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Prof. Alexander N. R. Weber
Eberhard-Karls-Universität Tübingen
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SCL 1 - Innate immunity across organisms

SCL 1-1

Ancient origin of inflammatory cell death in bacterial immune systems

Tanita Wein[1]
[1]Weizmann Institute of Science, Department of Systems Immunology, Rehovot, Israel

Inflammatory cell death is a key immune strategy across life. In animals, caspase recruitment domains (CARDs) and pyrin domains activate caspases, which cleave gasdermin pore-forming proteins to induce pyroptosis. We show that CARD domains are also present in bacterial anti-phage defense systems, where they are essential for protease-mediated activation of bacterial gasdermins, leading to cell death. Additionally, multiple bacterial defense systems utilize CARDs to activate diverse cell death effectors, highlighting their broad role in immunity. Our findings suggest that CARD domains represent an ancient component of innate immunity, with CARD-dependent gasdermin activation conserved from bacteria to humans. More broadly, our lab explores the evolution of immune mechanisms across domains of life, focusing on bacterial immune systems, programmed cell death, and host-microbe interactions.

SCL 1-2

Evolution of immunity across domains of life

Aude Bernheim[1]
[1]Institut Pasteur, Paris, France

Immune defence mechanisms exist across the tree of life in such a wide diversity that the immune mechanisms of bacteria (antiphage systems) were considered unrelated to immunity of eukaryotes. However, recent discoveries unveiled hundreds of novel antiphage systems. Among this diversity of novel bacterial immune mechanisms, it emerged that a subset of antiphage defense systems are conserved in eukaryotes and are major actors of diverse immune pathways, leading us to revisit this paradigm. I will discuss the evolutionnary dynamics of immunity across domains of life and how the conservation of immune modules in bacteria, plants and animals can lead to discoveries in prokaryotes and eukaryotes.

SCL 1-3

cGAS/STING-dependent immunity : insights from Drosophila

Jean-Luc Imler[1]
[1]Université de Strasbourg, Institut de Biologie Moléculaire et Cellulaire, Strasbourg, France

The cGAS-STING pathway plays a central role in the detection of DNA in the cytosol of mammalian cells, leading to activation of immunity. This pathway is evolutionarily conserved and cGAS-like receptors (cGLRs) define a novel family of pattern recognition receptors sensing nucleic acids in animals. In flies, two such receptors, cGLR1 and cGLR2, sense the presence of viral RNAs in infected cells and trigger the production of at least four different cyclic dinucleotides (CDNs), 2'3'-cGAMP, 3'2'-cGAMP, 2'3'-c-diAMP and 2'3'-c-diGMP. These CDNs bind to STING, triggering activation of Relish, a transcription factor of the NF-κB family initially characterized for its role in the antibacterial IMD pathway. We are exploiting the Drosophila model to shed light on the function and regulation of the cGAS-STING pathway, with the aim of (i) unravelling previously hidden aspects of this important pathway in mammals, and (ii) exploring the portfolio of STING-regulated genes for new antiviral genes that may reveal original strategies to counter viral infections.

SCL 1 - Innate immunity across organisms

SCL 1-4

Characterization of a novel invertebrate DNA sensor with ancient roots in prokaryotic immune defense

Leonard Drees[1]

[1]Max Planck Institute for Multidisciplinary Sciences, Tissue Dynamics and Regeneration, Göttingen, Germany

The planarian flatworm Schmidtea mediterranea can regenerate its whole body from small pieces of tissue which is facilitated by pluripotent stem cells that are distributed throughout the body. While regeneration and stem cells are extensively studied in S. mediterranea, relatively little is known about their innate immune system.
Planarians may have unique requirements for their innate immune system compared to less- or non-regenerative animals, due to exposure of their inner organ systems to the environment during amputation, the need to protect their extensive stem cell population and the ability to replace any damaged tissue.
In an ongoing effort to characterize the planarian immune system, we identified a novel DNA-sensing protein. This protein is highly conserved across Protostomia. Using RNAi-mediated knockdown in planarians and CRISPR/Cas9-mediated knockout in Drosophila melanogaster, we demonstrate that this gene is essential in both species.
Interestingly, a recent study revealed that this protein evolved from a bacterial immune effector Purine Nucleoside Phosphorylase (PNP) which blocks phage infection by depleting cellular ATP in infected cells. While the bacterial version is triggered by secondary metabolites and lacks a DNA-binding domain, our biochemical and structural analyses show that the planarian protein binds DNA via a winged-helix-like domain, which leads to subsequent activation of its PNP domain.
Immunofluorescence microscopy shows that this DNA sensor localizes to the plasma membrane and intracellular vesicles. Consistently, immunoprecipitation followed by mass spectrometry identified interacting proteins from the endo-lysosomal system. Together with in vivo infection assays in D. melanogaster, these findings implicate a role as a pattern recognition receptor of the innate immune system.
We therefore propose the identification of a novel DNA sensor with a conserved role in innate immunity across invertebrates.

SCL 2 - Pattern recognition receptors - then and now

SCL 2-1

How Pattern Recognition Receptors signal

Clare Bryant[1]

[1]Addenbrooke's Hospital, Cambridge Biomedical Campus, Department of Medicine, Cambridge, United Kingdom

Pattern recognition receptors include membrane and cytosolic proteins, for example Toll-like receptors (TLRs) and NOD-like receptors (NLRs), broadly signal by forming large protein platforms or supramolecular organising centres (SMOCs). Understanding the mechanistic basis of PRR signalling has been hampered by limited availability of selective and specific antibodies challenging the robustness of standard cell biology/signal transduction biology techniques such as immunolocalization or immunoprecipitation leading to often contradictory cell population data sets. Elegant structural biology studies have identified how PRRs bind to their ligands in vitro and the protein-protein interactions involved in SMOC formation, but how these proteins behave in cells is only now being studied at a single protein resolution. Here I will show our recent data on TLR signalling including single cell imaging and single molecule fluorescence studies to explore the mechanisms of how these receptors signal within cells.

SCL 2-2

UNC93B1 prevents human lupus by restraining uncontrolled TLR7 activation

Min Ae Lee-Kirsch[1]

[1]Universitätsklinikum Carl Gustav Carus, Dresden, Germany

UNC93B1 is critical for the trafficking and function of nucleic acid-sensing Toll-like receptors (TLR), which are essential for antiviral immunity. Overactive TLR7 signaling induced by self-nucleic acid recognition has been implicated in systemic lupus erythematosus (SLE). The identification of UNC93B1 variants causing selective TLR7 hyperactivation in patients with early-onset SLE has unraveled novel functions of UNC93B1 in regulating TLR subtype-specific mechanisms of ligand recognition, receptor turnover, and signaling termination. These findings establish a central role of UNC93B1 in TLR7-dependent autoimmunity and highlight the therapeutic potential of targeting TLR7 in SLE.

SCL 2-3

The NLRP3 inflammasome senses cellular danger directly via cytosolic potassium

Alexander N. R. Weber[1]

[1] University of Tübingen, Institute of Immunology, Department of Innate Immunity, Tübingen, Germany

The NLRP3 inflammasome is a sentinel of cellular homeostasis and its activation triggers the assembly of a molecular machinery to drive inflammation in infections, cardiovascular, metabolic and neurodegenerative disease. The broad range of pathophysiological conditions leading to NLRP3 activation correlates with a plethora of different triggers, including pore-forming toxins, aggregated proteins or inhibitors of mitochondrial oxidative phosphorylation. The unifying concept of how most of these triggers induce an NLRP3 conformational change that leads to the subsequent formation of an inflammasome, release of IL-1 cytokines and cell death (pyroptosis) is potassium (K+) efflux from the cell and a consequent drop in intracellular potassium ion concentration. However, how precisely NLRP3 senses potassium efflux as the most pivotal step in its activation cascade has been highly enigmatic. Here we provide evidence that potassium induces conformational changes in NLRP3 itself: Thus, in both cell lines and primary blood immune cells potassium stabilized a compact structure resembling inhibitor-bound NLRP3, whereas low potassium or the presence of a potassium chelator favored an open, more flexible structure. Using exogenous expression and highly purified NLRP3 proteins, we demonstrate that NLRP3 conformationally responds to potassium directly, i.e. without cellular co-factors. We map conformational changes to the FISNA-NACHT module of NLRP3 but high-performance computation additionally identifies conformationally flexible loop regions as putative potassium binding sites which, mechanistically, stabilize the inhibited face-to-face but not back-to-back NLRP3 dimer interface. Collectively, our study suggests that NLRP3 is a direct potassium sensor and operates independently of co-factors to sense cellular danger.

SCL 2 - Pattern recognition receptors - then and now

SCL 2-4

Proteasome-mediated core histone degradation drives immune tolerance in human monocytes

Judith Austermann[1], Ihab Azzam[1], Theresa Ortkras[1], Achmet Imam Chasan[1], Alina Burghard[1], Jonas Wolf[1], Thomas Vogl[1], Johannes Roth[1]

[1]Institute of Immunology, University of Münster, Münster, Germany

The concept of innate immune memory comprises two opposing mechanisms, the increased "trained" and the attenuated immune response called immune tolerance. In sepsis immune tolerance can be triggered by a prolonged endotoxin-stimulation of Toll-like receptor 4 (TLR4). During immune tolerance monocytes have been described to undergo a major gene reprogramming. Thereby inflammatory genes were shown to be inhibited (tolerant genes), while for example antimicrobial genes were either up-regulated or remained inducible (non-tolerant genes) upon further LPS challenge. Gene expression is tightly controlled by dynamic chromatin remodeling. During immune tolerance distinct epigenetic modifications of specific histone residues have been described. In contrast, we now demonstrate that immune tolerance of monocytes is associated with extensive proteasomal degradation of core histones. This results in significantly increased open chromatin regions and alterations of chromatin condensation and nuclear elasticity which is driven by the chaperon nucleolin. Nucleolin inhibition prevented histone degradation and subsequent induction of tolerance. Transcriptome and proteome analysis confirmed major changes in chromatin, ubiquitin and proteasome metabolism. Analysis of monocytes during cardiopulmonary bypass surgery confirmed these alterations during the development of immune tolerance in a clinically relevant setting. This novel mechanism of global chromatin reorganization in immune tolerant monocytes offers new molecular targets for treatments of immune paralysis in monocytes.

SCL 3 - Nucleic acid-mediated immunity

SCL 3-3

RNA and DNA sensing pathways in the innate immune response are largely coordinated by human endogenous retroviruses

Sergey Iordanskiy[1], Eric Russ[1], Natallia Mikhalkevich[2], Clifton Dalgard[3]

[1]Uniformed Services University of the Health Sciences, Pharmacology & Molecular Therapeutics, Bethesda, MD, United States
[2]National Cancer Institute, Center for Cancer Research, Bethesda, MD, United States
[3]Uniformed Services University of the Health Sciences, Anatomy, Physiology & Genetics, Bethesda, MD, United States

Human endogenous retroviruses (HERVs) comprise ~8.3% of the human genome and are normally inactive. When reactivated, their expression products can prime pattern recognition receptors to induce virus-like innate immune response. Our studies suggest that certain HERVs upregulated by various external stimuli are essential for modulation and coordination of cytokine expression. We found that interferon gamma (IFNγ) signaling triggers transcription of the intergenic provirus HERV-K102, a member of the HERV-K HML-2 subfamily, through recruitment of the IFNγ-induced transcription factor, IRF1. Alongside, DNA damage and transcriptional disruption by ROS, which are enhanced by various infections and environmental stressors, increase the number of dsDNA and RNA:DNA duplexes, which in turn activate HML-2 expression via the cGAS-STING pathway. Viral transcripts in the cytoplasm trigger the RNA-sensitive RIG-I/MDA5-MAVS pathway, resulting in enhanced type I interferon (IFN-I) signaling and stimulation of the innate immune response through upregulation of genes containing an interferon-stimulated response element (ISRE) in their promoter, such as ISG15, ISG20, and CXCL10. Additionally, HML-2 modulates the expression of multiple other HERVs, multiplying the effect of RNA signaling, as silencing of this clade results in decreased expression of numerous HERV subsets in ionizing radiation-activated macrophages. Knockout experiments demonstrate that MAVS deficiency results in increased cGAS-STING signaling, particularly by RNA:DNA hybrids, and uncontrolled inflammatory activation of macrophages, whereas in wild-type and STING-deficient cells, even upon HML-2 upregulation, the inflammatory response is mitigated in comparison. Collectively, this suggests that specific HERV-activated factors such as OAS2, OASL, and IFIT1 are critical for the reduction of both DNA- and RNA-sensing signaling and hence for a balanced regulation of the inflammatory response and resolution of inflammation.

SCL 3 - Nucleic acid-mediated immunity

SCL 3-4

The single-cell epigenomic and transcriptional landscape of immunity to 6 antiviral vaccines

Florian Wimmers[1], Ruchir Rastogi[2], Nir Yosef[3], Bali Pulendran[4]
[1]Uni Tübingen, Molecular Medicine, Tübingen, Germany
[2]UC Berkeley, Berkley, CA, United States
[3]Weizmann Institute of Science, Rechovot, Israel
[4]Stanford University, , Stanford, CA, United States

Recent analyses have highlighted the ability of vaccines to induce lasting functional and epigenetic changes tothe innate immune system (Trained Immunity). However, a comprehensive comparison of different vaccine types with respect to their ability to induce Trained Immunity is lacking. Here, we compared the epigenetic and functional changesin innate immune cells after vaccination with six distinct antiviral vaccines, covering a range of vaccine types (inactivated, mRNA, adjuvanted, recombinant, live-attenuated).

To construct the transcriptional and epigenetic immune cell landscape, we collected PBMCs from healthy participantsbefore and after immunization with six antiviral vaccines and analyzed them with scRNA-seq and scATAC-seq. In parallel, we measured in vitro cytokine production and resistance to unrelated viruses in matched PBMC samples. Our analyses reveal that most antiviral vaccines induce lasting epigenetic changes, especially in CD14+ monocytes.The induced epigenetic patterns differ between vaccines but employ the same transcription factor (TF) motifs.Importantly, TF motif changes did behave not independently but concertedly, revealing TF motif super-clusters thatchange together. Subclustering analysis of integrated CD14+ monocytes from all vaccine cohorts revealedtranscriptionally defined subsets of cells with distinct functional phenotypes whose relative contribution to themonocyte pool shifts during vaccination. Linking epigenetic and functional data across vaccines, AP-1 accessibilityemerged as a central regulator associated with monocyte functionality.

Together, we show that many, but not all, vaccines can induce epigenetic modifications in the innate immune system.The coordinated alterations of specific TF motif families and the identification of distinct monocyte subpopulationsimply the existence of a broader regulatory framework. Understanding this framework will be critical to rationallydesigning interventions inducing Trained Immunity.

SCL 4 - Inflammasome-mediated immunity

SCL 4-1

Inflammasome signalling is terminated by a proteolytic timer to enforce homeostasis and prevent disease

Kate Schroder[1]
[1]The University of Queensland, Institute for Molecular Bioscience, Brisbane, Australia

A central tenet of innate immunology is that potential threats to organismal homeostasis trigger inflammatory programs to eliminate the threat, after which inflammation is silenced to restore homeostasis and normal tissue functions. We understand how inflammatory programs are silenced for many innate immune pathways (e.g. toll-like receptors, common signalling modules such as those activating NF-kB). Here, switching off inflammatory programs in vivo generally involves transcriptional programs that feedback to inhibit regulated gene expression. Inflammasomes are signalling complexes that trigger potent inflammatory responses by regulating proteolysis, rather than transcription. Unlike transcriptional changes, which are inherently reversible, protein cleavage is not reversible. It has remained a major challenge to identify fundamental mechanisms that silence proteolytic signalling cascades and their resultant biological programs. This study provides the first in vivo mechanism for inflammasome signal termination. We generated a knock-in mouse (Casp1.CDL) in which the caspase-1 CARD domain linker (CDL) is mutated to prevent autocleavage-induced protease deactivation, and examined these mice during steady-state and upon major physiological challenges. We discovered that CASP1 CDL autocleavage self-limits inflammasome activity to enforce homeostasis in the steady state, temper inflammatory programs during organ challenge, and reinstate tissue homeostasis following the removal of a major challenge to organ function. The critical importance of CASP1 silencing for homeostasis and restoring tissue function highlights CASP1 as an emerging target for new anti-inflammatory drugs.

SCL 4-2

Concomittant induction of the NLRP3 inflammasome and cGAS-STING in Alzheimer's disease

Michael T. Heneka[1], Masahiro Itakura[1], Paula Matorell[1], Frederic Brosseron[2]
[1]University of Luxembourg, Luxembourg Centre for Systems Biomedicine, Esch-Belval, Luxembourg
[2]German Centre for Neurodegenerative Disease, Bonn, Germany

The accumulation of neurotoxic amyloid beta peptides along with neurofibrillary tangle formation are key pathological hallmarks of Alzheimer's disease. The brain has been considered as an immune-privileged organ, however, increasing evidence from translational, genetic, and pathological studies suggests that activation of distinct innate immune pathways represent a third important disease hallmark which actively contributes to disease progression and chronicity.
Microglia play a pivotal role in this immune response and are activated by binding of aggregated proteins or aberrant nucleic acids to pattern recognition receptors. This immune activation leads to the release of inflammatory mediators but also distracts microglia cells from their physiological functions and tasks. Downstream of receptor activation, NLRP3 inflammasome activation and cGAS-STING activation interact and mount a a hyperinflammatory microglial reaction that terminates in pyroptosis and the release of ASC specks. The latter contributes to seeding of pathology by enhancing the propensity of beta-amyloid peptides to aggregate. This mechanism may account for the spread of pathology within a brain region, but also from one brain area to another. Beyond accelerating the spread of beta-amyloid pathology, NLRP3 inflammasome and cGAS-STING activation negatively impacts on the survival and functioning of neurons and specifically drive tau pathology. Interfering with both immune pathways may protect the brain from neurodegeneration and therefore holds potential as future therapy for Alzheimer's disease

SCL 4 - Inflammasome-mediated immunity

SCL 4-4

OXPHOS inhibition triggers NLRP3 and suppresses apoptosis

Olaf Groß[1]

[1]University Medical Center Freiburg, Institute of Neuropathology, Freiburg i.Br., Germany

How mitochondria balance their roles in the functionally distinct cell death pathways of apoptosis and NLRP3 inflammasome-mediated pyroptosis remains unclear, as is their precise role in NLRP3 activation, the danger signals conveyed to NLRP3, and its evolutionarily conserved physiological function. In this study, we observed that when cells were simultaneously challenged, apoptosis was suppressed, and NLRP3 activation dominated. The inhibition of apoptosis by structurally diverse NLRP3 activators, such as nigericin, imiquimod, extracellular ATP, particles, and viruses, was not a direct result of inflammasome activation. Instead, it was due to their impact on mitochondrial function. We found that NLRP3 activators act as inhibitors of oxidative phosphorylation (OXPHOS), disrupting the architecture of mitochondrial cristae and causing cytochrome c to become trapped. Although this effect alone was insufficient to activate NLRP3, OXPHOS inhibitors became effective triggers for NLRP3 activation when combined with resiquimod or Yoda-1. This suggests that NLRP3 activation requires two concurrent cellular signals, one originating from mitochondria. Consequently, the inhibition of OXPHOS and apoptosis by NLRP3 activators provides stringency in cell death decision-making. NLRP3 may have evolved to detect the ability of pathogens to suppress apoptosis, with the need for a second concurrent signal acting as a safeguard.

SCL 5 - Visualizing innate immunity

SCL 5-1

Visualising neutrophil behaviour and fates during inflammation

Milka Sarris[1]
[1]University of Cambridge, Department of Physiology, Development and Neuroscience, Cambridge, United Kingdom

Neutrophils rapidly infiltrate injured tissues to provide antimicrobial functions. The scale of neutrophil recruitment must be tightly tuned to physiological demands, to provide sufficient defence from infection but also timely resolution. A key control point is the paracrine release of chemoattractants by neutrophils, which escalates their recruitment into large aggregates (referred to as "swarms"). However, it remains unclear how neutrophils coordinate the generation of chemical gradients and how these group behaviours adapt to different immunological triggers. Using live imaging in zebrafish larvae in vivo and human neutrophils in vitro, we reveal a conserved role of coordinated calcium signals in initiation of neutrophil swarming. These calcium signals are stimulated by homotypic neutrophil contacts at the target region (e.g. wound core) and by damage signalling molecules. We describe the generation of new fluorescent biosensors to probe the damage signal release by neutrophils as an amplification mechanism for swarming. We further introduce silico modelling approaches, indicating that juxtacrine or contact-mediated stimulation of attractant production in clustering cells, can recapitulate key aspects of neutrophil swarm dynamics. Finally, we interrogate the impact of neutrophil swarm dynamics on the evolution of wound infections by opportunistic pathogens (Pseudomonas aeruginosa) and examine strategies to enhance neutrophil swarming in disease settings.

SCL 5-2

Innate immune cells regulate megakaryopoiesis

Florian Gaertner[1]
[1]Medizinische Klinik und Poliklinik, LMU Klinikum, Munich, Germany

Platelets are anucleate cell fragments crucial for maintaining vascular integrity in both health and disease. They originate from megakaryocytes, their bone marrow precursors, through a process known as thrombopoiesis. During this process, megakaryocytes undergo complete fragmentation to release platelets, necessitating continuous replenishment through megakaryopoiesis. However, the precise cellular and molecular mechanisms that regulate megakaryocyte homeostasis remain largely unclear. Using an intravital imaging approach with two-photon microscopy, we tracked the cellular dynamics of megakaryopoiesis and investigated the regulatory mechanisms that sustain megakaryocyte homeostasis within the bone marrow niche. Our study identified plasmacytoid dendritic cells (pDCs) as key regulators of megakaryopoiesis. These cells act as homeostatic sensors, detecting platelet-producing megakaryocytes and releasing IFN-alpha within the megakaryocytic niche. IFN-alpha promotes the proliferation and maturation of megakaryocyte progenitors, ensuring a balanced interplay between platelet production and megakaryopoiesis under both steady-state and stress conditions. Thus, our findings reveal a pDC-dependent homeostatic circuit that integrates innate immune sensing with the demand-driven release of inflammatory mediators, ultimately maintaining megakaryocytic lineage homeostasis.

SCL 5 - Visualizing innate immunity

SCL 5-3

Gasdermin E Pore structure and its impact on mitochondrial integrity during apoptosis

Nadine Gehle[1], Katia Cosentino[1]
[1]CellNanos University Osnabrück, Osnabrück, Germany

Gasdermins (GSDMs) are a family of pore-forming proteins that execute cell death pyroptosis by forming pores at the plasma membrane (PM), leading to the release of inflammatory cytokines and causing an immune response. The family member Gasdermin E (GSDME) is cleaved by apoptotic caspase-3, potentially bridging apoptosis and pyroptosis. GSDME has been implicated in the permeabilization of both PM and mitochondrial membranes. However, its precise membrane-targeting preference and downstream effects remain unresolved.
To address this, we investigated the real-time sequence of GSDME-driven membrane permeabilization during apoptosis, focusing on its localization dynamics in the presence and absence of BAX and BAK, key regulators of mitochondrial outer membrane permeabilization (MOMP). Using live-cell imaging and super-resolution microscopy, we tracked mitochondrial fragmentation, PM integrity, and GSDME pore formation at a nanoscale resolution.
Our findings reveal that mitochondrial fragmentation occurs prior to PM permeabilization, with GSDME targeting both the outer and inner mitochondrial membranes. GSDME pore formation at the mitochondria enhances the release of mitochondrial DNA (mtDNA), a known activator of the cGAS-STING inflammatory pathway. Using dual-color DNA-PAINT microscopy, we provide the first direct visualization of GSDME pores forming at mitochondrial membranes with nanometer resolution.
These insights position GSDME as a key player in mitochondrial permeabilization, linking apoptotic caspase activity to mitochondrial immune signaling. Understanding the interactions between GSDME, BAX, and BAK could shed light on novel mechanisms of cell death-driven inflammation, with implications for immune regulation and disease pathogenesis.

SCL 5-4

Zombies and immune cells – the interplay between intracellular pathogen proliferation and host cell death induction during L. major infection

Leon-Alexander Dewitz[1], Andreas Müller[1]
[1]Otto-von-Guericke-Universität Magdeburg, Institute of Molecular and Clinical Immunology, Magdeburg, Germany

Leishmania major (L. major) is an intracellular pathogen which is located mainly in macrophages during an established infection. It has already been shown that L. major parasites can be divided into high and low proliferating subpopulations, which seem to be correlated with differential host cell death induction in vitro and in vivo.
Whether activation of host cell death pathways is causally linked with the different proliferation rates of the infecting L. major parasites has remained unclear. To elucidate this question, we have generated killed but metabolically active (KBMA) L. major parasites, which are not able to proliferate, but retain their metabolic functions and the differentiation capacity from promastigote into amastigote upon infection.
By infecting macrophages with proliferation-competent versus KBMA parasites, we have determined the influence of the parasite proliferation capacity on the host cell. Using flow cytometry and live cell imaging, significant differences in host cell death could be observed dependently of the intracellular pathogen proliferation. We observed a differential activation of Caspase-1 versus Caspase-3 upon infection with proliferation-competent pathogen in vitro and in vivo by flow cytometry. In addition, we have stably expressed a fluorescently labeled ASC protein within monocytes. This biosensor allows us to follow NLRP3 inflammasome activation and therefore subsequent pyroptotic cell death in live cell imaging in vivo and in vitro.
The results suggest a differential influence of the proliferation rate of L. major parasites on their host phagocytes, highlighting the proliferation as an important factor influencing the host-pathogen interaction during an infection with L. major.

SCL 6 - Host-Microbiota Interactions

SCL 6-1

Host-microbiome interaction in health and disease

Eran Elinav[1]
[1]Weizmann Institute of Science, Department of Systems Immunology, Rehovot, Israel

The mammalian intestine contains trillions of microbes, a community that is dominated by members of the domain Bacteria but also includes members of Archaea, Eukarya, and viruses. The vast repertoire of this microbiome functions in ways that benefit the host. The mucosal immune system co-evolves with the microbiota beginning at birth, acquiring the capacity to tolerate components of the community while maintaining the capacity to respond to invading pathogens. The gut microbiota is shaped and regulated by multiple factors including our genomic composition, the local intestinal niche and multiple environmental factors including our nutritional repertoire and bio-geographical location. Moreover, it has been recently highlighted that dysregulation of these genetic or environmental factors leads to aberrant host-microbiome interactions, ultimately predisposing to pathologies ranging from chronic inflammation, obesity, the metabolic syndrome and even cancer. We have identified various possible mechanisms participating in the reciprocal regulation between the host and the intestinal microbial ecosystem, and demonstrate that disruption of these factors, in mice and humans, lead to dysbiosis and susceptibility to common multi-factorial disease. Understanding the molecular basis of host-microbiome interactions may lead to development of new microbiome-targeting treatments.

SCL 6-2

Secreted mucus is an outer innate defence barrier

Malin E. V. Johansson[1]
[1]University of Gothenburg, Medical Biochemistry and Cell biology, Gothenburg, Sweden

The intestine experiences several hazards including chemicals, mechanical stress, and microbes. To maintain a good environment at the epithelium mucus is secreted forming a barrier which can filter, modulate diffusion, provide binding sites, serve as a microbial niche, and reduce mechanical force. I will explain some of the biochemical features rendering mucus these properties as glycosylation, network formation, and modulation of the mucus by different mucus proteins. Mucus is produced by and secreted from goblet cells, which have a specific biosynthesis machinery. The goblet cells and the mucus they produce vary depending on site and requirement coupled to functions as protection and absorption. Both the mucus and the goblet cells are affected in inflammatory conditions which could be part of the pathogenesis. In conclusion mucus is a very dynamic part of our innate defence system in the intestine.

SCL 6-3

What AHR we talking about? Host Aryl Hydrocarbon Receptor (AHR) spying on bacterial communication and quorum

Pedro Moura-Alves[1]
[1]i3S, Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Immune Sensing and Signaling Dynamics, Porto, Portugal

The interaction between a bacterial pathogen and its host can be viewed as an "arms race" in which each participant continuously responds to the evolving strategies of the other partner. A mechanism allowing bacteria to rapidly adapt to such changing circumstances is provided by density-dependent cell-to-cell communication known as Quorum Sensing (QS). QS involves a hierarchy of signaling molecules, which in pathogenic bacteria is associated with biofilm formation and virulence regulation. We hypothesized that if a host sensor can detect and differentiate between bacterial QS molecules and their expression patterns, hosts can customize their immune responses according to the stage and state of infection.

Methods and Results: Taking advantage of different in vitro and in vivo (e.g., zebrafish and mouse) model systems, we demonstrate that infected hosts show differential modulation of the Aryl Hydrocarbon Receptor (AHR) signaling throughout a bacterial infection. AHR modulation depends on the relative abundances of different QS molecules, whereby their quantitative assessment enables the host to sense bacterial community densities that may have distinct gene expression programs and infection dynamics. The AHR can sense and bind to diverse microbial-derived ligands and regulate different host defence mechanisms, including ligand degradation, expression of pro-inflammatory mediators, immune cell recruitment, and bacterial clearance. By sensing infection dynamics, the AHR regulates diverse host defense mechanisms and impacts bacterial clearance.

Conclusions: We propose that by spying on bacterial quorum, the AHR acts as a major sensor of infection dynamics, capable of orchestrating host defense according to the status quo of infection. Importantly, AHR modulation and activation status impact antibiotic therapeutic efficacy, potentially driving antimicrobial resistance and microbial adaptation strategies. We unveiled a role for the AHR as a Pattern Recognition Receptor.

SCL 7 - Innate immunity in the skin

SCL 7-2

The skin commensal yeast Malassezia promotes tissue homeostasis via the aryl hydrocarbon receptor

Salomé Leibundgut-Landmann[1]
[1]Section of Immunology, Vetsuisse Faculty, and Institute of Experimental Immunology, University of Zurich, Switzerland

As an abundant fungal colonizer of human skin, Malassezia has long been associated with pathological skin conditions, yet its role in skin homeostasis remain poorly understood. Here, we demonstrate that Malassezia furfur plays an active role in maintaining epidermal integrity by producing tryptophan-derived metabolites that activate the aryl hydrocarbon receptor (AhR), a key regulator of keratinocyte differentiation and inflammation. Using a fungal mutant defective in indole production, we show that M. furfur-derived AhR activation is required to restore barrier function and control inflammation in diseased skin. AhR-deficient mice fail to benefit from M. furfur-mediated barrier protection, underscoring the importance of microbial-derived AhR agonists in skin physiology. These findings establish a previously unrecognized mutualistic role for Malassezia in epidermal homeostasis, challenging its perception as solely a pathogenic fungus and expanding our understanding of the skin microbiota's influence on barrier function and immune regulation.

SCL 7-3

Ingestion of mast cell granules boosts macrophages and drives atypical programming

Konstantinos Katsoulis-Dimitriou[1], Anne Dudeck[1]
[1]Institute for molecular and cellular immunologie, Immune regulation, Magdeburg, Germany

Macrophages (Mphs) exhibit high heterogeneity and plasticity, which is essential for their multifaceted roles in host defense and tissue regeneration. Mast cells (MCs) respond rapidly to injury or infection by releasing intact secretory granules with a plethora of mediators, thereby initiating and potentiating innate and adaptive immunity. Since, MCs and Mph reside in close proximity in the skin, we tracked the fate of MC granules in vivo and in vitro and decoded their impact on Mph phenotype and functions by. We found that intact MC granules are engulfed in vivo by skin Mph in murine models, and in vitro by bone marrow-derived Mph in vitro. ,. MC granule ingestion boosted Mph functional capacities and resulted in a plasticity representing both alternatively activated and classically activated Mph features suggesting increased efficiency in their multifaceted roles. . In addition, the engulfment of intact MC granules by Mph led to a specific transcriptome reprogramming. Importantly, both the process of MC granule ingestion and its functional impact could be confirmed for human MCs and Mphs in vitro and in situ [DA1] in healthy human skin explants and in psoriatic patient lesional skin. Collectively, ingestion of intact exocytosed MC granules by Mph and its boosting of Mph function may support the emerging view that MCs act beyond acute inflammation and are important regulators of both host defense and tissue regeneration by their intercellular communication properties.

SCL 8 - Innate immune cell cross-talk

SCL 8-1

Stromal-Immune Interactions Regulating Pulmonary Immunity

Clare Lloyd[1]
[1]NHLI, Imperial College, London, United Kingdom

Many lung diseases are characterized by airway inflammation in conjunction with pathological tissue remodeling. There is growing recognition of the importance of type2 inflammation in tissue healing, with archetypal type2 cells thought to be integral to the tissue repair process. We have determined that the local environment provides directional cues that dictate movement of cells within the lung, and this impacts the course and magnitude of local tissue inflammation. This presentation will focus on the cellular and molecular interactions that govern development of inflammation and remodeling in the lung.

SCL 8-2

Harnessing macrophage efferocytosis to promote tissue repair and remodeling

Lidia Bosurgi[1]
[1]Universitätsklinikum Hamburg-Eppendorf, Medizinische Klinik und Poliklinik, Hamburg, Germany

The clearance of apoptotic cells by phagocytes is crucial for restoring tissue homeostasis after damage. We recently described that the identity of the dying cells engulfed by macrophages significantly influences the macrophages' capacity to acquire a tissue-remodeling function. However, the mechanism by which the nature of dying cells shapes efferocytic macrophage function is still not fully understood.
In autoimmune liver diseases such as primary sclerosing cholangitis, cell death is triggered by the accumulation of toxic bile acids within parenchymal cells. Whether the content of the dying cells contributes to altered macrophage function—and in particular, whether bile acid-laden dying cells affect the efficiency of phagocytosis in restoring tissue balance—remains unexplored.
Our recently generated data demonstrate that apoptotic hepatocytes loaded with bile acids in vitro can act as "Trojan horses," delivering bile acids into efferocytic macrophages. Consequently, in a murine model of cholangitis, bile acids accumulate in a subpopulation of macrophages exhibiting pro-inflammatory features—contrasting with macrophages that engulf apoptotic parenchymal cells devoid of bile acids.
Overall, this delineates a system in which the contents of phagocytosed dying cells—specifically, bile acid accumulating in hepatocytes—induce a pro-inflammatory signature in efferocytic macrophages, likely contributing to chronic hepatic inflammation.

