next, we translated the protein sequences into feature vectors consisting of global properties, amino acid and dipeptide composition and combination of them. Subsequently, we trained different machine learning classification models on the training dataset using WEKA. The best models were obtained using random forests, which in 10-fold cross-validation experiments achieved an accuracy of 69.95 %± 1.81 and AUC value of 0.76 ± 0.02. This random forest model was also tested in an independent dataset, including 2346 proteins that are only annotated in EXOCARTA and where not used in the training dataset, reaching an accuracy of 75.73 % and an AUC of 0.840. Finally, we developed an online tool, ExoPred, available at http://imath.med.ucm.es/exopred, which implements the model here generated.

Keywords: Big data, endo- and exocytic vesicles in immunity, immune communication, omics technologies

P-1119

Effect of outer membrane vesicles derived from the probiotic strain *E. coli* O83 on the immune system

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It has been shown that certain probiotic bacteria can reduce allergic sensitization in mice and humans. The aim of this study was to explore the effect of outer membrane vesicles derived from the probiotic bacterial strain *E. coli* O83 (EcO83-OMVs) on the immune system *in vitro* and *in vivo*. The effect of EcO83-OMVs was studied using HEK293 cells expressing NOD1/NOD2 and bone marrow-derived derived dendritic cells (BMDCs) from naïve and TLR4KO BALB/c mice. Immunotherapeutic effect of EcO83-OMVs was studied using a mouse model of ovalbumin (OVA)-induced allergic airway inflammation. The production of IL-8 increased upon stimulation of HEK NOD1/NOD2 cells with EcO83-OMVs stimulation, suggesting that NOD receptors may be involved in response to EcO83-OMVs stimulation. Stimulation of BMDCs with EcO83-OMVs increased the production of IL-23, IL-12, TNFα and IL-10, while BMDCs from TLR4KO mice exhibited lower cytokine production. *In vivo*, intranasal application of EcO83-OMVs reduced the level of eosinophils and airway hyperresponsiveness in comparison to allergic group. Treatment with EcO83-OMVs caused increased neutrophil infiltration into the lungs in comparison to naïve mice. Here we have shown that EcO83-OMVs induce TLR4/NOD1/NOD2-dependent production of pro- and antiinflamatory cytokines and reduce the development of experimental allergy.

Keywords: Adjuvants and vaccines, allergic disorders, dendritic cells, immune regulation and therapy, immunotherapy

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POSTER PRESENTATIONS

P-1122

Teriflunomide ameliorates disease severity and immunopathology of experimental autoimmune myasthenia gravis induced by acetylcholine receptor immunizations in mice

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Myasthenia Gravis (MG) is an autoimmune disease characterized by skeletal muscle weakness which increases with exercise. There is a need for new drugs effective in refractory MG patients. We aimed to test the potential of teriflunomide, an immunomodulatory drug with low side effects, in a murine experimental autoimmune myasthenia gravis (EAMG) model. EAMG was induced by immunizations with recombinant acetylcholine receptor (AChR) protein. Teriflunomide treatment (10 mg/kg/day, intraperitoneal) was initiated to one group of mice (n=21) after the last immunization and continued for five weeks. Disease control group (n=19) did not receive medication. Naïve mice (n=10) received only mock immunization. The clinical disease scorings and flow cytometric assay examinations of the number and cytokine profile of T cells, B cells were performed. Anti AChR specific antibodies in the peripheral blood serum were quantified by ELISA assays. Teriflunomide significantly reduced clinical disease scores, the absolute number and cytokine production by CD4+ T cells. IFN-γ+CD4+ or IL-2+CD4+ T cells were more drastically reduced both in absolute number or percentage among all CD4+T cells in the spleen and lymph nodes. The thymic CD4+ T cells were also reduced. Teriflunomide mostly spared CD8+ T cells number and cytokine production. Teriflunomide treatment also reduced CD138+CD19+(kappa or lambda+) plasma B cells' percentages among total B cells or their absolute numbers and CD138 mean fluorescent intensities. Consequently, antibodies generated in the myasthenic mice shifted from IgG to IgM type with teriflunomide use. Teriflunomide appears to have potential for the treatment of MG in humans.

