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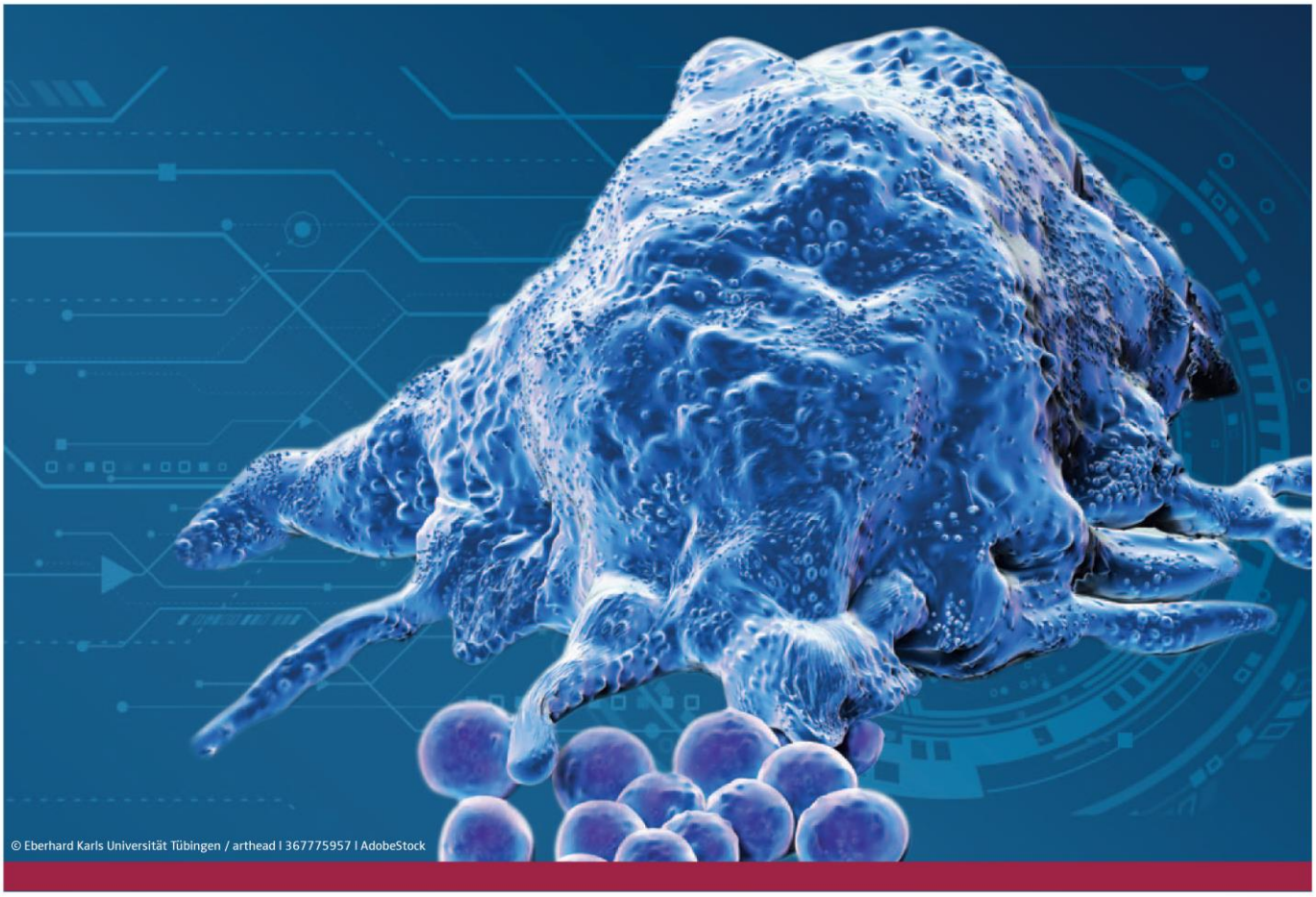

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NOVEL CONCEPTS IN INNATE IMMUNITY

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ABSTRACTS

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SCL 1 I Pattern recognition receptors and inflammasomes

SCL 1-1

How cells defend their cytosol against invasive bacteria

Felix Randow[1]
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Intracellular pathogens colonize specific subcellular niches determined by the pathogen's requirement for host-derived nutrients and antagonized by compartment-specific immunity. Most intracellular bacteria dwell in phagosomes and only few species have succeeded in conquering the cytosol, a perhaps counterintuitive situation given the abundance of nutrients freely available in the cytosol. Potent cytosolic defense mechanisms must therefore exist. I will discuss how cells defend their cytosol against bacterial invasion through autophagy, triggered either upon sensing membrane damage caused by bacterial entry into the cytosol or through the deposition of ubiquitin on the bacterial surface.

SCL 1-2

Activation of antitumor immunity by bacteria-derived signals - pyroptosis & beyond

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Pyroptosis is a proinflammatory cell death executed by the gasdermin-family pore-forming proteins. Among the family, gasdermin D (GSDMD) is cleaved by inflammasome-activated caspase-1 and LPS-activated caspase-11/4/5. The cleavage unmasks the pore-forming domain in GSDMD that perforates plasma membrane. Using a bioorthogonal chemical biology approach allowing controlled delivery of active gasdermin into tumors in mice, we found that pyroptosis of < 15% tumour cells could clear the entire 4T1 mammary tumourgraft, which was absent in immune-deficient mice or upon T-cell depletion. Thus, pyroptosis stimulates potent and effective antitumour immunity. In antitumor immunity, cytotoxic lymphocyte relies on granzymes to kill target cells. We found that natural killer cells and cytotoxic T lymphocytes kill GSDMB-positive cells through pyroptosis, mediated by granzyme A (GZMA) cleavage of GSDMB. IFN-γ upregulates GSDMB expression and promotes pyroptosis of cancer cells including that by CAR-T/TCR-T cells. Thus, gasdermin-executed pyroptosis serves as a cytotoxic lymphocyte killing mechanism, playing an important role in cancer immunotherapy. We recently discovered a novel cytosolic innate immune receptor alpha-kinase 1 (ALPK1) that recognizes a bacterial metabolite ADP-heptose. ADP-heptose-activated ALPK1 phosphorylates the TIFA adaptor, thereby stimulating the NF-κB signaling and proinflammatory cytokine production. I will also discuss the function of ALPK1-TIFA axis in cancer immunity.

SCL 1-3

Human Th17 cells engage gasdermin E pores to release IL-1a upon NLRP3 inflammasome activation

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There is evidence that innate immune responses coopt adaptive properties such as memory. Whether T cells harness innate immune signaling pathways to diversify their repertoire of effector functions remains unknown. Here, we found that human T cells expressed gasdermin E (GSDME), a membrane pore-forming molecule that has recently been shown to execute pyroptotic cell death and thus to serve as a potential cancer checkpoint. In T cells, GSDME expression was, in contrast, associated with durable viability and was repurposed for the tunneled release of the alarmin IL-1a. This property was restricted to a subset of human Th17 cells with specificity for *C. albicans* and was regulated by a T cell-intrinsic NLRP3 inflammasome and its engagement of a proteolytic cascade of successive caspase-8, caspase-3 and GSDMEcleavage following T-cell receptor stimulation and calcium-licensed calpain maturation of the pro-IL1a form. Our results propose GSDME pore formation in T cells as a mechanism of unconventional cytokine release through harnessing of innate signaling platforms in response to adaptive stimuli. This finding diversifies the functional repertoire and mechanistic equipment of T cells with implications for anti-fungal host defense.

SCL 1 I Pattern recognition receptors and inflammasomes

SCL 1-4

NLRP3 membrane-bound decamer is dispensable for K^+ -dependent but critical for K^+-independent inflammasome activation

María Mateo-Tórtola[1], Inga V. Hochheiser[2], Jelena Grga[1,3], Jana Müller[1], Matthias Geyer[2], Alexander N. R. Weber[1,3,4], Ana Tapia-Abellán[1,3,4]

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NLRP3 belongs to the family of NOD-like receptors and is the inflammasome-forming protein with the most disease associations. NLRP3 can be activated by a great variety of potassium (K^+)-dependent or -independent stimuli, and both interactions with cellular organelles/membranes as well as assembly into a decameric "cage" were proposed as critical for NLRP3 activation. However, the relative mechanistic requirements of membrane association and oligomerization for K^+-dependent vs -independent stimuli have not been investigated in a spatially and dynamically highly resolved manner in human macrophages. To address this question, we developed an NLRP3 construct with deletion of the short exon 3 of *NLRP3* that encodes a disorder linker region located between its PYD and FISNA domains. This Δlinker construct, when purified, formed smaller NLRP3 species than WT NLRP3 that lost the ability to form decameric cages and bind membranes in cells. Surprisingly, endogenous deletion of the linker by exon skipping in cells did not prevent inflammasome activation in human macrophages to the K^+-dependent stimulus, nigericin. Rather, we observed that the non-decameric Δlinker NLRP3 engaged ASC in membrane-independent specks that enacted cell death- and IL-1β release, albeit with delayed kinetics compared to WT NLRP3 which formed both membrane-bound and membrane-independent specks. For K^+-independent stimuli, however, Δlinker NLRP3-expressing macrophages failed to form specks and activate IL-1β processing. Thus, decameric membrane-bound NLRP3 "cages" emerged as critical for K^+-independent NLRP3 inflammasome activation in general but K^+-dependent stimuli activate NLRP3 independent of membrane phenomena and decamer formation. Our results indicate that NLRP3 decameric cage formation and membrane engagement are strictly linked and that there are at least two cell biologically distinct inflammasome pathways.

SCL 1-5

Inflammasomes at the crossroads of basic science and therapeutic intervention

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In this talk, I will give an overview on inflammasome biology, delve into the NLRP3 inflammasome as well as the downstream pore-forming protein GSDMD, and discuss both the basic mechanistic understandings and therapeutic potential.

SCL 2 I Innate immune and tissue cell interaction

SCL 2-1

A transient and spatially-restricted subset of resident Kupffer Cells induced by lipid uptake

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In recent years the plasticity of tissue-resident macrophages (mϕs) has been questioned. Fitting with the idea that resident mϕs may not be as plastic as once thought, we have recently demonstrated that liver-resident Kupffer cells (KCs) do not display any overt activation in the context of fatty liver disease. However, this has been challenged using different models of NAFLD and whether this holds true in acute inflammation has also not been assessed. To investigate this, we employed a combination of spatial proteogenomics techniques to assess hepatic mϕ heterogeneity over time during non-alcoholic fatty liver disease (NAFLD) induced by feeding a choline deficient high fat diet, previously reported to induce KC activation and acetaminophen-induced liver injury (AILI) and repair. Consistent with previous reports, we observed a significant infiltration of monocyte-derived mϕs in these models, that were lost upon return to homeostasis. However, unlike previous reports, we did not observe a decrease in KCs. This discrepancy was due to the digestion method used as *in vivo* perfusion of enzymes to digest the livers liberated equal numbers of KCs while *ex vivo* digestion, as previously reported, identified a reduction in KC numbers. Further examination of the KC pool revealed the emergence of a subpopulation of resident KCs present only in the *in vivo* digested samples. This subset of KCs expressed a selection of genes previously associated with recruited lipid-associated macrophages (LAMs) including *Trem2* and *Cd36*. These LAM-like KCs were uniquely positioned around damaged tissue likely explaining the difficulties in isolating these cells. Further analysis of the LAM-like KC phenotype did not reveal any increased activation in terms of pro or anti-inflammatory cytokine expression. Fitting with the LAM-like signature, we found that lipid uptake, primarily through efferocytosis is important for the generation of this phenotype. We are currently investigating the relevance of the different macrophage subsets in disease progression.

SCL 2-2

Release of the pre-assembled naRNA-LL37 composite DAMP re-defines neutrophil extracellular traps (NETs) as intentional DAMP webs

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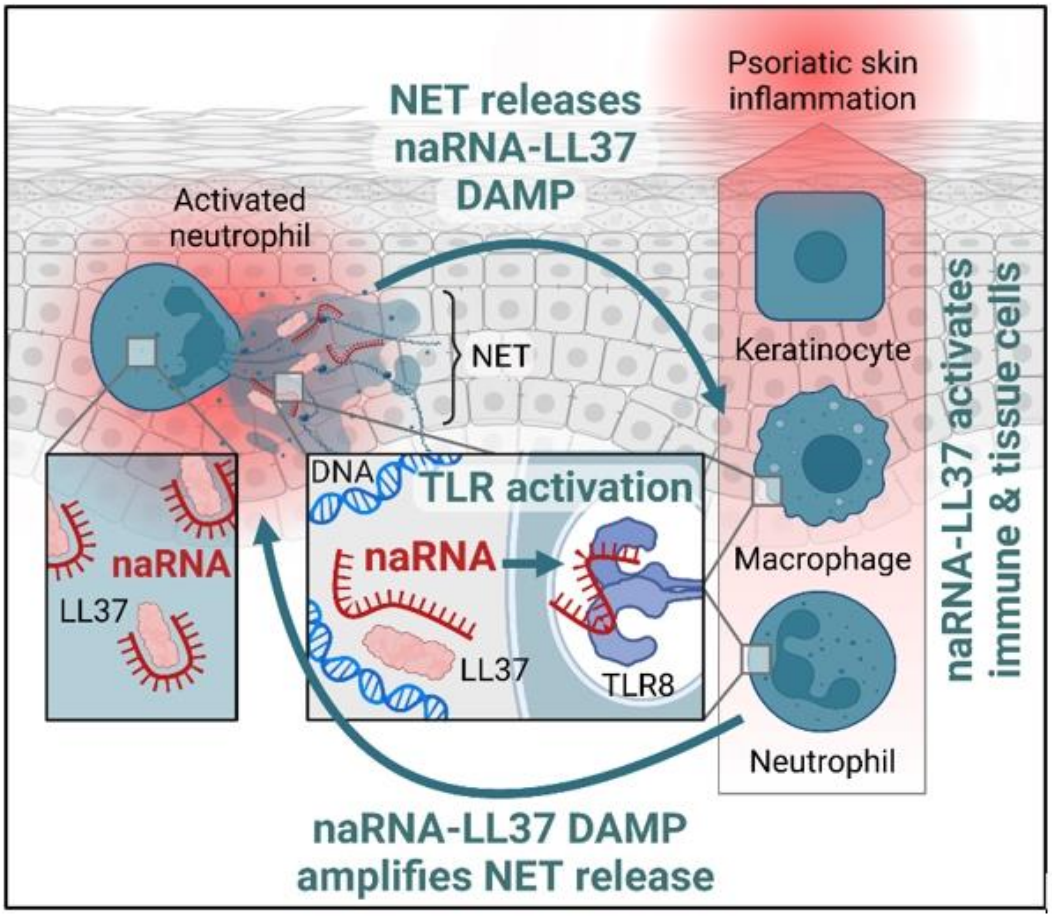
Neutrophil extracellular traps (NETs) are a key antimicrobial feature of cellular innate immunity mediated by polymorphonuclear neutrophils (PMNs), the primary human leukocyte population. NETs trap and kill microbes but have also been linked to inflammation, e.g. atherosclerosis, arthritis or psoriasis by unknown mechanisms. We here characterize naRNA (NET-associated RNA), as a new canonical, abundant, and unexplored inflammatory NET component. naRNA, upon release by NET formation, induced both further NET formation in naïve PMN and macrophage responses via TLR8 in humans and Tlr13 in mice, in vitro and in vivo. In neutrophils, this involved a novel NLRP3 inflammasome-dependent pathway. Keratinocytes also responded to naRNA, with expression of psoriasis-related genes (e.g. *IL17, IL36*). Importantly, in vivo naRNA strongly drove skin inflammation, whereas genetic ablation of RNA sensing drastically ameliorated psoriatic skin inflammation. Rather than accidentally assembling with LL37 on the NET, naRNA was intracellularly pre-associated with LL37 in resting neutrophils as a "composite DAMP", thus highlighting NET formation as a DAMP release process. This re-defines sterile NETs as an intentionally inflammatory agent, signaling and amplifying neutrophil activation in early immune responses. Moreover, in the many conditions previously linked to NETs and extracellular RNA, TLR-mediated naRNA sensing may represent both potential cause and new intervention target.

SCL 2 I Innate immune and tissue cell interaction

Fig. 1



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SCL 2 I Innate immune and tissue cell interaction

SCL 2-3

Arginine methylation promotes monocyte extravasation driving chronic obstructive pulmonary disease

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Histone arginine methylation by protein arginine methyl transferases (PRMTs) is one of the most important epigenetic controls and recently implicated in the regulation of the immune system and inflammatory responses. PRMT7, a unique member of the PRMT family, catalyzes the addition of a methyl group to arginine residues resulting in monomethylation, the only PRMT that does not methylate the arginine residue further. Here, we investigate the role of arginine methylation via PRMT7 as an important determinant of monocyte driven inflammatory responses in chronic obstructive pulmonary disease (COPD).

Transcriptomics data analysed by gene set enrichment analysis (GSEA) and qPCR of additional cohorts revealed enrichment of arginine methylation and PRMT7 in the lungs of COPD patients, correlating with disease susceptibility. Crucially, PRMT7 co-localized to macrophages, with scRNA-Seq revealing greatest expression in a unique CS-induced macrophage population that originated from classical inflammatory monocytes, with NF-κB/RelA activation regulating PRMT7 expression. *Prmt7*+/- heterozygous mice with reduced expression of *Prmt7* demonstrated impaired recruitment of macrophages to the lungs following CS exposure, which prevented emphysema development. Mechanistically, using ATAC-Seq and ChIP-qPCR, we discovered that PRMT7 induced methylation of histones, in particular H3R2me1 and H3R2me2, at enhancers regulating chromatin accessibility and *Rap1a* expression, which is crucial for MAPK signaling, integrin activation and the subsequent adhesion and migration ability of monocytes.

Taken all together, inhibition of arginine mono-methylation might offer therapeutic potential in monocyte-driven inflammatory conditions that result in tissue damage if left untreated.

SCL 3 I Trained innate immunity

SCL 3-1

Fungal β-glucan and the regulation of innate immune responses

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Advances in the field of immunological memory show that innate immune cells can recall a previous encounter with microbes or microbial products to exhibit a modified immunological response called innate memory. Our previous work established that fungal β-glucan from yeast such as *Candida albicans* induces innate memory in human primary monocytes leading to an enhanced immunological status towards several non-related pathogens (trained immunity). *In vitro*, in just a few hours, monocytes are imprinted transcriptionally, epigenetically, and metabolically by purified β-glucan. However, while it has been demonstrated that monocytes can be imprinted by β-glucan, bloodstream classical monocytes are not themselves effector cells during infection, but rather mostly circulating intermediates. Once recruited to peripheral tissue, following conditioning by local products, monocytes differentiate into macrophages that undergo activation or "polarisation" along a wide spectrum of phenotypes. We thus investigated the effect of β-glucan imprinting on monocyte polarisation into macrophages We observed that responsiveness to secondary challenges is oppositely regulated and that in contrast to the current model of trained immunity, β-glucan imprinting does not always enhance macrophage function but can also reduce it depending on the environment. With this respect, we further characterized macrophage responses focusing on the cytokine IL-1β, a unique cytokine that requires inflammasome activation and is associated with autoinflammatory disorders. Our work demonstrates that β-glucan-induced innate memory represses IL-1β-mediated inflammation through a novel mechanism by regulating the early activation events upstream of NLRP3 inflammasome and supports its potential beneficial and clinical use in NLRP3-driven diseases. Altogether we uncovered important new features that are essential to fully understand the potential biological impacts of β-glucan induced innate memory.

SCL 3-2

Trained immunity in microglia identifies regulators of disease-associated activation states

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Microglia are the brain's major resident macrophage population. We previously demonstrated that microglia are capable of innate immune memory, which is sufficient to modulate neurological disease severity (Wendeln et al., Nature, 2018). In line with previous reports on different macrophage populations, we found that immune training in microglia enhanced hypoxia-inducible factor-1alpha (HIF-1a) signalling, which correlated with increased disease severity in mouse models of Alzheimer's disease pathology. Using single cell epigenetic and transcriptional profiling, we now delineate the role of microglial HIF-1a signalling in promoting disease-associated microglial activation states and their impact on brain pathology.

SCL 3 I Trained innate immunity

SCL 3-3

Bacteria-derived nucleic acids promote long-term hyperreactivity in macrophages by endosomal TLR stimulation

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Innate immunity is the frontline of host defense against different pathogens. Innate cells utilize evolutionary conserved Toll-like receptors (TLRs), recognizing bacterial or viral components, thereby initiating inflammatory responses. Recent studies revealed innate cells can acquire an antigen-independent "memory" by engaging certain receptors, e.g. Dectin-1. This process has been named trained immunity (TI). During infection, disrupted pathogens release pathogen-derived nucleic acids, which can be sensed by intracellular nucleic acids sensors. Our previous studies have identified TLR8 or TLR13 contributing to bacterial RNA (bRNA) sensing in human and murine monocytes upon *S. pyogenes* infection, respectively.

Here, we studied the contribution of bRNA on generating TI in human $CD14^+$ monocytes or murine BMDM over a time-period of 7 days. First, we observed that live $Gram^+$ bacteria infection sensitized BMDM toward secondary TLR-agonists stimulation, resulting in higher secretion of proinflammatory cytokines. This phenotype could be recapitulated by priming monocytes with total bRNA from two different $Gram^+$ bacteria thus showing bRNA is sufficient to induce long-lasting hyperreactivity. Furthermore, interrupting glycolysis or cholesterol synthesis upon priming reversed the phenotype, indicating bRNA stimulation rewired BMDM metabolism and may imitate TI.

Next, we used a 19-mere synthetic oligos, containing a conservative sequence of rRNA from *S. aureus* (Sa19) to prime cells. Interestingly, the priming effect of Sa19 was comparable to bRNA, implying the sensitizing macrophage only requires limited length of oligos. Finally, priming of hematopoietic progenitor cells with Sa19 skewed differentiation towards a hyperreactive BMDM in a TLR13-dependent manner, suggesting a long-term memory effect of RNA sensing. Overall, our findings not only decipher the important role of nucleic acid sensing on innate memory, but may also provide new insights on mRNA-based vaccination.

SCL 4 I Innate immunity in the skin

SCL 4-2

Neutrophil extracellular traps promote *S. aureus* skin colonization by induction of oxidative stress in the skin and downregulation of epidermal barrier genes

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Introduction: *Staphylococcus aureus* is the main cause of skin infections in humans. It is usually absent from healthy human skin but abundantly found on the skin of atopic dermatitis patients where it actively contributes to the disease. Our group previously showed that on inflamed skin *S. aureus* colonization is enhanced by the interaction of keratinocytes with neutrophil extracellular traps (NETs) released by infiltrating neutrophils. However, the mechanism behind this is not yet understood.

Objectives: In this work, we studied the role of neutrophils and NETs in *S. aureus* skin colonization and persistence further.

Methods: Using an *in vitro* co-culture model we investigated the interaction of NETs and keratinocytes by studying the induction of inflammatory responses via ELISA and qPCR and activated signaling pathways by western blot. We further analyzed neutrophil recruitment and *S. aureus* colonization in the skin of different knock-out mice using an epicutaneous colonization model. Moreover, the role of neutrophils in the persistence of *S. aureus* on inflamed and not inflamed skin was investigated by neutrophil depletion *in vivo*.

Results: We show that neutrophils actively contribute not only to the initial colonization but also to the enhanced persistence of *S. aureus* on inflamed skin. A crosstalk between infiltrating neutrophils and keratinocytes primes neutrophils for NET formation which correlates with enhanced *S. aureus* skin colonization. Further experiments revealed that NETs induce oxidative stress in the skin leading to the secretion of DAMPs and downregulation of epidermal barrier genes.

Conclusion: Our data indicate a functional role of neutrophils in shaping *S. aureus* skin colonization and persistence. We propose that in inflamed skin, infiltrating neutrophils are primed for NET formation which induces oxidative stress and downregulation of epidermal barrier genes in the skin thus promoting a skin barrier dysfunction which drives *S. aureus* colonization and persistence.

SCL 4 I Innate immunity in the skin

SCL 4-3

Expansion microscopy of neutrophil nuclear structure and extracellular traps

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Neutrophils are the first responders to injury and are critical to the inflammatory process and tissue homeostasis. The lobulated nuclei of these cells contain a unique membrane composition, giving a deformable structure that is both responsive to the environment and facilitative of extravasation to the site of inflammation. Neutrophils possess an impressive arsenal of antimicrobial defense mechanisms including neutrophil extracellular traps (NETs), expelled nuclear DNA studded with cellular and antimicrobial proteins. Dysregulated neutrophil activation is implicated in many inflammatory diseases, however, highlighting the need to better understand their biology. To date, thorough study of neutrophil morphology and particularly of their nucleus has been hindered by the resolution limits inherent in microscopy. This typically requires expensive and technically challenging super-resolution equipment. Therefore, we have implemented expansion microscopy for imaging of neutrophils, as this technique is inexpensive and easily performed using conventional microscopes. We used this novel technique to characterize the distribution of chromatin, histone H1, and nucleophosmin (NPM1) in neutrophils and NETs. We further labeled lamins B1 and B2 as nuclear envelope markers and myeloperoxidase (MPO) as a neutrophil cytoplasmic protein. The gain in resolution was reflected by changes in histone H1 and chromatin colocalization, which was assessed using Pearson's coefficient. Line scan analysis of histone H1 and NPM1 distribution throughout the nucleus showed a marked increase in detail. In conclusion, expansion microscopy is a novel tool to study neutrophil nuclear dynamics and image neutrophil proteins beyond the resolution limit using conventional microscopes. This method can be easily adapted to other cells of the immune system and is thus a valuable tool to analyze cellular morphology in the context of inflammation.

SCL 4-4

Mast cell secretory granules serve as endogenous C-type lectin receptor ligands skewing dendritic cell function towards type II immunity

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Question: Mast cells (MCs) are best known as key effector cells of type I allergic reactions. Despite increasing evidence for an additional, critical impact of MCs on adaptive immunity, the underlying mechanisms are poorly understood. We recently reported that, upon skin inflammation, dermal dendritic cells (dDCs) engulf MC secretory granules (MCG), which boost DC functionality and thereby adaptive immunity remotely (Dudeck et al. 2019). Based on this study, we intended to identify aspects of underlying molecular mechanisms and consequences on DC functions.

Methods: Different transgenic mouse lines, were used to clarify mechanisms that underlie MCG uptake. Mouse models of inflammatory disorders, basically hapten-induced skin inflammation and allergic airway inflammation, were used to determine the relevance of involved mechanisms for the immune response.

Results: We could show, that immature DCs sense/engulf MCG in a Card9-dependent manner. Consistently, Card9 deficiency resulted in a reduced T cell priming/differentiation upon hapten sensitization and diminished T cell driven adaptive ear swelling response upon elicitation. Questioning the MCG sensing DC surface receptors, we could show that the c-type lectin receptors (CLRs) MCL and SIGNR3 bind to MCG and mediate their uptake. Most importantly, MCG uptake by DCs modulated their antigen-presenting capacity towards a type II immune response. Hence, mice that lack *Mcl*/*Signr3* expression showed reduced allergic airway inflammation, in line with reduced cDC2 numbers and Th2 cytokine production.

Conclusions: Herein, we show that MCG are endogenous CLR ligands that translate (MC degranulation inducing) danger signals into adjuvant effects promoting lymph node-borne adaptive immunity. Understanding the uptake mechanism and DC modulating properties of MCG may give rise to therapeutic strategies to either intentionally boost adaptive immunity or dampen elevated immune responses.

