

ABSTRACTS – MESIA 2022

MAIN HALL (A) PRESENTATIONS

AUSTRIA

From allergen-specific to innate concepts in the management of allergic diseases

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According to the European Academy of Allergy and Clinical Immunology, allergies will affect up to 50% of the European population in 2050. In Austria allergies already ranked second place among chronic diseases, with 1,7 million affected people. This is due to the climate change, global warming and pollution, with higher pollen counts, mold growth and invasive alien species. All together lead to barrier disruption, dysbiosis, and the promotion of Th2 immune responses. Th2 immune responses and atopy are accentuated by sterile food and modern malnutrition. There are several conservative options to manage allergies, but first precise diagnosis is pivotal. Since the detection of IgE in 1967 the cloning of allergens has improved the precision of allergy diagnosis by introducing molecular allergens allowing determination of IgE crossreactivities and a risk assessment, which can be done today by micro- and macroarrays. Symptomatic treatments of IgE-mediated allergies include adrenalin, beta-mimetics, antihistamines, leukotriene antagonists, and cortisone. They are not disease modifying, but alleviate symptoms. The era of biologics introduced antibodies and small molecules which improve allergic inflammation by inhibition of the hyperactivated Th2 immune pathways. However, the gold standard among allergy treatments is allergen-specific immunotherapy (AIT). Based on exact diagnosis, the specific allergen is selected and introduced into the patient by the subcutaneous or sublingual route. These therapies are most effective against pollen, house dust mite, and insect venom allergies, but take 3-5 years. The long duration of AIT is a challenge for doctors and patients and renders high dropout rates, which were even higher in the pandemic. Moreover, today's patients increasingly explore alternative, non-evidence-based treatments, such as bioresonance, homeopathy and more. Therefore, whenever AIT is not possible, it is advantageous to offer alternative, but evidence-based strategies. Considering the fact that innate cells such as NK cells and macrophages have a strong immunomodulatory potential, the innate arm of the immune defense could be harnessed in the fight against allergies. The evidence-based novel concepts for management of allergies presented include i) probiotics to complement dysbiotic microbiota, ii) targeted micronutrition to nourish exhausted allergic innate immune cells using the cowshed pill, and iii) strategies to physicochemically support the barrier function of a leaky mucosal barrier. Complementary to the power of allergen-specific immunotherapy, we need to exploit also the innate immune defense to prepare for the raising numbers of allergies.

Allergens at the crossroad: the lack of ecological interactions as a trigger for atopic diseases

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Although iron is one of the most abundant elements on earth, about a third of the world's population are affected by iron deficiency. Main drivers of iron deficiency are beside the chronic lack of dietary iron, a hampered uptake machinery as a result of immune activation. Macrophages are the principal cells distributing iron in the human body with their iron restriction skewing these cells to a more pro-inflammatory state. Consequently, iron deficiency has a pronounced impact on immune cells, favoring Th2-cell survival and priming mast cells for degranulation. Thus, iron deficiency increases the risk of atopic diseases, whereas an improved iron status seems to protect against allergy development. Major allergens such as lipocalins and the pathogenesis-related proteins have their natural function in nutritional immunity. They are capable to bind to iron via secondary metabolites such as siderophores or flavonoids and deprive the local environment from this precious micronutrient. With the whey protein beta-lactoglobulin we demonstrate that iron is bound to beta-lactoglobulin (holoBLG), transported to host immune cells (holoBLG) and thereby promotes tolerance and protects against allergy. Since 2019, several clinical trials have also been conducted in allergic subjects using holoBLG as a food for special medical purposes, leading to a reduction in the allergic symptom burden. Supplementation with nutrient-carrying lipocalin proteins can circumvent the mucosal block and nourish selectively immune cells, therefore representing a new dietary and causative approach to compensate for functional iron deficiency in allergy sufferers.

Host-Microbiota interaction in health and allergic disease

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Background: Probiotic bacteria have been shown to reduce allergic sensitization in mice and humans. *Escherichia coli* A034/86 (EcO83; serotype O83:K24:H31) is a Gram-negative probiotic bacterium, which has been commercially available. Oral application of EcO83 to newborns reduced the development of sensitization later in life but had no beneficial effects on allergic lung disease. Recently, we have shown that the intranasal application of EcO83 reduced several hallmarks of allergic airway inflammation in mice. Importantly, EcO83-treatment was effective in prophylactic and therapeutic settings and signalling through the TLR4 was required for the allergy-preventive effects. Extracellular vesicles derived from bacteria have been recognized as efficient carriers for the delivery of biomolecules to recipient cells, and to efficiently regulate host pathophysiology. The potential of probiotic bacteria-derived outer membrane vesicles (OMVs) for the prevention or treatment of allergy is unclear. Herein, we isolated OMVs from EcO83 (EcO83-OMVs) and studied their effect in a model of experimental allergic airway inflammation.

Methods: EcO83-OMVs were isolated by ultracentrifugation. HEK293 cells expressing NOD1, NOD2, TLR2, and TLR4 and bone marrow-derived dendritic cells (BMDCs) from wild-type (WT) and TLR4KO BALB/c mice were stimulated with EcO83-OMVs. The production of cytokines was measured by ELISA. A mouse model of ovalbumin-induced airway inflammation was used to study the immunotherapeutic effects of EcO83-OMVs.

Results: Stimulation of HEK293 NOD1, NOD2, TLR2, and TLR4 cells with EcO83-OMVs increased the production of IL-8 suggesting the involvement of these receptors in the signalling by EcO83-OMVs. Stimulation of WT BMDCs with EcO83-OMVs increased the production of IL-23, IL-12, TNF α , IL-1 β , and IL-6, while BMDCs from TLR4KO mice exhibited reduced production of these cytokines. Intranasal application of EcO83-OMVs reduced allergic airway hyperresponsiveness and the level of eosinophils in the lungs in comparison to sham-treated controls, and increased the numbers of pulmonary neutrophils, indicating that EcO83-OMVs might cause a shift from Th2 towards Th1 response.

Conclusion: Here we have shown that i) EcO83-OMVs are recognized by NOD1, NOD2, TLR2, and TLR4, ii) EcO83-OMVs induce production of pro- and anti-inflammatory cytokines in a TLR4-dependent manner, and iii) intranasal application of EcO83-OMVs reduce the development of experimental allergy. Despite numerous attempts to cure allergic diseases, there are few viable treatment options. Our research suggests that probiotic-derived OMVs might be a novel treatment option for allergic diseases in humans.

What makes a protein an allergen or hypoallergen

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Allergens are defined as immunoglobulin E- (IgE-)binding proteins able to induce allergic symptoms such as allergic rhino-conjunctivitis, asthma or even life-threatening anaphylaxis in sensitized individuals. Although the effector phase of the allergic disease is well understood, it remains unclear why certain proteins are becoming allergens while others are not. What are the specific properties making a protein an allergen? Based on current knowledge, the main properties of allergens can be grouped into (i) biochemical and structural features, or based on (ii) their ability to interact with the innate immune system in order to activate a T helper class II-biased adaptive immune response resulting in disease development and progression. Among common biochemical features for allergens, enzymatic or proteolytic activity, size (5–100 kDa), high solubility, abundance, thermal and proteolytic stability, and post-translational modifications (glycosylation) have been proposed. However, for most allergens only a few of these properties apply. Regarding the interaction with the innate immune system several immune receptors were proposed to play a role including Toll-like receptors, C-lectin receptors as well as protease-activated receptors. Apart from these allergen-inherent features, lately it became evident that the context in which allergens are being delivered plays a pivotal role not only in the process of allergic sensitization but also in the effector phase of the allergic disease. This context can for instance encompass (i) natural ligands modulating basic structural features of allergens (as its stability, protein fold, or endolysosomal processing), or (ii) non-allergen immunomodulatory molecules possessing an adjuvant function, which are co-delivered with the allergens as part of the allergenic source (i.e. pollen). Such non-allergen adjuvants can enable T helper cell class 2 polarization and, subsequently, allergic sensitization to allergens. The hypoallergen-concept was introduced to provide non- or less-allergenic alternatives that potentially replace the IgE-reactive wild type allergens within therapeutic products used for allergen-specific immunotherapy. The aim of creating a hypoallergen is to produce a molecule combining reduced allergenicity, i.e. via destruction of IgE-epitopes, but favorably retaining a high immunogenicity in order to guarantee the induction of an adaptive T cell response leading to immunotolerance and the reduction of allergic symptoms. This response comprises the production of specific IgG blocking antibodies (mainly IgG4) and the recruitment of specific regulatory T cells. But are hypoallergens really the solution? Novel therapeutic approaches

are on the rise combining the hypoallergen-concept with increased immunogenicity for an enhanced and more rapid induction of immunotolerance. Among others, such approaches include hypoallergen-displaying nano- and bio- particles. In this talk, main properties of allergens as well as the development, necessity and applicability of hypoallergens will be discussed.

Medical Specialization Allergology in Austria: Essential measure to improve care of allergic patients

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Since the EAACI 2019 position paper by Fyhrquist et al. on the training opportunities in allergology knows that Austria (along with Norway, Ireland, Denmark and Belgium) has long been a blank spot on the "allergology" map of Europe. While most European countries offered in-depth training in allergology (e.g. specialization in allergology), this was not possible in Austria until June 2022. Several attempts and initiatives had been necessary over the past 15 years to achieve the seemingly impossible in Austria: on July 1, 2022 the specialization in allergology became a reality. The specialization in allergology includes the deepening of knowledge, experience and skills in the prevention, diagnosis and therapy of allergic diseases and their consequences. Eight medical specialties joined together to offer an 1,5 year curriculum after residency to specialize in allergology. In my talk, I will give an overview of the new medical specialization in allergology and a brief outlook on the training alliances that will be needed for training of new specialists in the field.

Immunology of COVID-19 and Long-COVID

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The ongoing SARS-CoV-2 pandemic represents a major global health challenge. Health care systems have to cope not only with increasing prevalence and new variants of concern, but also with patients suffering from long-term sequelae, termed post-acute COVID-19 syndrome or Long-COVID.

There is increasing evidence that both, the acute and the post-acute disease course, are largely influenced by the patient's specific immune response. Already at the beginning of the pandemic, differences in immune responses were defined, such as T-cell exhaustion and hyper-inflammation distinguishing severely affected acute COVID-19 patients from mild disease courses. However, even after mild or asymptomatic disease, patients develop long-term sequelae with persisting or new symptoms. Characterizing the immune signatures of Long-COVID patients might be a game changer in identifying long-term sequelae prediction markers. Recent findings suggest reduced numbers of innate and adaptive immune cells in Long-COVID patients, but also increased immune cell counts, elevated cytokine levels and complement activation. Moreover, there is increasing evidence on the impact of SARS-CoV-2 infections on the microbiome, mycobiome and also on the virome composition along mucosal barriers, which might have a substantial influence on the immune response. We have previously reported EBV reactivation, which was detected by EBV qPCR in throat washing samples, in a sub-group of Long-COVID patients suffering from post-exertional malaise.

Gaining further insights in mechanisms of disease is crucial to correctly interpret recent data. Defining immune characteristics presents a powerful tool to predict the risk and the progression of long-term sequelae after viral diseases, as well as to identify novel treatment approaches.

Immunological determinants of COVID-19 severity

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SARS-CoV-2 infection manifests in a highly diverse way, ranging from almost asymptomatic to life-threatening disease with lung failure. While certain risk factors such as age or obesity are well described, mechanistic insights into molecular and cellular disease-driving factors remain sparse. To model COVID-19 in mice we generated the mouse-adapted SARS-CoV-2 strain maVie16, which potently infects mice via murine ACE2 and induces key features of human disease such as lymphopenia, pneumonia and inflammation-induced immunopathology. While BALB/c mice are highly sensitive to MaVie16 and succumb to low infection doses, C57BL/6 mice develop profound but transient pneumonia. Interestingly, 129/sv mice are fully resistant and only develop murine COVID-19 with advanced age. Aside from the genetic background, disease severity is, similar to humans, highly age dependent in all mouse strains and can be improved by simultaneous blockade of IFN γ and TNF or inhalative treatment with

soluble ACE2. Thus, our model serves as a novel and useful tool to study both, antiviral effector mechanisms and COVID-19 associated immunopathology.

Proteins involved in SARS-CoV-2 infection

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) requires a permissive host cell to replicate and release progeny virus. Viral entry is the first step of infection and a main target for vaccines and therapeutics against COVID-19. This process is executed by the S protein on the envelope of SARS-CoV-2, which recognizes the key host cell receptor angiotensin-converting enzyme 2 (ACE2), and mediates membrane fusion to allow the viral genome to be released into the cytoplasm. In addition to ACE2, several other host cell proteins, such as proteases (TMPRSS2, cathepsin L) and multiple alternative/auxiliary receptors have been reported to interact with the S protein and facilitate SARS-CoV-2 entry. Also in our hands, neutralizing antibodies targeting the S protein that arose in response to COVID-19 or vaccination or various TMPRSS2 inhibitors effectively prevented infection of ACE2-positive cells by SARS-CoV-2. In addition to viral entry mediated by the S protein, a low level of infection might be achieved by host cellular proteins incorporated into mature SARS-CoV-2 virions, as such an infection was described for several other enveloped viruses, including SARS-CoV-1. To learn whether this mechanism is also employed by SARS-CoV-2, we developed a strategy to obtain highly pure SARS-CoV-2 virions and analysed their protein content by mass spectrometry. Using this approach, we identified a multitude of host cellular proteins hijacked by the virus. This data set will allow us to systematically dissect the significance of the virus-associated cellular proteins for SARS-CoV-2 cell entry or other stages of the viral life cycle. This work was supported by the Austrian Science Fund (P 34253-B).

Protective features of anti-COVID-19 immune response

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Rationale: Currently, the world is again challenged with rising break-through infections caused by SARS-CoV-2 Omicron BA.4/5. Although, various studies were conducted investigating systemic immunity provided by neutralizing antibodies in sera, mucosal immunity was barely evaluated.

Objectives: Along with systemic immunity, we here also investigate mucosal immunity, since neutralizing antibodies at mucosal entry sites play a fundamental role in disease limitation. In addition, comparing vaccinated and convalescent individuals enables deciphering natural and vaccine-induced immune defenses in mucosa and serum.

Methods: Humoral immune responses and virus neutralization of vaccinated and/or BA.1/BA.2-convalescent individuals were investigated. Serum and saliva samples were used to determine SARS-CoV-2 Spike-specific IgG and IgA titers and neutralizing activity against replication competent SARS-CoV-2 wildtype virus and Omicron BA4/5 variant.

Results: Besides strong induction of serum IgG/IgA, salivary IgA and neutralization against SARS-CoV-2 wildtype in vaccinated/convalescent subjects, we detected a 10-fold reduced, yet positive serum neutralization, against BA.4/5. While most vaccinated or unvaccinated/BA.1 convalescent individuals had no sufficient neutralization against BA.4/5, vaccinated and BA.2-convalescent patients demonstrated the best serum neutralization against BA.4/5. In addition, salivary neutralization against wildtype virus was also strongest in vaccinated and BA.2-convalescent subjects, but this advantageous neutralizing effect was not observed against BA.4/5.

Conclusions: Our data support the contention that current COVID-19 vaccines are very efficient against severe or critical disease progression. Moreover, these results prompt an adjustment of the current vaccine strategy to adapted and alternative vaccine delivery such as mucosal booster vaccinations to establish robust sterilizing immunity against novel SARS-CoV-2 variants.

Sabine Altrichter - Diagnosis of allergy to COVID vaccines

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CROATIA

Congenital cytomegalovirus infection–neuroinflammation and brain pathology mechanisms

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Congenital cytomegalovirus (cCMV) infection is the leading viral cause of mental retardation and sensorineural hearing loss in infants and children. Since human CMV is species-specific, research of cCMV infection rely heavily on animal models. Infection of newborn mice with mouse CMV recapitulates the major hallmarks of human cCMV infection: virus dissemination to brain parenchyma, neuroinflammation, altered brain development and sensorineural hearing loss. We have previously established that virus-induced inflammation in the brain is the driving force behind CNS damage after cCMV infection (Kosmac et al PLoS Pathog 2013, Kveštak et al J Exp Med 2021). Thus, it is crucial to elucidate the mechanisms that lead to neuroinflammation, developmental abnormalities and other pathological changes associated with cCMV infection. Many aspects of the pathogenesis of cCMV infection are still not well defined, which limits the possibility of developing potential therapeutic interventions to prevent CNS sequelae. In my talk I will present our recent studies describing the role of microglia and other immune response mechanisms in driving CNS pathology in the mouse model of congenital CMV infection.

Early life cytomegalovirus infection extensively reshapes the transcriptional profile and functionality of NK cells

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Infections during early life can have substantially different outcomes and consequences than infections occurring in adulthood. In this study, we have used newborn mice infected with mouse cytomegalovirus (MCMV) to investigate the immunomodulatory effects of a perinatal beta-herpesvirus infection on NK cells. We found that MCMV infection causes a significant shift of NK cell population towards the terminally mature phenotype and severely compromises their functionality, as demonstrated by the reduced ability of NK cells from infected mice to produce cytokines. Such extensive reshaping of NK-cell phenotype occurred only in mice infected during early life and required active virus replication. Remarkably, even infection with heavily attenuated MCMV strains induced NK cell hyporesponsiveness, suggesting that NK cell dysfunction is not due to impaired control of the virus in newborn mice. Mechanistically, the infection caused suppression of principal transcription factors governing NK cell fate and function, such as TCF-1 and Eomes, and resulted in dysregulation of numerous genes and impairment of NK cell function. Altogether, our data indicate that early life perinatal cytomegalovirus infection can have profound adverse effects on the functional abilities of NK cells.

Human thymus contains epithelial cells with stem cell features long after birth

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Thymus function regarding T lymphocyte development and selection is mainly considered to end at early age in human. Therefore when life saving cardiac surgery in children born with a complex heart disorders is needed, it is part of the procedure to perform thymectomy. Several groups have shown that thymus is active during the whole life, although it involutes at puberty and its function to keep the organism healthy decreases with age. Therefore the reactivation of the thymus either in vivo or by reassembling it in vitro from thymic epithelial stem cells would bring considerable benefits to patients with damaged thymus function either by age, injury or iatrogenically induced. By means of immunohistochemistry and confocal microscopy methods we attempted to find cells from the human thymus with stem cell characteristics. Also we analysed thymi of children of different age, from newborn to 11 years old. We describe here a marked difference in histology sections of thymus from children of

different ages and our results indicate that although there are specific non hematopoietic CD45- progenitor populations in postnatal human thymus, the aging is an important factor for cell recovery and possibly functionality. When cultured in vitro their ability to support lymphocyte differentiation depends strongly on the age of the patient. Our work shows that in the human thymus there are rare populations of epithelial stem cells and although very rare they can be the initial population that can be isolated and used for the thymus regeneration. This work has received funding from the Croatian Science Foundation under grant agreement HRZZ-2020-02-2431. Thyminnova

NKG2D-mediated immunosensing of metabolically stressed hepatocytes by innate-like T cells is essential for initiation of NASH and fibrosis

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Metabolic-associated fatty liver disease (MAFLD) comprises a spectrum of clinical entities ranging from benign steatosis to cirrhosis. A key event in the pathophysiology of MAFLD is the development of non-alcoholic steatohepatitis (NASH) that may lead to fibrosis and hepatocellular carcinoma. What triggers inflammation in MAFLD is unknown. We find that lipid accumulation in hepatocytes induces expression of ligands for the activating immune receptor NKG2D. Tissue-resident innate-like T cells are activated through NKG2D and secrete IL-17A. IL-17A licenses hepatocytes to produce chemokines that recruit pro-inflammatory cells into the liver, causing NASH and fibrosis. NKG2D-deficient mice did not develop fibrosis in a dietary model for NASH and had a marked decrease in the incidence of hepatic tumors. Importantly, the frequency of IL-17A+ $\gamma\delta$ T cells in the blood of MAFLD patients correlated with liver pathology. Our findings identify a key molecular mechanism through which stressed hepatocytes trigger inflammation in context of MAFLD.

Transcriptome profiling of CCR2hi and CCR2lo osteoclast progenitor subpopulations associated with arthritis

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As the existence of different osteoclast progenitor (OCP) subsets has been confirmed, their detailed characterization is required to understand the pathophysiology of increased osteoclast activity causing periarticular and systemic bone resorption in arthritis. We previously defined OCP subsets based on the level of CCR2 expression, as circulatory-like committed CCR2hi OCPs, substantially expanded in arthritis, and marrow-resident CCR2lo OCPs of immature phenotype and behaviour. We sorted CCR2hi and CCR2lo OCPs of mice with collagen-induced arthritis (CIA) and control mice (n=4 for each group) and performed next-generation RNA sequencing. We confirmed a disparity between the transcriptomes of CCR2hi and CCR2lo OCP subsets, and identified pathway enrichment for osteoclast differentiation, chemokine and NOD-like receptor signaling in the CCR2hi OCP subset and ribosome biogenesis in eukaryotes and pyrimidine metabolism in the CCR2lo OCP subset. Genes associated with the osteoclastogenic pathway (Fcgr1, Socs3), and genes involved in cell adhesion and migration (F11r, CD38, Lrg1) were used to both identify the CCR2hi subset and distinguish CIA from control group, as validated by qPCR (n=6 for control, n=9 for CIA). The latter set positively correlated with arthritis clinical score and percentage of CCR2hi OCPs, indicating potential disease markers. Moreover, osteoclast pathway-identifying genes remained upregulated in committed preosteoclasts cultured for two days, suggesting their importance for enhanced osteoclastogenesis of CCR2hi OCPs in arthritis. Our approach identified differentially expressed genes that allow detection of distinct OCP subsets associated with arthritis and well as indicate possible therapeutic targets aimed to modulate progenitor migration, proliferation, differentiation, or activity.

Allergic diseases in urban environment in Croatia

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Background. Rapid climate changes and low biodiversity threaten human health by contributing to the increasing number of allergic persons in countries with westernized lifestyle. We have to expand our understanding of the mechanisms by which these environmental changes induce harmful immune reaction in order to prevent events in which large number of people seek emergency treatment (e.g. in Melbourne in 2016, when 9 people died of thunderstorm asthma).

Some epidemiological studies that have been monitoring the trends in prevalences of allergic diseases have reported that allergy epidemic in developed countries has reached its plateau, while a rise is expected in developing ones. Also, different impact of rural and urban milieu has been gaining considerable scientific interest because of its role in immune system education.

Objective. Our aim was to compare prevalence of allergic diseases and associated causative factors among schoolchildren from the city of Zagreb (Croatia, a low-income European country) sixteen years after first results collected by same methodology and on the population of same age. This is a part of the bigger study which involved also children living in rural setting of Natural Park Lonjsko polje.

Methods. Symptoms of asthma, allergic rhinitis (AR) and atopic dermatitis (AD), along with prenatal, early-life, and current exposures were assessed using The International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. An allergic profile was determined by the skin prick test.

Results. Prevalence of current, ever-in-a-lifetime and diagnosed AR of 35.7%, 42.5% and 14.9%, and AD of 18.1%, 37.1% and 31.1% demonstrated a significant increase. Asthma prevalence of 5.7% remained unchanged. Maternal smoking in pregnancy, dampness and mould in homes, day-care facility and pertussis vaccination increased during the observed period. Allergen sensitivity rate remained similar, but pollens became dominant. Exposures to mould and dog allergens were risks for asthma (OR 14.505, OR 2.033, respectively). Exposure to cat allergens appeared as protective in AR (OR 0.277). Fast food consumption was rare, not contributing to the AD increase. Parental history of allergy was a risk factor in all conditions.

Conclusion. Over sixteen-year-period, prevalences of AR and AD in Zagreb considerably increased accompanied with the high proportion of children's atopy, which was not affected by the passage of time. Prevalence of asthma remained the same. During this observed period, lifestyle factors like maternal smoking in pregnancy, dampness and mould in homes, day-care facilities and pertussis vaccination also increased, but were identified as risks only in relation with asthma together with dog exposure. Rapidly progressing AR was related only with cat exposure but in a protective way. Our explanation for high AR prevalence is based on data of contemporary observed higher proportion of children with pollen sensitisation in comparison to previous historic period. Reported higher PM10 concentration in city of Zagreb could not be eliminated as confounding factor. This two causative factors are obviously in relationship with continuously registering climate changes. Further research is necessary to provide an explanation for the longstanding question about unique role of cat allergen in allergic individuals. Stable asthma trend remains insufficiently understood. We speculate that it could be a result of decreased sensitisation to indoor allergens whose role is more important than the role of outdoor ones in this allergic disease. We did not provide explanation for high prevalence of 31.1% of physician-diagnosed AD in our urban children cohort. Strong genetic background in all three conditions is evident, but influence of environmental factors on gene functioning could not be examined by this type of studies.