Keywords: Animal models, autoimmunity, drugs for immune modulation

P-1136

Sw71 sheroids as a model to study the immune interactions during human implantation

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It is estimated that 40 to 60% of human conceptions fail, with the majority of the losses occurring just prior to or during implantation, a process that is initiated soon after the blastocyst attaches to the uterine wall at about day 6 post-conception. Implantation and immune homeostasis establishment remain the rate-limiting steps for the success of native and *in vitro* fertilization. Many regulations prohibit *in vitro* studies using human blastocysts and there is an urgent need of functional experimental models to study earliest stages of human implantation. Established *in vitro* 3D models such as Sw71 spheroids were capable of self-organization and resembling human blastocyst functionality during the peri-implantation period (Holmberg J, Mor G, et al, 2012). The model was created using cell line isolated from human placenta (7gest. week, SWAN 71) cultured in 3 dimensions and/or embedded within extracellular hydrogel matrix as well as co-cultured with endometrial epithelial cells (You Y, Mor G et al. 2019). We built the model and checked whether Sw71 spheroids express/produce HLA-C - the only classical HLA molecule responsible for the immune tolerance establishment during implantation and human early pregnancy. We confirmed that models based on Sw71 trophoblastic spheroids as embryo surrogates resembled human blastocyst in size, shape and function providing similar composition and architecture to primary trophectoderm/trophoblast tissue. Importantly, strong HLA-C expression/secretion was detected as first signal to maternal immune system for immune tolerance establishment. These models were suitable for dynamic and fully automated quantitative imaging and analysis.

Keywords: Cellular interactions, modelling, reproductive immunology

P-1150

Connection of CXADR gene and ACE2 gene in COVID-19

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Coronavirus symptoms and myocarditis symptoms are similar to each other such as chest pain. To distinguish between in two diseases are very important to prevent or to treat of disease. In this study we aimed to describe connection of CXADR gene and ACE2 in COVID-19. We were found similar genes with CXADR gene and ACE2 using STRING and GENEMANIA databases. Then we attained pathway of viral myocarditis using KEGG Pathway Database and we got probably damaging SNPs on CXADR gene using Exome Variant Server and PolyPhen2 databases. We showed expression levels of CXADR gene using IGV Browser, Ensembl Genome Browser, and UCSC Genome Browser. Our results may be support to explain the pathogenesis of high-risk group diseases in COVID-19. In the future, this study may contribution to solve physiopathology of COVID19 linked with myocarditis.

Keywords: Cardiovascular diseases, infectious disease, inflammatory disease, viral infections

P-1152

Using proteogenomics to identify tumor-specific antigens in breast cancer-derived cell lines

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The search for tumor-specific and -associated antigens (respectively TSAs and TAAs) has considerably accelerated during the past decade due to the improvement of proteogenomic detection methods. This provides new opportunities for the development of novel antitumoral immunotherapies to mount an efficient T cell response against one or multiple types of tumor. While patients with breast cancer have not yet benefitted from such a development, the opportunity of enlarging the repertoire of targetable/actionable TSAs by looking at antigens arising from both coding and non-coding regions of the genome opens up interesting possibilities for immunotherapeutic developments. Here, using a well-established pipeline based on sample-personalized proteome databases and state-of-the-art mass spectrometry, we analyzed the immunopeptidome of breast cancer and non-tumorigenic cell lines. By filtering peptides based on the expression of their source sequence in medullary thymic epithelial cells (mTECs) and normal tissue transcriptomes (GTEx), we respectively identified 119 TSA and 12 TAA candidates. Most of these antigens do not arise from somatic mutations but from the cancer-specific or aberrant expression of coding (exon) and non-coding (introns, UTRs, or intergenic) regions, thus representing novel candidates for the development of anti-tumoral vaccines.

Keywords: Adaptive immunity, anti-cancer vaccine, antigen processing and presentation, cancer immunology, cancer immunopeptidome, omics technologies

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POSTER PRESENTATIONS

P-1153

In silico design and analysis of next-generation multi-epitope based vaccine against non-typhoidal *Salmonella enterica*

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Nontyphoidal *Salmonella enterica* infection is the most common cause of acute bacterial diarrhea and accountable for a significant fraction of the global infectious disease burden. Currently, there is no licensed vaccine against nontyphoidal Salmonella available. In this study, we have used *in silico* methods to develop a multi-epitope vaccine that promotes strong mucosal protection against Salmonella infection. We chose the PrgI protein from the type 3 secretion system of *Salmonella typhimurium* for vaccine development. Using the immune epitope databases and prediction tools, we found 12 linear T cell epitopes and three linear B cell epitopes. We selected several epitopes after in silico antigenicity, allergenicity, and toxicity analyses and linked them with specific linkers. The FliC sequence was attached to the construct to stimulate the class switching to IgA and affinity maturation, resulting in long-lasting mucosal immunity. Server-based in silico tools used to ensure the final structure's antigenicity, toxicity, and allergenicity. We used numerous prediction tools to predict, refine, and validate the 3D protein structure of the proposed vaccine. Finally, to ensure the effectiveness of cloning and expression, we did a codon optimization and inserted a sequence into a plasmid. In conclusion, we used several *in silico* algorithms to design a multi-epitope vaccine construct containing multiple B-cell and CTL epitopes. This construct, expected to effectively stimulate mucosal immunity and is promising for fighting Salmonella gastroenteritis, can be evaluated in clinical trials.