SCL 5 I Metabolic regulation of innate immunity

SCL 5-1

Fumarate hydratase controls macrophage cytokine production

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Metabolic changes triggered during innate immunity have become a particular focus for researchers interested in inflammation. Mitochondrial disturbance is a feature of inflammatory cells and we have been analysing the Krebs cycle intermediates succinate and fumarate, as well as the Krebs cycle-derived metabolite itaconate, in activated macrophages in response to signalling by Toll-like receptors. Broadly speaking succinate is pro-inflammatory via several processes, whilst itacoante is anti-inflammatory effects, acting to limit inflammatory cytokines such as IL-1beta but also limiting M2 macrophage polarisation via the targetting of JAK1. Fumarate is also proving to be a very interesting metabolite. It is generated via repression of the enzyme FH and also induction of the argininosuccinate shunt. Fumarate suppresses IL10 produciton which in turn leads to increased TNF. The decrease in FH however also leads to mitochondrial disturbance, which involves release of double-stranded mitochondrial RNA. This is sensed by the RNA sensors RIG-I, MDA-5 and TLR-7 driving production of Type I Interferons. All of these studies indicate that metabolites are acting as signals and impacting on signalling pathways in unexpected ways. These insights are providing a new view of metabolism in immunity and inflammation and might indicate new therapeutic approaches.

SCL 5-2

Macrophages inhibit *Coxiella burnetii* by the ACOD1-itaconate pathway for containment of Q fever

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Infection with the intracellular bacterium *Coxiella (C.) burnetii* can cause chronic Q fever with severe complications and limited treatment options. *C. burnetii* is sensed by Toll-like receptors (TLR) and in a recently established Q fever model in *Myd88*$^{-/-}$ mice, we noticed the induction of IFNγ and several of its target genes in a Myd88-dependent manner. Here, we have investigated the role of MyD88-/IFNγ-induced genes (eg. *Acod1, iNOS, Gbp2, Ido*) in controlling bacterial replication and macrophage reprogramming during infection with *C. burnetii* and identified the mitochondrial enzyme ACOD1 and its product itaconate as protective host immune pathway in Q fever. Aconitate decarboxylase 1 (ACOD1 or IRG1) is emerging as a regulator of immunometabolism in inflammation and infection which catalyzes the decarboxylation of the TCA cycle metabolite cis-aconitate to itaconate (ITA) in activated macrophages. ITA has recently received a lot of attention for its immunoregulatory effects, but it can also act as an antimicrobial agent. In macrophages, *Acod1* was essential for restricting *C. burnetii* replication, while other antimicrobial pathways were dispensable. Intratracheal or intraperitoneal infection of *Acod1*$^{-/-}$ mice caused increased *C. burnetii* burden, weight loss, and stronger inflammatory gene expression. Exogenously added itaconate restored pathogen control in *Acod1*$^{-/-}$ mouse macrophages and blocked replication in human macrophages. In axenic cultures, itaconate directly inhibited the growth of *C. burnetii*. Finally, treatment of infected *Acod1*$^{-/-}$ mice with itaconate efficiently reduced the tissue pathogen load. Thus, ACOD1-derived itaconate is a key factor in the macrophage-mediated defense against *C. burnetii* and may be exploited for novel therapeutic approaches in chronic Q fever.

SCL 5 I Metabolic regulation of innate immunity

SCL 5-3

Identification of an endogenous lipid driving metaflammation

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Chronic low-grade inflammation, called metaflammation, is associated with prevalent non-communicable diseases, such as atherosclerosis. Anti-inflammatory therapy provides a clinical benefit in patients, but the triggers inciting metaflammation remain largely unknown. To uncover non-genetic inflammatory factors influencing atherosclerosis severity, we performed an unbiased *in vivo* screen measuring thousands of host- and microbe-derived molecules in murine atherosclerosis. Machine learning-supported analyses identified, next to known pro- and anti-atherogenic factors, an endogenous sphingolipid positively associated with atherogenesis. This sphingolipid activated macrophage innate immune signaling, metabolic reprogramming and pro-inflammatory gene transcription. Human interventional and observational trials demonstrated that dietary changes altered the concentrations of this sphingolipid and that increased circulating this sphingolipid was associated with carotid plaque development in obese individuals. These findings identify an inducible endogenous sphingolipid that drives metaflammation, providing the rationale for preventative approaches and pharmacological interventions that may curb detrimental inflammation secondary to Western-type lifestyle habits.

SCL 6 I Host-microbiota interactions

SCL 6-1

Intestinal barrier cell remodelling in IBD

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Understanding the cellular pathology and molecular pathways dysregulated in conditions such as inflammatory bowel disease (IBD), which affect several million people globally is required to improve medical treatment, which fails in around 40% patients. We charted the cellular anatomy of the human intestine through development, in health and in IBD to define the molecular circuits that drive acute inflammation and dysregulated repair using multimodal single cell technologies. We defined new intestinal cell states, differentiation hierarchies and the functional properties of rare cell types of the intestine in development and in health. Comparing these with cellular states occurring in IBD revealed mechanisms of barrier breakdown, loss of anti-microbial protective pathways and pathological immune and mesenchymal cellular microdomains that fuel disease.

SCL 6-2

Airway commensal bacteria isolated from cystic fibrosis patients can modulate *P. aeruginosa*-induced lung inflammatory response via the production of acetate

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The lung pathology in cystic fibrosis (CF) is characterized by a vicious cycle of infection and inflammation which ultimately results in lung damage and respiratory failure. Infection by *P. aeruginosa* remains the most predominant infection with an overall European average of 41% of adult patients infected. We have previously demonstrated that certain airway commensals that were isolated from CF sputum samples mediate protective effects by modulating the *P. aeruginosa*-induced inflammatory response in commensal-host-pathogen interactions. Here, we now demonstrate that short-chain fatty acids (SCFA) like acetate produced by these protective commensals can mediate these protective responses.

Protective and nonprotective commensals were directly cocultured with *P. aeruginosa* PA01 for 24 h and the resulting pathogen/commensal-conditioned media were sterile-filtered and analyzed via high-performance liquid chromatography for their SCFA profiles. Human airway epithelial cell lines (BEAS-2B) and mouse precision cut lung slices (PCLS) were either treated with LPS or infected by PA01 for 2 h and 4 h respectively in the presence of varying acetate concentrations. Inflammatory cytokines released into the supernatants after overnight incubation were measured via ELISA.

The SCFA profiles revealed that significant amounts of acetate (>0.5 mg/ml) were produced by protective commensals in direct cocultures with PA01 while nonprotective commensals did not produce acetate levels that were different from PA01 alone. Exogenous addition of acetate significantly lowered both the LPS-induced and the PA01-induced inflammatory response in human BEAS-2B cells and mouse PCLS. Given that the therapeutic use of whole bacteria as probiotics in immunocompromised patients like in CF possesses several challenges, the use of bacterial metabolites, like acetate, which may also produce the commensal-mediated protective effects presents a safer, easier, and more practical alternative.

SCL 6 I Host-microbiota interactions

SCL 6-3

Bacterial toxin driven cytosolic sodium accumulation is a cellular danger signal triggering endocytic dysfunction and NLRP3 inflammasome activation

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The concentration of sodium in the cytosol is highly regulated to ensure both the osmotic balance of the cell and the correct function of organelles. Here we demonstrate that aberrant accumulation of cytosolic sodium is a cellular danger signal that triggers activation of the NLRP3 inflammasome. We determined that the large clostridial toxin family, including toxin B (TcdB) from *Clostridioides difficile*, caused translocation of sodium from the endo-lysosomal network into the cytoplasm through insertion in endo-lysosomal membranes. This led to accumulation of sodium in the cytosol, which triggered cell swelling and subsequent drop in cellular potassium, leading to NLRP3 activation. Notably, sodium accumulation also blocked endo-lysosomal trafficking through osmotic perturbation of the passive gradients required to drive loss of tension on endosomal membranes and enabling NLRP3 activation. Furthermore, we find that sodium is increased by other NLRP3 activators and osmotic perturbation of endocytic trafficking is able to enhance NLRP3 inflammasome activation by other NLRP3 activators. These findings demonstrate that cytosolic sodium accumulation is a danger signal that triggers the NLRP3 inflammasome activation through osmotic perturbation of endocytic trafficking.

SCL 7 I Nucleic-acid driven innate immunity

SCL 7-1

The central role of RNase T2 in RNA recognition in the endolysosome

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Toll-like receptors (TLRs) are key pattern recognition receptors of the innate immune system. Among them, TLR7 and TLR8 are ssRNA sensors located in the endolysosomal compartment. Structural studies suggested that these RNA-sensing TLRs contain two distinct binding pockets that are engaged by RNA degradation products rather than long single-stranded RNA molecules. Consistent with this notion, we and others have recently found that the endolysosomal endonuclease RNase T2 plays a critical role in processing exogenous RNA into fragments that can engage TLR8. A key to this function is its exquisite substrate specificity, which generates unique fragments required for binding and activation of the two TLR8 binding pockets. In this talk, I will provide an update on this paradigm and discuss how structural features and common RNA modifications influence TLR8-mediated immunogenicity. In addition, I will provide insights into how RNase T2 deficiency affects the activity of the innate immune system in vivo.

SCL 7-2

SLC15A4 and TASL form a druggable signalling complex required for TLR7-9-induced proinflammatory responses

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Nucleic acid sensing by endolysosomal Toll-like receptors (TLRs) plays a crucial role in innate immune responses to invading pathogens. In contrast, aberrant activation of these pathways is associated with several autoimmune diseases, including systemic lupus erythematosus (SLE). The endolysosomal solute carrier family 15 member 4 (SLC15A4) is required for TLR7, TLR8 and TLR9-induced inflammatory responses and for disease development in different SLE murine models, highlighting its potential as drug target. SLC15A4 has been proposed to affect TLR7-9 activation through its transport activity, as well as by assembling in an IRF5-activating signalling complex with the innate immune adaptor TASL, but the relative contribution of these different functions remains unclear. Here we show that the essential role of SLC15A4 is to recruit TASL to the endolysosomal compartment, while its transport activity is dispensable. Targeting of TASL to the endolysosomal compartment is sufficient to rescue TLR7-9-induced IRF5 activation and proinflammatory responses in SLC15A4-deficient cells. These findings support interfering with the SLC15A4-TASL complex as a potential therapeutic strategy for SLE. We therefore performed a chemical screen and identified a compound, feeblin, which affects the assembly of the SLC15A4-TASL complex, leading to efficient degradation of TASL and ablation of downstream IRF5 activation. Consequently, feeblin blocks endolysosomal TLR-induced responses in disease-relevant human immune cells. Altogether, our study reveals that the SLC15A4-TASL complex, independently of SLC15A4 transport function, is essential for TLR7-9-mediated inflammatory responses and provides proof-of-concept that chemical interference with this module could represent a novel therapeutics strategy for SLE and related diseases.

SCL 7 I Nucleic-acid driven innate immunity

SCL 7-3

Antagonistic nanobodies reveal mechanism of GSDMD pore formation and unexpected therapeutic potential

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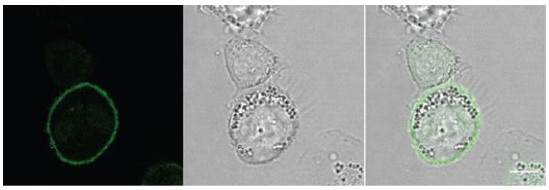
Activation of various inflammasomes converges on the cleavage of gasdermin D (GSDMD) by pro-inflammatory caspases, followed by oligomerization of its N-terminal domain ($GSDMD^{NT}$) and the assembly of pores penetrating target membranes. Yet, it remained unclear what triggers the conformational changes that allow membrane insertion, as methods to study pore formation in living cells are limited.

We raised nanobodies specific for human GSDMD and identified two nanobodies that prevent pyroptosis and IL-1β release when expressed in the cytosol of human macrophages. Nanobody binding prevented $GSDMD^{NT}$ oligomerization, while assembly of inflammasomes and GSDMD processing by caspases were not affected. Cytosolically expressed nanobodies thus stabilized monomers of $GSDMD^{NT}$, which still efficiently partitioned into the plasma membrane (see Fig. 1). This suggests that pore formation is initiated by insertion of monomers, followed by oligomerization in the target membrane. When GSDMD pore formation was inhibited, cells underwent caspase-1-dependent apoptosis, likely due to the substantially augmented caspase-1 activity in the absence of pores. This hints at a novel layer of regulation of caspase-1 activity by GSDMD pores. Moreover, we revealed the unexpected therapeutic potential of antagonistic GSDMD nanobodies: Recombinant nanobodies added to the medium prevented cell death by pyroptosis, likely by entering through GSDMD pores and curtailing the assembly of additional pores. We suspect that the first pores are rapidly removed by membrane repair processes. GSDMD nanobodies may thus be suitable to treat the ever-growing list of diseases caused by activation of the (non-) canonical inflammasomes.

Taken together, we show that customized nanobodies stabilize informative intermediates of protein assemblies, reveal novel mechanistic insights of GSDMD pore formation, and lastly reveal a novel strategy for therapeutic intervention.

Fig. 1



SCL 8 I Innate lymphoid cells

SCL 8-2

Attenuated Salmonella blocks cancer metastasis by activating NK cells in an IFN-γ-dependent manner

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Bacterial cancer therapies represent an alternative approach to targeting solid tumors for evoking anti-tumor immune responses. As a facultative anaerobic pathogen that can penetrate and colonize solid tumors, Salmonella typhimurium exert its intrinsic antitumor activity by directly inducing systemic immune responses. Metastasis accounts for 90% of cancer related deaths and blocking of metastatic cascade has critical clinical impact. However, the clinical drug development for cancer treatment, including cancer immunotherapies, is evaluated largely depending on their ability to cause tumor shrinkage and ignores the effect on metastasis as it has proven challenging to target. Therefore, there is an urgent need for novel therapeutic strategies and agents targeting metastasis.

Using an attenuated Salmonella typhimurium strain YB1 engineered by our lab, we have found a potent suppressive effect of attenuated salmonella on cancer metastasis, regardless of cancer types and genetic background, by evoking strong anti-metastatic immune response. Further study showed that suppression of cancer metastasis by attenuated Salmonella only requires the innate immune response. Among the many induced cytokines, we identified IFN-γ as an indispensable factor for inhibiting cancer metastasis. Based on CyTOF (mass cytometry or cytometry by time of flight) analysis of the innate immune responses after Salmonella treatment and antibody-mediated cell depletion, we further demonstrated that NK cells are the major factor involved in Salmonella-provoked metastasis suppression. We found that IFN-γ was mainly produced by NK cells during early Salmonella infection, and in turn, IFN-γ promoted the accumulation, activation and cytotoxicity of NK cells. The IFN-γ-dependent NK cells directly eliminated newly accumulated cancer cells in the lung to block the cancer metastasis cascade in response to the Salmonella treatment.

SCL 8-3

Reprogramming of NK cells by disseminated tumor cells as a barrier to anti-metastatic immunity

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Natural killer (NK) cells are key effector cells in the control of metastasis. This role reflects the ability of NK cells to rapidly detect and eliminate metastasizing tumor cells through targeted cytotoxicity and to produce immunostimulatory cytokines and chemokines that further regulate anti-metastatic immunity. Recent work has demonstrated a critical role for the bioactive lipid prostaglandin E2 (PGE2) in immune evasion of primary tumors via suppression of intratumoral immunity and identified NK cells as a key cellular target of PGE2 signaling (Böttcher et al., Cell, 2018, Bonavita et al., Immunity 2020). However, how PGE2 signaling regulates NK cell biology and function and its consequences for NK cell-mediated control of metastasis remain poorly understood. By using transcriptional and epigenetic analyses, we show that PGE2 signaling on NK cells imprints a dysfunctional program characterized by the inability of NK cells to use key intercellular communication pathways upon their activation. Mechanistically, this program is governed by a transcription factor network downstream of PGE2 receptor signaling that is conserved between mouse and human NK cells. In vivo, PGE2-mediated programming of NK cell dysfunctionality by disseminated tumor cells in the lung is critical for outgrowth of macrometastases. Targeting PGE2-signaling in NK cells overcomes this inhibitory mechanisms and restores anti-metastatic immunity. Taken together, our data reveal a novel mechanism that could be exploited for therapy of cancer metastasis.

SCL 9 I Innate immunity and oncogenesis

SCL 9-1

The NLRP3/eIF2 axis in acute myeloid leukemia

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The NLR family pyrin domain containing 3 (NLRP3) inflammasome has lately attracted great attention in various biomedical fields, as excessive activation of the NLRP3 inflammasome plays an important role in many different pathologies including hematologic diseases. In this context, our study highlights a novel function of the NLRP3 inflammasome in Acute Myeloid Leukemia (AML). We demonstrate that NLRP3 inflammasome components and the inflammasome-related cytokines IL-18 and IL-1b are overexpressed in AML patients, resulting in poor survival rates. Using shotgun proteomics, we could identify the eIF2 pathway as an important novel NLRP3 target. Genetic knockdown or pharmacological inhibition of NLRP3 results in enhanced eIF2a phosphorylation, which in turn was linked to the inhibition of cell cycle progression and apoptosis in vitro and in vivo. Additionally, a strong decrease in the cyclin-dependent kinases CDK4 and CDK6 was observed, also accompanied by an upregulation of the CDK inhibitor p21 (CDKN1A), resulting in pronounced cell cycle arrest in the G0/G1 phase and increased apoptosis. Taken together, these findings suggest that NLRP3 overexpression, as observed in many AML patients, blocks apoptosis of leukemic cells by dysregulating the eIF2 signaling pathway. Our data suggest that the NLRP3/eIF2 axis acts as a novel driver of cell cycle progression in AML. As AML is a disease with extremely low survival rates and limited treatment options, a better understanding of the molecular mechanism linking inflammation to excessive cell proliferation may open new paths for novel treatment strategies.

SCL 9-2

A novel immunoTracer platform for non-invasive visualization of distinct immune cell infiltrates in cancer and inflammation using PET/MRI

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Immune cell infiltration, their activation state, and the interaction between different populations dictate the development and progression of various diseases. Thus, we validated a set of immunoPET tracers based on full-length monoclonal antibodies (mAb) and mAb fragments targeting different subsets of innate and adaptive immune cells or their functional state. Our goal was to implement an imaging platform for the detection of rare immune cell populations in cancer and inflammation mouse models.

For this purpose, αCD4, αCD8, αCD69, αOX40, and αSIRPa PET tracers radiolabeled with Zr-89 or Cu-64 were validated in vitro for immunoreactivity, specific binding, and impact on cellular function. PET/MRI was conducted on syngeneic MC38, B16, and PyMT tumor models treated with different cancer immunotherapy (CIT) regimens. A mouse model of delayed-type hypersensitivity reaction (DTHR) was used to investigate immune cell alterations during chronic ear inflammation.

CD4 and CD8 PET were applicable to differentiate tumor immune infiltrates based on the tumor model and during CIT. In the DTHR model, minimal changes in CD4+ cell densities could be detected by PET/MRI during the inflammatory processes, correlating with ex vivo CD4 quantification by IHC. Furthermore, longitudinal imaging of T cell activation dynamics during cancer immunotherapy allowed the visualization of early (CD69+) and late (OX40+) immune responses during checkpoint blockade in tumor-bearing mice. Importantly, mice responding to CIT revealed significantly higher tumor uptake of CD69- and OX40-tracers when compared to non-responding ones.

Finally, in vivo imaging of SIRPa showed high tracer uptake in organs known to home myeloid cells, enabling their detection in MC38 tumor tissue.

In summary, this novel set of immunoPET tracers allows the visualization of rare immune cell subsets and their functional state within diseased tissue, giving hints on mechanisms involved in immunotherapy response.

SCL 9 I Innate immunity and oncogenesis

SCL 9-3

Neutrophils' regenerative power and cancer progression

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Neutrophils play an important role in cancer initiation and they are deeply influenced by the growth of cancer in turn shaping its progression and metastatic outcome.
We showed that the pro-metastatic activities of neutrophils are in part to be attributed to their physiologic role in tussive repair. Indeed, in the context of radiation injury, we revealed that neutrophil engagement and activation after lung injury supports the tissue response to the insult by enhancing regenerative pathways in alveolar cells. Importantly, this is in turn, unintentionally enhancing the metastatic potential of cancer cells seeding in the port-injured lung.

SCL 9-4

APC-like activity of tumor-draining lymph node neutrophils in cancer

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Tumor-associated neutrophils contribute to cancer progression via multiple mechanisms, including the support of angiogenic and metastatic processes, but also suppression of anti-tumor immune responses. However, these cells are highly heterogeneous and strongly reactive to environmental cues. Therefore, under certain circumstances, neutrophils can play an anti-tumoral role. Next to direct tumor cell killing, neutrophils are able to stimulate anti-tumoral T cell activity. These processes have been suggested previously for the primary tumor site.

Here we describe neutrophil-mediated initiation of effective anti-tumor immune responses in tumor-draining lymph nodes (TDLNs). We observed that neutrophils that carry tumor antigens transmigrate to lymph nodes early after tumor establishment, develop antigen (AG)-presenting phenotype and stimulate T cell proliferation. Therefore, the accumulation of neutrophils in T-rich zones of TDLNs in N0 stage cancer patients constitutes a positive predictor for 5-years survival. Unfortunately, this immunostimulatory role of neutrophils is limited to the early metastasis-free (N0) stage of cancer. At later, metastatic (N1-3) stages, the microenvironment of LN metastases triggers the development of immunosuppressive PD-L1^{hi} neutrophils that suppress anti-tumoral T-cell responses. Hence, increased numbers of neutrophils in TDLNs of late (N1-3) cancer stage patients serve poor prognosis predictor. Importantly, we could demonstrate that suppressive activity of neutrophils is reversible and could be therapeutically targeted.

These results have important implications for the prediction of cancer patient prognosis, but also confirm a dual-edged role of neutrophils in the regulation of anti-cancer immune responses and argue for approaches fostering anti-cancer activity of these cells during cancer immunotherapy.

LT 1 I Lightning talks I

LT 1-1

Enhanced airway epithelial response to SARS-CoV-2 infection in children is critically tuned by the crosstalk between immune and epithelial cells

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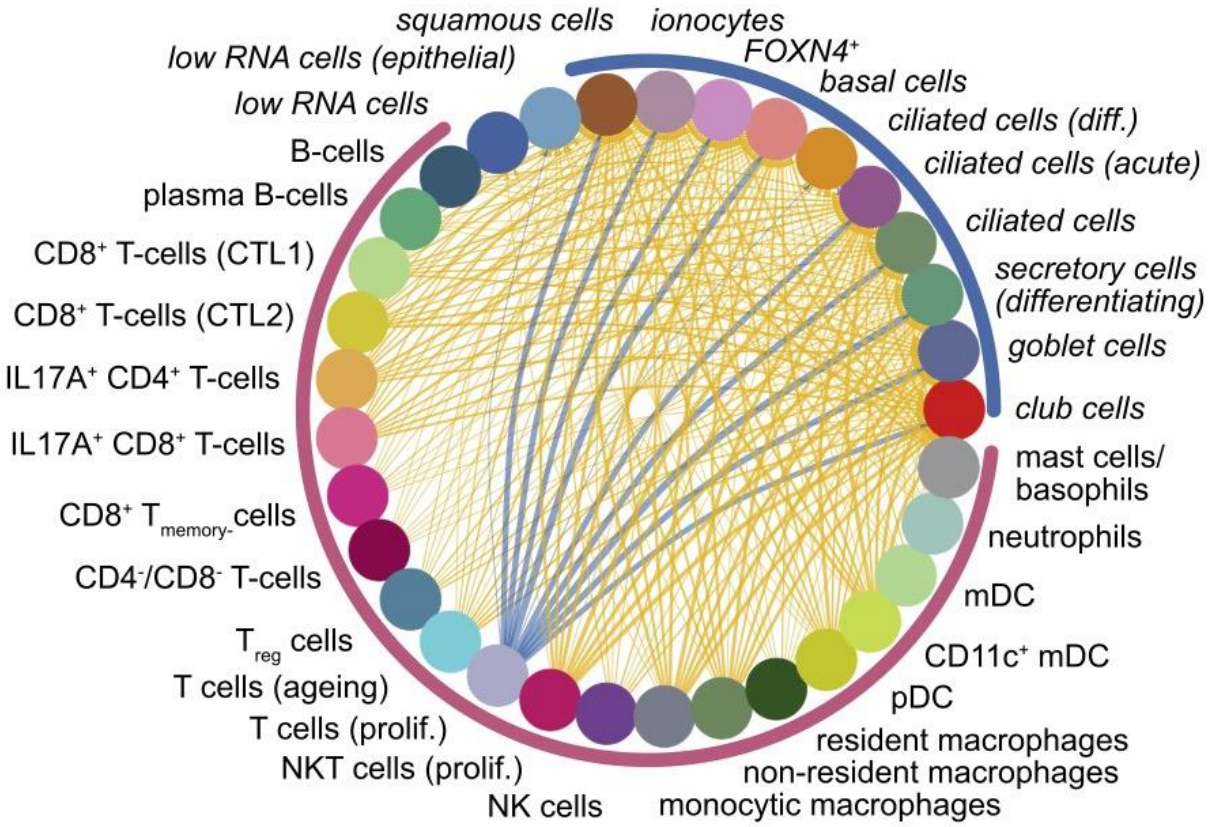
To cope with virus infections to which no prior adaptive immunity exists, the body relies on the innate immune system. In such cases, including SARS-CoV-2, children tend to fair better than adults. In the context of COVID-19, it became evident that a rapid interferon (IFN) response at the site of primary infection is key for successful control of the virus and prevention of severe disease. The airway epithelium of children was shown to exhibit a primed state already at homeostasis and to respond particularly well to SARS-CoV-2 infection. However, the underlying mechanism remained elusive. Here we show that interactions between airway mucosal immune cells and epithelial cells are stronger in children, and via cytokines and IRF-1 lead to upregulation of viral sensors. Based on an *in vitro* model we show stimulated human PBMCs can induce a robust IFN-b response towards SARS-CoV-2 in a lung epithelial cell line otherwise unresponsive to this virus. This is mediated by type I IFN, IFN-g and TNF, and requires induction of both, RIG-I and MDA5. In single cell-analysis of nasal swab samples the same cytokines are found to be elevated in mucosal immune cells of children, correlating with elevated epithelial expression of viral sensors. *In vitro* analysis of PBMCs derived from healthy adolescents and adults confirm that immune cells of younger individuals show increased cytokine production and potential to prime epithelial cells. In co-culture with SARS-CoV-2-infected A549 cells, PBMCs from adolescents significantly enhance the antiviral response. Taken together, our study suggests that innate immune cells in the airway mucosa of children tune the set-point of the epithelial antiviral system. This likely is a major contributor to the robust immune response to SARS-CoV-2 in children. Our findings shed light on the molecular underpinnings of the stunning resilience of children towards severe COVID-19, and may propose a novel concept for immunoprophylactic treatments.