SCL 8 - Innate immune cell cross-talk

SCL 8-4

The dichotomous roles of S100A8 and S100A9 in experimental autoimmune encephalomyelitis in mice

Sandra Bachg[1], David Popp[1], Achmet Imam Chasan[1], Arjan Boltjes[2], Femke van Wijk[2], Frank Rühle[3], Stefanie Warnat-Herresthal[4], Wilco de Jager[2], Joachim Schulze[4], Melanie Eschborn[5], Luisa Klotz[5], Boris Skryabin[6], Thomas Vogl[1], Thomas Ulas[4], Monika Stoll[3], Gerd Meyer zu Hörste[5], Johannes Roth[1]

[1]University of Münster, Institute of Immunology, Münster, Germany
[2]Center for Translational Immunology, Utrecht, Netherlands
[3]University of Münster, Institute of Human Genetics, Münster, Germany
[4]German Centre for Neurodegenerative Diseases, Bonn, Germany
[5]University of Münster, Department of Neurology with Institute of Translational Neurology, Münster, Germany
[6]University of Münster, Transgenic Animal and genetic Engineering Models, Münster, Germany

S100A8/S100A9 complexes, released from myeloid cells during acute inflammation, activate Toll-like receptor 4 (TLR4) signaling. While their serum levels serve as biomarkers of inflammation, prolonged exposure induces hyporesponsiveness in monocytes, suggesting also a regulatory role. This study investigates these dual functions and the distinct contributions of S100A8 and S100A9 in experimental autoimmune encephalomyelitis (EAE) in mice.

Using conditional S100A8 knockout mice we demonstrate a pro-inflammatory role for S100A8. In contrast, S100A9 exhibits regulatory functions dependent on the stage of EAE. S100A9 transgenic mice are protected during the antigen presentation phase but develop severe disease in the effector phase of adoptive transfer EAE, highlighting a priming-specific regulatory effect of excess S100A9 on the immune response.

To assess S100 protein effects on antigen-presenting cells, we differentiated wild type HoxB8 monocytes into dendritic cells (DCs) in the presence of S100 proteins. Early S100 exposure during differentiation induces a tolerized DC phenotype with reduced MHCII and co-stimulatory molecules on CD11c+ DCs, impairing T-cell activation. In contrast, late S100 exposure promotes DC maturation and clonal T-cell expansion. Additionally, S100A9 transgenic DCs fail to mature upon LPS/TLR4 stimulation, reveal high levels of iNOS, IL-10 and TGFβ, and correspondingly low TNFα levels in cell culture supernatants, when compared to wild type DCs. We confirm that human S100-proteins show the same dichotomous effect on human DC differentiation.

In an in vivo study we now demonstrate that the administration of S100A8-tolerized DCs or S100A8 proteins in the preclinical phase of EAE prevents the development of severe disease progression.

These findings highlight the dual roles of S100A8/S100A9 in promoting acute inflammation while preventing chronic adaptive immune activation and suggest potential therapeutic strategies for treating autoimmune disorders.

SCL 9 - Innate immunity and cancer

SCL 9-1

PRECIOUS-01: A PLGA-Based Nanomedicine for Immunomodulation in cancer patients

Jolanda de Vries[1]
[1]Radboudumc, Department of Medical BioSciences, Nijmegen, The Netherlands

Induction of immune responses with dendritic cell (DC) vaccination has been effective in cancer patients. However, production of these patient-specific cells is a complex production process with interindividual variability. PRECIOUS-01 is a synthetic, off-the-shelf nanomedicine based on PLGA nanoparticles, designed to induce antigen-specific immune responses. It co-encapsulates NY-ESO-1 peptides and IMM60, a potent iNKT cell agonist, enabling direct in vivo activation of immune cells.
Preclinical studies show that PRECIOUS-01 not only elicits strong CD8 and CD4 T-cell responses but also enhances broader innate immune activation, including NK cells and iNKT cells. These findings suggest that PRECIOUS-01 can induce trained immunity, promoting a sustained immune response.
Initial clinical data from a phase I trial (NCT04751786) demonstrate that PRECIOUS-01 is safe, with no severe toxicity, and induces measurable immune responses in patients with solid tumors. This makes PRECIOUS-01 a promising candidate for further clinical development in cancer immunotherapy, with potential applications in infectious diseases and inflammatory disorders.

SCL 9-3

Myeloid cell-expressed endothelial protein C receptor (EPCR) regulates tumor immunity

Jennifer Pott[1], Sven Pagel[1], Petra Wilgenbus[1], Wolfram Ruf[1], Claudine Graf[1]
[1]Center for Thrombosis and Hemostasis (CTH), Mainz, Germany

Question: The coagulation system plays a crucial role in innate immunity but also drives immune evasion in malignancy. We recently demonstrated that macrophage-derived factor Xa as part of the ternary complex EPCR-TF-FVIIa-FXa cleaves protease activated receptor (PAR)2 thereby promoting tumor growth. However, whether the endothelial protein C receptor (EPCR) which is expressed by tumor-associated macrophages (TAMs) and dendritic cells (DCs) directly interferes with the generation of anti-tumor immune responses is unknown. We aimed to delineate the functional contributions of EPCR expressed by TAMs and DCs to tumor progression.
Methods: We evaluated tumor growth in the spontaneous breast cancer model PyMT and the transplantable tumor model T241 in immunocompetent mice with cell-type specific deletions of EPCR in TAMs (EPCRfloxLysMcre) or in DCs (EPCRfloxCD11ccre) with or without concomitant anti-PD-L1 treatment. We characterized macrophages and DCs in the tumor microenvironments (TME) and tumor-draining lymph nodes (dLN) by multicolor-flow cytometry and mRNA-expression profiling.
Results: EPCR deletion in TAMs phenocopied the attenuated tumor growth that we previously observed with FX deletion. In contrast, EPCR deletion in DCs enhanced tumor progression. These effects were not exacerbated or suppressed by addition of checkpoint blockade. Further, EPCR deletion in DCs led to lower abundance of CD103+ DCs, and decreased IL12 production. Consistently, in vitro-generated DCs from EPCR-recycling deficient mice also displayed impaired IL12 production and decreased uptake of antigens.
Conclusion: EPCR exhibits cell-type specific functions in tumor progression. EPCR+ TAMs mediate immunosuppression in the TME favoring tumor growth, whereas EPCR on DCs regulates IL12 production and antitumor immunity. These results highlight the complex interactions between coagulation proteins, immune cells, and the TME in cancer progression.

SCL 10 - Innate immunity in the brain

SCL 10-1

NLRP3 regulates microglial metabolism impacting cell function in models of alzheimer's disease

Roisin McManus[1]
[1]DZNE, Bonn, Germany

Question: We have shown that the NLRP3 inflammasome has a critical role in Alzheimer"s disease (AD). Here, we set out to uncover the pathways under the influence of NLRP3, beyond IL-1β, that might contribute to disease progression.

Methods: Single cell RNA sequencing was performed on microglia from aged wildtype, APP/PS1, NLRP3-/- and APP/PS1.NLRP3-/- mice. Microglia were assessed for real-time metabolic and phagocytic function. Targets were investigated in the post-mortem brain tissue of those with and without AD. Microglial cells and APP/PS1 mice were treated with novel NLRP3-targeted inhibitors to confirm findings.

Results: Single cell RNA sequencing analysis found a unique cluster of microglia with pathways related to phagocytosis and glutamate metabolic signaling in APP/PS1.NLRP3-/- mice, with a specific increase in the glutamate transporter Slc1a3. Metabolically, NLRP3-/- microglia had increased utilization of glutamine with elevated levels of the downstream metabolite a-ketoglutarate, which was associated with epigenetic changes. NLRP3-/- microglia had greater Aβ phagocytosis than the wildtype cells, which was strictly connected with cellular utilization of glutamate and glutamine. Importantly, we could replicate these findings in human cells and using NLRP3-specific inhibitors in vitro and in vivo (McManus et al., Immunity 2025).

Conclusions: We have identified a new mechanism where loss of NLRP3 influences glutamine/glutamate metabolism, which triggers epigenetic changes that in turn enhance phagocytic activity. This pathway is conserved between mouse and human cells. Critically, we can mimic this effect pharmacologically using NLRP3-specific inhibitors. Together, our data strengthens NLRP3 as an important target in the treatment of AD and dementia.

SCL 10-2

Brain Border Regions - Mount Strong Anti-Glioblastoma Immune Response but Fail to Overcome Immunosuppression

Roman Sankowski[1], Jonathan Cahueau[1], Adria Dalmau Gasull[1], Dieter Henrik Heiland[2], Marco Prinz1[1]
[1]Institute of Neuropathology, Medical Faculty, University of Freiburg, Freiburg, Germany
[2]Clinic for Neurosurgery, Faculty of Medicine, University of Freiburg, Freiburg, Germany

Introduction: Glioblastoma are the most common and most lethal primary brain tumor. It is characterized by a profound immunosuppression that has resisted clinical interventions. Unfortunately, the mechanisms behind these failures remain unclear. The brain contains two distinct
compartments: the parenchyma that contains only microglia and border regions (perivascular space, meninges, and choroid plexus) which host diverse immune cells.

Hypothesis: We hypothesized that leptomeninges would show reduced glioblastoma-associated immune suppression due to their separation from brain parenchyma.

Methods: We anatomically dissected the brain tissue and leptomeninges from histologically normal and glioblastoma samples. Samples were obtained from patients undergoing surgery and mouse models. Tissues were analyzed using single-cell RNA sequencing, protein profiling,
and spatial analyses, with interventional and mechanistic studies were conducted in GL261 glioblastoma mouse models.

Results: Human brain border regions contained diverse myeloid and lymphoid immune cells, confirming mouse model findings. In glioblastoma-associated border regions, immune composition shifted from tissue-resident to blood-derived cell phenotypes. Comparative analyses
of immune checkpoint inhibitor-treated tissues revealed enhanced myeloid-lymphoid crosstalk in border regions, accompanied by increased TGF-beta and IGF2-associated and regulatory lymphoid cell responses.

Conclusions: Glioblastoma-associated border regions showed more extensive immune cell interactions than tumor cores. However, this immune crosstalk failed to overcome tumor-induced immune suppression, explaining why immunotherapies remain ineffective against glioblastoma.

SCL 10 - Innate immunity in the brain

SCL 10-3

Innate immunity in the brain – focus on microglia

Rosa Chiara Paolicelli[1]

[1]University of Lausanne, Department of Biomedical Sciences, Lausanne, Switzerland

Once considered dormant brain cells, microglia are now recognized as key regulators of both physiological and pathological processes. These long-lived, yolk-sac-derived cells migrate into the neuroepithelium during early embryonic stages, where they proliferate, colonize the brain, and develop increasing morphological and functional complexity. As the innate immune cells of the CNS, microglia serve as resident macrophages — highly motile, phagocytic, and capable of releasing inflammatory mediators. They contribute to gliogenesis, neurogenesis, vasculogenesis, synaptic refinement, and myelination, playing a crucial role in brain development and homeostasis throughout life. Microglia express a diverse set of surface receptors, collectively termed the "sensome," which enable them to detect extracellular signals, neuronal activity, and microenvironmental changes. This allows them to integrate and respond to both physiological and pathological cues. Traditionally viewed as passive responders to injury or infection, microglia were long mischaracterized as "resting" cells that "activate" only upon pathological insults. However, advances in live imaging and genetic tools have overturned this notion, revealing that microglia are constantly active and, when dysfunctional, may contribute to -and even drive- disease onset and progression. Genome-wide Association Studies (GWAS) have identified numerous genetic variants linked to neurodegenerative disease risk, many of which are predominantly expressed in microglia. This suggests that microglial dysfunction can predispose individuals to neurodegeneration. Here, we will discuss how microglia shape brain function beyond immunity, and we will explore how microglial risk genes associated with neurodegeneration may influence brain maturation and contribute to disease later in life.

LT 1 - Lightning talks I

LT 1-1

Peroxisomes assemble the building blocks of inflammation

Francesca Di Cara[1], Yizhu Mu[1], Neal Silverman[2]
[1]Dalhousie University, Microbiology and Immunology, Halifax, Canada
[2]University of Massachusetts Chan Medical School, Worcester, MA, United States

Immune and inflammatory pathways must relay signals from upstream sensors to downstream effectors in an efficient and controlled manner. A highly-conserved mechanism of signal propagation used by many pathways involves the prion-like oligomerization of adapter proteins. Various immune receptors, such as TNFR, upon ligand recognition, recruit the downstream adaptor proteins to form large assemblies for a finely tuned immune or inflammatory response.
The importance of adaptor protein oligomerization for TNF pathway signaling has been demonstrated. However, regulators of the process are undefined.
We use the Drosophila melanogaster, a sophisticated genetic model with low genomic redundancy and conserved innate immune signaling to reveal the molecular determinants of prion-like oligomerization. One particularly well-characterized pathway in Drosophila, the Immunodeficiency pathway (IMD), is extensively conserved with the mammalian TNF pathway, and the prion-like oligomerization of the adaptor protein, IMD, mirrors that of its homology, RIPK1/3.
We found that IMD oligomerization relies on the presence of functional peroxisomes in macrophages. Peroxisomes are central organelles in lipid metabolism. Our group determined that peroxisomes are core immune metabolic organelles in Drosophila, mice, and humans as they regulate key innate and adaptive immune functions.
We performed lipidomics and transcriptomics of peroxisome-deficient macrophages under steady-state conditions and during microbial stimulus and determined that peroxisome lipid metabolism a) produces distinct lipids required for the assembly of the IMD in Drosophila and RIPK1/3 in humans; b) triggers chaperons proteins that regulate the assembly and activation of the signaling; c) modulates the prion-like oligomerization in other immune pathways such as NLRP3-ASC inflammasome.We proved the relevance of our finding in patients affected by inflammatory diseases.

LT 1-2

Signal peptides are essential players for innate immune cell responses to bacteria

Heiko Heilmann[1], Christoph Porten[2], Nadine Schmid[3], Christin Intzen[3], Isabell Theisohn[4], Lukas Busch[1], Fabian Panter[2], Christian Herr[4], Robert Bals[4], Daniel Krug[2], Sabryna Junker[1,5], Rolf Müller[2], Frank Zufall[3], Markus Bischoff[5], Bernd Bufe[1]
[1]University of Applied Sciences Kaiserslautern, Zweibrücken, Germany
[2]Helmholtz Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI), Saarland University, Department of Pharmacy, Saarbrücken, Germany
[3]Saarland University Medical Center, Center for Integrative Physiology and Molecular Medicine (CIPMM), Homburg, Germany
[4]Saarland University Medical Center, Department of Internal Medicine V – Pulmology, Allergology, Respiratory and Environmental Medicine, Homburg, Germany
[5]Saarland University Medical Center, Institute for Medical Microbiology and Hygiene (IMMH), Homburg, Germany

Formylated peptides in supernatants of bacterial cultures represent one of the oldest pathogen-associated molecular patterns (PAMPs) known in the field of innate immunity. Although it has been known for decades that these peptides induce a potent activation of innate immunity via formyl peptide receptors (FPRs), neither the precise biosynthetic origin of formylated peptides, nor their exact release mechanism, nor their biological significance in comparison to other bacterial PAMPs is well understood yet. N-terminal fragments of bacterial signaling peptides are required for the protein export via the secretory protein translocation machinery. We previously proposed, that they could be the origin of these peptides (Bufe et al. JBC 2015). Consistent with this hypothesis, we demonstrate here that the release mechanism of formylated peptides in E. coli is SecA-depended. Using HPLC, size exclusion chromatography and mass spectrometry, we could unambiguously identify and quantify several formylated peptides in E. coli and S. aureus supernatants that originate from bacterial signal peptide fragments. We found that many of these peptides robustly and sensitively activate FPRs even at low picomolar concentrations. Furthermore, we observed that signal peptides in bacterial supernatants trigger several important pro-inflammatory responses in different primary isolated immune cell types that could not be replaced by other PAMPs. Taken together, these results suggest an important role for bacterial signal peptides in mediating the host's defense against invading bacteria.

LT 2 - Lightning talks II

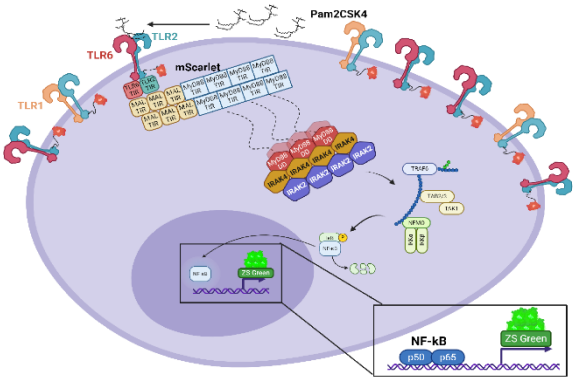
LT 2-1

Molecular analysis of Toll-like receptor 2 signalling

Prerna Mudai[1], Parimala Vajjhala[1], Sara Thygesen[1], Bostjan Kobe[2], Katryn Stacey[1]
[1]University of Queensland, Innate Immunity Lab, School of Chemistry and Molecular Biosciences, Brisbane, Australia
[2]University of Queensland, Structural Biology, School of Chemistry and Molecular Biosciences, Brisbane, Australia

QUESTION: Toll-like receptors (TLRs) detect pathogens and activate innate immunity. TLR2 interacts with TLR1 or TLR6 to form heterodimers and is activated by distinct bacterial lipoprotein subsets. This activates a signalling pathway involving adaptors MAL and MyD88, leading to inflammatory cytokine release. TLR2 signalling is associated with diseases such as septicaemia, Alzheimer's, etc. Crystal structures of TLR2 heterodimers have been elucidated but the dimerization of TIR domains and recruitment of signalling adaptors remains unclear.
METHODS: In prior work, we elucidated in-vitro filamentous structures of MAL, MyD88, and a MAL-TLR4 complex through cryo-EM and microcrystal electron diffraction. We integrated cell-based assays of TLR4 signalling with structural data to formulate a model of the TLR4 signalling complex. This project posits that the TLR4 signalling complex provides a template for TIR domain interactions of TLR2 with TLR1 or TLR6, as well as with downstream adaptors MAL and MyD88. We employed a dual-fluorophore system to tag both NF-kB-driven reporter gene and the TLR proteins in cell-based assays to investigate TLR2 and TLR6 mutants, with subsequent analysis performed via flow cytometry.
RESULTS: The mutation of TLR2 TIR surfaces interacting with TLR6 TIR diminishes, but does not abolish signalling, unlike modifications to TLR4 TIR which result in null signals. A divergent role of a TLR2 mutant in signalling across different heterodimers has been identified. Additionally, we have developed chimeric constructs of TLR2 and TLR6 to investigate TIR homodimer signalling and the TIR structural arrangement within the TLR2 signalling complex.
CONCLUSION: TLR2-TLR6 TIR interaction may not exhibit a defined polarity, contrasting with the TLR4 homodimer. It is improbable that TLR2 signals via a homodimeric configuration of TIRs. Investigating the molecular architecture of the TLR2 signalling complex and affirming its functional significance in cellular contexts is crucial for understanding the role of TLR2 signalling in pathological conditions.

Fig 1



hEKBlue-MD2-CD14- NFκB Zs Green reporter cell
A ZsGreen-tagged NFκB reporter was stably integrated in hEK cells lacking TLR2 but expressing MD2, CD14, TLR1 and TLR6 endogenously. TLR2 mutants tagged with mScarlet-I were transiently transfected in the cells to reconstitute signalling upon TLR2 ligand treatment.

Fig 2.

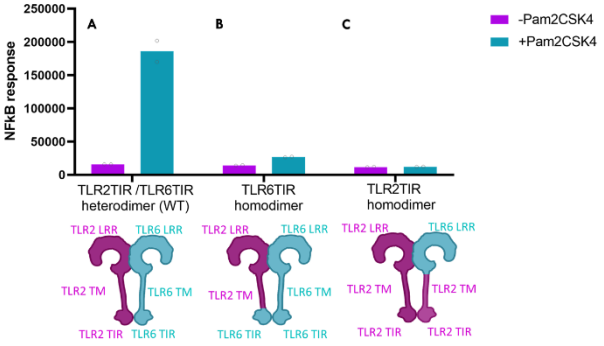


Figure 2: TLR2^{TIR} homodimers do not induce signalling, whereas TLR6^{TIR} homodimers have minimal signalling. WT and chimeric constructs were transfected into TLR1-6 double knockout cells and treated with TLR2/6 ligand Pam_2CSK_4.
A) TLR2^{TIR} /TLR6^{TIR} heterodimer (wild-type), **B)** TLR6^{TIR} homodimer and **C)** TLR2^{TIR} homodimer.

LT 2 - Lightning talks II

LT 2-2

A novel regulatory role of Toll signaling pathway on cellular immunity in Drosophila melanogaster

Electra Nenedaki[1], Ilias Kounatidis[2], Agostinho Carvalho[3], Alexandros Galaras[4], Pantelis Hatzis[4], Zoe Veneti[1], Georgios Chamilos[1]

[1]IMBB-FORTH, School of Medicine, UOC, Heraklion, Greece
[2]The Open University, School of Life Health and Chemical Sciences, Milton Keynes, United Kingdom
[3]Life and Health Sciences Research Institute (ICVS), , Braga, Portugal
[4]Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece

Drosophila melanogaster (Dm) is a model organism to study evolutionarily conserved aspects of innate immunity. Phagocytosis, an ancient host defense mechanism, protects humans against the major fungal pathogen Aspergillus fumigatus. Activation of pattern recognition receptors, including TLRs, triggers NOX-2-dependent production of ROS in the phagosome lumen to promote elimination of fungal spores inside macrophages via LC3 associated phagocytosis (LAP). LAP is a non-canonical form of autophagy that results in single membrane LC3 lipidation upon integration of WD40 domain of Atg16L1 protein on the phagosome membrane. The role of cellular immunity and LAP in Dm antifungal host defense is uncharacterized. Herein, we found that Dm hemocytes rapidly phagocytosed the vast majority of A. fumigatus spores within minutes of systemic infection, followed by the delayed (≈ 48h) activation of humoral immunity. Importantly, genetic or functional ablation of hemocytes resulted in heightened susceptibility of Dm to A. fumigatus infection, demonstrating a non-redundant role of cellular immunity in antifungal defense. Studies in Atg16L1 WD40 Dm mutants and transgenic lines with conditional inactivation of NOX and other components of LAP in hemocytes revealed a crucial role of LAP in cellular immunity against A. fumigatus. Furthermore, we identified that immunometabolic rewiring towards glycolysis during A. fumigatus infection in hemocytes regulates NOX-dependent activation of LAP via the HIF1α/LDH signaling. Strikingly, we discovered that this immunometabolic mechanism of NOX-dependent activation of LAP is under the control of Toll signaling in fat body. Collectively, our findings demonstrate a novel mechanism of immunometabolic cross-talk of humoral and cellular immunity orchestrated by Toll signaling with a fundamental role in antifungal host defense.

LT 3 - Lightning talks III

LT 3-1

A tissue-intrinsic mechanism sensitizes HIV-1 particles for TLR-triggered innate immune responses

Samy Sid Ahmed[1], Liv Zimmerman[2], Andrea Imle[1], Katrin Wuebben[3], Nadine Tibroni[1], Lena Rauch-Wirth[4], Jan Münch[4], Petr Chlanda[2], Frederik Graw[3], Oliver T. Fackler[1]

[1]Heidelberg University, Department of Infectious Diseases, Integrative Virology, CIID, Heidelberg, Germany
[2]Heidelberg University, Schaller Research Groups, Department of Infectious Diseases, Virology, Heidelberg, Germany
[3]Heidelberg University, BioQuant-Center for Quantitative Biology, Heidelberg, Germany
[9]Ulm University Medical Center, Institute of Molecular Virology, Ulm, Germany

Untreated infection with HIV-1 is characterized by a progressive depletion of CD4+ T cells accompanied by chronic inflammation. While many molecular mechanisms of HIV-target cell interactions are well characterized, important aspects of the complex immune pathology of HIV-1 infection in vivo remain unknown. This knowledge gap includes mechanism and relevance of lymph node fibrosis caused by abnormal deposition of extracellular matrix components (mainly collagen).
We previously identified that tissue-like 3D collagen matrices exert a potent environmental restriction to the infectivity of cell-free HIV-1 particles (ERVI) (Imle et al., 2019, Nat Commun). Here we report that ERVI is implemented by different adhesive tissue-like 3D matrices within minutes, is saturable, and reduces the infectivity of a wide range of primary HIV-1 strains and virions bearing distinct viral glycoproteins by preventing fusion with target cells. Importantly, the uptake of particles subjected to ERVI triggers pronounced pro-inflammatory cytokine secretion by monocyte-derived macrophages. Mechanistic analyses reveal that this increased innate immune recognition is driven by conformational changes in the viral glycoprotein Env resulting from transient contact with collagen fibers. Env proteins subjected to ERVI are recognized by toll-like receptor (TLR) 2, which drives routing of viral particles into TLR8-positive endosomes for increased sensing of viral RNA genomes. These findings reveal that ERVI employs a two-pronged mechanism that affects virus spread directly by reducing the fusogenicity of cell-free virions and sensitizes them for innate immune recognition. The biophysical properties of the extracellular matrix in tissue thus represent a broadly acting barrier that impairs virus spread and promotes inflammation. This tissue-intrinsic mechanism may act as a previously unrecognized arm of antiviral innate immunity.

LT 3-2

Chronic ER stress in myotonic dystrophy type 2 promotes autoimmunity via mitochondrial DNA release

Sarah Rösing[1], Fabian Ullrich[2], Susann Meisterfeld[1], Franziska Schmidt[1], Laura Mlitzko[1], Marijana Croon[3], Ryan G Nattrass[4], Nadia Günther[1], Julia Mahlberg[2], Martin Schlee[2], Anja Wieland[2], Philipp Simon[2], Daniel Hilbig[5], Ulrike Reuner[6], Alexander Rapp[7], Julia Bremser[4], Peter Mirtschink[8], Stephan Drukewitz[9], Thomas Zillinger[2], Stefan Beissert[1], Katrin Paschke[2], Gunther Hartmann[2], Aleksandra Trifunovic[3], Eva Bartok[2], Claudia Günther[1]

[1]University Hospital Carl Gustav Carus, TU Dresden, Dermatology, Dresden, Germany
[2]University Hospital Bonn, Clinical Chemistry and Clinical Pharmacology, Bonn, Germany
[3]CECAD Research Center, Mitochondrial Diseases and Aging, Faculty of Medicine, Cologne, Germany
[4]University Hospital Bonn, Experimental Haematology and Transfusion Medicine, Bonn, Germany
[5]University Hospital Bonn, Oncology, Haematology, Rheumatology and Immune-Oncology, Bonn, Germany
[6]University Hospital Carl Gustav Carus, TU Dresden, Neurology, Dresden, Germany
[7]Technical University of Darmstadt, Biology, Cell biology and Epigenetic, Darmstadt, Germany
[8]Faculty of Medicine, TU Dresden, Clinical Chemistry and Laboratory Medicine, Dresden, Germany
[9]University of Leipzig Medical Center, Human Genetics, Leipzig, Germany

Myotonic dystrophy type 2 (DM2) is a genetic disorder characterized by expansive tetranucleotide CCTG repeats in the CNBP gene, manifesting as myopathy and an elevated prevalence of autoimmune conditions. Intriguingly, DM2 patients exhibit a pronounced type-I interferon (IFN) signature in both blood samples and cultured fibroblasts, accompanied by a notable accumulation of repeat RNA in the cytosol of patient fibroblasts. Contrary to initial expectations, this cytosolic RNA fails to activate innate immune RNA sensors. Instead, the cytosolic RNA-Repeats are translated by a process called repeat-associated non-AUG (RAN) translation, resulting in the accumulation of the aberrant protein LPAC within DM2 fibroblasts. This accumulation triggers chronic endoplasmic reticulum (ER) stress, evidenced by dysregulation of key stress response proteins: pancreatic ER kinase (PKR)-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activation of the activating transcription factor 6 (ATF6) pathway. The chronic ER stress induces mild mitochondrial stress, prompting the release of mitochondrial DNA (mtDNA) into the cytoplasm of affected cells. This extracellular mtDNA serves as a potent activator of the cytosolic DNA sensor cGAS, thereby initiating the cGAS-STING signaling pathway. The activation of this pathway culminates in heightened IFN production, establishing a predisposition to autoimmune disorders in DM2 patients. Altogether, these findings demonstrate a novel mechanism by which large repeat expansions induce a type-I IFN response predisposing DM2 patients to autoimmunity.

LT 4 - Lightning talks IV

LT 4-1

NINJ1-mediated plasma membrane rupture during cell death is a two-step process requiring cell swelling

Elliott Bernard[1], Ella Hartenian[1], Giulia Ammirati[2], Hubert Leloup[3], Magalie Agustoni[1], Lukas Bissegger[4], José Santos[1], Ivo Glück[5], Stefania Mari[2], Christian Sieben[5], Virginie Petrilli[3], Sylvain Monnier[6], Daniel Müller[2], Petr Broz[1]

[1]University of Lausanne, Immunobiology, Epalinges, Switzerland
[2]ETH Zurich, Biosystems Science and Engineering, Basel, Switzerland
[3]University of Lyon, Centre for Cancer Research, Lyon, France
[4]University of Basel, Biomedicine, Basel, Switzerland
[5]Braunschweig Technical Unviersity, Helmholtz Centre for Infection Research, Braunschweig, Germany
[6]University of Lyon, Insitute of Light and Matter, Lyon, France

The induction of an inflammatory response during infection and tissue injury can be mediated by the release of damage-associated molecular patterns (DAMPs) from dying cells. To release large DAMPs, necrotic cells must rupture their plasma membrane, which is achieved through the oligomerization of the protein NINJ1 into amphipathic filaments. It is not known how cell death triggers NINJ1 oligomerisation nor how NINJ1 filaments rupture the plasma membrane.
In this work, we demonstrate that NINJ1 oligomerisation and plasma membrane rupture (PMR) are two experimentally separable events. We first show that during cell death increases in cytosolic Ca2+ concentration and cell swelling coincide with NINJ1 oligomerisation and PMR. We next find that Ca2+ influx can trigger NINJ1 oligomerisation but is not required for NINJ1 activation during ferroptosis. On the other hand, blocking cell swelling during ferroptosis inhibited PMR while still allowing NINJ1 oligomerisation, demonstrating that cell swelling is required to endow NINJ1 filaments with lytic properties. Using atomic force microscopy, we find that there is no increase in intracellular pressure during cell swelling, suggesting this is not the cause of lesion formation. Rather, we speculate that cell swelling increases tension in the plasma membrane leading to lesion formation by NINJ1 filaments.
We go on to show that the close homologue NINJ2 oligomerises during cell death but fails to form lesions during cell swelling. Co-expression of NINJ1 and NINJ2 reduced PMR, implicating NINJ2 as a negative regulator of NINJ1. NINJ1-NINJ2 chimeras identified an amino acid in helix α1 that differs between NINJ1 and NINJ2, mutation of which endows NINJ2 with NINJ1-like membrane rupturing properties.
Overall, we propose a 2-step model for NINJ1-mediated PMR with signal 1 causing NINJ1 oligomerisation and signal 2, cell swelling, functionalising these NINJ1 filaments to form lesions.

LT 4-2

Identification and characterization of potent and selective allosteric caspase-4/5 inhibitors

Elad Elkayam[1], Patrick Cyr[1], Marie-Anne Germain[1], Samuel Gaudreault[1], Vasiliy Sysoev[1], Francois Gervais[1], Kelly Pike[1], Michael Crackower[1]

[1]Ventus Therapeutics, Waltham, MA, United States

The biological role of the non-canonical inflammasome lacks sufficient understanding. This is owing to the absence of specific tool molecules and the absence of caspase-4 in rodents. Furthermore, development of caspase inhibitors for therapeutic use has proven elusive given the very high fractional inhibition required for cellular and in vivo efficacy. Here we report the discovery of novel, potent and selective allosteric inhibitors of the inflammatory caspases 4 and 5. These compounds are specific, non-active site binders to caspase-4/5. They block cleavage of natural substrates gasdermin D (GSDMD) and IL-18, potently and selectively inhibit non-canonical inflammasome activation, including IL-18, IL-1β and pyroptosis in endothelial cells, gut epithelial cells and numerous other immune and non-immune cell types. These compounds have both biochemical and cellular potencies in the single digit nanomolar and do not display the large potency shift seen with active site caspase inhibitors. Using mice expressing human caspase-4, we show that, in response to LPS, these compounds can significantly inhibit IL-18 and IL-1β release as well as pyroptosis. In addition, caspase-4 inhibition results in significant reduction of overall inflammation, and attenuation of vascular permeability. This first-ever discovery of an allosteric caspase inhibitor suggests an important therapeutic role for caspase-4/5 inhibition in numerous diseases of high unmet need including sepsis, inflammatory bowel disease (IBD) and hidradenitis suppurativa (HS).

LT 5 - Lightning talks V

LT 5-1

Exploring The Neutrophil and Platelet Dynamics in Psoriasis Skin Autoinflammation.