Ragweed pollen and allergic symptoms in children: Results from a three-year longitudinal study

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Ambrosia artemisiifolia L. (Ambrosia elatior L., common ragweed) is a type of short ragweed has been spreading invasively in many European countries. Hypersensitivity reactions to ragweed pollen manifest as rhinitis, rhinoconjunctivitis and/or allergic asthma. As ragweed is one of the most potent allergenic pollen species in Croatia, sensitization rates are increasing in all age groups- ranging from 15% to nearly 20% throughout in the eastern region of Croatia, according to the results of a three-year longitudinal study as part of the ATOPICA project. According to the results of the ATOPICA study involving more than 4000 children in Croatia, the sensitization prevalence rates to ragweed were up to 15% (depending on the region examined- Mediterranean- about 1,5 % or continental- 15-18 %). The cumulative 3-year de novo sensitization rates to Ambrosia also varied depending on the region- from 2,5 % in the Mediterranean region to over 7 % in the continental areas. Moreover, sensitization to ragweed seemed to increase the risk for sensitization to other allergens as well as the risk for allergic symptom manifestation in children (especially symptoms of allergic rhinitis), compared to other allergens. Because allergens

of Ambrosia are airborne not only by pollen grains but also by other particles of less than 10 µm (PM10) in diameter, they are able to contribute to out-of-the pollination-season symptoms in sensitised subjects and to penetrate in the lower respiratory tract, which is why Ambrosia is also highly asthmogenic.

Allergic reactions to COVID-19 vaccine in Croatian high-risk patients

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Introduction. Shortly after the implementation of mass vaccination for coronavirus disease 2019 (COVID-19), concerns have been raised regarding allergic reactions to messenger ribonucleic acid (mRNA) COVID-19 vaccines. Patients with anaphylaxis to mRNA vaccines frequently report a history of prior allergic reactions to drugs. We aimed at estimating the rate of immediate allergic reactions to mRNA vaccines in patients with a history of drug allergies or other allergic disorders. **Patients and methods.** We included adult patients with a history of drug allergies or other allergic disorders (asthma, allergic rhinitis, atopic dermatitis, food or insect venom allergy, systemic mastocytosis, idiopathic anaphylaxis, acute or chronic urticaria and/or angioedema) who received at least one dose of an mRNA vaccine at the vaccine referral center of the Special Hospital for Pulmonary Diseases in Zagreb, Croatia, between March 1, 2021, and October 1, 2021. Immediate allergic reactions occurring within four hours of vaccination were recorded. **Results.** Among 1679 patients, 1432 (85.3%) reported a history of drug allergy. In our cohort, six immediate allergic reactions to mRNA vaccines were identified (0.36%). One patient (0.06%) experienced anaphylaxis that resolved after epinephrine administration, and other reactions were mild and easily treatable. Of note, two patients with systemic mastocytosis and seven patients with idiopathic anaphylaxis tolerated mRNA vaccine. **Conclusion.** Most patients with a history of drug allergies and other allergic disorders can safely receive mRNA COVID-19 vaccines. However, adequate observation periods and preparedness to recognize and treat anaphylaxis are indispensable.

Effect of the COVID-19 pandemic and Zagreb earthquake on the psychological stress level and disease condition of atopic dermatitis patients.

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Background: Stress, including psychological stress, can affect various chronic diseases, including atopic dermatitis (AD). Thus, we wanted to examine how the COVID-19 pandemic and the March 2020 earthquake in Zagreb, Croatia, affected the psychological stress levels and disease severity of AD patients.

Subjects and methods: We conducted a cross-sectional study that involved 150 AD patients (three groups with 50 patients each): 1) patients not exposed to either the COVID-19 pandemic or the earthquake; 2) those who only experienced the pandemic; and 3) those who experienced both the pandemic and the earthquake. Patients' data from the Perceived Stress Scale (PSS), data on AD severity (SCORAD), and data on disease-related behaviors (answers from our custom designed questionnaire) were examined and statistically analyzed.

Results: The patients who experienced both disasters had greater PSS scores than those who experienced only the pandemic (especially women), and they also had higher SCORAD results than those in the other two groups. Also, 59% of patients reported stress during the pandemic, mostly caused by the possibility of infection (31%), a changed work life and possible loss of income (23%), general pandemic-related conditions (17%), worry about physical survival (11%) and other (6%). PSS scores significantly positively correlated with psychological experience of the earthquake and with intensity of sleep disturbances.

Conclusion: The COVID-19 pandemic influenced AD patients' stress levels, and the combined stress caused from both disasters affected patients' AD condition/severity. This supports the idea that psychoneuroimmunological factors affect the course of AD

CZECH REPUBLIC

Smouldering inflammation: from tissue remodeling to cancer niche

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Long lasting chronic inflammation induces progressive fibrosis of the involved tissues. Chronic inflammation, a leading factor in many pathological processes, can address cancer establishment and evolution. Local changes of immune activation inside a tissue, if maintained and supported by the environment, can induce structural remodeling. Reciprocally, collagen accumulation can affect the local immunity. We have shown that the colonization of germ-free (GF) mice colon by conventional mice (CV) intestinal microflora quickly modifies the local and systemic immunity. Contemporarily, it induces a fast remodeling of the collagen scaffold in the intestinal mucosa. Using a rat model of chronic colitis (dextran sodium sulphate – DSS – induced colitis) and of carcinogenesis (using azoxymethane – AOM - carcinogen for the colon) we have shown that, in both models, inflammation activates remodeling of the collagen scaffold organization even when the mucosa appears recovered from the acute induction. Multi-photon confocal microscopy of CV and GF animal mucosa resulted with higher complexity in structure in the CV rats (with microbiome). The immunological data suggest that the response to the microbiota presence elicit effective homeostatic regulation in the healthy CV rats, to avoiding inflammation and maintaining cytokine levels near the spontaneous production found in the GF animals. These conditions establish what we define as “inflammatory threshold” of the mucosa, allowing a range of tolerance to the continuous immune activation. The results also indicated that the collagen scaffold adapts to the immune microenvironment conditions, and quickly it can be altered if the immune threshold is overcome. The dis-balance between pro-inflammatory and regulatory signals, found even under apparently normal or reduced levels of microenvironmental cytokines, generates a smouldering inflammation still capable to damage the tissue structure. Moreover, in a mouse pancreatic cancer model, IL-17 expression resulted important to address the evolution of profibrotic collagen organization. Concluding, inflammatory-threshold changes producing smouldering inflammation predisposes to chronic inflammation and/or cancer niches establishment. Cytokine levels and collagen scaffold remodeling measured in the tissue can offer possibly new diagnostic markers.

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Immunological Configuration of Ovarian Carcinoma: The Impact on Disease Outcome and Response to Immunotherapy

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Ovarian carcinoma (OC) is among the top five causes of cancer-related death in women. At odds with other neoplasms, OC is poorly sensitive to immune checkpoint inhibitors, correlating with a tumor microenvironment that exhibits poor infiltration by immune cells and active immunosuppression. Thus, novel strategies are needed to overcome the lack of pre-existing immunity, potentially including immunogenic chemotherapies, adoptive T cell transfer strategies, and/or vaccination approaches. As most immunotherapies only benefit a small percentage of patients, it is also imperative to discover biomarkers that prospectively identify patients potentially achieving benefits from specific immunotherapeutic regimens, in the setting of personalized cancer immunotherapy.

Cellular machinery for antigen distribution in the thymus

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The presentation of self-antigens in the thymus by medullary thymic epithelial cells (mTECs) and thymic dendritic cells (DCs) is fundamental for the establishment of T cell central tolerance and prevention of autoimmunity. While mTECs produce and present self-antigens in an autonomous manner, DCs can acquire these self-antigens from mTECs by the process of Cooperative antigen transfer (CAT). While CAT is required for effective T cell selection, many aspects remain enigmatic. Given the recently described heterogeneity of mTECs and DCs, we have recently shown that each subset of thymic DCs preferentially targets a distinct mTEC subset(s).

Importantly, XCR1+ activated DC subset represented the most potent subset in CAT. Thymic DCs can also acquire antigens from more than one mTEC, and of these, monocyte-derived DCs (moDC) were determined to be the most efficient. moDCs also represented the most potent DC subset in the acquisition of antigen from other DCs. These findings suggested a preferential partnership between specific subsets of TECs and DCs for CAT. To identify molecular determinants that mediate such specific cellular pairing, we employed single-cell RNA sequencing approach. Using fluorescent tdTomato protein as a mTEC-produced model antigen, we compared the transcriptome of each thymic DC subset which acquired the tdTomato protein from mTECs to those cells of the same DC subsets which failed to do so. We found that tdTomato+ conventional DC type 1 (cDC1) expressed high levels of the tight junction-associated proteins, Claudin-1 and EpCAM. Remarkably, when either Claudin-1 or EpCAM were genetically ablated, the capacity of cDC1 subset to participate in CAT was significantly diminished. Taken together, our data shows that Claudin-1 and EpCAM are key molecular determinants of CAT in regards to the cDC1 subset and suggests that each DC subset possesses a distinct set of signature genes that mediates CAT. Revealing the entire set of molecular determinants which control and modulate presentation of self-antigens will be next important step towards a unified view of how the universe of self-antigens and its cellular distribution in thymus is functionally coupled to the T cell selection process.

Intranasal application of defined bacterial components in prevention of airway allergic inflammation

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The currently observed high prevalence of allergic diseases is associated with changes in microbial exposure in industrialized countries. Defined bacterial components represent a new strategy for modulating the allergic immune responses. We have recently investigated the effect of *Lactocaseibacillus* (L.) mixture (L. rhamnosus LOCK900 and LOCK908 and L. paracasei LOCK919) on allergic sensitization in gnotobiotic mouse model showing that colonization by lactobacilli-mixture dampens the allergic sensitization by stimulation of TGF- β and IgA responses [1]. Next, we have isolated and characterized two exopolysaccharides (L900/2; 830 kDa and L900/3; 18 kDa) produced by L. rhamnosus LOCK900 strain and proved their potential to modulate immune response to a bystander antigen in bone marrow-derived dendritic cells [2]. Here we investigate the potential of the intranasal application of exopolysaccharide L900/2 (EPS) to prevent the development of the airway allergic immune response upon challenge with allergen in sensitized mice. Using ovalbumin murine model we show that intranasal administration of EPS downregulates the development of allergic airway inflammation and Th2 cytokine response in sensitized individuals. Simultaneously, EPS treatment significantly increased the level of total, OVA-specific, and also bacteria-specific IgA in bronchoalveolar lavage and the number of IgA-producing B-cells in lung tissue of these mice. The EPS activated polyspecific IgA production through a T-cell-independent pathway by induction of TGF- β , BAFF and NOS2 costimulatory factors in lung tissue. Thus, the EPS derived from L. rhamnosus LOCK900 can be considered as a safe candidate for prevention of lung allergic symptoms development in sensitized individuals upon exposure to allergen.

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Unique roles of LCK in helper and cytotoxic T cells

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The kinase LCK and CD4/CD8 coreceptors are crucial components of the T cell antigen receptor (TCR) signaling machinery leading to key T cell fate decisions. Despite decades of research, the role of CD4- and CD8-LCK interactions in the TCR triggering in vivo remained unknown. In this study, we created animal models expressing endogenous levels of modified LCK to resolve whether and how coreceptor-bound LCK drives TCR signaling. We demonstrated that the role of LCK depends on the coreceptor to which it is bound. The CD8-bound LCK is largely dispensable for anti-viral and anti-tumor activity of cytotoxic T cells in mice. However, it facilitates CD8+ T cell responses to suboptimal antigens in a kinase-dependent manner. In contrast, the CD4-bound LCK is required for

efficient development and function of helper T cells via a kinase-independent stabilization of surface CD4. Overall, our findings reveal the role of coreceptor-bound LCK in T cell biology, show that CD4- and CD8-bound LCK drive T cell development and effector immune responses using qualitatively different mechanisms, and identify the coreceptor-LCK interactions as promising targets for immunomodulation

Sensitization to inhalant allergens in allergy patients in Central Europe

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Molecular diagnosis of allergy and microarray technology have opened a completely new avenue of insight into sensitization profiles from both the clinical and the epidemiological points of view. We used this innovative tool in the description of sensitization patterns in allergy patients in Central Europe. Immunoglobulin E detection using 112 different allergenic molecules was carried out employing the ImmunoCAP ISAC microarray system.

Sera from 1255 allergic patients were subjected to analysis. The highest observed sensitization rate was 70.0% to grass-specific molecules (the most frequent being Phl p 1; 60.2%). The second most frequent sensitization was 47.4% to Betulaceae-specific molecules (Bet v 1; 46.8%). Together, grasses and Betulaceae components (and their cosensitizations with other components) comprised the vast majority of pollen sensitizations. Unexpectedly frequently observed sensitizations were those to Cupressaceae-specific molecules (12.2%), Oleaceae-specific molecules (9.3%), and the plane tree-derived molecule Pla a 2 (13.4%). The sensitization rates for all other molecules were within the expected range (Art v 1, 11.8%; Pla l 1, 8.3%; Che a 1, 7.3%; Par j 2, 0.8%; Amb a 1, 0.7%, and Sal k 1, 0.4%). Sensitization rates of pollen-derived cross-reacting molecules were found to be 10.7% for profilins, 4.3% for polcalcins, and 5.5% for lipid transfer proteins.

The major sources of allergens in the indoor air include house dust mites, dander derived from domestic animals and rodents, cockroach, and several fungi. Mites are the main cause of allergies in some countries with a warmer climate. The sensitization to at least one mite-specific molecule (Der p 1, 2, Der f 1, 2) was observed relatively frequently in 32.7% of patients. Specific IgE to mite group 2 molecules is almost fully cross-reactive. Group 1 allergens are also cross-reactive, but in some patients, a species-specific response was observed. A relatively high rate of sensitization both to group 1 and 2 allergens in our patients indicates the greater role of co-sensitizations. Isolated sensitizations to molecules derived from glycyphagid mites Lep d 2 and/or Blo t 5 without sensitization to other mite-derived molecules were observed only exceptionally (in 0.6% of cases). True sensitization to at least one cockroach-specific molecule (Bla g 1, 2, 5) was very rare (in 0.6% of cases), and nearly all of them were co-sensitizations with other noncockroach-derived molecules. Sensitization to an inhaled tropomyosin was observed rarely in 2.2% of patients (Der p 10 in 1.9% and Bla g 7 in 1.5%). Co-sensitization of inhaled tropomyosins with the respective mite- or cockroach-specific molecules was observed only in the minority of patients suggesting the different route of sensitization being more frequent.

Among domestic animals, the highest sensitization rates were observed for uteroglobin Fel d 1 (31.8%) and kallikrein Can f 5 (16.4%), followed by animal lipocalins Can f 1 (13.9%), Equ c 1 (6.2%), Fel d 4 (5.3%), Can f 2 (4.2%) and Mus m 1 (4.1%). Sensitization rates to serum albumins Fel d 2, Can f 3, Equ c 3, and Bos d 6 were very low, with the highest being 3.2% for Fel d 2. Detailed subanalysis confirmed the dominant role of Fel d 1 or Can f 5 and/or Can f 1 in cat- or dog- sensitized patients, respectively. Further analysis focused on lipocalins and albumins confirmed a high rate of cosensitizations within both groups.

Molecular diagnosis of allergy gives a more precise and comprehensive insight into sensitization patterns than extract-based testing, allowing a better understanding of the sensitization process and regional differences. The data presented here may help to improve the diagnostic and allergen-specific treatment procedures in the respective region.

Immune contextures of the tumor microenvironment: Solid tumor pre-conditioning to improve cancer immunotherapy

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The immunotherapy with immune checkpoint inhibitors (ICIs) has become the first line treatment option in metastatic diseases. However, the response to immunotherapy varies among diverse cancer types and also, among individuals, and the mechanisms of primary and acquired immunotherapy resistance are poorly understood. Currently, a careful stratification of patients and proposing an algorithm to guide the use of immunotherapy strategies is crucial to allow outcome improvements in the clinical practice. Only clear scientific rationale with clearly documented preclinical steps may allow development of novel combination therapies for metastatic diseases, including soft tissue sarcomas and esophageal carcinomas. With the aim to establish consecutive steps to sensitize these tumors to immunotherapy, we have investigated the proportions and phenotypes of cells

infiltrating the tumor microenvironment (TME) and the paratumoral tissue compartments. We have also tested the combinatorial treatment strategies to increase the immune cell activation the TME. As a result, we have shown that alterations of the TME imposed by chemotherapy and other pre-treatments have a prognostic value and that the biopsy samples should be assessed at multiple time points. Moreover, the tumor-immune system interplay extends beyond the tumor which highlights the importance of compartmental tissue analyses.

Immunotherapy of solid tumors

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Purpose: We developed autologous dendritic-cell - based immunotherapy for prostate, ovarian and lung cancer.

Patients and methods: Between 2014-2021, we conducted 2 phase II clinical trials in patients with platinum sensitive ovarian cancer in first and second line of the treatment, one clinical trial phase II in patients with unresectable stage III-IV non-small cell lung cancer and phase III in patients with metastatic hormone-refractory prostate cancer.

Results: More than 1000 patients were enrolled in these trials. Overall safety of the treatment was very favorable with minimum side effects. There was a general tendency for the improvement of PFS across all diagnoses and statistically significant overall survival benefit in patients with lung cancer, ovarian cancer and a subgroup of patients with prostate cancer. In the detailed analysis of immune infiltrate in patients with ovarian cancer, most pronounced clinical benefit was seen in patients with low mutation burden and scarce CD8+ T-cell infiltration in the primary tumor („cold“ tumors).

Conclusions: Our findings confirmed clinical benefit of DC-based immunotherapy across several types of solid tumors with extremely favorable safety profile. The analysis of immune infiltrate in the primary tumors of ovarian cancer helped to identify subgroups of patients most benefiting from the immunotherapy highlighting the personalized approach in selecting patients responding to immunotherapy.

Granulocytes in patients with common variable immunodeficiency (CVID)

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Common variable immunodeficiency (CVID) is the most frequent symptomatic primary defect of antibody production. Abnormalities of various segments of the immune response were described in CVID. Besides a plethora of abnormalities in T- and B-lymphocyte numbers or function, also abnormalities in various cells of non-specific immunity were described. Non-specific activation of various components of the immune system is frequently observed.

The role of myeloid cells in CVID pathogenesis was a topic of our several studies in CVID patients.

To address the mechanisms of chronic immune activation in CVID, we performed an analysis of immune cell populations and serum levels of soluble (s)CD14, soluble CD25 (sCD25), and lipopolysaccharide in CVID patients and control healthy persons. In CVID subjects, the concentration of serum sCD14 was significantly increased and correlated with the level of sCD25, C-reactive protein, and the extent of T cell activation. However, no increase in serum lipopolysaccharide concentration was observed.

In a subsequent study, we showed that CVID is associated with elevated serum levels of TNF- α , IL-10, IL-12(p40), G-CSF, CCL-2/MCP-1, CXCL-10/IP-10, IL-1R antagonist, and CCL-11/eotaxin. The detected cytokine signature is consistent with an ongoing activation of cells of myeloid lineage. In contrast, the levels of cytokines typically produced by CD4+ T helper cells of Th1 (IFN- γ , IL-2), Th2 (IL-9, IL-13), and Th17 (IL-17) subtypes were suppressed in CVID patients compared to healthy donors. These data suggest that the altered cytokine profile observed in patients with CVID may be attributed to the activation of monocyte-macrophage and granulocyte lineages, possibly driven by the translocation of bacterial components, not necessarily containing lipopolysaccharide across the gastrointestinal or respiratory tracts' mucosal barriers.

Granulocyte activation markers elastase and myeloperoxidase and activation neutrophil gelatinase-associated lipocalin (NGAL) plasma levels were determined in CVID patients in a stable state with no apparent acute infection. In addition, granulocyte activation markers' plasma levels in CVID patients were determined before and one hour following intravenous immunoglobulin (IVIg) administration. Neutrophil elastase, NGAL, and myeloperoxidase plasma levels were significantly higher in CVID patients than in healthy controls. Systemic elastase levels were further increased following IVIg administration. The data presented here indicate that CVID is associated with chronic granulocytic activation which is further exacerbated by administering IVIg.

Our most recent study was focused on the suppressive effect of granulocytes in CVID patients. Neutrophils from CVID patients exhibited elevated surface levels of CD11b and PD-L1 and decreased levels of CD62L, CD16, and CD80, consistent with a phenotype of activated neutrophils with suppressive properties. CVID neutrophils actively suppressed T cell activation, proliferation, and production of IFN- γ . Furthermore, CVID was associated with an increased frequency of low-density neutrophils (LDNs)/granulocytic myeloid-derived suppressor cells (G-MDSCs). LDN/G-MDSC frequency in CVID patients correlated with reduced T cell responsiveness.

The data presented here demonstrate that neutrophils in the blood of CVID patients acquire an activated phenotype and exert potent T cell suppressive activity. Specific targeting of myeloid cell-derived suppressor activity represents a novel potential therapeutic strategy for CVID.

In general, our studies showed activation of granulocytes in CVID, however, this activation may be associated with secondary immunosuppression.

Inflammation and metabolism in autoinflammatory diseases

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Autoinflammatory diseases represent a rapidly growing group of disorders characterized by the activation of inflammatory mechanisms. In addition to the already classical diseases associated with increased IL-1 secretion and inflammasome activation, such as cryopyrinopathies, Familial Mediterranean Fever and others, other categories are now defined, such as disorders associated with dysregulation of the NF κ B pathway and disturbances in antigen processing mechanisms, interferonopathies and others. The inflammatory manifestations of many autoinflammatory diseases are associated with marked activation of the immune system, but also with changes in the metabolic state of cells. The prototype of diseases combining immune and metabolic changes is hyperIgD syndrome. This is primarily a metabolic disease caused by impaired mevalonate kinase function, which causes a block in the metabolic pathway of cholesterol synthesis. The name hyperIgD syndrome is misleading in this respect and the disease should be referred to as mevalonate kinase deficiency.

In our Department of Immunology we are following 4 patients with MVK deficiency, with different mutations in the compound heterozygous state, p.Glu93Glnfs*38 + p.R Arg277His, p.Val310Met + p.Val377Ile, p.Leu29Pro+p.Val377Ile and p.Cys152Trpfs*5 + Val377Ile. All of them present with a variable, but typical course of the disease, with flares of excessive inflammatory attacks. Their immune profile shows variable, but always increased IgD, but no other abnormalities including their rather normal B cell profile.

Their metabolic profiles according to available parameters of lipid and glucose metabolism are unremarkable. Detailed metabolic examination at the cell level is ongoing.

The clinical course of the disease is difficult to influence by therapy, with limited response to many antiinflammatory strategies. IL-1 blockade shows the greatest efficacy, with two of our patients requiring long-term regular therapy. The other two patients use IL-1 blockade only on-demand.

Here, we report the course of pregnancy in one of our patients who was covered throughout her pregnancy with the use of Anakinra preventive treatment. There are only very limited references in the literature on the safety and efficacy of IL-1 blockade in pregnancy in general, and there is as yet no publication on pregnancy in a patient with MVK deficiency treated in pregnancy with IL-1 blockade. In our patient we have used the daily administration of Anakinra, including the day of delivery by cesarean section. The delivery was also covered prophylactically with antibiotics for previous proctal abscesses in the patient. Pregnancy and delivery were uneventful. The healthy girl is heterozygous for the MVK mutation.

HIDS is a metabolic disease properly termed MVK deficiency, where the link between immune-mediated inflammation and metabolic changes is still poorly understood and awaits the results of further research, including our ongoing study.

HUNGARY

Intracellular signaling in inflammatory disease models

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Inflammatory diseases such as autoimmune joint, skin and kidney diseases and autoinflammatory diseases such as gout place a major burden on the health care systems and the affected individuals worldwide. During the last several years, we have tried to identify novel intracellular signaling components in myeloid cells that are critical for in vivo animal and ex vivo human models of inflammatory diseases. Using genetically modified mouse strains, we have shown that myeloid Src-family kinases, Syk, PLC γ 2 and CARD9 are all required for autoantibody-induced inflammatory reactions such as K/BxN serum-transfer arthritis or autoantibody-induced skin blistering disease models. The genetic deficiency of those signaling molecules also prevented accumulation of myeloid cells at the site of inflammation, likely through their involvement in the generation of the inflammatory microenvironment. Using lineage-specific deletion approaches, we have shown that Syk, PLC γ 2 and CARD9 are likely critical within the neutrophil compartment. Ongoing experiments indicate the additional role of Src-family kinases in autoantibody-induced glomerulonephritis and in an in vivo mouse model of gout. Pharmacological studies in an ex vivo human model suggest a role for the above signaling molecules in bullous pemphigoid, as well. Taken together, the Src-family–Syk–PLC γ 2 pathway plays a critical role in various autoimmune and autoinflammatory disease models, raising the possibility of the exploitation of this pathway as a potential therapeutic target.