Fig. 1



LT 1 I Lightning talks I

LT 1-2

Non-canonical chemokine receptor ACKR3/CXCR7 regulates immunothrombotic platelet response

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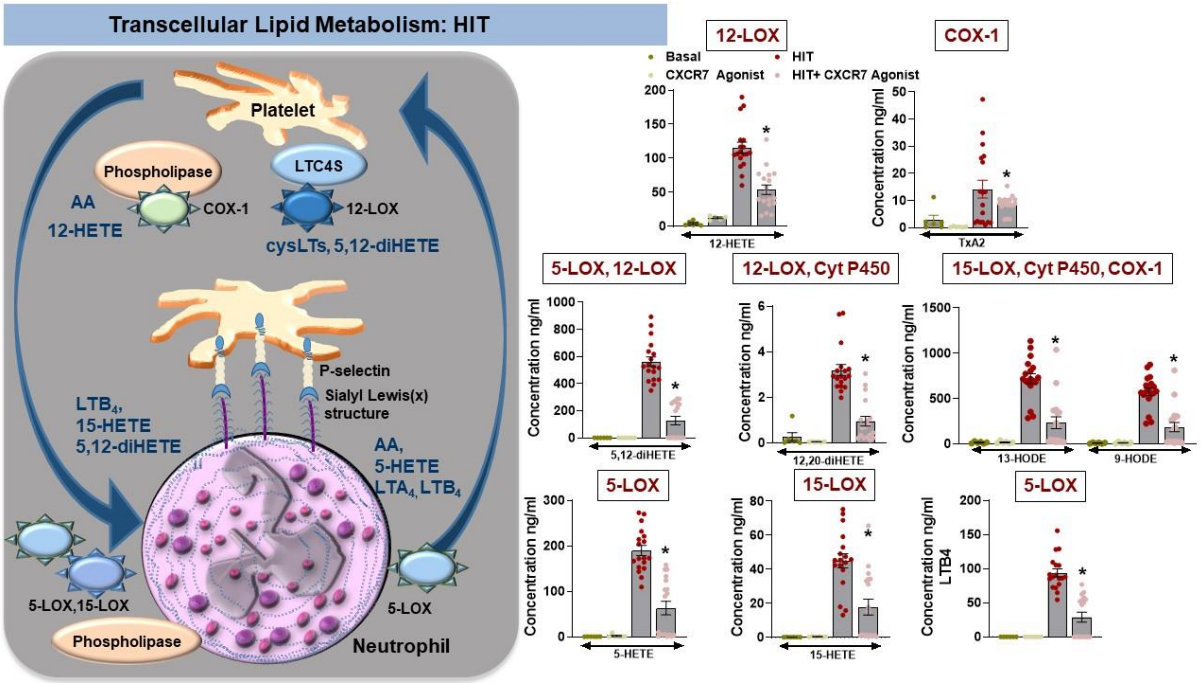
Immunothrombosis resulting from FcγRIIA mediated platelet activation in heparin induced thrombocytopenia-(HIT) and other acute immune response like COVID-19 drives thrombotic and hypercoagulatory complications, necessitating antiplatelet therapy. Current investigation explores the therapeutic potential of CXCR7 in modulating immunothrombotic platelet response.

We have employed lipidomics analysis by UHPLC-QTOF-MS/MS. Platelet degranulation, αIIbβIII-integrin activation, procoagulant phosphatidylserine exposure, platelet-neutrophil aggregate formation were estimated by flow cytometry. We detected thrombo-inflammatory release from platelets by cytometric bead array; thrombin generation by calibrated automated thrombinoscopy; phosphorylation of SykTyr525/526 , SykTyr323 by immunoblot analysis.

Pharmacological CXCR7-agonist-(VUF11207) decreased platelet degranulation, αIIbβIII-integrin activation, heparin induced platelet aggregation (HIPA assay) and thrombotic response induced by HIT and COVID-19 sera and IgGs respectively. VUF11207 also reduced neutrophil active-CD11b surface expression and platelet-neutrophil aggregate formation induced by IgG from HIT and COVID-19 patients. Platelet procoagulant activity induced by IgGs was significantly counteracted by CXCR7-agonist decreasing phosphatidylserine exposure and thrombin generation. CXCR7-agonist countered SykTyr525/526 , SykTyr323 phosphorylation induced downstream of FcγRIIA mediated platelet activation triggered by IgGs from HIT and COVID-19 patients. Presence of CXCR7-agonist reduced the generation of thrombo-inflammatory lipids metabolised through platelet COX-1-(TxA2), 12-LOX-(12-HETE) and leukocyte 5-LOX-(5-HETE), 15-LOX-(15-HETE); furthermore, intercepted transcellular lipid metabolism between platelet and leukocytes (5,12-diHETE, 12,20-diHETE, 13-HODE, 9-HODE).

Therefore, therapeutic targeting of CXCR7 may modulate immunothrombotic complication mediated through FcγRIIA induced platelet activation as in HIT.

Fig. 1



LT 1 I Lightning talks I

LT 1-3

Retroviral cores trigger innate immune responses through a cryptic sensor in human hematopoietic stem cells and primary macrophages

Erika Valeri[1], Francesco Piras[1], Sara Valsoni[1], Ivan Cuccovillo[1], Roberta Caccia[1], Alice Reschigna[1], Angelo Lombardo[1], Anna Kajaste-Rudnitski[1]
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Although hematopoietic stem and progenitor cells are resistant to viral infections, the mechanisms by which they defend themselves remain poorly elucidated. We have shown that while HIV-1-derived lentiviral vectors (LV) escape innate immune sensing, MLV-based ɣ-retroviral vectors (ɣRV) activate robust type I interferon (IFN) responses. ɣRV recognition is independent of reverse-transcribed DNA and occurs also with empty viral particles devoid of genomic RNA, suggesting that IFN-induction is mainly driven by recognition of viral structural components. Here we show that human primary macrophages and PMA-differentiated U937 cells recapitulate this nucleic acid (NA)-independent activation of type I IFN responses and exploit them to better dissect mechanisms of yRV recognition. In agreement with structure-mediated sensing, PMA-U937 cells knock-out for the canonical NA sensors cGAS, STING, RIGI, or MAVS still up-regulated type I IFN responses upon yRV transduction, excluding their role in vector recognition. To further dissect the role of the structural components in ɣRV sensing, we produced chimeric LV/ɣRV by replacing the matrix (MA) and capsid (CA) of the LV with those of the ɣRV. Remarkably, a full MA-CA chimeric LV induced type I IFN responses in MDM, while MA only chimeric LV did not, highlighting a role of the ɣRV capsid in viral recognition. Interestingly, ɣRV-mediated induction of type I IFN was abrogated by inhibition of the central immune kinase TBK1. To identify host factors involved, we performed a CRISPR/Cas9 screening targeting interferon-stimulated genes and are currently assessing the role of the most relevant candidate factors. In parallel, we fused the APEX2 sequence to the MLV CA to perform proximity biotinylation of capsid interactors. Overall, our work uncovers the existence of a novel innate immune sensor of retroviral cores in human primary cells, informing the development of novel antiviral strategies and improved gene therapies.

LT 1-4

Therapy for cancer-specific MyD88^{L265P} signaling based on exon skipping using LNP-mediated ASO delivery

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Introduction: MyD88 is a central signaling adaptor in innate immunity, mediating signaling via TLRs and IL-1R. Somatic *MYD88^{L265P}* mutation enables its spontaneous oligomerization, leading to constitutive activation of the proinflammatory signaling pathway. This mutation is frequent in several types of B cell lymphoma, e.g. Waldenström`s macroglobulinaemia (WM).

MyD88 signaling is regulated in several ways e.g. alternative splicing. MyD88$_S$, which is upregulated upon LPS stimulation, lacks exon 2. MyD88$_S$ is inhibitory as it prevents binding of IRAK4 and forms signaling-incompetent myddosome complex. Vickers et al (J Immunol, 2006) showed that antisense oligonucleotides (ASO) designed to skip exon 2 increased translation of MyD88$_S$, which inhibited NF-κB signaling, but no one has shown whether short variant MyD88$_S^{L265P}$ can be inhibitory as well.

Methods: We designed several ASOs targeting different sites in introns and exons to improve exon skipping. ASOs were transfected/electroporated to HEK293 or MWCL cells and mRNA transcripts, cytokines, apoptosis were analyzed. Lipid nanoparticles (LNPs) with different compositions for ASO delivery were prepared and tested in mice.

Results: We showed that MyD88$_S^{L265P}$ variant efficiently inhibits MyD88^{wt} and MyD88^{L265P} signaling. ASOs targeting different segments of intron and exon sequences improved exon skipping and inhibited cytokine release from IL-1β stimulated HEK293 cells. Decrease of exon 2 containing mRNA transcripts and inhibition of cytokine expression were also observed in WM cells. WM is B cell lymphoma with the main pathology in the bone marrow. By changing the composition we prepared LNPs that increased targeting of B cells in vivo.

Conclusions: MyD88^{L265P} presents a survival signal for cancer cells, therefore inhibition of its signaling could be used for treatment. By improving alternative splicing and in vivo targeted delivery of ASOs we deliver novel therapy for cancers that involve MyD88-dependnt signaling.

LT 2 I Lightning talks II

LT 2-1

TRK-fused gene (TFG) regulates macrophage inflammation and pyroptosis via TRAF3/ULK1 axis

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Question: TRK-fused gene (TFG) is known to be involved in protein secretion and plays essential roles in antiviral innate immune response. However, its function in LPS induced inflammation and pyroptotic cell death is still unknown.

Method and Results: Our results showed that TFG-deficient THP-1 macrophages exhibits higher mitochondrial ROS production. LPS/Ng stimulation triggers a much higher level of ROS and induces pyroptotic cell death. ULK1 undergoes a rapid turnover in TFG-deficient THP-1 cells. TFG forms complex with an E3 ligase, tumor necrosis factor receptor-associated factor 3 (TRAF3), and stabilizes ULK1 via disturbing ULK1-TRAF3 interaction. Knockdown of TFG facilitates interaction of ULK1 with TRAF3 and subsequent K48-linked ULK1 ubiquitination and proteasome degradation. Rescue of ULK1 expression blocks LPS/Ng induced cell death in TFG-deficient THP-1 macrophages. Finally, myeloid cell-conditional TFG knockout mice (TFG-MKO) were established and peritoneal derived macrophages (PEMs) were isolated from TFG-MKO mice and wild type (WT) control mice. RT-PCR results showed that the expression of pro-inflammatory factors, iNOS, IL-6 and TNF-α, significantly increased in TFG-MKO PEMs. Furthermore, DSS-induced acute ulcerative colitis model were established in TFG-MKO and WT mice, and the results showed that significantly shorter colons and higher DAI scores were observed in TFG-MKO group.

Conclusions: Taken together, TFG plays an essential role in LPS/Ng induced pyroptotic cell death via regulating K48-linked ULK1 ubiquitination in macrophages. Myeloid cell-conditional TFG knockout facilitates DSS-induced acute ulcerative colitis in mouse model.

LT 2-2

The ER-resident chaperone CNPY3 is essential for TLR trafficking and inflammasome activation

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Toll-like receptors (TLRs) mediate the recognition of microbial and endogenous insults encountered on the cell surface or exposed inside the cell. The subcellular distribution of the different TLR members reflects this strategy: some TLRs are localised at the plasma membrane, while others accumulate at intracellular vesicles and endomembranes. Since PAMP surveillance by TLRs is highly dependent on the correct subcellular localisation their proper maturation, folding, glycosylation and trafficking are critical for the function of TLRs and other PRRs. The chaperone Canopy FGF Signaling Regulator 3 (CNPY3) is essential for trafficking of several TLRs, including TLR1/2/4/5/9 but not TLR3. Loss of CNPY3 function prevents TLR exposure at the cell surface, precluding in turn PAMP recognition and inflammatory signalling. We have recently discovered an additional function of CNPY3 in the control of inflammasome activation, suggesting a more versatile and dedicated role of CNPY3 in the inflammatory response than previously thought. This latter function of CNPY3 is independent of its role as TLR chaperone, as CNPY3-deficient macrophages primed via the TLR3 receptor, which is fully functional in these cells, show severely impaired inflammasome activation in response to various triggers. Interestingly, rare pathogenic variants of CNPY3 are associated with cases of paediatric early infantile epileptic encephalopathy (EIEE). Originally assumed to be a loss-of-function condition, our recent analysis of new pathological EIEE variants challenges this assumption. Furthermore, it is unclear whether defects in the known functions of CNPY3 (i.e. TLR trafficking and inflammasome activity) underlie the clinical pathology or whether EIEE instead results from defects in other, as yet unknown, substrates of CNPY3. We will present our findings from proteomic screening approaches aimed at deciphering the cellular substrate(s) and function(s) of CNPY3 as well as the molecular causes of EIEE.

LT 2 I Lightning talks II

LT 2-3

RBM39 regulates the transcription and alternative splicing of IRF3 to maintain innate immune response

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Question: If there are some factors specifically regulate innate immunity in hepatocytes? If so, how do they play their function and if these factors are associated with liver diseases, especially chronic hepatitis virus infection?

Methods: CRISPR/Cas9, RT-qPCR, luciferase assay, western blot, RNA-seq, Mass spectrometry.

Results: We validated 50 candidates of a CRISPR/Cas9 screen on TLR3 immune response to poly(I:C) in hepatocytes by siRNA and analyzed RBM39 in-depth. Knockdown of RBM39 affected the induction of type I interferons and ISGs via IRF3, but not NF-κB, which was rescued by ectopic expression of RBM39 siRNA-resistant mutant. The function of RBM39 in innate immunity was not restricted to TLR3 or hepatocytes, suggesting an ubiquitous impact on the interferon pathway. Mechanistically, RBM39 knockdown reduced IRF3 protein expression by transcriptional regulation and alternative splicing, resulting in a switch from the functional IRF3 isoform to other non-functional mRNAs. Indisulam, a sulfonamide drug with anticancer activity promoting degradation of RBM39, suppressed IRF3 expression in all tested human but not murine cell lines, including primary human hepatocytes. Genome-wide transcriptome and proteome analysis upon knockdown of RBM39 or indisulam treatment identified additional innate immune factors affected by RBM39 downregulation, such as RIG-I, MDA5 and the Interferon λ receptor.

Conclusion: Our CRISPR/Cas9 screen identified RBM39 as a crucial factor of innate immune pathways. RBM39 regulates IRF3 promoter activity and modulates the ratio of functional/non-functional IRF3 isoforms via alternative splicing, as well as the expression of other factors of cell intrinsic innate immunity. Since specific drugs degrading RBM39 are available, it could provide a promising target to modulate innate immune responses.

Fig. 1

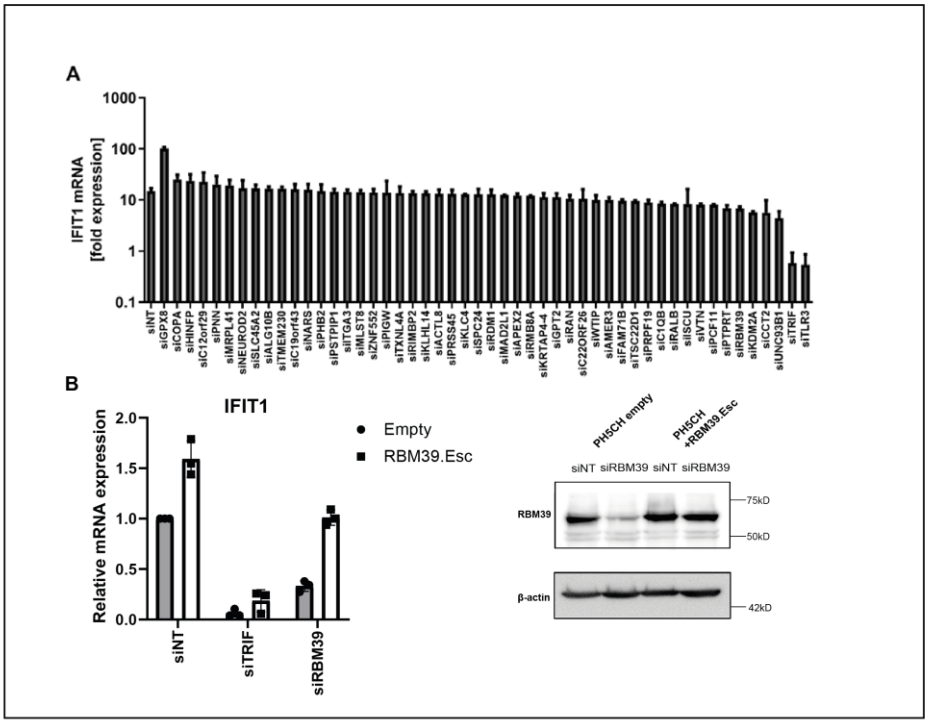

Figure 1. RBM39 was identified as a novel innate immune factor. A. siRNA validation of 50 candidate genes. PH5CH cells were stimulated by poly(I:C) supernatant feeding, IFIT1 as an ISG was measured by RT-qPCR upon knockdown of each genes. B. Rescue of RBM39 knockdown. RBM39 siRNA-resistant mutant (RBM39.Esc) was expressed to PH5CH cells by lentivirus transduction, IFIT1 was measured upon siRNA knockdown and poly(I:C) supernatant feeding. RBM39 knockdown and rescue efficiency was measured by immunoblot.

Fig. 2

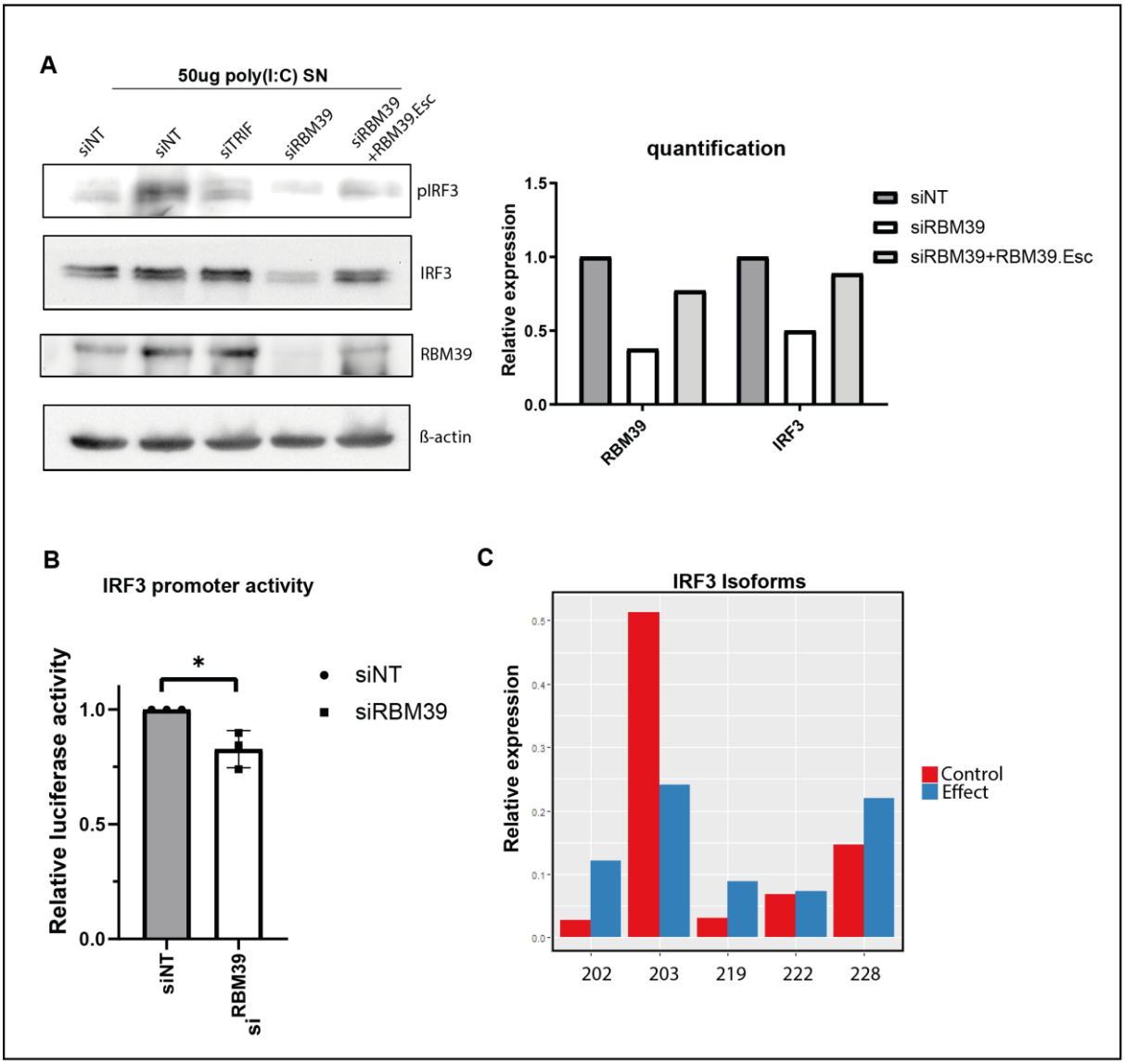

Figure 2. RBM39 affect IRF3 expression via transcriptional regulation and alternative splicing. A. RBM39 knockdown reduced IRF3 protein expression. protein level was measured by immunoblot (left) and quantified (right). B. IRF3 pro -moter activity slightly decreased upon RBM39 knockdown. Promoter sequence was inserted upstream firefly luciferase gene and measured by luciferase assay. C. IRF3 isoforms relative expression in RNA sequencing. IRF3-203 is the major and functional isoform. Control means combination of siNT and DMSO treatment, Effect means combina -tion of siRBM39 and indisulam treatment.

LT 2 I Lightning talks II

LT 2-4

Dysregulation of NLRP3 inflammasome affects Valboro-Pro (Talabostat)-induced pyroptosis in CD8+ T cells of ART-treated HIV patients

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Introduction: HIV-infected (HIV) patients exhibit immune dysregulation independently of antiretroviral therapy. The inflammasome, a cytosolic complex responsible for the cleavage of the inflammatory cytokines IL -1β and IL -18, and pyroptosis are highly activated in peripheral blood mononuclear cells of HIV patients, suggesting its involvement in cell dysfunction. While monocytes, B cells, and CD4+ T cells have been studied, little is known about CD8+ T lymphocytes. Therefore, we proposed to characterize the inflammasome activation in these cells, both the NLRP3 and NLRP1/CARD8 pathways, which were partially described in T cells.

Methods: The study is in accordance with the ethical committees. CD8+ T lymphocytes from non-HIV healthy donors (HD;n=17) and HIV patients (HIV;n=14) were analyzed ex vivo and stimulated in vitro with known activators of NLRP3 (α-CD3/α-CD28), NLRP1 and CARD8 (DPP9 inhibitor ValboroPro, VbP) to assess inflammasome activation by caspase-1 activation, cytokines release, and pyroptosis. Immunofluorescence staining was performed to identify the assembly of the complex and the receptor(s).

Results: HIV CD8+ T cells present a constitutively activated caspase-1 which positively correlates with the cell activation state (Fig.1). These cells do not produce IL -1ß or IL -18 but show increased pyroptosis. HIV CD8+ T cells were more activated and more resistant to NLRP1-induced pyroptosis compared with HD. Immunofluorescence analysis detected NLRP3 in α-CD3/α-CD28-induced pyroptosis in HIV but not in HD CD8+ T cells, and NLRP1/CARD8 as receptors involved in VbP-induced pyroptosis only in HD(Fig.2).

Conclusion: CD8+ T lymphocytes from HIV patients showed higher pyroptosis under NLRP3 agonists compared with HD, suggesting that the patient's inflammatory state influences the hyperregulation of this receptor. On the other hand, we found that pyroptosis in CD8+ T lymphocytes from HIV patients is mainly induced by NLRP3 and in HD mainly by NLRP1 or CARD8.

Fig. 1

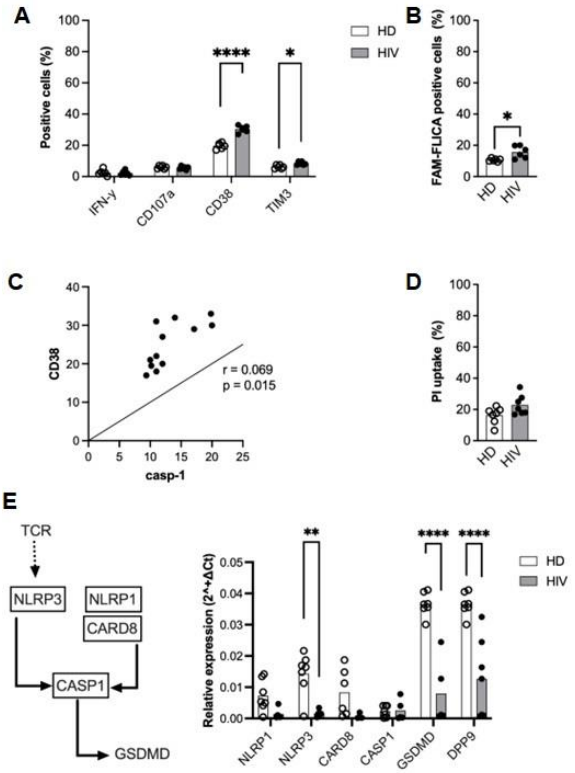

Fig. 1. Caspase-1 is activated in CD8+ T cells of HIV patients
500.000 CD8+ T cells isolated from healthy donors (n = 6) and HIV patients (n = 6).
Surface markers and IFNg **(A)** and activated caspase-1 **(B)** were analysed by flow cytometry. Pearson Correlation test was realized for CD38 surface expression and activated caspase-1 (**C**). The Propidium Iodide (PI) up-take was measured and expressed as the percentage of PI positive cells related to Triton-treated cells (100%) **(D)**. Relative expression was measured in HD and HIV CD8+ T cells and expressed by 2-ΔCt **(E)**.
Two-way ANOVA test was applied to compare all the conditions in A and E graphs. T test was used in B and D graphs. *: p< 0.05; **: p< 0.01; ****:p< 0.0001 .

