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Skin colonization with *S. aureus* can lead to increased inflammasome activation in patients with atopic dermatitis

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Atopic dermatitis (AD) and psoriasis (PS) are inflammatory skin diseases and one of the features of AD is dominant colonization with *Staphylococcus aureus* (*S. aureus*). Inflammasomes are protein complexes that contribute to host defense against pathogens and cleave the cytokines pro-interleukin (IL)-1 β and pro-IL-18 into their secreted active forms. In the current study, we measured the expression of IL-1 family members and inflammasome components in the skin of PS and AD patients and studied their regulation by cytokines, *S. aureus* and *S. epidermidis* in human primary keratinocytes by RT-qPCR, ELISA, and immunofluorescence. We show that the expression of *IL1B* and *IL18* is increased in the lesional skin of PS and AD patients, respectively, while inflammasome components, *NLRP1*, *NLRP3*, *PYCARD*, and *CASP1* are increased in these diseases. A positive relationship between *NLRP1* and *IL1B* was found both in AD and PS, but between *NLRP1* and *IL18* expression, only in AD. Accordingly, increased caspase-1 activity in the skin of AD patients was detected. Interestingly, in keratinocytes, the inoculation with *S. aureus*, but neither *S. epidermidis* nor tested cytokines led to the increased secretion of IL-1 β and IL-18. In addition, we performed colocalization analysis using datasets from two GWAS and 112 eQTL studies to identify variants that are associated with AD and can alter gene expression. Among 148 genes identified to be associated with AD, we detected *IL1RL1*, *IL18*, *IL18R1*, *IL18RAP* as AD-related genes with expression in multiple immune-system-related cell types. Together, our data indicate the increased inflammasome activation in the skin of AD patients, which could be a result of higher levels of inflammasome genes in the skin, colonization with *S. aureus*, and genetic variations in the IL-1 family cytokines and their receptors.

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Profiling the effects of acne therapeutics, including the novel narrow-spectrum antibiotic sarecycline, on the human microbiota

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Non-invasive skin-surface biomarkers for psoriasis monitoring

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In clinical dermatology there is a need for non-invasive efficient and accurate diagnostic tools that can objectively measure psoriasis (PS) activity and can be used to monitor therapeutic effects of PS treatment. This exploratory study aimed to determine whether non-invasive measurements of proteins from PS lesional skin can be used to assess disease severity and measure treatment efficacy. BM measurements from skin were performed using non-invasive Transdermal Analysis Patch (TAP) capture antibody microarrays detecting IL-1 α , IL-1RA, CXCL-1/2, hBD-1 (ng/ml). Captured BM were visualised using spot-ELISA. TAPs were applied to non-lesional (NL) and lesional (L) skin of PS patients (N=30). PASI and local inflammation scores of lesions were documented; thickness of epidermis, dermis, subepidermal low-echoic band (SLEB) was analysed using ultrasound (US) at the same skin site as TAP measurements and clinical scores. Potential of these skin-surface BM was further studied by monitoring skin lesions of PS patients (N=14) undergoing UVB (311nm) therapy using TAP together with clinical scores for PS severity. Skin surface measurements of PS patients revealed clear differences of IL-1 α , IL-1RA, CXCL-1/2 on L skin when compared to NL. Comparing BM expression patterns with clinical scores of PS skin following correlations were observed: between CXCL-1/2 and lesion thickness ($p < 0.05$), between CXCL-1/2 and SLEB thickness ($p < 0.001$) on L; IL-1RA and SLEB thickness ($p < 0.05$) of analysed NL skin. During UVB treatment, clear patterns towards normalisation of IL-1RA and CXCL-1/2 levels were noted on L which was in accordance with clinical parameters. Skin surface analysis of IL-1RA and CXCL-1/2 displayed a different profile than achieved by visual scoring of local inflammation or US - confirming that measuring the 'molecular root' of inflammation appears to have value as an objective, non-invasive BM measurement for scoring disease severity in its own right.

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Impaired clearance of senescent fibroblasts from aging skin: Role of compromised natural killer cells