Baher Zalat[1], Miriam Rengel[2], Martina Giampetraglia[2], Tabea Bieler[3], Annika Lehmann[4], Susanne Karbach[4], Madhumita Chatterjee[5], Katharina Kommoss[3], Mathias Heikenwälder[4], Bettina Weigelin[2], Alexander N.R. Weber[1]

[1]University of Tübingen, Institute of Immunology, Department of Innate Immunity, Tübingen, Germany
[2]Eberhard Karls University Tübingen, Werner Siemens Imaging Center, Department of Preclinical Imaging and Radiopharmacy, Tübingen, Germany
[3]German Cancer Research Center (DKFZ), Division of Chronic Inflammation and Cancer, Heidelberg, Germany
[4]University Medical Center Mainz, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany
[5]University Hospital Tübingen, Institute for Clinical and Experimental Transfusion Medicine, Tübingen, Germany

Psoriasis is a chronic immunological disorder characterized by skin lesions, a complex inflammatory response, and often cardiovascular co-morbidities. Whilst significant infiltration of the skin by polymorphonuclear neutrophils (PMNs) is a well-known and dominant feature, the mechanisms by which neutrophils home to the skin and how their activity is modulated are not fully understood. In previous work we observed that in the imiquimod (IMQ)-induced mouse model of psoriasis and in human psoriasis samples, platelets were found in close proximity to PMNs in the skin, a phenomenon not observed in healthy skin. Moreover, circulating platelet-neutrophil-complexes (PNC) were increased. Interestingly, in vivo depletion of platelets significantly prevented skin inflammation, PMN skin homing and neutrophil extracellular trap (NET) release. This indicated platelets play a critical role in psoriatic skin inflammation. However, their precise role in infiltration and the activity of neutrophils in the skin are not well understood. To address these questions in a physiologically relevant setting, we established multiphoton intravital microscopy (MPM) and light-sheet microscopy (LSM) to respectively capture both, dynamic and spatial parameters of cell-cell interactions in the mouse ear skin following IMQ treatment or the genetic induction of psoriasis-like skin disease. We can observe joint extravasation events probably related to aggregation of PMN and platelets in specific inflammatory hot-spots. Moreover, conventional high-resolution confocal imaging of tissue sections was used for additional validation. Collectively, this integrated approach of multiple imaging modalities advances our understanding of PMN and platelet functions in psoriasis and may be relevant for other diseases driven by cellular interactions.

LT 5-2

Structural insights on higher-order assembly of the TLR4 signalosome complex

Hyoyoung Kim[1], Timothy Muusse[1], Jeffrey Nanson[1], Thanh-Binh Nguyen[1], Oliver Hughes[1], Weixi Gu[1], Parimala Vajjhala[1], Bostjan Kobe[1], Katryn Stacey[1]

[1]University of Queensland, School of Chemistry and Molecular Biosciences, St Lucia, Australia

Toll-like receptors (TLRs) play a critical role in driving innate immune inflammatory pathways in response to the invasion of microbial pathogens and intrinsic danger signals. Over decades, the ectodomains of TLRs and their ligands have been characterised, yet how their signalling TIR domains associate to form the signalosome complex in the cytosol remains elusive. We have solved the co-filament structure of TLR4 TIR domain with its adaptor, MAL in vitro and validated the structure in the cellular environment through robust mutagenesis in interfacing residues and a flow cytometric functional assay. The combination of a GFP-conjugated target protein and a NF-κB-driven Scarlet reporter gene allows us to analyse only those cells expressing the protein at an appropriate level, excluding irresponsive cells to stimuli due to insufficient expression or spontaneously active over-expression. Our studies demonstrated that TLR4 TIR domain is dimerised exclusively in an asymmetric head-to-tail manner and recruits its adaptor MAL through the lateral interfaces. MAL then recruits MyD88 adaptor, and MyD88 TIR assembles into a two stranded filament involving a major change to the conformation of the BB loop region. We examined the effect of disease-associated variants of MyD88 found in immunodeficiency and B cell lymphoma on signalosome assembly through a flow cytometric functional assay, confocal microscopy and molecular dynamics simulation. Lymphoma-associated mutants are spontaneously active at various levels and molecular dynamics predicts altered mobility of the loops involved in assembly formation. Activating mutants lead to greater stability of the active form of the BB-loop. An understanding of the structure of TLR signalling complexes is critical to targeting inflammatory disorders and may aid development of new approaches for lymphoma treatment.

LT 6 - Lightning talks VI

LT 6-1

Flagellin in the human gut microbiome is a diet-adjustable adjuvant for vaccination

Kelsey Huus[1], µHEAT Clinical Study Group[2], Yi Han Tan[1], Hirohito Abo[3], Ronald Keller[1], Ezgi Atay[1], Silke Dauser[1], Dai Long Vu[1], Dennis Jakob[1], Alina Prokipchuk[1], Alexander Tyakht[1], Nicholas Youngblut[1], Benoit Chassaing[4], Sang-Moo Kang[3], Julie Parsonnet[5], Peter Kremsner[2], Lisa Maier[6], Andrew Gewirtz[3], Meral Esen[2], Ruth Ley[1]

[1]Max Planck Institute for Biology, Microbiome Science, Tübingen, Germany
[2]University of Tübingen, Institute for Tropical Medicine, Tübingen, Germany
[3]Georgia State University, Institute for Biomedical Sciences, Atlanta, GA, United States
[4]Institut Pasteur, Microbiome-Host Interactions, Paris, France
[5]Stanford University, Department of Medicine, Stanford, CA, United States
[6]University of Tübingen, Interfaculty Institute of Microbiology and Infection Medicine, Tübingen, Germany

The intestinal microbiota is thought to modulate immune responsiveness to vaccines. Human studies on this topic, however, have yielded inconsistent results. We hypothesized that the microbiome would influence innate immune responses, and thus vaccine reactogenicity, more directly than vaccine immunogenicity. To test this, we established the µHEAT (Microbial-Human Ecology And Temperature) study, which longitudinally profiled the fecal microbiota, oral body temperature and serum antibody responses of 171 healthy adults (18-40 years old) before and after vaccination for SARS-CoV-2. Increased temperature after vaccination (ΔT) was associated with habitual diet and with baseline metabolic and immune markers. The microbiomes of ΔT-high (ΔThi) participants were characterized by high expression of flagellin and an overabundance of the flagellated bacterium Waltera. Fecal samples from ΔThi participants induced more inflammation in human cells via toll-like receptor 5 (TLR5) and the NLRC4 inflammasome, and induced stronger post-vaccine temperature responses in mice compared to ΔTlo samples, suggesting a causal role for the microbiome. Moreover, Waltera flagellin replicated the inflammatory phenotypes in vitro and was modulable via a dietary additive. Overall, these data suggest that flagellin from the gut microbiome stimulates innate immunity and vaccine reactogenicity, and that this axis can be manipulated via diet. These findings have implications for improving human vaccine tolerance and immunogenicity.

LT 6-2

LukAB promotes Staphylococcus aureus escape from macrophages by triggering an unknown cell death pathway.

Calla Jickeli[1], Lukas Funk[1], Xiao Liu[1], Janina Bayer[2], Christiane Wolz[2], Alexander N.R. Weber[1]

[1]University Tübingen, Institute of Immunology, Department of Innate Immunity, Tübingen, Germany
[2]University Tübingen, Interfaculty Institute for Microbiology and Infectious Medicine, Tübingen, Germany

Staphylococcus aureus is a facultative pathogen that causes diverse diseases worldwide, some life-threatening. To evade host immunity, S. aureus expresses virulence factors including the pore-forming Leukocidin A/B (LukAB). Secreted LukAB is cytotoxic for various immune cells, acts via cell surface integrins, CD11b/CD18, and a hydrogen voltage channel, HVCN1, and potently triggers NLRP3 inflammasome activation, IL-1β secretion and pyroptosis. Although LukAB is critical for S. aureus killing of infected phagocytes and consequent bacterial exit, the receptors, signaling and cell death pathways activated by LukAB expression during infection, i.e. "from the inside", are unknown. Using genetically modified bacteria and host cells we found that CD11b, CD18 or HVCN1 KO cells were protected against LukAB-expressing S. aureus, implicating these factors also in intracellular LukAB killing. Infection also triggered NLRP3 activation and IL-1β release, albeit independently of LukAB or gasdermin D, and cell death was hence non-pyroptotic. Apoptosis was also ruled out but RIPK3 and MLKL phosphorylation were observed 3 h after infection and MLKL KO THP-1 cells were partially resistant to S. aureus cytotoxicity. However, both effects were also observed in LukAB-negative bacteria. Thus, LukAB-mediated cell death is also independent of conventional necroptosis. Interestingly, the necroptosis inhibitor, nec-1, and extracellular K+ blocked MLKL phosphorylation and IL-1β secretion, respectively, suggesting that K+ efflux via MLKL pores, despite not mediating LukAB-related cell death, activates the inflammasome. Collectively, S. aureus skillfully avoids conventional cell death pathways to enact the demise of the phagocyte via LukAB and a non-conventional novel cell death and exit pathway.

LT 7 - Lightning talks VII

LT 7-1

Inflammatory citrullinated neutrophil extracellular traps accumulate in skin and blood of hidradenitis suppurativa patients and are therapeutic targets for anti-citrullinated histone antibody CIT-013

Josephine Stein[1], Stephanie van Dalen[1], Maarten van der Linden[1], Kelsy Waaijenberg[1], Eline Zwiers[1], Annemarie Kip[1], Sangeeta Kumaari[1], Martyn Foster[2], Jacek Szepietowski[3], Piotr Krajewski[4], Kelsy van Straalen[5], Errol Prens[5], John Ingram[6], Renato Chirivi[1], Eric Meldrum[1]

[1]Citryll, Bio-analytics, Oss, Netherlands
[2]Experimental Pathology Consultancy, Benfleet, United Kingdom
[3]Wroclaw University of Science and Technology, Faculty of Medicine, Wroclaw, Poland
[4]Wroclaw Medical University, Department of Dermatology, Wroclaw, Poland
[5]Erasmus MC, Rotterdam, Netherlands
[6]Cardiff University, Division of Infection and Immunity, Cardiff, United Kingdom

Introduction: Hidradenitis suppurativa (HS) is an inflammatory skin disease characterized by neutrophil infiltration in nodules, abscesses and tunnels. Upon specific activation, neutrophils release extracellular traps (NETs) which are the cell"s citrullinated chromatin decorated with granule contents. NETs are DAMPs that associate with a wide range of disease pathologies. The therapeutic antibody CIT-013, which targets citrullinated histone H2A and H4, has shown therapeutic efficacy in pre-clinical models of NET-associated inflammation. Given the prominent role of neutrophils in HS, the aim of this study was to assess the distribution of NETs in tissue and their presence in serum in HS thereby validating HS as a therapeutic indication for CIT-013.

Method: NETs were detected in skin and serum of HS patients by quantifying citrullinated histone H3 using immunohistochemistry and ELISA respectively. Confocal microscopy was used to assess CIT-013"s mechanisms of action. CIT-013"s target engagement was examined in healthy subjects dosed with 2 ng/kg LPS to induce low-grade inflammation by monitoring NET levels.

Results: Compared to unaffected skin, HS patient lesional and perilesional skin showed increased presence of NETs. Moreover, elevated NET levels were detected in serum of HS patients and correlated with disease activity. Targeting NETs with CIT-013 resulted in potent inhibition of NETosis in a concentration dependent manner and enhanced Fc-mediated phagocytosis of NETs by human macrophages. To confirm CIT-013's target engagement in humans, LPS nano-dosing in healthy subjects induced a significant level of circulating NETs which was completely inhibited by IV administration of 0.3mg/kg CIT-013.

Conclusion: These data demonstrate that NETs are present in HS serum and tissue, reinforcing the potential of CIT-013 with its dual mechanism of action as a promising novel therapeutic for HS. A phase 2a clinical study in HS patients, CITY LIGHTS, will begin in 2025.

LT 7 - Lightning talks VII

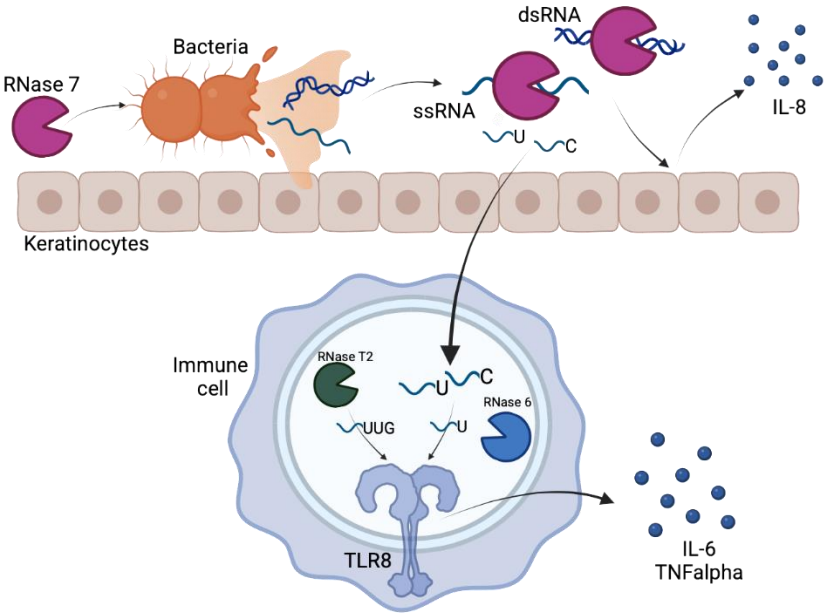
LT 7-2

Keratinocyte-derived RNase 7 is involved in RNA-mediated skin innate immune responses

Luisa Breitenbach[1], Amélie-Electra Ehnle[1], Alexander N.R. Weber[2], Alexander Dalpke[1], Lan-Sun Chen[1]
[1]Uniklinikum Heidelberg, Medical Microbiology and Hygiene, Heidelberg, Germany
[2]Tübingen University, Institute of Immunology, Tübingen, Germany

During infections, RNA serves as an important activator of innate immune responses. Particularly, bacterial RNA (bRNA) can stimulate Toll-like receptor 8 (TLR8), a pattern recognition receptor (PRR) for single-stranded RNA. Recognition requires processing of RNA by endosomal RNases, such as RNase T2 and RNase A family members (including RNase 6 and RNase 2). These enzymes generate short RNA fragments suitable for TLR8 binding and activation. In the skin, the RNase A family member RNase 7 is secreted by epidermal keratinocytes, where it functions as an antimicrobial peptide and facilitates DNA sensing, thereby contributing to skin immunity. However, the overall role of RNase 7 in RNA-mediated PRR activation and skin immunity remains unclear. This study aims to investigate the role of keratinocyte-derived RNase 7 in RNA recognition of skin-resident immune cells.
For this, we generated RNASE7-/- HaCaT keratinocytes which showed a decreased cytokine response to stimulation with both bRNA and PolyI:C. In Wild Type cells, RNase 7 was also upregulated upon stimulation. Ex-cellulo digestions confirmed that RNase 7 is able to efficiently break down both bRNA of different species, and PolyI:C. Of note, bRNA from some skin commensals was more resistant to digestion. Using short synthetic oligoribonucleotides of known sequences, we determined the preferential cleavage sites of different RNases and found that both RNase 7 and 6 can cleave after uridines, thereby creating fragments that might bind to the TLR8 apex pocket. Consistently, pre-digesting bRNA by RNase 7 could partially compensate for the loss of RNase 6 when transfected into BLaER1 RNASE6-/- monocytes. Interestingly, this pre-digestion also led to an increased cytokine response to bRNA in Wild Type BLaER1 cells.
Overall, these results suggest that the ribonuclease activity of RNase 7 is involved in RNA-mediated PRR activation, which warrants further investigation in the context of skin immunity.

Fig 1.



LT 8 - Lightning talks VIII

LT 8-1

Increased platelet-macrophage interactions and altered platelet and megakaryocyte phenotypes in a mouse model of liver fibrosis

Carsten Deppermann[1], Martina Casari[1], Friedrich Reusswig[1], Bettina Mros[1], Olga An[1], Dominik Siegl[2], Detlef Schuppan[2]
[1]University Medical Center of the Johannes Gutenberg University, Center for Thrombosis and Hemostasis, Mainz, Germany
[2]University Medical Center of the Johannes Gutenberg University, Institute of Translational Immunology, Mainz, Germany

Question: Liver fibrosis is characterized by the accumulation of extracellular matrix proteins, including collagen, that occurs during chronic liver disease and can compromise liver functionality and result in hepatocellular carcinoma (HCC). Liver macrophages maintain liver homeostasis and can promote progression of liver diseases. Besides their role in hemostasis, platelets and megakaryocytes are increasingly being recognized as important players in immune responses. However, their functional role in liver fibrosis and HCC remains incompletely understood.
Methods: Mdr2-/- mice which develop a cholestatic liver disease resembling human primary sclerosing cholangitis were studied. Bone marrow, spleen and liver tissue were analyzed by immunofluorescence staining and flow cytometry to characterize immune cells, platelets and megakaryocytes. Platelet activation, receptor expression, phosphatidylserine exposure and platelet-leukocyte aggregate (PLA) formation were measured by flow cytometry using whole blood from Mdr2-/- mice.
Results: Infiltration of monocyte-derived macrophages (MDMs) and other immune cells was observed in Mdr2-/- livers. Intrahepatic platelet accumulation and enhanced colocalization between platelets and MDMs and Kupffer cells was found. Analysis of blood from Mdr2-/- mice revealed increased platelet count and size, number of young platelets and reduced expression of the platelet collagen receptor GPVI. Moreover, Mdr2-/- platelets showed increased activation, phosphatidylserine exposure and PLA formation upon stimulation with collagen-related peptide. Analysis of Mdr2-/- spleen and bone marrow exhibited increased number and ploidy of megakaryocytes.
Conclusions: Taken together, we observed increased immune cell infiltration and platelet-macrophage interactions in the liver as well as altered platelet functionality and megakaryocyte phenotype in Mdr2-/- mice.

LT 8-2

Methylmalonate accumulation suppresses macrophage interferon responses

Alexander Hooftman[1], Matteo Lunghi[1], Luc Reymond[2], Olga Rosspopoff[1], Florian Traversi[3], Alexander Keller[1], Pauline Melenec[1], Ming Yang[4], Christian Frezza[4], D Sean Froese[3], Didier Trono[1], Andrea Ablasser[1]
[1]Swiss Federal Institute of Technology Lausanne (EPFL), Life Sciences (SV), Lausanne, Switzerland
[2]Swiss Federal Institute of Technology Lausanne (EPFL), Biomolecular Screening Facility, Lausanne, Switzerland
[3]University of Zurich, Division of Metabolism and Children's Research Center, Zurich, Switzerland
[4]University of Cologne, CECAD Research Centre, Cologne, Germany

Changes in the abundance of specific mitochondrial metabolites can influence immune cell behaviour during an infection or immune response. Here, we describe the accumulation of a novel immunometabolite, methylmalonate (MMA), during macrophage activation and provide a comprehensive first report of its role in regulating cytokine responses. We show that MMA is the most highly upregulated metabolite in cGAS-STING-activated macrophages, derived from branched-chain amino acid (BCAA) degradation. In order to manipulate MMA levels, we have designed potent cell-permeable derivatives which deliver MMA intracellularly. This approach, combined with the use of unmodified methylmalonic acid and genetically targeting BCAA degradation enzymes, has revealed that methylmalonate suppresses the type I interferon (IFN) response in murine and human macrophages. Mechanistically, MMA does not affect signalling events immediately downstream of pattern recognition receptor (PRR) ligation, but rather suppresses transcription of Ifnb1 and interferon-stimulated genes (ISGs). We found this transcriptional suppression to be linked to reduced acetylation of H3K27, an activating epigenetic mark which supports macrophage activation. MMA also suppresses type I IFN responses in human monocytes and macrophages, including those isolated from patients suffering from systemic lupus erythematosus (SLE), an inflammatory disease characterised by dysregulated type I IFN release. These results are of relevance to the human disease methylmalonic aciduria, an inborn error of metabolism which is characterised by MMA accumulation. Transcriptomics performed on fibroblasts isolated from methylmalonic aciduria patients exhibited similar defects in the type I IFN response. In summary, we present evidence supporting the role of MMA as a novel immunometabolite which orchestrates macrophage function and raises the potential of harnessing this metabolite in the treatment of inflammatory diseases.

LT 9 - Lightning talks IX

LT 9-1

Overcoming chemotherapy-induced immunosuppression in TNBC – TLR3/4 activation reprograms macrophages and enhances pirarubicin efficacy

Jian-Hong Shi[1], Ruobing Zhang[1], Nai-Peng Cui[2], Jing-Hua Li[1], Yanqiu He[3]
[1]Affiliated Hospital of Hebei University, Central Laboratory, Baoding, China
[2]Affiliated Hospital of Hebei University, Breast Surgery, Baoding, China
[3]Affiliated Hospital of Hebei University, Baoding, China

Triple-negative breast cancer (TNBC) lacks effective therapeutic targets and is prone to relapse, necessitating novel strategies to enhance treatment efficacy. Tumor-associated macrophages (TAMs), key immune components in the tumor microenvironment (TME), exhibit dynamic polarization states influenced by Toll-like receptor (TLR) signaling. This study investigates the therapeutic potential of combining the anthracycline chemotherapeutic agent pirarubicin (THP) with TLR3/TLR4 agonists to modulate innate immunity and improve anti-tumor outcomes.
Clinical analysis revealed reduced TLR3 and TLR4 expression in breast tumor tissues compared to adjacent normal tissues, correlating with poorer patient prognosis. In vitro co-culture models mimicking TME interactions demonstrated that cross-talk between breast cancer cells (MDA-MB-231, E0771) and macrophages (THP-1, iBMDM) suppressed TLR3/4 expression. THP further exacerbated this suppression, impairing innate immune signaling. However, combining THP with TLR3 agonist Poly IC or TLR4 agonists LPS/MPLA reversed TLR3/4 downregulation, restored macrophage activation via TBK1/NF-κB pathways, and enhanced pro-inflammatory cytokine production (IFN-α/β, IL-6, TNFα). This combination synergistically inhibited cancer cell proliferation and migration ($p<0.001$) compared to monotherapy.
In murine TNBC models, THP combined with Poly IC, LPS, or MPLA significantly reduced tumor volume and weight ($p<0.01$) while upregulating intratumoral TLR3/4 expression ($p<0.05$). Immunohistochemistry confirmed enhanced TLR3/4 levels in treated tumors, suggesting restored innate immune activation.
These findings highlight the dual role of THP-TLR agonist combinations in directly targeting cancer cells and reprogramming TAMs toward anti-tumor M1 phenotypes via innate immune pathways. This strategy offers a promising approach to overcome chemotherapy-induced immunosuppression and improve TNBC prognosis by leveraging TLR-mediated innate immunity.

LT 9-2

Cell cycle dynamics modulate inflammatory responses

Pujan Engels[1], Julius Lingnau[1], Dorothee Lapp[1], Florian N. Gohr[1], Kim Hebbel[2], Alexander N.R. Weber[2], Florian I. Schmidt[1]
[1]Insitute of Innate Immunity, Bonn, Germany
[2]Department of Innate Immunity, Tübingen, Germany

Inflammasomes are crucial mediators of innate immune responses, detecting cellular stress and initiating inflammation. Although NLRP1 and NLRP3 inflammasomes have been extensively studied, we do not sufficiently understand how the cell cycle and senescence influence their activation. We observed that inflammasome assembly is substantially increased during mitotic arrest and cellular senescence in both myeloid cells and keratinocytes. Kinetic inflammasome activation assays using live-cell microscopy revealed that inflammasome activation in mitotically arested cells occurs more rapidly and at a higher frequency. To investigate these interactions in a physiologically relevant context, we have developed an ex vivo model using epidermal sheets from human skin explants. This system allows us to culture primary epidermal cells for several days, offering a unique platform to study inflammasome dynamics in a native tissue environment. Notably, we correlated the expression of cell cycle regulators with ASC specking frequency, revealing that NLRP1 inflammasome activation predominantly occurs in mitotically inactive cells. This highlights the critical role of cell cycle status in regulating immune responsiveness. By elucidating the interplay between cellular arrest and inflammasome activation, our study offers novel insights into mechanisms driving age-associated inflammation, barrier dysfunction, and chronic immune disorders.

LT 10 - Lightning talks X

LT 10-1

Peripheral cGAS -STING-mediated inflamm-aging drives neurodegeneration

Caitlyn Myers[1], Najmeh Saffarzadehghandehari[1], Maria Oberg[1], Nelson Gekara[2], Anetta Hartlova[1]
[1]Gothenburg University, Microbiology and Immunology, Gothenburg, Sweden
[2]Stockholm University, Department of Molecular Biosciences, Stockholm, Sweden

Aging occurs universally, yet its progression varies across individuals, tissues, and cells. Neurodegeneration, a hallmark of aging, often manifests long after peripheral aging markers emerge. However, the genetic determinants linking systemic aging to neurodegeneration remain poorly understood. Mutations in leucine-rich repeat kinase 2 (LRRK2) are major genetic risk factors for Parkinson"s disease (PD). The most prevalent and best studied is the G2019S mutation which increases LRRK2 kinase activity. However, the pathogenic mechanism behind LRRK2-driven PD progression and its link to aging remains unclear. By analyzing PD patients and mice with LRRK2 gain-of-function mutation (LRRK2GoF), we demonstrate that PD is an accelerated aging disease characterized by age-associated inflammation (inflamm-aging). This STING-dependent inflamm-aging first manifests in the periphery, then disrupts the blood-brain barrier and progresses to the brain, resulting in neurodegeneration as characterized by the loss of dopaminergic neurons. We further demonstrate that, mechanistically, a primary consequence of aging or LRRK2GoF is a decline in the endo-lysosomal system. This decline results in cytosolic build-up of extraneous self-DNA and subsequent increase in DNA-containing extracellular vesicles which trigger the cGAS-STING pathway. Taken together, this study unveils the cGAS-STING pathway and LRRK2GoF as key determinants and potential targets for preventive or therapeutic strategies against accelerated aging, inflamm-aging, and neurodegeneration.

LT 10-2

Toll-like receptor 9 activation disrupts microglial lysosomal integrity to promote Alzheimer disease pathology in APP23 mice

Moise de Lavergne[1], Bénédicte Manoury[1], Lucie Mazzola[1], Marie-Claude Potier[2], Sheela Vyas[3]
[1]INEM, U1151-CNRS UMR 8253, University Paris City, Paris, France
[2]ICM, Paris Brain Institute CNRS UMR7225-INSERM U1127–Sorbonne University, Paris, France
[3]Paris Seine Sorbonne University, Institute of Biology, Paris, France

Purpose: Genetic studies in Alzheimer's disease (AD) reveal fundamental role of innate immune responses in neurodegenerative processes stemming from amyloid beta and Tau pathologies. These responses either counteract or exacerbate AD pathology, thus understanding their actions is essential. Toll-like receptors (TLRs), major initiators of immune activation, are potentially decisive in modulating AD pathology, yet their exact roles are unknown. TLR9 is an endosomal receptor stimulated upon recognition of CpG sequences found in DNA from pathogens but also in mitochondrial DNA released from dying neurons. TLR9 is interesting in AD: phospholipase D3 and progranulin, whose genes are linked to predisposition of AD, are controlling TLR9 activation in immune cells and repeated injection of CpG protects against the development of AD. We therefore study the role of TLR9 in AD pathology.
Methods: We deleted TLR9 in a mouse model of AD (APP23) and monitored 1) amyloid beta deposition, microglial, neuron and astrocyte activation, 2) inflammatory responses and 3) mood and cognitive changes. We also measured amyloid beta trafficking and lysosome fitness in microglia from APP23 and APP23xTLR9 deficient mice.
Results: In contrast to APP23 mice, APP23xTLR9 KO mice show significantly less 1) deficit in novel object recognition object and Y-maze behaviour tests, which are both hallmarks of AD pathology, and 2) amyloid beta deposits and microglia activation in the brain. Mechanistically, stimulation of TLR9 by amyloid beta peptides and/or mitochondrial DNA in microglia from APP23 mice leads to increased inflammation and impaired amyloid beta peptides proteolysis due to lysosome disfunction.
Conclusion: Altogether, our results have identified TLR9 as a critical innate immune receptor which, by participating in lysosomal leakage to facilitate amyloid beta spreading, contributes to the development of AD in APP23 mice.

Poster session

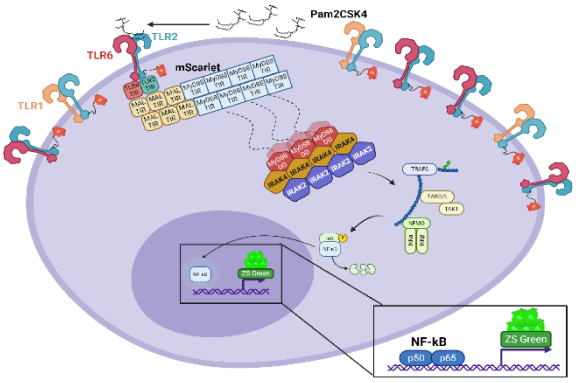
P 001

Guardians of the immune galaxy – toll-like receptors

Prerna Mudai[1], Parimala Vajjhala[1], Sara Thygesen[1], Bostjan Kobe[2], Katryn Stacey[1]
[1]University of Queensland, Innate Immunity Lab, School of Chemistry and Molecular Biosciences, Brisbane, Australia
[2]University of Queensland, Structural Biology, School of Chemistry and Molecular Biosciences, Brisbane, Australia

QUESTION: Toll-like receptors (TLRs) detect pathogens and activate innate immunity. TLR2 interacts with TLR1 or TLR6 to form heterodimers and is activated by distinct bacterial lipoprotein subsets. This activates a signalling pathway involving adaptors MAL and MyD88, leading to inflammatory cytokine release. TLR2 signalling is associated with diseases such as septicaemia, Alzheimer's, etc. Crystal structures of TLR2 heterodimers have been elucidated but the dimerization of TIR domains and recruitment of signalling adaptors remains unclear. METHODS: In prior work, we elucidated in-vitro filamentous structures of MAL, MyD88, and a MAL-TLR4 complex through cryo-EM and microcrystal electron diffraction. We integrated cell-based assays of TLR4 signalling with structural data to formulate a model of the TLR4 signalling complex. This project posits that the TLR4 signalling complex provides a template for TIR domain interactions of TLR2 with TLR1 or TLR6, as well as with downstream adaptors MAL and MyD88. We employed a dual-fluorophore system to tag both NF-kB-driven reporter gene and the TLR proteins in cell-based assays to investigate TLR2 and TLR6 mutants, with subsequent analysis performed via flow cytometry. RESULTS: The mutation of TLR2 TIR surfaces interacting with TLR6 TIR diminishes, but does not abolish signalling, unlike modifications to TLR4 TIR which result in null signals. A divergent role of a TLR2 mutant in signalling across different heterodimers has been identified. Additionally, we have developed chimeric constructs of TLR2 and TLR6 to investigate TIR homodimer signalling and the TIR structural arrangement within the TLR2 signalling complex. CONCLUSION: TLR2-TLR6 TIR interaction may not exhibit a defined polarity, contrasting with the TLR4 homodimer. It is improbable that TLR2 signals via a homodimeric configuration of TIRs. Investigating the molecular architecture of the TLR2 signalling complex and affirming its functional significance in cellular contexts is crucial for understanding the role of TLR2 signalling in pathological conditions.

Fig 1



hEKBlue-MD2-CD14- NFκB Zs Green reporter cell
A ZsGreen-tagged NFκB reporter was stably integrated in hEK cells lacking TLR2 but expressing MD2, CD14, TLR1 and TLR6 endogenously. TLR2 mutants tagged with mScarlet-I were transiently transfected in the cells to reconstitute signalling upon TLR2 ligand treatment.

Fig 2.

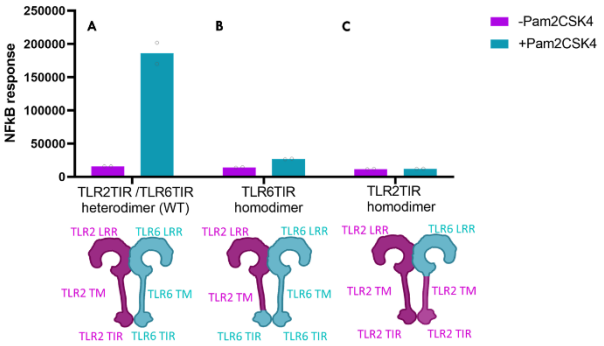


Figure 2: $TLR2^{TIR}$ homodimers do not induce signalling, whereas $TLR6^{TIR}$ homodimers have minimal signalling. WT and chimeric constructs were transfected into TLR1-6 double knockout cells and treated with TLR2/6 ligand Pam_2CSK_4.
A) $TLR2^{TIR}$ /$TLR6^{TIR}$ heterodimer (wild-type), **B)** $TLR6^{TIR}$ homodimer and **C)** $TLR2^{TIR}$ homodimer.