Fig. 2

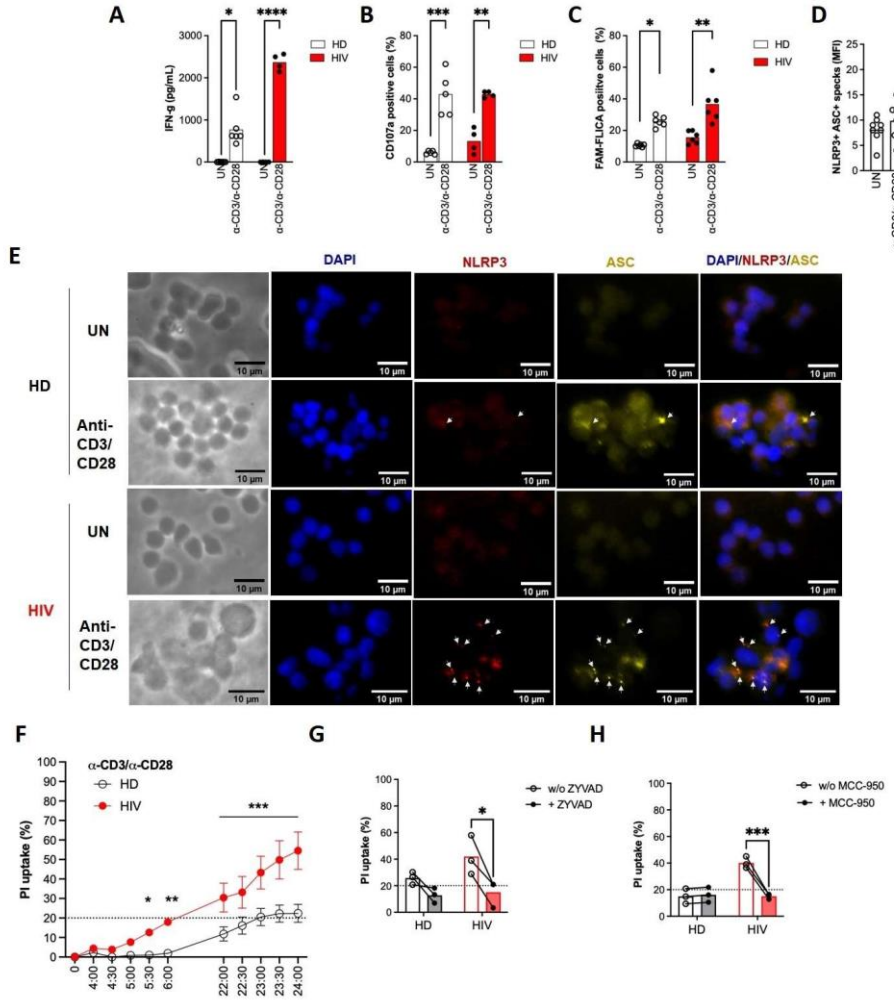

Fig. 2 In HIV CD8+ T cells the TCR stimulation triggers NLRP3 inflammasome and pyroptosis.
25.000 CD8+ cells isolated from healthy donors (n = 6) and HIV patients (n = 6) were treated with anti-CD3 and anti-CD28 for 24 hours. The release of IFN-g was measured in culture supernatants and expressed as pg/mL.**(A),** The surface expression of CD107a/LAMP **(B)** and caspase-1cleavage/activation **(C)** were analyzed by flow cytometry. ASC+ NLRP3+ specks are stained with red fluorescence (Goat anti-human NLRP3) and yellow fluorescence (Rabbit anti-human PYCARD/ASC). DAPI dye (Blue fluorescence) was used for nuclei coloration. Data were reported as microscopy photographs (100 µm) for one representative experiment **(D)** and bar graphs showing the MFI of ASC+NLRP3 positive CD8+ T cells as a mean of the three experiments ± standard error **(E).**The Propidium Iodide (PI) up-take was measured and expressed as the percentage of PI positive cells related to Triton-treated cells (100%) in a real time curve **(F).** In some experiments, cells were pre-treated with caspase-1 inhibitor ZYVAD **(G)** or NLRP3 inhibitor MCC-950 **(H)** and PI up-take was measured at 2 hours.
Two Way ANOVA was used to compare traeayed and untreated conditions in the two groups in A-D. Repeated-measures 1 Way ANOVA was used to compare all the conditions in F graph. Paired T test was used to compare treated and untreated groups in the G and H graph. *: p< 0.05; **: p< 0.01; ***: p< 0.001; . ****: p< 0.0001.

LT 3 I Lightning talks III

LT 3-1

Hyperglycemia amplifies TLR-mediated inflammatory response of human healing macrophages to dyslipidemic ligands

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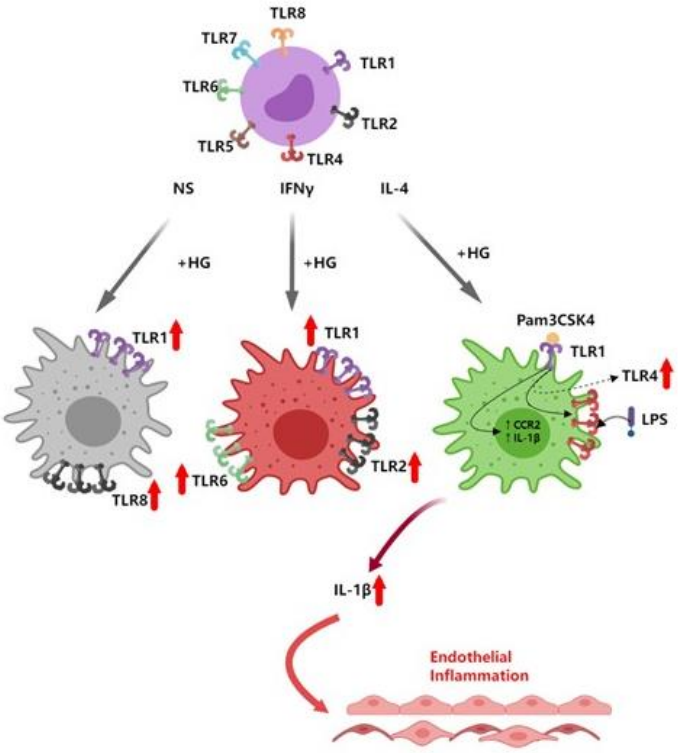
Question. Hyperglycemia (HG) is a hallmark of diabetes and is a critical factor in the initiation of diabetic complications. Macrophages are key innate immune cells that regulate inflammatory responses which are responsible for the development of micro- and macrovascular complications. Increased expression of toll-like receptor 4 (TLR4) has been inked to type 2 diabetes (T2D). Here for the first time, we systemically addressed the role of hyperglycemia in the regulation of TLR system in primary human macrophages. Methods and Results. Expression of TLR 1-9 was examined in primary human monocytes-derived homeostatic M(NS), inflammatory M1(IFNgamma) and healing M(IL4) macrophages in normoglycemic and hyperglycemic (HG) conditions by RT-PCR and flow cytometry. HG induced upregulation in expression of TLR1 and TLR8 in M0 macrophages, TLR1, TLR2 and TLR6 in M1, and TLR4 and TLR5 in M2. HG potentiated TLR4-mediated response of M2 to LPS and significantly enhanced production of IL1beta. In M (IL4), HG in combination with PAM3CSK4 (PAM3), synthetic triacylated lipopeptide, ligand for TLR1/TLR2 amplified expression of TLR4, enhanced production of IL1beta, and supressed production of IL10. Conclusions. We found that hyperglycemia alone enhances inflammatory potential of homeostatic, inflammatory, and healing macrophages by increasing specific profiles of TLRs. In combination with dyslipidemic ligands, hyperglycemia can switch the inflammatory program in healing M(IL4) macrophages towards supporting vascular inflammatory complications in diabetic.

Fig. 1



LT 3 I Lightning talks III

LT 3-2

NLRP3 inflammasome activation in cochlear macrophages causes sensorineural hearing loss in Chronic Suppurative Otitis Media

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Chronic suppurative otitis media (CSOM) is the most common cause of permanent hearing loss in children in the developing world. We have created a validated Pseudomonas Aeruginosa (PA) CSOM mouse model and observed outer hair cell (OHC) loss in the cochlear basal turn. We also revealed that macrophages are associated with this OHC loss. The NLRP3 inflammasome is an intracellular immune sensor that is expressed in monocytes, macrophages and dendritic cells. In the current study, we investigate the NLRP3 inflammasome activation in the cochlear macrophages in CSOM sensorineural hearing loss (SNHL). We depleted the resident macrophages using the colony stimulating factor 1 receptor (CSF1R) inhibitor PLX 5622. We observed that there are both more macrophages and more OHC loss in the control group compared to the macrophage depletion group at 14 days CSOM. Interestingly, the NLRP3 inflammasome associated factors including NLRP3, PYCard, Caspase1 and IL-1β are upregulated in the control group compared to the macrophage depletion group at 7d CSOM. We further generated CSOM in NLRP3-/- mouse and assessed OHC loss and macrophage numbers. OHC are protected in NLRP3-/- CSOM cochleae with no change of macrophage numbers comparing with control CSOM. Taken together, the data suggest that NLRP3 inflammasome activation in cochlear macrophages causes sensorineural hearing loss in Chronic Suppurative Otitis Media.

Reference: Xia et al, Chronic suppurative otitis media causes macrophage-associated sensorineural hearing loss. Journal of Neuroinflammation (2022) 19:224

LT 3-3

Bone-targeting bisphosphonates may boost immune responses to pulmonary infection by inhibiting the mevalonate pathway in alveolar macrophages

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The mevalonate pathway is a ubiquitous metabolic pathway required for sterol and isoprenoid lipid biosynthesis. This pathway is inhibited by bisphosphonate (BP) drugs, the gold standard treatment for metabolic bone diseases. Due to their high affinity for calcium, BPs are considered to only act in bone-resident cells (osteoclasts). However, epidemiological studies suggest that BP therapy is also associated with reduced risk of infection and mortality from pneumonia. To better understand how BPs may have beneficial effects in the lung, we employed multicolour flow cytometry, liquid chromatography-mass spectrometry and single-cell RNA sequencing and identified alveolar macrophages (aMac) as novel targets of BPs in mice.

Analysis by flow cytometry of lung tissue digests, after a single intravenous (*iv*) injection of fluorescently-tagged BP (AF647-ZOL), revealed BP uptake by >97% of aMac ($CD11b^{lo/-}CD11c^{hi}CD64^{+}$). The intracellular buildup of the metabolite isopentenyl diphosphate (IPP) and unprenylated protein are hallmarks of BP action in cells. Consistent with intracellular uptake of AF647-ZOL, using LC-MS/MS and a customised prenylation assay we detected a clear accumulation of IPP and unprenylated Rab GTPases in aMac 48 hours after a single *iv* dose of BP. After challenging mice with intranasal endotoxin, scRNA-seq of aMac isolated from BP-treated mice revealed enhanced gene expression of proinflammatory cytokines and chemokines (including *IL1b and Cxcl2*) in a subpopulation of homeostatic aMac. This was accompanied by elevated proinflammatory cytokine/chemokine levels in the bronchoalveolar lavage fluid.

These studies dispel the dogma that BP drugs act only in the skeleton and reveal that BP also inhibits the mevalonate pathway in aMac and enhances production of proinflammatory cytokines/chemokines in response to endotoxin. We propose that BP treatment may boost the response of homeostatic aMac to pulmonary infections, thereby conferring protection from pneumonia.

LT 3 I Lightning talks III

LT 3-4

Bruton"s tyrosine kinase (BTK) and matrix metalloprotease 9 (MMP9) regulate NLRP3 inflammasome-dependent cytokine and NET responses in primary neutrophils

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Background: Bruton's tyrosine kinase (BTK) has a central role in the maturation and function of neutrophils, the primary leukocyte population in humans. As BTK recently emerged as a positive NLRP3 inflammasome regulator in macrophages, we explored the regulatory function of BTK in NLRP3 inflammasome activation in neutrophils, especially regarding a prototypical neutrophil function, the release of neutrophil extracellular traps (NETs).

Methods: NLRP3 inflammasome activation was evaluated in primary bone-marrow derived neutrophils, and matched macrophages, isolated from Btk KO, Btk-mutated "X-linked immune defect" (XID), NLRP3 KO and C57BL6 WT mice. Moreover, neutrophils and matching PBMC were also purified from healthy donors (HD) and X-linked agammaglobulinemia (XLA) patients with verified clinical *BTK* deficiency. The isolated cells were treated with well-known NLRP3 activators and the release of myeloperoxidase (MPO), Interleukin (IL)-1β and NETs quantified. Pharmacological inhibitors for BTK, NLRP3, caspase-1, gasdermin D and matrix metallopeptidase 9 (MMP-9), an abundant component of neutrophil granules, were used to establish the role of these factors in neutrophil NLRP3 inflammasome signaling.

Results: In both mouse and human primary neutrophils we observed a significant increase in NLRP3 inflammasome-dependent IL-1β and NETs when BTK was absent or inhibited, whereas IL-1β release was decreased in corresponding primary macrophages or PBMC. This suggests an unexpected negative regulatory role of this kinase in terms of neutrophil NLRP3 activation. Both IL-1β and NET release in mouse and human primary neutrophils were strictly dependent of NLRP3, caspase-1, gasdermin D and, surprisingly, MMP-9.

Conclusion: Our study identifies BTK as a cell-type-dependent rheostat of NLRP3 inflammasome activation and NET release, and MMP-9 activity as a novel requirement of neutrophil IL-1β and NET release.

Key words: Neutrophils, NLRP3, BTK, MMP-9.

PS 1 I Poster session I

P 001

Modulation of S1PR1-expression on neonatal T-cells via breast milk ILCs in women with gestational diabetes

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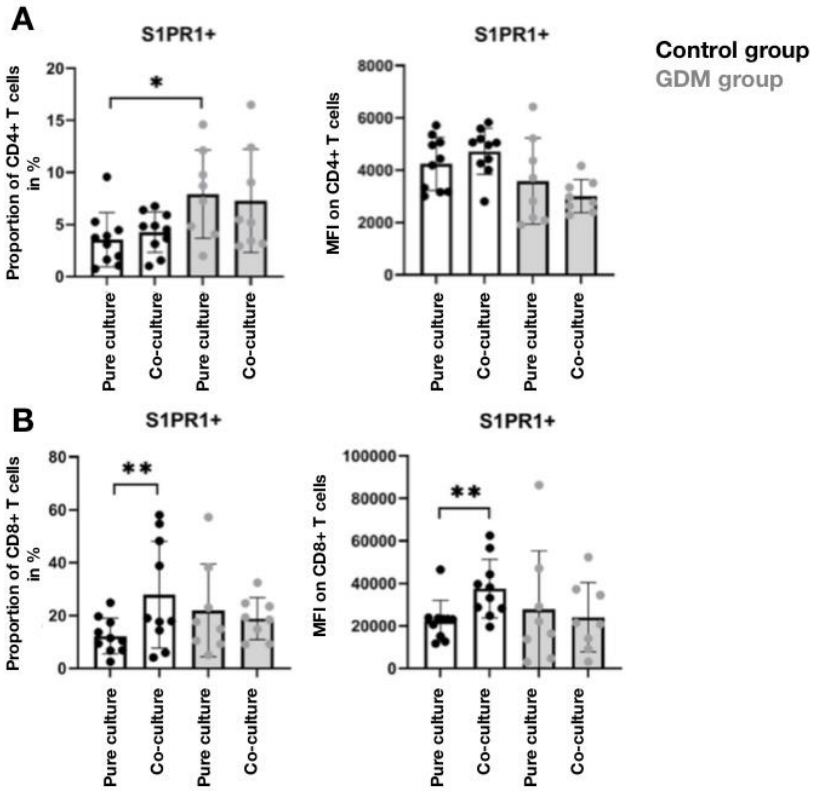
Background: Gestational diabetes (GDM) is associated with neonatal complications like hypoglycemia and a higher risk of metabolic syndrome. Breast milk (BM) is an effective immunological protection postnatal. Proinflammatory innate lymphoid cells-1 (ILC1), has been detected in BM from mothers with GDM. Migration of proinflammatory lymphocytes is regulated by the sphingosine 1 phosphate receptor 1 (S1PR1). Thus this study aims to analyse whether BM ILC1 of mothers induce a proinflammatory T cell activation of cord blood due to the induction of S1PR1on lymphocytes of term infants.

Method: In this prospective study, the T cell activation status of term infants born from mothers with GDM (n=8) was compared to a control group (n=10). Isolated ILC (lin-, CD127+) were incubated with pan T-cells of cord-blood after 4-5d in a 24-h co-culture. The percentage of T cells expressing S1PR1 and the mean expression density (MFI) were determined by flow cytometry.

Results: Percentage of S1PR1 of T cells, mainly Th cells, is higher in GDM samples than healthy controls in pure culture. (median 8.8% vs. 3.4%) (p=0,0155)(figure 1 A), In the control group, more CD8+ T cells express S1PR1 after co-culture than in pure culture (median 11.0% vs. 18.5%)(p=0.0314), this effect is also seen in MFI (median 23.2 vs. 35.7)(p=0.0052)(figure 1 B).

Conclusion: GDM leads to an increased S1PR1 expression on Th cells postnatal, which might induce a proinflammatory metabolic state in newborn due to an induced migration of T-cells from the lymphatic tissue in the newborn circulation. While coculture with ILCs has no significant effect on S1PR1 T cell expression in the GDM group, S1PR1 expression in the control group is significantly higher in coculture than in pure culture. Thus, breast milk ILCs do not induce a proinflammatory status due to the induction of S1PR1 in infants. This may contribute to the mechanism that breastfeeding reduces the risk of metabolic syndrome in children of mothers with GDM.

Fig. 1



PS 1 I Poster session I

P 003

Salmonella-delivered COBRA-HA1 antigen derived from H1N1 hemagglutinin sequences elicits broad-spectrum protection against influenza A subtypes

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A universal vaccine is in great demand to address the uncertainties of antigenic drift and mismatch of current influenza vaccines. In this study, a strategy called computationally optimized broadly reactive antigens (COBRA) was used to generate a consensus sequence of the hemagglutinin globular head portion (HA1) collected from 1918 to 2021 to trace evolutionary changes and reflect them in the designed constructs. Constructs carrying different regions of HA1 were delivered into eukaryotic cells by *Salmonella*-mediated bactofection using a Semliki Forest virus RdRp-dependent eukaryotic expression system, pJHL204; and protein expression was confirmed by western blot and immunofluorescence assays. Mice immunized with the designed constructs produced a humoral response, with a significant increase in IgG level and cell-mediated immune response, including a 1.5-fold increase in CD4+ and CD8+ T cells. The protective efficacy was shown by up to 4-fold higher production of neutralizing antibodies. A broad protective immune response was conferred by a notable reduction in viral titer and minimal inflammation in lungs challenged with the influenza A/PR8/34, A/Brisbane/59/2007, and A/California/07/2009 strains. This opens a new horizon for influenza vaccine design and delivery based on *Salmonella*-mediated COBRA-HA as a highly efficient *in vivo* antigen presentation strategy against seasonal influenza and pandemic outbreaks.

P 005

Association of a *TLR7* variant with spontaneous clearance of hepatitis B virus infection in Caucasians

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Background: Toll-like receptor 7 (TLR7) is a major part of the innate immune system generating immune responses against hepatitis B virus (HBV). Recently, TLR7 activation has been shown a promising target for the treatment of chronic hepatitis B. However, polymorphisms within the *TLR7* gene might alter immune responses and therapy efficiency. Therefore, we investigated the impact of the *TLR7* polymorphisms rs179008, rs864058 and rs2302267 on the course of HBV infection.

Method: In this study, 530 Caucasian patients with chronic hepatitis B virus infection and 195 individuals with spontaneous HBV surface antigen (HBsAg) seroclearance (SC) were enrolled. Genotyping of the *TLR7* SNPs was performed. Serum cytokine levels were measured in 193 patient samples.

Results: The genotype distribution of the *TLR7* SNP rs179008 was significantly different between patients with chronic HBV infection and HBsAg SC (p=0.003). The AT/TT genotypes were significantly associated with an increased likelihood of HBsAg SC in adjusted forward logistic regression analysis (OR=1.48 [95% CI: 1.01-2.17] p=0.045). Carriers of the rs179008 AT/TT genotypes with HBsAg SC showed lower serum levels of IL-1 beta (p=0.03), TNF alpha (p=0.01), IFN alpha (p=0.0008), IFN beta (p=0.019) and IL-10 (p=0.029) than carriers of the AA genotype. Additionally, there was a significant association of *TLR7* rs2302267 TG/GG genotypes with HBeAg-negative infection (OR=1.51, p=0.039), and an association of *TLR7* rs864058 GG genotype with an increased risk of liver cirrhosis development (OR=3.48, p=0.019).

Conclusion: We confirmed the previously reported association of the *TLR7* rs179008 variant with HBsAg seroclearance in a Caucasian cohort and the effect on cytokine production. Furthermore, we identified new effects of *TLR7* rs2302267 and rs864058 on disease progression. Our findings further strengthen the relevance of the genetic predisposition in individualized medicine and the development of new TLR7-directed therapies.

PS 1 I Poster session I

P 007

Extracellular histones H1 and H3 have differential actions as the damage-associated molecular patterns of the central nervous system - pro-inflammatory effects on microglia and direct neurotoxicity

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Question: Histone proteins organize DNA within cellular nuclei, but can be released from activated, damaged, or dying cells. Extracellular histones are known to act as damage-associated molecular patterns (DAMPs) in peripheral tissues inducing pro-inflammatory activation of immune cells, but very limited studies have considered DAMP-like activity of histones in the central nervous system (CNS). We hypothesized that different isoforms of histones have distinct pro-inflammatory effects on microglia, which are the professional CNS immunocytes, and also act as neurotoxins.

Methods: The core histone H3 and the linker histone H1 were applied to BV-2 murine or THP-1 human microglia-like cells alone or in combination with the inflammatory stimuli lipopolysaccharide (LPS) and interferon (IFN)-γ. Cultured murine cortical neurons, NSC-34 murine and SH-SY5Y human neuronal cells were used to assess the direct neurotoxicity of histones.

Results: H1, but not H3, downregulated phagocytic activity of microglia. Both histone types induced or enhanced inflammatory activation of microglia-like cells by upregulating their secretion of nitric oxide (NO) and cytokines, including tumor necrosis factor (TNF) and C-X-C motif chemokine ligand 10 (CXCL10). In most cases, histones acted synergistically with IFN-γ, but not with LPS, which implicated the activation of microglial toll-like receptor (TLR) 4. The selective inhibitor TAK-242 was used to confirm the role of TLR4 in H1-induced NO secretion by BV-2 microglia. H1, but not H3, was directly toxic to all neuronal cell types studied.

Conclusions: Extracellular H1 and H3 have the potential to act as CNS DAMPs by inducing TLR4-dependent pro-inflammatory activation of microglia, as well as direct neurotoxicity. Therefore, inhibition of the DAMP-like and neurotoxic activity of extracellular histones represents a novel strategy for combating neuroinflammatory diseases characterized by the adverse activation of microglia and death of neurons.

P 009

Role of TRAF6 adaptor in the immune responses to murine polyomavirus

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Polyomaviruses are associated with many diseases due to their ability to persistently infect the host. Since 2008, twelve new human polyomaviruses have been discovered. Among the new species, Merkel cell polyomavirus (MCPyV) was identified as the etiological agent of skin Merkel cell carcinoma. Unfortunately, the cell type productively infected by MCPyV is unknown and therefore in vitro studies of the MCPyV are still a challenge. Nevertheless, its closest related member, Murine polyomavirus (MPyV) has been used per decades as in vitro model to understand fundamental questions about the biology of polyomaviruses. We demonstrated that MPyV activates innate immunite responses via GAS-STING and TLR4. It is known that the transcription factors, NF-Kβ and IRF3 are activated during launching of the above pathways. However, NF-Kβ is mainly activated by TLR4 for pro-inflammatory cytokines production and IRF3 by cGAS-STING for interferon (IFN) production.

Here, we focus on TRAF6, because, apart from its well-known role as one of the possible adaptors in the TRL4 pathway, recently it was suggested to play some role in the cGAS-STING pathway. We used TRAF6 knockout mouse embryo fibroblast and inhibitor of TLR4 to follow activation of NF-Kβ, the levels of IFN β and levels of pro-inflammatory cytokines (CXCL10, IL6) in response to MPyV infections. We found that the knockout of TRAF6 or the inhibition of TLR4 lead to almost complete inhibition of the translocation of NF-Kβ to the nucleus and downregulation of the production of pro-inflammatory cytokines in response to the infection. Truly suggesting a major role of TRAF6 as the adaptor for TLR4. Finally, the interferon responses to the virus were decreased in absence of TRAF6. Since the promoter of IFN-β gene has binding sites for both NF-Kβ and IRF3, our results so far indicate a cross talk between TLR4-TRAF6 and cGAS STING via NF-Kβ.

PS 1 I Poster session I

P 011

Mycobacterial infection uncovers Kupffer cell plasticity

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Kupffer cells (KC) are resident tissue macrophages in the liver. Due to their anatomical positioning, they play important roles in clearance of senescent erythrocytes, particles, as well as blood-borne pathogens. In this project, we used intravenous infection of the low pathogenic *Mycobacterium bovis* BCG (Bacille Calmette-Guerin) to investigate the role of macrophage origin and plasticity in a disease context.

A hallmark of mycobacterial infections is the formation of granulomas – organized immune cell aggregates that mainly consist of macrophages. Using the *Clec4fCre-td-Tomato*:R26-yfp reporter mouse, which labels specifically KCs, we were able to identify that liver granulomas were of Kupffer cell origin but downregulated *Clec4f* in the granuloma core. This observation allowed us to sort these spatially distinct KC populations and perform bulk RNA sequencing. We found that granuloma core macrophages downregulated typical KC markers but acquired the expression of inflammatory genes. Irradiation experiments with bone-marrow transplantations validated that granuloma core KCs were indeed derived from bona fide resident macrophages and not of monocyte origin.

Due to the low abundance of monocyte-derived macrophages within the granulomas we wondered about their impact during the infection. Therefore, we infected CCR2-/- mice, which lack Ly6Chigh monocytes in the blood. These mice had granulomas reduced in size and amount, while the bacterial burden was comparable to WT mice.

Taken together, the data indicates that monocytes are required for granuloma induction, while tissue resident Kupffer cells are needed for the granuloma core formation. Further work is needed to unravel the particular signals required for the induction of granulomas, as well as the functional consequences of granuloma macrophage heterogeneity.

P 013

^{64}Cu-αGPIX-PET for the non invasive *in vivo* detection of platelets

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Platelets activation and aggregation can cause thrombosis and consequently serious diseases. Besides their critical functions in hemostasis, thrombosis and wound healing, the innate immune response is remarkably affected by the crosstalk with platelets. However, tools for the non-invasive *in vivo* examination and diagnosis of platelets related pathologies remain limited and are urgently needed. We have developed a novel antibody-based PET tracer, ^{64}Cu-αGPIX, which targets GPIX on platelets and progenitor cells. We aimed to evaluate ^{64}Cu-αGPIX for the *in vivo* detection of platelets aggregation and smallest lesions in mice using a relevant mouse model of thrombosis. *In vitro*, ^{64}Cu-αGPIX has a high radiochemical purity(>94%), remained stable in mouse serum for at least 24h and exhibited an immunoreactive fraction of 50%. First-*in vivo* PET scans of naïve mice revealed suitable whole-body distribution of ^{64}Cu-αGPIX with low uptake in the brain. Next, we evaluated ^{64}Cu-αGPIX in a recent mouse model for cerebral (sinus) venous thrombosis (CVST) by PET/MRI. CVST was induced by anti-CLEC-2 small antibody treatment after ^{64}Cu-αGPIX injection and shortly after start of the dynamic PET/MRI acquisition. The reconstructed PET images were colocalized to MRI scans to determine the uptake in the brain (vessels). After CVST induction, mice (n=7) rapidly developed CVST, which was accompanied by an immediate increase in the cerebral ^{64}Cu-αGPIX signal. In comparison, sham-treatment (n=4) resulted exclusively in a low background signal from circulating platelets in the brain. PET Quantification of the final minutes of the scan revealed a strong cerebral platelet-aggregation due to CVST (P<0.04). In conclusion, this study revealed the enormous potential of ^{64}Cu-αGPIX-PET which is a promising tool for non-invasive *in vivo* examinations of thrombus formation with even smallest lesions as well as the interplay of platelets and (innate) immune cells using multiparametric imaging setups.