Factor H family proteins as modulators of complement activation for better or worse

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As a major arm of innate immunity, the complement system plays important roles in the protection against infections, removal of immune complexes, apoptotic cells and debris, regulates inflammatory processes and modulates cellular activities. Since complement activation is rapid and occurs in a cascade-like manner, its proper regulation is essential to avoid unwanted damage to the host. Misdirected complement activation or defective complement regulation may contribute to or cause diseases, including infectious, (auto)inflammatory and autoimmune diseases. Factor H is the main soluble regulator of the alternative pathway and the amplification loop, acting at the level of the central component C3. In addition, in humans five factor H-related (FHR) proteins exist, whose role is less characterized. Recent evidence suggests that, in contrast to factor H, the FHRs rather promote complement activation and compete with factor H for binding to certain ligands. Variations in factor H and FHR protein levels, genetic changes and autoantibodies to factor H have been linked to a number of diseases, including particularly kidney diseases such as C3 glomerulopathy, IgA nephropathy and atypical haemolytic uremic syndrome, as well as the eye disorder age-related macular degeneration. The complex role of factor H family proteins in complement regulation and beyond will be discussed in the presentation in the context of health and disease.

Extracellular vesicle-mediated intercellular communication in cancer

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Evolutionary process of solid tumors highly relies on the small extracellular vesicle (sEV)-mediated cross-talk between malignant cells and healthy cells of the body. sEVs released by cancer cells, tumor stroma cells or leukocytes during the tumor development and immunoediting enter the circulation and may reflect the pathophysiological state of patients. Therefore, interest on investigating molecular composition of blood EVs for tumor diagnostic purposes has dramatically increased. As diagnosis of central nervous system (CNS) malignancies is especially challenging, we attempt to reveal the potential role of circulating sEVs in monitoring CNS tumors.

A total of 230 serum samples were obtained from four patient groups (malignant glioblastoma, and non-small-cell lung cancer brain metastasis, benign meningioma and lumbar disc herniation as control). After the isolation and characterization of sEVs, LC-MS was performed on six-sample-pools of whole serum and serum sEVs as well. Proteomics data were subjected to in silico analysis using the Ingenuity Pathway Analysis (IPA). Then, individual sEV samples were further investigated by ELISA and Raman spectroscopy to measure their MMP9 level and whole molecular composition, respectively. Statistical analyses were completed to compare the proteome of the two investigated sample types, the MMP9 levels and the Raman spectral signature of serum sEVs derived from the four

patient groups. From the 311 identified proteins, 10 whole serum proteins and 17 sEV proteins showed the highest intergroup differences, and sEVs resulted a more accurate classification of patients than whole sera. In silico analysis of sEVs, but not whole serum proteomics data showed a clear separation of meningioma from the malignant tumors in the tumor-related and activated immune functions. Significant differences were observed in the MMP9 levels between the patient groups with a correlation with tumor aggressiveness. We observed an impact of MMP9 level on overall survival (OS) as well; patients with low MMP9 level (<30 ppm) showed a significant OS benefit of 8 months. Using Raman spectra, the compared groups were distinguishable with AUC scores in the range of 0.82-0.9 suggesting excellent classification performance. Our results support that isolated serum sEVs have greater potential to distinguish CNS tumors, than whole sera, their MMP9 levels correlate with tumor aggressiveness and patient survival, and Raman spectroscopy of serum sEVs is a promising tool for CNS tumor diagnosis. This study was supported by GINOP-2.2.1-15-2017-00052; "National Brain Research Program NAP 2.0"; Szent-Györgyi Albert Research Fund (2021) provided by the University of Szeged and TKP2021-EGA09.

MSC-like cells increase the ability of monocyte-derived dendritic cells to polarize IL-17/IL-10-producing T cells via CTLA-4

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Based on the ongoing clinical trials, mesenchymal stem/stromal cells (MSCs), due to their multipotent differentiation and efficient immunomodulatory potential, are promising candidates for cell-based therapies of immune-mediated diseases. One of the strategies to overcome the limitation of MSCs with different origins is the use of MSC-like (MSCI) cell lines generating unlimited numbers of early passage MSCs with consistent quality and immune-modulatory features. In vitro generated MSCI cells seemed to be an appropriate model to examine the functional properties of MSCs. We investigated the effect of MSCI cells on the dendritic cells (DC) functions, notably on the monocyte differentiation process, as very plastic DCs are critical players in the communication between innate and adaptive immune cells. As it is still uncovered how MSCI-conditioned medium (MSCI-CM)- or MSCI cells-conditioned monocyte-derived dendritic cell (moDC) populations drive the polarization of helper T cells (Th), we investigated the MSCs-driven immune modulation via the alteration of monocyte-derived cell differentiation. We induced the generation of moDCs in co-culture with MSCI cells or in the presence of MSCI-CM, IL-4, and GM-CSF. MoDCs were characterized by flow cytometry while their cytokine production was measured by Human XL Cytokine Array Kit or ELISA. The polarization of T cell response was monitored by ELISPOT and flow cytometry. Here, the effects of MSCI cells on the in vitro differentiation of human monocytes into DCs were investigated. MSCI cells promote the differentiation of CD14, DC-SIGN, CD86, MHC-II, and CTLA-4 expressing monocyte-derived cells partially via all-trans retinoic acid production (ATRA) functioning as a ligand of RAR α , a key nuclear receptor in DC development. The presence of MSCI cells or MSCI-CM during the moDC differentiation resulted in the altered secretion of a wide array of inflammatory and immunosuppressive cytokines as well. These semi-matured DCs exhibit an ability to activate allogeneic, naive T cells and polarize them into IL-10⁺ IL-17⁺ double-positive T helper cells in a CTLA-4-dependent manner. Thus, these data outline a novel mechanism mediated by MSCI cells, which drives moDC differentiation to a regulatory phenotype. Additionally, mapping the molecular mechanisms of MSC-mediated indirect modulation of DC differentiation may help to expand MSCs' clinical application in cell-free therapies.

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Extramedullary hemopoiesis is decreased in the absence of Nkx2-3 transcription factor in mice

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Introduction: The red and white pulps as two main parts of the spleen perform significantly different functions in both humans and mice. While the immunological functions related to the white pulp have been thoroughly investigated, much less is known about the regulation of hematopoietic events restricted to the splenic red pulp. In this work, we investigated the role of Nkx2-3 homeodomain transcription factor as major morphogenic element for spleen in the extramedullary hematopoiesis. **Materials and methods:** BALB/c and Nkx2-3^{-/-} mice were used

throughout the experiments. Blood cell counts, bone marrow and splenic Ter119+ erythroid cells (flow cytometry), and presence of CD29+ hematopoiesis-supporting stroma (histology) was analyzed at steady state and after bleeding. Megakaryocyte differentiation was induced using Romiplostim and CD41+ or IBL-17+ splenic megakaryocytes were counted (histology). *Results:* Already at steady state we observed significantly lower red blood cell, hematocrit, and hemoglobin values in Nkx2-3^{-/-} mice and acute blood loss aggravated anaemia. While blood loss led to higher Ter119+ cell counts in both the bone marrow and the spleen of wild-type mice, we observed no induction of hematopoiesis in Nkx2-3^{-/-} spleens. This was coupled with an altered distribution of hematopoiesis-supporting stroma in these mice. Similarly, while Romiplostim treatment could significantly enhance splenic megakaryocyte colony formation in wild-type mice, the absence of Nkx2-3 completely prohibited the splenic expansion of megakaryocytes. *Conclusions:* Our findings suggest a role for Nkx2-3 in establishing the reserve hematopoietic capacity of the splenic red pulp, without influencing the bone marrow. Detailed investigation of the splenic hematopoietic niche in the absence of Nkx2-3 might can give more insight into stroma – hematopoietic stem cell interactions. Key words: Spleen, Nkx2-3, stress hematopoiesis, erythropoiesis, megakaryocyte

New aspects of HAE pathomechanism

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Background: Hereditary angioedema (HAE) with C1 inhibitor deficiency (C1-INH-HAE) is a rare autosomal dominantly inherited disease that belongs to bradykinin-mediated angioedemas. It is characterized by recurrent subcutaneous and/or submucosal swelling episodes (HAE attacks). The mutations in the SERPING1 gene result in decreased C1-INH function. C1-INH is involved in the regulation of the complement, contact-kallikrein-kinin, coagulation, and fibrinolytic plasma enzyme systems. Deficiency of C1-INH leads to the activation of these cascade systems, generation of bradykinin resulting increased vascular permeability and angioedema. Endothelial cells have a great variety of receptors for controlling permeability. During HAE attacks, increased peripheral white blood cell (WBC) count is observed without apparent inflammatory signs.

Aim: The focus of our research was to investigate the role of mannan-binding lectin-associated serine protease-1 (MASP-1), protease activated receptors (PARs) and WBC in the pathomechanism of C1-INH-HAE.

Results: We found that MASP-1 could increase endothelial paracellular permeability, could directly activate endothelial cells via PARs, could induce cytoskeletal rearrangement, could rearrange molecules involved in endothelial cell adhesion. MASP-1 was able to cleave high molecular weight kininogen resulting in bradykinin production, and it was able to modify the transcriptional pattern of inflammation-related genes and enhance adhesion between endothelial cells and neutrophils. These effects of MASP-1 could be blocked by C1 inhibitor. Investigating the WBC, we found that the elevation of WBC count is significantly greater than expected based on hemoconcentration alone, and it involves specifically the peripheral blood neutrophil granulocytes (NGs) which were shown to be activated. The activation of NGs in symptom-free periods and a further increase observed during attacks suggests that NGs may be involved in the pathomechanism of C1-INH-HAE. NGs may contribute to the dysregulation of kinin-kallikrein system, since this system can also be activated on the surface of neutrophils and modify the C1-INH molecule, thereby further attenuate its already impaired function. Postmortem macroscopic, microscopic and immunohistological assessments of the upper airway tissues of a patient with C1-INH-HAE, who died from laryngeal HAE attack, were compared with a non-HAE patient, who died from other condition without any signs of angioedema, showed that PAR1 expression was strongly reduced in the C1-INH-HAE patient, suggesting the overactivation of this hyperpermeability inducing receptor.

Conclusion: These observations strongly support the hypothesis that C1-INH-HAE has multifactorial pathomechanisms, beyond the monogenetic mutation in SERPING1 gene.

The pathogenetic role of altered microRNA expression profiles in circulating follicular T helper and B cells in systemic lupus erythematosus

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Systemic lupus erythematosus (SLE) is a prototypical chronic systemic autoimmune disease that affects various organ systems, such as the renal, cutaneous, cardiopulmonary or musculoskeletal systems. The imbalance of B cell

subsets, the pronounced activation of autoreactive B cells and the production of antinuclear autoantibodies are of crucial importance in the pathogenesis of the disease. Based on the critical role of follicular T helper (TFH) cells in B cell activation and antibody production, their failure to maintain self-tolerance potentially leads to the development of autoreactive immune processes. For the better understanding of the molecular mechanisms and pathways behind the pathological changes of B and TFH cell functions in SLE, in our study, we focused on the role of the altered expression profiles of microRNAs (miRNAs).

Circulating CD19+ B and CD4+CXCR5+ TFH (cTFH) cells were magnetically isolated from the peripheral blood of 13 SLE patients and 11 healthy individuals for the analysis of miRNA expression profiles by Illumina next-generation sequencing technique. Differentially expressed microRNAs (DEmiRs; FC>1.5, p<0.05) were identified using StrandNGS software. MirDIP database was used to find predicted miRNA-target interactions (MTIs). Experimentally validated MTIs were downloaded from miRTarBase database. miRNA-target gene network was visualized by Cytoscape 3.9.1 software. Hub miRNAs were identified using the CytoHubba plugin of Cytoscape. Functional enrichment analysis of the identified target genes was performed using ClueGO plug-in of Cytoscape.

A total of 102 DEmiRs (17 upregulated and 85 downregulated) were identified in the cTFH cells, and 94 DEmiRs (24 upregulated and 70 downregulated) were found in the B cells of SLE patients, compared to healthy controls. 19.259, and 16.218 predicted MTIs with 6.760, and 6.235 identical targets were found in cTFH cells and B cells by mirDIP database, respectively. According to miRTarBase database, we identified 1.681, and 1.108 experimentally validated MTIs with 1.031, and 783 targets in cTFH cells and B cells, respectively. Functional enrichment analysis showed that these DEGs were mainly involved in myeloid cell differentiation, complement activation, immunoglobulin mediated immune response, antimicrobial humoral immune response and B cell receptor signaling pathway. The observed changes in the gene expression in B and cTFH cells may help elucidate the molecular pathways contributing to the development SLE; furthermore, individual miRNAs may serve as potential biomarkers or therapeutic targets in the future.

Regional differences in the activity of the skin immune system

A. Kapitány, Zs. Dajnoki, O. Somogyi, L. Szabó, K. Gáspár, E. Janka, A. Szegedi:

The distinct healthy skin regions differ in their anatomy (e.g. number of sebaceous and apocrine glands), chemical milieu and microbiota composition, and can be divided into 3 main regions: sebaceous gland-rich (SGR), apocrine gland-rich (AGR) and sebaceous and apocrine gland poor (GP) areas. Since microbiota and chemical-milieu have a significant impact on skin immune function, our research group investigated, whether regional differences in skin immune function could be detected. We have demonstrated significant differences in the number and activity of immune cells (DCs, T cells) in SGR and GP skin regions, and we have shown that AGR skin also has a specific immune milieu compared to GP skin. We have highlighted that SGR and AGR regions are characterized by a non-inflammatory IL-17/IL-10 environment, associated with significantly higher T cell and DC presence (non-activated) and elevated levels of IL-17-related chemokines and antimicrobial peptides. We also detected differences in the permeability barrier of the three skin regions, with reduced expression of its main components, tight junction and desmosome components, in SGR and AGR skin regions. Our results revealed that our skin is not unified, and can explain the characteristic localization of certain immune mediated skin diseases.

Reconstruction of polyreactive B-cell lineages helps understanding autoimmunity in RAG1/2 deficiency

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Emergence, survival, growth and maintenance of autoreactive (AR) B-cell clones hallmarking humoral autoimmunity leave their footprints in B-cell receptor repertoires. Collecting immunoglobulin heavy chain (IgH) sequences related to polyreactive (PR) ones from adaptive immune receptor repertoire (AIRR) datasets make the reconstruction and analysis of AR/PR B-cell lineages possible. We developed an approach (ImmChainTracer) to extract members and visualize representative lineages of such clones. This method was successfully applied on the IgH repertoires of patients suffering from hypomorphic RAG1/2 deficiency (pRD). We were able to find relatives of AR IgH sequences and track their fate in AIRRs of pRD patients. Signs of clonal expansion, diversification and class-switching events could be revealed in PR and non-PR B-lineages of pRD patients. Extension of our approach towards different autoimmune conditions may further aid our understanding the microevolution of AR/PR lymphocytes.

The trend of change of allergic rhinitis and risk factors between 2007-2019 (Three repeated surveys)

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Objective: To assess time trends in the prevalence and risk factors of allergic rhinitis and other allergic diseases we carried out three repeated cross-sectional surveys, as well as compared the observed findings of these studies. **Methods:** 6335 ISAAC-based and additional questionnaires related to possible risk factors were distributed in 2007, 6441 in 2013 and 6869 in 2019 to the parents of 6-12-year-old children who completed them. Pupils visited the same 21 randomly selected primary schools in Budapest. The data were processed by standard descriptive statistics: frequencies (percentages) and means for categorical and quantitative data, respectively.

Results: Response rate was 62.8% in 2007, 52.9% in 2013 and 56.6% in 2019. The prevalence of physician-diagnosed allergic rhinitis (AR) was 11.6% (n=413) in 2007, 11.1% (n=380) in 2013 and 9.7% (n=373) in 2019. The prevalence of current AR symptoms (children presenting clinical symptoms of AR in the past 12 months) was 14.9% (n=530) in 2007, 23.3% (n=796) in 2013 and 29.3% (n=1043) in 2019 ($p < 0.0001$ CI 1.062-1.083). This way the number of cumulative AR (physician-diagnosed + current) increased too from 26.5% (n=943) to 34.5% (n=1176) and in 2019 to 36.2% (n=1289) ($p < 0.0001$ CI 1.029-1.046). The other physician-diagnosed allergic diseases changed the following way: eczema 10.2%, 15.4%, 12.8%, food allergy 4.8%, 6.2%, 6.3%, asthma 6.2%, 5.7%, 6.5%. Comparing the answers of all pupils suffering from AR with the healthy children, in the former group all of the three studies a family history of atopy, antibiotics given in the first year of life and visible mould in the bedroom were found to be significant risk factors of cumulative AR.

Conclusion: Comparing the three surveys the authors conclude, that in our capital the prevalence of allergic rhinitis in children seems to be increasing, the prevalence of physician-diagnosed food allergy and asthma is stagnating. The prevalence of physician-diagnosed eczema - after an impermanent increase - stagnates. Family history of atopy, antibiotics given in the first year of life and visible mould in the bedroom can be the risk factors of AR.

SLOVAKIA

Milky way of immunity - galactic functions of lactoferrin in host defence

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Lactoferrin, a member of the lactotransferrin family of iron-binding glycoproteins, is present in most human exocrine fluids, particularly mother milk. Lactoferrin exhibits a plethora of biological activities, including iron sequestration and modulation of immune cells. Recently the evidence has been gathered by us that lactoferrin directly binds and block plasminogen, a serine protease which is physiologically implicated in fibrinolysis and cell migration, but also, which may be harnessed in pathologies, e.g. tumour cell dissemination, bacteria invasion, or virus priming. The focus of the presentation will be put on the potential therapeutic role of lactoferrin in COVID-19. First, natural and synthetic peptides derived from lactoferrin block the activity transmembrane protease serine 2, a homologue of plasminogen, responsible for proteolytic priming of the spike protein of SARS-CoV-2 which causes COVID-19. Second, lactoferrin and derived peptides block the interaction of spike protein with the heparan sulfate proteoglycans upon virus-cell attachment. This makes lactoferrin a cheap and widely available candidate for supplementary therapy in management of COVID-19.

Blood biomarkers that could aid screening and predict survival in glioma patients

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Objective: The majority of central nervous system tumors are diagnosed after clinical symptoms become apparent. The markers of survival prognosis in patients with gliomas are still evaluated. In this study we attempt to analyze multiple factors associated with anti-tumor immunity to identify profiles associated with gliomas and their prognosis. **Patients and Methods:** In 63 adult patients with gliomas and 31 healthy controls the expressions of TREM-1 and TREM-2 on CD14+ blood cells (flow cytometry), the levels of soluble immune markers (Elisa tests) and the serum levels of 25(OH)D (electrochemiluminescent binding test) were analyzed. **Results:** We demonstrate that the ROC curve for three selected markers, sTREM-1, sHLA and BDNF, possesses the best discriminatory capability for glioblastoma patients (AUC=0.9534), sensitivity 79.2, specificity 94.1. These markers could be used for a potential capture of patients with some unspecific neurological symptoms for earlier indication of neuroimaging tests. Cox Proportional Hazard Analysis showed that TREM-1/TREM-2 ratio (expression on blood monocytes) was associated with reduced overall survival (OS) in high-grade gliomas (HR=1.001, P=0.023). A significant difference in OS in patients with severe VD deficiency, VD deficiency and insufficiency in grade IV of gliomas was found.

CD33 rs3865444 polymorphism: a possible genetic link between multiple sclerosis and Alzheimer's disease?

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Introduction: Microglia and monocyte-derived macrophages play an essential role in phagocytic uptake and removal of protein aggregates, cellular and myelin debris, and apoptotic cells accumulating in the brain. Their dysregulation may negatively affect remyelination and regeneration processes and thus contribute to the development of neurodegenerative disorders, such as multiple sclerosis (MS) or Alzheimer's disease (AD). Polymorphisms in genes encoding membrane receptors that modulate phagocytic activity are therefore potential genetic susceptibility candidates for various complex immune-related neurological pathologies. Interestingly, a well-established AD risk polymorphism rs3865444:C>A in the gene coding for the immunoregulatory receptor CD33 was recently reported as a possible MS susceptibility variant. In the current study, we aimed to 1) validate the association between rs3865444 and MS risk, 2) assess the effect of the strongest MS risk allele HLA-DRB1*15:01 on this association, and 3) analyze the correlation of rs3865444 with selected clinical phenotypes.

Material and methods: CD33 rs3865444 and HLA-DRB1*15:01 were genotyped in a cohort of 579 MS patients and 1,145 controls. The association of rs3865444 with MS risk and clinical phenotypes was analyzed by logistic and linear regression analysis, respectively. **Results:** Statistical analyses revealed that the rs3865444:C>A substitution reduces the risk of MS in the HLA-DRB1*15:01-positive subpopulation (codominant CA vs. CC model: P = 0.028, OR = 0.64, 95% CI = 0.44–0.95) but not in the cohort negative for HLA-DRB1*15:01 (P = 0.50, OR = 1.10, 95% CI = 0.82–1.47). Moreover, a significant antagonistic epistasis between rs3865444 A and HLA-DRB1*15:01 alleles in the context of MS risk was detected by the interaction synergy factor (SF) analysis (SF = 0.62; P = 0.032). CD33 rs3865444 was also associated with a decreased risk of developing the secondary progressive MS form, irrespective of the HLA-DRB1*15:01 carrier status. On the other hand, no correlation could be found between rs3865444 and the age of disease onset or MS severity score. **Discussion:** Our results suggest that CD33 rs3865444 contributes to susceptibility to MS but its protective effect is limited to subjects positive for HLA-DRB1*15:01 allele. The association of rs3865444 with MS risk could be possibly explained by its effect on CD33 exon 2 splicing. The protective A allele is linked to an increased production of a short CD33m isoform lacking the ligand-binding Ig V-set domain at the expense of the functional full-length CD33M. The resulting decrease in inhibitory signaling by CD33 would lead to enhanced phagocytic clearance capacity of microglia and macrophages. This research was supported by the VEGA/1/0738/20 grant.

Multisystem inflammatory syndrome in adults (MIS-A) – our experiences

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Multisystem inflammatory syndrome in adults (MIS-A), is a rare complication of COVID-19. According to the observations so far, it occurs 2-12 weeks after overcoming the SARS-CoV2 infection, while most often it is a gap of 4-6 weeks. Rarely (also from our experience), MIS-A can also arise as a result of vaccination with mRNA vaccine against SARS-CoV2 disease. However, vaccination is considered a protective factor in the development of this serious hyperinflammatory complication. MIS-A is associated with the involvement of several organ systems, while one of the most feared is cardiac manifestations. The etiology of the syndrome is unclear, likely to be a combination of several factors - the presence of persistent infection, endothelial dysfunction, dysregulation of the RAAS system and coagulopathy. The facts about the persistent infection are also recorded by the fact that some of our patients had a positive COBAS liat PCR rapid test upon admission, while the classic PCR was negative. COVID-19 thus causes a dysregulation in the immune system with the emergence of a hyperinflammatory state, which affects several organ systems, by a mechanism not yet fully understood. Since MIS-A can occur even after an asymptomatic SARS-CoV2 infection, in case of suspicion it is necessary to examine antibodies after overcoming the disease (e.g. against nucleocapsid – N protein). Proposed diagnostic criteria for MIS-A include the presence of fever $\geq 38^{\circ}\text{C}$ lasting at least 24 hours, elevation of at least two inflammatory markers (CRP, procalcitonin, IL-6, ferritin, etc.), the need for hospitalization, and multiple organ systems must be affected. In addition, 3 clinical criteria must be met, at least one of which must be primary. The primary clinical criteria include cardiac involvement (myocarditis, pericarditis, coronary artery dilatation/aneurysm, newly discovered right or left ventricular dysfunction, atrioventricular block II or III, or ventricular tachycardia) or skin exanthema (most often erythema multiforme rash) or bilateral conjunctivitis. Secondary clinical criteria include neurological impairment, shock or hypotension, gastrointestinal symptoms and thrombocytopenia. The presence of lymphadenopathy, leukocytosis, lymphopenia or anemia in the blood count is also common. In the treatment, the use of immunosuppressants is necessary, in addition to corticoids, intravenous immunoglobulins in a high dose (2g/kg divided into two days) or anticytokine treatment (anakinra). Anticoagulant treatment can't be forgotten. We present a case report of two cases of diagnosed MIS-A from a total of 5 patients treated at Department of Internal Medicine in Trnava. 4 out of 5 patients are men.

Monoclonal antibodies neutralizing circulating SARS-CoV-2 variants of concern

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Background: Variants of the SARS-CoV-2 virus emerge while it circulates within human population. They carry mutations mostly localized to the Spike protein, which drives viral entry into mammalian cells, and thus allow the virus to escape the neutralizing activities of the host's immune system. The repeated emergence of the variants raised concern about the activities of current vaccines and therapeutic antibodies, some of which have shown drastically reduced efficacy. **Methods:** We employed hybridoma technology, ELISA-based and cell-based S-ACE2 interaction assays combined with authentic virus neutralization tests to develop antibodies specifically selected for their ability to neutralize the new variants of SARS-CoV-2. **Findings:** AX290 and AX677, two monoclonal antibodies with non-overlapping epitopes, exhibit subnanomolar or nanomolar affinities to the receptor binding domain of the viral Spike protein carrying amino acid substitutions found in the circulating virus variants. The antibodies showed excellent neutralization of an authentic SARS-CoV-2 virus representing strains circulating in Europe in spring 2020 and also the variants of concern B.1.1.7 (Alpha), B.1.351 (Beta) and B.1.617.2 (Delta). In addition, AX677 has preserved its ability to bind and neutralize the current variant B.1.1.529 (Omicron) just like

the wild type Spike. The combination of the two antibodies prevented the appearance of escape mutations of the authentic SARS-CoV-2 virus. Prophylactic administration of AX290 and AX677, either individually or in combination, effectively reduced viral burden and inflammation in the lungs, and prevented disease in a mouse model of SARS-CoV-2 infection. *Acknowledgements:* The work was supported by AXON COVIDAX a.s. and by grants from the Slovak Research and Development Agency APVV 20 0478 and APVV 20 0585.