Keywords: Adjuvants and vaccines, bacterial infections, infectious disease

P-1156

Withaferin A inhibits neutrophil functions and promotes apoptosis in stimulated neutrophils: multifaceted potential of withaferin a as a therapeutic for neutrophil-mediated diseases

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Dysregulation of neutrophil functions and delayed neutrophil apoptosis can exacerbate disease pathology. Though modulation of neutrophil functions and lifespan represent promising therapeutic targets, current pharmacologic options are limited. Withaferin A (WFA), derived from Withania somnifera, has anti-inflammatory properties and induces tumor cell apoptosis, but effects on neutrophil function and apoptosis are unknown. We hypothesized that WFA would inhibit adhesion, chemotaxis, and respiratory burst and promote apoptosis in equine neutrophils. For functional assays, neutrophils from healthy horses were pre-treated with varying WFA concentrations or vehicle control. Respiratory burst in response to granulocyte-macrophage colony-stimulating factor (GM-CSF)/lipopolysaccharide (LPS), phorbol 12-myristate 13-acetate (PMA), or insoluble immune complexes (IIC) was detected via luminol-enhanced chemiluminescence. Calcein-AM-loaded neutrophils were used to determine adhesion induced by IIC, PMA, or interleukin-8 (IL-8) and to quantify chemotaxis towards IL-8. To assess apoptosis, neutrophils were simultaneously treated and stimulated with GM-CSF or left unstimulated for 2 or 24 hours, followed by annexin V/propidium iodide staining and flow cytometry. Effects of treatments were evaluated using one-way repeated measures ANOVA with Holm–Sidak testing. WFA markedly inhibited neutrophil respiratory burst, adhesion, and chemotaxis for all stimuli evaluated (n=4-6, $p<0.05$). After 24-hour incubation, WFA significantly increased neutrophil apoptosis under GM-CSF stimulation (n=6, $p<0.05$). There was no effect of WFA on percentages of live or apoptotic neutrophils following 2-hour incubation, the maximum duration of the functional assays (n=3-4). Our data demonstrate that WFA suppresses neutrophil effector functions, without comprising short-term viability, and promotes timely apoptosis of activated neutrophils, suggesting multifaceted therapeutic potential for neutrophil-mediated diseases.

Keywords: Drugs for immune modulation, granulocytes, immune regulation and therapy, inflammatory disease, neutrophils, veterinary immunology

P-1163

Prime editing in primary human T cells using improved nucleofection protocol

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Despite rapid advances in immunotherapy, lack of efficient non-viral gene delivery methods for primary human T cells poses a major hurdle for clinical applications. Electroporation/nucleofection is a commonly used gene transfer method, albeit with lower transfection efficiency and viability post-nucleofection, especially for large DNA molecules. Here, we show that incubating T cells in fetal bovine serum (FBS) or CTS Immune Cell Serum Replacement (ICSR) (Thermo Fisher Scientific), immediately post-nucleofection can increase nucleofection efficiency. We tested this approach by transfecting 3 plasmids to achieve genome editing using prime editing system. 1. pCMV-PE2-P2A-GFP (~10.6 kb): expresses prime editor 2 and GFP, 2. pU6-Sp-pegRNA-RNF2_+5GtoT (~2.3 kb): expresses prime editing guide RNA, and 3. pU6-sp-sgRNA-RNF2_+41nick (~2.3 kb): expresses nicking-guide RNA. Co-transfection with these 3 plasmids can create P28H mutation in ring finger protein 2 in human embryonic kidney 293T cells with an editing frequency of about 80%, based on previous findings. Incubating T cells in 40% FBS or ICSR immediately post-nucleofection for 15 min at 37°C, increased nucleofection efficiency (GFP+) to ~75%. However, viability 24 hours post-nucleofection was <25%. Among several nucleofection protocols, we found that T-007 provided optimal nucleofection efficiency and viability (>50%) after 24 hours. Editing frequency among GFP+ cells was 8-10%, consistent with previous reports of prime editing in human primary cells. Altering the temperature to 30°C or 39°C did not improve efficiency. In conclusion, we provide an optimized non-viral electroporation-based method for efficient delivery of DNA into primary T cells, revealing a limited capacity for prime editing in these cells.