PS 1 I Poster session I

P 015

Skin mast cells require integrin β1 for perivascular alignment and a sufficient immune response during contact hypersensitivity

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Mast cells (MCs) have a critical sentinel function in the innate immune response due to the immediate release of active mediators such as TNF and histamine upon activation. Particularly, perivascular MCs are essential for the onset and kinetics of inflammation through the directional release of these mediators into the blood stream. Here, we studied the role of the adhesion molecule integrin β1 (Itgb1) expression on perivascular MC vessel attachment and intravascular degranulation using a mouse model of conditional Itgb1 deletion in connective tissue-type MCs (CTMCs). Quantitative high-throughput image analysis of fluorescence microscopy images of integrin β1-deficient mice ($MC^{\Delta Itgb1}$) compared to Cre- littermate controls showed that Itgb1 is crucial for the spatial distribution of MCs in the ear skin. In detail, the spindle-like morphology, perivascular alignment and homogeneous distribution of MCs along the perivascular niche is impaired in $MC^{\Delta Itgb1}$ mice, particularly along arterioles. Moreover, we studied the functional consequence of Itgb1 deficiency in MCs in the model of contact hypersensitivity (CHS). Notably, $MC^{\Delta Itgb1}$ mice show a drastically diminished ear swelling upon hapten elicitation. In fact, there is a significant reduction of leukocyte infiltration into the challenged ear skin of $MC^{\Delta Itgb1}$ mice, in particular for neutrophils, macrophages and $CD8^+$ T cells. Importantly, the lack of Itgb1 in MCs resulted in an impaired degranulation efficiency. These findings indicate that integrin β1 expression is vital for skin MC distribution, morphology, perivascular alignment and their function in the CHS model.

P 017

Embryonic microglial colonization of the central nervous system is dependent on a distinct integrin expression pattern

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Microglia arise early during embryogenesis from the first erythromyeloid progenitors (EMPs) in the yolk sac and colonize the central nervous system (CNS) around embryonic day (E) 9.5. However, the precise molecules involved in this recruitment process are not characterized yet. Here, we combined unbiased transcriptomic proteomic approaches to define signals in the developing CNS facilitating microglial progenitor immigration. We found that especially extracellular matrix (ECM)-associated proteins are upregulated in the embryonic CNS during microglial colonization. Interestingly microglial progenitors and microglia during different developmental stages revealed a distinct and dynamically regulated integrin expression profile, indicating the integrin-ECM interaction might be an essential mechanism to enable microglial progenitors to colonize the CNS during embryogenesis.

PS 1 I Poster session I

P 019

Analysis of Yersinia-mediated cell death pathways in human macrophages

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Infection with *Yersinia enterocolitica* mediates cell death and inflammation in macrophages. This is triggered by the effector protein YopP, which induces apoptosis through the deactivation of several TLR-induced signalling pathways. Inhibition of TAK1 through YopP activates RIP1 that in turn activates caspase-8. Studies in mouse macrophages show that caspase-8 can activate gasdermin D, resulting in pyroptosis and release of interleukins. GSDMD plays an important role in cell death response upon infection. The related gasdermin E however, doesn"t have similar effects. Even though it"s cleaved into its active fragment, there are no signs of GSDME influencing either cell death or IL-1β release. While the roles of gasdermins during *Yersinia* infection in murine macrophages have been studied in depth, their relevance in human macrophages has not yet been extensively investigated.

Our research focusses on the involvement of gasdermins in primary human macrophages and PMA-differentiated THP-1 cells infected with *Yersinia*, with emphasis on the role of YopP. This is accomplished by studying protein processing, interleukin release and cell death rates. GSDME-deficient THP-1 cells were generated using CRISPR-Cas9 systems.

Our data shows that while caspase-8 was activated during infection, GSDMD was not processed. GSDME however, was processed into its active form along with caspase-3, which can cleave GSDME. We also investigated cell death rates and interleukin release and found that absence of YopP decreased cell death and increased the release of IL-1β and TNF-α. Absence of GSDME in THP-1 macrophages decreased *Yersinia*-induced cell death.

The absence of active GSDMD and the processing of GSDME suggests that *Yersinia* infection leads to the activation of different cell death pathways in human and murine macrophages. Further studies are required to address whether GSDME triggers pyroptosis in human macrophages and how it affects cell death or interleukin release.

P 021

Catecholamines as modulators of immune functions in neutrophils

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The catecholamine neurotransmitters dopamine, epinephrine and norepinephrine are essential for intercellular communication within the nervous and the endocrine system. Additionally, there is growing evidence pointing to a critical role of catecholamines as immunomodulators within the immune system. Neutrophils are the most abundant immune cells and key effector cells in the innate immune system. However, dysregulated neutrophils can also contribute to uncontrolled inflammation and tissue damage and are implicated in various inflammatory and autoimmune diseases. It is therefore crucial to understand the regulation of neutrophil activation. Here, we have investigated the dopaminergic machinery in human neutrophils using flow cytometry and immunofluorescence staining and have been able to identify enzymes for dopamine production and transport. We have found direct evidence for the uptake and storage of dopamine by neutrophils using HPLC. These findings suggest that neutrophils play a role in catecholamine transport and metabolism within the immune system. Furthermore, we found that human neutrophils express the dopamine receptors DRD2 and DRD3. We could show that dopamine, but not epinephrine or norepinephrine, inhibited the formation of neutrophil extracellular traps (NETs), an important immune defense mechanism, in a concentration-dependent manner. These results show that dopamine is an immune modulator that most likely exerts its effect via auto- and paracrine pathways and illustrate the significance of a neuro-immunological axis within the (innate) immune system. This important topic merits further studies and could revolutionize our understanding of how inflammatory processes are modified in a context-dependent manner.

PS 1 I Poster session I

P 023

Murine leukemia virus (MLV) and human immunodeficiency virus (HIV) differ in their sensitivity against the antiretroviral guanylate-binding proteins (GBPs) from humans, mice and rabbits

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Guanylate binding proteins (GBPs) represent an evolutionarily ancient protein family that is widely distributed among eukaryotes. They belong to the superfamily of dynamin-like, IFN-inducible guanosine triphosphatases and are part of the cell-autonomous innate immune response against various bacterial, parasitic and viral infections. Recently, the human orthologs GBP2 and GBP5 were shown to limit human immunodeficiency virus (HIV) replication by interfering with furin-mediated activation of the viral envelope (Env) protein.

The aim of this study is to gain deeper insights into the cross-species conservation of human, mouse and rabbit GBPs, as well as the mode of action, breadth and evolution of their activity against retroviruses.

While the *GBP* gene clusters of humans, mice and rabbits differ substantially in the number and arrangement of their paralogs, the respective protein sequences are well-conserved. Immunofluorescence microscopy showed that different GBPs localize to distinct subcellular compartments (Golgi, cytoplasm, nucleus, vesicles). Similar to human GBP2 and GBP5, several mouse and rabbit orthologs (e.g., mouse GBP3/5/10 and rabbit GBP4L2/4sg) significantly inhibited human furin activity in an *in vitro* reporter assay, suggesting that they also suppress furin-mediated Env maturation. Furthermore, overexpression of several GBPs impaired proteolytic HIV-1 Env processing (e.g., mouse GBP3/5, rabbit GBP4sg/5L2) and reduced the amount of functional Env at the cell surface (e.g., mouse GBP3/5/10, rabbit GBP4L4/7). Finally, co-expression of GBPs significantly reduced infectivity of newly formed HIV particles (mouse GBP3/5/10, rabbit GBP4L2/4sg/7, human GBP1/2/5). In contrast, the infectivity of murine leukemia virus (MLV) was only reduced by murine GBP3/5 and human GBP5.

Our findings show that the ability of GBPs to interfere with Env maturation and to reduce retroviral particle infectivity is conserved among humans, mice and rabbits.

PS 1 I Poster session I

P 025

Mast cell granule uptake polarizes macrophages toward a specialized phenotypic and functional state

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Inflammation, resolution of inflammation and tissue regeneration are tightly regulated processes dependent on the functional state of tissue resident and infiltrating macrophages. In the skin, mast cells and macrophages reside in high density and close proximity, particularly at the blood vessels. Mast cells act immediately at sites of injury and infection by releasing both intact secretory granules, which contain a plethora of up to 400 mediators, as well as soluble factors which impact neighboring immune cell function. However, the functional consequences of mast cell-macrophage interactions have been poorly studied.

We found that intact mast cell granules, released upon activation into the extracellular matrix (ECM), are engulfed by tissue resident macrophages and infiltrating monocytes. We experimentally delineated the functional consequences for macrophages in vitro and in vivo and, to our surprise, we found granule uptake alone is insufficient to commit macrophages toward an expected classical, proinflammatory state. Instead, mast cell granule uptake results in an atypical phenotypic marker expression, such as Arginase 1, MHCII and Mannose receptor, metabolic changes and gain of functions. In detail, both phagocytic and efferocytic functions are enhanced in mast cell granule-bearing macrophages. Transcriptome analysis of granule-bearing macrophages compared to those untreated indicated multiple pathway alterations including genes involved in cytokine-cytokine receptor interactions and ECM receptor interactions.

We have observed that mast cell granule uptake results in an atypical, non-classical macrophage activation phenotype. This distinct response by macrophages may support the emerging view that mast cells act beyond acute inflammation and are additionally important regulators of inflammatory resolution and healing.

P 027

Infiltrative monocyte derived adenosine deaminase is associated with SARS-CoV2 induced acute respiratory distress syndrome

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Acute respiratory distress syndrome (ARDS) is a severe complication of COVID-19 characterized by immune dysregulation and fibrotic changes in the lung. Emerging studies highlighted the involvement of innate immune cells as critical drivers of severe inflammation in COVID-19. However, the pathogenetic mechanism behind this remains unclear. Extracellular ATP is released during inflammation and hypoxia and rapidly converted into the immunosuppressive adenosine. Adenosine is further metabolized into inosine by adenosine deaminase (ADA). ADA2 is an extracellular isoform of ADA secreted by infiltrated CD16+CXCR3+ monocytes and is associated with liver fibrosis.

Herein, we aim to study the impact of ADA2 derived from circulating infiltrative monocytes in the pathogenesis of COVID-19 associated ARDS. Critically ill COVID-19 patients were assigned to the ARDS group based on Berlin criteria, while the non-ARDS group included COVID-19 patients without any or with low-flow oxygen therapy.

We noted elevated ADA activity in the serum of ARDS patients, reflecting increased level of the secreted ADA2 protein, when compared to the non-ARDS group. Notably, correlation analysis showed a significant association between circulating ADA activity and lactate level, a marker for ongoing tissue hypoxia. Chemokine profiling revealed increased serum level of the chemokine for CXCR3+ monocytes (CXCL10) in ARDS patients, which positively correlated with ADA activity. Post-mortem bulkRNAseq analysis of lung tissues showed increased expression of the ADA2 gene, CECR1, suggesting intrapulmonary accumulation of ADA2 positive macrophages. These observations were further supported by the analysis of single-nuclear and spatial transcriptomic atlas of lung tissues of severe COVID-19 patients, which demonstrated a strong expression of ADA2 in CD16+ monocytes.

In summary, our data suggest a potential role of infiltrative monocyte-derived ADA2 in modulating the immune response in COVID-19 related ARDS.

P 029

An improved organotypic cell culture system to study tissue-resident macrophages *ex vivo*

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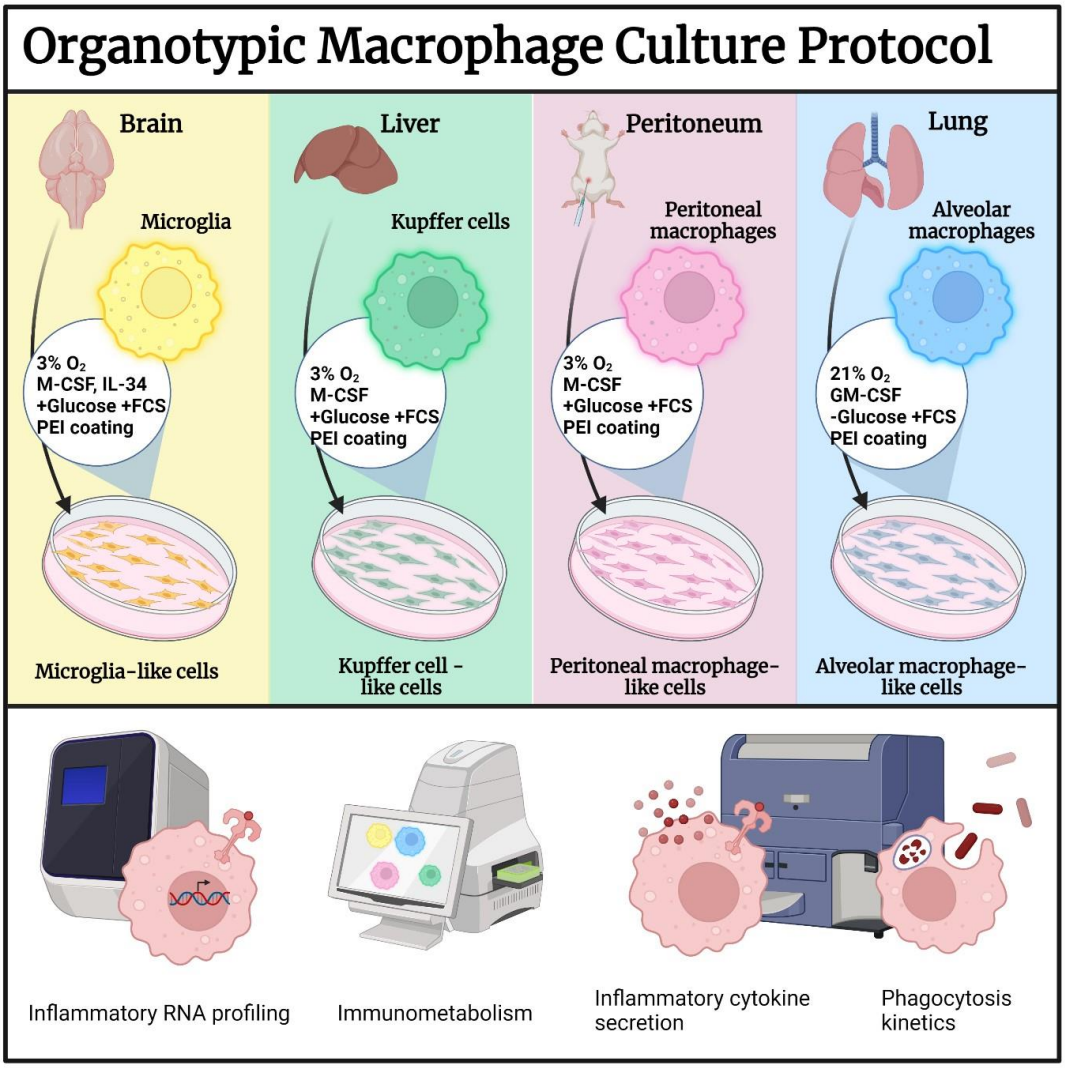
Tissue-resident macrophages (TRM) perform organ-specific functions that are dependent on factors such as hematopoietic origin, local environment and biological influences. A diverse range of *in vitro* culture systems has been developed to decipher TRM functions. However, these models often rely on bone marrow-derived macrophages (BMDMs), immortalized cell lines or macrophages from induced pluripotent stem cells (iPSC), which all have certain limitations including different origin, artificial activation or highly sophisticated differentiation protocols. Further, attempts to culture primary macrophages from different organs often require purification of these cells, lack a high cell yield and consistent phenotype and do not allow a long-term culture approach. Here, we aimed to address these practical limitations by establishing a new organotypic primary cell culture protocol. We obtained long-term monocultures of macrophages derived from distinct organs without FACS or MACS purification using specific growth factors and tissue physoxic conditions that largely conserved a TRM-like identity *in vitro*. Further on this protocol offers a multi-species approach by using species specific cytokines. Thus, this new organotypic system offers an ideal screening platform for primary tissue-resident macrophages from different organs which can be used for a wide range of assays and readouts across different species, including human macrophages.

Fig. 1



PS 1 I Poster session I

P 031

The role of TMEM176B, negative regulator of NLRP3 inflammasome, in colorectal cancer prognosis

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Question: Colorectal cancer (CRC) outcome after surgery is often different from the predicted one, highlighting the need for a more precise prognostic marker. Inflammation has been associated with the CRC malignancy and overall patients" survival. Inflammasome, the cytosolic complex responsible for IL-1ß and IL-18 release, can contribute to colon inflammation, oncogenesis, and tumor progression, being expressed in gut epithelium, stromal and immune cells. We therefore hypothesized that the activation of inflammasome may represent a possible prognostic marker for patients" post-surgery follow-up.

Methods: A multi-omics approach was used to identify inflammasome-related markers. Immunohistochemistry (IHC) and target gene expression (TGE) analysis were realized in fresh and paraffin-embedded CRC biopsies to describe the expression of inflammasome components according to tumor stage. Functional variants in inflammasome genes were analyzed in a cohort of CRC patients submitted to surgery and followed-up for 10 years, by allele-specific assays and qPBR. In vitro assays were used to confirm our findings.

Results: IHC and TGE analysis revealed the significant and inverse correlation between the inflammasome receptor NLRP3 and the CRC stage. Accordingly, the loss-of-function variant Ala134Thr (rs2072443) in the NLRP3 inhibitor *TMEM176B* was more frequent in CRC patients with high overall survival. As the expression of NLRP3 resulted quite restricted to tumoral cells, while its inhibitor in both tumoral cells and immune infiltrate, we evaluated the effect of chemical inhibition of TMEM176B and of rs2072443 *in vitro.* As expected both the chemical inhibition of TMEM176B and rs2072443 leads to increased IL-1ß release.

Conclusion: Our findings described the NLRP3 inflammasome and its inhibitor TMEM176B as a possible prognostic marker for CRC, and pointed out that the activation of the complex and IL-1ß release by immune infiltrate is possibly responsible for a better CRC outcome.

P 033

S100A8/A9 regulate SYK tyrosine kinase dependent NLRP3 inflammasome activation

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S100A8/A9 are the most abundantly expressed alarmins by monocytes. They are highly released in various inflammatory diseases locally and systemically and are pivotal mediators of inflammation. Extremely high expression and secretion of S100A8/A9 can be found in the autoinflammatory Familial Mediterranean Fever (FMF) syndrome. In FMF gain of function mutations in pyrin result in an increased caspase-1 activity which leads to an increased secretion of S100-alarmins, IL-1ß and IL-18. However, S100A8/A9 are also known to exert intracellular functions, where they are implicated in cytoskeleton. The aim of the present study was to determine the impact of intracellular S100A8/A9 and its interaction to SYK for the activation of NLRP3/Pyrin inflammasome. *In vitro* models with specific knockout cell lines and pharmacological inhibitors were used to investigate SYK and S100A8/A9 functions. Inflammasome activation was analyzed by measuring cytokines (ELISA) and western blotting using Hoxb8 murine monocytes. Stimulation with LPS led to increased S100A8/A9 and SYK mRNA and protein levels. We observed that S100A8/A9 positively regulate the expression of SYK because S100A8/A9 knock out resulted in a reduced SYK expression. Inhibition and SYK knock out, as well as S100A8/A9 knock out, impaired Caspase-1 cleavage after inflammasome activation and prevented the secretion of S100-alarmins, IL-1ß and IL-18. Therefore, our analyses clearly revealed a SYK dependent activation of the NLRP3 inflammasome. Whereas S100A8/A9 knock out resulted in a diminished SYK expression followed by reduced NLRP3 inflammasome activation, which could not be rescued by extracellular addition of S100 proteins. Moreover, SYK inhibition decreased the secretion of S100-alarmins, IL-1ß and IL-18 in FMF cells, indicating its involvement in pyrin inflammasome. To conclude, S100A8/A9 act as a master regulator of NLRP3 and Pyrin inflammasome activation by regulating the SYK dependent cleavage of caspase-1.

PS 1 I Poster session I

P 035

Triclosan and its substitutes alter the immune response on the molecular level in human macrophages

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Since the bacteriocidic Triclosan (TCS) was banned by regulatory agencies in Europe, substitution products for TCS have come into focus. Recently, TCS has been shown to reprogramme immune metabolism in human macrophages, but knowledge of substitutes is scarce. Hence, we aim to examine the potential adverse effects of TCS and alternatives on the molecular level in human macrophages.

LPS-stimulated macrophages were treated with TCS or the substitutes, including Benzalkonium chloride, Benzethonium chloride, Chloroxylenol, Chlorhexidine (CHX) and Cetylpyridinium chloride, to gain insights into their mode of action. The cytokine release showed induction of TNF by TCS as well as significantly higher IL-1β release than the control, suggesting the activation of the NLRP3-inflammasome, which was verified by the MCC950 inhibitor experiment. IL-6 was reduced in all treatments. In contrast, the alternative CHX completely abolished cytokine release.

For detecting molecular pathways, we used untargeted LC-MS/MS-based proteomics and subsequent pathway analysis by Ingenuity Pathway analysis. TCS and CHX showed the strongest response at the protein and signalling pathway levels with inhibition of oxidative phosphorylation and the TCA cycle, while NRF2-mediated oxidative stress response was upregulated in TCS-treated cells. CHX was associated with down-regulation of signalling pathways related to translation, like EIF2 signalling. Although TCS and CHX act differently on activated macrophages, both caused significant changes, either triggering the inflammasome pathway or a combination of increased oxidative stress and reduced immune metabolism.

Taken together, the data obtained suggest modulation of the immune response in macrophages by TCS and novel substitution products and illuminated underlying molecular effects. These results illustrate that molecular immunotoxicity should be included in future test strategies for hazard assessment.

P 037

Calcium ionophore-induced extracellular vesicles mediate cardioprotection against simulated ischemia/reperfusion injury

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Introduction: Cardioprotection against ischemia/reperfusion injury is still an unmet clinical need. The transient activation of Toll-like receptors (TLRs) has been implicated in cardioprotection via the activation of an adaptive response, which may be achieved by treatment with blood-derived extracellular vesicles (EVs). However, the EVs isolated from blood can contain various impurities, the methods involved are cumbersome and yields are low. Our aim was to establish a cellular model from which cardioprotective EVs can be isolated in a reproducible manner. We investigated the capability of extracellular vesicles, released after oxidative stress (stressEVs), to induce TLR dependent signaling in H9c2 and AC16 heat-derived proliferating cell lines and to assess if such EVs are capable of inducing cardioprotection against acute ischemia/reperfusion injury.

Methods: EV release was induced in HEK293 cells with calcium ionophore A23187. EVs were isolated using ultracentrifugation and characterized by DLS, NTA, WB and electron microscopy. Changes in gene expression and signaling after stressEV stimulation were determined by dual luciferase assay, qPCR, WB and ELISA. Cytotoxicity after hypoxia/reoxygenation was assessed by LDH activity assay and by calcein staining.

Results and conclusions: Cardioprotection afforded by EVs and its mechanism were investigated after 16 h simulated ischemia and 2 h reperfusion. In both H9c2 and AC16 cells, stressEVs induced the downstream signaling of TLR4 and heme oxygenase 1 (HO-1) expression. StressEVs decreased necrosis due to simulated ischemia/reperfusion injury in H9c2 and AC16 cells, which was independent of TLR4 activation, but not that of HO-1. These results suggest that calcium ionophore-induced EVs exert cytoprotection by inducing HO-1 in a TLR4 independent manner and may offer novel avenues for cardioprotective treatments against ischemic cardiac disease.

PS 1 I Poster session I

P 039

Optimising the polarisation of macrophages with disease-relevant stimuli for drug development

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Macrophages are key for the detection and response to infection and the modulation of inflammation. Given increasing evidence of their involvement in many autoimmune diseases and immune disorders, *in-vitro* primary macrophage assays can be used for drug development in these areas. To do so, monocytes must be differentiated into macrophages and stimulated to take on particular phenotypes. Commonly used pro-inflammatory stimuli include lipopolysaccharide (LPS), which binds toll-like receptor 4 (TLR4), and R848 (Resiquimod), which binds TLR7 and 8; these are receptors typically associated with the detection of bacterial and/or viral components. Given the range of macrophage phenotypes identified, it is beneficial to optimise stimuli that polarise macrophages into a similar state to those seen in autoimmune diseases, and so increase the disease-relevance of assays used in drug testing.

Tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) are cytokines associated with the pathogenesis of several autoimmune diseases and are known to alter macrophage phenotypes. Additionally, in some autoimmune diseases such as systemic lupus erythematosus, there is evidence for elevated cyclic guanosine monophosphate adenosine monophosphate (cGAMP), a key second messenger in the cyclic GMP-AMP synthase - stimulator of interferon genes (cGAS-STING) signalling pathway. Therefore, human monocyte-derived macrophage (hMDM) culture and stimulation protocols were optimised for TNF-α, IFN-γ, and cGAMP, with cytokine profiling suggesting differences in the induced phenotypes. This is exemplified in the low interleukin-6 and TNF- α production but very high CXCL10 production by cGAMP stimulated hMDMs, contrasting to the high levels of all the above cytokines produced by LPS stimulated hMDMs. These results demonstrate the development of more disease-relevant macrophage-based assays at Sitryx, aiding in drug discovery and development for autoimmune diseases and immune disorders.

P 041

Molecular mechanisms of antiviral NLRP1 responses in different skin models

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Inflammasomes are cytosolic multiprotein signaling complexes which assemble upon activation and regulate inflammation. NLRP1 is the only inflammasome sensor expressed in keratinocytes of the human skin. It is activated by diverse stress signals leading to caspase-1 activation and maturation and release of pro-inflammatory cytokines.

We recently discovered that NLRP1 can be activated by direct phosphorylation by MAP kinase p38, which is initiated by triggers of the ribotoxic stress response as well as by alphavirus infection [1]. Now, we aim to dissect the p38-dependent molecular mechanism of NLRP1 activation by arthropod-borne RNA viruses. We are applying pooled CRISPR/Cas9 screens to identify the crucial ubiquitin ligase machinery for p38-dependent NLRP1 activation, as well as potential additional upstream kinases. To visualize NLRP1 inflammasome assembly in primary cells and physiological tissues samples, we have generated different recombinant alphaviruses, such as Semliki-Forest-Virus and Sindbis-Virus, encoding caspase-1CARD (C1C) fused to the fluorescent emiRFP670 reporter under a second subgenomic promoter. Using clinical samples of healthy skin, we plan to examine the physiological role of alphavirus-induced NLRP1 activation and the coordination of the ensuing inflammation between different cell types.

[1] Lea-Marie Jenster, Karl-Elmar Lange, Sabine Normann, Anja vom Hemdt, Jennifer D. Wuerth, Lisa D.J. Schiffelers, Yonas M. Tesfamariam, Florian N. Gohr, Laura Klein, Ines H. Kaltheuner, Stefan Ebner, Dorothee J. Lapp, Jacob Mayer, Jonas Moecking, Hidde L. Ploegh, Eicke Latz, Felix Meissner, Matthias Geyer, Beate M. Kümmerer, Florian I. Schmidt; P38 kinases mediate NLRP1 inflammasome activation after ribotoxic stress response and virus infection. J Exp Med 2 January 2023

PS 1 I Poster session I

P 043

Dissecting the antiviral innate immune response in the skin

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Inflammasomes are large cytosolic signaling complexes, which assemble to mount a rapid innate immune responseto infection. Recognition of diverse signatures by various inflammasome sensors nucleates the polymerization and cross-linking of adaptor ASC to form a single macroscopic ASC speck per cell. Pro-caspase-1 is recruited to this ASC speck, leading to its autocatalytic activation. Caspase-1 subsequently matures the pro-inflammatory cytokines IL-1β and IL-18 and pore-forming molecule gasdermin D, leading to cell death and release of these cytokines by pyroptosis.

While reductionist experimental systems have been useful to study inflammasome activation in itself, the interplay of inflammasome activation with other innate signaling pathways is likely more complex in the physiological environment. In this context, preliminary data from *in vivo* infected murine ear skin suggests that only few cells within a vaccinia virus (VACV) lesion assemble inflammasomes and presumably coordinate further immune responses.