Monogenic autoinflammatory disorders in Slovakia – unexpected experience and data

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Introduction and objective: Autoinflammatory diseases (AID) represent a specific category of inborn errors of immunity. Up to now, more than 40 various diseases very described and identified and the majority of them are from the category of rare diseases. The most common monogenic AID worldwide is familial Mediterranean fever (FMF). Its prevalence in the central Europe was expected to be very low (1:465.500). The true prevalence data from CEE region and clinical experience are usually scarce. *Methods:* We analyzed the data from the National Registry of Periodic Fever Syndromes in Slovakia. We focused especially on the Slovakian cohort of FMF patients followed by the National Centre for Periodic Fever Syndromes in University Hospital in Martin, Slovakia. The patients were collected from the whole are of Slovakia through the collaboration with the rheumatologists and clinical immunologists. Current population of Slovakia is 5 449 270 inhabitants (2021).

Results: Altogether, we identified 115 patients (estimated prevalence of 1:47 385; 25.2% children; 46.1% males; aged 36.42±18.91 years) with the clear clinical FMF phenotype and genetic confirmation. 5.2% were homozygous for pathogenic mutation in MEFV gene, 14.8% compound heterozygotes, 67.8% heterozygotes and 12.2% with the genetic finding of unknown significance. The mean age of first symptoms was 14.86±14.9 years, age of diagnosis confirmation 32.65±19.22 years. With the diagnostic delay of 17.41±15.63 years. The most prevalent clinical symptoms were abdominal pain (88.7%), recurrent fever (86.9%), accelerated fatigue (72.2%), arthralgia (70.4%), chest pain (45.2%), cervical lymphadenopathy (36.5%) and tonsillitis (33.0%). 86.1% were treated with colchicine, 17.4% with anakinra on demand and 15.7% with canakinumab. Elevation of serum amyloid A outside the flares was detected in 41.7% patients before the therapy initiation and elevated IgD in 5 patients.

From the other AID we identified 10 patients with cryopyrinopathies; 2 patients with familial cold autoinflammatory syndrome type 2 and 3; 6 patients with TRAPS; 4 patients with mevalonate kinase deficiency (hyper IgD syndrome); 2 patients with DADA2, 2 patients with Yao syndrome; 2 with Blau syndrome; and 1 patient with Majee syndrome. *Conclusions:* Our experience strongly encourages the need for FMF awareness even in the countries with the expected low prevalence. Early diagnosis could lead to appropriate therapy with improved prognosis and quality of life. The prevalence of the other rare AID was as expected.

Keywords: autoinflammation, familial Mediterranean fever, periodic fever syndromes, disease awareness, biological therapy, monogenic autoinflammatory disorders

Immunological predictive markers for COVID-19 outcome

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Over the past two years, the SARS-CoV-2 virus has spread around the world, causing the disease called COVID-19 of varying severity and associated with several complications. Clinical deterioration is related to an exaggerated

immune response and cytokine storm. Since the beginning of the pandemic, there have been many successful efforts to identify clinical or laboratory prognostic markers, including immunological ones.

We analyzed immune profile in 823 hospitalized COVID-19 patients on admission and after one week of hospitalization to identify predictive markers of fatal outcome. The investigated immune profile included the differential blood cell counts, serum concentration of immunoglobulins and basic complement compounds C4 and C3, flow cytometric lymphocyte subsets phenotyping and the measurement of selected activation (CD38, HLA-DR) and inhibition (PD1, Tim-3, NKG2A) markers. The best predictive values were obtained for the total number of lymphocytes, neutrophils, CD4+, CD8+ and CD19+ cells after one week of hospitalization, however, various combinations of immune parameters have shown promising results in identifying fatal outcome even on admission. Severity of COVID-19 correlated with neutrophilia, thrombocytopenia, depletion of leukocyte (except for neutrophils) and lymphocyte subsets. In contrary, survival was associated with higher concentration of C3 and IgM, lower proportion of CD8+CD38+ cells, higher proportion of CD8+NKG2A+ and NK NKG2A+ cells on admission and with the significant increase in the expression on HLA-DR on both CD3+ and CD8+ cells over the first week.

The examination of the immune profile of hospitalized COVID-19 patients may be helpful in identifying patients at risk of the unfavourable outcome who potentially require more intensive care and/or therapy. On the other hand, detection of the changes in the immune profile is also essential to understand the pathophysiology of this still relatively new disease.

Hrubisko M., Zuzulova M., Mrazova V. (Bratislava): Benefits of immunity profile measuring in oncology patients with radiotherapy
(recorded presentation)

Polymorphisms in vitamin D binding protein gene and allergic diseases

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Introduction: Vitamin D deficiency has been recognized as an important cofactor for many immunopathological conditions, including allergic diseases. However, there is still debate as to whether the deficiency may be directly related to the onset and/or progression of the disease, or whether it is just an epiphenomenon. At least two polymorphisms (rs4588, rs7041) of Gc gene directly affect the structure of vitamin D binding protein (DBP) and concentration of vitamin D. Specifically, the minor allele (T) of rs7041 determines the Gc2 and Gc1F isoform and the major (G) Gc1S isoform of DBP. In the case of rs4588, the minor allele (A) determines the Gc2 isoform and the major one (C) forms Gc1S and Gc1F. **Objectives:** We determined 4 polymorphisms in the Gc gene and investigated their relationship to the presence or severity of allergic diseases. **Patients and Methods:** The cross-sectional observational study of 403 outpatient, 47% of whom were under 15 years of age. The patients were examined by an allergist for the occurrence of atopy (155 patients, 39,5 %) and bronchial asthma (41 patients, 10,2 %).

Results: Representation of the diplotypes Gc in our study group was as follows: 1S-1S: 33,50 %, 1S-2: 36,72 %, 2-2: 7,94 %, 1F-2: 7,44 %, 1F-1F: 1,74 % and 1S-1F: 12,66 %. The minor homozygotes (T/T) for rs7041 had lower concentrations of 25(OH)D compared to homozygotes G/G ($22,15 \pm 10,64$ ng/ml vs. $28,45 \pm 13,17$ ng/ml, $p < 0,01$). In the atopic group, the prevalence of T/T homozygotes was 23.9% compared to 12.7% in the non-atopic group (OR = 2.18 (95% CI 1.25-3.81), $p = 0.006$). The simultaneous presence of minor alleles of both polymorphisms (T for rs7041 and A for rs4588) increased the probability of the need for nasal corticoids in atopic subjects more than 2-fold (OR = 2,21, 95% CI 1,12 – 4,39; $p = 0,024$). Although major homozygotes for rs4588 (C/C) had higher concentrations of 25(OH)D than A/A homozygotes ($28,84 \pm 12,52$ ng/ml vs. $21,25 \pm 11,15$ ng/ml $p < 0,01$) the probability of bronchial asthma was higher in C/C group (OR = 2,12 (95% CI 1,06 – 4,16; $p = 0,029$)). However, this association only became significant after adjusting for vitamin D concentration, where lower levels indicate higher risk. **Conclusions:** Minor alleles rs4588 and rs7041 are associated with lower concentrations of vitamin D. In allergic rhinitis, these alleles increase the likelihood of the need for intranasal corticoids. Low vitamin D concentrations increase the likelihood of inhaled corticosteroid usage in C/C homozygotes for rs4588, who usually belong to the group with higher vitamin D concentrations. **Key words:** vitamin D, atopy, rhinitis, asthma, corticosteroids, Gc, rs4588, rs7041

Hulinkova I., Soltysova A., Medova V., Dobsinska V., Dolnikova D., Ciznar P. (Bratislava): Analysis of T-cell receptor excision circles in newborn from tertial clinical centre

SLOVENIA

Deconstructing inflammasomes

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Multiprotein complexes inflammasomes are central components of the early inflammatory response to invading pathogens. Upon sensing infection or disrupted tissue homeostasis, inflammasome sensors through oligomerization lead to the assembly of inflammasomes and activation of inflammatory caspases. Caspase-1 afterward proteolytically activates proinflammatory cytokines IL-1 β and IL-18. Gasdermin D is another substrate of inflammatory caspases. In a dormant state, gasdermin D molecules are present in autoinhibited form, while cleavage and recognition of negatively charged phospholipids enable the N-terminal domain of Gasdermin D to form pores in the membranes and facilitate pyroptotic cell death. Despite enormous progress that has been achieved in the past 20 years since the discovery of inflammasomes, there are still major gaps in our understanding of how inflammasomes are activated and the regulatory processes involved.

Our work mainly focuses on the NLRP3 inflammasome that is connected to versatile sterile inflammatory conditions such as metabolic and neurodegenerative diseases, and has recently been shown to drive inflammation in COVID-19. We investigate the regulation of the initial steps of NLRP3 inflammasome assembly and downstream responses that are common to all inflammasomes. We designed peptides that inhibited NLRP3 inflammasome activation at various stages with different levels of specificity. We defined the shortest NLRP3 molecule that is able to sense inflammasome triggers and induce inflammasome assembly. While the current emphasis of NLRP3 inflammasome research is focused more on its detrimental involvement as a major driver of inflammation, the canonical role of inflammasomes is nevertheless to protect the organism against threats. Cancer is an example of a disease where the immune system fails to detect the threat and several types of tumors have been shown to downregulate inflammasome components. Using the synthetic biology approach combined with the mechanistic insight into inflammasome assembly, we are currently deconstructing inflammasomes to provide efficient priming of immune responses in cancer immunotherapy.

Oxidative stress derived extracellular vesicles promote inflammation in chronic diseases

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Chronic diseases (e.g. atherosclerosis, rheumatoid arthritis) have inflammatory characteristics involving oxidative stress, where lipids with unsaturated fatty acids chains are particularly prone to oxidation with reactive oxygen species and might be released from cells with extracellular vesicles (EVs). These oxidized species represent endogenous damage associated endogenous ligands (DAMPs) and can be recognized by innate immune receptors such as Toll-like receptors and promote the so called sterile inflammation. We showed that EVs isolated from patients with RA or released under oxidative stress in cell cultures activated Toll-like receptor 4 (TLR4/MD-2) receptor complex. The activity of EVs was dependent on oxidation. 15-lipoxygenase oxidized lysophospholipids (lysoPLs) were characterized as TLR4 ligands. Their formation was dependent on 15-LO and secreted phospholipase (sPLA2) activity, which we detected in the synovial fluid from patients. Furthermore, sPLA2-IIA promoted K/BxN serum induced arthritis in mice in TLR4-dependent manner.

EVs released under oxidative stress activate TLR4 with a gene profile different from bacterial lipopolysaccharide (LPS), which is its PAMP (pathogen associated molecular pattern). Several proinflammatory cytokines (e.g. IL-23, Ccl24) as well as antiinflammatory proteins (e.g. Tnfaip6) are expressed after EV stimulation of TLR4, underlying the differences between pathogen-induced and sterile inflammation. Dendritic cells, which contribute to initiation and sustainment of several chronic diseases, respond better to EVs than macrophages.

The transient activation of TLRs has been implicated in cardioprotection. Oxidized PLs are formed in cardiomyocytes after ischemia/reperfusion (I/R) and can induce cytoprotective effects through Nrf2-mediated redox-regulatory genes such as heme oxygenase-1 (HO-1) in cells when added in low concentrations thus protecting the cells from further oxidative stress. This is called adaptive response. We showed that EVs released under oxidative stress stimulated expression of HO-1 in heart-derived proliferating cell line H9c2 but not in macrophages. Pretreatment of H9c2 cells with EVs before they had been subjected to simulated I/R decreased the cell death in HO-1-dependent manner.

Immune response in acute and post-acute multisystem inflammatory syndrome (MIS-C)

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Although children appear to be less susceptible to COVID-19 with usually mild symptoms, some of them develop a rare but serious condition called multisystem inflammatory syndrome (MIS-C). In our study, immunological characteristics of patients in acute and post-acute phases of MIS-C were analysed and compared to healthy controls to better understand the dynamics of MIS-C recovery after treatment. Peripheral blood was analysed for lymphocytes populations, T and B cell differentiation, as well as the phenotype of antigen presenting cells (APC). The results of 14 children with MIS-C in acute and post-acute phase were compared between each other and with healthy controls. Similarly to other studies we observed decreased concentrations of major lymphocyte populations (B cells, CD4 + and CD8 + T cells and NK cells) and recent thymus immigrants (RTE) in the acute phase of MIS-C indicating decreased T lymphocyte production. The percentages of activated T cells were significantly increased in acute-phase, followed by an increased proportion of double negative T cells (DNTs). Impaired differentiation of B cells was also observed as a reduced proportions of B cells expressing CD21, activated/memory B cells and class-switched memory B cells, but the proportion mostly returns to normal range in the post-acute phase. Antigen-presenting cells (APC) and their shaping of the immune response play an important role in MIS-C pathology. In the acute phase we observed significantly reduced levels of plasmacytoid dendritic cells and classic monocytes, as well as an increased proportion of conventional dendritic cells type 1, non-classical monocytes and intermediate monocytes compared to post-acute phase and to the group of healthy children. In parallel, mitochondrial respiration and glycolysis of peripheral blood mononuclear cells (PBMCs) obtained from MIS-C patients in the post-acute phase were measured by Seahorse Extracellular Flux Analyzer. Results were compared to the group of healthy children. The MIS-C patients in post-acute phase had similar mitochondrial respiration and glycolysis rates as healthy controls. While both, immunophenotyping and immunometabolic analyses showed that immune cells in the post-acute MIS-C phase returned to normal in many parameters, there were some significant alterations in terms of lower expression of T cell co-receptors (CD3, CD4 and CD8), a high percentage of $\alpha\beta$ DNT and $\gamma\delta$ DNT in post-acute phase, a high proportion of plasmablasts and more metabolically active CD3/CD28 stimulated T cells. Importantly, we have still observed in post-acute phase low proportion of plasmacytoid dendritic cells which are important in defence against viral infections. This suggests that some changes in the immune system persist in the post-acute phase of MIS-C two to three months after acute MIS-C.

Therapeutic potential of antigen receptor-engineered regulatory T cells for immunotherapy of autoimmune diseases

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Autoimmune diseases affect people of all ages and often significantly impair their quality of life. Patients often require intensive, lifelong medical interventions that result in only partial remissions. In the search for safe and effective therapies, there is great motivation to develop cell-based immunotherapies. Ideally, such treatments would reduce dependence on immunosuppressive drugs, restore immune response, and potentially stimulate tissue repair. Modulation of the immune system by immunotherapies using autologous, genetically engineered immune cells is at the forefront of several paradigm-changing therapeutic approaches for the treatment of serious diseases such as cancer and autoimmune disorders, offering a solution to conditions for which there are limited or no therapeutic options. Due to their multiple suppressive mechanisms affecting a broad spectrum of immune cells, CD4⁺FOXP3⁺ regulatory T cells (Treg) are currently being explored to develop cell therapies for autoimmune diseases and solid organ transplant rejection. Clinical studies have shown that polyclonal Treg cells can control autoimmune responses after adoptive transfer. In preclinical models, antigen-specific Treg cells were superior to polyclonal ones, which has driven the development of antigen receptor-engineered Treg cells. To understand the molecular mechanisms involved in the protective role of genetically engineered Treg cells and to evaluate the strengths and limitations of this approach, we used a preclinical mouse model of central nervous system (CNS) autoimmune disease - experimental autoimmune encephalomyelitis (EAE) induced by immunization with a peptide derived from myelin oligodendrocyte glycoprotein (MOG). Treg cells engineered to express TCRs that recognize the MOG35-55 peptide, protected mice from developing EAE. After disease onset, they initially accumulated in draining lymph nodes and upregulated LAG-3, CTLA-4, and PD-1. The genetically engineered Treg cells also significantly reduced the acute autoreactive CD4⁺ T cell response in the treated mice, as evidenced by the decreased number of MOG-reactive proinflammatory CD4⁺ T cells (identified by the expression of CD40L, GM-CSF, IL-17A, IFN- γ) in the blood at day 9 after immunization. They also persisted in target organs for more than 30 days after immunization and showed an effector/memory CD44^{high}CD62L^{low} phenotype compared with endogenous Treg cells. To define the molecular characteristics of autoreactive Treg cells in the CNS of diseased mice, we performed single RNAseq and bulk transcriptome analyzes of autoreactive Tregs and conventional T cells (Tconv) at the peak of the EAE. Among other genes, Il10, Lag3, Ctla4, and Areg were highly expressed in Treg cells in the CNS compared with their Tconv counterparts. Using genetic approaches we generated Treg cells in which genes were knocked out (Lag3) or silenced (Il10, Ctla4, Areg). We demonstrated that Il10, Ctla4, and to a lesser extent Lag3 were nonredundantly required for protection against EAE, whereas Areg had no effect. Engineered Treg cells thus protect against autoimmunity in the CNS via multiple immune mechanisms. This highlights their immense potential for intercepting autoimmune responses and developing cell-based therapies.

Antitumor immunity boosted by GSDMD-induced necrosis

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Pyroptosis is a programmed form of immunogenic cell death mediated by gasdermin D (GSDMD) pore formation downstream of inflammasome activation. Induction of immunogenic cell death has proved promising in the context of cancer therapy. In the present work, we investigate the effect of GSDMD-induced pyroptosis of cancer cells on boosting anti-tumor immunity of solid tumors. We have designed various GSDMD variants with different pore-forming activities and divergent modes of activation, where GSDMD variants are either transcriptionally regulated or GSDMD cleavage is chemically regulated. GSDMD variant pore-forming activity was tested in vitro and in vivo in a B16 mouse melanoma model, where tumors were injected with GSDMD variant-encoding plasmids and subsequently electroporated. Pyroptosis-induced inflammation via tightly regulated GSDMD pore formation resulted in cytotoxic CD8⁺ T-cell tumor infiltration, tumor regression, increased survival rate and remission. GSDMD treatment in combination with IL-1 β or IL-18, which have known function in T-cell mediated antitumor immunity, further increased survival rate of treated mice. In conclusion, stringently controlled formation of GSDMD pores leads to cancer cell death through release of immunostimulatory components, promotes infiltration of immune cells into the tumor microenvironment and stimulates a potent antitumor response.

Immunological and inflammatory profiles in hospitalized patients with COVID-19

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It was recently suggested that the pathobiology of Covid-19 pneumonia is distinct from pneumonia caused by other respiratory viral pathogens demonstrating that SARS-CoV-2 causes a slowly unfolding, spatially limited alveolitis in which alveolar macrophages harbouring SARS-CoV-2 and T cells form a positive feedback loop that drives persistent alveolar inflammation and promotes the recruitment of T cells to the alveolar space

(doi:10.1038/s41586-020-03148-w). This enrichment of T cells in the alveolar space may correlate with lymphocytopenia (doi:10.1038/s41586-020-2355-0) and it was demonstrated that moderate or severe Covid-19 is accompanied by an overall reduction in blood T cell number (doi: 10.1038/s41586-020-2588-y). However, the longitudinal immunological T cell correlates of disease outcome remain unclear. Here we serially analyzed blood T, B, and natural killer (NK) cell counts in hospitalized Covid-19 patients and examined correlations between lymphocyte immune profiling, 28-day mortality, and corticosteroid treatment (doi:10.1056/NEJMoa2021436) at a quaternary hospital providing care to patients with Covid-19. Data were obtained regarding consecutive patients hospitalized with Covid-19, excluding those who were died, intubated, or discharged within 48 hours after the presentation (study baseline). The primary outcome was 28-day mortality. Of the 268 consecutive patients, 14 patients were excluded from the analysis. Of the remaining 254 patients, 45 (18%) died within 28 days after the presentation, with a median follow-up of 9 days. Longitudinal immune profiling revealed that the patients who progressed to the primary endpoint displayed a marked reduction of CD4+ T cells, with an overall difference of 247 CD4+ cells per cubic millimetre (95% CI, 184 to 293; $P < 0.0001$) in comparison to those discharged from the hospital alive. Mortality at 28 days was significantly higher in patients presenting with less than 200 and 200 to 500 CD4+ cells per cubic millimetre, with deaths reported in 38% (21 of 55) and 17% patients (22 of 130), respectively than in patients with more than 500 CD4+ cells per cubic millimetre with deaths reported in 3% (2 of 69) of patients (age and level of respiratory support at the time of presentation adjusted hazard ratio, 0.996, 95% confidence interval [CI], 0.994 to 0.999; $P < 0.01$). There was no significant association between CD8+ T cell, B cell, or NK cell counts and the primary endpoint. In the secondary analysis, we compared CD4+ counts in patients who received corticosteroids (doi: 10.1038/s41586-020-2588-y) with those who did not, weighting according to the propensity-score matching. The use of corticosteroids resulted in a significant increase in CD4+ counts ($R = 0.5$; $P < 0.0001$). This raises the possibility that immunological intervention may target inflammatory markers predictive of worse disease and help identify individuals with whom these therapies may benefit most. Herein, we have demonstrated that loss of peripheral-blood CD4+ cells is independently and proportionally associated with progression to respiratory failure and death, suggesting that CD4+ cells may contribute to divergent Covid-19 disease trajectory. Further, host immune response is thought to play a key role in the pathophysiology of Covid-19 organ failure. These findings raise new questions about how inflammatory organ injury occurs in severe Covid-19.

Gene therapy with interleukin-12; from bench to phase one clinical trial

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Immune therapies are currently the subject of intensive research as they often provide excellent responses in a variety of tumours. A possible approach for immunotherapy is targeted intratumoural delivery of interleukin-12 (IL-12), a cytokine with known antitumour efficacy. Due to its immunomodulatory action, it can be used to stimulate an immune response that may enhance the in situ vaccination effect of local ablative therapies. To comply with EU regulatory requirements for clinical application, which recommend the use of antibiotic-resistance-gene-free plasmids, we constructed and developed a production process of clinical grade quality for antibiotic-resistance-gene-free plasmids encoding human IL-12 (pIL12) and its orthologue encoding murine IL-12 (pmIL12). To demonstrate the suitability of the pIL12 or pmIL12 plasmid for the first-in-human clinical trial, the biological activity of the expressed transgene, its level of expression and plasmid copy number were determined in vitro in the human squamous cell carcinoma cell line FaDu and murine colon carcinoma cell line CT26, and antitumor effectiveness, toxicity and immunogenicity were determined in vivo in BalbC male and female mice bearing CT 26 tumors. The positive results of nonclinical evaluation formed the basis for the application of the first human clinical trial for pIL12 gene therapy (ISRCTN15479959, ClinicalTrials NCT05077033). Electroporation was used as a delivery system for pIL12 employing a clinically approved pulse protocol that is used in electrochemotherapy. The study was designed to assess the safety and tolerability of pIL12 gene therapy in the treatment of basal cell carcinoma in patients with operable tumors in the head and neck region. The study is a phase I study with dose escalation of the pIL12 plasmid to determine the safety and tolerability of the treatment and to determine the dose of the pIL12 plasmid that is safe and elicits its biological activity. Currently, the first cohort of patients receiving the lowest dose of pIL12 is included in the study. No local or systemic side effects were observed. Thus, the next cohort of patients will be included. The results of this first-in-human clinical trial will provide a basis for the use of pIL12 gene therapy as an adjuvant treatment to local ablative therapies with the aim of enhancing their local response and eliciting a systemic response.

IL-6, suPAR AND MR-proADM AS A PROGNOSTIC FACTOR OF OUTCOME IN COVID-19 PATIENTS

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Background: Less specific markers like suPAR and MR-proADM, related to systemic response and with other diagnostic tools, proved useful in stratification and escalating appropriate treatment as early as possible. Since the beginning of SARS-CoV-2 pandemics, it has been a worldwide struggle for clinicians to stratify the phase of illness, possible deterioration and mortality risk in patients with SARS-CoV-2 infection admitted to the hospital, all for the purpose of initiating the proper treatment as soon as possible. suPAR is a soluble receptor derived from enzymatic cleavage of the membrane uPAR receptor at the surface of blood mononuclear and endothelial cells in response to a vast array of inflammatory and immunomodulatory stimuli. ADM is a key hormone secreted by endothelial and vascular smooth muscle cells, playing an endothelial barrier function-stabilising agent. IL6 is an inflammatory interleukin mainly produced by macrophages and T lymphocytes in response to pathogens and is key to controlling several viral infections. Our study aimed to determine if any given biomarkers elevate earlier than other well-known biomarkers or predict deterioration sooner than others, which will help clinicians act as quickly as possible. **Methods and results:** From October 2021 until January 2022, we examined nine subjects with SARS-CoV-2 infection who were hospitalised in our hospital. IL-6 levels were assessed by Immulite 2000 assay, while suPAR and MR-proADM levels with different ELISA kits. Our samples were serum or EDTA plasma. Based on their status, the patients were assigned to either survival or non-survival group. For biomarkers MR-proADM and suPAR we also added health controls in our study. Average suPAR values in non-survivors are higher than in survivors. IL-6 increases much more slowly in survivors than in non-survivors (Figure 1). Many patients have high values of biomarkers already before admission to the hospital. From previous studies, we know that there are many 2 parameters and co-morbidities, such as cardiovascular diseases, diabetes, obesity, respiratory diseases, hypertension, smoking and age, which affect higher initial or baseline values. Therefore, not all elevated baseline values are necessarily related to SARS-CoV-2 infection. Due to these factors, it is important to monitor the dynamics of the parameters (follow-up) and act upon their significant or exponential increase. We also compared measurements of all biomarkers on days 1, 3, 5 and 7 after admission between survivors and non-survivors (Figure 2). Results showed no significant difference between survivors and non-survivors in IL-6, suPAR and MR-proADM variables (P-value > 0,05). None of the examined biomarkers elevates earlier or predicts deterioration sooner than others. Also, patients with lower MR-proADM values need intensive treatment. **Conclusions:** We found out that none of the investigated inflammatory biomarkers elevates earlier than others. New biomarker MR-pro ADM did not predict deterioration sooner than suPAR and IL-6. Since we only have treatment available for the first two biomarkers, only these two are measured in further diagnostics and follow-up in routine practice. We established the importance of monitoring the parameters' dynamics from admission and starting appropriate drug therapy in concordance with the patient's current clinical status.