Keywords: Cell based therapies, immunological techniques, immunotherapy, molecular immunology

P-1168

Proteogenomic analyses of colorectal cancer reveal tumor-specific antigens across microsatellite status

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Colorectal cancer is the second leading cause of cancer death worldwide, and the incidence is expected to increase as global socioeconomic changes occur. Immune checkpoint inhibition therapy effectively treats a minority of colorectal cancer tumors; however, microsatellite stable tumors do not respond well to this treatment. Emerging cancer immunotherapeutic strategies aim to activate a cytotoxic T cell response against tumor-specific antigens (TSAs) presented exclusively on the surface of cancer cells. These rare antigens are best identified with a mass spectrometry-based approach which directly samples and sequences these peptides. While the few TSAs identified to date derive from coding regions of the genome, recent findings indicate that a large proportion of TSAs originate from allegedly non-coding regions. Here, we employed a novel proteogenomic approach to identify tumor antigens in a collection of colorectal cancer-derived cell lines and matched tumor and normal adjacent tissue biopsies. Personalized cancer databases paired with mass spectrometry analyses enabled the identification of over 30 000 unique MHC I-associated peptides. We identified over 20 putative TSAs in both microsatellite stable and unstable tumors, over 75% of which derived from non-coding regions. These peptides derived mainly from source genes involved in colorectal cancer progression, suggesting that antigens from these genes could have therapeutic potential in a wide range of tumors. These findings could benefit T cell-based vaccine development, which could be used in tandem with existing immune checkpoint inhibition therapies to bridge the gap in treatment efficacy across subtypes of colorectal cancer with varying prognoses.

Keywords: Anti-cancer vaccine, antigen processing and presentation, cancer immunology, cancer immunopeptidome, immunotherapy, mass spectrometry

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POSTER PRESENTATIONS

P-1179

In silico designed multi-epitope vacinne against *Brucella melitensis*

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Brucella melitensis is an intracellular pathogen transmitted from animals by infected food ingestion, direct contact, or inhalation. Antibiotic regimens for brucellosis patients can last several months and are not always completely effective, so the search for ideal brucellosis vaccines continues today. The goal of our research was to create a novel multiepitope vaccine against Brucella melitensis. We choose four antigenic proteins, omp2b, omp16, omp31, and BP26, for the vaccine. These proteins had analyzed in silico via different tools to explore antigenic motifs. T cell epitopes of these antigens were determined by using The Immune Epitope Database by assessing proteasomal cleavage, TAP transport, and MHC class I scores. We used several algorithms for predicting both linear and discontinuous B cell epitopes. We chose peptides with high presentation properties that are non-toxic, non- allergenic, and antigenic. Epitopes were connected using the particular linker GPGPG, and flagellin segments, an effective adjuvant, were introduced to the beginning and end of the construct. The final vaccine construct was analyzed to find out antigenic, allergenic, and toxic properties. Physicochemical properties of vaccine protein were computed via Expasy Protparam tool, confirmed that our peptide is stable. The vaccine construct's 3D structure had been predicted, refined, and validated. We evaluated the final construct for probable autoimmune induction using both sequence and structure-based methods, revealed that the vaccine protein had no expected autoimmunity risk. Overall, we designed the novel multiepitope vaccine against Brucella melitensis with enhanced immunity and confirmed its safety and efficacy with multiple computational algorithms.

Keywords: Adjuvants and vaccines, bacterial infections, infectious disease

P-1182

How important is the kit in the diagnosis of myopathies?

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Currently there are several commercial assays for detection of autoantibodies associated to IIM (IIMAA), but there are concerns about their reliability because of their low sensitivity for some antibody specificities and their high percentage of false positives for others. Some of these antibodies are associated with cancer, which has important implications for the diagnosis and management of patients. To compare the diagnostic concordance of 2 commercial IIMAA immunoblot kits from different manufacturers and identify differences in the results obtained, serum samples were analyzed. Eighteen positive and seven negative samples analyzed with kit 1 were selected and analyzed subsequently with kit 2. The concordance between the results of both kits was analyzed, as well as between the results of each kit and the diagnosis of the patients. The results obtained were that eighteen samples (72%) had some positive antibody with kit 1, but only 5 (20%) with kit 2. 10 samples (40%) obtained concordant results, 6 of them negative. 15 samples (60%) were discrepant, 14 of them with some positive antibody with kit 1 but negative with kit 2. Kit 1 showed greater concordance with diagnosis, especially those positive with kit 1 but negative with kit 2, since those patients had not been diagnosed with IIM. Notably, 5 patients with positive anti-TIF1γ antibodies with kit 1 but negative with kit 2 had not been diagnosed with dermatomyositis or cancer. The concordance between the kits is low, where Kit 1 appears to have false positive results, especially for anti-TIF1γ antibodies.

Keywords: Antibody, autoimmunity, biomarkers

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