This project aims to establish *ex vivo* skin infection models utilizing murine skin derived from split mouse ears and human skin removed during surgery. Utilizing genetically engineered viruses encoding the novel inflammasome reporter caspase-1^{CARD} together with staining of cell-specific and immune-specific markers, we aim to reveal which cells assemble inflammasomes and how this is coordinated with other innate immune responses in the skin. We have successfully infected murine skin explants with VACV and HSV-1. Furthermore, infection of isolated primary human keratinocytes with the poxviruses VACV or monkeypox virus (MPXV, clinical isolate from 2022 outbreak), leads to an AIM-2 dependent inflammasome response after IFN-γ pretreatment. Interestingly, this response is significantly lower in MPXV infected cells. We will further present our efforts to optimize human *ex vivo* skin infection models.

P 045

Escherichia coli Nissle 1917 flagellin outer sheath domains are dispensable for TLR5 recognition but modulate bacterial motility

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The onset and course of different diseases like type I diabetes, rheumatoid arthritis and inflammatory bowel disease (IBD) can be influenced by the composition of the intestinal microbiota. In case of IBD, microbiome interventions via the probiotic *Escherichia coli* Nissle 1917 (EcN; Mutaflor®) prolongs the remission phases in IBD patients by strengthening the epithelial barrier. In a dextran sodium sulfate (DSS)-induced intestinal inflammation model in mice, disease amelioration could be achieved by the unique EcN flagellin (FliC). Flagellin is a microbe associated molecular pattern (MAMP) recognized by Toll-like receptor 5 (TLR5) on immune and epithelial cells. FliC is the structural subunit of the bacterial flagellum and crucial for bacterial motility. Here, the conserved D0 and D1 domains form the filament core and a hypervariable region (HVR) forms a so-called an outer sheath. However, the structure of the EcN HVR and its role in TLR5 recognition or motility are not known. Here, we report the structure of the EcN flagellin to 1.65 Å resolution, showing the HVR to adopt two canonical (D2 and D3) and one additional D4 domain. Using both recombinant proteins and gene-edited EcN strains expressing mutant flagellins, we found that human and mouse TLR5 recognition was unaffected by removal of the D4 or a unique D1-D2 linker. However, they affected bacterial motility. In future, bacterial fitness, colonization and symbiotic effects will be studied in the DSS-induced colitis mouse model. Collectively, these data suggest that the HVR of EcN is not important for Flagellin-TLR5 complex formation but for flagella stability and motility.

PS 1 I Poster session I

P 047

Discovering novel therapeutic targets for drug-induced senescence in liver cancer

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Therapy-induced senescence (TIS) is a state of cell cycle arrest induced by chemotherapeutics and other antitumor treatments. While the secretome of senescent cancer cells has been extensively studied, changes in innate immune pathways and cellular surface marker expression during cancer senescence are not well described. Here we identify novel therapeutic senescence targets and delineate ways in which specific TIS selection combined with immunotherapy might be used to promote certain anti-tumor effects. We compared the effects of three therapy-induced senescence agonists – etoposide, alisertib and CX5461 –on innate immune pathways, surface marker expression, tumor cell-neutrophil interactions and natural killer (NK)-cell mediated killing in different hepatocellular carcinoma cell (HCC) lines. Furthermore, we characterized the senescent surface-ome to identify global potentially therapeutically-tractable TIS markers using a flow cytometry-based screening approach. We observed striking differences between TIS agonists for several clearance-relevant properties including secretion of SASP factors and expression of surface proteins. Furthermore, different TIS treatments conferred disparate sensitivity to innate immune modulation via the cGAS-STING pathway, tumor cell-neutrophil interaction and NK-cell mediated tumor cell killing. By characterizing the surface-ome of senescent HCC cell lines we identified CD340 (Her2) and CD95 (Fas) as upregulated TIS markers. Expression of these therapeutic targets was strongly increased in HCC cells upon senescence induction with different TIS agonists andare currently being validated in a HCC in vivo mouse model with CX5461 TIS induction. We hypothesize that specific TIS treatments combined with CD95- and/or CD340-focussed biological and/or immunotherapy could be used to augment anti-tumor effects in HCC and selectively target senescent pre-malignant cells.

P 049

LukAB is essential for *Staphylococcus aureus* exit from within macrophages

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Staphylococcus aureus is a notorious facultative pathogen that causes a diverse range of illnesses world-wide. *S. aureus* has different strategies to avoid the host immune response, e.g. hiding inside of the phagocytic cells (here referred as "PersistStaph"). However, "ExitStaph" *S. aureus* can escape from and kill macrophages by the induction of a so far unknown type of cell death. The pore-forming Leukocidin A/B (LukAB) when induced from within macrophages is considered the main factor required for exit and host cell death. LukAB when added exogenously potently triggers the activation of the NLRP3 inflammasome, promotes IL-1ß secretion and eventually kills primary human monocytes. Conversely, the role of LukAB, when expressed intracellularly by *S. aureus*, and its effects on the cell death pathways and NLRP3 inflammasome are not well understood. We found that NLRP3 inflammasome activation after phagocytosis of macrophages was LukAB-independent, since IL-1ß and IL-18 release could be potently triggered by both USA300WT ExitStaph and PersistStaph mutants deficient in LukAB/PVL synthesis (agr/sae, lukAB mutants). Moreover, intracellularly induced LukAB results in cell-death independent of pyroptosis and apoptosis. Rather, necroptosis seems to be critical since p-MLKL was observed from 3 h after infection, and a potent necroptosis inhibitor, nec-1, could block MLKL phosphorylation as well as ExitStaph exit. However, the levels of p-MLKL in WT and arg/sae mutant infected macrophages were comparable up until 6 h post-infection, when WT *S. aureus* causes cell disintegration and starts to appear extracellularly. Our findings suggest that toxin-mediated inflammasome activation and cell death are decoupled in *S. aureus* infections of human macrophages. Furthermore, whilst necroptosis features are triggered by both PersistStaph and ExitStaph, they are insufficient for cell death/exit or can be overcome by infected cells. For WT ExitStaph LukAB thus appears to either enroll additional processes causing full execution of necroptosis, or to block cellular repair mechanisms.

PS 1 I Poster session I

P 051

Recruitment of Mast cells and subsequent Bruch"s membrane remodelling is a common early precursor in both 1q32 and 10q26 disease-risk allele patient groups for age-related macular degeneration

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Purpose: To identify and compare the protein content of human Bruch"s membrane (BrM) from human donor eyes stratified for genetic risk of age-related macular degeneration (AMD).

Methods: Human BrM was enriched from donor eyes without AMD but who were carrying homozygous genetic risk at either: the 1q32 allele only (Chr1, n=8); the 10q26 allele only (Chr10, n=8); or who were homozygous protective at both alleles (n=8). We used untargeted mass spectrometry-based quantitative proteomics to compare the protein content of each donor risk group compared to the protective controls. One eye from each donor was used for proteomic studies with the contralateral eye processed for histological analysis.

Results: Both Chr1 and Chr10 risk groups shared commonalities when compared to the low-risk group, particularly the increased accumulation of mast-cell specific proteases (*i.e*. tryptase, chymase and carboxypeptidase A3). Histological analyses of submacular tissue from contralateral eyes confirmed increased choroidal mast cell accumulation with genetic risk, as well as evidence of their active degranulation and corresponding collagen denaturation within BrM.

Conclusion: Increased mast cell inner choroidal infiltration, degranulation and subsequent BrM remodelling are early events in AMD pathogenesis and represents a unifying mechanistic link between Chr1 and Chr10 mediated AMD.

P 053

Dissecting single-stranded DNA sensing

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The molecular determinants of single-stranded DNA (ssDNA)-associated inflammatory responses are poorly resolved. Yet, immunogenic ssDNAs are frequently associated with (patho-)physiological conditions, such as DNA repair or stalled replication. Mutations in *TREX1* and *SAMHD1* lead to accumulation of cytosolic ssDNA and to cGAS-STING-dependent type I Interferon (IFN) response. Since cGAS has very low affinity for ssDNA, backfolding of complementary ssDNAs is assumed to promote cGAS activation. However, this was not formally demonstrated. Unraveling the rules governing ssDNA sensing would pave the way towards a better understanding and treatment options for ssDNA-associated diseases.

To this aim, we analyzed the kinetics of innate immune responses to synthetic ssDNA in several cell types. HeLa and T98G cancer cell lines responded to ssDNA by activating NF-κB and IFN signaling pathways. Importantly, T98G do not express cGAS, indicating the presence of cGAS-independent ssDNA detection routes. In contrast, myeloid-like THP-1 cells were unresponsive to ssDNA stimulation, further suggesting cell-type specific mechanisms. To identify ssDNA binding proteins involved in ssDNA sensing, we performed in vitro pulldown assays using biotinylated ssDNA baits and cytoplasmic fractions followed by mass spectrometry. This allowed the generation of cell type specific interaction maps of ssDNA, that were subsequently validated following ssDNA transfection in cells. Our analyses allowed the detection of known interactors such as RPA1 and TREX1, confirming the validity of our approach. Interestingly, we did not detect cGAS-ssDNA interaction in our assays, supporting the hypothesis that cGAS-independent routes are at play. Furthermore, neither expression of TLR9 nor IFI16 correlated with responsiveness towards ssDNA. Using a combination of biochemical and functional assays, we will now characterize novel ssDNA interactors, ultimately providing new insights into ssDNA sensing.

P 055

The Impact of Peripheral Inflammation on the Brain"s Immune Response

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Acute and chronic inflammation in the periphery have been shown to prompt immune responses in the central nervous system. As demonstrated previously, the brain's primary innate immune cells, microglia, can develop long-lasting innate immune memory that alters neuropathology in mouse models of neurological diseases. The innate immune memory status of microglia includes an enhanced immune response, also known as "immune training", and a suppression of immune responses, known as "immune tolerance". It is mediated by lasting epigenetic changes leading to gene expression alterations upon cell activation. However, the molecular signaling pathways engaged in the formation of innate immune memory in the brain and how exactly the brain reacts to different stimuli remain unknown. In this study, we aim to investigate the brain's responses to peripheral inflammation by challenging mice with various peripheral stimuli, and molecularly characterize the acute and long-term effects of peripheral inflammation on the brain. To assess the molecular profiles of individual cells in depth, we are planning to apply SHARE-seq, a recently-developed method enabling the analysis of both gene expression and chromatin accessibility in the same cell, on sorted nuclei from frozen mouse brains as well as human brain samples. The single-nuclei transcriptomic and epigenetic data may pave the way for identifying key molecules as potential targets for therapeutic modulation of the brain's immune responses.

P 057

The anti-citrullinated histone antibody CIT-013 as a dual action therapeutic for neutrophil extracellular trap associated diseases

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Question: Since their discovery, neutrophil extracellular traps (NETs) have been associated with an increasing number of inflammatory and autoimmune pathologies. Though NETosis-targeting therapeutics have shown potential as effective treatments, currently there are no NET-specific therapies available. We have developed a monoclonal antibody, CIT-013, showing therapeutic efficacy in multiple mouse models of NET-driven disease. This leaves us with the question, how does CIT-013 induce this therapeutic effect? Here we elucidate CIT-013"s mechanism of action on a cellular level.

Methods and results: CIT-013 binds with picomolar affinity to citrullinated histones H2A and H4, which are known to be present in NETs. With live imaging confocal microscopy, we demonstrate that CIT-013 prevents NET release during the final stage of NETosis, when plasma membrane rupture occurs. Other anti-histone antibodies lack this unique mechanism of inhibition, for which antibody bivalency is required. Besides inhibition of NET release, CIT-013 enhances macrophage phagocytosis of netting neutrophils and already existing NETs in an Fc-receptor dependent manner. This dual mechanism of CIT-013 was confirmed *in vivo*, where diminished tissue NET burden leads to anti-inflammatory responses and reduced tissue damage. Finally, we demonstrate CIT-013's therapeutic potential in rheumatoid arthritis (RA), a disease associated with citrullinated NETs, confirming that RA can be a target disease for CIT-013. A clinical phase 2a proof-of-concept study is due to commence in 2024.

Conclusion: Altogether, CIT-013"s unique therapeutic mechanism provides new insights for the development of NET antagonists and strengthens the promise of CIT-013 as therapy for NET-associated diseases with unmet therapeutic needs.

PS 2 I Poster session II

P 002

Expanding the maxpar direct immune profiling assay to enable comprehensive antigen-specific immune analysis

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The complex nature of the immune system requires deep interrogation at the single-cell level. Mass cytometry, which uses CyTOF® technology, utilizes antibodies tagged with monoisotopic metals, resulting in discrete signals that enable highly multiparametric characterization on a single-cell level. CyTOF can currently enable cellular phenotyping of over 50 single-cell parameters simultaneously, including phenotypic and functional markers, thereby significantly increasing the ability to comprehensively evaluate immune responses.[1]

The Maxpar® Direct™ Immune Profiling Assay™ is a pre-titrated, dried-down, 30-marker antibody cocktail for immune profiling of human whole blood and peripheral blood mononuclear cells (PBMC) by CyTOF (Figure 1). Using Maxpar Pathsetter™ software, stained samples are automatically resolved into 37 immune populations. This assay was designed with customization in mind, with 18-plus open channels available for panel expansion.

This study highlights the use of the Maxpar Direct T Cell Activation Expansion Panel and live-cell barcoding to add 15 parameters to the backbone Maxpar Direct panel. The array of key surface and intracellular functional parameters in this expanded panel enables comprehensive analysis of immune cell activation and antigen-specific recall responses. Such phenomena are hallmarks for research on infection, vaccine development, and immunotherapy.

P 004

Bacterial lysate imprints an interesting anti-inflammatory profile on human monocytes expressing CD56 - promising immunomodulatory effects in respiratory infections

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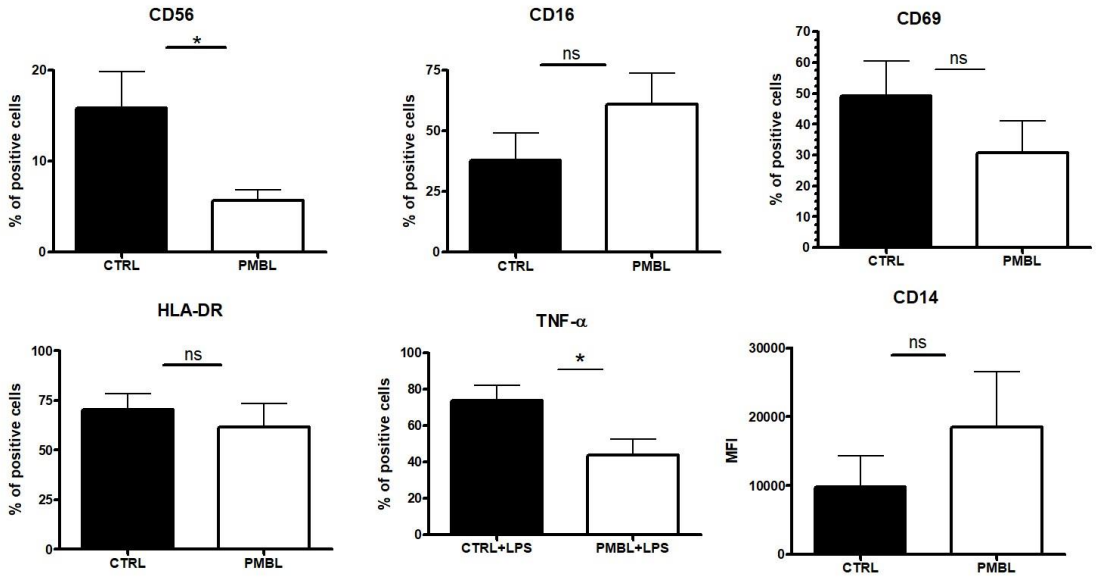
Polyvalent mechanical bacterial lysate (PMBL) has been reported to be effective in the treatment of recurrent respiratory tract infections since improves immune response thanks to its immunogenic capabilities. Monocytes play a key role during infection and their recruitment is essential for effective control and clearance of pathogens, although it has been described that recruited-inflammatory monocytes can also contribute to disease pathogenesis. Therefore, in our study, we investigated the potential immunomodulatory effects of bacterial lysates in regulating monocyte immune responses. Starting from circulating monocytes of healthy donors, we generated *in vitro* monocytes expressing high levels of CD56, marker of a monocyte subset with inflammatory features, and analysed their phenotype and functions after PMBL treatment. Our data reveal that inflammatory monocytes $CD56^{+}$exposed to PMBL down regulate CD56 expression contextually to the up-regulation of CD16, marker of an anti-inflammatory monocyte subset. Accordingly, when we analysed their functional capabilities, we observed that PMBL-exposed monocytes, following stimulation with TLR4-agonist LPS, produce significantly lower levels of inflammatory cytokine TNF-α although maintaining almost unaltered levels of expression of the activation markers,CD69 and HLA-DR, compared to their inflammatory counterpart. In addition, PMBL-treated monocytes show a reduced phosphorylation of p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK1/2), critical intracellular signal transduction pathway involved in the inflammatory response. Our findings suggest that PMBL might modulate monocyte immune responses by inducing a phenotypic and functional switching toward a non-inflammatory monocyte subset and interfering with the intracellular signaling cascade that regulates monocyte-mediated inflammation, thus providing new insights into the mechanism of action of PMBL.

Fig. 1


CD56
*
% of positive cells
20
10
0
CTRL
PMBL
CD16
ns
% of positive cells
75
50
25
0
CTRL
PMBL
CD69
ns
% of positive cells
70
60
50
40
30
20
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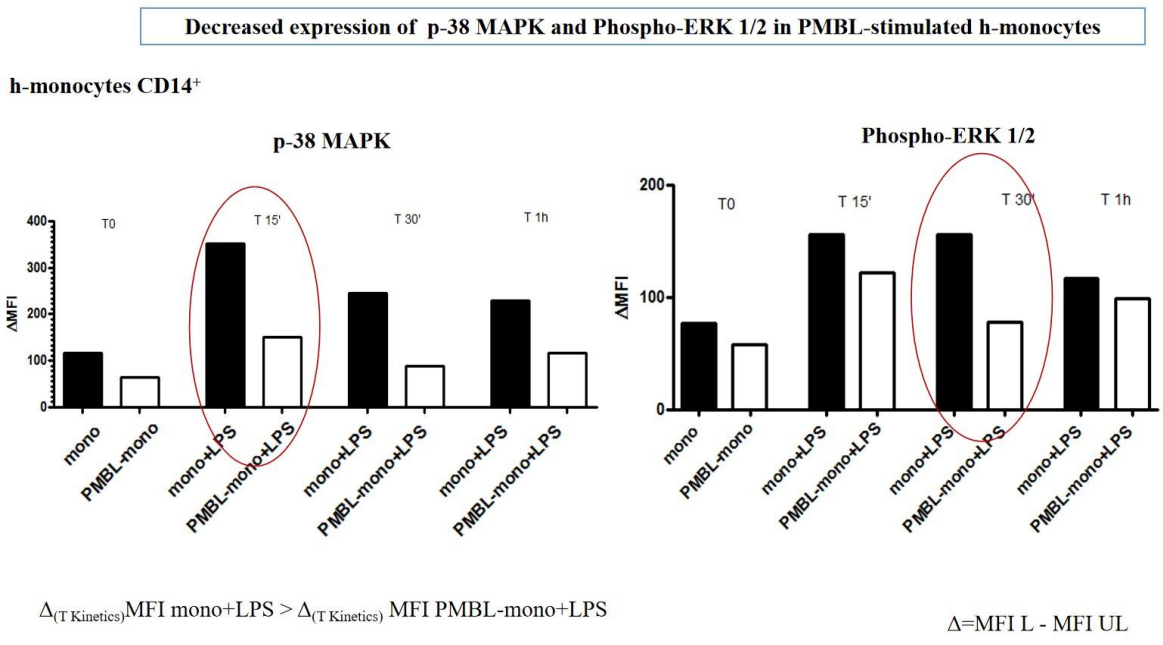
0
CTRL
PMBL
HLA-DR
ns
% of positive cells
100
75
50
25
0
CTRL
PMBL
TNF-α
*
% of positive cells
100
80
60
40
20
0
CTRL+LPS
PMBL+LPS
CD14
ns
MFI
30000
20000
10000
0
CTRL
PMBL

Fig. 2


Decreased expression of p-38 MAPK and Phospho-ERK 1/2 in PMBL-stimulated h-monocytes
h-monocytes CD14+
p-38 MAPK
ΔMFI
400
300
200
100
0
T0
T 15'
T 30'
T 1h
mono
PMBL-mono
mono+LPS
PMBL-mono+LPS
Phospho-ERK 1/2
ΔMFI
200
100
0
T0
T 15'
T 30'
T 1h
mono
PMBL-mono
mono+LPS
PMBL-mono+LPS
Δ(T Kinetics)MFI mono+LPS > Δ(T Kinetics) MFI PMBL-mono+LPS
Δ=MFI L - MFI UL

PS 2 I Poster session II

P 006

Impact of exogenous, endogenous and mixed-type asthma on microbiota composition and secretory immunity in the human nasopharynx

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Transepithelial transport of secretory immunoglobulin A (SIgA) into the mucosal lumen by the polymeric immunoglobulin receptor (PIGR) is an important mechanism of mucosal host defense. Human asthma is associated with impaired secretory immunity and increased risk for severe pneumococcal infections. While nasal microbiota is altered in asthmatics, it is unclear whether this is related to altered secretory immunity in the upper airways. The impact of allergy on nasal microenvironment in asthma is unknown. The aim was to analyze the impact of asthma on nasal secretory immunity and microbiota composition.

112 male and female subjects (30 controls, 33 exogenous asthma, 24 endogenous asthma, 25 mixed-type asthma) between 18-82 years were analyzed. *PIGR* gene/PIGR protein expression in nasal epithelial cell biopsies were determined by qRT-PCR and Western Blot. Relative SIgA and total IgE/IgG levels in nasal lavage fluid were determined by ELISA and bead-based immunoassay. Bacterial DNA isolated from nasal swabs was used for gene sequencing to analyze microbiota composition.

PIGR gene/PIGR protein expression and SIgA/IgE levels were unaltered between healthy and asthmatic individuals as well as between asthma endotypes. Exogenous and endogenous asthma patients showed elevated IgG2 levels compared to healthy subjects. Microbial diversity was increased in exogenous and mixed-type asthma compared to healthy individuals. There was no correlation between microbial diversity and IgG2 levels. Chitinophagaceae, Burkholderiaceae and Prevotellaceae were more abundant in asthma patients than in healthy controls, while Corynebacteriaceae and Carnobacteriaceae were reduced.

PIGR-mediated secretory immunity in the human nasopharynx is not affected by asthma or the reactivity to aeroallergens. Nasal microbial diversity is not affected by increased amounts of IgG2 and *vice versa*. Microbial equilibrium of the upper respiratory tract is disrupted in asthma.

PS 2 I Poster session II

P 008

Influence of formyl-peptide receptor activation on skin inflammation and *Staphylococcus aureus* skin colonization

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The skin acts as a barrier with over 90% keratinocytes as the predominant cell type in the epidermis. Keratinocytes respond to pathogenic microorganisms and injury by producing antimicrobial peptides and cytokines that promote the immune responses and wound healing. Skin commensals and pathogens such as *Staphylococcus aureus* secrete high amounts of phenol-soluble modulin (PSM) peptides, agonists of formyl-peptide receptor (FPR)2. It is known that FPR2 is crucial for the recruitment of neutrophils in local infections and can influence inflammation. Although it has been shown that various epithelial cells express FPRs, the consequences of FPR activation regarding keratinocytes were unclear. Since an inflammatory environment influences *S. aureus* skin colonization, e. g. on the skin of patients with atopic dermatitis (AD), we hypothesized that FPRs influence bacteria-induced skin inflammation and colonization of the skin.

To verify this, we investigated FPR activation and inhibition in keratinocytes regarding the consequences on cytokine release, proliferation, and wound healing. In addition, we treated primary human keratinocytes as well as N/TERT-1 cells with *S. aureus* and analyzed the influence of FPR2 inhibition on bacterial colonization. In an in *vivo* tape-stripping model using wild-type (WT) and Fpr2-/- mice, we analyzed cytokine release and skin colonization by *S. aureus* with or without FPR2 inhibition by WRW4.

We could show that the activation of FPR1 and FPR2 in keratinocytes promotes inflammation via the release of cytokines and chemokines. In addition, FPR2 activation enhances cell proliferation and wound healing in a receptor-dependent manner. Furthermore, we demonstrated that FPR2 inhibition prevents inflammation but promote thereby skin colonization of *S. aureus*.

Our data suggest that moderate keratinocyte-induced inflammation is necessary to prevent *S. aureus* colonization of the skin.

P 010

FMF monocytes and neutrophile granulocytes show an altered phenotype in functional key features

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The Familial Mediterranean Fever (FMF) is a hereditary, auto-inflammatory disease caused by various mutations in the Mediterranean fever gene encoding a 781 amino acid protein called pyrin. The synonyms periodic peritonitis, periodic fever and recurrent polyserositis highlight key symptoms of the disease, which result in multiple somatic complications like developing amyloidosis or vasculitis-related diseases. Triggers for attacks include physical activity, an infection or stress. Furthermore, the effect on the psychological health poses additional potential for further reduction of the patients life quality.

The underlying molecular mechanism of the disease lies in the gain of function mutations of the pyrin inflammasome. Opposed to the physiological necessity of proper stimulation by pathogen or damage associated molecular patterns (DAMP or PAMP), the pyrin inflammasome shows a near spontaneous caspase-1 activation followed by a Gasdermin D mediated release of Il-1ß, Il-18 and S100A8/A9. These pro-inflammatory cytokines mediate a local inflammatory response as well a systemic reaction.

In this study we seek to shed light on the role of monocytes and neutrophile granulocytes in the pathophysiology of FMF. Previous results suggest different behavior upon stimulation with Lipopolysaccharide, Adenosintriphosphate or Clostridium difficile toxin. We extend the findings towards mechanisms of phagocytosis, ROS production, adhesion, migration and differentially expressed differentiation markers in HoxB8 monocytes and neutrophile granulocytes carrying the FMF mutation V726a compared to cells lacking the pyrin protein completely. Additionally we extend our research to monocytes and neutrophile granulocytes carrying a mutation responsible for the PAPA syndrome, an autoinflammatory disease not unlike the FMF syndrome, but with interesting key differences though sharing a similar pathophysiology.

PS 2 I Poster session II

P 012

Intranasal allergic sensitization to birch pollen extract is reduced in NLRP3-deficient animals

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Allergies against inhalant antigens such as birch pollen affect already up to 20% of the population in Europe and the prevalence is predicted to increase even further. The lack of safe, effective as well as timesaving curative treatments emerges as one of the main problems, making it crucial to not only understand the allergic immune response, but to examine how sensitization occurs in the first place.

In the past years, it has become increasingly evident that not necessarily the major allergen, but the pollen-intrinsic and extrinsic context influences an immune response on various levels. Amongst these factors, birch pollen contains its own microbiome, mainly Gram-positive bacteria, which might shape the allergen-dependent immune response by activating pattern recognition receptors including the family of intracellular nucleotide-binding domain, leucine-rich repeat receptors (NLRs), in particular NLRP3.

To study the role of NLRP3 in an adjuvant-free birch pollen extract (BPE) - induced allergic airway model, we sensitized C57BL/6 wildtype and NLRP3-/- mice with BPE via different administration routes and monitored BPE-specific antibody production, allergic lung inflammation and the systemic immune response.