Poster session

P 002

Ecklonia cava extract attenuates the particulate matter exacerbated Th2/Th17 immune response and alternative activation of macrophages in murine model of asthma

Youngheun Jee[1], Hyo Jin Kim[2], Jiwon Yang[1], Kalahe Hewage Iresha Nadeeka Madushani Herath[3], Sulakshani Thilakarathnad Manathunge Kumudu[1], Prabhavi Dayaddrad Gamaralalage[1]
[1]Jeju National University, Jeju, South Korea
[2]Harvard University, Boston, MA, United States
[3]Wayamba University, Gonawila, Sri Lanka

Environmental factors, particularly airborne particulate matter (PM), have been implicated in exacerbating inflammatory responses associated with asthma. The research focuses on the ethanol extract of Ecklonia cava (ECE), an edible brown alga known for its anti-inflammatory properties, and investigates its alveolar macrophages (AMs) polarization in PM-exacerbated asthma using Th2/Th17 inflammation mouse model incorporating PM as the central allergen. ECE attenuated the PM-exacerbated histopathological changes like infiltration of inflammatory cells and mast cell degranulation in the trachea and lung. ECE restrained the secretion of Th2- and Th17-mediated cytokines (IL-4 and IL-17a, respectively), which suppressed the accumulation of respective eosinophils in the trachea and lung. Furthermore, ECE suppressed oxidative stress in polarized AMs and restored AM polarization, which stimulated Th1- or Th2-like responses in the PM-exacerbated asthmatic mice. ECE specifically reduced the presence of CD80+ M1 and MDA dual-positive macrophages and influenced M2 macrophages under oxidative stress, as shown by CD206 and MDA expressions. Importantly, phagocytosis by M2 macrophages was reduced, as shown by a decrease in MPO activity and fewer histone-H3 positive cells. These suggest a potential role of ECE in alleviating asthma symptoms exacerbated by environmental triggers and highlight ECE as a promising candidate for future exploration in clinical settings. The study also contributes to our understanding of natural remedies for the management of PM-exacerbated asthma.
This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Government of Korea (MSIT) (No. 2023R1A2C1005930)
Keywords: Ecklonia cava, Particulate matter, Macrophage polarization, phagocytosis, Th2/Th17 responses

P 003

Toll-like receptor 4 deficiency alleviates interleukin-17-mediated airway inflammation in PM-exacerbated allergic asthma mice

Jiwon Yang[1], Hyo Jin Kim[2], Olupathage Indrachapa Udayanganie[1], Manathunge Kumudu Sulakshani Thilakarathna[1], Prabhavi Dayaddrad Gamaralalage[1], Youngheun Jee[1]
[1]Jeju National University, Jeju, South Korea
[2]Harvard University, Boston, MA, United States

Particulate matter (PM) is increasingly recognized as a significant contributor to allergic inflammation in asthma. Recent studies have elucidated the exacerbation of airway inflammation in allergic asthmatic mice characterized by Th2/Th17 responses, primarily mediated via TLR4 and the MyD88-dependent NF-κB pathways. In this study, we investigated the role of TLR4 in PM-exacerbated allergic asthma using a mouse model in which TLR4−/− and wild-type (WT) C57BL/6 mice were sensitized with ovalbumin (OVA, 20 μg) and exposed to PM (5 mg/m^3) via inhalation for seven consecutive days. WT mice showed a marked alveolar macrophage (AMs) polarization disparity depending on applied agents: PM exposure increased CD80+ M1 macrophages while OVA exposure elevated CD206+ M2 macrophages. Additionally, PM exposure in OVA-induced WT mice markedly amplified oxidative DNA damages in both M1 and M2 macrophages. Concurrently, the mRNA expression of TLR4, the Th17 transcription factor RORγt, and Th17-differentiating cytokines TGF-β and IL-23, as well as their related cytokines IL-17A, IL-17F, and IL-22 was upregulated, indicating their involvement in airway inflammation upon PM exposure. Intriguingly, however, the secretion of cytokines by M1 (IL-1β, TNF-α, IL-6) and M2 (IL-4, IL-10) macrophages, as well as the secretion of Th17 differentiation-inducing and Th17-derived inflammatory cytokines were reduced in TLR4-/- mice compared to those in WT mice when exposed to PM. Inflammatory cell infiltration, mucus hypersecretion, and mast cell activation in lung tissues of TLR4-/- mice were also reduced in TLR4-/- mice compared to those in WT mice when exposed to PM. Specifically, we confirmed the attenuation of Th17-mediated neutrophil infiltration in TLR4-/- mice compared to that in WT when exposed to PM. These results suggest that TLR4 plays a crucial role in the development of asthma upon PM exposure by modulating M1/M2 macrophage polarization and Th17 cell differentiation and cytokine secretion. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Government of Korea (MSIT) (No. 2023R1A2C1005930)

Poster session

P 004

Diagnostic yield of genome-wide genetic testing and demographic distribution in 556 index patients with suspected monogenic autoinflammatory disorders

Darja Gauck[1], Marc Sturm[1], Ute Grasshoff[1], Stefanie Beck-Wödl[1], Bernd-Ulrich Keck[2], Martin Kehrer[1], Olaf Rieß[1], Jasmin Kümmerle-Deschner[3], Tobias Haack[1]

[1]University of Tübingen, Institute of Medical Genetics and Applied Genomics, Tübingen, Germany

[2]Diakonie Hospital Schwäbisch-Hall, Department of Pediatrics, Schwäbisch-Hall, Germany

[3]University Hospital Tübingen, University of Tübingen, Division of Pediatric Rheumatology and autoinflammation reference center Tübingen (arcT), Tübingen, Germany

Background: Monogenic autoinflammatory diseases (AID) are a genetically heterogenous group of disorders characterized by dysregulation of the innate immune system, leading to recurrent episodes of sterile inflammation. In this report we provide an overview of the diagnostic yield and demographics in a multi-center cohort of 556 index patients with a suspected clinical diagnosis of monogenic AIDs.

Methods: Sequencing libraries were generated from genomic DNA for genome or exome sequencing and sequenced on a NovaSeq6000 System (Illumina).

Results: In our cohort of pediatric and adult patients suspected with monogenic cause of autoinflammatory disorders (AIDs), genetic analysis revealed a definitive molecular diagnosis in a modest subset of cases. Among pediatric patients, 4.7% (n=21/451) received a firm genetic diagnosis, with 42.8% (n=9/21) harboring disease-causing biallelic variants in the MEFV gene. In the adult cohort, a definitive molecular diagnosis was established in 12.5% (n=10/80) of cases, predominantly associated with pathogenic variants in the NLRP3 gene (40%, n=4/10). For 13.3% (n=78) of the entire cohort, variants in known AID genes were identified, but their significance remained uncertain. Notably, in 81.2% (n=476) of cases, the underlying genetic cause of the autoinflammatory phenotype remained unsolved.

Conclusion: Our findings highlight the significant diagnostic challenges in patients with suspected monogenic AIDs. The substantial proportion of variants of uncertain significance in known AID genes underscores the need for robust functional assays to elucidate their pathogenic potential. Moreover, the high frequency of genetically undiagnosed cases emphasizes the importance of ongoing research to identify novel candidate genes. Integration of multi-omics technologies and advanced sequencing methods, such as long-read sequencing, may expand our understanding of the genetic models underlying the autoinflammatory phenotypes.

P 005

Adventures on human myddosome assembly in yeast – setting up a model

Victor Cid[1], Elba del Val[1], Alejandro Fernández-Vega[1], Julia Maria Coronas-Serna[1], María Molina[1]

[1]Universidad Complutense de Madrid, Dpt. of Microbiology and Parasitology. School of Pharmacy, Madrid, Spain

The yeast Saccharomyces cerevisiae is a ready eukaryotic model, widely used as a chassis for Synthetic Biology approaches. We have challenged this heterologous model to to study diverse aspects of protomyddosome and myddosome assembly in TLR signaling by engineering yeast cells to produce the human adaptors TIRAP, MyD88, TRAM and TRIF, as well as the IRAK kinases and TRAF6.

In yeast, TIRAP and TRAM localized at the plasma membrane, the latter forming long filaments. TIRAP could readily recruit MyD88 to the plasma membrane. However, in the absence of TIRAP, MyD88 formed tight aggregates at the endoplasmic reticulum-mitochondria encounter sites (ERMES). In the absence of the yeast dynamin-like DRP1 homolog Dnm1, an ERMES-associated protein, MyD88 was largely unstable. We found that association of MyD88 with the yeast ERMES relied on its Death Domain, and that stability and self-interaction of the MyD88 protein could be greatly enhanced by deleting its 20-amino acid N-terminal extension. Pull-down experiments revealed that myddosome components MyD88-IRAK4-IRAK1/2 and TRAF6 maintained their interactions in this heterologous model, and that a kinase-dead version of IRAK4 provides a more robust interaction that WT IRAK4, hinting for a role of the kinase in the dynamics of the complex.

Based on these results, we have developed a tripartite GFP-based system in yeast to titrate interactions of MyD88 with itself, as well as with TIRAP and IRAK4. Furthermore, we have devised and validated two different yeast-based platforms ready to screen for IRAK4 kinase activity inhibitors, one based on growth and the other based on a fluorescent reporter. We hope that these tools will be useful for researchers in the field.

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Poster session

P 006

Interaction of TLR-mediated and TGFβ-induced pathways trigger inflammatory programs in macrophages in diabetic conditions

Julia Kzhyshkowska[1], Quan Liu[1], Christina Schmuttermaier[1], Fangrong Xu[1]
[1]Heidelberg University, Medical Faculty Mannheim, Institute of Transfusion Medicine and Immunology, Mannheim, Germany

Question. Diabetes is one of the most common metabolic diseases worldwide, characterized by hyperglycaemia (HG) and dyslipidemia. Macrophage-mediated chronic inflammation accelerates progression of diabetes and its complications. We asked the question how TLR and TGFβ-mediated pathways in macrophages interact in diabetic conditions. Methods. We used the model of primary human monocyte-derived macrophages cultivated in hyperglycaemic (HG) conditions, supplemented by the PAM3CSK4, ligand of TLR1/TLR2 that mimic dyslipidaemic conditions. Results. We demonstrated that HG stimulated expression of TLRs on RNA and protein levels, and patterns of TLR upregulation were specific for the inflammatory and tolerogenic macrophages. HG amplified the response of tolerogenic M(IL4) to LPS by elevation of IL1β and suppression of IL10 production. Addition of PAM3CSK4 in HG conditions, amplified expression of TLR4, and production of IL1β. For the TGFb-induced pathway, HG inhibited phosphorylation of SMAD2/3, while TLR1/2 ligand PAM3CSK4 supressed phosphorylation of SMAD1/5. TLR1/2 affected levels of TGFβ1-driven p-SMAD1/5 by regulating the ubiquitination and degradation of MyD88. NGS data demonstrated that the addition of PAM3CSK4 reverses the anti-inflammatory function of TGFβ and promotes expression of pro-inflammatory CXCL9, CXCL12 and IL32. KEGG enrichment analysis and Western Blot showed that the combination of PAM3CSK4 and TGFβ1 significantly promoted the activation of the NFκB pathway. Conclusions. Hyperglycaemia was identified as a sensitizer of tolerogenic macrophages to the dyslipidemic ligands by increasing expression of TLRs. TGFβ1 promoted the pro-inflammatory effect of the dyslipidemic ligand in HG conditions. TLR and TGFb1 pathways can cross talk via the MyD88"s ubiquitin-degradation process. The pro-inflammatory crass-talk of TLR and TGFβ pathways in macrophages open perspective for identification of new molecular targets to prevent diabetic vascular complications.

Poster session

P 007

The role of toll-like receptor 7 agonist in improving COVID-19 vaccine immunology

Yujing Fan[1], D. Liu[1], K.Y. Leung[1], R. Zhang[1], K.H. Chan[1], F.N.I. Hung[1]
[1]The University of Hong Kong, Hong Kong, China

Background: COVID19 infection is not ended up and the rapid mutation of SARS-CoV-2 reduced the effectiveness of COVID-19 vaccines obviously, resulting in many vaccines have stopped production. Although novel variants showed lower pathogenicity in healthy people, it still threatens immunocompromised population and elderly people. Therefore, to explore a novel adjuvant for COVID-19 vaccination is still needed. toll-like receptor agonists, as vaccine adjuvants, have been attempted in HBV and influenza vaccines. In this study, we evaluate the effects of toll-like receptor 7 agonist, as COVID-19 vaccine adjuvant, on humoral and cellular immune response following vaccination on animal models.
Methods: TLR7 agonist, imiquimod, combined with COVID-19 vaccine, was evaluated the efficacy in hamsters after lethal virus challenge and the long-term immunological memories in mouse models.
Results: In the group of vaccine combined with imiquimod (Vac_IM), the hamsters only showed slightly body weight reduction after inoculation of lethal dose virus (10^5 PFU/50ul), compared with control groups. The viral load in lung and nasal lavage fluid was significantly lower than control groups ($p < 0.05$) at all timepoints post infection. The neutralizing antibody response was more rapid and robust to be induced after infection in the group of Vac_IM Additionally, the effects of imiquimod was better in females, compared with males, although male hamster in the Vac_IM showed increased immune responses as well. As for the long-term immunological memories after vaccination combined with imiquimod, sex-based antibody difference was observed in the long term after stratifying sex. In males, the group of Vac_IM was induced higher neutralizing antibody titers to wild-type strain than control group at Day 42 ($p < 0.05$). And at Day 90 after 2 doses, Vac_IM group of males was remained 4 times as many as control group in neutralizing antibodies to wild-type strain ($p < 0.05$) But there were not obvious differences in female groups at all timepoints. Similarly, only male Vac_IM group was elicited obviously higher neutralizing antibodies to Delta strain in serum at Day 42, in comparison with Vac_PBS group ($p < 0.05$). At Day 90, the GMT of Vac_IM group in males sightly outweighed Vac_PBS group ($p = 0.06$). The ELISA results demonstrated Vac_IM group tended to have higher IgG titers, in comparison with Vac_PBS group following 2-dose administration, expected for Day 28 post 2 doses, which was consistent with neutralizing titers at Day 28 post 2 doses. At Day 14 post 2 doses, anti-RBD IgG titers in Vac_IM group was higher obviously than Vac_PBS group ($p < 0.05$). These results showed the function of imiquimod on improving antibody response to vaccines. Although there were no noticeable difference in total T cells, the results suggested Vac_IM group has obviously increase on population of CD8+ cells at Day 7 after vaccination, than Vac_PBS group, which presented Vac_IM group was obviously induced cytotoxic function of CD8+ cells.
Conclusion: Imiquimod, as COVID-19 vaccine adjuvant, can improve vaccine protection, and enhance the immune responses of hosts. Therefore, imiquimod can be expected to improve immune response in the people who are immunocompromised post COVID-19 vaccination. The mechanism of imiquimod induced immune protection is worth to research further and make it can be used clinically.

Poster session

P 008

Signaling Network Perturbations Induced by Novel MYD88 and IRAK4 Variants

Min Zhou[1], Martin C. Moncrieffe[2], Andras Szolek[1], Maria Mateo Tortola[1], Gaopeng Li[1], Lip Kun Tan[3], Benedict Yan[3], Frank Lichtenberger[4], Nicholas J. Gay[2], Alexander N.R. Weber[1]

[1]University of Tübingen, Institute of Immunology, Department of Innate Immunity, Tübingen, Germany
[2]University of Cambridge, Department of Biochemistry, Tennis Court Road, Cambridge, United Kingdom
[3]National University Hospital, Department of Laboratory Medicine, Singapore, Singapore
[4]Piedmont HealthCare, , Mooresville, United States

Myeloid differentiation 88 (MyD88) is a pivotal adaptor protein within the signaling pathways of Toll-like receptors (TLRs), interleukin-1 receptor (IL-1R), and transmembrane activator and CAML interactor (TACI). Certain gain-of-function (GOF) MYD88 variants have been implicated in lymphomagenesis by promoting aberrant B cell proliferation, whereas GOF variants in IRAK4, a kinase downstream of MyD88, have not been described. In this study, we investigated novel clinical MYD88 and IRAK4 variants to elucidate their biological effects and underlying mechanisms. Our research first focused on characterizing a MyD88 C229S mutation, identified in a myelofibrosis patient. Using transient transfection assays and THP-1 monocytic cells stably reconstituted with MyD88 C229S, responses to TLR ligands were evaluated. We observed MyD88 C229S to hyperactivate NF-κB signalling and enhanced IL-8 and TNFα expression were measured, despite MyD88 comparable protein expression levels. Additionally, immunostaining with an HA tag revealed increased protein aggregation for the C229S variant. This suggests C229S is the first non-lymphoma GOF mutation of MYD88. We also investigated IRAK4 mutations, V11G, C13Y, V11G/C13Y, identified in a patient with lymphoplasmocytic leukemia. Based on purified protein analysis, we found that the mutated IRAK4 highly aggregated. We hypothesize that these mutated proteins might exhibit GOF properties, but further research is ongoing. To investigate molecular mechanisms of different MyD88/IRAK4 variants at endogenous levels, we developed a nanobody-based tracking system. This innovative method enabled real-time monitoring of MyD88 complex assembly and dynamics. These findings shed light on the mechanisms underlying variants in MYD88 and IRAK4, and expand their roles in oncogenesis and immune regulation.

P 009

Predicting flagellin-host interactions – insights from gut microbiome and IBD

Anna Bogdanova[1], Andrea Borbón[1], Ruth Ley[1], Alexander Tyakht[1]

[1]Max Planck Institute for Biology, Microbiome Science, Tübingen, Germany

Bacterial flagellins are key modulators of the immune system through their interaction with human TLR5 (hTLR5). While many flagellins elicit strong immune responses, others remain undetected or bind hTLR5 without activation, suggesting an evolutionary adaptation for immune evasion. This phenomenon, particularly observed in gut commensals like Roseburia hominis and other Lachnospiraceae, raises questions about the role of flagellins in gut immune homeostasis and inflammatory diseases.

To investigate whether flagellin immunostimulatory potential can be predicted from sequence and structural features, we developed a machine learning model trained on experimentally validated flagellins with known hTLR5 binding and activation properties. Using this model, we annotated a reference set of gut bacterial flagellins and quantified them across metagenomes and metatranscriptomes of inflammatory bowel disease (IBD) patients.

We found that structural constraints on hTLR5-binding residues are major determinants of immunostimulatory capacity. Flagellomes - the total pool of flagellins in a sample - were more diverse in healthy individuals than in the patients. Several flagellins were also differentially expressed between the clinical groups, suggesting their potential immunoregulatory roles.

These results support the feasibility of computationally predicting flagellin immunogenicity and suggest that flagellome profiling provides informative features potentially applicable for microbiome-based disease prediction.

Poster session

P 010

Gracilaria verrucosa-derived Polysaccharide enhances gut barrier function and modulates microbiota-immune interactions

Yunkyoung Lee[1,3], Eunyoung Kim[1], Zemin Li[2], Jiamei Cui[1], Kayeon Ko[1], Minhyeok Kang[3], Yunpeng Wang[2], Hyo Jin Kim[1], Jiwon Yang[3], Tatsuya Unno[4], Youjin Jeong[5], Youngheun Jee[1], Guiguo Zhang[2]

[1]Jeju National University, Jeju, South Korea
[2]Shandong Agricultural University, Tai'an, China
[3]Jeju National University, Interdisciplinary Graduate Program in Advanced Convergence Technology & Science, Jeju, South Korea
[4]Chungbuk National University, Cheongju, South Korea
[5]Korea Research Institute of Bioscience and Biotechnology (KRIBB), Cheongju, South Korea

Gracilaria verrucosa-derived polysaccharide (GVPS) has demonstrated metabolic health benefits, yet its role in gut barrier protection and host-microbiota interactions remains unclear. This study investigated GVPS"s impact on intestinal integrity, mucosal immunity, and microbial composition under metabolic and inflammatory stress using in vivo and in vitro models. GVPS administration in a diet-induced obesity model reduced body weight gain, improved insulin sensitivity, and alleviated hepatic lipid accumulation. Colon histological analysis showed that GVPS inhibited immune cell infiltration and downregulated inflammatory markers (Tnf-α, F4/80, Mcp1, Cd11c), while partially restoring mucus synthesis, reinforcing barrier integrity. Furthermore, GVPS mitigated weight loss, preserved colon length, and protected against mucosal injury, allowing a focused evaluation of its role in barrier repair and immune modulation in dextran sulfate sodium colitis obese mice. We next evaluated that GVPS restored barrier integrity by counteracting inflammation-induced permeability increases in an intestinal co-culture model. Correlation analyses, integrating in vivo and in vitro findings, identified core microbial taxa (Lactobacillus, Bacteroides) and core host genes (Gnb4, galnt4, Cxcr3) associated with GVPS-mediated gut protection. Since physicochemical properties influence functionality, we analyzed structural characteristics. GVPS is a long-chain polysaccharide with highly branched, helical domains, composed of over 90% galactose and a molecular weight of 8.1 × 10^4 g/mol. Although its direct link to bioactivity remains unclear, further studies are needed to clarify its structural relevance to observed effects. Taken together, our findings highlight GVPS as a promising functional agent that reinforces gut barrier integrity, alleviates intestinal inflammation, and modulates microbiota composition, offering insight into its molecular mechanisms and therapeutic potential for gut health.

P 011

Hematopoietic stem cell manipulation for greater relevance of human-focused research in innate immunity

Xiao Liu[1], Lena Erlebach[2], Deborah Kronenberg-Versteeg[2], Alexander N.R. Weber[1]

[1]University Tübingen , Institute of Immunology, Department of Innate Immunity, Tübingen, Germany
[2]University Tübingen, Hertie-Zentrum für Neurologie, Hertie-Institut für klinische Hirnforschung, Tübingen, Germany

Inflammation and cancer are among the leading causes of mortality worldwide. Pattern recognition receptors (PRRs) play a central role in regulating immune responses, cellular fate, differentiation, migration, and disease outcomes. Thus, PRRs are crucial targets for therapeutic interventions to address a range of unmet medical needs. Despite their critical roles, most existing knowledge of PRRs is derived from studies using artificial cellular systems, such as immortalized cancer cell lines or murine models, which do not always reflect the complexity of human primary immune cells and tissues. This limitation is particularly evident for microglia or neutrophils, which are difficult to culture and resistant to genetic manipulation in laboratory settings. However, recent advances in gene editing technologies, such as CRISPR-Cas9, have enabled the manipulation of human stem cells, facilitating the study of PRRs in human immune cells. Our research focuses on utilizing these cutting-edge techniques to improve the translational relevance of PRR-based therapies. In our lab, we have adopted the well-characterized KOLF2.1J iPSCs and generated iPSC-derived microglia and macrophages to investigate NLRP3 inflammasome activation in response to various agonists and inhibitors. Additionally, we have successfully established a MyD88-overexpressing KOLF2.1J model through lentiviral transduction and created ASC (PYCARD) knockout KOLF2.1J cells using CRISPR-Cas9 editing. These cellular models have been applied to PRR pathway analysis in human immune cells and may help to enhance the translational potential of PRR-targeted research.

Poster session

P 012

In vivo and in silico investigation of novel bioactive peptides-based therapy targeting multiple parallel hits in Western diet-induced MAFLD

Chang-Chi Hsieh[1], Vipul Wayal[2], Shulhn-Der Wang[1]
[1]Tunghai University, Animal Science and Biotechnology, Taichung, Taiwan
[2]China Medical University, School of Post-Baccalaureate Chinese Medicine, College of Chinese Medicine, Taichung, Taiwan

Multiple parallel hits have contributed to the progression of metabolic-associated fatty liver disease (MAFLD) from a normal liver condition to chronic liver disease. This condition is characterized by an abnormal accumulation of fat within liver, inflammation and oxidative stress. In this study, we investigated the potential therapeutic effects and underlying interfered targets by novel bioactive peptides (EWYF and EWFY) on Western diet (high-fructose and high-fat) induced MAFLD in C57BL/6J mice. Mice fed a normal chow diet (ND group) and Western diet (WD and treatment groups) for 8 weeks. Treatment groups received bioactive peptides in low (10 mg/kg/day) and high (50 mg/kg/day) doses. WD-induced body weight gain and increased liver weight along with visceral adiposity, which were markedly reversed by bioactive peptides in a dose-dependent manner. Additionally, bioactive peptides significantly reduced hepatic steatosis, liver injury and proinflammatory response. Western diet-induced glucose tolerance and insulin resistance, whereas bioactive peptides significantly improved glucose tolerance and insulin sensitivity. Persistent intake of WD triggered chronic inflammation and severe oxidative stress, which were significantly alleviated by bioactive peptides treatment via inhibiting NOD-like receptor protein 3 (NLRP3) inflammasome activation, oxidative stress and pyroptosis by modulating TLR4/NF-κB, Keap1/Nrf2/HO-1 and IL-1β/Caspase1/GSDMD signaling pathways. Furthermore, molecular docking studies suggest that bioactive peptides act as fructokinase and TLR4 antagonists, which potentially modulated fructose metabolism and downregulated WD induced multiple parallel hits in MAFLD. Collectively, these findings highlight bioactive peptides as promising candidates for MAFLD treatment due to their potent antioxidant, anti-inflammatory and mitigate pyroptosis viatargeting specific molecular inhibition.

Fig 1.

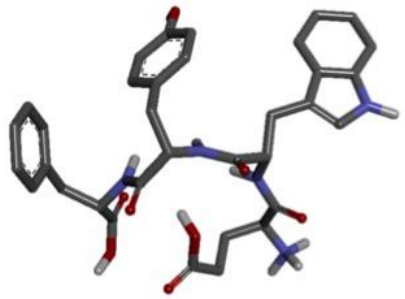
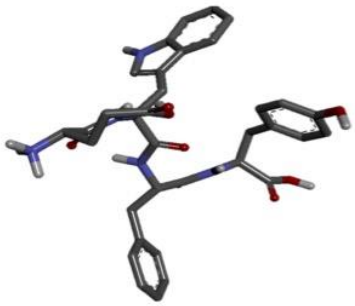


Fig 2.



P 013

Extracellular inflammasome complexes (ASC specks) in post-sepsis immunosuppression

Nathalia Sofia Rosero Reyes[1], Ibrahim Hawwari[1], Salie Maasewerd[1], Lukas Roßnagel[1], Bernardo Franklin[1]
[1]Universitätsklinikum Bonn, Institut für angeborene Immunität, Bonn, Germany

Sepsis, a life-threatening condition caused by a dysregulated immune response to infection, affects around 50 million people globally and leads to 11 million deaths each year. While acute-phase treatments have improved survival, long-term complications persist. Approximately 75% of survivors experience ongoing inflammation and immunosuppression, often leading to hospitalization and death.
Recent research highlights pyroptosis—an inflammatory lytic form of cell death—as a key driver of sepsis mortality. This process is driven by inflammasomes, molecular platforms that activate and release pro-inflammatory cytokines, including IL-1β and IL-18, and lipid mediators, both of which can reproduce the clinical features of sepsis. Upon activation, the inflammasome adaptor ASC forms specks that are released into the bloodstream and accumulate in tissues, perpetuating low-grade inflammation. We hypothesize that this build-up of extracellular ASC specks (eASC) in sepsis contributes to post-sepsis immunosuppression, and that removing these eASC could alleviate this condition.
To address this hypothesis, we will monitor and quantify eASC in plasma and peripheral blood mononuclear cells (PBMCs) from pediatric and adult sepsis patients across four European countries, as part of the EU-funded BEATSepsis consortium. Using flow cytometry and confocal microscopy, we will analyze samples during acute sepsis, at ICU discharge, and after 3-12 months of discharge. To identify pyroptotic cell populations, we will employ ImageStream. Additionally, we will test whether novel ASC nanobodies can clear eASC from tissues and prevent post-sepsis immunosuppression in mouse models. Our ultimate goal is to establish the causal role of eASC in sustaining post-sepsis immune dysfunction and explore its therapeutic potential to mitigate this condition.

Poster session

P 014

Analysis of membrane-binding features of NLRP10

Christoph Winterberg[1], Dennis de Graaf[2], Eicke Latz[2], Matthias Geyer[1]
[1]Institute of Structural Biology, Bonn, Germany
[2] Institute of Innate Immunity, Bonn, Germany

The mammalian immune system is composed of two major parts, the innate and the adaptive immune system. Pattern recognition receptors (PRRs) present in the innate immune system are able to recognize pathogens via pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). Nucleotide-binding domain, leucine-rich repeat and pyrin domain containing receptor 10 (NLRP10) is a PRR that is activated by mitochondrial damage, which leads to NLRP10 inflammasome activation, ASC speck formation and caspase-1-dependent cytokine release.1 Also poly(I:C) has been hypothesized to be an activator of the hNLRP10 inflammasome.2 By exchanging several lysine and arginine residues to glutamines in the C-terminal tail region, we removed the overall positive charge resulting in a highly reduced activity of NLRP10. This was shown by reduced ASC speck formation leading us to the hypothesis that either nucleotide or membrane binding is involved in the NLRP10 inflammasome activation. Our results are consistent with the observation that mitochondrial damage and release of distinct molecular entities leads to NLRP10 activation.1 We will further test different lipid compositions and nucleic acids (DNA or RNA) in direct binding assays to get a deeper view in the activation mechanism of NLRP10.

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P 015

Neutrophil NLRP3 assembles an inflammasome independently of the MTOC

Atousa Hashemi[1], María Mateo-Tórtola[1], Gaopeng Li[1], Francesca Bork[1], Lukas Funk[1], Alexander N.R Weber[1]
[1]University of Tübingen, Institute of Immunology, Department of Innate Immunity, Tübingen, Germany

The NLRP3 inflammasome is a key regulator of innate immunity and is implicated in various chronic and autoinflammatory diseases, including cryopyrin-associated periodic syndrome (CAPS), gout, and Alzheimer's disease. Using high-resolution live-cell imaging, we previously identified two distinct NLRP3 activation pathways in human macrophages: a pathway that relies on decameric cage-like structure and their trafficking from the trans-Golgi network (TGN) to the microtubule-organizing center (MTOC); and a second, parallel pathway that proceeds independently of the cage, TGN, and MTOC. To assess whether these pathways are conserved in other primary human immune cells, we isolated and stimulated human primary neutrophils and analyzed the relative localization of ASC specks and gamma tubulin or pericentrin as markers for the MTOC. Confocal microscopy results suggested the existence of MTOC independent activation pathway in neutrophils, evidenced by exclusively MTOC-distal ASC specks. Furthermore, live-cell imaging showed that microtubule-disrupting agents such as colchicine and nocodazole strongly disrupt microtubule filaments in neutrophils but do not impair IL-1 β secretion after stimulation. In conclusion, the neutrophil NLRP3 inflammasome appears to follow the newly discovered MTOC-independent activation pathway. Not only will this contribute to a more comprehensive understanding of human NLRP3 function; because neutrophils are the most abundant type of leukocytes in humans, considering how to target their NLRP3 may also be essential for the development of new therapeutic strategies for NLRP3-driven diseases in patients.

P 016

Innate immune tolerance and activation – distinct NLRC4 inflammasome responses to bacterial flagellins

Ezgi Atay[1], Kelsey Huus[1], Ruth Ley[1]
[1]Max Planck Institute for Biology, Microbiome Science, Tübingen, Germany

Bacterial flagellins are produced by both pathogenic and commensal gut bacteria, presenting a challenge for the innate immune system in distinguishing between harmful and harmless microbes. Our recent research revealed that gut commensals produce "silent" flagellins, which interact with the flagellin receptor Toll-like receptor 5 (TLR5) but induce only a weak immune response. However, innate immune recognition of flagellins occurs not only through TLR5 but also via the intracellular NLR family CARD domain-containing protein 4 (NLRC4) inflammasome. Whether silent flagellins can activate the NLRC4 inflammasome remains unknown. To investigate this, we are performing in vitro experiments on human monocytic leukemia cells (THP-1), exposing them to a panel of bacterial flagellins either extracellularly or intracellularly. Inflammasome activation is assessed by measuring the production of IL-1ß and IL-18 and via cell death. Although ongoing, preliminary results indicate that silent flagellins might evade NLRC4 activation, even when in contact with the inflammasome. In contrast, extracellular stimulatory flagellins are capable of entering the cell and triggering inflammasome activation in a TLR5-dependent manner. These findings emphasize the innate immune system's ability to tolerate silent flagellins while remaining vigilant against pathogenic flagellins.

Poster session

P 017

Toward potent and selective inhibition of NOD-like receptors by small molecules

Dominic Ferber[1], Maria Zyulina[1], Nico Kirsch[2], Hannes Buthmann[1], Jane Torp[1], Tim Keuler[3], Michael Gütschow[3], Günther Weindl[2], George Hartman[4], Kevin Wilhelmsen[4], Matthias Geyer[1]
[1]Institute of Structural Biology, Bonn, Germany
[2]Pharmaceutical Institute, Pharmacology and Toxicology, Bonn, Germany
[3]Pharmaceutical Institute, Pharmaceutical & Medicinal Chemistry, Bonn, Germany
[4]BioAge Labs, Richmond, CA, United States

Inhibition of the cytoplasmic pattern recognition receptor NLRP3 (NLR family pyrin domain containing 3) can be achieved using the potent and selective small molecule MCC950 (CRID3) and its sulfonylurea derivatives.1-3 Recently, a new compound class of indazole-containing small molecules (BAL-compounds) has been described to effectively inhibit the NLRP3 inflammasome.4,5 Here, we characterized a series of new BAL-compounds by biochemical and biophysical means. BAL-compounds are highly specific in targeting NLRP3 with dissociation constants in the low nM binding regime. Their binding mode, which differs from MCC950 binding to NLRP3, allows for mechanistic insights into NLRP3 biology and makes BAL-compounds a versatile and valuable alternative in NLRP3-mediated inflammasome inhibition. At the same time, the inhibition of other NOD-like receptors such as NLRP1 remains an open task: apart from the dual NLRP1 and NLRP3 inhibitor ADS032, there is no public report on selective small molecule NLRP1 inhibitors.6 Therefore we aimed to develop biochemical and cellular screening approaches for small molecule candidates based on either MCC950-related sulfonylurea derivatives or indazole-containing BAL-compounds. With the work presented here, we want to pave the way toward potent and selective inhibition of the NOD-like receptors NLRP3 and NLRP1 by small molecule compounds.

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P 018

Assessing differences in pro-inflammatory M1 macrophage-like cell models

Celine Buchmann[1], Gilles Gasparoni[2], Ann-Katrin Wentz[1], Julia Schulze-Hentrich[2], Jörn Walter[2], Bernd Bufe[1]
[1]University of Applied Sciences Kaiserslautern, Department of Informatics and Microsystems Technology, Zweibrücken, Germany
[2]Saarland University, Institute of (Epi-) Genetics, Saarbrücken, Germany

Monocytic cell lines are often used to study macrophage function in vitro because of limited availability and high donor variability of primary cells. Currently, a multitude of protocols for their differentiation exists. For pro-inflammatory macrophage-like cells, protocols using phorbol 12-myristate 13-acetate (PMA) followed by polarization with lipopolysaccharide (LPS) and interferon gamma (IFNy) are most common. However, systematic studies of their global effects on gene expression and cell function are still lacking.
We therefore compared the influence of varying concentrations of PMA alone or in combination with LPS and interferon gamma on the differentiation of the monocyte-like cell line THP-1. First, we performed RNAseq to study the genome-wide variability on gene expression. We found that genes that are correlated with immune system function are highly upregulated when using low concentrations of PMA and LPS. Focusing on M1 marker genes we found drastic protocol-dependent differences. High PMA concentrations in combination with LPS lead to an up to 100-fold stronger expression in a third of the 160 M1 marker genes that are associated with inflammation. On the other hand, low concentrations of PMA and LPS are associated with changes in genes related to antigen processing and presentation. To further assess the effects of these alterations on protein expression and cell function, we next monitored the protein expression of typical macrophage markers and performed functional assays for phagocytosis, MMP9 release and chemotaxis. Currently, the strong alterations in gene expression only resulted in moderate protocol-dependent influences on protein expression and functional responses. Since our assays so far only focus on few physiological readouts, we now plan to use cell painting and calcium imaging in combination with a large panel of specific stimulations for a more comprehensive analysis.