Maša Bošnjak - Immunogenicity of plasmid DNA electrotransfer in different skin cell types

Novel combination of experimental and bioinformatic approaches for epitope identification in allergic diseases

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Background: IgE-mediated allergic diseases are a consequence of allergen binding and consequently cross-linking IgE antibodies attached to high affinity IgE receptors (FcεRI) on the surface of mast cells and basophils. For this to occur, IgE antibodies need to bind specific regions on allergen, called epitopes. Such binding sites are called conformational or B-cell epitopes. Preventing the antibodies from binding to these allergen regions could hence prevent the onset of an allergic reaction. So far, proper diagnosis and treatment of allergic diseases remains challenging, and mechanisms of IgE binding are still not completely understood. With identification of epitopes we could get an in-depth insight into molecular mechanisms involved in allergic diseases. This knowledge could among others help us better understand the common case of allergen cross-reactivity. Another important aspect is the therapeutic potential of epitopes in immunotherapy treatment. Since immunotherapy is currently the only effective treatment for insect venom allergies, epitope-based peptides could present a novel approach for allergy treatment. This study focuses on insect venom allergies, specifically on identifying epitopes of major wasp venom allergen Ves v 5. **Materials and methods:** Phage display libraries, containing phages that express cyclic or linear, 7

or 12 amino acid long peptides (10^9 different variants) on their coat protein, underwent three rounds of selection (binding of peptides to α -Ves v 5 antibody, washing unbound phages, elution of bound phages, amplification of eluted phages), to obtain peptides with strongest binding affinity for the antibody. DNA isolated from those phages was subjected to next generation sequencing (NGS). Bioinformatic analysis of sequenced data included computational tools such as: BBDuk for high-quality sequence retrieval, PuLSE to obtain fragments coding for antibody binding sequences, MEME for motif identification, SAROTUP for non-target binding sequence elimination and Pepitope for epitope localization on the allergen. *Results:* We obtained sequences of most abundant peptides recovered after three panning rounds. The non-target binders were identified and removed from the set of sequences, resulting in only potential true binders. We detected between 8 and 15% non-target binders in linear and less than 1% in cyclic library. We identified three distinct motifs, indicating the main regions of potential antibody binding. Finally, we localised most prominent regions of antibody binding, on the allergen, based on the peptides and motifs obtained after the analysis. *Conclusion:* With phage display followed by NGS, we identified peptides, carrying motifs, which interact with α -Ves v 5 antibodies and determined their location on the allergen.

LOUNGE (B) PARALLEL SESSION PRESENTATIONS

SELECTED ABSTRACTS FOR ORAL PRESENTATION

Ritonavir derivate bound to HPMA copolymer carrier has potent antitumor activity which synergizes with IL-2 based immunotherapy

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This study describe a polymer conjugate composed of the antiretroviral drug ritonavir derivative (5-methyl-4-oxohexanoic acid ritonavir ester; RD), covalently linked to HPMA copolymer carrier via a pH-sensitive hydrazone bond (P-RD). RD possesses considerable cytostatic activity in six mice (IC₅₀ ~ 2.3 - 17.4 μ M) and six human (IC₅₀ ~ 4.3 - 8.7 μ M) cancer cell lines, and it inhibits the migration and invasiveness of cancer cells in vitro. RD inhibits STAT3 phosphorylation in CT26 cells in vitro and in vivo, and expression of the NF- κ B p65 subunit, Bcl-2 and Mcl-1 in vitro. RD also dampens chymotrypsin-like and trypsin-like activity of proteasome and induces ER stress as documented by induction of PERK phosphorylation and expression of ATF4 and CHOP. P-RD conjugate has significant antitumor activity in CT26 and B16F10 tumor-bearing mice with negligible toxicity. This antitumor activity moreover synergizes with immunotherapy based on the use of IL-2/anti-IL-2 mAb S4B6 complexes with strong stimulatory activity for memory and activated CD8⁺ T cells as well as for NK cells. P-RD possesses very promising therapeutic activity also in human FaDu xenografts. Further, we show that RD is more potent inhibitor of P-glycoprotein in comparison to ritonavir and HPMA copolymer conjugate bearing both RD and doxorubicin is effective in the treatment of P-gp expressing chemoresistant tumors.

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Clinical significance of tumor-infiltrating conventional and plasmacytoid dendritic cells in pancreatic ductal adenocarcinoma

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Dendritic cells (DCs) play a key role in the orchestration of antitumor immunity. Activated DCs efficiently enhance antitumor effects mediated by natural killer cells and T lymphocytes. Conversely, tolerogenic DCs essentially contribute to an immunosuppressive tumor microenvironment. Thus, DCs can profoundly influence tumor progression and clinical outcome of tumor patients. To gain novel insights into the role of human DCs in pancreatic ductal adenocarcinoma (PDAC), we explored the frequency, spatial organization, and clinical significance of conventional DCs type 1 (cDC1s) and type 2 (cDC2s) and plasmacytoid DCs (pDCs) in primary PDAC tissues. A higher density of whole tumor area (WTA)- and tumor stroma (TS)-infiltrating cDC1s was significantly associated with better disease-free survival (DFS). In addition, an increased frequency of intraepithelial tumor-infiltrating cDC2s was linked to better DFS and overall survival (OS). Furthermore, an increased density of WTA- and TS-infiltrating pDCs tended to improve DFS. Moreover, a higher frequency of WTA- and TS-infiltrating cDC1s and pDCs emerged as an independent prognostic factor for better DFS and OS. These findings indicate that tumor-infiltrating DCs can significantly influence the clinical outcome of PDAC patients and may contribute to the design of novel treatment options that target PDAC-infiltrating DCs.

Gut microbiota manipulation with oral antibiotics enhance the therapeutic response of MC-38 tumours to anti-PD1

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Background and aim: Gut dysbiosis and host immune system play important roles in tumour progression and response to cancer immunotherapy. Here, we investigated how gut microbiota manipulation with oral antibiotics influence the outcome of cancer treatment with anti-PD1 (aPD1) and which gut factors are important for systemic carcinogenesis using immunological techniques. **Methods:** Conventionally-bred C56BL/6 mice were treated with mixed or single oral antibiotics ad libitum (250mg/ml of vancomycin, 7.5mg/100ml of colistin, 5g/100ml of streptomycin) and 300 µl of metronidazole by 3 times weekly oral gavage. MC-38 adenocarcinoma was inoculated subcutaneously on the 18th day of antibiotics administration, and on days 7, 10, and 13 post-MC-38 inoculations, aPD1 treatment was given intraperitoneally. The growth of the tumours was followed for 18 days and then the intratumoral immune cell infiltrate and immune reactions in the mesenteric lymph node and spleen were analysed by flow cytometry and ELISA. Cytokine production in the Peyer's patches (Pp) and colon were also measured by ELISA. Next, similar experiment was repeated using germ-free (GF) mice colonized by gut microbiota from either control or colistin-treated mice. **Results:** While either antibiotic mixture or colistin alone improved the effectiveness of aPD1 treatment, slightly different immune changes were observed for these different treatments. Antibiotic mixture decreased production of IL-33, IL-6 in the Pp and increased Th1, CD4/IFNγ+, CD8/IFNγ+ cells within the tumour microenvironment (TME), colistin mainly increased S100A8 production in the Pp and Th17, CD4/IFNγ+ cells in the TME. Mice colonized with colistin-changed microbiota had limited effect with aPD1 treatment of MC-38 tumours, but showed some immune reactions similar to that observed in colistin-treated mice. **Conclusions:** Treatment of MC-38 tumours with mixed oral antibiotics enhances the efficacy of aPD1 through Th1, IFNγ, and IL-6 immune mediations, while colistin use improves aPD1 treatment by Th17, IFNγ and S100A8 immune responses. In both treatments, the immune reactions within the Pp suggest that immune synergy between the antibiotics and the changed microbiota is essential for effective treatment.

Bristol-Myers-Squibb presentation: Marek Šťastný - Why immunotherapy represents (r)EVOLUTION in cancer therapy?

The impact of early postnatal administration of probiotic strain *Escherichia coli* O83:K24:H31 on maturation of immune system

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The early postnatal period is crucial for the maturation of the newborn's immune system and the establishment of balanced mutual interactions between the developing microbiota and the host's immune system. Colonization of the neonatal gastrointestinal tract by pioneering microorganisms provides the basis for the formation of a microbiota important for the activation of the immune system as well as for the induction of appropriate tolerance to relatively harmless environmental antigens and compound of the healthy microbiome. Early postnatal administration of appropriate probiotics could support this process. Previously, it has been reported that early postnatal administration of *Escherichia coli* O83:K24:H31 reduces the incidence of allergies and supports immunoregulatory functions, therefore we have focused on clarification of the possible modes of action of the beneficial effect of EcO83 using an experimental mouse model. In the current study, the immunomodulatory capacity of EcO83 was characterized both in vitro and in vivo. Firstly, the immunomodulatory abilities of EcO83 with and without hemolytic activity was compared in vitro on both human peripheral blood cells and dendritic cells derived from mouse bone marrow precursors. Activating markers on dendritic cells and cytokines were determined using flow cytometry and quantitative real-time PCR, respectively. The probiotic EcO83 was administered within 24 hours after birth and the persistence of EcO83 in the neonatal gastrointestinal tract was monitored using bioimaging. Both strains (with and without hemolytic activity) showed a comparable capacity to induce dendritic cell maturation while maintaining the ability to express key immunoregulatory cytokine interleukin 10 (IL-10) in dendritic cells and T cells cocultured with EcO83-primed dendritic cells. Early postnatal administration of EcO83 resulted in massive but transient colonization of the neonatal gastrointestinal tract as detected by in vivo bioimaging. Compared to adults, neonatal mice have significantly higher and prolonged presence of EcO83 in stomach. Early postnatal administration of EcO83 promoted intestinal barrier function by increasing tight junction protein expression. Expression of key immunoregulatory cytokine IL-10 has been increased in neonatal intestine of mice treated with EcO83 compared to non-supplemented mice.

Early postnatal administration of EcO83 supports the maturation of the neonatal immune system, including promotion of immunoregulatory and gut barrier functions.

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Role of FAM83H in immune system homeostasis

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Fam83h is a keratin-associated protein, expressed mainly in epithelial cells and has been suggested to be responsible for intracellular transport, regulation of cytoskeletal networks, and enamel formation. Defects in FAM83H are a cause of amelogenesis imperfecta type 3 (AI3), a soft enamel disease. Interestingly, two AI patients from one family in Czech Republic with confirmed FAM83H mutation developed juvenile rheumatoid arthritis. To understand the role of Fam83h in immune system homeostasis, we have generated Fam83h mutant mice (Fam83htg/tg) with an 88aa N-terminal deletion. Fam83htg/tg animals exhibit decreased body size, sparse and scruffy coat, scaly skin, weakness and hypoactivity. While we have not observed any obvious dentin-related phenotype, Fam83htg/tg pups show severe swelling of their forepaws accompanied by severe bone deformation at as early as 3 weeks of age. Majority of the Fam83htg/tg animals present increased neutrophil as well as G-CSF and inflammatory cytokine levels in their peripheral blood. We have shown that Fam83h is expressed in immune organs and bone marrow (BM), where the source of its expression is in stromal cells. As a result, Fam83htg/tg mice show decreased thymus size and altered T cell selection and BM hematopoiesis. These findings will contribute to the unraveling of the role of Fam83h in the development of arthritic lesions and, in general, regulation of the immune system.

Effect of Pseurotin D on MEC-1 human lymphoma cells

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Chronic lymphocytic leukemia (CLL) is the most prevalent lymphoid malignancy in many geographical regions of the world. The current treatment consists mainly of a combination therapy, which is focused on inhibition of DNA replication, BCR signalization, induction of apoptosis and, more recently, mitochondrial metabolism. Pseurotin D is a secondary metabolite from the pseurotin family of bioactive natural products with a newly ascribed range of interesting biological activities. The purpose of this study was to bring new insights into the mechanism behind the effects of pseurotin D on CLL cells, with a particular focus on targeting mitochondria metabolism. The effect of pseurotin on viability was measured by counting number of cells and MTT assay. Apoptosis was detected by flow cytometry (ANX/PI, active casp3/PI staining), Western blot (pro-apoptotic proteins) and DNA fragmentation. Cell cycle was measured by flow cytometry (Click-iT EdU kit) and Western blot (cyclins expression). We measured mitochondrial respiration by Aligent Seahorse analyzer and BIOLOG MitoPlates. Mitochondrial reactive oxygen species (ROS) production was measured by flow cytometry (MitoSOX and DHR123 probes) and fluorimetry (AmplexRed). Flow cytometry was also used for detection of mitochondrial membrane potential (TMRE probe). Phosphorylation of STAT, p70 S6 and MAPK proteins was determined by Western blot. Our results showed that pseurotin D was able to significantly inhibit the proliferation of CLL cells, arrested them in the G2/M cell cycle phase and induced apoptosis. We found that these effects were associated with a change in mitochondrial membrane potential and the production of mitochondrial ROS. We showed for the first time that pseurotin D suppresses CLL cell proliferation and induces apoptotic cell death via induction of the collapse of the mitochondria respiratory chain and the ROS-related caspase pathway. Therefore, they could represent a new potent group of drugs for therapeutic treatment associated with lymphomas.

Metabolic changes of in vitro expanded NK cells

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NK cells are one of the key immune cell subsets responsible for malignant or virus infected cells surveillance and removal. Because of their high cytotoxic potential and low risk of GvHD are NK cells promising tool in immunotherapy of haemato-oncological disorders. During transition from steady state to highly proliferating and activated state the NK cells switch their metabolism towards glycolysis in order to support high energetic and proteosynthetic demands linked with cytotoxic and effector functions. However, this transition is further challenged after adoptive transfer of immunotherapeutic NK cells to patients. Major aim of the project is to understand the metabolic demands of NK expansion during the immunotherapeutic product preparation and later changes after the adoptive transfer to patients. In order to clarify molecular mechanism linking metabolism and NK cell cytotoxicity we have expanded primary NK cells with selected metabolic modulators and performed cytotoxicity, gene expression and metabolic analysis. Our results showed that the expanded NK cells have higher killing ability compared to freshly isolated cells. Furthermore, metabolically modulated NK cells sustain changed metabolic and expression profile after 14 days of expansion compared to control cells. The changes in gene expression included effector molecules such as Granzyme B and IFN γ in a case of 2-deoxyglucose and metformin. Our data suggest that this ability to change metabolism in long term opens window for metabolic intervention during expansion and for further direction to superior phenotype of immunotherapeutic NK cells. These results confirmed that metabolic priming of NK cells does have long term effect on expanded NK cells in a matter of gene expression. Our research thus suggests possible modulators in a regulation of cytotoxic functions of NK cells, and thus helps understanding the regulation of NK cell cytotoxic machinery.

CaN-NFAT controls immunometabolic reprogramming of human monocytes and contributes to immunoparalysis in sepsis

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Energy metabolism of monocytes in steady-state relies on fatty acid oxidation, while exposure to environmental stimuli as invading pathogens is followed by monocyte activation leads to a metabolic switch, represented by

increased glycolysis linked with anabolic processes. This immunometabolic switch ensure the gain of required amount of energy and intermediate metabolites for defensive processes, which are vital for host protection. These processes are altered in many pathologies including sepsis progression.

The immunometabolic status and its subsequent transformations are regulated by various signaling pathways (e.g. mTOR or HIF-1a). Calcineurin (CaN) – NFAT is signaling pathway, which role is well established in the context of immune response and inflammation. CaN-NFAT signaling pathway is targeted by immunosuppressives – Cyclosporin A and Tacrolimus, with surprising roles in myeloid cells as we described earlier.

Interestingly our data suggests, not yet described direct link between CaN-NFAT axis and the regulation of immunometabolism. Our results indicate the altered immunometabolic status in the monocytes treated with CaN-NFAT inhibitors. These changes may affect also effector defensive functions of immune cells. We observed changes in functionality of monocytes induced by immunosuppressive treatment and show significant decrease in ROS production. This might lead to the impairment of important immune-protective function of myeloid cells as monocytes use ROS for killing of phagocytosed pathogens. We also found several crosstalks in patients with septic shock. In summary, we showed a novel aspect of the CaN-NFAT in the monocyte metabolism and their effector functions, indicating possible role of this signaling pathway in increased susceptibility of immunoparalyzed patients to various secondary infections.

Recombinant cytomegaloviruses expressing NKG2D ligands as candidates for T-cell based vaccines and vaccine vectors

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Cytomegalovirus (CMV) is a major cause of morbidity and mortality in congenitally infected and immunocompromised individuals and development of an effective human CMV vaccine has been ranked as a top priority. Besides, the immunobiology of this virus makes it a very attractive viral vector. CMV establishes lifelong latency with periodic reactivation, which lead to accumulation of large amounts of effector memory CD8 T cells in peripheral tissues, a process called memory inflation. NKG2D is a potent activating NK cell receptor and has also a costimulatory role on CD8 T cells. Its importance in CMV immune surveillance is indicated by viral evasion mechanisms evolved to avoid NKG2D. We have generated recombinant mouse CMV (MCMV) expressing NKG2D ligand RAE-1 γ , MULT-1 or H60 as experimental CMV vaccines and tested their vaccine properties. Despite tight innate immune control and their profound attenuation, MCMVs expressing NKG2D ligands elicited strong inflammatory response to some CD8 T cell epitopes. Thus, we investigated the contribution of NKG2D costimulation in CD8 T cell response upon CMV infection and its role in CD8 T cell inflation. In addition, we have constructed MCMV vaccine vectors expressing NKG2D ligand RAE-1 γ and foreign CD8 T-cell epitope and found that such vectors induce expansion of epitope-specific phenotypically distinct CD8 T cells as compared to the control vector. Overall, our results indicate that a recombinant CMVs encoding a ligand for NKG2D receptor might provide a new, powerful approach for development of a safe and immunogenic CMV vaccine as well as CMV-based vaccine vector.

Functional characterization of unconventional naïve CD8+ 4-1BB+ T cells using a novel inducible monoclonal TCR mouse model

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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. After antigenic stimulation, naïve cells give rise to potent short-lived effectors as well as different kinds of memory cells. However, it remains unclear whether the cell fate is determined by intrinsic properties of naïve cells, TCR specificity, or the environmental stimuli.

In this study, we performed scRNAseq and scTCRseq on more than 50 000 murine CD8+ T cells. Alongside established cell subsets, we revealed an unconventional population of naïve CD8+ T cells (TPAM), characterized by the expression of 4-1BB (Tnfrsf9) and upregulation of the polyamine metabolism (Odc1, Srm, Eif5a).

To study TPAM cells in vivo, we developed an inducible OT-I TCR mouse model (TCR-switch). In TCR-switch mice, T cells develop a polyclonal TCR repertoire but are forced to express OVA-reactive TCR after switching. We show that upon infection with Lm-OVA or LCMV-OVA, TCR-switch TPAM cells develop into unique long-lived CX3CR1+ memory T cells. Targeting the axis establishing long-lived memory T cells might be beneficial in diverse therapeutic approaches, such as anti-cancer therapies or T-cell vaccination.

MABTECH presentation: Avinash Ravindran - A new dimension of immune profiling with FluoroSpot analysis on Mabtech IRIS

Immunomodulatory effect of metabolites secreted by probiotic strain of *Escherichia coli* O83:K24:H31

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Bacteria and their metabolites have a profound effect on the immune system, particularly when encountered early during life. It has been demonstrated that postnatal supplementation of neonates by suitable probiotic strains can be beneficial for immune system development and can ameliorate various diseases, including infections and allergy. Nevertheless, the exact beneficial modes of actions of these probiotics, including their metabolites, are yet to be fully elucidated. In our study, the immunomodulatory effect of metabolites produced by probiotic strain of *Escherichia coli* O83:K24:H31 (EcO83) cultured under aerobic and anaerobic conditions was tested in vitro on human mononuclear cells from peripheral blood (PBMC), as well as mouse splenocytes and bone marrow derived dendritic cells (BMDC). We have observed strong and concentration dependent effect of EcO83 metabolites on selected cytokines. Notably, EcO83 metabolites were able to promote the production of immunoregulatory cytokine IL-10 on both mRNA and protein level in all cell types tested. EcO83 metabolites also significantly promoted IFN- γ gene expression and secretion. Interestingly, in PBMC the supernatants from aerobic culture of EcO83 exerted a more profound effect on Th1 cytokines (IL-2, IFN- γ) while the supernatants from the anaerobic culture had a greater effect on Th2 cytokine IL-13. No striking difference between aerobic and anaerobic EcO83 culture supernatants was observed upon stimulation of murine cells; both the aerobic and the anaerobic EcO83 supernatants promoted BMDC maturation. Our data suggest that the beneficial effect of probiotics may be mediated via their metabolites, not merely by probiotic bacteria itself. Furthermore, acetate and propionate were detected in both aerobic and anaerobic supernatants, and our study is thus the first published confirmation of the production of these SCFA by EcO83. The project was supported by the research programmes Cooperatio IMMU 207032 (Charles University), GAUK 478119 (Grant Agency of Charles University) and 8J19AT010 (Ministry of Education, Youth and Sports).

TNBS colitis severity is influenced by gut microbiota composition

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TNBS colitis is one of the animal models influenced by gut microbiota composition. For our experiment we used mice from two animal facilities – Institute of Microbiology (IMIC) and 1st Faculty of Medicine (FoM). Our main goal was to compare the sensitivity of mice to TNBS-induced colitis, both toxic and hapten-type. Toxic-type was induced with intra-rectal TNBS dissolved in 50% ethanol. Hapten-type colitis included skin pre-sensitization with TNBS dissolved in olive oil 7 days before induction. 16S rRNA sequencing was used to characterize the gut microbiota composition of both groups of mice. We also tested the ability to transfer the phenotype by colonizing germ-free (GF) mice. The cells isolated from mesenteric lymph nodes and spleens were analyzed by flow cytometry. Our results indicates that FoM mice are significantly more sensitive to toxic TNBS colitis based on Wallace score and that pre-sensitization helped to increase colitis severity in IMIC mice, but not in FoM mice. We were also able to identify the differences in the composition of gut microbiota. Although the main classes like Bacilli, Clostridia and Bacteroidia are similarly abundant, classes Campylobacteria and Verrucomicrobiae are more abundant in both conventional and colonized IMIC mice, while class Saccharimonadia is more abundant in FoM conventional and also colonized mice. When we analyzed the cytokine production and T-cell composition in those groups, we found out that conventional mice from FoM have significantly more IFN- γ +CD4+ T cells and less naive T cells in their spleen than mice from IMIC. Ex-GF mice colonized with microbiota of FoM mice have more TNF- α +CD4+ T-cells in their mesenteric lymph nodes than mice colonized with IMIC microbiota. FoM conventional mice also have significantly more effector memory T-cells (TEM) in their spleen. It is possible that those cells are crucial for successful TNBS colitis induction since they are reacting fast to pathogen, which may be represented by bacteria translocated by

induction itself. This suggests that gut microbiota influences acute TNBS colitis severity by modulating T-cell functions. This study was supported by the project "Grant Schemes at CU" (reg. no. CZ.02.2.69/0.0/0.0/19_073/0016935).

The role of skin and intestinal microbiota in pathogenesis of atopic dermatitis and food allergy in mouse model

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Background: Atopic dermatitis (AD), one of the most common skin disorders, has its onset during the first year of life in 20% of children in industrialized countries. AD is very often accompanied by the subsequent development of food allergy (FA) due to epicutaneous sensitization (EC) by food allergens. Environmental factors such as skin and intestinal microbial dysbiosis contribute to its development. We aimed to assess the role of skin and intestinal microbiota in the development of the AD and FA symptoms in experimental mouse model.