We observed that intranasal but not intraperitoneal administration induced an allergic response in wildtype mice indicated by high BPE-specific IgG, IgG1 and IgE levels, increased infiltration of eosinophils, neutrophils, T cells and B cells into the lung and elevated Th2-related cytokine production by splenocytes. Very strikingly, antibody production, cell infiltration and cytokine secretion was significantly reduced in NLRP3-deficient animals.

These findings identify the intranasal route of sensitization as a promising adjuvant-free animal model of allergic airway inflammation and demonstrate that the absence of NLRP3 in C57BL/6 mice might protect from allergic sensitization to BPE.

PS 2 I Poster session II

P 014

Acidosis promotes immune escape through the IFN-γ-induced induction of PD-L1 transcription in cancer cells

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[5]University of Tübingen, Department of Dermatology, Tübingen, Germany
[6]University of Tübingen, Department of Pathology, Tübingen, Germany
[7]University of Tübingen, Interfaculty Institute of Biochemistry, Tübingen, Germany
[8]The John Hopkins University, Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD, United States
[9]The John Hopkins University, Department of Radiation Oncology and Molecular Radiation Sciences, Baltimore, MD, United States
[10]German Cancer Consortium (DKTK) and German Cancer Research Center, Heidelberg, Germany
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Question: In the tumor microenvironment (TME), the expression of programmed cell death ligand-1 (PD-L1) on cancer cells is mainly regulated by IFN-γ and induces T cell exhaustion, enabling tumor immune escape.

Methods: We investigated murine and human cancer cell lines, treated them with IFN-γ and/or acidic cell culture media and studied PD-L1 expression *in vitro*. Then, we silenced signal transducer and activator of transcription 1 (STAT1) and the eukaryotic initiation factor 4F (eIF4F) complex by specific inhibitors. *In vivo*, extracellular tumor pH (pH_e) was neutralized by sodium bicarbonate ($NaHCO_3$) treatment and confirmed by non-invasive *in vivo* acido chemical exchange saturation transfer magnetic resonance imaging (CEST-MRI).

Results: Here, we show that acidosis, a frequent feature of solid tumors, significantly increased IFN-γ-induced PD-L1-expression on cancer cells. This phenomenon was mediated by increased genomic expression and phosphorylation of STAT1 and translation of the Stat1 mRNA by eIF4F. The acidosis-mediated increase in IFN-γ-induced PD-L1-expression was observed in anti-PD-L1-responsive MC38 and CT26 adenocarcinoma cells, but not in anti-PD-L1-nonresponsive B16-F10 melanoma or 4T1 mammary carcinoma cells. Neutralization of the acidic extracellular tumor pH (pH_e) by $NaHCO_3$ treatment suppressed IFN-γ-induced PD-L1-expression in cultured MC38 cancer cells and MC38 tumors. In anti-PD-L1-responsive tumors, $NaHCO_3$ treatment not only increased the tumor pH_e but also increased immune cell infiltration and reduced tumor growth. In contrast, in anti-PD-L1-nonresponsive tumors, $NaHCO_3$-mediated pHe neutralization did not modulate PD-L1-expression in the tumor and failed to reduce tumor volume.

Conclusion: In summary, we propose an acidosis-mediated increase in IFN-γ-induced PD-L1-expression on cancer cells via enhanced STAT1 phosphorylation as an immune escape mechanism that might serve as a novel biomarker for the anti-PD-L1/PD-1 treatment response.

PS 2 I Poster session II

P 016

The role of mitochondrial CLPP protease in the regulation of innate immunity

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The ablation of the caseinolytic peptidase proteolytic subunit, CLPP, the catalytic component of a barrel-like mitochondrial matrix protease, ClpXP, has a surprising beneficial effect on systemic glucose metabolism, renders animals resistant to diet-induced obesity, without affecting lifespan. Remarkably, our recent transcriptome data revealed a predominant upregulation of innate immunity and type I Interferon (IFN-I) pathways in heart, brown and white adipocyte tissues of CLPP deficient animals. The activation of innate immune response is not linked to the level of mitochondrial respiratory chain deficiency as similar response was not observed in when LONP1, second mitochondrial matrix protease was depleted in heart, leading to stronger OXPHOS deficiency. **Questions:** Our preliminary results suggested contribution of both mitochondrial DNA and dsRNA, as DAMPs for the activation of innate immunity in CLPP-deficient cells. While the release of mitochondrial DAMPs, mtDNA in particular, into the cytosol is well established, the mechanisms allowing its transfer from mitochondria to the cytosol are less clear. It is not clear what safeguard mechanism sense and control the release of mitochondrial DAMPs. **Methods:** By using different CLPP-deficient cell lines and primary cells cultured from Lung, Bone marrow of the CLPP KO mice, we aim to figure out the triggers and cellular mechanism in the regulation of innate immune response in CLPP KO cells. **Results:** Our data shows no significant change of cytosolic mtDNA or mitochondrial dsRNA, but with a clear involvement of VDAC and the canonical cGAS-STING dependent pathways in the activation of ISG response in CLPP KO cells. **Conclusion:** Although the exact forms of mitochondrial DAMPs remain to be further identified via profiling of the mitochondrial nucleoic acid species, our results clearly imply the involvement of ClpXP machinery in this process, making it a perfect model to dissect these pathways further.

P 018

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

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Mast cells (MC) are key initiators of vasoactivation and immune cell infiltration upon allergic contact dermatitis. We recently identified MCs as a prominent source of receptor activator of NFκB (RANKL), which is mostly known for being involved in bone resorption, with more information coming to light about its qualities as an immune regulator. However, the relevance of MC-derived RANKL in skin inflammation is completely unknown. Using a conditional RANKL knockout in connective tissue type MCs mouse line, we identified a crucial role of MC-derived RANKL in contact hypersensitivity.

Surprisingly, mice lacking MC-derived RANKL displayed massive lymphocyte hyperplasia in inguinal lymph nodes (LN) 24h after DNFB challenge, accompanied by profound blood lymphopenia. This was a temporal effect, suggesting that lymphocyte egress is delayed in the absence of MC-derived RANKL. However, despite an increased immune cell infiltration at 48h after challenge, skin inflammation remained markedly dampened. Strikingly, MC depletion and reconstitution with RANKL deficient MCs only locally in the ear skin resembled inguinal LN hyperplasia, blood lymphopenia and reduced lymphocyte infiltration to inflamed skin 24h post DNFB challenge. Importantly, the phenotype of lymph node hyperplasia and blood lymphopenia could be rescued by intravascular injection of Shingosine-1-phosphate (S1P) 2h after challenge.

Consequently, RANKL release by peripheral skin MCs upon DNFB challenge exerts a long-distance effect, involving S1P signalling, that is a prerequisite for the timely lymphocyte egress from remote LNs, which is in turn critical for the onset and severity of allergic skin inflammation.

P 020

Characterization of pulmonary cytotoxic immune cells in lung fibrosis by spectral flow cytometry

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Interstitial lung disease (ILD) is a fibrotic disease that can occur in different conditions like rheumatoid arthritis (RA), systemic sclerosis (SSc) or severe COVID-19 infections. It is driven by overshooting repair mechanisms and chronic inflammation. ILDs lead to irreversible symptoms like dyspnea, chronic cough and death. Therapeutic options are very limited. NK cells may be involved in the pathogenesis of severe COVID-19-associated lung fibrosis (Krämer et al., 2021). Characterization of pulmonary immune cells of ILD patients may help to identify their role in fibrosis and to better understand the mechanisms of lung fibrosis.

We sampled bronchoalveolar lavage (BAL) from patients with RA- and SSc-associated ILD undergoing bronchoscopy. To characterize the cells, we evaluated the expression of 28 different surface markers using spectral flow cytometry. We compared the pulmonary immune cells to peripheral blood cells from healthy donors and ILD patients, to synovial fluid cells from patients with arthritis and to peripheral blood cells from patients with arthritis.

Live NK cells and CD8+T cells were found in the BAL samples from ILD patients. NK cell subgroups, defined by their CD56 and CD16 expression, varied greatly between peripheral blood and lung cells. The phenotype of pulmonary NK cell subgroups resembled the findings in synovial NK cells. We detected increased expression of CD69 and HLA-DR, and concomitantly a low expression of CD16, CD8, CD38, CD27 and CD57 on pulmonary NK cells.

Cytotoxic lymphocytes reside in airways from ILD patients and possess a defined phenotype different from blood and similar to infiltrates in arthritis. The expression patterns of surface markers on pulmonary NK cells indicate a phenotype of activation and/or tissue residency, limited differentiation and intermediate cytotoxic potential.

Krämer et al. "Early IFN-α signatures and persistent dysfunction are distinguishing features of NK cells in severe COVID-19." *Immunity* (2021)

PS 2 I Poster session II

P 022

Impact of canonical and non-canonical NF-κB signaling pathway on acute and chronic cutaneous delayed-type hypersensitivity reaction (DTHR)

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The transcription factor NF-κB is a crucial regulator of inflammation. While canonical NF-κB (NF-κB1) activation is commonly linked to innate immune responses, the noncanonical NF-κB (NF-κB2) activation is more associated with the adaptive immunity. Here we investigated the role of canonical and noncanonical NF-κB signalling during the acute and chronic cutaneous DTHR by employing the NF-kB1-/- and NF-κB2-/- mice. We determined ear-swelling responses and conducted extensive *ex vivo* analyses of inflamed ears focusing on impairments in immune cell homing and differences in the inflammatory microenvironment (adhesion molecules, angiogenesis, chemokines, cytokines).

We observed reduced ear swelling response in NF-kB1-/- mice during the acute inflammation, while no differences were observed in NF-kB2-/- mice when compared to the WT mice. Histopathological analysis of the inflamed ears with acute DTHR revealed similar inflammation score and immune cell infiltration in all experimental groups. In contrast, during the chronic phase NF-kB1-/- exhibited only a moderate reduction in the ear swelling response, while the ear swelling response in the NF-κB2-/- mice was heavily impaired. In addition, we detected less acanthosis, hyperkeratosis, strongly reduced infiltration of T cells, neutrophils and macrophages as well as reduced blood vessel formation in inflamed ears of NF-κB2-/- mice during chronic DTHR. Furthermore, immunofluorescence microscopy analysis and proteome analysis of inflamed ears of NF-κB2-/- mice displayed impairments in expression of multiple chemokines, endothelial adhesion molecules and angiogenesis promoting mediators during the chronic DTHR.

Our results demonstrate that disruption of the noncanonical NF-κB-signalling attenuates the cutaneous DTHR by impairing chemotaxis, expression of endothelial adhesion molecules and angiogenesis resulting in a reduced recruitment of the immune cells into the inflamed ear tissue.

P 024

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

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Mast cells (MCs) are tissue resident immune cells, which are well known for their detrimental role in allergy. However, MCs also contribute critically to inflammation by orchestrating innate and adaptive immune responses. The underlying mechanisms are still barely understood.

During contact hypersensitivity (CHS) induced skin inflammation, MCs promote the innate and adaptive immune response. In detail, MC-derived TNF promotes $CD8^+$ DC maturation and migration and consequently $CD8^+$ T cell priming in the sensitization phase. Importantly, upon reexposure to the hapten, skin inflammation was reduced in the acute phase, but the ear thickening did not recede at later time points in absence of MC-derived TNF. Questioning the underlying mechanism we found that in the acute phase, skin infiltrating inflammatory monocyte and $CD8^+$ T cell numbers were reduced. At later time points of CHS, skin leukocyte numbers and pro-inflammatory cytokine levels were not altered in the absence of MC-derived TNF. However, we observed reduced skin levels of the matrix-metalloproteinases (MMP)-8 and proMMP-9, while the level of collagen I was increased indicating an impact of MC-derived TNF on tissue recovery. Of note, the adoptive transfer of primed $CD8^+$ T cells from sensitized wild type mice could rescue the monocyte recruitment and prevented the ear thickening at later stages in the absence of MC-derived TNF. Hence, MC-derived TNF is required for proper tissue recovery by promoting the priming of $CD8^+$ T cells, which guide monocyte recruitment. Intriguingly, the treatment of WT mice with an anti-TNF antibody at the beginning of CHS led to the same defect in resolving the ear swelling as seen in absence of MC-TNF, thereby suggesting a potential detrimental effect of anti-inflammatory TNF therapies in tissue recovery.

In conclusion, a prompt and sufficient initiation of inflammatory immune responses by MC-derived TNF is crucial for subsequent resolution and tissue regeneration.

PS 2 I Poster session II

P 026

Drosophila macrophages control systemic cytokine levels in response to oxidative stress via a non-canonical DNA damage repair signaling cascade

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[2]University of Freiburg, Faculty of Biology, Freiburg, Germany
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[5]Imperial College London, MRC Centre for Molecular Bacteriology and Infection, London, United Kingdom
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Environmental factors, infection, or injury, cause oxidative stress in diverse tissues, resulting in immune activation and loss of tissue homeostasis. Effective stress response cascades, conserved from invertebrates to mammals, ensure the re-establishment of homeostasis and tissue repair. Plasmatocytes, the *Drosophila* macrophage-like cells, are thought to respond to oxidative stress by immune activation. However, the signaling cascades involved in oxidative stress sensing and subsequent immune activation are yet to be defined. Furthermore, their role in modulating and controlling oxidative stress response to facilitate tissue repair and survival of the organism is not resolved. Here we describe the responses of hemocytes in adult *Drosophila* to oxidative stress and the essential role of non-canonical DNA damage repair activity in direct "responder" hemocytes to control JNK-mediated stress signaling, systemic levels of the cytokine upd3 and subsequently susceptibility to oxidative stress. Our results point to an essential systemic role of hemocytes in controlling systemic oxidative stress response in *Drosophila*, including energy mobilization for potential tissue repair.

P 028

The anti-inflammatory lipid mediator PGE1 boost inflammasome activation in human macrophages

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Background: Prostaglandins are small lipid inflammatory mediators produced at sites of tissue damage or infection. Prostaglandin E1 (PGE1), is a naturally occurring prostaglandin with widespread use as a medication. PGE1 is well known for its anti-inflammatory effects, and it is on the World Health Organization´s List of Essential Medicines. In this study, we investigated the effects of PGE1 on the activation of the NLRP3 Inflammasome, a signaling platform required for the maturation of the highly inflammatory cytokines of the IL-1 family.

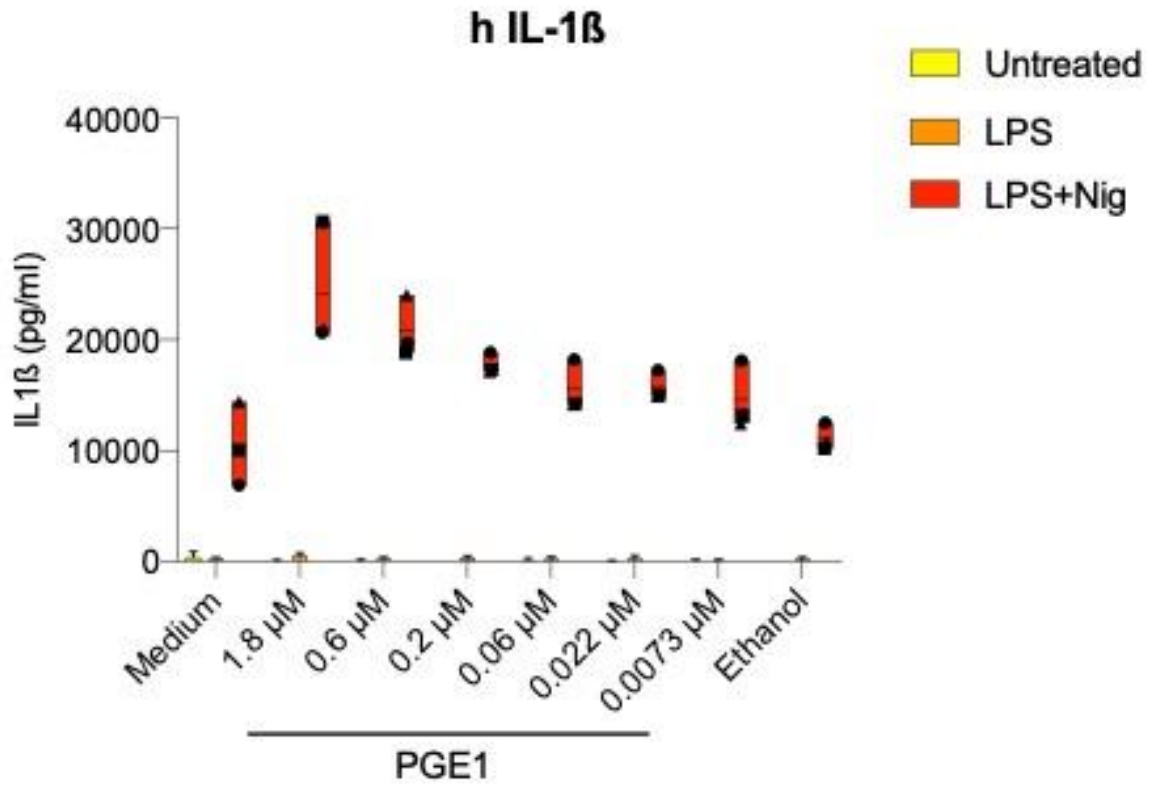
Methods: In this study, GM-CSF macrophages isolated from Buffy coat were used. PGE1 was added at different concentration (1.8µM or 0.6µM) in macrophages before LPS stimulation or after 3 hours priming. NLRP3 Inflammasome was classically induced with LPS for 3 hours and Nigericin for 90 minutes. HTRF ® technology was used to assess cytokines levels. Cell viability was quantified with the Cell-Titer Blue Cell viability assay and Lactate dehydrogenase assay. Protein expression was estimated by western Blot analysis.

Results: In line with an anti-inflammatory effect of PGE1, we observed that addition of PGE1 to macrophages diminished their secretion of TNF alpha in response to LPS stimulation. In contrast, priming GM-CSF macrophages with rhPGE1 and LPS before activating NLRP3 with Nigericin boost the secretion of inflammatory cytokines IL-1ß and IL-18 compared to priming with LPS alone. Activation of NLRP3 with rhPGE1 and Nigericin after LPS priming did not lead to increased cytokine secretion compared to activation with nigericin alone. Hence, PGE1 affects inflammasome activity during the priming phase.

Figure 1. levels of hIL-1B (a) and hTNFα (b) were measured by HTRF

Conclusion**:** Our findings reveal an unexpected proinflammatory feature of PGE1 that acts as a synergistic priming signal to license inflammasome activation in vitro. These finding call for considerations regarding the use of PGE1 as a medication.

Fig. 1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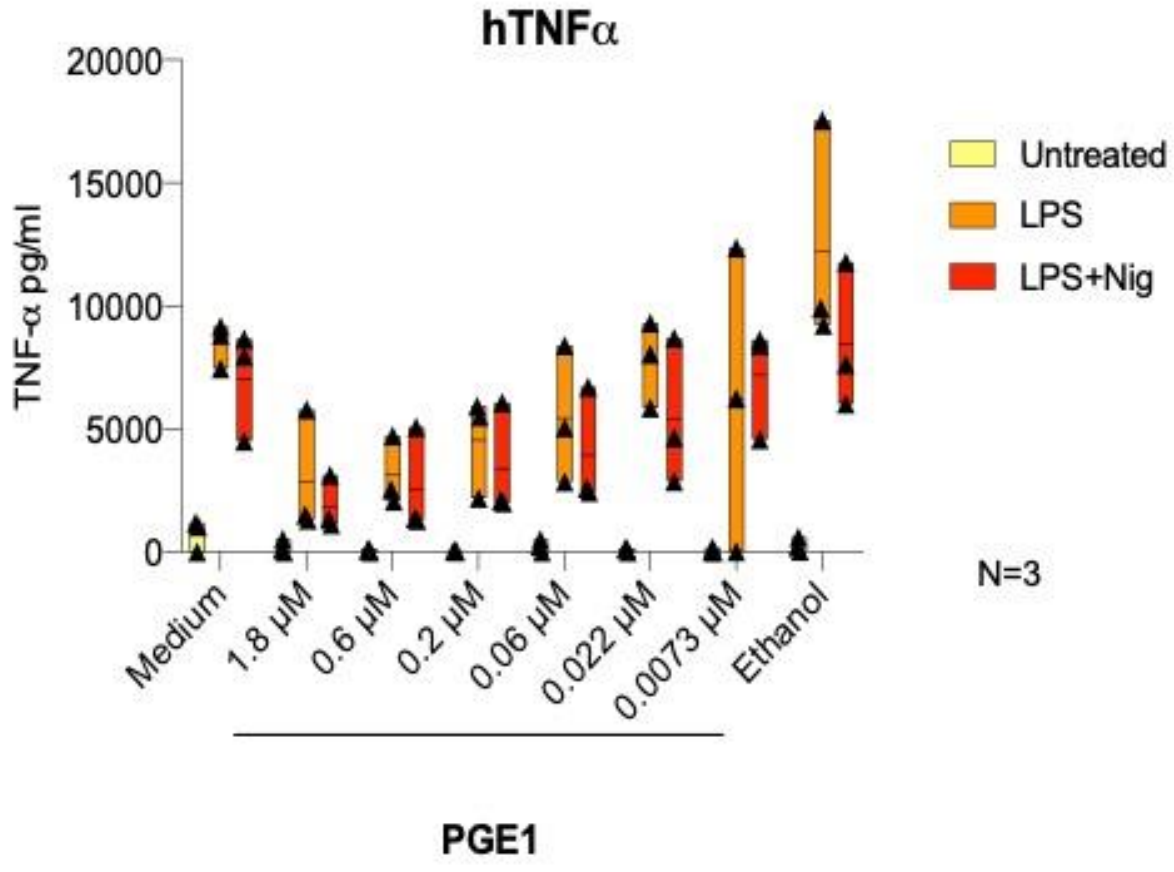
Ethanol
PGE1

Fig. 2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Ethanol
N=3
PGE1

PS 2 I Poster session II

P 030

Role of crosstalk between γδ T cells and Langerhans Cells in driving their mutual differentiation in the postnatal niche

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Epithelial differentiation and barrier formation are essential steps to shield the organism from the environment upon birth. Therefore, epithelial barriers need to undergo a rapid perinatal differentiation and adaptation process, including anatomical differentiation but also acquisition of a specialized barrier immune compartment. Epithelial barriers, including epidermis and oral mucosa, harbor specialized subsets of resident immune cells: the Langerhans cells (LC) and T cells expressing a γδ T-cell receptor (γδTCR) called γδ T cells. LCs constitute a distinct immune cell type with features of Mϕ and DCs. Specialized resident γδ T cells known as dendritic epidermal T cells (DETC) are the only T cells present in the mouse epidermis. Both LCs and DETCs critically contribute to the immune defense and tissue homeostasis of epithelial barriers throughout life. In the mouse, DETCs and LCs colonize the epidermis and oral mucosa during late embryonic development followed by their rapid differentiation in the early postnatal phase. Hence, this perinatal phase represents a crucial time window where multiple factors can influence DETC and LC differentiation, thereby contributing to the establishment of a healthy epithelial barrier. In our work, we aim to decipher how LCs and DETCs co-evolve in the developing epidermis and which factors influence the establishment of the healthy adult LC and DETC network. Here we show a detailed flow cytometric and immunohistochemical characterization of LCs and DETCs throughout development. We analyzed the colonization dynamics of the early postnatal epidermal niche and investigated the differentiation of both immune cell populations. Furthermore, we found that mice lacking DETCs showed subsequent effects on the evolving LC compartment. Together our data give first insights on interconnected epidermal immune cell development and creates a basis for further investigation of the unique co-maturation of DETCs and LCs in epithelial tissues.

P 032

Ribonuclease 6 processes bacterial RNA thus generating uridine-terminated products for toll-like receptor 8 stimulation

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Mechanistic studies have revealed that TLR8, an endosomal single-stranded RNA (RNA) sensor, requires simultaneous binding of uridine and di- or trinucleotides, respectively, on the apex and concave pockets for activation. Thus, ssRNA needs to be processed to become a TLR8 ligand and induce downstream NF-κB-dependent cytokines. Meanwhile, a synergistic cleavage by ribonucleases (RNase) T2 and RNase 2 - a member of the RNase A family - has been shown as their breakdown products are sensed by TLR8. However, further RNase A family members may contribute to this process. In this family, RNase 6 is a promising candidate as it is involved in the innate immune response acting as an antimicrobial peptide and overlaps TLR8 expression in classical monocytes and dendritic cells. Yet, RNase 6 role in bacterial RNA (bRNA) processing for TLR8 activation has not been studied so far. To decipher the role and cleavage mechanism of RNase 6, BLaER1 cells *TLR8*-/- or *RNASE6*-/- were generated by CRISPR-Cas9 and initially tested regarding their proinflammatory response upon live bacterial infection or total bRNA stimulation. Analysis of the supernatant by ELISA revealed a significant decrease in TNF and IL-6 release in *RNASE6*-/- cells suggesting that RNase 6 was required to sense certain bacteria. Also, bRNA that was *in vitro* digested by RNase 6 could stimulate TLR8 in the absence of endogenous RNase 6 showing the immunostimulatory potential of those fragments. Interestingly, the first data using the same *in vitro* digestion approach shows that 2"-O-methylation RNA modification - that had been shown to impede TLR8 stimulation - in between uridine and adenosine (preferential site of RNase 6 cleavage) could impair the upstream processing by RNase 6 but also dampened TLR8 stimulation as IL-6 release was abrogated. Taken together, these data suggest the role of RNase 6 for bRNA processing by the generation of immunostimulatory breakdown products which are uridine-terminated for TLR8 activation.

P 034

More than NLRP3 - B cells show high sensitivity to DPP9 inhibition

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Recents findings demonstrated caspase-1 activation and IL-1ß production in B lymphocytes promoting a novel vision and perspective about a proinflammatory activation of lymphoid compartment and suggesting an important contribution in physiologic and pathologic immune response. Among inflammasome receptors, NLRP1, NLRP3, CARD8 and AIM2 are highly expressed in peripheral blood CD19+ lymphocytes, however the complete landscape of the activation pathways and exact role of inflammasome in B cells is not fully understood. Here we report the response of B cells against the DPP-9 inhibitor ValBoro-Pro (VbP; Talabostat), known activator of NLRP1 and CARD8. Our preliminary data shown that, differently from NLRP3-dependent activation, VbP triggers IL-1ß release just in primed cells and seems not to affect IgM secretion **(Figure 1)**. VbP induces caspase-1-dependent Propidium Iodide (PI) up-take in CD19+ cells **(Figure 2)**. Both NLRP1 and CARD8 can be activated by VbP in B lymphocytes. Thus, our study identifies NLRP1 and CARD8 as important contributors for inflammasome activation in B lymphocytes, and suggests that an exacerbate activation of this pathway may promote a pathologic pro-inflammatory milieu in a variety of diseases such as infections, autoimmune or metabolic diseases, cancer.