Poster session

P 019

Dissecting the regulation of human inflammasome forming NLRs with proximity proteomics

Lukas Jacobi[1], Tabea Klein[1], Kanishka Kumar[1], Felix Meissner[1]
[1]Institute of Innate Immunity, Bonn, Germany

Inflammasomes play an important role in surveilling cellular homeostasis, and initiate innate immune responses in immune and barrier cells. Despite major advances in recent years, it is still incompletely understood how dynamic protein assemblies and post-translational modifications regulate Nod-like receptor (NLR) activity and inflammasome assembly.
Here we set out to dissect regulatory mechanisms of NLR activation such as NLRP3 and 1 using proximity proteomics in human monocyte and keratinocyte cell lines. To identify transient protein assemblies, we harness the fast biotinylation kinetics of APEX2-based proximity labeling of tagged NLR bait proteins. APEX2 generates highly reactive phenoxy radicals and labels proximal proteins that are then enriched by streptavidin-coated beads and quantified by mass spectrometry. We devised a streamlined approach to compare NLR activation kinetics across agonists and with suited cellular compartment controls, to reveal context-dependent interaction partners of inflammasome sensors. Advanced mass spectral searches further provide agonist-induced post translationally modified sites of NLRs and proximal proteins.
Taken together, our study establishes an experimental framework to screen dynamic NLR protein assemblies that can aid to our understanding of regulatory mechanisms up- and downstream of inflammasome forming NLRs.

P 020

Advancing autophagy research – a nanobody toolkit to dissect LC3-Mediated inflammasome regulation

Jan-Patrick Hetzel[1], Birte Albrecht[2], Wiebke Aderhold[2], Sabine Neumann[1], Steffen Pritzl[1], Florian I. Schmidt[1]
[1]University Bonn, Institute for Innate Immunity, Bonn, Germany
[2]University of Bonn, Nanobody Core Facility, Bonn, Germany

Hyperactive inflammasomes drive chronic inflammation in diseases such as inflammatory bowel disease, Alzheimer's disease, and multiple sclerosis. Recent studies highlight autophagy as a regulator of inflammasome activity by degrading inflammasome components or complexes, or by mitigating mitochondrial damage, thereby preventing or curtailing excessive inflammatory responses. LC3 proteins are core components of the autophagy machinery, and guide autophagosome formation, membrane elongation, and cargo recruitment for degradation. Humans express six LC3 homologs (LC3A, LC3B, LC3C, GABARAP, GABARAPL1, and GABARAPL2), each contributing to distinct steps of autophagosome formation. However, studying individual LC3 proteins in endogenous settings is challenging due to their structural similarity and genetic redundancy. To address this, we developed and characterized camelid-derived nanobodies targeting various combinations of the human LC3 homologs. These nanobodies exhibit distinct binding properties and either specifically bind individual LC3s, several LC3s, or all family members. They provide a versatile toolkit for investigating autophagy dynamics, perturbing LC3-mediated pathways, and targeting LC3-binding proteins for degradation. Our future work will leverage these tools to dissect the role of LC3-mediated autophagy in the regulation of inflammasomes and other signalosomes, aiming to uncover novel insights and therapeutic strategies for inflammasome-driven diseases.

Poster session

P 021

Inhibiting NLRP3 in stroke mitigates inflammation and slows infarct growth already before recanalisation

Maximilan Bellut[1,2], Lukas Roßnagel[1], Guido Stoll[2], Michael Schuhmann[3]
[1]University of Bonn, Institute of Innate Immunity, Bonn, Germany
[2]University Clinic Würzburg, Neurology, Würzburg, Germany

Question: The NLRP3 inflammasome is a multiprotein complex that regulates the activation of the innate immune system. In experimental ischaemic stroke (IS), NLRP3 inhibition attenuated ischaemia/reperfusion injury in the first 24 h and secondary infarct expansion >24 h after recanalisation. In the present study, we investigated whether NLRP3 inhibition can halt stroke progression in large vessel occlusion (LVO) prior to recanalisation.
Methods: We occluded the middle cerebral artery in Bl6N mice for up to 4 hours and examined NLRP3 mRNA expression under LVO. In a subsequent experiment, Bl6N mice were prophylactically and therapeutically treated with the NLRP3 inflammasome inhibitor MCC950. We assessed stroke volume, clinical outcome, caspase 1, gasdermin D, IL1b levels (immunoblot), neuronal survival and blood-brain barrier (BBB) integrity (IHC).
Results: We detected a 5-10 fold upregulation of NLRP3 mRNA expression already under LVO (n=10/group). Its prophylactic and therapeutic inhibition significantly reduced stroke size [vehicle/prophylactic/therapeutic MCC950: 67.4±16.4/37.2±10.2/42.9±8.4 ml] while improving clinical outcome. As a result, caspase 1, gasdermin D and IL1b release decreased, while BBB integrity and neuronal survival improved significantly. We observed these improvements in both prophylactic and therapeutic settings.
Conclusions: We show that NLRP3 inhibition attenuates cerebral inflammation and infarct growth already under LVO. In doing so, it partially preserves the ischaemic penumbra and "buys time" for recanalisation. This may have important clinical implications for future IS therapies, as infarct progression under LVO is a major risk factor for poor clinical outcome despite technically successful recanalisation.

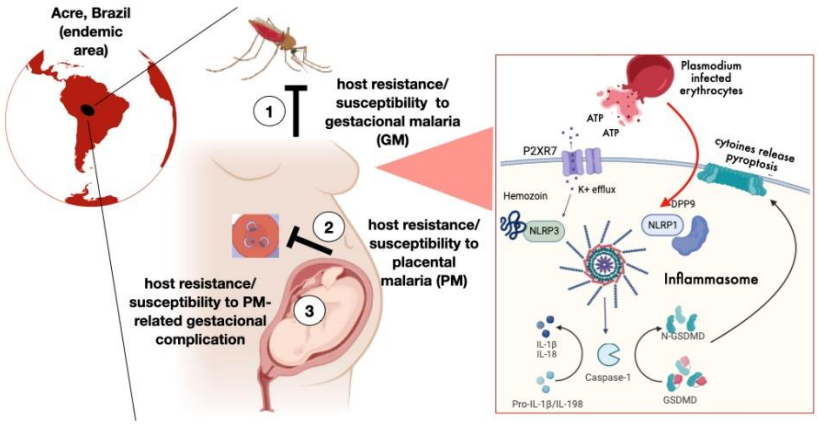
P 022

Impact of NLRP1 Met1154Val and IL1B variants on gestational malaria – an unexplored role of NLRP1 in inflammasome activation by plasmodium spp.

Alessandra Pontillo[1]
[1]Universidade de Sao Paulo (USP), Departamento de Imunologia, Insituto de Ciencias Biomedicas, Sao Paulo, Brazil

We hypothesized that variants in inflammasome-related genes could influence susceptibility to gestational malaria (GM). To test this, we conducted an association study in a cohort of pregnant women from a malaria-endemic region in Northern Brazil, assessing whether specific functional single nucleotide variants (SNVs) in inflammasome genes affect (1) resistance to Plasmodium infection, and (2) the development of placental malaria.
Our findings revealed that the NLRP1 Met1154Val variant was associated with increased resistance to Plasmodium infection. Moreover IL1B SNVs appeared more prevalent in severe cases. Additionally, multivariate analyses incorporating placental blood cytokines, growth factors, and immunohistochemical features revealed that the NLRP1 Met1154Val variant correlated with a healthier placental state, highlighting a potential protective role of the NLRP1 inflammasome in GM.
For the first time, we showed that infected red blood cells induce NLRP1- and Caspase-1-dependent pyroptosis in BeWo trophoblast cells, identifying a novel inflammasome pathway involved in GM pathogenesis.
Our study identifies a genetic variant underlying NLRP1 contribution to GM and suggests that NLRP1 may be an underexplored inflammasome receptor in malaria and infected erythrocytes sensing.

Fig 1.



Poster session

P 023

CRISPR screening reveals novel E3 ubiquitin ligases mediating ZAKα-dependent NLRP1 activation in keratinocytes

Andreas Dumortier[1], Seth Masters[2], James Vince[1], Cassandra Harapas[3], Amber Alsop[1], Katja Hrovat[1], Paul Baker[2], Shouya Feng[2], Matthias Geyer[4]

[1]Walter and Eliza Hall Institute, Inflammation Division, Parkville, Australia
[2]Hudson Institute, Clayton, Australia
[3]German Rheumatology Research Center (DRFZ), Berlin, Germany
[4]Bonn University, Institute of Structural Biology, Bonn, Germany

NLRP1 is an innate immune sensor predominantly expressed in the skin. It is activated by diverse stimuli, including ultraviolet B (UVB) radiation and ribotoxins, which induce ribosome stalling and trigger a ribotoxic stress response (RSR) mediated by the kinase ZAKα. Upon activation, ZAKα and its downstream effector p38 phosphorylate the intrinsically disordered linker region of NLRP1 (1), leading NLRP1 inflammasome formation.
Proteasomal degradation of the auto-inhibitory N-terminal fragment (NT) of NLRP1 is essential for releasing the C-terminal fragment (CT) and assembling the NLRP1 inflammasome. However, the precise mechanism by which ZAKα-driven phosphorylation accelerates NT degradation remains unclear (1, 2, 3).
To address this, we used automated robotics to perform an arrayed CRISPR knockout screen targeting 646 E3 ubiquitin ligases in human keratinocytes and quantified NLRP1 inflammasome activation using high-throughput microscopy. Using N/TERT cells expressing ASC-GFP and Cas9, we knocked out each E3 ligase with lentiviral-delivered gRNAs in duplicates. The cells were then stimulated with anisomycin, a ribotoxin known to induce ZAKα-dependent NLRP1 activation (1). Automated high-throughput microscopy allowed quantification of ASC speck formation as a functional readout of inflammasome activation.
Our findings reveal that knockout of specific E3 ligases, among which FBXO11, completely abolishes NLRP1 inflammasome formation, implicating these ubiquitin ligases as critical regulators of NLRP1 activation.
These results provide new insights into how the ubiquitin-proteasome system regulates NLRP1 inflammasome activation. While further studies are needed to determine whether these E3 ligases directly target NLRP1 NT for degradation, our results highlight key candidates that may link ZAKα signaling to NLRP1 activation, paving the way towards future therapeutic targets.
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P 024

Impact of IL-10, and IL-33, polymorphisms on atherosclerosis risk

Ammar Ali Deeb[1], Kawther Amawi[1], Amjad Hamedallah[1], Tareq Al Ramadneh[1]

[1]Zarqa University, Allied Medical Science, Zarqa, Jordan

This study investigates the relationship between polymorphisms in the IL-10 and IL-33 cytokine genes and their association with atherosclerosis in a Jordanian population. Previous research indicates that genetic variations in these cytokines may influence disease progression. Our analysis revealed a significant prevalence of the T allele and T/T genotype at the IL-33 rs7044343 position in the control group compared to patients, suggesting a potential protective effect against coronary artery disease (CAD). Although no significant differences were found in IL-33 genotype frequencies between groups, deviations from Hardy-Weinberg equilibrium indicated possible population stratification. Furthermore, serum levels of IL-33 were significantly lower in atherosclerosis patients, aligning with its proposed protective role. For IL-10, the -1082 A>G (rs1800896) polymorphism displayed borderline significance in genotype distribution, indicating its potential involvement in atherosclerosis progression. Our findings underscore the importance of understanding genetic diversity and the intricate relationship between cytokine polymorphisms and atherosclerosis. Future studies should further explore the functional implications of these genetic variations and their interactions with environmental factors to develop more targeted prevention and treatment strategies for atherosclerosis in diverse populations.

Poster session

P 025

Endogenous sulfatides trigger the canonical and non-canonical NLRP3 inflammasomes

Cecil Zera Otieno[1], Paula Gutierrez Fajardo[1], Joan Anyango Otieno[1], JM Elisabeth van Hooij[1], Tamar Zelig[1], Iris Ben Dror[1], Clinton J. Bradfield[2], Iain D.C Fraser[2], Tsaffrir Zor[1]

[1]Tel Aviv University, Biochemistry and Molecular Biology, Tel Aviv, Israel

[2]National Institute of Health, National Institute of Allergy & Infectious Diseases, Laboratory of Immune System Biology, Bethesda, MD, United States

Macrophages sense LPS through TLR4 and caspase-11/4/5, initiating pro-inflammatory activities aimed at pathogen clearance and tissue damage repair. Also, these receptors detect DAMP molecules from damaged cells, initiating sterile inflammation. Recently, we reported C16:0-sulfatide as a natural ligand for the TLR4/MD-2 complex, mimicking LPS and acting as an agonist and antagonist in murine and human macrophages, respectively. We examined whether sulfatides mimic LPS also in activation of caspase-11/4/5. Cell-delivered sulfatides stimulate IL-1α/β release in TLR2-primed human and murine macrophages. This trigger led to the cleavage of caspase-11 and GSDMD, increased PI uptake, suggesting pyroptotic cell death. The release of IL-1β was dependent on caspase-1, as demonstrated using caspase-1-deficient BMDMs; NLRP3-deficient BMDMs and THP-1 macrophages. Inhibition with the caspase-1 inhibitor VX765 and NLRP3 inhibitor MCC950 blocked sulfatide-stimulated IL-1β release and significantly reduced IL-1α release. Notably, neither the deletion of caspase-11 nor the dual knockout of caspase-4/5 abrogated sulfatide-stimulated IL-1β release, in contrast with LPS-stimulated responses. Interestingly, neither the single knockout of caspase-1 nor caspase-11 inhibited sulfatide-stimulated IL-1α release, whereas LPS-stimulated activity predominantly required caspase-11. Strikingly, dual knockout of caspase-1/11 completely abolished IL-1α release in response to sulfatide. In conclusion, sulfatides trigger the NLRP3 inflammasome in TLR2-primed human and murine macrophages both via caspase-11-independent and -dependent mechanisms. This activity results in GSDMD pores formation, IL-1α/β release and pyroptotic cell death.

P 026

Human NLRC4 Serves as the Direct Sensor for Cytosolic Flagellin

Gaopeng Li[1], Xiao Liu[1], Nadya Panagides[1], Florian Ingo Schmidt[2], Clare Elizabeth Bryant[3], Liudmila Andreeva[1], Alexander N. R. Weber[1]

[1]University of Tübingen, Institute of Immunology, Department of Innate Immunity, Tübingen, Germany

[2]University of Bonn, Institute of Innate Immunity, Medical Faculty, Bonn, Germany

[3]Addenbrooke's Hospital, Department of Medicine, Cambridge, United Kingdom

For immune defense against bacteria, humans employ PRRs (pattern recognition receptors) expressed by host immune cells to detect MAMPs (microbe-associated molecular patterns) and then initiate immune responses. Flagellin, a critical component of the bacterial motility apparatus, is a MAMP recognized by both TLR5 (Toll-like receptor 5) and intracellular receptors: Cytosolic Naip5/6 (NLR family apoptosis inhibitory protein 5/6) are well established as murine cytosolic flagellin receptors that induce conformation-dependent activation of the Nlrc4 (NLR family CARD-containing protein 4) inflammasome, leading to pyroptosis or interleukin-1 family cytokine release. However, human NAIP has mainly been characterized as a receptor for cytosolic needle protein, a component of bacterial type III secretion systems, whereas its role as a flagellin sensor is far less clear. We therefore sought to investigate potential mechanisms underlying cytosolic flagellin sensing in human cells. Immunoprecipitation from transfected HEK293T cells revealed that human NLRC4, and not NAIP, bound to different flagellins directly, most strongly Legionella FlaA. Ectopic FlaA expression also induced NLRC4 oligomerization and IL-1β release in the absence of NAIP. Unexpectedly, the presence of NAIP diminished both the binding of NLRC4 to flagellins and IL-1β release. Similarly, a stable interaction between NAIP and NLRC4 was detectable in resting THP-1 macrophage-like cells. Moreover, in the absence of NAIP, THP-1 responses to FlaA were increased but still completely NLRC4-dependent. Collectively, our data highlight the intriguing possibility that in human cells NLRC4, and not NAIP, is the direct sensor for cytosolic flagellins, whereas NAIP acts as a negative regulator of flagellin sensing.

Poster session

P 027

Cold exposure influence innate immunity against LPS-induced inflammation by modulating TLR4 pathway

Chen Min-Hui[1], Yuan-I Chang
[1]National Yang Ming Chiao Tung University, Institute of Physiology, Taipei, Taiwan

In recent years, climate change has caused severe and extreme challenges, with weather-related issues occurring frequently. Additionally, frequent exposure to cold environments, such as cold-water swimming, ice swimming, and cold air, has raised concerns about the effects of cold on the human immune response. Therefore, our research focuses on exploring the effects of long-term moderate-to-low temperature cold exposure on the immune response of wild-type (WT) mice. The results indicate that, compared to the thermoneutral (TN) group, chronic cold exposure (CC) led to a reduction in the proportion of myeloid cells in the bone marrow, spleen, and peripheral blood. Subsequently, through cell experiments, CD11b+ cells were isolated and placed in a mildly cold environment, revealing that Toll-like receptor 4 (TLR4) appears to be involved in the process, with significant changes in the cytokines produced by immune cells. After simulating infection with lipopolysaccharide (LPS), the downstream pathways and immune responses demonstrated that long-term cold exposure helps regulate the immune system, promotes immune tolerance, inhibits excessive inflammatory responses, and may improve the control of chronic inflammation or autoimmune diseases. These findings contribute to understanding of human defense mechanisms against various degrees of cold exposure and provide insights for future cold therapy.

P 028

NLRP3 is a thermosensor that is negatively regulated by high temperature

Rebecca Coll[1], Wei Wang[1], Damien Bertheloot[2], Junya Zhang[3], Marcia Munoz[4], Shangze Xu[3], Amelia Stennett[3], Chloe McKee[1], Ryan Knight[1], Melanie Cranston[1], Bernardo Franklin[2], Michael Rogers[4], Agnieszka Bronowska[3]
[1]Queen's University Belfast, Wellcome-Wolfson Institute for Experimental Medicine, Belfast, United Kingdom
[2]University of Bonn, Institute of Innate Immunity, Bonn, Germany
[3]Newcastle University, School of Chemistry, Newcastle, United Kingdom
[4]UNSW Sydney, Garvan Institute of Medical Research, Sydney, Australia

Inflammasome signalling drives local inflammation and systemic responses like fever, but our understanding of how inflammasome signalling is negatively regulated is limited. Mutations in the inflammasome sensor NLRP3 cause Cryopyrin Associated Periodic Syndromes (CAPS) characterised by recurrent fevers. A subgroup of NLRP3 mutations cause Familial Cold Autoinflammatory Syndrome (FCAS) where NLRP3 activation is triggered by cold temperature. In health NLRP3 is activated by a vast number of stimuli and senses perturbations of cytoplasmic homeostasis. As temperature is a fundamental environmental stressor, we hypothesised that NLRP3 inflammasome signalling would be sensitive to increased temperatures.

We investigated the effects of high temperatures on NLRP3 in macrophages. Short-term incubation at high fever range temperatures inhibits NLRP3 activation, while secretion of inflammasome-independent cytokines is much less affected. High temperature blocks NLRP3 inflammasome formation, and NLRP3 is highly sensitive to temperature-mediated inhibition relative to the NLRC4, AIM2, and NLRP1 inflammasomes. Using cellular and in silico assays we show that the effect of high temperature on NLRP3 is protein intrinsic. The activation of NLRP3 is associated with a decrease in the thermal stability of the protein and molecular dynamics simulations identify a peptide in the C-terminal of the FISNA domain (COFI) that undergoes a significant conformational shift at high temperature. Cellular assays demonstrate that the COFI regulates NLRP3 stability and is required for activation.

We demonstrate in vivo that elevation of mouse body temperature negatively regulates LPS-induced inflammatory cytokine production. We further demonstrate that FCAS NLRP3 is attenuated by high temperature. Our studies thus reveal that high temperatures associated with fever limit NLRP3 activity in a classical negative feedback mechanism and identify a novel role for NLRP3 as a protein thermosensor.

Poster session

P 029

Engineering human NLRP1 and NLRP3 inflammasomes in Saccharomyces cerevisiae

Óscar Barbero-Úriz[1], Marta Valenti[1], María Molina[1], Teresa Fernández-Acero[1], Víctor J. Cid[1]
[1]Facultad de Farmacia, Universidad Complutense de Madrid, Microbiología y Parasitología, Madrid, Spain

Inflammasomes are cytosolic multi-protein complexes that mediate the inflammatory response and coordinate host defences against threats that emerge during infections, tissue damage or metabolic imbalances. These hazards are detected by sensor proteins, with two key examples being NLRP1 (Nucleotide-binding oligomerization domain, Leucine-rich repeat and Pyrin domain-containing protein 1) and NLRP3 (NLR family Pyrin domain-containing protein 3). Upon activation, they recruit the adaptor protein ASC (Apoptosis-associated Speck-like protein containing a CARD), which bridges the receptor and the effector protein of the pathway, caspase-1 (CASP-1). CASP-1 remains inactive under basal conditions and becomes activated upon inflammasome assembly.
In this work, we study inflammasome components and different versions of NLRP1 and NLRP3 using Saccharomyces cerevisiae as a platform. We have co-expressed ASC adaptor with diverse versions of the NLRP1 and NLRP3. Interestingly, both proteins co-localize with ASC in speck-like cytoplasmic spots in yeast. Besides, we have over-expressed CASP-1, leading to yeast growth inhibition due to its auto-processing activity.
We have attempted to reconstitute NLRP1 and NLRP3 inflammasomes in yeast by expressing different versions of these receptors, the ASC adaptor and an engineered low-expression version of CASP-1 to prevent its auto-activation in yeast. We have observed that the human NLRP1 inflammasome is specifically activated upon co-expression of NLRP1 Ct fragments, ASC and low-expression CASP-1, causing yeast growth inhibition.
Our approach aims to provide novel humanized yeast-based tools to gain knowledge into the molecular aspects of inflammasome assembly and to screen for new molecules capable of inhibiting this process.
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P 030

NLRP3 directly senses intracellular potassium efflux to initiate inflammation

Lukas Funk[1], Ana Tapia-Abellán[1], Tobias Schäfer[1], Jelena Grga[1], Jane Torp[2], Corinna Gehring-Khav[1], Inga V. Hochheiser[2], María Mateo-Tórtola[1], Helmut Bischof[3], Robert Lukowski[3], Liudmila Andreeva[4], Christopher Farady[5], Martin Frank[6], Matthias Geyer[2], Alexander N.R. Weber[1]
[1]University of Tübingen, Institute of Immunology, Department of Innate Immunity, Tübingen, Germany
[2]University of Bonn, Institute of Structural Biology, Bonn, Germany
[3]University of Tübingen, Institute of Pharmacy, Department of Pharmacology, Tübingen, Germany
[4]University Hospital Tübingen, Department of Internal Medicine VIII, Tübingen, Germany
[5]Novartis Pharma AG, Novartis Institutes for BioMedical Research, Basel, Switzerland
[6]Biognos AG, Göteborg, Sweden

The NLRP3 protein is a pivotal cytosolic sentinel of cellular homeostasis and its activation triggers the assembly the inflammasome, a molecular machinery that drives inflammation in infections, cardiovascular, metabolic and neurodegenerative disease. This broad range of pathophysiological conditions prompting NLRP3 activation correlates with a plethora of triggers, including pore-forming toxins such as nigericin, ATP, crystals and aggregated proteins. The unifying concept of how most triggers induce NLRP3 activation and inflammasome formation is the efflux of intracellular potassium ions (K+) due to compromised plasma membrane integrity. However, a central enigma in the field remains unsolved: whether NLRP3 directly (i.e. independently of other cellular factors) senses the drop in cytosolic K+ concentration and, if so, how? Using limited proteolysis and thermal shift assays, we show that the physiological intracellular concentration of K+ stabilized a compact NLRP3 structure resembling inhibitor-bound NLRP3, whereas low K+ or the presence of a K+-chelator favored an open, more flexible conformation. What we observed for NLRP3 in primary human PBMCs and macrophage-like THP-1 cell lysates also applied to exogenous expression of NLRP3 in Drosophila Schneider 2 cells and, importantly, highly purified NLRP3 proteins. Thus, we concluded that NLRP3 conformationally responds to K+ as a direct sensor. We further mapped K+ sensitivity to the FISNA-NACHT module of NLRP3 and, through molecular dynamics simulations, identified specific regions with K+ binding potential. Collectively, our study shows that, similar to the bacterial cytosolic K+ sensing protein, Kbp, NLRP3 directly responds conformationally to K+ perturbations, and this enables the initiation of inflammasome formation.

Poster session

P 031

Transcriptomic landscape of recurrent fever syndromes enables disease delineation

András Szolek[1], Fehime Eroglu[1,2], Özlem Satirer[2], Jasmin B. Kuemmerle-Deschner[2], Alexander N. R. Weber[1]
[1]University of Tübingen, Institute of Immunology, Department of Innate Immunity, Tübingen, Germany
[2]University Hospital Tübingen, Pediatric Rheumatology and Autoinflammation Reference Center, Department of Pediatrics I, Tübingen, Germany

Recurrent fever syndromes are a subset of autoinflammatory diseases (AIDs) driven by dysregulated innate immunity pathways that are also implicated in broader inflammatory conditions. In the absence of pathogenic mutations distinguishing between AIDs remains challenging due to overlapping clinical phenotypes, complicating both diagnosis and treatment. Identifying disease-specific transcriptomic signatures may enhance classification and inform therapeutic strategies. We performed whole-blood RNA sequencing on 45 patients diagnosed with periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA); familial Mediterranean fever (FMF); cryopyrin-associated periodic syndrome (CAPS); and unclassified AID during both flare and remission. Gene expression profiles were analyzed using differential expression analysis, gene set enrichment analysis (GSEA), gene set variation analysis (GSVA), and Ingenuity Pathway Analysis (IPA). To evaluate disease specificity, we compared our findings with five additional blood transcriptome datasets from other inflammatory conditions, namely rheumatoid arthritis, psoriasis, and surgery-induced systemic inflammation. Despite the absence of known genetic drivers, PFAPA exhibited the most distinct and reproducible transcriptomic signature, independent of clinical severity. The FMF transcriptomic profile closely resembled PFAPA, with minimal uniquely differentially expressed genes, despite its established genetic association with pyrin inflammasome hyperactivation. In contrast, CAPS displayed greater variability in transcriptomic and clinical changes between flare and remission. Our findings indicate that transcriptomic signatures of AIDs are distinct from generalized inflammatory responses. These disease-specific molecular patterns may refine transcriptome-based classification and support precision medicine approaches in the management of autoinflammatory diseases.

P 032

Characterizing inflammasome activation by bacterial amyloid curli complexes from biofilms in dendritic cells

Shrutika Mintri[1], Guangnan Hu[1], Anukriti Mathur[1], Brian Le[1], Kaitlyn Grando[2], Çagla Tükel[2], Katherine A. Fitzgerald[1], Stefania Gallucci[1]
[1]University of Massachusetts Chan Medical School, Department of Medicine, Worcester, MA, United States
[2]Temple University, Philadelphia, PA, United States

Question: Bacteria protect themselves from stresses by embedding in an extracellular matrix to form biofilms. Biofilms from bacterial species such as E. coli and Salmonella contain an amyloid protein, curli that can act as a biofilm-specific PAMP,but the mechanisms involved remain elusive (1). Understanding the innate immune responses to curli may assist in development of new therapeutic targets for treatment of biofilm associated bacterial infections.
Methods: We have utilized biochemical and molecular techniques to study molecular mechanisms involved in inflammasome activation by curli in bone marrow derived dendritic cells (BMDCs) from WT and several genetic knockout mice.
Results: Previously reported in macrophages (2), our own data reveals that both murine DCs (Figure 1A) and human DCs release high levels of IL-1β without undergoing lytic cell death (Figure 1B) in response to curli. Using genetic knockouts of caspases involved in inflammasome activation, we have further characterized their significance in curli mediated inflammasome regulation. Lastly, curli driven IL-1 β release requires GSDMD processing.
Conclusions: Our findings highlight Curli as a novel activator of the inflammasome complex. Interestingly, IL-1 β release with curli is dependent on GSDMD but these cells do not undergo the conventional inflammasome driven pyroptosis. As GSDMD is the executioner of pyroptosis, understanding the mechanism of IL-1β release in the absence of cell death will clarify how curli and more broadly biofilms can hyperactivate DCs, revealing a novel immune strategy induced by bacterial biofilms in infections.

Reference

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Fig 1

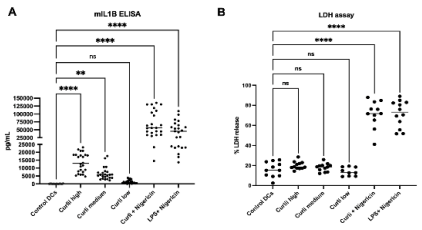


Figure 1: Curli induces IL-1 β release without mediating cell death in wild-type bone marrow derived dendritic cells (BMDCs). (A) IL-1 β ELISA in cell supernatants from DCs stimulated with PBS (control), titrating doses of Curli (high, medium, low) for 22hrs or Curli (3hrs) + Nigericin (2hrs) or positive control LPS (3hrs) + Nigericin (2hrs). **(B)** Lactate Dehydrogenase (LDH) was measured in DCs supernatants to assess cell death after treatment with similar conditions as (A).

Poster session

P 033

Linking FHR-3/1 deficiency to malaria severity – insights from a ghanaian cohort

Gabriele Pradel[1], Lucy Wahler[1], Alliyah Byrd[1], Timo Reiß[1], Abdullatif Rnjbal[1], Julius Müller[1], Anna Bachmann[2]
[1]RWTH Aachen University, Cellular and Applied Infection Biology, Aachen, Germany
[2]Bernhard-Nocht Institute for Tropical Medicine, Cellular Parasitology, Hamburg, Germany

Plasmodium falciparum, an obligate intracellular parasite, is the causative agent of malaria tropica, which leads to over 597,000 deaths annually. Malaria presents with symptoms such as fever, headaches, body aches, and diarrhea, while severe cases can result in anemia, cerebral malaria, multiple organ failure, and ultimately death. Over the course of host-parasite evolution, P. falciparum has developed mechanisms to evade human complement attacks, including binding to the complement regulator Factor H (FH). Previously, we demonstrated that in cultured P. falciparum, Factor H-Related Protein 1 (FHR-1) competes with FH for binding sites, thereby preventing FH-mediated immune evasion. Given that up to one-third of the African population carries a CFHR-3/1 gene deletion, we investigated a potential link between FHR-1 deficiency and malaria severity.
Our analysis was based on data from a previous cohort study of Ghanaian children infected with P. falciparum (Timmann et al., 2012: doi: 10.1038/nature11334). All participants provided informed consent before being categorized according to malaria severity—coma, anemia, acidosis, multisymptomatic, or asymptomatic. Serum samples were collected and analyzed via Western blot to detect the presence of FHR-1. Among the 300 serum samples tested, approximately one-third of the patients were FHR-1-deficient, particularly those with multisymptomatic malaria. Additionally, FHR-1-deficient patients, especially children under one year of age, exhibited poorer hematological parameters. While our previous studies on smaller cohorts suggested that FHR-1 deficiency correlated with improved hematologic values and a milder disease course, our current findings indicate an association between FHR-1 deficiency and more severe malaria progression.

P 034

Immunomodulatory role of CD69 on neutrophil functions

Lisa Krafft[1], Johannes Roth[1], Thomas Vogl[1]
[1]University of Münster, Institute of Immunology, Münster, Münster, Germany

The receptor CD69 is involved in the regulation of immune responses in many cells including T cells and monocytes through a variety of mechanisms. Ligands are the alarmins S100A8 & S100A9, which comprise around 40% of neutrophil cytosolic proteins. S100A8 and S100A9 form heterodimers, whose activity is short-lived, locally restricted and abrogated by tetramerization in the presence of calcium. Recent studies have shown that while the dimers exhibit a pro-inflammatory response via TLR4, the binding of S100A8/A9 tetramers to CD69 leads to dampened monocyte dynamics. With the functions of S100A8/A9-dimers and TLR4 being well documented, the effect of the CD69-S100A8/A9-tetramer binding on neutrophil functions remains unknown. To investigate this, we created immortalized ER-Hoxb8 neutrophils of murine wildtype, CD69 KO and S100A9 KO cells. Here, we show that CD69 is absent on resting neutrophils but upon TLR4 activation with low doses of LPS, the expression is quickly upregulated from an intracellular CD69 pool. Further, we show that CD69 deficiency results in reduced ROS production, phagocytosis and transmigration, whereas S100A9 KO neutrophils have increased responses compared to the wildtype. To analyze the effect of the extracellular alarmin complexes, we added S100A8/A9-dimers and tetramers before inducing ROS production. An increased response is induced by S100A8/A9-dimers binding to TLR4, whereas the S100A8/A9-tetramers have no effect on effector functions likely due to the absence of CD69 on the cell surface. Thus, we next induced the CD69 expression with LPS prior to the incubation with the S100A8/A9-tetramers. We observed that the S100A8/A9-tetramers dampens the response of wildtype and S100A9 KO but not CD69 KO neutrophils. In future experiments, we will perform ATAC and bulk mRNA sequencing to further gain insight into the underlying mechanisms that determine the opposite functions of the S100A8/A9 dimers and TLR4 versus tetramers and CD69.