Methods: Three weeks old germ-free mice were gavaged with fecal microbiota from the infant with manifested AD symptoms or healthy control. Swab of infant skin microbiota was applied on the mouse skin before and after EC sensitization on the half of the experimental mice (FS group) only. Mice were three times EC sensitized by one-week exposures to ovalbumin (OVA, 2mg/ml) applied as a patch to tape stripped skin, separated by two-weeks rest intervals. Then, mice were orally gavaged three times a week for 2 weeks with 50 mg OVA. Fifteen minutes after the gavage, drop in body temperature was assessed. Microbiome analysis was performed in samples of skin swabs and feces collected throughout the experiment. The specific OVA antibody response was measured in sera by ELISA or by rat basophile leukemia cell-based assay. The levels of cytokines (IL-4, IL-5, IL-10, IL-13 and INF-gamma) were measured in OVA stimulated spleen cell culture supernatants and jejunum homogenates by Luminex. Histopathological changes and mast cell infiltration in skin and jejunum were evaluated.

Results: In AD-microbiota colonized mice, epicutaneous sensitization and oral challenge by OVA led in FS group to non-significantly higher manifestation of anaphylactic hypothermia compared to F group. Similarly, these mice exhibited trend to increased levels of OVA-specific antibodies in sera and cytokines in splenocyte cultures. On the other hand, histological analysis showed the significant occurrence of mast cells and tissue structure changes in skin and jejunum of these mice. On the other hand, fecal and skin microbiota from healthy children led to mild symptoms of disease, such as anaphylactic hypothermia and mast cells occurrence in jejunum.

Conclusion: The colonization of mice by fecal microbiota together with skin microbiota from pediatric AD patient have impact on skin and intestinal morphology as well as number of mast cells in mice after the EC sensitization and FA induction.

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Gut microbioma can influence the function of even the thymus

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Objective. Thymus is considered as an immune-privileged organ, where the blood-thymus barrier prevents the blood circulating antigens from reaching the developing T-cells. In the absence of blood-thymus barrier, a specific clone of T-cells could bind a foreign antigen and be considered as self-reactive, leading to its undue clonal deletion during the negative selection process. The objective of the work was to evaluate if inflammatory and immunological changes in the colon would influence the thymus and elicit a response. *Materials and methods.* Animals raised in absence of the microbiome (germ-free, GF), were exposed to a conventional food/environment to produce the gut colonization (conventionalization, CV). Different groups of animals were sacrificed at different time points after starting the conventionalization (12hrs, 24hrs, 48hrs, 72hrs, 7days); GF and CV mice were used as controls. The thymic tissue of each group was evaluated in RT quantitative PCR to determine the differential gene expression of a panel of cytokines and genes involved in inflammation and fibrosis.

Results. Intra-thymic expression of inflammatory cytokines during the conventionalization increased of 1.5-2 fold change than in germ-free animals, generally peaking at 48-72h. A similar pattern was observed in the immunoregulatory cytokines (especially IL-10), with a possible mitigation meaning, with the exception of TGFb that, aside a temporary spike of 1.1 fold change at 48h, during the conventionalization maintained a lower expression than the germ-free baseline, and similar to conventional animals. Remarkably, gene expression of collagen and cross-linking collagen enzymes such as LOX and LOXL-2, resulted increased of several fold changes.

Conclusions. These results suggest that changes in the colon immunological conditions might influence even distant lymphoid organs. Colonic antigens might reach the thymus despite its immune-privileged status and elicit central

tolerance mechanisms towards the commensal microflora. Acknowledgements: RVO 61388971, GENERALI-CP Foundation CZ; UniCredit Bank CZ&SK

Clinical significance of MMP-2, MMP-9, their tissue inhibitors TIMP-1 and TIMP-2 and innate immune cells in patients with colorectal cancer

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Background: Colorectal cancer (CRC) is one of the primary causes of cancer-related mortality worldwide with approximately one million new cases every year and is accompanied with high mortality rate. The number of CRC patients is increasing progressively. The human response to malignancies includes an alteration in immune response, observed as decreases in natural killer (NK) cell cytotoxicity, lower antibody titers and decreased lymphocyte proliferation. The family of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) play very important role in colon carcinogenesis. MMPs were initially believed to produce and secrete tumor cells and degrading basal and extracellular components, but MMPs also produce surrounding stromal cells, including fibroblasts and infiltrating inflammatory cells. They have the ability to break down the extracellular matrix important for tumor growth, invasion, and metastasis. Tumor cells produce proteolytic enzymes that can destroy the matrix around the tumor, allowing invasion into the surrounding connective tissues. MMPs are capable of degrading almost all components of the extracellular matrix and connective tissue surrounding the tumor cells and the basement membrane. Matrix metalloproteinase-9 (MMP-9) has a big role in the progression of CRC and may be accompanied with distant metastasis associated with high mortality rates. TIMP-2 may inhibit metastasis and on that way predicts prognosis of colorectal cancer via regulating MMP-9.

AIM: The purpose of this study was to examine the changes in percentage of innate immune cells: NKT and regulatory T cells (Tregs) in peripheral blood and the correlation with the levels of MMP-2 and 9 and TIMP 1 and 2 in urine of patients with colorectal cancer in comparison to healthy volunteers.

Subjects and methods: A total of 145 patients with CRC and 50 healthy control subjects were included in this study. Human peripheral blood mononuclear cells (PBMC) from healthy adult volunteers and from patients with colorectal cancer, classified as Dukes' B or Dukes' C, were taken before the operation and were labeled with monoclonal antibodies. Analysis was performed on flow cytometer (FACSCalibur, Becton Dickinson) using CELLQuestPro Software (Macintosh, Quadra 650). Patients were selected from a stratified population sample of adult patients of both sexes. The method of enzyme immunoassay (ELISA) was used to determine enzyme expression of MMP-2 and 9, TIMP 1 and 2. **Results:** our results showed a large increase in the concentration of both enzymes in the urine of patients with colorectal cancer. The concentration of MMP 2 and 9 correlate with the stage of tumor. High expression of MMP-9 and lower TIMP-2 expression correlated with the progression of tumor. In the same time in these patients are noticed the increased percentage of NKT cells and lower expression of Tregs. **Conclusion:** New possibilities for better monitoring of the disease are very important. We verified the activity of MMPs in the urine of patients with diagnosed colorectal cancer in different stages of disease.

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3D cell models and their use in the study of immunological changes associated with human colorectal carcinoma

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3D cell models provide a wide scope for studying immunological changes in the tumormicroenvironment. The structure of the tumor microenvironment can be modulated by local immunity.

Aim of the work: In our research, we focus on the development of a suitable method of 3D tumor cell culture (spheroids, organoids) from mouse and human cell lines. In the HT29 (human colon cancer) we have studied the modulation of the expression of the immune checkpoint ligand PD-L1 and ACE2 receptor in different phases of tumor development. Another goal was to develop 3D mixed cell cultures (tumor cell line and fibroblasts of mouse) as a model of tumor-stroma interaction. **Material and methods:** Mouse (CT26) and human (HT29) colorectal

carcinoma cell lines were cultured in microgel form (The 3D Petri Dish®, Microtissues™) to form spheroids. After 3 and 7 days, RNA was isolated from the spheroids and retrotranscribed into cDNA. The expression of the PD-L1 and ACE2 genes was subsequently determined using qRT-PCR. In the following experiments, mouse fibroblasts (3T3) were stained with the Vybrant CFDA SECell Tracer kit (living cell staining), and after 24 hours we added three-day-old CT26 spheroids to them. After subsequent one-day cultivation, fixation and staining (Hoechst 33258), the samples were analyzed by confocal microscopy. *Results:* According to preliminary qRT-PCR results from 3D cell line HT29, PD-L1 gene expression appears higher in 7 day spheroids and ACE2 gene expression appears higher in 3 day spheroids. Confocal microscopy results indicate physiologically increased 2D 3T3 fibroblast redistribution at the interface with 3D CT26 spheroids, reminiscent of defensive clustering in front of tumor invasion.

Conclusions: Modeling the tumor microenvironment in vitro using 3D cellular methods allows a better investigation of PD-L1 and ACE2 gene expression during tumor development in its early stages, and also describes its physiological behavior. *Acknowledgment:* grants RVO 61388971 (CZ), Generali/Česká pojišťovna s. (CZ&SK), UniCredit Bank s.r.o., Praha (CZ), Ponticelli Donation (CZ), CAMIC (CZ), Eurinox s.r.o (CZ) and ARPA Foundation (IT), The project National Institute for Cancer Research (Programme EXCELES, ID Project No. LX22NPO5102) - Funded by the European Union –Next Generation EU, The work was supported by MEYS CR (Large RI Project LM2018129 Czech-Biolmaging) and ERDF (project No. CZ.02.1.01/0.0/0.0/18_046/0016045).

Key words: tumor microenvironment, confocal microscopy, gene expression, PD-L1, ACE2

Immunogenicity of plasmid DNA electrotransfer in different skin cell types

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Skin, the largest organ in the body, consists of three main layers: the outermost layer, the epidermis, followed by dermis and the lowest subcutaneous layer. Skin has three main functions: protection, thermoregulation and sensation supported by various cells including epithelial tissue cells such as keratinocytes and sebaceous gland cells, connective tissue cells such as fibroblasts and endothelial cells, immune cells such as leukocytes and Langerhans cells, and ectoderm-derived cells such as melanocytes and Merkel cells. Keratinocytes are a major cell type of the epidermis, in dermis, the predominant cells are fibroblasts. The diversity of cell types in skin could impact gene therapies because DNA transfection could elicit different responses. Previously, we observed the upregulation and activation of cytosolic DNA sensing pathways in several non-tumor and tumor cell types in vitro, as well as in vivo in tumors after electrotransfer of plasmid DNA. Based on this research and the innate immunogenicity of skin, here we correlated the effects of electrotransfer of plasmid DNA (pDNA) in mouse skin to fibroblasts and keratinocytes in vitro using reverse transcription real time PCR (RT-qPCR) and ELISA, multiplex bead array, and immunofluorescent staining. After electrotransfer of pDNA, the mRNAs of interferon beta (Ifn-β1), tumor necrosis factor alpha (Tnfα), interleukin 1 beta (Il-1β), interleukin-6 (Il-6) and the putative DNA sensors DEAD (AspGlu-Ala-Asp) box polypeptide 60 (Ddx60), DNA-dependent activator of interferon regulatory factor/ Z-DNA binding protein 1 (Dai/Zbp1), interferon activated gene 202 (Ifi202); interferon-inducible protein 204 (Ifi204) were upregulated in keratinocytes, while the cytokine mRNAs Ifn-β1, Tnfα, Il-6 and DNA sensor mRNAs Ddx60, Zbp1 and Ifi204 were upregulated in fibroblasts. Secretion of significant levels of IFN-β protein was detected by both cell lines after pDNA electrotransfer. Keratinocytes secreted IL-6, while fibroblasts did not secrete detectable levels. Skin experiments confirmed our in vitro results with increased expression of the mRNAs of putative DNA sensors Ddx60, Zbp1, Ifi202 and Ifi204 along with several cytokines and chemokines. Finally, we demonstrated that skin keratinocytes, fibroblasts and macrophages contribute to the immune response observed after electrotransfer of pDNA. In conclusion, our results confirm the essential immune functions of both immune and non-immune cells in the skin, which are essential for vaccination and gene therapy using pDNA electrotransfer.

In vitro and in vivo evaluation of ferric-based nanoparticles in theranostic perspective

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Cancer diseases represent second leading cause of death in OECD countries. The most common cancer treatments may include surgery, radiation, chemotherapy, immo-therapy, hormonal therapy and targeted therapy or their combinations. With the progress in targeted therapies, a variety of nanoparticles (NPs) have been studied for possible theranostic approaches. Based on their unique properties, their growing use as carriers in targeted localization, MRI and/or thermotherapy requires thorough assessment of their possible short- and long-term

toxicity and immunotoxicity. In our study, tumour and non-tumour cell lines (CT26 colorectal carcinoma and 3T3 fibroblasts) were challenged with 4 types of NPs. γ -Fe₂O₃-based NPs (with or without nickel) (polymer coated/naked) were tested in vitro for effects on cell viability (MTT, crystal violet assay) and also on apoptosis and ROS production (FACS). Their effect on immune cells in vivo was evaluated by FACS on murine splenocytes. Their diagnostic properties were tested in nuclear magnetic resonance (NMR).

Our data showed small to no reduction in cell viability and negligible changes in ROS production in most in vitro conditions. Preliminary data showed almost no significant changes 24h after systemic administration in vivo. Only a slightly reduced percentage of NK/NKT cells, mild activation of B lymphocytes and small recruitment of naïve macrophages into the spleen was observed in some conditions. All NPs produced signal similar to the standard contrast agent in NMR. In conclusion, our NPs showed no significant toxicity/immunotoxicity in vitro and in vivo, and they generated signal in NMR in vitro, which makes them appropriate candidates for further testing in theranostic approaches. Acknowledgment: grants RVO 61388971 (CZ), Generali/Česká pojišťovna a.s. (CZ&SK), UniCredit Bank Czech Republic and Slovakia, a.s., Praha (CZ), PPT ABRASIVES s.r.o. (CZ), CAMIC (CZ), Italinox s.r.o. (CZ) and ARPA Foundation (IT). The work was partially supported by MEYS CR (Large RI Project LM2018129 Czech-Biolmaging), ERDF (project No.CZ.02.1.01/0.0/0.0/18_046/0016045), and Next Generation EU (NPO Exceles NCI, reg.n. LX22NPO5102).

Dexamethasone based polymer therapeutics for treatment of rheumatoid arthritis

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Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease caused by alteration of both the innate and adaptive immune system. Current therapy includes various approaches, however it is often connected with various side effects. The conjugation of the drug to polymeric delivery system improves its therapeutic properties. Therefore we aimed to evaluate the biological effects of nanopolymer drug conjugates based on biocompatible N-(2-hydroxypropyl) methacrylamide carrying glucocorticoid dexamethasone (DEX). The drug was derivatized prior the attachment to polymer with the aim to introduce suitable functional groups for the attachment via stimuli sensitive spacers. The biological effects of polymer conjugates were evaluated both in vitro and in vivo. Acute toxicity and anti-inflammatory properties were tested using murine peritoneal macrophages RAW 264.7 activated by lipopolysaccharide. Adjuvant induced arthritis mouse model was used for evaluation of therapeutic efficacy of nanopolymer conjugates. Acute toxicity was not observed for any tested compound. All tested compounds decreased production of inflammatory mediators (NO and IL-6) by stimulated RAW 264.7 cells which suggests that anti-inflammatory activity was retained after DEX modification and its attachment to polymer. All tested polymer conjugates showed better therapeutic efficacy in comparison to free DEX which was observed as a decrease in paw swelling using in vivo mouse model. The data suggest that the conjugation of DEX to polymer carrier did not affect its biological activity and the therapeutic potential was retained and even higher than free DEX. Therefore, the conjugation of DEX to nanopolymer carrier by stimuli sensitive spacers is a suitable strategy for improvement of RA therapy. This work was supported by the Ministry of Health of the Czech Republic (project NU20-08-00255). Daniela Rubanová is a Brno Ph.D. Talent Scholarship Holder – Funded by the Brno City Municipality.

Optimization of in-vitro differentiation and activation of monocytes before the analysis of PLAUR gene expression

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The aim of our research is to define PLAUR gene expression after activation of fully differentiated monocytes in patients with hereditary angioedema, asthma bronchiale and rheumatoid arthritis. PLAUR gene codes a multifunctional protein uPAR (CD87) which affects fibrinolysis following an interaction with urokinase plasminogen activator (uPA), influences blood coagulation and bradykinin production through the binding of FXII, which is activated at cell surfaces, and modulates cell adhesivity and migration through the interactions with extracellular matrix proteins. The cornerstone of this project was to optimize the process of isolation, differentiation and activation of monocytes. The effort was to achieve maximum yields of monocytes from the sample of peripheral blood, with purity as highest as possible and the lowest possible primary cell activation. By choosing the appropriate concentration of cytokines and incubation time, we tried to achieve in-vitro differentiation of

monocytes into M1 type macrophages. Monocytes were isolated from the samples of peripheral blood using the magnetic separation method. Obtained monocytes were activated by a combination of cytokines M-CSF and INF γ , which promoted their differentiation into M1 macrophages. The purity of isolated monocytes, their differentiation and activation were determined using the flow cytometry and optical microscopy.

Higher yields of monocytes were achieved using PBS in concentration 2x higher than usually, when the average yield of monocytes was around 150,000 cells from 1 ml of peripheral blood with purity above 95 %. Low activation of monocytes was achieved by using glass tubes and maintaining a low temperature (< 8°C) of the cell suspension throughout the isolation process. Microscopic observation determined the most appropriate length of monocyte incubation and the concentration of MCSF and INF γ cytokines. Monocyte differentiation and activation was determined by flow cytometer measurements of surface expression of CD86, CD38 and CCR7 markers. Monocytes differentiated and activated in this way proved to be suitable for further processing.

The result of the performed experiments is an optimized and verified protocol for the isolation and activation of monocytes necessary for the precise analysis of the expression of the PLAUR gene.

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Could we use degradation capacity of histamine as a laboratory marker for diagnostic and monitoring histamine intolerance ?

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Introduction: Nowadays, adverse reactions to food are much more subjectively perceived than objectively verified. Although clinical, diagnostic or therapeutic procedures are constantly advancing, scientific knowledge about non-allergic adverse reactions, including histamine intolerance (HIT), can be considered insufficient. The study and scientific evidence supporting the supposed connection between increased intake of histamine in foods, its high level in body, and its adverse scientific reactions are not yet verified in such detail. Moreover, there is no perfectly reliable laboratory test for the objectively diagnosis of this disease. Priority indicated parameters are quantitative and functional determination of diamine oxidase (DAO), however, determination of total histamine degradation (THDC) with using ELISA methods seems to be a promising parameter for the diagnosing and monitoring of patients with HIT. *Material and methods:* We determined THDC, functional activity of DAO and its quantity in 40 patients in the outpatient department of the Allergy and Clinical Immunology Unit and in 20 patients in the outpatient department of Gastroenterology Unit, with symptoms of HIT. *Results:* In the entire group of patients, we were able to find a correlation between activity of DAO and the ability of the serum to degrade histamine. Patients with DAO activity < 3 U/mL ($2,1 \pm 1,0$ U/mL) had a low the THDC value ($8,9 \pm 9,4$ %) while patients with DAO activity > 10 U/mL ($13,6 \pm 3,4$ U/mL) reached normal values of histamine degradation ($75,6 \pm 17,8$ %). The presence of allergy increased the values of DAO activity and decreased the ability of total histamine degradation (DAO activity in patients with allergies $6,8 \pm 5,8$ U/mL vs. Without allergy $6,0 \pm 5,0$ U/mL, THDC with allergies $40 \pm 28,6$ % vs. without allergy $44,2 \pm 27,1$ %), the presence of GIT symptoms significantly reduced the monitored parameters (DAO activity in patients with symptoms $4,2 \pm 1,8$ U/mL vs. without symptoms $8,5 \pm 7,2$ U/mL, THDC with symptoms $11,7 \pm 24,2$ % vs. without symptoms $45,9 \pm 26,9$ %). *Conclusion:* The routine use of this new marker and the determination of the concentration and activity of DAO can be considered as a more accurate indicator of the state of histamine metabolism in the human body and can serve to clarify the diagnosis of HIT.

Studying the potential of “trained immunity” in human oral epithelial cells using Candida species

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The term „trained immunity” refers to the immunological memory of the innate immune system. Trained immunity – unlike classical immune memory – lasts up to only a few months, is regulated by epigenetic modifications, and because no specific antibodies are involved in memory formation, the secondary immune response may be activated not only against pathogen inducing the first immune response but also against pathogens whose antigens are recognised by the same pattern recognition receptors. The number of fungal infections has been increasing in recent decades. These infections are often caused by members of the genus *Candida*. Systemic candidiasis, vulvovaginal candidiasis and oral thrush are different manifestations of such infections. Since oral candidiasis may frequently develop independent of genre or medical interventions (e.g., catheter insertion), in the followings we

aimed to study the first line of the human oral niche, thus the response of oral epithelial cells. Based on our research group's previous work, the natural immune responses induced by *C. albicans* and *C. parapsilosis* are fundamentally different as in *C. albicans* initiates a rapid and excessive inflammatory cascade, whereas *C. parapsilosis* induces only a moderate host response. Taking advantage of the difference in the pathobiology of the two species, we aimed to investigate whether trained immunity can be achieved by pre-treatment of human host cells with *C. parapsilosis* in order to allay future *C. albicans* infections. For our experiments, we used the oral epithelial cell line OKF6/TERT-2, - derived from healthy individual – as the host, and two distinctive strains of *C. albicans* (SC5314 and WO-1) and *C. parapsilosis* (GA1 and CLIB214) as pathogens, to examine species- as well as strain-dependency. OKF6/TERT-2 cells were pre-treated for 24 h with live *C. parapsilosis* cells, then fungal cells were eliminated using the antifungal agent nourseothricin. After that, we stimulated host cells with nourseothricin-resistant *Candida albicans* cells on the fifth day for 24 hours and examined the extent of host cell damage. Our results indicate that the preincubation with the less pathogenic species, *C. parapsilosis* has the potential to decrease host cell damage during infection with the more pathogenic and more frequent cause of oral candidiasis, *C. albicans*, hence suggest a potential preventative effect. *Acknowledgments:* EU's Horizon 2020 research and innovation program under grant agreement No. 739593 Momentum Grant by the Hungarian Academy of Sciences (LP2018-15/2018)

YOUNG IMMUNOLOGISTS' SESSIONS

Immune regulatory programs in human dendritic cells upon immunization with live and UV-inactivated *Chlamydia trachomatis*

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Background: Infections with *Chlamydia trachomatis* (Ct) are the leading cause of bacterial sexually transmitted infections worldwide. However, to date there is no vaccine approved against Ct, as vaccination trials with inactivated Ct did not show long-lived protection against subsequent infection. It was shown in a mouse model that upon mucosal immunization with live and UV-inactivated Ct (UV-Ct), antigens are taken up by immunogenic CD103⁺ and tolerogenic CD103⁺ dendritic cells (DC) mediating activation of regulatory T cells and effector T cells, respectively. We want to explore further, why live and UV-Ct alter the subsequent immune responses by DC and if we can observe a similar phenotype in human antigen presenting cells.

Methods: To investigate different mechanisms between DC immunized with live and UV-Ct, we performed flow cytometric analysis as well as bulk RNA-Seq of monocyte-derived human DC (moDC).

Results: Upon stimulation with live and UV-Ct, moDC upregulate activation and maturation markers on their surface without significant differences between both conditions. However, signaling pathways regulating immune function and apoptosis are differentially expressed on RNA level between live Ct- and UV-Ct-stimulated moDC. Amongst genes significantly upregulated in DC infected with live Ct were CCL17 and IL23A, which are involved in helper T cell migration and activation.

Conclusion: Our results suggest that even though the main co-stimulatory molecules are similarly expressed in moDC stimulated with live and UV-Ct, differential transcriptional programs are operative. The capacity of moDC to prime T cells needs to be evaluated to identify mechanisms being inevitable to convey the opposing immune response observed in Ct/UV-Ct immunized mice.

CMTM4 is a subunit of the IL-17 receptor and mediates autoimmune pathology

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Interleukin-17A (IL-17A) is a key mediator of protective immunity to yeast and bacterial infections but also drives the pathogenesis of several autoimmune diseases, such as psoriasis or psoriatic arthritis. Here, we show that the

tetra-transmembrane protein CMTM4 is a subunit of the IL-17 receptor (IL-17R). CMTM4 constitutively associated with IL-17R subunit C (IL-17RC) to mediate its stability, glycosylation and plasma membrane localization. Both mouse and human cell lines deficient in CMTM4 were largely unresponsive to IL-17A, due to their inability to assemble the IL-17 receptor signaling complex. Accordingly, CMTM4-deficient mice had a severe defect in the recruitment of immune cells following IL-17A administration and were largely resistant to experimental psoriasis, but not to experimental autoimmune encephalomyelitis. Collectively, our data identified CMTM4 as an essential component of the IL-17 receptor and a potential therapeutic target for treating IL-17-mediated autoimmune diseases.

The challenges and limitations of choosing targets for CAR-T cell therapy

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CAR-T cell therapy is a novel autologous treatment for blood cancers. It is based on transfecting patient's own T-lymphocytes with a chimeric antigen receptor (CAR) which targets antigens specific for tumour cells. Upon contact with tumour cells, the receptor binds to target antigen and induces specific T-lymphocyte response.

CAR receptor is a recombinant chimeric protein consisting of distinct domains that each have their specific function. Extracellular domain is usually derived from scFv antibody fragment and ensures specificity against the antigen of choice. The spacer domain and transmembrane domain assure the proper positioning of the receptor. Intracellular activation and costimulatory domains are responsible for activation of T-lymphocytes.