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Aging is a physiological process characterized by progressive loss of integrity, impairment in tissue/organ function and increase susceptibility to age-related diseases. Cellular senescence, a state of permanent cell cycle arrest, is thought to be one of the causative processes of tissue aging and age-related disorders. Fibroblasts, which constitute a principal component of connective tissue rich organs, play a pivotal role in organ homeostasis and aging. We previously showed that senescent fibroblasts profoundly accumulate in the skin during aging. Under some transient conditions in mammals and amphibians with high regenerative potentials, senescent cells can be successfully removed by cells of the innate immune system such as Natural Killer (NK) cells. We here wanted to understand whether senescent fibroblasts in aging skin are resistant to removal by NK cells or, alternatively, NK cells themselves are compromised to remove senescent fibroblasts. Using a newly developed NK cell mediated killing assay, we found that NK-92 cell line and primary human and mouse NK cells preferentially kill senescent fibroblasts, while primary NK cells isolated from old human donor (~70 years) or old mice (~700 days) are profoundly less efficient in killing senescent fibroblasts as opposed to those isolated from young human donor (~23 years) and young mice (~100 days), respectively. Of note, there is no change in the absolute number of NK cells in peripheral blood and skin between young and old healthy individuals. Furthermore, at the synapse between target cells and NK cells the release of granzyme B is significantly reduced in aged NK cells. This is relevant as granzyme B, the effector molecule, which through pores formed by perforin on the target cell membrane enter the target cell to initiate their apoptosis. These data contribute to advancing our understanding of mechanisms underlying senescent cell accumulation, and may be exploited for future senolytic therapies.

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The role of PLC γ 2 in the *in vitro* activation of neutrophils by type VII collagen immune complexes

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Phospholipase C γ 2 (PLC γ 2) is a protein expressed predominantly in hematopoietic cells and has a great importance in immunoreceptor signaling. Our group previously found that PLC γ 2 deficient mice were protected from autoantibody-mediated skin blistering model induced by the systemic injection of antibodies against type VII collagen (C7), which is located in the basement membrane. The inflammation mediated mainly by neutrophils was completely abrogated in PLC γ 2 deficient mice, but the mechanism is presently unknown. We aimed to examine the expression of PLC γ 2 in the main circulating leukocyte populations and investigate the *in vitro* activation of PLC γ 2 deficient neutrophils by C7-anti-C7 immune complexes. Expression of PLC γ 2 in circulating leukocyte populations was measured by flow cytometry after intracellular labelling. We used bone marrow derived neutrophils to examine *in vitro* cell activation plated onto immobilized C7-anti-C7 immune complex surfaces. Type VII collagen or anti-C7 served as control surfaces. Cell spreading was evaluated using phase-contrast microscopy. Production of reactive oxygen species (ROS) was measured in a cytochrome-C based reduction assay. PLC γ 2 was present in neutrophils, eosinophils, monocytes and B cells but not in T cells of the wild type mice. While examining the effector functions of neutrophils activated by C7-anti-C7 immune complexes, we found that nearly a third of the cells of the wild type mice spread and a great amount of ROS was produced. On the contrary, both neutrophil spreading and ROS production were completely absent in immune complex-treated PLC γ 2 deficient cells. Our results show that the PLC γ 2 is expressed in the major myeloid cell populations-expression of the circulating immune cells and plays a crucial role in the spreading and ROS-production of neutrophils activated by C7-anti-C7 immune complexes *in vitro*, which can explain why PLC γ 2 deficient mice are protected in autoantibody mediated skin blistering.

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Primary Changes in the Development of Hidradenitis Suppurativa

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Hidradenitis suppurativa (HS) is a chronic, inflammatory skin disease of the apocrine gland-rich (AGR) skin region. Although the immune alteration in lesional HS skin is well-characterized the initial steps of the disease are not fully understood. We aimed to investigate the immune characteristics of non-lesional HS skin, representing the first stage of HS development, and to compare that with lesional HS skin as well as healthy AGR region. Our main focus was to study the immune components showing significantly different levels between AGR and lesional HS skin samples in our previously published report. Immune cell counts (T cells, dendritic cells, macrophages), T helper (Th)1/Th17-related molecules (IL-12B, TBX21, IFNG, TNFA, IL-17, IL-10, IL-23A, TGFB1, RORC, CCL20), keratinocyte (KC)-related sensors, mediators (S100A7, S100A8, S100A9, DEFB4B, LCN2, CAMP), proinflammatory molecules (TLR2, TLR4, IL-1B, IL6, TNFA, IL-23A) and chemokines (CCL2) were investigated in the three sample groups by multiple mRNA (RNAseq and RT-qPCR) and protein-based (IHC and IF) methods. According to our findings, when comparing non-lesional HS and AGR skin, KC-derived antimicrobial peptides were highly upregulated in the non-lesional HS skin. The epidermal occurrence of IL-1 β , TNF- α and IL-23 were also significantly increased in the non-lesional HS epidermis but not in the dermis. On the contrary, no significant differences were detected regarding the dermal cellular components and Th1/Th17-related mediators. In summary, our results indicate KCs as crucial players in the first stages of HS development since all the investigated KC-derived molecules' expression were increased even in non-lesional HS skin in contrast to the (adaptive) dermal components which showed similar levels in AGR and non-lesional HS skin but were prominently elevated in lesional HS skin in parallel with the further enhancement of the innate immune components.