Poster session

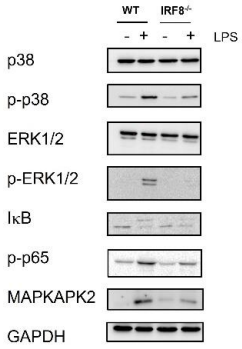
P 035

Regulation of the function and phenotype of neutrophils by interferon regulatory factor 8

Janna Carina Grimm[1], Laura Polmann[1], Johannes Roth[1], Katarzyna Barczyk-Kahlert[1]
[1]University Münster, Institute of Immunology, Münster, Germany

Sepsis is a poorly understood condition with a high mortality rate. Neutrophils are the most prevalent type of circulating leukocytes performing effector functions to eliminate threads causing systemic infections. The NF-kB and mitogen-activated protein kinase (MAPK) pathways regulate neutrophil responses to proinflammatory mediators like LPS. The transcription factor interferon-regulatory-factor-8 (IRF8) plays a pivotal role during differentiation of myeloid cells, directing development into monocytes while inhibiting neutrophil differentiation to ensure homeostasis. Mice lacking IRF8 display enhanced neutrophil amounts and survive longer during endotoxic-shock.
Our aim is therefore to investigate the IRF8 mediated impact on neutrophil phenotype and function during systemic infections. Neutrophil functions with a focus on cytokine secretion, ROS production and phagocytosis in steady state and in response to LPS were examined via ELISA, RT-qPCR and flow cytometry and potentially altered pathways were analyzed via Western Blot.
HoxB8 and primary IRF8ko neutrophils exhibited significantly lower cytokine levels as well as transcription of TNF-α, IL-1β, IL-6 and IL-12a compared to WT counterparts upon LPS treatment. HoxB8 and primary neutrophils in WT further exhibited significantly higher ROS levels and phagocytic capacity than IRF8ko equivalents leading to the hypothesis of a hyporesponsive state in IRF8ko neutrophils. Analysis of NF-kB and MAPK pathway components revealed a defective activation of p38 and ERK1/2 and revealed lower p65 phosphorylation in HoxB8 and primary IRF8ko-neutrophils upon LPS stimulation compared to WT counterparts. This indicates significant alterations in the activation and signaling transduction of MAPK and NF-kB signaling pathways upon LPS stimulation.
Identification of molecular events is required to elucidate the role of IRF8 in the function and phenotype of neutrophils and may be beneficial for developing novel sepsis therapies.

Fig 1.



P 036

An alternative mechanism for the detection of formylated peptides by innate immune cells

Jennifer Doberaß[1], Bernd Bufe[1], Markus Bischoff[2]
[1]University of Applied Sciences Kaiserslautern, Department of Informatic and Microsystems Technology, Zweibrücken, Germany
[2]Saarland University Medical Center, Institute for Medical Microbiology and Hygiene (IMMH), , Homburg, Germany

Formylated peptides that are present in the supernatants of bacterial cultures represent one of the oldest pathogen-associated molecular patterns (PAMPs) known in innate immunity. Formyl peptide receptors (Fprs) are currently the only identified receptors capable of recognising formylated peptides, such as bacterial signal peptides (SPs). However, chemosensory cells in the lung of mice can sense formylated peptides via a yet unidentified independent mechanism (Perniss et al. Immunity 2020). The identification of its molecular basis is difficult because the majority of mammals possess Fprs. However, Fprs are absent in the genome of all Cetaartiodactyla species i.e. cloven-hoofed animals and whales. Consequently, immune cells of such animals offer ideal conditions for investigating alternative recognition mechanisms for formylated peptides. We therefore isolated peripheral blood mononuclear cells (PBMCs) from pigs and analyzed their innate immune responses to ten different bacterial SPs using a combination of high-throughput microscopy, binding studies, chemotaxis and calcium imaging experiments. While challenging the porcine PBMCs with these SPs did not alter the calcium release, they were able to induce a migratory response to all bacterial SPs in low nanomolar concentrations. Pertussis toxin inhibited the SP-induced PBMC migration, indicating a Gi/o protein-dependent recognition mechanism. Taken together, our data demonstrate that bacterial SPs can trigger at low concentrations an innate immune response via a yet unidentified GPCR in pigs. We now plan to use RNA-Seq and Cell Painting assays to further investigate the molecular basis of this mechanism that might also exist in Fpr-positive mammals.

Poster session

P 037

Impact of chronic idiopathic neutropenia on neutrophil serine proteases – insights into innate immune dysfunction

Angelika Mazur[1], Joanna Skrzeczynska-Moncznik[1], Irene Mavroudi[2], Christina Maria Perraki[2], Helen Papadaki[2], Joanna Cichy[1]
[1]Jagiellonian University, Immunology, Kraków, Poland
[2]School of Medicine & University Hospital of Heraklion, Hematotolgy, Heraklion, Greece

Haematopoiesis is a tightly regulated bone marrow process, and its disruption can alter blood composition, including leukocyte count and phenotype. One such condition is neutropenia, characterized by reduced neutrophils, which are critical for innate immunity. Neutrophils store serine proteases (e.g., elastase, cathepsin G, proteinase 3) essential for antimicrobial defence. Studies on congenital neutropenia show that elastase mutations impair protease expression, degranulation, and NET formation. We hypothesized that acquired neutropenia similarly alters neutrophil function by affecting serine protease expression, localization, and activity.
To test this, we isolated circulating neutrophils from patients with chronic idiopathic neutropenia (CIN) and healthy controls. Flow cytometry and ELISA were used to assess the levels and immunostaining of key serine proteases (e.g., elastase, cathepsin G) and their primary inhibitor, SLPI. Additionally, proteolytic activity was quantified using fluorometric assays.
Our results revealed significant differences in serine protease immunostaining between neutropenic patients and healthy controls, with the most pronounced changes observed in elastase. A subset of patients also displayed altered proteolytic activity. Interestingly, while the total amount of elastase remained unchanged in neutrophils, its serum levels were reduced in CIN patients. Moreover, no differences were observed in the immunoreactivity of PBMCs, and the proportion of low-density neutrophils (LDNs) was comparable between groups.
These findings suggest that neutropenia affects neutrophil function beyond cell count reduction, altering serine protease expression and activity. This may impact degranulation, NET formation, and immune regulation. Overall, this study provides new insights into how CIN alters innate immunity, paving the way for further research to improve clinical management and reduce infection risk in neutropenic patients.

P 038

Inflammatory monocytes (not neutrophils) command venous blood clotting and clot resorption

Christian Becker[1], Fatemeh Shahneh[1], Verena Katharina Raker[1]
[1]University Hospital Münster, Dermatology, Münster, Germany

Despite the fact that thrombosis represents the most prevalent cause of mortality on a global scale, the cells and mechanisms involved are only partially understood. Using inflammatory monocyte-deficient mice, adoptive transfers and reporter animals we show in a venous thrombosis mouse model that
· peripheral blood Ly6Chi inflammatory monocyte counts (but not splenic monocytes) correlate with clot frequencies and sizes,
· Inflammatory monocyte numbers determine clot growth and resolution speed,
· A single transfer of tissue factor (TF)-competent Ly6Chi monocytes restores clotting in mice with low monocyte counts,
· Reduction in the number of inflammatory monocytes through forced peripheral monocyte conversion by treatment with a NR4A1 agonist reduces venous thrombus formation in wild-type mice,
· Forced peripheral monocyte conversion accelerates monocyte-mediated clot resorption and is accompanied by faster myeloid differentiation in thrombi,
· Neither mice with low peripheral monocyte counts nor NR4A1 agonist-treated mice showed changes in neutrophil numbers,
· NR4A1 agonists enforce human CD14+CD16neg monocyte conversion in vitro
We conclude that the number of inflammatory Ly6Chi monocytes controls deep vein thrombosis formation, growth, and resolution and can be therapeutically manipulated with a NR4A1 agonist at all disease stages.

Poster session

P 039

Soluble uric acid suppresses neutrophil-mediated host defense in sepsis

Stefanie Steiger[1], Quibo Li[1], Juliane Anders[1], Kailey Flora[1], Liang Yang[1], Louisa Ehreiser[1], Carolin Wendling[2]
[1]LMU Hospital Munich, Division of Nephrology, Department of Internal Medicine IV, Munich, Germany
[2]LMU Munich, Max von Pettenkofer Institute, Munich, Germany

Neutrophils are essential in host defense and sterile inflammation. However, neutrophil dysfunction is a hallmark of acquired immunodeficiency in kidney disease, contributing to an increased susceptibility to infections such as peritonitis, sepsis, and pneumonia. We speculated that the impaired renal clearance of the metabolite soluble uric acid (sUA) may account for neutrophil dysfunction. Indeed, hyperuricemia (HU, serum UA of 9-14 mg/dL) related or unrelated to kidney disease significantly exacerbated the inflammatory immune response to infection by impairing neutrophil functions in mice with mono- and polybacterial sepsis. This aggravated inflammatory response was partially reversible by lowering UA levels with febuxostat. We validated these findings in vitro using either neutrophils or serum from healthy individuals or patients with kidney disease-related HU. Depleting UA partially restored the defective phagocytic capability and oxidative burst. Mechanistically, sUA impaired the phagocytic capability, bacterial clearance and oxidative burst in neutrophils by modulating cytoskeletal dynamics and degranulation, processes essential for host defense. However, sUA had no impact on neutrophil extracellular trap formation in neutrophils from healthy subjects exposed to LPS or E.coli. Our findings reveal an unexpected immunoregulatory role of HU related or unrelated to kidney disease in exacerbating the inflammatory response during sepsis. This effect is primarily mediated by impaired neutrophil phagocytosis, pathogen clearance, and oxidative burst. Targeting UA may help to overcome the acquired immunodeficiency during infection, while aggravating sterile forms of inflammation.

P 040

The complement protein C3b facilitates erythrocyte invasion by Plasmodium falciparum merozoites

Alliyah Byrd[1], Timo Reiß[1], Lucy Wahler[1], Gabriele Pradel[1]
[1]RWTH Aachen University, Cellular and Applied Infection Biology, Aachen, Germany

The parasite Plasmodium falciparum causes severe malaria, which is particularly acute in the WHO African region. With over 597,000 deaths each year, malaria is still one of the deadliest infectious diseases in the world and has a dramatic impact on childhood mortality in endemic countries.
Clinical manifestations of Plasmodium falciparum infection are induced by the asexual stages that develop inside erythrocytes. Following red blood cell invasion, the parasites develop within 48 h from the ring stage to the mature schizont, which form ~32 new merozoites. These are then released into the bloodstream to rapidly reinvade new red blood cells. During this phase, the parasite faces two critical challenges: evading the immune system, while successfully invading erythrocytes.
Our previous research revealed that P. falciparum hijacks factor H to evade complement-mediated lysis, while replicating in erythrocytes. Now we have uncovered a further proliferation strategy involving proteins of the complement system: P. falciparum utilizes C3b to facilitate its invasion of erythrocytes. Using merozoite invasion assays, we showed that C3b, but not inactivated C3b, significantly enhances erythrocyte invasion by P. falciparum in an otherwise complement-free system, a finding reinforced by growth assays showing increased parasitemia.
Experiments conducted in a C5-deficient serum system, which allows C3b activation without subsequent formation of the terminal complement complex and thus cell lysis, confirmed that C3b facilitates the invasion process in a concentration-dependent manner.
We hypothesize that P. falciparum exploits C3b to enhance its binding to the erythrocyte surface, potentially utilizing interactions between C3b, the erythrocyte receptor CR1, and the merozoite surface protein PfRh4. Further investigations will focus on characterizing these interactions to uncover their precise role in parasite invasion.

Poster session

P 041

The C-terminal domain of NLRP10 controls aggregation and signalling

Timo-Daniel Voß[1], Adrian Beck[1], Christoph Winterberg[2], Matthias Geyer[2], Thomas Kufer[1]
[1]University of Hohenheim, Institute of Nutritional Medicine - Immunology, Stuttgart, Germany
[2]University of Bonn, Institute of Structural Biology, Bonn, Germany

NOD-like receptor (NLR)-proteins are pattern recognition receptors, which contribute to both innate and adaptive immune responses in mammals. The pyrin domain (PYD)-containing NLRP10 is highly expressed in human and mouse skin. Unlike the other human NLR-proteins that contain a PYD, NLRP10 lacks the leucine-rich repeat (LRR) domain, usually associated with pattern recognition. NLRP10 contributes to bacterial-induced inflammatory response and recent data now suggest that NLRP10 can also form an inflammasome in epithelial cells upon mitochondrial stress. The function of NLRP10 and its activation mechanism(s), however, are still poorly understood. To investigate the activation of NLRP10, we generated stable cell lines in a NLRP10-knockout background with inducible expression of human and murine eGFP-NLRP10. Treatment of these cells with the mitochondrial damaging agent m-3M3FBS and analysis by live-cell imaging showed formation of cytosolic NLRP10 complexes for both human and murine NLRP10. Aggregation dynamics thereby differed between human and murine NLRP10. Human and mouse NLRP10 differ in their C-terminal domain. We showed that the C-terminus of NLRP10, but not its PYD, was required for NLRP10 oligomerisation. Deletion of distinct C-terminal regions inhibited complex formation of human and murine NLRP10. Moreover, we identified specific interactors of activated human NLRP10, that link its function to different cellular signalling pathways. In ongoing work, we now explore the functional and structural details of the C-terminus of NLRP10 and of evolutionarily conserved amino acids in this domain.
Taken together, our results showed that the C-terminus of NLRP10 is necessary for protein aggregation upon activation and evolutionary sequence diversity in this domain might define functional differences in NLRP10 from different species.

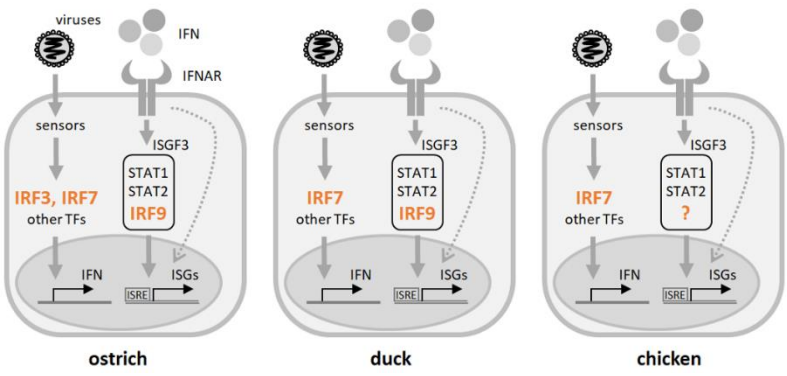
P 042

Birds do it their way – avian interferon regulatory factors (almost) resolved

Lenka Ungrová[1], Josef Geryk[1], Marina Kohn[2], Dana Kučerová[1], Veronika Krchlíková[1], Tomáš Hron[1], Vladimír Pečenka[1], Petr Pajer[1], Eliška Gáliková[1], Ľubomíra Pecnová[1], Bernd Kaspers[2], Jiří Hejnar[1], Jiří Nehyba[1], Daniel Elleder[1]
[1]Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic
[2]Ludwig-Maximilians-University, Faculty of Veterinary Medicine, Department of Veterinary Science, Planegg-Martinsried, Germany

Interferon regulatory factors (IRFs) are key transcription factors in vertebrate antiviral immunity. IRF3 and IRF7 activate expression of IFN-β which is together with other type I interferons responsible for forming ISGF3, a complex of STAT1, STAT2 and IRF9. This complex then binds in the nucleus to the interferon-stimulated response element (ISRE) and activates expression of interferon-stimulated genes (ISGs). While well-studied in mammals, limited information is available in birds, where IRF9 and IRF3 are supposedly missing.
Using de novo assembly, we were able to annotate IRF3 in paleognath birds and IRF9 in multiple avian species. We examined the role of duck IRF9 in the IFN pathway and its role in regulation of ISGs expression after interferon stimulation. Our results show a decrease in the expression of ISGs in the absence of duck IRF9. Additionally, we tested the effect of duck IRF9 deficiency following a viral infection. In duck IRF9 knockout (KO) cells, we observed reduced inhibition of the cytopathic effect after IFN treatment and infection.
Interestingly we were not able to annotate IRF9 in chicken or other galliform birds. To explore potential alternative proteins in the ISGF3 complex we used mass spectrometry. Analysis of potential interactors with STAT2 after interferon stimulation suggested IRF8 as a possible substitute. To test this hypothesis, we generated a chicken IRF8 knockout cell line and we observed decreased ISGs expression after interferon stimulation. A luciferase promoter assay further revealed that in chicken, ISGs transcription activation depends on both ISRE and IRF8. Additionally, we predicted the ISGF3 complex structure in chicken with IRF8, supporting the hypothesis that IRF8 has a regulatory role in the interferon pathway. These findings suggest a possible role for IRF8 in the chicken type I IFN response in contrast to other vertebrates, highlighting the need for further investigation of its function and evolutionary significance.

Fig 1.



Poster session

P 043

Design and characterization of a novel FPR1 targeting bacterial signal peptide radiotracer for radiopharmaceutical applications

Zukaa Altaleb[1], Bernd Bufe[1], Mark Bartholomä[2]
[1]University of Applied Sciences Kaiserslautern, Department of Informatics and Microsystems Technology, Zweibrücken, Germany
[2]Saarland University, Medical Center, Department of Nuclear Medicine, Germany

Formyl peptide receptors (FPRs) are G protein-coupled receptors that are enriched on the cell surface of several tumor types, including glioblastoma, colon cancer and ovarian cancer. Bacterial signal peptides represent a large group of high-affinity ligands for FPR. In this study, we evaluated the potential of bacterial signal peptides, a relatively unexplored class of high-affinity FPR1 agonist, for radiopharmaceutical applications.
We first used calcium imaging and confocal microscopy to test a selection of bacterial signal peptides and their fluorescently labeled derivatives to identify peptide residues that allow chemical modification without a significant loss of affinity. We then developed a selective fluorescent peptide derivative with over 1000-fold selectivity toward FPR1, which binds rapidly at low nanomolar concentrations. This probe forms stable receptor-ligand complexes that persist for up to 72 hours and effectively penetrate spheroids of human glioma U87 cells. Additionally, we designed a formylated signal peptide radiotracer by introducing a metal chelator (DOTA) via a 6-aminohexanoic acid spacer (Ahx), enabling the radiolabeling with the positron-emitter 68Ga and the beta-emitter 177Lu. Notably, the corresponding metal-chelate-peptide conjugate exhibited similar affinity and was efficiently taken up by both FPR1-transfected HEK293T cells and naturally FPR1-expressing U87 cells. A pilot study using planar scintigraphy in a healthy mouse showed no significant uptake or retention in healthy organs, suggesting that this peptide probe could serve as promising tools for radiopharmaceutical development.
Taken together, our findings suggest that radioligands targeting FPR1 could provide a unique opportunity to track FPR1 expression and tumor associated FPR1 activity in vitro and in vivo. This study holds strong candidate for molecular imaging techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT).

P 044

Characterize the temporal dynamics of immunophenotypes using mass cytometry in immunotherapeutic-treated hepatocellular carcinoma

Ying-Po Yang[1], Jang Jang Jin[2], Sepideh Babaei[1], Tamara Jasmin Krpicak[1], Simona Ursu[4], Sarah Warth[4], Jens-Peter Betz[1], Jonas Bochem[1], Christine Geisler[1], Janine Spreuer[1], Michael Bitzer[1], Ruth Ley[5], Nisar Peter Malek[1], Manfred Claassen[1], Kilian Wistuba-Hamprecht[2], Ronald Keller[5], Benjamin Ruf[1]
[1]University Hospital Tübingen, University of Tübingen, M3 Research Center, Tübingen, Germany
[2]University of Tübingen, Department of Computer Science, Faculty of Science, Tübingen, Germany
[3]M3 Forschungszentrum - Universitätsklinikum Tübingen, , Tübingen, Germany
[4]University of Ulm, Core Facility Cytometry, Faculty of Medicine, Ulm, Germany
[5]Max Planck Institute for Biology Tübingen, Microbiome science, Tübingen, Germany

Introduction: Hepatocellular carcinoma (HCC) remains a leading cause of cancer-related mortality, characterized by high recurrence rates and limited treatment efficacy. Although immune checkpoint blockade (ICB) has enhanced patient outcomes, therapeutic resistance remains a challenge due to the immunosuppressive nature of the tumor immune microenvironment (TiME). This study seeks to analyze the components of the TiME and identify immune phenotypes that may play a critical role in mediating ICB resistance in advanced HCC. Methods: We analyzed PBMCs from 34 patients with advanced HCC treated with atezolizumab (anti-PD-L1) and bevacizumab (anti-VEGF), with samples collected before treatment and three weeks after initiation. Patients were categorized as responders or non-responders based on their six-month tumor outcomes. A 32-color mass cytometry panel was designed for frozen PBMC samples, complemented by an in-house single-cell analysis pipeline to identify immune phenotypes associated with ICB response and overall survival. Results: Mass cytometry profiling of $CD45^+$ cells from HCC patients identified distinct immune phenotype clusters, highlighting significant immune dynamics in ICB-treated HCC. These analyses comprehensively characterized the dynamic changes in innate, innate-like, and adaptive immune cell subsets throughout combination immunotherapy. Our in-house single-cell analysis pipeline offers critical insights into the evolving immune landscape of HCC under ICB treatment, potentially guiding the development of more effective, personalized immunotherapeutic strategies. Conclusion: This study explores dynamic changes in the circulating tumor environment of HCC patients undergoing combination immunotherapy. By characterizing these immune phenotype dynamics, this study provides key insights into resistance mechanisms and might pave the way for novel immunotherapeutic strategies to enhance treatment efficacy in HCC.

Poster session

P 045

Development of cellular and nanobody-based tools to unravel the regulation of GSDME

Lena Wolf[1], Sabine Normann[1], Florian I. Schmidt[1]
[1]University of Bonn, Institute of innate immunity, Bonn, Germany

Gasdermin E (GSDME) is a ubiquitously expressed member of the gasdermin family, which can all assemble pores when the N-terminal domain is released from the inhibitory C-terminal domain. This causes pyroptosis, a highly inflammatory type of programmed cell death. GSDME, is evolutionary older than the best-studied family member GSDMD, but remains incompletely understood. GSDME is cleaved and thus activated by caspase-3, the executioner caspase of apoptosis, and thus changes the mode of cell death from immunologically silent apoptosis to proinflammatory pyroptosis. How GSDME is regulated is unclear and the level of GSDME expression in cells may determine the mode of cell death. In line with this, GSDME is silenced in many tumors, but well expressed in somatic cells. This makes it an intriguing yet complex target for cancer therapy. To address the molecular regulation and assembly of GSDME pores, we are developing and screening GSDME-specific nanobodies and will display first characterizations. These nanobodies will be employed to inhibit or activate GSDME pore formation, image GSDME to identify its target membranes, and explore therapeutic applications. We have meanwhile also established cellular systems to quantify GSDME-induced pyroptosis with overexpressed and endogenous GSDME.
By advancing our understanding of GSDME function and regulation, this research aims to contribute to new mechanistic insights and the development of novel therapeutic strategies targeting inflammation, cancer or GSDME-mediated hearing loss.

P 046

Profiling chemokine receptor expression on unconventional T cells of healthy donors

Sarah Beyer[1], Nicola Herold[1], Jens-Peter Betz[1], Noah Schmidt[2], Kristin Bieber[3], Janine Spreuer[1], Benjamin Ruf[1], Kilian Wistuba-Hamprecht[2]
[1]University Tübingen, M3 Research Center for Malignome, Metabolome and Microbiome, Faculty of Medicine, Tübingen, Germany
[2]DKFZ, German Cancer Research Center, Heidelberg, Germany
[3]University Hospital Tübingen, University of Tübingen, Department of Internal Medicine, Tübingen, Germany

Introduction: Chemokine receptors play a crucial role in regulating T cell trafficking to tissues, with their expression patterns potentially determining the successful infiltration of tumors by T cells. Accurately quantifying the expression of multiple chemokine receptors on immune cells is essential for understanding their potential functions and migration routes to various tissues. However, the chemokine receptor expression, particularly on unconventional T cells (γδ T cells, mucosal-associated invariant T (MAIT) cells, and natural killer T (NKT) cells), remains poorly understood.
Methods: Here, we established an advanced 22-marker spectral flow cytometry antibody panel to assess chemokine receptor, memory differentiation and replicative senescence marker expression across conventional αβ T cells, MAITs (MR1 tetramer), NKT cells (CD1d tetramer) and γδ T cells (Vδ1, Vδ2, Vδ3).
Results: Based on own previous work and literature research, we selected informative chemokine receptors and established their quantification on unconventional and conventional T cells (CD4, CD8, MAITs, NKTs, γδ T cells) and their subsets that can further be classified using memory differentiation- and senescence markers (CD45RA, CD27, CD28, CD57). Multiple antibody clones and fluorophore conjugations were compared and various stimulation experiments with isolated T cell populations performed. PD-L1 expression was used to identify potential regulatory unconventional T cell subsets.
Conclusions: The antibody panel described here will enable us to study comparatively chemokine receptor, memory differentiation-, senescence and checkpoint receptor expression profiles on carefully selected subsets of conventional and unconventional T cells in, for example, peripheral blood and tumor infiltrating lymphocytes (TILs). Thereby, we aim at generating novel insights in migrations routs of T cells in tissues and deviate putative functional patterns including regulatory features in malignant tissues.

Poster session

P 048

Uridine diphosphate promotes the differentiation of monocytes into dendritic cells

Caterina Giordano[1], Debora Gentile[1], Emilio Straface[1], Costanza Maria Cristiani[1], Raffaella Gallo[1], Antonio Abatino[1], Ilenia Aversa[1], Selena Mimmi[1], Iaccino Enrico[1], Camillo Palmieri[1], Giuseppe Fiume[1]

[1]Magna Græcia University of Catanzaro, Department of Experimental and Clinical Medicine, Catanzaro, Italy

Uridine diphosphate (UDP) is an extracellular nucleotide signaling molecule that regulates biological processes through the pyrimidinergic receptor P2Y6. Recently, we found that UDP is highly enriched in melanoma tumor interstitial fluid, leading us to study its role in immune response. We found that PBMC treatment with UDP reduced CD14+ monocyte proliferation and increased the rate of HLA-DR+ CD11c+ cells, suggesting that UDP induces monocyte differentiation into dendritic cells. To confirm this, we analyzed the differentiation of CD14+ monocytes into dendritic cells induced by GM-CSF and IL-4, with or without UDP. The addition of UDP significantly increased the number of differentiated immature dendritic cells, as shown by an increase in CD14- and HLA-DR+ CD11c+ cells compared to treatment with the cytokines alone. We then induced dendritic cell maturation using a cytokine cocktail (IL-6, IL-1β, Poly I:C, TNF-α) with or without UDP. These experiments revealed that UDP increased not only the percentage of HLA-DR+ CD11c+ cells but also CD80+ CD86+ mature dendritic cells.

Then, we assessed that UDP treatment influences the functionality of PBMCs by enhancing the phagocytosis of both E. coli bacteria and necrotic cells (efferocytosis). Additionally, we studied gene expression kinetics induced in PBMCs treated with UDP, identifying eight distinct gene clusters activated. Bioinformatics analysis highlighted the involvement of transcription factors such as NF-κB, IRF3, and BATF as primary regulators. ELISA and EMSA confirmed increased activity of NF-κB subunits p65, p50, RelB, and p52, and Western blot analysis showed increased p-ERK, p-STAT1, and p-STAT3.

In conclusion, our findings highlight that UDP may play a key role in shaping immune responses within the tumor microenvironment by regulating immune cell differentiation and function, paving the way for new therapeutic strategies. Whether or not this function is mediated by P2Y6 activation needs to be further evaluated

Fig 1.

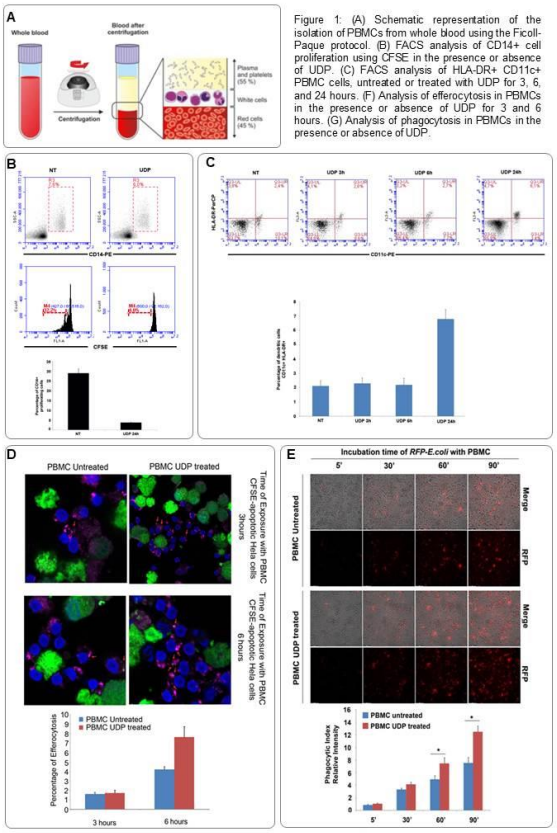


Figure 1: (A) Schematic representation of the isolation of PBMCs from whole blood using the Ficoll-Paque protocol. (B) FACS analysis of CD14+ cell proliferation using CFSE in the presence or absence of UDP. (C) FACS analysis of HLA-DR+ CD11c+ PBMC cells, untreated or treated with UDP for 3, 6, and 24 hours. (F) Analysis of efferocytosis in PBMCs in the presence or absence of UDP for 3 and 6 hours. (G) Analysis of phagocytosis in PBMCs in the presence or absence of UDP.

Fig 2.

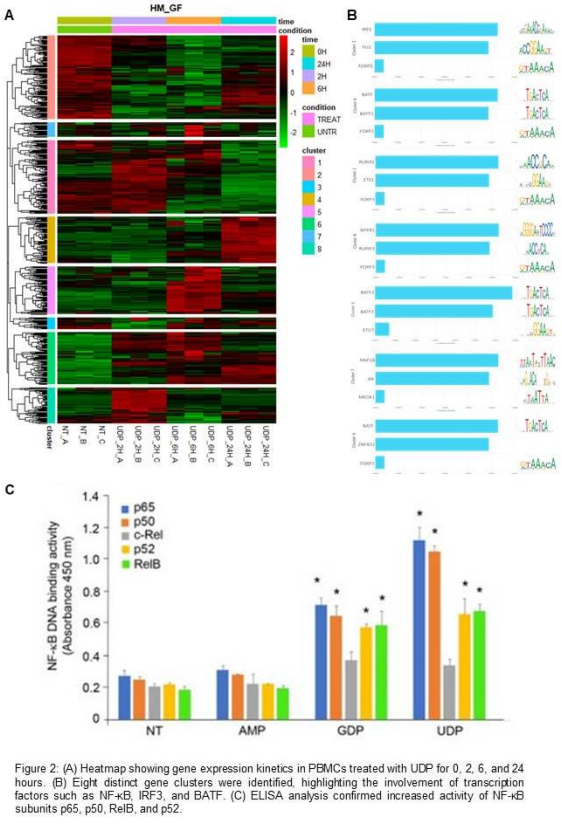


Figure 2: (A) Heatmap showing gene expression kinetics in PBMCs treated with UDP for 0, 2, 6, and 24 hours. (B) Eight distinct gene clusters were identified, highlighting the involvement of transcription factors such as NF-κB, IRF3, and BATF. (C) ELISA analysis confirmed increased activity of NF-κB subunits p65, p50, RelB, and p52.

Poster session

P 050

How the microbiome shapes S. aureus colonization of healthy and inflamed skin

Jule Riebelmann[1], Birgit Schittek[1], Nicole Kienzle[1]
[1]University Hospital Tübingen, University of Tübingen, Dermatology, Tübingen, Germany

Introduction: Staphylococcus aureus (S. aureus) is the main cause of bacterial skin infections, yet it is typically absent from healthy human skin. Skin barrier defects, such as those seen in atopic dermatitis, can favour S. aureus colonization. We have previously shown that in healthy skin, commensal bacteria protect against S. aureus skin colonization. This protective effect depends on the integrity of the skin barrier. This study investigates the microbiome's protective mechanism in healthy skin and their absence in inflamed, barrier-compromised skin as seen in AD patients.
Methods: We analyzed immune responses induced by microbiome-secreted factors in human keratinocytes, skin explants, and 3D skin models using Legendplex analysis, RT2 Profiler PCR arrays, and western blotting to identify signaling pathways. To understand how microbiome-secreted factors reduces S. aureus colonization, we used inhibitor studies targeting especially the aryl hydrocarbon receptor (AHR) pathway. To study the effect of the microbiome on inflamed skin, we used human skin explants with induced barrier defects through tape-stripping, and 3D human skin reconstructs with an AD-like phenotype.
Results: In healthy skin, microbiome secreted factors induce a protective immune response, promote an anti-inflammatory environment, enhance antimicrobial defences, and strengthen the skin barrier. Bacteria-conditioned medium pretreatment reduces S. aureus colonization and its immune effects, partly via AHR signaling. However, in inflamed skin, these factors worsen skin inflammation and trigger the secretion of damage-associated molecular patterns (DAMPs) in the skin.
Conclusion: These data suggest that in healthy skin, skin secreted factors of the skin microbiome protect against S. aureus skin colonization by promoting a strong anti-inflammatory and antimicrobial environment, partially through AHR signaling. In contrast, in inflamed skin, these factors exacerbate inflammation, potentially contributing to skin barrier defects and further promoting S. aureus colonization.