One of the most important aspects of designing CAR receptor and CAR-T cell therapy is choosing the target antigens. The challenges of choosing targets are finding target proteins that are expressed only or at least in much higher quantity on target cells. If antigens are present on other tissues there can be severe on-target off-tumor toxicity. Besides that, target antigen needs to be vital for the cell, because otherwise, the cells will down-regulate or even completely stop expressing the antigen – antigen escape. The first registered CAR-T cell therapy was aimed at CD19 and later on CD20 on B-lymphocytes but due to antigen escape new targets are needed. B-cell maturation antigen (BCMA) is also expressed on B-cells, more specifically plasma B cells. It is also overexpressed on malignant cells of multiple myeloma, which makes it a promising target for development of CAR-T cell therapy.

In the scope of my PhD project we will design a novel CAR receptor based on monoclonal antibodies produced by hybridoma technology. So far we have created 13 hybridoma cell lines which produce monoclonal antibodies specific for extracellular domain of BCMA. We tried to determine the epitopes by binding antibodies to short peptide sequences, but discovered that epitopes are conformational. We are currently testing if these antibodies bind to BCMA expressed on B-cells in native form. Once we finish with antibody characterisation, we will prepare cDNA sequences for selected antibodies and use them to create scFv fragments and then entire CAR constructs to be tested in vitro and in vivo.

The epigenetic state of IL-4-polarized macrophages enables activated NFkB-p65 cistrome expansion and extended synergistic responses to LPS/TLR ligands resulting in hyperinflammatory gene expression

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Prior exposure to signals can fundamentally change the response of innate immune cells to subsequent stimuli underlying phenomena such as cellular memory, tissue type-specific reactions, cytokine storm, or anergy/tolerance. Our understanding of the molecular nature of such interactions, especially at the epigenomic and gene expression level, is fragmented. It is believed that alternative macrophage polarization and inflammatory signals-activated transcriptional programs largely antagonize each other, and no significant convergence has been identified between them. In contrast, here we show that IL-4 polarized macrophages irrespective of their origin establish a unique inflammatory gene expression program upon lipopolysaccharide exposure. This interaction, we termed extended synergy, is dependent on IL-4-induced STAT6-directed epigenomic remodeling, the vast expansion of the LPS-activated NFκB-p65 cistrome, increased chromatin accessibility, and enhancer activity. The EGR2 transcription factor contributes to the regulation of the LPS-induced de novo and enhanced NFκB-p65 binding and synergistic gene activation in a macrophage subtype-specific manner. Consequently, the previously alternatively polarized macrophages produce extreme levels of immune-modulatory factors, including CCL17, CCL22, CCL2, and EDN1, both in vitro and in vivo in a murine Th2-type airway inflammation model upon LPS exposure. Thus, our findings establish that the IL-4-induced epigenetic reprogramming is responsible for the development of robust inflammatory hyperresponsiveness to TLR activation and likely contributes to lung pathologies, such as exacerbation of Th2 inflammatory disease or pneumonia.

OUR EXPERIENCES WITH CLINICAL MANIFESTATIONS OF PATIENTS WITH COMMON VARIABLE IMMUNODEFICIENCY FROM SPECIALISED CENTRE FOR PRIMARY IMMUNODEFICIENCIES IN MARTIN, SLOVAKIA

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Background and aims: Common variable immunodeficiency (CVID) is heterogeneous group of disorders that belongs to Predominantly antibody deficiencies with various clinical and immunological features. There are four classification systems based on B cell phenotypic profiling. The genetics supplies clinical diagnostic criteria and often changes the definite diagnosis.

Methods: This is the retrospective clinical study of clinical manifestation, laboratory findings, autoimmune, granulomatous, lymphoproliferative complications of patients followed up with the diagnosis of CVID. It also compares the clinical manifestation of patients in different groups according to EuroCLASS classification and tries to find some correlations and perspective diagnostic markers. We also study the genotype-phenotype correlations in patients with confirmed causal pathogenic variant in genes associated with Primary immunodeficiencies.

Results: We follow up 58 patients (28 males, 30 females) with CVID diagnosis. The mean age of patients is 40 years. Infectious complications are present in 88 % (n=51) of patients. Autoimmune complications are present in 43,1 % (n=25) of patients. Lymphoproliferative disease is found in 8,6 % (n=5), splenomegaly in 22,4 % (n=13) and granulomatous complications in 17,2 % (n=10) of patients of our cohort. We perform molecular-genetic testing in 22 % (n=13) of patients and find causal pathogenic variant in 30,8 % (n=4) of this group.

Conclusions: Clinical diagnostic criteria for CVID and classification systems are still not sufficient to predict clinical manifestation and complications. Genetic testing can be helpful in this issue.

COVID-19 postinfection and postvaccination immune response in paediatric patients with primary immunodeficiencies

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Since the first description in December 2019, the SARS-CoV-2 virus causes a COVID-19 disease which evolved into a still ongoing global pandemic.

Primary immunodeficiencies, also known as inborn errors of immunity, are caused by various genetic defects that affect at least one component of innate or adaptive immunity. The clinical course of disease in patients with primary immunodeficiency (PID) may vary from asymptomatic to death. Although effective cellular immunity is indispensable for control of COVID-19, dysregulated activation of T-cells may contribute to disease severity in patients. COVID-19 is generally a mild disease in children and adolescents, however fraction of them including patients with inborn errors of immunity may develop into severe disease.

In this retrospective study we summarized humoral and cell immune response in patients with severe clinical manifestation of PID in our centre, Diagnostic and research centre for primary immunodeficiencies in Bratislava. We analyzed immune response after infection with SARS-CoV-2 virus or after vaccination against COVID-19 disease. To evaluate humoral response, we performed serologic tests for titres of IgM, IgG, IgA and neutralising anti-SARS-CoV2 antibodies. Specific memory T-cells were measured to analyse cell immune response.

The control group consisted of patients with allergic rhinitis or bronchial asthma who did not meet criteria for inborn errors of immunity and were otherwise healthy.

Humoral and memory T-cell response in CVID patients was significantly lower when compared to control group. Results also showed that postvaccination response in CVID patient is significantly higher than response after overcoming the COVID-19. Patients with agammaglobulinemia were not able to produce adequate titres of antibodies, vaccination also significantly increases antibody responses when compared to postinfection. However ability to produce T-memory cells was maintained. Group of patients with CID/SCID demonstrated no neutralising response, T-cell response was also weakened. Only vaccinated patient with CID/SCID showed adequate T-cell response. Although we see a decline in COVID-19 cases, there are still groups of patient that may be at risk of severe clinical course of disease. PID represent a heterogenous group of rare diseases that affect various components of immune system. Despite initial concerns, multiple studies suggest that patients with antibody disorders tend to have mild COVID-19 infection. Effective T-cell response is associated with milder course of infection. Inborn errors of immunity that associate with alterations in type 1 interferon pathways may lead to possibly fatal COVID-19 pneumonia.

PID patients, including the ones who do not respond to classical vaccines by measurable antibody titers should still be vaccinated, as the vaccines have been shown to activate T-mediated cellular immunity. Vaccination also significantly increases antibody responses when compared to postinfection.

COVID-19 SEVERITY AND POSTVACCINATION OUTCOMES AFTER BNT162b2 VACCINE ADMINISTRATION IN PATIENTS WITH INBORN ERRORS OF IMMUNITY

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Background: Inborn errors of immunity (IEI) are characterized by higher susceptibility to infections and a broad spectrum of non-infectious complications. Therefore, IEI patients may be regarded as a high-risk group for severe COVID-19, and response to anti-SARS-CoV-2 is questionable, particularly in patients with antibody deficiency. Thus, we initiated the studies focused on the severity of COVID-19 disease and postvaccination response.

Methods: In the first part, the multicenter retrospective survey-based study, the demographic, clinical, and laboratory data were collected by investigating physicians from 8 national referral centers. In the second part, the prospective observational study focused on vaccination outcome, safety, and dynamics of postvaccination response to the BNT162b2 vaccine in Common variable immunodeficiency (CVID) patients.

Results: In the first part, 81 IEI patients were found to have a 2.3-times higher risk for hospital admission and a higher mortality ratio (2.4% vs. 1.7% in the general population). COVID-19 severity was associated with clinically relevant comorbidities, lymphopenia, and hypogammaglobulinemia. In the second part, 21 CVID patients were included in the prospective study. At the end of follow-up (month 6), the humoral response was observed in 33.3% and T-cell immune response was measurable in 50% of CVID patients. RT-PCR confirmed infection in 3 patients (14.3%), and all of them had a mild course. We also demonstrated a favorable safety profile.

Conclusion: IEI are associated with a higher risk for a severe course of COVID-19 disease and mortality ratio. BNT162b2 vaccine may elicit a measurable humoral and T-cell immune response in CVID patients. Vaccination also showed a favorable safety profile and good clinical postvaccination outcomes.

SARS-CoV-2 cell entry mechanism and its inhibition using antioxidant compounds

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After almost three years of the COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the molecular mechanisms of viral infection and host pathogenesis remain elusive. The Spike protein of SARS-CoV-2 plays a vital role in initiating viral infection. Spike protein is a homotrimer comprised of two subunits that require proteolytic processing for successful viral entry. Spike protomers are rich in cysteine residues, many of which form disulfide bonds that have an essential structural role in allowing a high-affinity interaction with the angiotensin-converting enzyme-2 (ACE2) receptor. Previously studied redox-sensitive viral fusion proteins had been shown to be impacted by antioxidants, making antioxidants beneficial not only in preventing oxidative stress-

related disorders but also in influencing viral cell entry. Described herein, we demonstrate the importance of disulfide-thiol balance within the spike protein for viral fusion and cell entry of SARS-CoV-2. By utilizing cell-cell fusion systems, we showed reduced viral cell entry and syncytia formation in the presence of several reducing compounds in a dose-dependent manner. Results were complemented with the pseudoviral infection model in vitro and in vivo, confirming the efficiency and potential therapeutic value of tested antioxidant and anti-inflammatory compounds. Understanding the cellular and inhibiting molecular mechanism of viral cell entry is essential in controlling the ongoing pandemic and possibly averting them in the future.

Evaluation of the role of free nucleic acids in IL-23 production of human keratinocytes and their connections to psoriasis pathogenesis

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Psoriasis is a multifactorial, chronic inflammatory skin disease, the development of which is affected by both genetic and environmental factors. Cytosolic nucleic acid fragments, recognized as pathogen- and danger-associated molecular patterns, are highly abundant in psoriatic skin. It is known that psoriatic skin exhibits increased levels of IL-23 compared to healthy skin. However, the relationship between free nucleic acid levels and IL-23 mRNA expression has not been clarified yet. Additionally, we have recently shown the role of PRINS long non-coding RNA (lncRNA) in keratinocytes' inflammatory responses by downregulating the expression of IL-6 and CCL-5 through sequence specific binding to their respective mRNAs, a regulatory process, which might have additional target genes, such as IL-23.

Our aim was to investigate how psoriasis-associated high free nucleic acid levels influence IL-23 production in keratinocytes, and whether IL-23 can be a subject to PRINS mediated regulatory events. To induce inflammatory processes, normal human epidermal keratinocytes (NHEK) were transiently transfected with synthetic dsDNA analogue poly(dA:dT) and dsRNA analogue poly(I:C) or were treated with psoriasis-specific cytokines (TNF α , IL-17A, IL-12). Both nucleic acid analogues and cytokine treatments caused elevated IL-23 mRNA expression, but to different degree and with different kinetics. To clarify which receptors and pathways play a role in nucleic acid induced IL-23 mRNA expression of keratinocytes, we silenced several nucleic acid receptors (TLR3, RIG-I, IFIH1, cGAS) and used specific inhibitors to decrease the activity of the signalling pathways (MAP kinases, STAT and NF- κ B). Detection of IL-23 mRNA levels by qPCR showed that silencing of TLR3 receptor and inhibition of MAP kinases and NF- κ B resulted in a decrease in the nucleic acid induced IL-23A mRNA expression in keratinocytes.

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.

Our results suggest that free nucleic acids induce epithelial IL-23 production through TLR3 receptor and specific signalling pathways thus contributing to the development of psoriatic inflammation. We propose that this process might be fine-tuned by PRINS non-coding RNA, but by which mechanism remains to be elucidated.

Innate immune response to different isolates of *Streptococcus agalactiae*

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A Gram positive, encapsulated, β -hemolytic *Streptococcus agalactiae* (group B *Streptococcus*, GBS) is commonly found as a commensal of the genitourinary and gastrointestinal tracts in healthy adults but also remains a major cause of neonatal infections due to its various virulence factors, usually manifesting as sepsis, meningitis or pneumonia. To date, 10 various serotypes (Ia, Ib, II – IX) differentiating by virulence factors and immune response have been described. To mitigate the risk of vertical transmission from mother to newborn, colonized women

receive intrapartum antibiotic prophylaxis during delivery, which has greatly reduced the incidence of early-onset disease. However, as no treatment is currently available to prevent the risk of late-onset disease, and because GBS is also increasingly recognized as a cause of invasive infections in the elderly and immunocompromised individuals, more studies, investigating the GBS-associated pathogenesis are needed. Since adaptive immunity is impaired in neonates, macrophages, as part of innate immunity, play a critical role in protection against pathogens. As phagocytes they are involved in pathogen recognition, phagocytosis and elimination, regulate further immune responses and are crucial for tissue homeostasis. Because the severity of the disease depends on both the GBS isolate and the immune status of the individual, we examined the influence of previously genotyped 12 distinct GBS isolates belonging to 6 different serotypes (Ia, Ib, II – V) on the immune response of THP-1 macrophages. Isolate-specific differences were observed in the phagocytic uptake, measured by flow cytometry, which ranged from 10% to over 70%. Macrophages, infected with distinct isolates also differed in the expression of costimulatory molecules and scavenger receptors at the protein level, with colonizing isolates expressing higher levels of CD86 and CD80 compared to invasive ones. In addition, we determined the percentage of cellular respiration and glycolysis of the infected macrophages as well as the ATP production using Seahorse Extracellular Flux Analyzer. Macrophages, stimulated with distinct isolates of GBS also differed in the expression of genes involved in inflammation and metabolism and were differentially resistant to GBS-mediated cell cytotoxicity as measured by LDH release. The differences are evident both between serotypes and between isolates obtained from different specimens (colonizing from pregnant women or invasive from blood, cerebrospinal fluid). Thus, the data so far have shown that different GBS isolates appear to have different potential to become invasive or to remain colonizing. Furthermore, colonizing isolates seem to be more cytotoxic, whereas invasive isolates appear to use macrophages to their advantage.

Upregulation of retinoic acid-inducible gene-I-like receptors after electrotransfer of plasmid encoding Interleukin-12 in vitro

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Once exogenous nucleic acids enter the cytoplasm, infected host cells can sense them by binding and activating endosomal and cytosolic nucleic acid-specific pattern recognition receptors. The retinoic acid-inducible gene-I-like receptors (RLRs) are a family of cytosolic pattern recognition receptors that are essential for detecting viral RNA in the cytoplasm of both immune and non-immune cells, initiating the innate immune response that can lead to antitumor effects. Intratumoral interleukin-12 (IL-12) gene therapy has proved to be very effective in local tumor control and has a systemic effect. The transcribed IL-12 mRNA can potentially activate RLRs, thus the aim of our study was to evaluate the effect of gene electrotransfer of plasmid DNA encoding IL-12 on some of RLRs.

Parallel electrodes with a 2-mm gap and a clinically used pulse protocol were used for gene electrotransfer; 8 times 1300 V/cm pulses of 100-microsecond duration at a frequency of 5 kHz were applied with CLINIPORATOR®. Plasmid DNA encoding murine IL-12 (1 mg/ml, pmIL12), noncoding backbone plasmid DNA (1 mg/ml, pScramble), and a plasmid coding for a green fluorescent protein (1 mg /ml pEGFP, BD Biosciences Clontech, Palo Alto, California) were used for gene electrotransfer into B16-F10 melanoma and CT26 colon carcinoma cells. Transfection efficiency was determined 2 days after electrotransfer of pEGFP using fluorescence microscopy. Cell viability was determined 3 days after treatment using the PrestoBlue™ Cell Viability Reagent. The expression of three RLRs, a retinoic acid-inducible gene I (RIG-I), melanoma differentiation-associated protein 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2), was determined using qRT-PCR 2 days after treatment.

Transfection efficiency of pEGFP after gene electrotransfer was 24% in the B16F10 cell line and only 6% in the CT26 cell line. Significant viability reduction was obtained after pmIL12 and pScramble gene electrotransfer. Furthermore, the RIG-I sensor was significantly upregulated after pmIL12 plasmid gene electrotransfer in both cell lines, however, the upregulation of MDA5 and LGP2 sensors was observed only in the CT26 cell line. In conclusion, gene electrotransfer led to upregulation of different RLRs, most likely due to the transcribed mRNA for IL-12, however, noncoding backbone pScramble caused RLRs upregulation as well, thus further studies are needed to decipher the underlying mechanisms.

Keywords: gene electrotransfer; retinoic acid-inducible gene-I-like receptors; MDA5; LGP2; RIG-I

1 P

Aberrant autophagy and skewed inflammatory and tolerogenic functions in STAT1 gain-of-function dendritic cells

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Signal transducer and activator of transcription 1 (STAT1) gain-of-function (GOF) mutations underlie an inborn error of immunity called chronic mucocutaneous candidiasis. Beyond the fungal susceptibility, attributed to Th17 failure, over half of the reported patients suffer from autoimmune manifestations, the mechanism of which has not been explained yet. Dendritic cells (DCs) have been implicated in the pathogenesis of various autoimmune disorders, however, to date they have not been studied in STAT1 GOF CMC. We hypothesized that the STAT1 mutations would affect DCs' properties and alter their inflammatory and tolerogenic functions. To test the hypothesis, we generated monocyte-derived DCs (moDCs) and tolerogenic DCs (tDCs) from freshly isolated STAT1 GOF patients' monocytes cultivated in the presence of IL-4 and GM-CSF (moDCs), and tolerogenic factors vitamin D2 and dexamethasone (tDCs). Functional and signaling studies, co-culture experiments and RNA sequencing demonstrated that STAT1 GOF DCs were profoundly altered in their phenotype and functions, characterized by defective autophagy, proinflammatory skew and loss of tolerogenic functions. The results suggested that DCs play an important role in the immune dysregulation in STAT1 GOF CMC and may contribute to the disease-associated autoimmune manifestations via alteration in various cellular mechanism, including autophagic processes.

2 P

The changes of innate immunity during antiinflammatory diet in obese patients

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Introduction: The activation of the immune system may have a key role in the pathogenesis of metabolic disorders related to obesity. Obesity represents a chronic low-grade inflammation, which may cause many different metabolic disease (type 2 diabetes, non-alcoholic fatty liver disease, metabolic syndrome), following with increased risk for developing cardiovascular disease (CVD) and accompanied with the activation of the immune system. Adipose tissue disorders initiate changes in adipose tissue distribution and function, with effects on cytokines, chemokines, hormone expression and on the immune cell populations present in adipose tissue. NKT cells represent a unique subpopulation of lymphocytes, which share the properties of natural killer cells and conventional T cells. The most specific marker that distinguishes regulatory from conventional T cells is forkhead box transcription factor (Foxp 3). Predominant cell types that expressed Foxp 3 are CD4+CD25+ and characteristic of subpopulations which are Foxp 3 negative are their regulatory function lacked. Natural Tregs have the possibility to suppress multiple cell types involved in immunity and inflammation by inhibition the proliferation, immunoglobulin production, the blocking of NK and NKT-cell cytotoxicity, as well as, the function and maturation of dendritic cells. The Dietary Inflammatory Index (DII) was developed and validated as a scoring algorithm of 45 food parameters to investigate the inflammatory potential of an individual's diet. It has been shown that DII is valuable tool for assessing a diet's inflammatory potential. **Objective of this study:** To analyses the association between diet inflammatory potential, inflammation and innate immune response of obese people. **Patients and methods:** 81 obese patients were monitored during anti-inflammatory diet nutritional intervention (intervention subjects IS) and during Clinical Hospital Center Rijeka standard education protocol and energy nutritional restriction of the diet (control group -CG) in period of six months. **Results:** Both studied groups statistically significantly reduced markers of inflammation, hs-CRP, IL-6 and TNF- α . The innate immune response (the percentage of NK, NKT cells and Tregs) is significantly decreased in intervention group. **Conclusion:** Intervention with anti-inflammatory diet showed the greatest reduction of these parameters in IS subjects with the highest obesity degree. **Acknowledgement:** This work is supported by grant from the University of Rijeka (uniri-biomed-18-220).

3 P

Mechanobiology of lymphocyte motility

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Motility is a key feature of lymphocyte biology. From maturation to activation and effector functions, nearly all steps depend on leukocyte migration and correct positioning in lymphoid and non-lymphoid tissues. Mature T cells exit the thymus, recirculate in blood, and home to secondary lymphoid organs - lymph nodes (LNs) and the white pulp of spleen. After transmigration from blood to LN parenchyma, those antigen-unexperienced - naïve T cells, rapidly migrate and scan dendritic cells for presented antigens. Lymphocyte motility is seen as chemically triggered process. Chemokines or bioactive lipids are recognized by receptors on lymphocyte surface which trigger signalling cascades leading to cytoskeleton reorganisation. However, if mechanical inputs regulate lymphocyte motility is not known. To study the role of mechanosensation in lymphocyte biology, we adopted a reductionistic in vitro system to confine primary murine T cells. Mechanical confinement is introduced by silicon pillars of defined height, which deform naïve T cells (5 µm in diameter) to 3 µm. We show that sole mechanical confinement can trigger morphological polarization and persistent random migration. The main goal of this work is to establish qualitative and quantitative description of lymphocyte response to confinement and identify a molecular mechanism.

4 P

Interleukin-31 (IL-31) serum levels are increased in kidney allograft rejection

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Interleukin-31 (IL-31) is a member of IL-6 cytokine family defined by pro-inflammatory properties and signaling pathway of gp130 receptor subunit. IL-31 has been extensively studied for its role in inflammation and neuroimmune interactions, particularly in allergic dermatitis and other pruritic disorders. There are no available data on a role IL-31 in organ transplantation. In our prospective study, serum values of IL-31 were evaluated in patients with kidney allograft transplantation. The samples were collected before and then one week, one month and one year after the transplantation. In subjects with acute rejection, serum samples were collected also at the day of biopsy showing characteristic morphological changes confirming this diagnosis. A total of 94 patients with uncomplicated outcome were compared with 44 patients with acute rejection (16 with cellular rejection and 28 with antibody-mediated rejection). Concentrations of IL-31 were measured by an immunoluminescence assay (Luminex). Serum levels of IL-31 in patients with acute rejection were increased at one month ($p<0.001$) and one year after transplantation ($p<0.001$) and particularly at the day of biopsy positive for acute rejection ($p<0.001$). In contrast, pretransplant concentrations of IL-31 were higher in patients with uncomplicated outcome in comparison with subjects with acute rejection ($p<0.01$) and decreased during the first month after the transplantation ($p<0.001$). No association between the type of rejection and serum concentrations of IL-31 was found. Our data suggested that increased serum levels of IL-31 might be involved in acute rejection of kidney allograft. High concentration of proinflammatory IL-31 in samples obtained in the day of biopsy positive for acute rejection raises a possibility to use this cytokine as a new biomarker of immune response against the kidney allograft. Supported by Ministry of Health, Czech Republic - conceptual development of research organization („Institute for Clinical and Experimental Medicine – IKEM, IN 00023001“)

5 P

High-risk neuroblastoma therapeutics, topotecan and 13-cis retinoic acid, modulate autophagy through epigenetic changes in cord blood-derived hematopoietic stem and progenitor cells and 13-cis-retinoic acid potentiates monocyte activity.

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The therapy of neuroblastoma, the most frequent extra-cranial solid tumor in early childhood, targets important functions of tumor cells. While induction of their replication arrest and/or further differentiation are the ultimate therapeutic goals, the damage of other cell types forms an eventual risk in long-term perspective. Senescence of immune cells is characterised by decline of both innate and adaptive immune functions. Immunosenescence is associated with a number of pathologies linked to aging, such as higher susceptibility to infections and cardiovascular diseases, and has been described in childhood cancer survivors. Therefore, we hypothesize that intensive therapy including treatment with topotecan, induction treatment before stem cell harvest, and 13-cisRA, serving as maintenance therapy after hematopoietic stem cell transplantation, serve as inducers of accelerated

aging of immune system. In this study, we focused on effects of topotecan and 13-cisRA on cord blood-derived hematopoietic stem and progenitor cells (HSPCs) and on monocytes from healthy donors. 13-cisRA treatment potentiated cytokine production and decreased the amount of ROS by monocytes. We show that topotecan and 13-cisRA change the HSPCs' differentiation profiles and the expression of cyclin-dependent kinases' genes related to senescence was ongoing after in vitro expansion. Moreover, both, topotecan and 13-cisRA, manipulate metabolism through autophagy and sirtuins 1 and 3 expression. Altered differentiation/expression profiles found in HSPCs in vitro may represent long-lasting effects of topotecan and 13-cisRA that involve epigenetic regulation in HSPCs and thus, these therapeutics might potentially alter the immune system function in survivors later in life.