Fig. 1

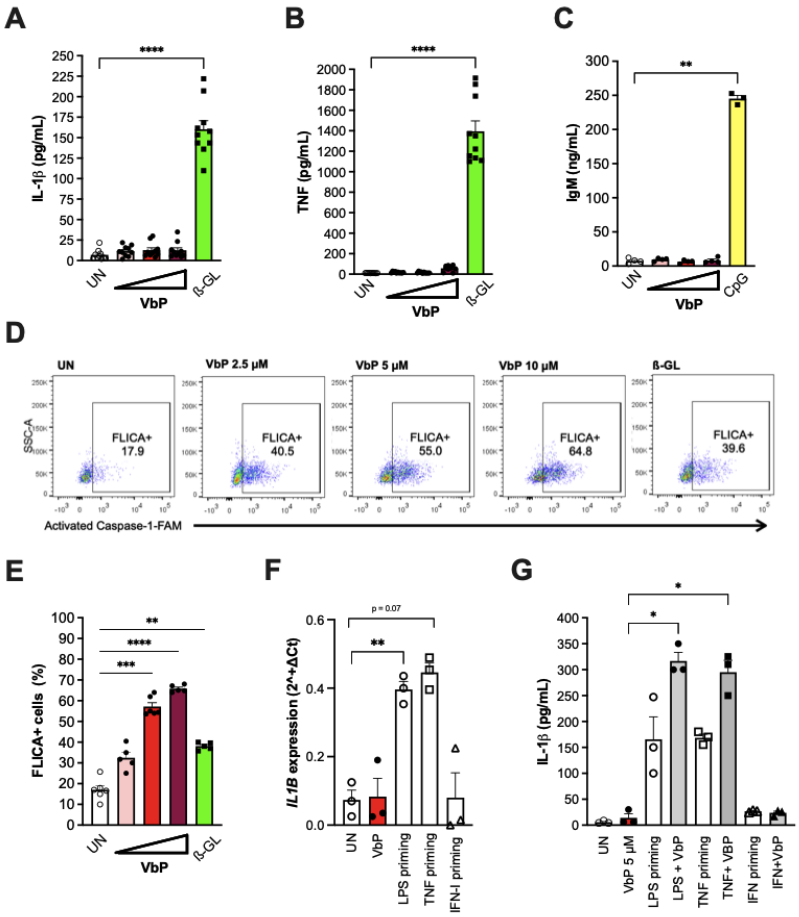

Figure 1. The DPP-9 inhibition activates caspase-1 but not IL-1ß release in human CD19+ cells. Healthy donors CD19+ cells were treated with Valboro-Pro (VbP; 2.5, 5 or 10 µM), or fungal ß-glucan (ß-GL; 200 µg/ml) or CpG (1µg/ml) for 24 hours. The release of IL-1ß (**A**), TNF (**B**) and total IgM (**C**) was measured in culture supernatants. Caspase-1 cleavage/activation in CD19+ cells was measured after 3 hours of VbP treatment by FAM-FLICA assay and flow cytometry (n = 5). Dot-blots of a representative experiment (**D**) and average percentage of FLICA positive cells (**E**) were reported. *IL1B* gene expression modulation by priming (0.1 µg/mL LPS, 0.1 µg/ml TNF or type I IFN (1000 U/ml) for three hours) is shown in (**F**) as well as the IL-1ß release in unprimed and primed CD19+ cells (**G**). Repeated-measures 1 Way ANOVA was used to compare all the conditions. *: p< 0.05; **: p< 0.01; ***: p< 0.001; ****: p<0.0001.

Fig. 2

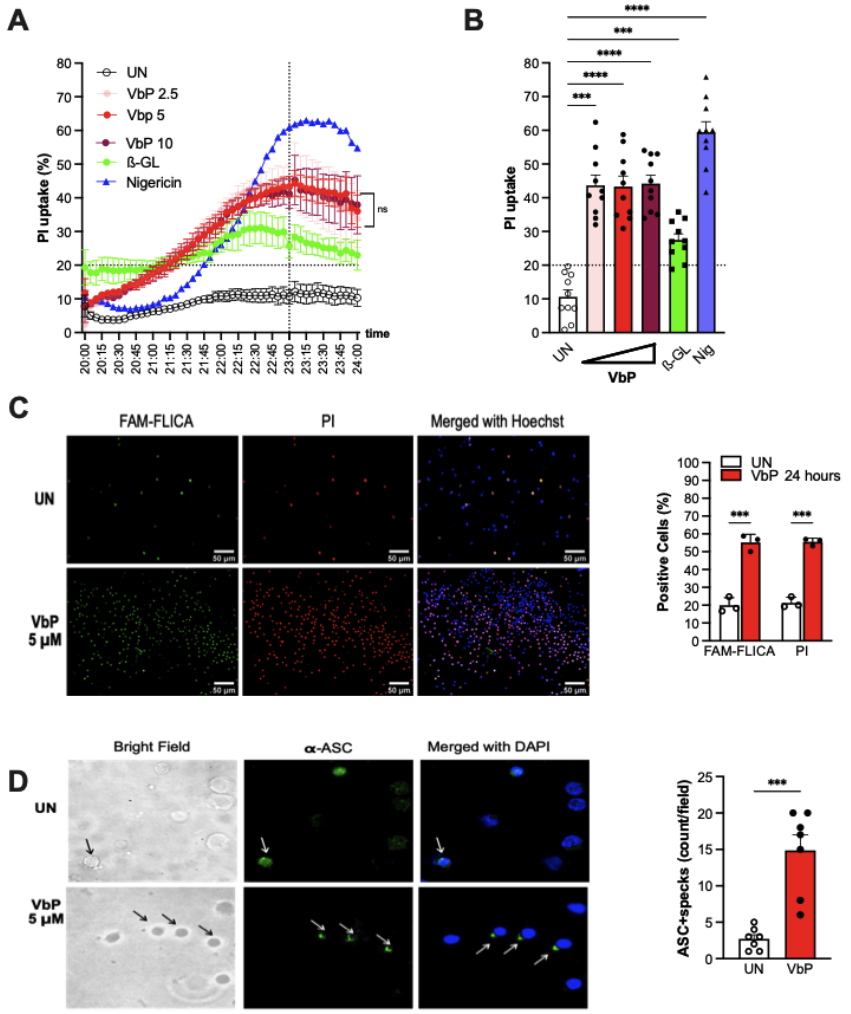

Figure 2. VbP induces pyroptosis in CD19+ cells.
Propidium Iodide (PI) up-take was measured in CD19+ cells during the last four hours of Valboro-Pro (VbP; 2.5, 5 or 10 µM) treatment (20-24 hours) and expressed as the percentage of PI positive cells related to Triton-treated cells (100%) in a real time curve (**A**) and as a graph bar at 3 hours of assay (**B**). CD19+ cells were treated with VbP 5 µM for 24 hours and (**C**). stained with FAM-FLICA kit (green fluorescence), and Hoechst nuclei dye (blue fluorescence) in the presence of PI (red fluorescence), or **(D)** stained for ASC+ speccks (green fluorescence) and DAPI nuclei dye (blue fluorescence). Data were reported as microscopy photographs (50 µm and 100 µm, respectively) for one representative experiment and bar graphs with data of three experiments (mean ± standard error)
Multiple t test was used in A. 1-Way ANOVA in B, Paired t test in C, D. *: $p < 0.05$; ***: $p < 0.001$; ****: $p < 0.0001$.

PS 2 I Poster session II

P 036

Dissecting MAVS signalosomes assembly and degradation using camelid nanobodies

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Viral dsRNA recognition is accomplished by the cytosolic RIG-I like receptors (RLRs), a group of cytosolic pattern-recognition receptors. During homeostasis, RLRs are auto-inhibited by their C-terminal regulatory domain (CTD). Binding of dsRNAs motifs by RLRs induces the release of the N-terminal tandem caspase activation and recruitment domains (CARDs), which are subsequently thought to nucleate the polymerization of MAVS into prion-like fibrils. Although the MAVS signalosomes are central hubs to coordinate type I interferon induction, their intrinsic signal processing and endogenous structure are still not fully understood.

We aim to use novel nanobody-based tools to unravel the molecular mechanism of MAVS signalosome assembly as well as its disposal when signaling is no longer needed. Nanobodies, single domain antibodies derived from the variable domain of camelid heavy chain-only antibodies (VHH), are easier to generate than regular antibodies and cheap to produce and modify. Nanobodies can be used in the cytosol of living cells to visualize, if fused to fluorescent proteins, and/or activate/disrupt endogenous proteins. These characteristics make them excellent tools to study MAVS signalosomes. We have established a panel of custom-generated nanobodies against MAVS, RIG-1, and MDA-5, which will be used to study signalosome activation, to interfere with MAVS function, and to microscopically analyze MAVS signalosome regulation using live cell imaging, super-resolution methods and proximity ligation assays. We have characterized nanobody binding to the respective targets as recombinant proteins and to the endogenous protein in living cells. We found that some of the nanobodies can be used to track endogenous MAVS, while others interfere with MAVS function or can be employed to degrade endogenous MAVS at will. Taken together, our aim is to provide a better understanding of the molecular mechanisms that regulate MAVS signalosome assembly and signal transduction.

P 038

Constitutive expression of interferon-kappa in keratinocytes promotes a primed state of their antiviral defense system

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Interferons (IFNs) are cytokines conferring host cell protection against infection through the induction of a wide range of interferon-stimulated genes (ISGs) with antiviral functions. Paracrine IFN signaling upon viral infection leads to the expression of ISGs, including the virus-sensing RIG-I-like receptors (RLRs), in bystander cells, putting them into an alert state. Such primed cells are prone to respond significantly faster and stronger to ensuing virus infection. In this study, we investigated the role of IFN-κ, a poorly studied type I IFN. In contrast to the classical virus-induced IFNs, expression of IFN-κ is constitutive, but largely limited to keratinocytes. This basal IFN-κ expression is thought to keep skin cells, being the body"s primary barrier towards microbes, in a constant state of alertness. In order to generate a suitable keratinocyte cell culture model, we knocked out (KO) IFN-κ by CRISPR/Cas9 technology in normal oral keratinocytes (NOK). We characterized the transcriptome of wildtype vs. KO cells and found a substantial downregulation of genes of the RLR pathway and ISGs in the absence of IFN-κ. This corroborated the role of IFN-κ as a mediator of the high homeostatic antiviral sensitivity of keratinocytes. Accordingly, on a functional level, we could show that constitutive IFN-κ expression in NOK cells establishes an alert state, conferring increased protection against Influenza A Virus and Rift Valley Fever Virus infection, which is lost upon KO. Our study highlights the importance of constitutive IFN-κ expression for maintaining high antiviral sensitivity of the epithelium. In ongoing studies, we aim to characterize biochemical properties of IFN-κ, including its capability to bind to cell surface heparan sulfates, and further investigate its role in skin immune homeostasis, both on epithelial cell-intrinsic pathways as well as on tissue-resident and patrolling immune cells.

PS 2 I Poster session II

P 040

Fcy-receptor IIIA activation by soluble immune complexes in chronic autoimmune diseases

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Autoantibodies play an important role in the diagnosis of chronic autoimmune diseases like systemic sclerosis (SSc), Sjögren's syndrome (SjS) and systemic lupus erytematosus (SLE). However, the link between occurrence of autoantibodies and the onset of clinically apparent disease is loose and ill defined. Fcγ-receptors such as FcγRIIIA are key to understand downstream effects of autoantibodies, but FcγRIIIA requires immune complexes for full activation. In this study, we investigated the presence of FcγRIIIA - activating soluble immune complexes (sICs) in patient serum.

We used a reporter cell model to screen for bioactive sICs in patient liquids. Reporter cells were BW5147 mouse thymoma cells stably transduced with a construct composing an extracellular domain of human FcγRIIIA, and a mouse CD3ζ intracellular domain. We stimulated these reporter cells over night with serum from patients with different autoimmune diseases and healthy donors. Mouse IL-2 secretion was measured by ELISA as a surrogate marker of FcγRIIIA activation.

Serum from patients with chronic autoimmune diseases activated the reporter cells, indicating the presence of FcγRIIIA-engaging sICs. We found that disease cohorts differ in their extent of FcγRIIIA activation. While SLE, SjS and SSC all show FcγRIIIA activation, it is stronger in SLE and SjS. In contrast to generalized SSC, serum from the local form of this disease, CREST syndrome, did not activate FcγRIIIA. Serum without autoantibodies e.g. from patients with psoriasis arthritis and healthy donors, does not induce FcγRIIIA activation. Additionally, the individual patients in each cohort, especially in SLE, showed a great heterogeneity in Fcγ-RIIIA activation.

FcγRIIIA-engaging sICs are present in the serum of SLE, SjS and generalized SSC. They link adaptive and innate immunity in chronic autoimmune diseases.

Chen et al. "Detection and functional resolution of soluble immune complexes by an FcγR reporter cell panel." *EMBO Mol Med* (2022)

PS 2 I Poster session II

P 044

Simultaneous PET/MRI using 64Cu-NOTA-anti-CD206 and 19F-PFC provides new insights into the role of macrophages in cancer immunotherapy

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Immune checkpoint Inhibitors (ICI) are novel and promising immunotherapeutics that improve the therapy outcome of many solid cancers, but clinical response rates are still low. Our study aimed to analyze the dynamics of immune-suppressive mannose receptor CD206 expressing TAMs (M2 macrophages) non-invasively *in vivo* by simultaneous positron emission tomography (PET)/magnetic resonance imaging (MRI) in a immunotherapeutic setting.

For this, we have employed a novel cross-reactive (human/murine) CD206-specific radiolabeled Nanobody (64Cu-αCD206) combined with 19F-Perfluorcarbon (19F-PFC) for simultaneous determination of TAMs and phagocytes via PET/MRI. OVA-MC38 colon adenocarcinoma bearing C57BL/6 mice were treated with bicarbonate and with αPD-L1 or isotype (Iso) mAbs. 64Cu-αCD206-PET/19F-PFC-MRI was performed 48h/24h after i.v. injection of 19F-PFC and 64Cu-αCD206 , respectively.

In vitro experiments revealed significant CD206 expression exclusively in M2 polarized (IL-4 and IL-13) but not in M1 or M0 macrophages. The *in vivo* signal of 64Cu-αCD206 in the OVA-MC38 tumor increased during two weeks of Bic + Iso mAbs-treatment, suggesting an increase of suppressive TAMs in the TME. Surprisingly, the addition of PD-L1 blockade exhibited a similar effect. In sharp contrast, the 19F-PFC signal within MC38 tumors dropped in both groups. *Ex vivo* flow cytometry of F4/80+ TAMs revealed an intratumoral increase in CD206+ expression in both experimental groups.

These findings emphasize the translational use of the cross-reactive 64Cu-NOTA-anti-CD206 Nb in the clinical setting as non-invasive *in vivo* imaging of the temporal dynamics of suppressive TAMs might serve as an early biomarker for tumor progression.

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P 046

The C229S *MYD88* mutation is a gain-of-function mutant in a patient with myelofibrosis

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Background: Myelofibrosis (MF) is a chronic myeloproliferative disorder, which exhibits biological and clinical heterogeneity. Here we report about a 70-year-old patient, diagnosed with MF and presenting a complex karyotype including a *TP53* mutation. Next-generation sequencing (NGS) identified one *MYD88* C229S variant among 700 patient samples undergoing clinical screening using a myeloid neoplasm NGS panel. In the Singaporean Chinese population, the *MYD88* C229S mutation frequency is around 0.0005. Although the C229S mutation maps to the critical Toll/Interleukin-1 receptor (TIR) domain of the critical Toll-like receptor (TLR) adaptor, Myd88, it is unclear whether the C229S *MyD88* mutation is functional or not. OF note, *MYD88* gain-of-function mutations have so far been found only in B cell malignancies.

Methods: HEK293T cells were transfected with different concentrations of plasmids encoding the *MYD88* WT and *MYD88* C229S variants. Dual luciferase Reporter assay (DLR) and immunoblot were conducted to investigate NF-κB signaling activation and protein expression. ELISA was conducted to explore IL-8 expression levels. Moreover, the position of this mutation in the structure of the MyD88 post-receptor complex was analyzed. [AW1]

Results: Compared to the WT, the *MYD88* C229S variant activated NF-κB-dependent luciferase production more highly and induced more IL-8 expression with comparable protein expression.

Conclusion: *MYD88* C229S is a gain-of-function mutant of MyD88 that is atypical in its occurrence in a myeloproliferative disorder. Further experiments are underway to determine the nature and mechanism behind this new mutation.

P 048

Aurora kinase inhibition induces senescence and enhances NLRP3-mediated inflammatory responses in THP-1 cells

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Background: The NLRP3 inflammasome is a cytosolic multiprotein complex, with a crucial role in releasing inflammatory cytokines and initiating innate immune responses. Although the NLRP3 inflammasome is a highly disease-associated inflammasome, its role in cancer, especially hematological malignancies, is not well described. Recent studies have indicated a functional role of the NLRP3 inflammasome during oncogene-driven cellular senescence. Senescence is a cellular process characterized by an irreversible and stable cell cycle arrest, terminating proliferation. Here we investigate the functional interplay between NLRP3 inflammasome activity and therapy-induced senescence in acute myeloid leukemia (AML) cell lines.

Methods: We compared the effects of the selective aurora kinase (AurK) inhibitors – Alisertib (AurK A inhibitor), Barasertib (AurK B inhibitor) and SNS-314 (Pan-AurK inhibitor) - in different AML cell lines. Senescence induction was quantified using senescence-associated β-Galactosidase (SA-β-Gal) expression, proliferation arrest and cell morphology. Furthermore, we stimulated inhibitor pre-treated THP-1 cells with the NLRP3 activators nigericin and imiquimod and investigated inflammatory responses by measuring secretion of pro-inflammatory cytokines IL-18 and IL-1β and analyzing expression and maturation of NLRP3 inflammasome components.

Results: We observed striking differences in cell morphology, SA-β-Gal expression and cell proliferation upon stimulation of THP-1 and U937 cells with selective aurora kinase inhibitors, suggesting the induction of a stable senescent phenotype. Comparison of NLRP3 stimulated senescent and non-senescent THP-1 cells revealed a strong increase in secretion of pro-inflammatory cytokines IL-18 and IL-1 β in senescent cells. Increased TNF-α secretion upon LPS treatment suggested a higher sensitivity to TLR-mediated priming, delineating ways in which aurora kinase inhibition increases inflammatory potential of THP-1 cells. Furthermore, we validated the NLRP3 specificity of these effects by treatment with the NLRP3-selective inhibitor MCC950. All observed effects were neither restricted to the specificity of the aurora kinase inhibitor (AurK A, B and C) nor to inflammasome activating compound (nigericin and imiquimod).

Conclusion: Here we identify selective aurora kinase inhibitors as potent senescence inducers in different AML cell lines and senescence induction as a novel priming pathway for the NLRP3 inflammasome. We hypothesize that combining senescence induction and NLRP3 stimulation for the release of immune-stimulatory cytokines could increase IL-18-mediated NK cell responses and improve innate immune clearance of senescent AML cells.

PS 2 I Poster session II

P 050

Evolutionary conservation of the role of STING in regulation of lipid metabolism

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Immunity and maintenance of metabolic homeostasis are tightly linked. The adaptor protein STING (Stimulator of Interferon Genes) is the central regulator of a variety of innate immune responses, most importantly, the induction of type I interferons in vertebrates. Beyond innate immunity, STING also modulates metabolic pathways at several levels. One of the ways in which STING exerts its control on metabolic homeostasis is by inhibiting FADS2 (Fatty Acid Desaturase 2), the rate-limiting enzyme in the omega-6 and omega-3 PUFA (polyunsaturated fatty acid) desaturation pathway. It has been reported that murine STING inhibits the enzymatic activity of FADS2 and leads to accumulation of PUFA derivatives, which in turn inhibits STING, thereby regulating type I IFN responses. This negative-feedback loop balances and fine-tunes inflammatory and metabolic homeostasis.

Both STING and FADS2 are highly conserved proteins with well-identified orthologues in ancestral invertebrates. Currently, there are reports confirming the conservation of the role of STING in metabolic regulation in humans, mice, and drosophila, although the mechanism through which this is achieved in drosophila is not well understood. We are investigating whether the role of STING in PUFA metabolism in general and specifically the STING-FADS2 interaction are evolutionarily conserved in invertebrates and non-mammalian vertebrates. To this end, we are assessing if STING from *Monosiga brevicollis*, *Nematostella vectensis* and *Danio rerio* can restore wild-type basal PUFA profiles by rescuing the metabolic alteration of PUFA derivatives witnessed upon STING ablation. Preliminary results from co-immunoprecipitation experiments show that murine FADS2 can bind to all of the three STING orthologues. Besides, we are also testing a wide range of known STING agonists against these orthologues to check if they can modulate downstream innate immune signaling pathways and metabolic responses.

P 052

Role of *Staphylococcus aureus* Nuc1 in immune stimulation

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Staphylococcus aureus is an opportunistic pathogen, causing a wide spectrum of acute and chronic infections. To better understand the success of this pathogen, it is necessary to know more about its interaction with the immune system. In this study, we investigated whether the secreted *S. aureus* nuclease Nuc1 (also called thermonuclease or NucA) contributes to immune stimulation and virulence. We compared *S. aureus* USA300-JE2 parent strain with its Nuc1-deficient JE2D*nuc*1 strain. Stimulation of RAW264.7 murine macrophages, MM6, and PBMCs with live *S. aureus* JE2 and JE2D*nuc*1 cells led to the production of TNF-α or IL-6. However, there was hardly any difference between the two strains. In order to identify the staphylococcal factor that exhibited immune-stimulatory activity, we compared the cytokine release in response to JE2, JE2D*nuc*1, JE2D*lgt* and the double mutant JE2D*lgt*/D*nuc*1. The D*lgt* mutant strains are deficient in lipidation of lipoproteins, which are well characterized microbe-associate molecular patterns (MAMPs). In all the strains lacking *lgt* the production of TNF-α or IL-6 was drastically decreased. This was observed in both murine macrophages and MM6. This result suggests that *nuc1* does not play a major role in immune stimulation in this setting and that lipopeptide sensing is dominan. However, when we investigated the internalization of *S. aureus* strains by HaCaT and RAW264.7, we observed that the D*nuc*1 mutant was significantly affected in internalization, and this effect was independent of *lgt* presence. Since bacterial internalization plays a role in virulence, we compared the behavior of *S. aureus* Newman with its D*nuc*1 mutant in a mouse model of infection. We observed that the mutant caused significantly lower weight loss and abscess score than its parent strain in infected mice. These findings confirm that Nuc1 is an important virulence factor in *S. aureus*.

P 054

Natural killer cells possess senolytic activity against non-malignant dermal myofibroblasts – a new therapeutic window in Systemic Sclerosis?

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New therapies are needed in Systemic Sclerosis (SSc). Fibrosis is driven by accumulation of myofibroblasts. Recent preclinical studies showed that fibrosis can be ameliorated by eliminating activated (FAP+) and/or senescent myofibroblasts. Here, we hypothesized that myofibroblasts accumulate due to impaired immune clearance and investigated the potential of natural killer (NK) cells to eliminate fibrotic myofibroblasts.

We used a co-culture system and flow cytometry to investigate NK cell cytotoxicity against primary skin myofibroblasts. Senescence was induced by radiation. Killing mechanisms were deciphered by blocking strategies. Peripheral blood mononuclear cells from 20 SSc patients and 15 healthy donors and surface ligands of NK cell receptors on 5 healthy and 3 fibrotic (morphea) primary skin myofibroblasts were analyzed.

NK cells preferentially killed senescent dermal myofibroblasts. This effect could be enhanced by pre-activating NK cells with IL-2. We identified granular exocytosis, thus natural cytotoxicity, as leading mechanism in myofibroblast elimination, while death receptor-mediated cytotoxicity played a minor role. Myofibroblasts expressed a set of activating ligands known to regulate granule exocytosis conserved across donors. The inhibitory ligand of natural cytotoxicity HLA-E was also expressed and significantly enhanced on myofibroblasts by NK cell-derived IFN-gamma. The corresponding receptors to the ligands found on myofibroblasts were expressed on NK cells from healthy donors and SSc patients. Interestingly, several cytotoxicity receptors were significantly enhanced on CD8+ T cells from SSc patients compared to healthy controls.

Elimination of fibrotic, including senescent, myofibroblasts by NK cells is performed by natural cytotoxicity. Cytotoxicity is plastic and may be targeted to therapeutically enhance immune clearance of myofibroblasts in fibrosis. HLA-E is a promising target as it may give negative feedback to activated NK cells.

P 056

Effects of genetic background on innate immunity-driven autoinflammation in mice

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Available inbred mouse strains significantly vary genetically, resulting in differences in immune responses. Two strains most commonly used in immunological studies include BALB/c and C57BL/6, which are known to be Th2 and Th1 prone, respectively. Less is known about differences in innate immunity of these mice. We investigated the impact of genetic background on the course and molecular mechanisms of the murine autoinflammatory disease Chronic Multifocal Osteomyelitis (CMO), caused by a mutation in Pstpip2 gene and driven exclusively by innate immunity.

We employed mouse strains carrying the CMO mutation on three genetic backgrounds BALB/c, C57BL/6J and C57BL/6NCrl. All three strains developed the disease and had similar levels of the main driver of the disease IL-1β. Paw swelling was slightly milder in BALB/c than in the other strains. This observation correlated with the higher surface expression of PIR-B, SIRP-α inhibitory receptors on neutrophils, and significantly lower levels of ATP in these cells compared with C57BL/6 strains. Interestingly, the composition of infiltrating cells at the site of inflammation was also different. C57BL/6NCrl mice showed the highest count of CD11b+ cells, in particular monocytes and neutrophils, whereas BALB/c mice showed the highest number of macrophages. Hind foot lysates were tested for the levels of neutrophil attracting chemokines (KC, CXCL2, MIP-1α). We observed that MIP-1α release was strongly affected by genetic background, and was significantly higher in C57BL/6NCrl compared to the other strains. However, neutrophils isolated from this mouse strain had the lowest migratory capacity towards MIP-1α. This correlated with lower expression of MIP-1αR mRNA. Hence, our data confirm a strong association between genetic background of the mouse and the course of development of autoinflammation driven by innate immunity.

P 058

The influence of the gp130 endocytosis motif on IL-6 signaling in bone marrow-derived macrophages

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Glycoprotein 130 (gp130) is the common signaling receptor subunit of all IL-6-type cytokines and one of the most ancient cytokine receptors. Its cell surface expression can be regulated by cross-talk and ligand-induced endocytosis depending on the internalization motif leucine 784, leucine 785 in its cytoplasmic region. The physiological importance of this process remains up to now unclear. Our group generated a transgenic gp130LLAA knock-in mouse line, harboring leucine to alanine point mutations, as a model to study the effect of gp130 endocytosis in an endogenous context.

Here, we evaluate the quantity and quality of IL-6-mediated signals via the classic or trans-signaling pathway in murine bone marrow-derived macrophages (BMMs) from wild-type and gp130-LLAA knock-in mice matured either by GM-CSF or M-CSF stimulation. To initiate classic signaling or trans-signaling, BMMs were stimulated either with IL-6 or hyper-IL-6, a fusion protein of IL-6 and soluble IL-6Rα, respectively.

We show that in wild-type mice M-CSF-stimulated BMMs display higher cell surface levels of gp130 and IL-6Rα than GM-CSF-stimulated BMMs. Both IL-6 and hyper-IL-6 activate STAT3 stronger than STAT1 in BMMs. In contrast to STAT3, STAT1 activation appears to be regulated by gp130 endocytosis. The strongest increase in STAT1 activation can be seen in hyper-IL-6-stimulated BMMs from gp130-LLAA mice matured by GM-CSF since low IL-6Rα cell surface expression appears to be the limiting factor for STAT1 activation by classic IL-6 signaling in GM-CSF stimulated BMMs.

Thus, we conclude that gp130 endocytosis might be involved in the regulation of the balance between STAT1 and STAT3 activation in BMMs, which might have an important impact to shape the later immune response.