P 051

Ki-67-Mediated regulation of NET formation through PAD4 inhibition and chromatin remodeling

Sangeetha Shankar[1], Jason Scott Holsapple[1], Nuria Andrés-Sanchez[2], Olga Schevchuk[3], Anna Lívia Linard Matos[4], Meike Steinert[1], Ana-Bella Aznar[2], Liliana Krasinska[2], Oliver Soehnlein[4], Sebastian Kruss[5], Daniel Fisher[2], Luise Erpenbeck[1]
[1]University of Münster, Department of Dermatology, Münster, Germany
[2]University of Montpellier INSERM, Institut de Génétique Moléculaire de Montpellier CNRS, Montpellier, France
[3]University of Essen, Proteomic facility, Essen, Germany
[4]University of Münster, Institute for Experimental Pathology (ExPat), Centre for Molecular Biology of Inflammation (ZMBE), Münster, Germany
[5]Ruhr University Bochum, Department of Chemistry and Biochemistry, Bochum, Germany

Neutrophils employ a remarkable arsenal of host defence mechanisms, including the formation of neutrophil extracellular traps (NETosis). NETosis involves the release of chromatin decorated with antimicrobial factors to trap and eliminate pathogens and relies on the enzyme peptidyl arginine deaminase 4 (PAD4) that citrullinates histones and enables chromatin decondensation. Many chronic inflammatory diseases in humans such as systemic lupus erythematosus (SLE), are characterized by increased or dysregulated NET formation, although the molecular mechanism behind this increase remains mostly elusive. Previous studies have demonstrated that neutrophils, although terminally differentiated, can hijack and express components of the mitotic machinery such as cyclin-dependent kinases 4 and 6 (CDK4/6) and the proliferation marker Ki-67, the role of which remains entirely enigmatic in the context of NET formation. In this study, we explore the role of Ki-67 in regulating NET formation and chromatin decondensation in both healthy individuals and patients with SLE. Our findings indicate that in healthy human and mouse neutrophils, Ki-67 accumulates upon NETosis induction while this accumulation is significantly reduced in SLE patients. Mouse neutrophils lacking Ki-67 show significantly increased NETosis rates upon stimulation. Our study suggests that, during NETosis induction, Ki-67 sequesters PAD4—a key enzyme in NET formation—by acting as a decoy target. The presence of Ki-67 appears to delay the access of histones, thereby impeding the onset of chromatin decondensation. Although these findings require further studies, downregulation of neutrophilic Ki-67 accumulation likely leads to enhanced PAD4 availability in SLE and may explain the accelerated NETosis in these patients. Thus, our results provide a novel rationale for non-enzymatic regulation of an important immunological mechanism through a protein that was previously regarded solely as a cell-cycle protein.

Poster session

P 052

SLPI protein as a potential regulator of neutrophil maturation and migration

Ivan Sinkevich[1], Joanna Cichy[1], Patrycja Kwiecinska[2], Joanna Skrzeczynska-Moncznik[1], Pawel Majewski[1], Monika Majchrzak-Gorecka[1], Michal Santocki[3], Elzbieta Kolaczkowska[3]

[1]Jagiellonian University, Department of Immunology, Kraków, Poland
[2]Jagiellonian University, Laboratory of Stem Cell Biology, Kraków, Poland
[3]Jagiellonian University, Laboratory of Experimental Hematology, Kraków, Poland

Background: Neutrophils are key effectors of the innate immune system, and their development and function are tightly regulated by various factors, including Secretory Leukocyte Protease Inhibitor (SLPI). This multifunctional protein is primarily recognized for its anti-inflammatory properties. However, our research demonstrates that SLPI also plays a significant role in regulating neutrophil maturation and migration.

Methods: SLPI knockout (KO) mice from two age groups (8–10 weeks and 14–16 weeks) were used in this study. Neutrophil maturation was analyzed using flow cytometry with surface markers c-kit and Ly6G to distinguish five developmental stages: myeloblast, promyelocyte, myelocyte, metamyelocyte, and mature neutrophil. Additionally, CD16/32, CD34, and CD48 markers were used to examine the Granulocyte-Macrophage Progenitor (GMP) population. Neutrophil migration was first assessed in fresh-frozen mouse skin tissue via fluorescence microscopy, utilizing specific markers: Ly6G, CD45, and Hoechst. These data were complemented by intravital microscopy-based skin imaging in early stages of psoriasis development.

Results and Conclusions: SLPIKO mice aged 14–16 weeks exhibited an increased number of mature neutrophil forms compared to wild-type mice, whereas no differences were observed in the 8–10-week-old group. Although both age groups of SLPIKO mice showed a trend toward an increased number of GMP cells, this difference did not reach statistical significance. The enhanced granulopoiesis observed in SLPIKO mice may be attributed to various factors, including altered bone marrow egress efficiency and compensatory mechanisms. Furthermore, under chronic inflammatory conditions in early stages of psoriasis, SLPI KO mice exhibited a delayed influx of neutrophils into the skin compared to WT mice. This aberrant neutrophil migratory behavior in SLPIKO mice may be associated with the role of SLPI in facilitating neutrophil migration across the vascular barrier.

Fig 1.

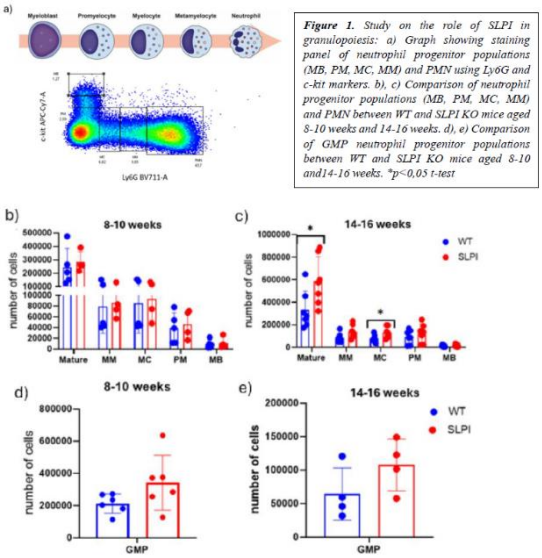


*Figure 1. Study on the role of SLPI in granulopoiesis: a) Graph showing staining panel of neutrophil progenitor populations (MB, PM, MC, MM) and PMN using Ly6G and c-kit markers. b), c) Comparison of neutrophil progenitor populations (MB, PM, MC, MM) and PMN between WT and SLPI KO mice aged 8-10 weeks and 14-16 weeks. d), e) Comparison of GMP neutrophil progenitor populations between WT and SLPI KO mice aged 8-10 and14-16 weeks. *p<0,05 t-test*

Fig 2.

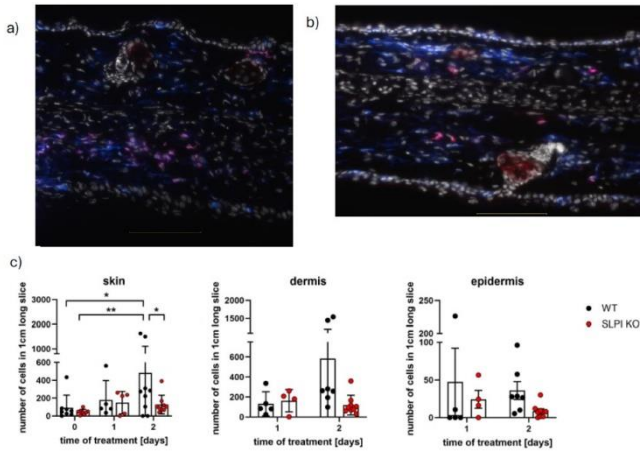


***Figure 2.** a), b) – fluorescence microscopy images. Colors: nuclei stained with Hoechst – white, CD45 – blue, Ly6G – red. a) WT mice ear in early stages of psoriasis, b) KO mice ear in early stages of psoriasis. Scale bar in both cases corresponds to 100 μm. c) results of automatic counting of Ly6G+ neutrophils in different parts of the skin at various stages of psoriasis development using Fiji software*

Poster session

P 054

ZBTB7B (ThPOK) is implicated in macrophage responses to intracellular pathogens

Julia Xiao Xuan Luo[1], Marija Landekic[2], Marine Leroux[3], Rebecca Bellworthy[1], Saumya Agrawal[4], Michiel de Hoon[4], Danielle Malo[2], Martin Olivier[3], David Langlais[1]
[1]McGill University, Human Genetics, Montreal, Canada
[2]McGill University, Medicine, Montreal, Canada
[3]McGill University, Infectious Diseases and Immunity in Global Health Program, Montreal, Canada [4], , ,
[4]RIKEN, Centre for Integrative Medical Sciences, Yokohama, Japan

ZBTB7B (or ThPOK) is a transcription factor well known to be essential for lineage determination in CD4 T cells; however, its role in innate immunity is unclear. Previous work in the lab has highlighted an essential role of ZBTB7B in intracellular pathogen disease models where macrophages are key for resolving infection via CD4 Th1-mediated IFNγ pathways. Thus, we aim to evaluate the role of ZBTB7B in macrophage immunity to intracellular pathogens. A preliminary ZBTB7B ChIP-seq analysis in murine bone marrow derived macrophages (BMDMs) and CD4 Th1 cells revealed approximately 4000 binding sites unique to BMDMs that are not found in Th1 cells. In vivo, we challenged WT and Zbtb7b-/- mice subcutaneously with 5x106 Leishmania major promastigotes in the right-hind footpad for up to 6 weeks. We found that Zbtb7b-/- mice had significantly larger skin lesions, higher parasite load, and sustained neutrophilia with reduced macrophage activation locally in the footpad at 6 weeks p.i. In vitro, we challenged WT and Zbtb7b-/- BMDMs with MOI10 S. typhimurium for 1, 4, 24 hours. We found that Zbtb7b-/- BMDMs have an altered transcriptomic profile with significantly decreased enrichment of autophagy pathways and fail to limit intracellular bacterial proliferation at 4 hours post-infection (p.i.). These findings confirm that Zbtb7b is an important contributor to the murine response to intracellular pathogens and suggest a cell-intrinsic role in macrophages. We are currently conducting additional functional and sequencing studies to understand the mechanism by which ZBTB7B regulates immune responses.

P 056

Skin mast cells require integrin β1 for their perivascular alignment and induction of contact hypersensitivity

Aaron Hoffmann[1], Konstantinos Katsoulis-Dimitriou[1], Jan Dudeck[1], Martin Voss[1], Anne Dudeck[1]
[1]Otto-von-Guericke-Universität Magdeburg, Institute of Molecular and Clinical Immunology, Magdeburg, Germany

Mast cells (MCs) play a pivotal role as innate sentinels by their immediate release of pro-inflammatory mediators, including TNF and histamine. Notably, perivascular MCs critically impact on the onset and kinetics of inflammation by their directional release of mediators into the bloodstream. However, the mechanisms underlying MC attachment to the vessel wall, as a prerequisite for the intraluminal degranulation, remain to be fully elucidated.
Using a conditional knockout in MCs, we studied the relevance of the adhesion molecule integrin β1 (Itgb1) for perivascular MC vessel attachment in vivo and intravascular degranulation upon skin inflammation. Fluorescence microscopy of murine Itgb1-deficient MCs (MCΔItgb1) revealed that Itgb1 is critical for the spindle-like morphology, homogeneous tissue distribution, and perivascular alignment of MCs in the perivascular niche along the blood vessels, particularly at arterioles, at steady state. Moreover, we observed a reduced capacity of MCΔItgb1 to degranulate into the blood vessels during skin inflammation. In the contact hypersensitivity (CHS) mouse model, MCΔItgb1 mice showed a dramatically reduced ear swelling, accompanied by a significant reduction of infiltrating neutrophils, monocytes, macrophages and CD8+ T cells in the ear skin. Notably, MCΔItgb1 mice exhibited an impaired activation of endothelial cells and a reduced immune cell extravasation from blood to the skin upon CHS. Furthermore, the lack of Itgb1 in MCs resulted in an impaired degranulation efficiency in vitro. Our findings reveal the pivotal role of Itgb1 in MC distribution and perivascular alignment along the dermal blood vessels. Additionally, we highlight its significance in MC degranulation capacity and pro-inflammatory functions in CHS responses, suggesting a possible bidirectional cross-talk between ear skin MCs and blood endothelial cells.

Poster session

P 057

Peptide selection for immunoregulatory protein promote IgA production via Th cells

Chang-Chi Hsieh[1], Te-Chia Feng[1], Cheng-Yao Yang[2]
[1]National Chung Hsing University, Graduate Institute of Veterinary Pathobiology, Taichung, Taiwan
[2]Tunghai University, Animal Science and Biotechnology, Taichung, Taiwan

In recent years, policies have focused on decreasing the use of antibiotics in feed additives, resulting in a swift increase in alternative products. Among these, immunomodulatory proteins and peptides have progressed significantly due to their effectiveness and reduced toxicity compared to conventional drugs. Prolactin-induced proteins (PIPs) are found in critical defense areas against pathogens, such as the lacrimal glands (eyes), sweat glands (skin), salivary glands (mouth), and ears. Although the precise roles of PIPs are not fully understood, growing evidence indicates their participation in both innate and adaptive immunity. To pinpoint the most active peptide sequences, an in silico analysis was performed to assess potential cleavage by gastrointestinal (GI) tract enzymes using the Expasy Peptide Cutter program. This evaluation included enzymes like pepsin (at pH levels above 1.3 and 2.0), trypsin (EC 3.4.21.4), and chymotrypsin (EC 3.4.21.1). Additionally, potential peptide synthesis and their interactions with target proteins were investigated through molecular docking studies. Before docking, energy minimization of proteins and compounds was conducted using AutoDock tools and AutoDock 4.2.6. The docking process employed the native binding pockets of homologous proteins, with GROMACS utilized to confirm the binding affinity of the protein-ligand complexes formed during docking. Ultimately, the most active peptide was selected for functional assays in both cell cultures and animal models. Using immunohistochemistry (IHC), enzyme-linked immunosorbent assay (ELISA), and flow cytometry for peptide functional analysis, results demonstrated that these active peptides enhance IgA production through interactions with dendritic cells (DCs) and Th cells, while also assessing T cell distribution via immunohistochemical staining indicated the signaling enhacement.

Fig 1.

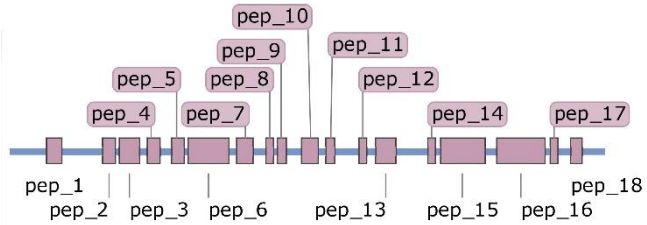
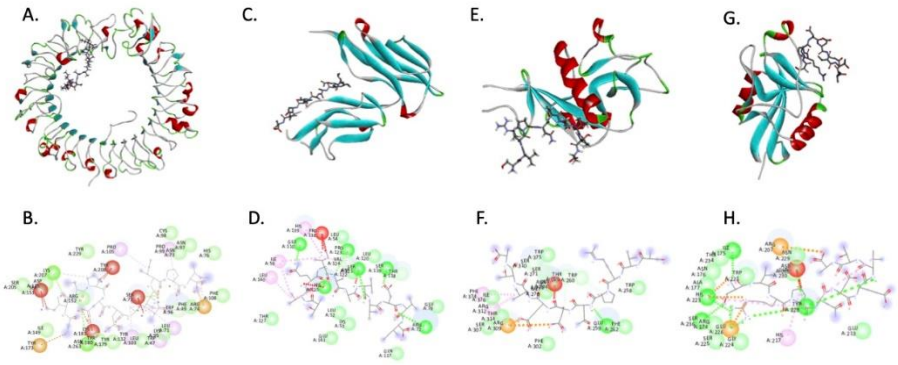


Fig 2.



Poster session

P 058

Innate T cell activities in venous thrombosis

Christian Becker[1], Doga Uncuer[1], Fatemeh Shahneh[2], Verena Katharina Raker[2]
[1]University Hospital Münster, Dermatology, Münster, Germany
[2]University Medical Center Mainz, Dermatology, Mainz, Germany

The capacity to respond independently of T cell receptor (TCR) stimulation to nonspecific danger signals or inflammatory cytokines, a phenomenon termed innate or bystander activation, allows memory T cells and regulatory T cells to participate in any type of inflammatory response.
Thromboses are the number one cause of death worldwide. While the role of innate immune cells in thrombogenesis and thrombus resolution has been known for decades, the possible involvement of adaptive immune cells has long been ruled out.
Employing a venous thrombosis mouse model, adoptive transfers, bulk & sc-RNA-Seq, reporter and TCR transgenic mice, together with selective manipulation of effector-memory T cells and regulatory T cells we provide experimental evidence for TCR-independent T cell activation in thrombosis. In particular we show that
-Effector memory T cells (TEM) infiltrate thrombi and adjacent vein walls, are activated in an antigen-independent manner, and delay thrombus resolution by mutual cytokine-mediated monocyte activation.
-Thymus-derived CD4+Foxp3+ regulatory T (Treg) cells accumulate in venous thrombi, become locally activated through cytokines and control clot resolution by potentiating monocyte recruitment, differentiation and metalloproteinase activity through the matricellular protein, secreted protein acidic and rich in cysteine (SPARC).
Together our observation identify T cells as integral part of the innate sterile immune response in thrombosis.

P 059

Search for metabolic therapeutic targets in a macrophage inflammatory transition model with a systems biology approach

Joel Rojas[1], Rodrigo Mora[2]
[1]University of Costa Rica, Postgraduate Program in Biology, San José, Costa Rica
[2]University of Costa Rica, Faculty of Microbiology, Lab of Tumor Chemosensitivity (LQT), Research Center for Tropical Diseases (CIET), San José, Costa Rica

It has been shown that macrophages can adopt various pro-inflammatory and anti-inflammatory phenotypes characterized by metabolic dynamics that lack clear boundaries but do exhibit a certain degree of overlap, indicating dynamic shifting states. The current consensus on the macrophage activation process is that the different pathways involved exhibit more than one steady state, a phenomenon known as multistability. Despite much work on this topic, there is a lack of experimental results of molecular switches controlling macrophage transitions with bistability in miRNA/TF-regulated gene expression networks.
By characterizing the metabolic fluxes and molecules that robustly control dynamic transitions, the molecular basis of disease progression can be elucidated. This characterization, approached through systems biology, enables the identification of gene expression circuits and the investigation of their role in bistability during pro-/anti-inflammatory transitions in macrophages. Achieving a proper construction of such circuits requires data sources such as proteomics, miRNA and mRNA transcriptomics, as well as data from single-cell experiments. Based on the above, a systems analysis approach to the metabolic networks of M1 and M2 macrophage phenotypes is proposed, with the aim of identifying the hypothesized metabolic switches derived from the loops present in the miRNA/TF interaction networks.
Transcriptomic data were collected from three patients 72 hours after macrophage stimulation for M1 and M2 states, and differential expression values were obtained using the DESeq2 package in R. Genes with an absolute log2FoldChange greater than 1.5 and a p-value less than 0.05 were selected. Those associated with metabolic processes were subsequently selected using the PANTHER Classification System. The selected genes were compared against the genes present in the Human1 genome-scale model (GEM), and only those present in it were retained. Using the 44 selected genes, a model was constructed with miRNA and TFs in the bioinformatic platform BioNetUCR.
The network was loaded into Matlab, where only nodes with more than 10 closed paths (loops) were selected. The network was visualized in Cytoscape after removing nodes in BioNetUCR that did not meet the minimum loop count. Expression values (for both genes and miRNAs from the same three patients) were added to better observe genes with the highest differential expression. Nodes that were not present in the miRNA and mRNA expression data and with a p-value greater than 0.05 were eliminated from the network. The model was later fitted using COPASI 4.45 (Build 298).

Poster session

P 060

The role of hypoxia affecting immune tolerance development in murine BV-2 Cells In Vitro

Alicia Chavero Vargas[1], Trim Lajqi[1], Natascha Köstlin-Gille[1], Reinhard Bauer[2], Christian Gille[1]
[1]Heidelberg University Hospital, Neonatology, Heidelberg, Germany
[2]University Hospital Jena, Institute for Molecular Cell Biology, Jena, Germany

Question: Microglia, the resident immune cells of the central nervous system (CNS), are essential for maintaining brain homeostasis and regulating neuroinflammation, particularly during early development1,2. Establishing immune tolerance in this period is crucial to prevent excessive inflammation and tissue damage3. Neonatal hypoxia, a major risk factor for neurodevelopmental impairments, may influence microglial immune tolerance, but its impact remains poorly understood4.
Methods: We employed a two-hit model using BV-2 microglial cells. Cells were primed with 100 ng/mL LPS on day 1, followed by a resting phase, then exposed to 24 hours of hypoxia during a second stimulation on day 3. Prior to experiments, we determined the optimal hypoxia and LPS conditions through dose- and time-dependent viability assays. Samples were analyzed for inflammatory markers, including cytokines (ELISA) and reactive oxygen species (ROS via H2DCFDA), while metabolic activity (glycolysis) and signaling pathways were assessed by Western blotting.
Results: Hypoxia-exposed cells exhibited an immune-tolerant phenotype, marked by reduced TNF-α, IL-6, and MCP-1 secretion upon LPS re-stimulation. This response was accompanied by lower ROS levels and decreased lactate production, suggesting a metabolic shift. Western blot analysis revealed alterations in immune signaling pathways, further supporting a hypoxia-driven modulation of immune tolerance in BV-2 cells.
Conclusions: These findings indicate that hypoxia modulates microglial immune tolerance, potentially serving a regulatory and neuroprotective function. The underlying mechanisms may enhance our understanding of microglial adaptation to hypoxic stress and its implications for neurodevelopment and inflammation-driven CNS pathologies.

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P 061

Dissecting metabolic control of innate immune sensing of DNA via genetic screens

Matteo Lunghi[1], Alexander Hooftman[1], Andrea Ablasser[1]
[1]Swiss Federal Institute of Technology Lausanne (EPFL), Global Health Institute, Lausanne, Switzerland

Following infection, innate immunity is initiated instantaneously to provide immediate protection and limit pathogen spread. At the front end of immune activation is the detection of pathogen-associated molecular patterns (PAMP) by distinct innate immune receptors. In immune cells, such as macrophages, pattern recognition receptors (PRRs) activate intracellular signalling pathways, leading to the production of pro-inflammatory cytokines, chemokines, and antimicrobial responses. Sustaining this process is a reprogramming of several metabolic pathways, including glycolysis, tricarboxylic acid (TCA) cycle, and fatty acid oxidation. Making use of disease variants and untargeted metabolomics several studies in recent years have identified key enzymes and immunometabolites capable of modulating macrophage biology. How different PRR stimulations affect metabolic rewiring, as well as all the mechanisms through which metabolic rewiring exert its function, still need to be fully elucidated.
In this study, we are leveraging CRISPR-Cas9 forward genetic screens in primary murine macrophages in the context of cGAS-STING mediated sensing of double stranded (ds)DNA and type I IFN signalling. By using gRNA sub-libraries targeting genes in metabolism and epigenetic regulation we are investigating: i. the mechanism of action of methylmalonate (MMA), a novel immunometabolite regulating type I IFN signalling; ii. Metabolic mechanisms underlying regulation of macrophage activation.

Poster session

P 062

Curcumin modulation of liver immune cell infiltration and inflammatory biomarkers in diabetic rat models

Inas Almazari[1], Kawther Amawi[1]
[1]Zarqa University, Clinical Pharmacy, Zarqa, Jordan

Question: This study investigates whether curcumin helps lower blood sugar and manage type 1 diabetes in diabetic rats. It also examines curcumin"s impact on proinflammatory cytokines and immune cell infiltration in the liver, focusing on IL-6, TNF-α, and macrophages.
Methods: Thirty albino rats were divided into three groups: control, diabetic, and curcumin (n=10 each). Type 1 diabetes was induced using an alloxan monohydrate injection. The curcumin group received curcumin post-diabetes induction. After one month, liver tissues were collected for histological and immunohistochemical analysis to assess inflammation markers (IL-6, TNF-α, and macrophages).
Results: Curcumin treatment significantly reduced IL-6 expression (p=0.000), while its effect on macrophages (p=0.415) and TNF-α (p=0.779) was not statistically significant. Biochemical analysis showed that glucose, triglyceride, and cholesterol levels were significantly higher in the diabetic group (p<0.001). Curcumin significantly lowered glucose levels (p<0.001) but had minimal effects on triglycerides and cholesterol. Histological analysis revealed that curcumin reduced diabetes-induced liver inflammation. Immunohistochemical findings confirmed increased TNF-α, IL-6, and macrophages in diabetic rats (p<0.005).
Conclusion: Diabetes leads to liver inflammation and metabolic disturbances. Curcumin effectively reduced glucose levels, liver inflammation, and IL-6 expression but had a limited effect on TNF-α and macrophages. Further research is needed to enhance curcumin"s therapeutic potential in managing diabetes-induced liver damage.

Fig 1.

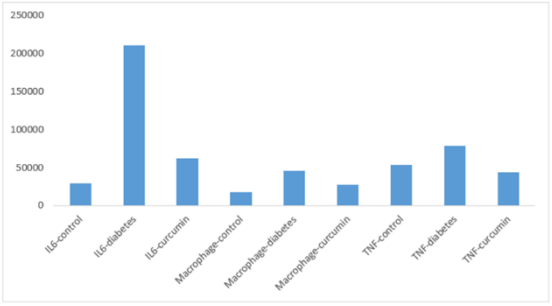


Figure 20: Quantitative Expression of Study Biomarkers in The Study Groups.

Fig 2.

Variable (M±SD)	Control group	Diabetic group	Curcumin group
Glucose (mg/dl)	97.5±8.4	250±24.3*	177.40±23.86*
Cholesterol (mg/dl)	72.4±7.6	130±15.6*	122.87±14.65
Triglyceride (mg/dl)	84.8±8.5	120.4±18.8*	114.76±16.37

P 063

Secretory leukocyte protease inhibitor (SLPI) as a regulator of macrophage-mediated immune responses

Mariia Tyshchenko[1], Anne F. McGettrick[2], Natalia Pocałuń[1], Kamila Kwiecień[1], Patrycja Kwiecinska[1], Anna Grochot-Przęczek[1], Joanna Cichy[1], Mieszko M. Wilk[1], Luke A. J. O'Neill[2], Ewa Oleszycka[1]
[1]Jagiellonian University, Faculty of Biochemistry, Biophysics and Biotechnology, Department of Immunology, Kraków, Poland
[2]Trinity College Dublin, Trinity Biomedical Sciences Institute, School of Biochemistry and Immunology, Dublin, Ireland

Secretory Leukocyte Protease Inhibitor (SLPI) is a multifunctional protein primarily recognized for its anti-inflammatory properties and role in maintaining tissue homeostasis. It is expressed in myeloid cells, where it modulates inflammatory signalling by inhibiting excessive pro-inflammatory responses. However, despite its known immunoregulatory functions, the specific expression patterns and roles of SLPI within distinct macrophage populations remain poorly defined. Moreover, the identification of molecules capable of enhancing SLPI expression and production under pathological conditions can hold a therapeutic potential.
To elucidate the role of SLPI in macrophage-mediated immune regulation, we employed a SLPI-knockout mouse model. Our investigation of tissue-resident macrophage populations revealed that peritoneal macrophages represent a primary source of SLPI. Notably, we identified SLPI as a negative regulator of matrix metalloproteinase-9 (MMP-9) expression. Furthermore, its expression was dynamically modulated in response to in vivo administration of LPS, particularly within various myeloid populations, including monocytes. Moreover, we demonstrate that SLPI production is regulated in NRF2-dependent, but in IRG1-independent manner. Additionally, macrophage exposure to itaconate and fumarate derivatives significantly enhanced SLPI expression and secretion. Preconditioning macrophages with immunomodulatory metabolites prior to immune stimulation showed their potential to modulate inflammation through SLPI-dependent pathway.
Overall, our findings redefine the role of SLPI, highlighting its function in macrophage-driven tissue remodelling rather than as a broad-spectrum anti-inflammatory mediator. Furthermore, we propose a novel mechanism by which metabolite derivatives regulate immune responses via SLPI, offering new insights into metabolic-immune crosstalk and potential therapeutic strategies.

Poster session

P 064

To investigate the impact of different cold exposure on the immune system

Chen Min-Hui[1], Yuan-I Chang[1]

[1]National Yang Ming Chiao Tung University, Department and Institute of Physiology, Taipei, Taiwan

The immune system is an important defense mechanism of the human body. It involves the self-renewal and differentiation of hematopoietic stem cells in the bone marrow, which are transported through the blood and lymph throughout the body. Its mechanism is divided into innate and adaptive immunity. In recent years, climate change has caused severe and extreme problems. Problems caused by the weather occur frequently. In addition, frequent exposure to cold environments such as cold-water swimming or ice swimming and cold air. The impact of these colds on the human immune response has yet to be determined. It is currently known that Uncoupling Protein 1 (UCP1), a protein related to thermogenesis, is increased due to sympathetic nerve activation during cold exposure. Therefore, in this experiment, we mainly explore the relationship between the body thermogenesis mechanism of wild-type (WT) mice and UCP1 whole-body knockout mice (UCP1 KO) and the impact of different cold exposures on the body's immune response. Our findings indicate that acute cold exposure has a more pronounced effect on UCP1 knockout (KO) mice compared to wild-type (WT) mice. This effect includes a reduction in the production of myeloid-derived cells and myeloid-derived suppressor cells (MDSCs), as well as an increase in helper lymphocytes in peripheral blood. In a Chronic mild exposure environment, the increase in hematopoietic stem cells and precursor lymphocytes is stimulated. Notably, the increase in precursor lymphocytes is more pronounced in UCP1 KO mice. In addition, chronic cold exposure can lead to a reduction in the proportion of hematopoietic cells in the bone marrow and spleen. These physical phenomena help to understand the body's defense mechanism against different degrees of cold exposure and provide information for future cold therapy—new insights.

Fig 1

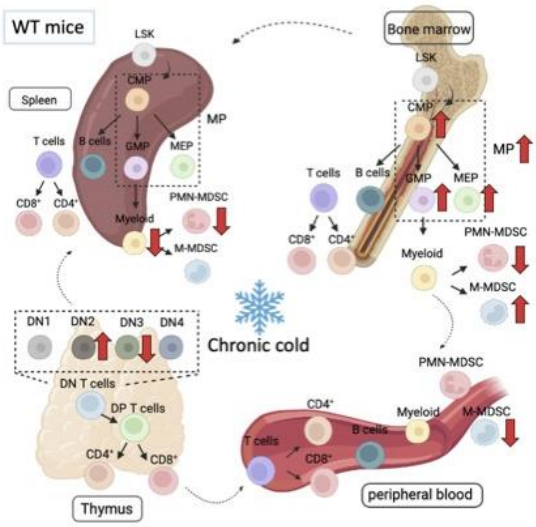
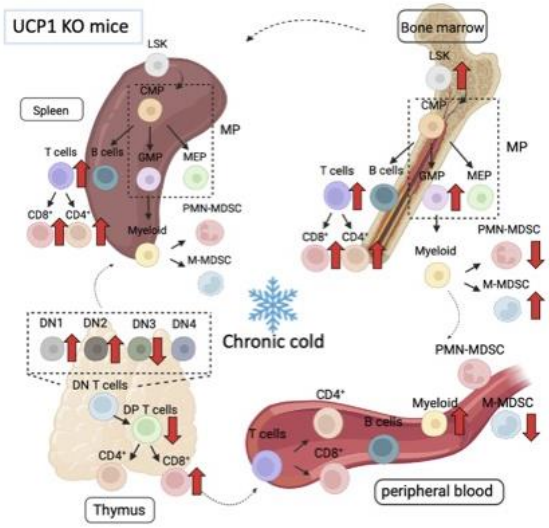


Fig 2.



Poster session

P 065

Metabolic priming of monocytes during endotoxin tolerance

Ricarda Jürgens[1], Nadine Bieß[2], Andrea Gerdemann[2], Matthias Behrens[2], Hans-Ulrich Humpf[2], Judith Austermann[1], Johannes Roth[1]
[1]University of Münster, Institute of Immunology, Münster, Germany
[2]University of Münster, Institute of Food Chemistry, Münster, Germany

Sepsis remains a significant global health threat with a high mortality rate despite advances in medical care. It is therefore necessary to understand the mechanisms underlying sepsis-induced immunosuppression, as deaths often result from insufficient defense against secondary infections during immune paralysis. Key players of the innate immune response are monocytes, which exhibit a dynamic phenotype closely linked to their metabolic profile. This study reveals the role of the antioxidant transcription factor Nuclear factor erythroid-2-related factor 2 (Nrf2) as a central mediator of the hypo-inflammatory state of tolerant monocytes and shows the relevance of glutamine metabolism in the establishment of endotoxin tolerance (ET).
Our findings challenge the current understanding of the IRG1-itaconate axis in ET in macrophages. We demonstrate that IRG1 knockout monocytes still establish ET, suggesting that disruption of the TCA cycle and itaconate accumulation are not essential for ET. Instead, our data indicate that alternative metabolic pathways contribute to the establishment of this hypo-inflammatory state. Further metabolic imbalances associated with ET affect the urea cycle, redox metabolism and mitochondrial health, emphasizing the complex interplay between metabolism and immune function. These imbalances act as potential activators of Nrf2, which dampens the immune response. Inhibition of Nrf2 and glutaminase 1 successfully reverses ET, offering promising therapeutic strategies for sepsis. In conclusion, unraveling the complex metabolic and redox regulation of ET provides valuable insights into sepsis and identifies potential therapeutic targets to restore immune function. These findings underscore that different differentiation stages of monocytes/macrophages and forms of ET rely on fundamentally different metabolic pathways, which may help refine therapeutic approaches targeting immune paralysis in sepsis.