6 P

Immunity following SARS-CoV-2 vaccination in autoimmune neurological disorders treated with rituximab or ocrelizumab

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Introduction: B cell-depleting therapy targeting CD20 molecule with rituximab (RTX) or ocrelizumab (OCR) affects the humoral immune response after vaccination. It remains unclear whether these therapies influence T-cell-mediated immune response against SARS-CoV-2 after immunization.

Aims: We aimed to evaluate the humoral and cellular immune response to the COVID-19 vaccine in a cohort of patients with multiple sclerosis (MS), neuromyelitis optica spectrum disorders (NMOSD), and myasthenia gravis (MG). **Methods:** Patients with MS (83), NMOSD (19), MG (7) under RTX (n=47) or OCR treatment (n=62) were vaccinated twice with mRNA BNT162b2 vaccine. Antibodies were quantified using the SARS-CoV-2- IgG chemiluminescence immunoassay targeting the spike protein. SARS-CoV-2-specific T-cell responses were quantified by interferon γ release assays (IGRA). Immunocompetent vaccinated individuals (n=41) were included as controls. **Results:** Almost all immunocompetent controls developed antibodies against the SARS-CoV-2 trimeric spike protein, but only 42.05 % of the patients under anti-CD20 (RTX or OCR) treatment were seroconverted. There was no correlation between circulating B cells and the levels of antibodies. Even patients with a low proportion of circulating CD19 B cells (<1%, 71 patients) had detectable SARS-CoV-2 specific antibody responses. This response was even higher in patients with longer than 3 weeks intervals of vaccination. SARS-CoV-2 specific T cell response measured by released interferon γ was detected in 94.39 % of the patients, independently of a humoral immune response. **Conclusions:** The majority of MS and NMOSD patients developed SARS-CoV-2-specific T cell response. The data suggest that vaccination can induce SARS-CoV-2-specific antibodies in a part of anti-CD20 treated patients. The response represented by levels of antibodies was better in individuals, who completed vaccination within more than 3 weeks.

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7 P

Sertoli cell – The cardinal intermediary of fertility

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Nowadays, it becomes harder and harder for couples to conceive, where men infertility accounts for approximately 45-50% of cases. In around 20% of these cases, the cause is unknown (idiopathic infertility). Corresponding factors could be lifestyle decisions, such as smoking and/or alcohol and drug abuse, but also infections or radiation.

SCs are a specialized type of somatic cells actively involved in spermatogenesis. Besides, they are also known for their immunomodulatory properties during homeostatic conditions within the testes, where they create an immunologically privileged site and protect germ cells. This protection and immune homeostasis is disrupted during infections or chronic inflammatory states, leading to reduced spermatogenesis. Since SCs possess anti-inflammatory properties, we performed a SCs transplantation in a mouse model of LPS-induced inflammation, in order to alleviate the inflammatory damage to the testicular tissue and preserve spermatogenesis. SCs decreased immune cells' infiltration, especially Ly6G+ cells. Using multi-color flow cytometry, the innate immune landscape within the testis was monitored with emphasis on the most abundant subpopulations, macrophages and monocytes. Markers for myeloid populations phenotyping included MHC II for M1 macrophages, and CD206 and MERTK for M2 macrophages. The positivity of cells for CD11b, F4/80, or Ly6C was used to discriminate infiltrating vs. resident subpopulations. The presence of inflammatory and anti-inflammatory cytokines together with testosterone (Leydig cell function) and inhibin B (SCs function) in serum and the testicular homogenate was assessed by ELISA tests. Germ cell viability and apoptosis were detected by phosphatidylserine positivity via Annexin V and Hoechst33258. To get information about mice fertility, the epididymis of each male was washed and spermiogram was evaluated by the CASA system. In the presence of SCs, the fitness of sperm cells improved. It is a long road to reach the "ideal" treatment for male infertility. In this study, we aimed to suppress the pathological effect of inflammation on testicular tissue and spermatogenesis via SCs transplantation. The effects of SCs transplantation included decreased immune cell infiltration and improved spermiogram results. We, therefore, propose that SCs may be the cell type of choice in treating testicular pathologies involving inflammation.

8 P

Changes in the immune system during cold acclimatization

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Thermogenesis is essential for maintaining a constant temperature and for preservation the physiological processes of organisms at a reduced temperature. The first phase of exposure to cold is associated with stress reaction; subsequently cold tolerance is induced. The acclimatization process is mainly related to the formation of brown adipose tissue and the white adipose tissue beiging, in which cytokines and immune cells play an essential role; however, immune cell dynamics at the systemic level have not yet been described. The study aims to describe the mechanisms of cold adaptation associated with the immune system. In this study, we monitored the presence and activation of individual immune cell populations at different time points of cold acclimatization. Significant changes in immune response to inflammatory stimuli indicated complex systemic modulation of immune reactions and were associated with altered metabolism of immune cells. We also described differences in cytokine production in various tissues, including spleen, white and brown adipose tissue, and systemically in the serum of cold-acclimatized rats. Control of thermogenesis has been associated with a homeostatic population of $\gamma\delta$ T-cells in brown adipose tissue. Accordingly, we observed an increase in the percentage of $\gamma\delta$ T-cells producing IL-17 and TNF α in the spleen, peripheral blood, and peritoneal cavity of cold-adapted rats. These results have also been confirmed in humans. Compared to controls, volunteers who regularly bathed in ice water showed an increased percentage of $\gamma\delta$ T-cells, including an altered phenotype of these cells and a difference in the levels of various cytokines. Altered cytokine production was also observed following inflammatory stimuli of PBMCs in vitro. Uncovering the immune-related processes associated with cold and subsequent metabolic changes may help develop therapeutic approaches to various diseases, such as obesity or metabolic disorders.

9 P

Acute- and late-stage Lyme disease patient's sera reactivities with distinct morphological forms of *Borrelia burgdorferi* sensu lato bacteria.

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Spirochetes of the species complex *Borrelia burgdorferi* sensu lato (s.l.), the causative agent of Lyme disease, could undergo transition into several morphological forms, such as spherical bodies (sometimes called „cysts“) or aggregates, in addition to the classic spirals. The possible role of these forms in the pathogenesis of the *Borrelia* infection is still unknown, but the subject of discussion is their role in the development of persisting symptoms in some patients. According to published hypotheses, these forms could assist the bacteria to escape from the mechanisms of the immune system or, on the contrary, could induce an immunopathological reaction.

In our study, we tested patients in the acute phase of *Borrelia* infection (n=30), patients after passing the late phase of infection (n=30) and persons with a negative Lyme disease laboratory (n=30) concerning to their serum reactivity with various in-vitro induced morphological forms of *B. burgdorferi* s.l. Spherical forms were obtained by cultivation in the presence of ampicillin, aggregates were obtained from aged cultures and helical forms were obtained from log phase culture. We used one strain each of the subspecies *B. burgdorferi* sensu stricto (s.s.), *B. afzelii*, *B. garinii* and *B. bissetti*. Specific IgG were determined by the ELISA using plates coated with detergent and sonication generated lysates of individual *Borrelia* forms.

The sera of both Lyme disease patients groups exhibited highest reactivity with *Borrelia* aggregates, with statistically significant difference detected for 2 out of 4 tested *Borrelia* strains. Sera of late phase Lyme disease patients showed higher reactivity than acute phase sera. The differences were significant for all but one subspecies (*B. burgdorferi*). The difference between the response of patient groups and negative controls was significant for all *Borrelia* strains and morphological forms.

Our work showed differences in the antigenicity of alternative morphological forms of spirochetes of the species complex *B. burgdorferi* s.l., however, due to the relatively small differences and variability of the results for individual strains, the clinical relevance of this phenomenon remains to be determined further.

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10 P

Immune response to gut fungal microbiota in inflammatory bowel disease.

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Inflammatory bowel disease (IBD) is a term for chronic inflammatory disorders, divided into Crohn's disease (CD) and ulcerative colitis (UC). Despite intensive efforts, IBD mechanisms are not fully understood. Gut barrier dysfunction, dysbiosis, and impaired immune response to gut microbiota appear to have a significant impact. While bacteria are the most studied gut inhabitants, research on the role of fungal microbiota (mycobiota) in IBD lags behind. The study aimed to reveal immune responses to gut mycobiota in IBD. Levels of antifungal antibodies and biomarkers relevant to gut barrier damage and microbial translocation were measured in the sera of patients with UC, CD, and IBD associated with primary sclerosing cholangitis (PSC-IBD). Since continuous intestinal macrophage activation is a feature of IBD, the effect of fungal mycobiota species on the reactivity of peripheral blood-derived macrophages from healthy donors was assessed in vitro. The levels of serum antibodies against mycobiota components were higher in IBD patients than in controls, and the most remarkable differences were detected in CD. Serum biomarker levels connected to microbial translocation were also elevated in IBD patients. Macrophages demonstrated no distinct polarization after stimulation with selected fungal species. However, increased production of pro-inflammatory cytokines was observed. Altogether, the results indicate the important role of mycobiota in IBD pathogenesis.

11 P

Early postnatal supplementation with probiotic strain *E. coli* O83:K24:H31 decreases inflammatory responsiveness of intestine-associated professional antigen-presenting cells and drives endotoxin tolerance.

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The hygiene hypothesis suggests that stimulation of the immune system during a postnatal period can prevent the development of autoimmune diseases and allergies. Interactions between the immune system and microbes in newborns are crucial for the adequate setting of immune response and tolerance later in life. Endotoxin tolerance is a phenomenon firstly described in septic shock caused by Gram-negative bacteria. The robust immune system activation after endotoxemia results in decreased inflammatory responsiveness of the immune system to another stimulus. It was shown that postnatal supplementation of children with the probiotic strain of *E. coli* O83:K24:H31 (EcO83) prevents the development of allergies later in life. In our previous work, we showed that the early postnatal colonization with EcO83 sets the tolerogenic environment in mice pups with increased expression of IL10 and genes involved in the formation of tight junctions. In this work, we want to test if the mechanism of the EcO83 tolerogenic function is caused by decreased responsiveness of intestine-associated immune cells to the secondary inflammatory stimulus after the first EcO83 encounter, in a similar manner to endotoxin tolerance. In vitro experiments are performed with bone marrow-derived dendritic cells. We isolate cells from mesenteric lymph nodes from treated and untreated newborn mice as well. We utilise ELISA, qRT-PCR, and flow cytometry to investigate the differences in the production of proinflammatory cytokines IL-1 β , TNF- α , IL-6 and IFN- γ after the secondary stimulus. We are also measuring the secretion of anti-inflammatory IL-10 and the surface expression of costimulatory molecules CD80 and CD86. It is possible that endotoxin tolerance is one of the mechanisms responsible for the EcO83 tolerogenic effect on the immune system of a newborn. This work was supported by Charles University research program GAUK 478119 and IMMU 207032.

12 P

Exopolysaccharide isolated from *Lactisacibacillus rhamnosus* LOCK900 modulate immune response in mouse model of allergic airway inflammation.

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Introduction: The increasing prevalence of allergic diseases in industrialized countries during recent decades has been correlated with reduced microbial burden in western population. Therefore, the use of probiotic bacteria and their bacterial cell components for modulation of allergic immune response has been introduced as an interesting concept. **Methods:** Two-month old BALB/c female mice were two-times sensitized by intraperitoneal injection of OVA and asthma-like symptoms were induced by intranasal application of four doses of OVA challenge. Exopolysaccharide from *Lactisacibacillus* (L.) *rhamnosus* LOCK900 (EPS) was applied intranasally before each ovalbumin challenge. One day after the last challenge, mice were sacrificed and asthma-like symptoms were evaluated by differential cell counting in bronchoalveolar lavage (BAL), histological staining of lung tissue, determination of humoral response in sera and cytokine response in splenocyte cultures and BAL fluid.

Results: Intranasal application of EPS before OVA-challenge decreased number of eosinophils in BAL and reduced the infiltration of cells in lung tissue assessed by histological staining. OVA-specific humoral immune response in sera of treated mice were not affected by EPS administration, whereas the production of OVA-specific cytokines IL-4, IL-5 and IL-13 was downregulated in spleen cell cultures and BAL fluid. Moreover, we detected significantly enhanced level of TGF- β and total IgA in BAL fluid and IgA-producing cells in lung tissue of EPS-treated mice.

Conclusion: Exopolysaccharide isolated from *L. rhamnosus* LOCK900 is able to modulate allergic immune response in mouse models of asthma and stimulate IgA production in lung tissue. Intranasal administration of these bacterial antigens with a defined structure and biological properties helps to elucidate the mechanism of bacterial-host interaction and represents a promising new strategy in the treatment of allergies.

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13 P

Ketogenic diet decreases severity of experimental autoimmune encephalomyelitis.

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Ketone bodies produced in the gut from dietary fiber by gut bacteria have potent anti-inflammatory properties. Ketogenic diet leads to production of ketone bodies, especially β -hydroxybutyrate, which interfere with

inflammatory processes in a similar way. Here, we studied the effect of the ketogenic diet in mouse model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE).

C57BL/6 mice fed with ketogenic or standard diet starting 14 days before EAE induction with myelin oligodendrocyte glycoprotein peptide (MOG35-55) mixed with complete Freund's adjuvant, together with intraperitoneal injection of Bordetella pertussis toxin. The disease development was monitored for three weeks, and the state of T cell activation was analyzed in mesenteric or lumbar lymph nodes by flow cytometry.

Ketogenic diet delays EAE onset and significantly decreases its severity. Nevertheless, this mitigation is not accompanied with significant increase in regulatory T cells or decrease in major pro-inflammatory cytokines employed in EAE (IFN- γ , IL-17A or TNF- α) produced by T cells. Polarization of CD4⁺ cells into Th1 and Th17 phenotypes, and populations of CD4⁺ and CD8⁺ T cells within mesenteric and lumbar lymph nodes also remain unaffected by the diet.

We conclude that ketogenic diet decreases severity of EAE by yet unrecognized immunomodulatory mechanism.

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14 P

ELANE mutations causing severe congenital neutropenia in humans, but induce only a weak neutropenic phenotype in mouse.

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Neutropenia is a hematological condition in which there is a reduced amount of neutrophils (PMN), which are one of the main cells of the immune system. One of the reasons for the reduction in the amount of PMN (below 1.5×10^9 / L of blood) in severe congenital neutropenia are genetic mutations usually within genes associated with serine proteases. Patients with neutropenia tend to suffer from severe, recurrent infections. In the case of genetic mutations, apart from the reduced number of PMNs, the effectiveness of neutrophils in combating pathological conditions might be also lower. We use the mouse model with the mutation C70W and G214R in ELANE gene, which are the same to those observed in patients with neutropenia, to describe impact of mutations on PMN number and functionality. Surprisingly, our results show that neutrophils from C70W and G214R mice mutants show weak phenotype. Only in C70W mutants we observe a modest 10% decrease of PMN. However, C70W mutant strain has higher baseline activation level in comparison to wild type. The additional conducted functional assays, so far, show no significant differences in functionality related to phagocytosis, NETs induction, ROS inductions, apoptosis or bacteria killing between neutrophils from mutant and wild-type strains. *Key Words:* Neutrophils, Neutropenia, Mice, mutation, phenotype

15 P

Viability Status-Dependent Effect of Bifidobacterium longum ssp. longum CCM 7952 on Prevention of Allergic Inflammation in Mouse Model.

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Background: The classical definition of probiotics states that bacteria must be alive to be beneficial for human organism. However, recent reports show that inactivated bacteria or their effector molecules can also possess such properties. *Methods:* Female BALB/c mice 6–8-week-old were i.p. sensitized with 10 μ g of OVA + Alum on days 1 and 14 of experiment, boosting dose of 15 μ g of OVA + Alum was administrated on day 21. Seven days after third immunization, the challenge with 100 μ g of OVA was performed for 4 consecutive days. Untreated (UN BI 7952) or heat-treated (HT BI 7952) Bifidobacterium longum ssp. longum CCM 7952 in dose 1×10^7 CFU/30 μ l or PBS were administered intranasally 4 h before each sensitization and challenge.

Results: In vivo experiments confirmed that untreated BI 7952 exhibited allergy-reducing properties when administered intranasally to OVA-sensitized mice, which manifested in significant suppression of airway inflammation. Untreated BI 7952 decreased local and systemic levels of Th2 related cytokines, OVA-specific IgE antibodies and simultaneously inhibited airway eosinophilia. In contrast, heat-treated BI 7952 was only able to reduce IL-4 levels in the lungs and eosinophils in bronchoalveolar lavage, but increased neutrophil and macrophage

numbers. *Conclusion:* We demonstrated that the viability status of BI 7952 is a prerequisite for the beneficial effects of bacteria, and that heat treatment reduces but does not completely abolish these properties.

16 P

Long-term Covid-19 antibody dynamics in fully vaccinated individuals

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Background: The efficacy of two doses of the severe acute respiratory coronavirus 2 (SARS-CoV-2) spike messenger RNA vaccines (BNT162b2 [Pfizer] and mRNA-1273 [Moderna]) in preventing symptomatic SARS-CoV-2 infection has been shown to be high. Vaccine-induced population immunity is a key global strategy to control SARS-CoV-2 infections. However, the potential waning of the vaccine-induced immune response through time and the emergence of new SARS-CoV-2 strains is making this goal more and more elusive.

Methods and Results: As part of the national vaccination strategy, all individuals working at University Clinic Golnik were offered to receive vaccination with Pfizer vaccine at December 2020. All were offered the measurement of IgG antibody levels after receiving the second dose. The quantitative measurement of SARS-CoV-2 anti S1 IgG antibodies was done using IDK anti-SARS-CoV-2 ELISA Kit (Imundiagnostik, Germany). Antibodies were measured in ng/ml and can be converted into international units e.g. binding antibody units per millilitre (BAU/mL), which are traceable to the WHO International Standard for anti-SARS-CoV-2 immunoglobulin. To obtain the concentration in binding antibody units (BAU)/ml, results in ng/ml were divided by 20 (20 ng/ml $\triangleq$ 1 BAU/ml).

The research group consisted of 285 healthy adults. They were fully vaccinated with Pfizer vaccine with second dose applied after 21 to 23 days after the first dose and consented to blood draws for IgG quantification. We evaluated the percentage of individuals with anti-S1-IgGs over the range of 20.000 ng/ml ($\geq$ 1.000 BAU/ml) since according to manufacturer recommendations individuals with antibody level over 1.000 BAU/ml has full protection against SARS-CoV-2. In the first subgroup (3-6 months after the complete primary course of vaccination), all 18 (100%) individuals with no prior Covid-19 infection had anti-S1-IgG levels lower than the cut-off 1.000 BAU/ml, among previously infected with Covid-19, 11 (37.9%) had IgGs higher than the cut-off value. In the second subgroup (6-10 months after the complete primary course of vaccination) one (0.5%) individual in the group with no prior Covid-19 infection reached level above 1000 BAU/ml (no positive PCR available in between to confirm additional infection with SARS-CoV-2 to prove high level of IgGs), whereas in the group with previously infected individuals 8 (15.1%) reached the level of anti-S1-IgG antibodies higher than 1.000 BAU/ml.

Both groups (3-6 and 6-10 months after the complete primary course of vaccination) reached statistical significance ($p < 0.0001$) when compared individuals with no prior infection vs prior infected with Covid-19 with median 4.023 ng/ml, 12.738 ng/ml and 2.933 ng/ml, 6.377 ng/ml, respectively. Statistical significance was also reached in a group overall (3-10 months after the complete primary course of vaccination), ($p < 0.0001$) with median 2.933 ng/ml and 8.775 ng/ml when compared no prior infected vs prior infected with Covid-19.

Conclusions: In the current study we present and confirm the evidence that prior COVID-19 infection affects long-term dynamics of anti-S1-IgG antibodies after full vaccination with mRNA (BNT162b2) vaccine. We demonstrated that vaccination with mRNA vaccines offers protection to avoid infection with SARS-CoV-2 virus or at least when infected, enables the severe outcome of the Covid-19 disease when followed adopted population-based vaccination program.

17 P

The effect of the p.o. Transfer factor on interferon- γ production

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Introduction: Leukocyte dialysate, used under the therapeutic name "Transfer factor", is an immunomodulatory drug that has been used for decades in the treatment of patients with secondary immunodeficiency in cell-mediated immunity. One of its most important antiviral mechanisms is the induction of interferon- γ production. The major sources of IFN- γ are natural killer (NK) cells, T cells and NKT cells, and its receptor is expressed ubiquitously on almost all cell types. Interferon- γ plays a central role in protection against viruses and other bacterial microorganisms. It activates macrophages and stimulates specific cytokine immunity. Its production begins in the early stages of infection and signals the initiation of an antigen-specific adaptive immune response. A new p.o. formulation is currently available with the potential to replace the older injectable form. *Methodology:*

The study included 124 patients examined in the Outpatient Clinic of Clinical Immunology with suspected secondary immunodeficiency in cellular immunity. For each patient, the "resting concentration" (without Transfer factor stimulation) of interferon- γ in serum was examined, followed by the concentration of IFN- γ after 24 hours of stimulation of the whole blood with the medicinal product. For the determination of interferon gamma concentration, the commercial kit of Euroimmune – IGRA SARS-CoV-2 was used in the study. The study was approved by an ethics committee of the NHH. *Results:* We demonstrated a statistically significant increase in interferon- γ concentration after stimulation of the whole blood with the medicinal product. This increase was observed in 95% of the patients examined. More than 3-fold increase in IFN- γ concentration after Transfer factor stimulation was observed in 92% of patients. *Conclusion:* We have demonstrated the efficacy of a novel form of immunomodulatory drug for induction of interferon- γ production. Thus, its successful use in the treatment of patients with secondary immunodeficiency in cell-mediated immunity can be expected.

18 P

Modulation of Notch signalling pathway in activated hepatic stellate cells has no influence on development or resolution of hepatic fibrosis

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We used transgenic mice to investigate the effect selective Notch inhibition or forced activation in activated hepatic stellate cells (aHSCs) has on liver fibrosis. We used two models, carbon tetrachloride (CCl₄) treatment and DDC-supplemented diet. The effect of Notch inhibition was investigated in SMACreERT2+/-/RBP-Jfl/fl mice, while Notch activation was studied using SMACreERT2+/-/RosaNICD1 mice. Mice were treated with 75 μ g/g of tamoxifen i.p. twice weekly. Identically treated Cre- littermates served as controls. CCl₄ and DDC caused a similar degree of fibrosis (Sirius red stained area, hydroxyproline content, aminotransferase activities, gene expression of Col1a1 and Acta2) in experimental and control groups in Notch-inhibition as well as Notch-activation experiments. We further tested whether forced Notch activation in aHSCs delays resolution of fibrosis. SMACreERT2+/-/RosaNICD1 mice were treated with either CCl₄ or DDC, and tamoxifen for 10 days, and then recovered for one month. Recovery was similar in both SMACreERT2+/-/RosaNICD1 and SMACreERT2-/-/RosaNICD1 mice, suggesting that forced Notch activation doesn't impair recovery. We also found that tamoxifen per se protects against DDC-induced fibrosis. This was evidenced by a lower Sirius red-stained area, lower hydroxyproline content, and further confirmed by qPCR showing a lower expression of genes for Col1a1, Acta2, Sox9, Pdgf, and Krt19, indicating the inhibitory effect on HSCs, collagen production, and biliary duct proliferation. This effect highlights the importance of a proper Cre- littermate control in this investigation. We conclude that modulation of Notch activity in aHSCs neither changes the degree of liver fibrosis nor does it impair recovery once the noxious stimulus is withdrawn.

19P

The role of IgG4 in immune response

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The IgG4 subclass represents less than 5% of total IgG in healthy subjects, individual level is very stable. The IgG4 molecule is characterized with several important structural and functional differences in comparison with other IgG subclasses and Ig isotypes: 1) "Fab-arm exchange" phenomenon, 2) inability to form classical immunocomplexes (suppression of pro-inflammatory immune response), 3) IgG4 Fc-part interaction with Fc-part of other IgG subclasses ("inactivating RF"?).

Hypothesis: "The importance of IgG4 as an anti-inflammatory factor of immune response".

IgG4 physiology: IgG4 synthesis during long-term antigenic activation in correlation with consideration of "blocking IgG4" in specific allergen immunotherapy. Regulatory T cells (Treg) discovery fundamentally changed theoretical background of understanding of immune response regulation, but the role of "IgG4 blocking antibodies" has remained. Pathophysiology of IgG4: IgG4-related diseases (IgG4-RD) have brought a lot of findings, where TGF β plays a pathognomic role (important for Treg differentiation, also fibrocytes differentiation with collagen production and tissue fibrotization). There was (in respect of cytokines expression) the significant difference

between Sjögren's syndrome and Mikulicz's disease (MD) found: elevated levels of Th2 cytokines are characteristic of both diseases, while IL-10 and TGF β were significantly expressed only in MD. Synergistic effect of Th2 polarization and Treg induction is expected with IgG4 increased production. Novelty in IgG4 pathophysiology area seems to be the group of autoimmune diseases, where pathognomic role of antigen-specific IgG4 is demonstrated (autoimmune encephalitis, blistering skin disease etc.), where IgG4 is blocking „receptor-ligand interaction“ of the immune system effector functions. In conclusion, IgG4 remains a mysterious antibody with unique characteristics, an antibody that can exhibit both protective and damaging effect.