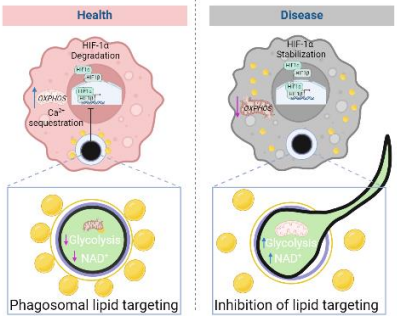
P 066

Rewiring of lipid metabolism upon sensing of fungal melanin is an essential host defense mechanism in alveolar macrophages

Vassilis Nidris[1,2], Ioannis Morianos[1,2], Evangelia Inze[2], Eva Papadogiorgaki[3], Georgios Chamilos[1,2]
[1]IMBB-FORTH, Clinical Microbiology, Heraklion, Greece
[2]University of Crete, Medical school, Heraklion, Greece
[3]University of Crete, Biology, Heraklion, Greece

Mucormycosis is a life-threatening respiratory disease caused by Mucorales fungi that uniquely affects patients with immunometabolic disorders, especially those with diabetic ketoacidosis (DKA), via unidentified mechanisms. Alveolar macrophages (AMs) constantly restrict inhaled Mucorales spores and confer protective immunity against mucormycosis. On the pathogen site, Mucorales spores induce phagosome maturation arrest following their uptake by AMs, via prolonged surface exposure of cell wall melanin. Importantly, the physiological pathways that inhibit Mucorales growth inside AMs remain uncharacterized.
Herein, we found that in contrast to other fungi, Mucorales triggers the selective recruitment of Lipid Droplets (LDs) to the phagosome, via rewiring macrophage metabolism towards oxidative phosphorylation (OXPHOS), upon sensing of fungal melanin. Of interest, free fatty acids (FFAs) derived from LDs accumulate inside Mucorales-containing phagosomes in AMs and inhibit fungal growth. Specifically, physiological FFAs induce mitochondrial damage within min of uptake by Mucorales spores, which results in redox imbalance and fungal growth arrest. Importantly, pharmacological inhibition of either LD formation or OXPHOS metabolism enables intracellular fungal germination. Furthermore, in the DKA mouse model of mucormycosis, stabilization of hypoxia inducible factor 1α (HIF1α) inhibits OXPHOS in AMs, abrogates LDs targeting to the phagosome, and results in invasive fungal growth. Accordingly, conditional deletion of HIF1α in AMs restores OXPHOS-mediated LD targeting to the phagosome and reverses susceptibility to mucormycosis in the setting of DKA. Collectively, our findings reveal a novel immunometabolic host defense mechanism, that relies on physiological lipids for selective inhibition of fungal growth with direct implication in pathogenesis and treatment of mucormycosis.

Fig. 1



Poster session

P 067

Hematopoietic reprogramming in MASH and ACLF – megakaryocytes in focus

Friedrich Reusswig[1], Cristina Ortiz[2], Nico Kraus[2], Bettina Mros[1], Gina Henle[2], Tim Krapoth[2], Emma Gerhold[1], Martina Casari[1], Christoph Welsch[2], Carsten Deppermann[1]
[1]University Medical Center of the Johannes Gutenberg-University, Center for Thrombosis and Hemostasis, Mainz, Germany
[2]Goethe University Frankfurt, University Hospital, Medical Clinic 1, Frankfurt a.M., Germany

Introduction: Metabolic dysfunction-associated steatohepatitis (MASH) is primarily a liver pathology, driving systemic inflammation upon induction of acute-on-chronic liver failure (ACLF). Megakaryocytes (MKs), known as platelet precursors, also show immune and niche supporting roles. Here, we wanted to analyze the impact of local and systemic metabolic dysfunction on megakaryopoiesis.
Methods: We examined a 7-week experimental MASH model in mice, by a high-fat, high-cholesterol diet with CCl4 injections to induce fibrosis. We analyzed bone marrow-derived MKs and blood platelets during this period. To induce ACLF, a single transient non-lethal transnasal stool infection (TNI) was administered to cirrhotic mice, and analyzed 72 hours later.
Results: During MASH progression and ACLF induction, bone marrow progenitors (LSK: $Lin^{-}Sca1^{+}c\text{-}Kit^{+}$) increased, while the hematopoietic stem cell population shifted towards enhanced myeloid output ($LSK^{+}CD48^{+}CD150^{-}$). Interestingly, megakaryocyte-erythroid progenitors population decreased, as MKs underwent phenotypic changes: The number of megakaryocyte progenitors increased, and the proportion of inflammatory MKs ($CD53^{+}$) expanded. MK ploidy analysis revealed a shift towards higher ploidy, indicating altered MK maturation in MASH and ACLF. Bone marrow cytokine profiling showed reduced TGF-β and myeloid growth factors IL-34, M-CSF, and SCF. In MASH mice, platelets initially show reduced glycoprotein expression, which increases after ACLF induction. Platelet activation shifts from enhanced P-selectin externalization in early MASH to a reduced activation response in ACLF.
Conclusion: Our data suggest that increased LSK output and altered niche factors indicate hyperactivation of hematopoietic stem cells (HSCs), driving differentiation toward the myeloid and megakaryocytic lineages. This shift in megakaryopoiesis suggests elevated platelet production and an increase in inflammatory MKs, contributing to the systemic inflammation in ACLF

P 068

Investigating the expression of Cyclin D1 and PAX5 in the liver of diabetic rats

Kawther Amawi[1], Inas Almazari[1]
[1]Zarqa University, Medical Laboratory Sciences, Zarqa, Jordan

Diabetes mellitus is a chronic metabolic disorder characterized by either insufficient insulin production by the pancreas or the inability of the body to effectively utilize the insulin produced, resulting in hyperglycemia (elevated blood glucose levels). The precise mechanisms underlying the onset and progression of diabetes remain unclear and require further investigation.
This study aims to evaluate the expression of PAX5 and Cyclin D1 in the liver tissues of diabetic and non-diabetic control groups. An animal model of diabetes was established by administering alloxan at a dose of 120 mg/kg. Blood glucose levels were monitored daily, and a glucose level of ≥200 mg/dl was used to confirm diabetes. After one month, the experiment concluded, and all rats were sacrificed. Blood samples and liver tissues were collected and preserved in 10% formalin. Biochemical analyses, including glucose and lipid profiles, were conducted, alongside histological and immunohistochemical studies.
The findings revealed a significant increase in blood glucose and lipid profile levels in the diabetic group compared to the control group. Histological analysis showed mild structural alterations in liver tissue. Immunohistochemical results demonstrated a significant upregulation of PAX5 and Cyclin D1 expression in the liver tissue of the diabetic group compared to controls.
The observed overexpression of PAX5 and Cyclin D1 in diabetic liver tissue indicates a potential inflammatory role of hepatic tissue in the pathogenesis and progression of diabetes. These findings suggest that PAX5 and Cyclin D1 could serve as key molecular markers in understanding diabetes-associated liver inflammation.

Fig 1.

Table 1: Biochemical profiles of glucose and lipids in study groups

Variable (M±SD)	Control group	Diabetic group	Significance
Glucose (mg/dl)	97.5±8.4	250 ± 24.3	<0.001
Cholesterol (mg/dl)	72.4 ± 7.6	130 ± 15.6	<0.001
Triglycerides (mg/dl)	84.8±8.5	120.4±18.8	<0.001

Poster session

P 069

Impact of CCR5 on aging-related myelopoiesis and cellular energy metabolism

Shuoh-Wen Chen[1], Yu-Xuan Wu[1], Yu-Suan Wei[1], Yuan-I Chang[1]
[1]National Yang Ming Chiao Tung University, Institute of Physiology, Taipei, Taiwan

Chemokine receptor 5 (CCR5) is integral to the inflammatory response, directing leukocytes to inflammation sites and modulating their activation. CCR5 signaling is critical in elevating IFNγ-dependent bone marrow (BM) failure, particularly in aging when CCR5 expression changes. Aging is highly associated with increased myelopoiesis, linked to a higher incidence of myeloid leukemias and myeloid-derived suppressor cells (MDSCs). However, the role of CCR5 in aging and myelopoiesis remains unclear. Here, our findings indicate that CCR5 expression in BM is lower in older wild-type (WT) mice than in younger ones. Aging-associated pro-inflammatory cytokines were absent in the plasma of CCR5 knockout aged mice. Deficiency of CCR5 increased MDSCs in young mice's peripheral blood (PB) and BM. However, CCR5 loss modulated age-related myelopoiesis, affecting the differentiation of hematopoietic stem cell (HSC) and MDSC production. Moreover, immunosuppressive gene expression also followed a similar pattern. Given the importance of cellular energy metabolism in HSC differentiation, we examined mitochondrial oxidative phosphorylation and glycolytic function in BM. Aging enhanced mitochondrial respiration, ATP production, and glycolytic capacity, but these were reversed by CCR5 knockout. Further, in vitro, CD11b+ myeloid cells treated with single-strand RNA (ssRNA) showed increased pro-inflammatory gene expression in both WT and CCR5 knockout young mice. Nevertheless, CCR5 loss significantly reduced age-induced pro-inflammatory gene expression under ssRNA activation. In conclusion, CCR5 plays a crucial role in aging-related myelopoiesis and inflammation. Its modulation affects hematopoietic differentiation and inflammatory responses. Additionally, CCR5 impacts cellular energy metabolism, reversing age-associated increases in mitochondrial and glycolytic function. These highlight CCR5 as a potential therapeutic target in age-related hematological conditions.

P 070

Investigating the role of DNA-repair protein Artemis in cytosolic DNA sensing

Abigail Burtner[1], Jiayu Chen[1], Brian Ferguson[1]
[1]University of Cambridge, Pathology, Cambridge, United Kingdom

Sensing of viral nucleic acids by pattern recognition receptors (PRRs) is critical for effective generation of host type-I interferon responses. The DNA-PK complex senses DNA in the cytosol and activates downstream innate immune responses via the canonical cGas/STING pathway. This complex of the proteins DNA-PKcs and Ku70/80 also has a well-defined role in the non-homologous end joining pathway (NHEJ) in repairing double-stranded DNA breaks (DSB) and generating antibody and T cell receptor diversity during V(D)J recombination. The contributions of other NHEJ pathway proteins to viral DNA sensing are still unknown. Recent work has found that a mutation in DNA-PKcs, L3062R, acts as a gain-of-function mutation in the cGas/STING pathway in human fibroblasts. This mutation presents clinically as a primary immune deficiency with hyperinflammation and severe-combined immunodeficiency (SCID), but only the latter can be directly explained by DNA-PKcs's role in V(D)J recombination. As the L3062R mutation sits at the interface between DNA-PKcs and another NHEJ protein, Artemis, we hypothesised that Artemis may have a previously unrecognized role in antiviral DNA sensing in the cytoplasm. As such, we set out to determine whether Artemis functions in the DNA-PK/cGAS/STING pathway. We found that Artemis is expressed in the cytoplasm of fibroblasts in the resting state, not just in the nucleus as previously assumed due to its DNA repair function. We then generated Artemis knockout (DCLRE1C-/-) human fibroblasts using CRISPR/Cas9 and used these to assess the impact of loss of Artemis function on sensing of exogenous DNA using qPCR and phospho-blotting of key downstream signaling molecules. This work has implications not only in understanding the innate immune response to DNA viruses (e.g., vaccinia and herpesviruses) but also in understanding the pathology of certain autoimmune diseases caused by DNA-induced sterile inflammation.

Poster session

P 071

Characterization of the transcriptional response to RLR stimulation of epithelial cells

Aleksandr Refeld[1], Guandi Wu[1], Carlos Ramirez[2], Carl Herrmann[2], Marco Binder[1]
[1]DKFZ, German Cancer Research Center, Virus-associated Carcinogenesis, Heidelberg, Germany
[2]Heidelberg University, Institute for Pharmacy and Molecular Biotechnology, BioQuant Center, Germany

RIG-I-like receptors (RLRs), including MDA5 and RIG-I, are essential for detecting viral dsRNA, activating the transcription factor IRF3, and triggering type I and III interferon (IFN) production. IFNs are then secreted and by an autocrine and paracrine signaling through a JAK/STAT cascade drive the expression of a large number of target genes, so-called interferon-stimulated genes (ISG), to establish a potent antiviral state of the cells.
However, our findings in A549 lung epithelial cells harboring functional knockouts of all three types of IFN receptors (IFNAR, IFNGR, IFNLR triple KO, "IFNR-TKO") challenge this canonical notion of sequential events. Upon millisecond electro-transfection of a RIG-I ligand dsRNA, we followed the transcriptional response of IFNR-TKO and wildtype (WT) cells over time. Interestingly, this transcriptomic analysis revealed almost identical transcriptional dynamics between the two cell lines from 4 to 24 hours post-dsRNA stimulation, regardless of the absence or presence of the IFN-JAK/STAT-signaling axis. This suggested that RLR-proximal IRF3 activation suffices to initiate a virtually complete antiviral transcriptional response, including genes that are only transcriptionally induced at late time points (12 – 24 hours). We are currently investigating transcription factors that might modulate or complement the initial IRF3 activity over time.
Interestingly, despite almost identical transcriptional profiles up to 24 hours, IFN-competent WT cells showed a reduced but sustained antiviral signature up to 72 hours post stimulation, whereas IFNR-TKO cells returned to baseline. In the future, we plan to investigate the exact nature of this sustained signature at the epigenetic, transcriptional and also functional level.

P 072

Immune complex activation of endosomal TLRs identifies a TLR7/8 endotype across autoimmune diseases

Stuart Hawtin[1], Cedric Andre[1], Sarah Dyball[2], Simone Appenzeller[3], Bettina Bannert[4], Hermine Brunner[5], Ian Bruce[2], Enrico Ferrero[1], Anne Gernand[1], Diego Kyburz[4], Britta Maurer[16], Richard Siegel[1], Tamas Shisha[1], Jonas Zierer[1], Tobias Junt[1]
[1]Novartis, Immunology, Basel, Switzerland
[2]University of Manchester, Manchester, United Kingdom
[3]University of Campinas, São Paulo, Brazil
[4]University Hospital Basel, Basel, Switzerland
[5]Cincinnati Children's Hospital, Cincinnati, OH, United States
[6]University Hospital Zurich, Zurich, Switzerland

Questions: Systemic autoimmune rheumatic diseases (SARDs) such as mixed connective tissue, Sjögren"s and systemic lupus erythematosus are clinically heterogeneous. Molecularly, they share common features such as expression of autoantibodies (AutoAbs) or IFN stimulated genes (ISGs). Our objective was to identify common molecular drivers of autoantibody-induced cytokine responses across SARDs.
Methods: AutoAbs were measured in sera of >320 patients. PBMCs or isolated cells from healthy donors were stimulated with immune complexes (ICs) of patient sera and necrotic cells or purified antigens, and cytokines measured. Patient blood transcriptomic profiles were probed with a TLR7/8 signature derived ex vivo.
Results: Using well defined inhibitors, we detected the activation of distinct molecular pathways of inflammation by ICs between patient sera and specific autoantigens. Of interest was the interferogenic activity of AutoAbs against ribonucleoproteins (RNPs), SS-A and RNP/Sm. We identified ssRNA as the interferogenic moiety of RNPs, which is protected from RNAse degradation by formation of ICs, and that ICs are internalized via Fcg receptors or phagocytosis by specific immune cells. We show a strong correlation of the interferogenic capacity of Ro60- and RNP/Sm-ICs and the respective AutoAb titers. Using a specific TLR7/8 antagonist MHV3701, we show that RNP-containing ICs activate TLR7 and TLR8 on plasmacytoid dendritic cells and monocytes, respectively. MHV370 was pharmacologically differentiated from other compounds used for SLE therapy, e.g. hydroxychloroquine or inhibitors of IFN signaling, suggesting that TLR7/8 are distinct contributors to pathology. We also detected a TLR7/8 transcriptomic signature in SARD patient blood.
Conclusion: Functional characterization of patient sera uncovered a TLR7/8 driven endotype across SARDs. These translational studies highlight the potential of targeting TLR7/8 and support clinical basket trials.
1Hawtin et al Cell Reports Medicine 2023

Poster session

P 073

P2Y4 modulated pDC cytokine production

Lena Rueschpler[1], Sebastian Schloer[1], Marcus Altfeld[1], Eva Tolosa[1], Friedrich Koch-Nolte[1]
[1]University Medical Centre Hamburg-Eppendorf, Institute for Immunology, Virus immunology, Hamburg, Germany

Nucleotide metabolites such as ATP, UTP and UDP are released during inflammation and act as DAMPs, binding to P2Y receptors as agonists. Interestingly, three of the human P2Y receptors are encoded on the X-chromosome: P2Y4, P2Y8 and P2Y10, of which P2Y4 is the only classical P2Y receptor with clearly defined nucleotide ligands. Through single cell expression profiling, we identified a significantly higher mRNA expression of those Xencoded P2Y receptors on plasmacytoid dendritic cells (pDCs) in females compared to males. We hypothesize that – like Tlr7 – the X-encoded P2RY genes partly escape Xchromosomal inactivation and are thus more highly expressed by female immune cells to potentially counterweight their TLR7-driven stronger pro-inflammatory profile. Using a flow cytometry approach, we investigated the functional relevance of sex-specific differential expression of P2Y receptors. Administration of P2Y4 antagonist resulted in increased production of pro-inflammatory cytokines TNF, IFNa, IL-6 and IL-8 by pDCs upon TLR7 stimulation, consistent with P2Y4-mediated inhibition of inflammation. To reflect the physiologic nucleotide release during inflammation, we furthermore performed in vitro nucleotide pre-treatments of healthy human pDCs and observed a significant downregulation of the pro-inflammatory cytokine production. This effect was more pronounced in female pDCs, suggesting sex-specific regulation. Taken together, these data indicate that the X chromosome-encoded P2Y receptor might mediate stronger inhibitory signals in female cells, and could be therapeutically targeted to either enhance antiviral immune response using selective P2Y antagonists or to dampen overshooting immune reactions using P2Y agonists.

P 074

The role of mitochondrial stress in dermatomyositis

Julian Steininger[1], Laura Mlitzko[1], Kristina Fischer[1], Susann Meisterfeld[1], Birgit Fehrenbacher[2], Martin Schaller[2], Sarah Rösing[1], Claudia Günther[1]
[1]Faculty of Medicine Carl Gustav Carus, Technische Universität Dresden, Department of Dermatology, Dresden, Germany
[2]University of Tübingen, Department of Dermatology, Tübingen, Germany

Dermatomyositis (DM) is a rare autoimmune disease characterized by proximal muscle weakness and skin inflammation. Several studies have found persistently elevated levels of type I interferon (IFN) in different tissues, indicating that DM is an IFN-driven disease. Here we asked whether disturbance of mitochondrial (mt) function can be involved in the induction of IFN in DM.

IncuCyte imaging system showed high overall rates of apoptosis in DM fibroblasts. Morphologically, electron microscopy revealed disrupted mt membranes. Furthermore, inflammation was reduced upon inhibition of Bcl-2-associated X protein (BAX), a key driver of mt outer membrane permeabilization and apoptosis. As performance measurements indicated a reduction in ATP levels, we evaluated the downstream effects of AMP-activated protein kinase (AMPK) as a marker of overall energy deficiency. The levels of pAMPK were significantly enhanced in DM fibroblasts in comparison to healthy controls. These levels were further increased by solar simulated irradiation mimicking UV exposure as a strong trigger of disease. In response to diminished ATP levels, AMPK is activated, stimulating catabolic pathways to enhance ATP production while inhibiting anabolic processes to conserve energy. In line with this, we also observed a significant increase in mitophagy. Quantification of key mitophagy markers (such as PTEN-induced kinase 1) via Western blot revealed markedly elevated levels in DM fibroblasts. Lastly, we could show that fibroblasts from DM patients experience mt stress, leading to mtDNA release that is detected by the cyclic GMP-AMP synthase (cGAS) / stimulator of interferon genes (STING) pathway, resulting in IFN upregulation. In conclusion, these data show evidence of chronic mt stress in patient fibroblasts that can explain low-level cytoplasmic mtDNA release and intrinsic IFN induction as a trigger of disease pathogenesis.

Poster session

P 075

Single stranded DNA sensing is associated with activation of inflammatory and DNA damage responses independently of canonical nucleic acid detection pathways

Roger-Junior Eloiflin[1], Moritz Schüssler[1], Adeline Augereau[1], Nahn Tran[1], Yasmine Messaoud-Nacer[1], Morgane Chemarin[1], Isabelle K. Vila[1], Nadine Laguette[1]
[1]IGMM, Université de Montpellier, CNRS, , Montpellier, France

Cytoplasmic DNA is recognized as danger signal by specific receptors, such as the cyclic GMP-AMP (cGAS) enzyme, which elicits prototypical type I Interferon (IFN) responses through the activation of the STING adaptor protein. The cGAS-STING axis is a central player in anti-tumoral and anti-viral immunity, thus attracting biomedical interest. However, several cell types do not express cGAS and/or STING, raising questions regarding their capacity to mount inflammatory responses to challenge. Furthermore, dsDNAs are the preferential substrates of cGAS, while ssDNAs have been repetitively reported as moieties present in a broad array of inflammatory pathologies.
While cGAS-STING-independent detection of dsDNA has been extensively explored, we here questioned the mechanisms governing – and the phenotypical output of – ssDNA detection. Under natural conditions, exogenous sources of ssDNA can be introduced into the cell by viral infection or damaged cells. During viral replication, ssDNA moieties can find their way into the cytoplasm. Using human and murine cell lines, we were able to establish that stimulation with ssDNA induces activation of a cGAS-independent inflammatory responses, as well as broad activation of DNA damage response pathways. Nucleic acid pulldown assays coupled to quantitative mass spectrometry were conducted to identify ssDNA interactomes that can be expected to comprise the primary sensors eliciting those cellular responses. In parallel, kinase activation assays were conducted to reveal the signaling pathways triggered by ssDNAs. The main targets identified through of these analyses are currently being investigated to delineate how ssDNA shape cellular responses and the associated functional consequences.
I will discuss how this study will bring novel insights into the field of nucleic acid immunity by identifying the key interactors and regulators that shape ssDNA-associated immunity.

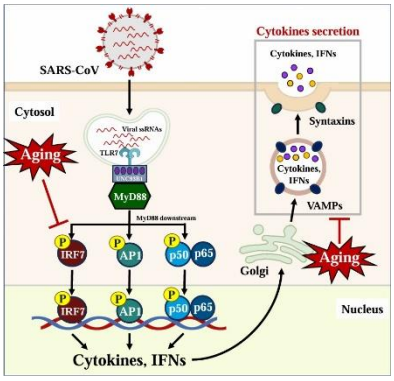
P 076

SARS-CoV ssRNA-induced immune dysfunction in aged mice – cytokine secretory defects and therapeutic rescue by young CD11b+ cell transfer

Yu-Xuan Wu[1], Chih-Wei Hu[2], Jui-Yu Chang[3], Yuan-I Chang[1]
[1]National Yang Ming Chiao Tung University, Institute of Physiology, Taipei, Taiwan
[2]National Defense Medical College, Institute of Preventive Medicine, New Taipei City, Taiwan
[3]National Yang Ming Chiao Tung University, Department of Biotechnology and Laboratory Science in Medicine, Taipei, Taiwan

Aging substantially compromises immune responses to viral infections, resulting in increased disease severity and mortality among elderly populations. We established a novel mouse model utilizing SARS-CoV-derived GU-rich single-stranded RNAs (ssRNAs) to investigate COVID-19-associated and age-dependent immune dysregulation by comparing responses in young versus aged mice.
Our findings reveal that aged mice experience significantly higher mortality rates, severe lung pathology, and prolonged immune recovery following ssRNA challenge. Importantly, we identified a critical mechanism underlying this vulnerability: aged CD11b+ myeloid cells exhibit preserved gene expression but severely impaired cytokine secretion, suggesting post-transcriptional trafficking defects. Molecular analyses uncovered disruptions in both IRF7 signaling pathways and SNARE-mediated vesicular transport machinery, which collectively contribute to the compromised immune responses in aged individuals.
Strikingly, adoptive transfer of young CD11b+ cells into aged mice dramatically rescued survival outcomes, mitigated inflammatory damage, and restored immune homeostasis following SARS-CoV ssRNA exposure. These results demonstrate the therapeutic potential of cellular rejuvenation strategies for addressing age-related immune senescence. Our study provides critical mechanistic insights into the cellular and molecular basis of age-associated viral susceptibility, offering promising targets for therapeutic interventions against SARS-CoV-2 and other viral pathogens affecting vulnerable elderly populations.

Fig 1.



Poster session

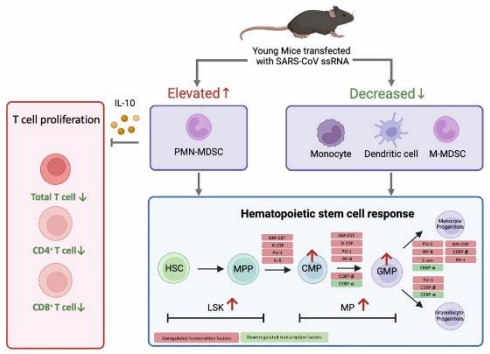
P 077

SARS-CoV ssRNA exposure reveals age-specific myeloid cell reprogramming – PMN-MDSC expansion and enhanced IL-10-mediated immunosuppression in young mice

Yu-Xuan Wu[1], Yuan-I Chang[1], Ching-Jung Teng[2]
[1]National Yang Ming Chiao Tung University, Institute of Physiology, Taipei, Taiwan
[2]National Yang Ming Chiao Tung University, School of Medicine, College of Medicine, Taipei, Taiwan

The emergence of highly pathogenic SARS-CoV strains in 2002 and 2019—with SARS-CoV-2 demonstrating enhanced transmissibility—underscores the critical need to elucidate viral pathogenesis mechanisms and age-dependent immune responses. This study investigates how age influences myeloid cell dynamics following SARS-CoV stimulation, with particular focus on PMN-MDSC functionality and their immunosuppressive effects.Using GU-rich single-stranded RNAs as viral surrogates, we comprehensively analyzed immune cell populations and signaling pathways across different age groups. Our findings reveal that SARS-CoV ssRNAs significantly reduce CD11b+ populations, including dendritic cells and M-MDSCs, while young mice uniquely exhibit robust PMN-MDSC expansion. This preferential myeloid reconfiguration correlates with increased hematopoietic stem cells and myeloid progenitors in younger subjects, accompanied by enhanced expression of critical transcription factors governing myelopoiesis (GM-CSF, G-CSF, IL-6, PU.1, c-Jun, C/EBPβ, and IRF8).Importantly, functional analyses demonstrate that these expanded PMN-MDSCs potently suppress T-cell proliferation, with young mice displaying significantly higher IL-10 expression levels compared to aged counterparts. This age-dependent divergence in PMN-MDSC accumulation and IL-10-mediated immunosuppressive capacity provides mechanistic insights into SARS-CoV immunopathology and may help explain the paradoxical age-related disparities in disease severity observed clinically. Our findings highlight how age fundamentally shapes myeloid responses to viral stimuli and establish a foundation for developing targeted immunomodulatory strategies against coronavirus infections.

Fig 1.



P 078

Fine-tuning the Saccharomyces cerevisiae cellular model for studying the cGAS-STING signaling pathway

Sara López-Montesino[1], María Molina[1], Víctor J. Cid[1]
[1]School of Pharmacy, Complutense University of Madrid, Microbiology and Parasitology department, Madrid, Spain

The cGAS-STING pathway is involved in immune response to cytoplasmic DNA, derived from viral infection or cell damage. Detection of DNA by cGAS generates a cyclic dinucleotide second messenger, cGAMP, which stimulates the adaptor STING to activate the TBK1 kinase and, in turn, the transcription factor IRF3 leading to the secretion of interferon and other inflammatory mediators. Some mutations on STING cause its aberrant activation triggering autoinflammatory diseases such as STING-associated vasculopathy with onset in infancy (SAVI). Saccharomyces cerevisiae has served as a model to understand many physiological processes of the eukaryotic cell. Although yeast do not present a homologous pathway to the mammalian cGAS-STING, in this work we aim to synthetically reconstitute features of this signaling module in the yeast cell.

Human STING, TBK1 and IRF3 genes were expressed under the control of the inducible GAL1 promoter and tagged with fluorescent proteins to assess their subcellular localization. Besides the wild-type versions, non-phosphorylatable versions of STING (S366A), IRF3 (harbouring 8 mutations to alanine in the signal response domain) and TBK1 (S172A), as well as kinase-dead TBK1 (K38A) were tested in the model.

In yeast cells, TBK1 but not its kinase-dead version was toxic. Interestingly, the co-expression of TBK1 with STING, but not with loss-of-function versions relieved TBK1-induced toxicity. STING co-localized with TBK1 at endoplasmic reticulum and both WT STING and IRF3 proteins were phosphorylated by the kinase in this model independently of the presence of cGAS.

We proposed the yeast model as a tool to study this important cell signaling pathway and perform pharmacological screenings to unveil inhibitors of the pathway.

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Poster session

P 079

Role of ATP in MDA5 sequence recognition and its regulation by phosphorylations

David Michalík[1], Aleksander Fedorov[2], Tanja Davis[2], Jan Rehwinkel[2], Carrie Bernecky[1]
[1]Institute of science and technology Austria (ISTA), Klosterneuburg, Austria
[2]University of Oxford, MRC Weatherall Institute of Molecular Medicine, Oxford, United Kingdom

MDA5 is a double-stranded (ds)RNA sensor important for sensing viral RNA through filament formation, activation of the MAVS pathway, and interferon production[1]. Previous reports used E. coli-expressed MDA5 to show that these filaments are destabilized upon ATP hydrolysis[2]. Furthermore, prior structural analysis suggested no direct base reading property[3].
We have identified phosphorylated serines in insect and human cells expressed and MDA5 and characterized them using structural, biochemical, and molecular biology methods. These phosphorylations switch the ATP-dependent protein behavior. Rather than destabilizing MDA5 filaments, ATP leads to an increase in the RNA binding affinity of phosphorylated MDA5 without inhibiting ATPase activity. Despite no direct base specific contact, the sequence of the bound RNA appears to modulate nucleotide occupancy in ATP binding pocket. Furthermore, we have investigated a difference in preferentially bound RNA molecules isolated from virus infected cells based on phosphorylation state and presence of ATP. Here, we report our progress in understanding the cross-talk between post-translational modifications, ATP hydrolysis and RNA sequence on MDA5 dependent signaling.

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Poster session

P 080

Immunomodulatory effects of long-acting rosuvastatin nanoparticles on inflamed macrophages in-vitro

Ramyadevi Durai[1], Petra Kluger[2], Svenja Nellinger[2], Sophia Nowakowski[2], Ramachandran Venkataraman[1], B Narayanan Vedha Hari[1]

[1]SASTRA Deemed University, Department of Pharmacy - School of Chemical & Biotechnology, Thanjavur, India

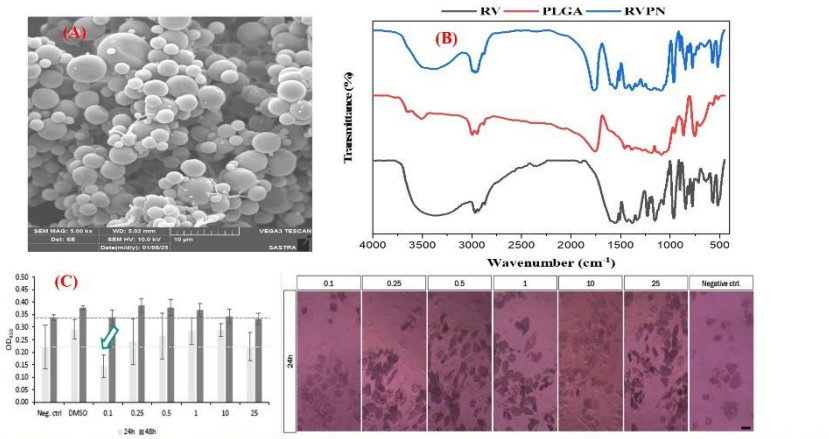
[2]Reutlingen University, Tissue Engineering and Bio-fabrication Laboratory, School of Life Sciences, , Germany

Inflammation is body"s innate defense mechanism against foreign pathogens, mediated by tissue-resident immune cells through Pattern Recognition Receptors (PRR). However, persistence of inflammation in absence of foreign agents leads to chronic low-grade inflammation, causing inflammatory diseases such as atherosclerosis, Crohn"s disease, diabetes, and autoimmune disorders. Statins category drugs are HMG Co-A reductase inhibitors, causing lipid-lowering effects, commonly prescribed to treat cardiovascular disorders and hyperlipidemia. Statins are recognized for their pleiotropic effects, which encompass anti-inflammatory, anti-thrombogenic, and antioxidant properties. Statins are known to reduce pro-inflammatory cytokines by downregulating the NFkB pathway and suppressing the expression of Toll-like receptor-4 (TLR4), a PRR on the surface of immune cells like macrophages and dendritic cells. Long-acting injectables are designed to overcome limitations associated with conventional statins, enhance bioavailability and treatment adherence, reduce drug toxicity, and prolong drug release, especially for managing chronic inflammation. In the current study, long-acting Rosuvastatin nanoparticles are developed using biodegradable polymer by spray drying technique. The optimized nanoparticles exhibit 355 nm ± 0.04 nm average particle size, -7.79 mV zeta potential, 91.62±7.73% drug loading and sustained release for 3 weeks under in-vitro conditions. The immune-modulatory effects of Rosuvastatin polymeric nanoparticles in-vitro are compared to pure drug to assess its impact on the secretion of pro-inflammatory cytokines (IL-6, TNF-α) and anti-inflammatory cytokines (IL-10) using an LPS-activated monocytic cell lines. Future studies will focus on testing the effects of Rosuvastatin nanoparticles within a 3D hydrogel environment incorporating adipocytes.

Keywords: Statin, Spraydry

Ref: Koushki et al. Clin Rev Allergy Immunol 2021;60:175 Xu et al. J Neuroinflammation 2017;14:167

Fig 1



(A) Scanning Electron Microscopy (Average Particle Size 355 nm); (B) Fourier Transform Infra-Red Spectroscopy (RV-Rosuvastatin, PLGA-Poly lactide co-glycolide polymer, RVPN-Rosuvastatin Polymeric Nanoparticles (C) Preliminary *In-vitro* Cell Viability Study of Differentiated Adipose derived Stem Cells treated with Statin

Poster session

P 081

Kinetic insights into TLR5 recognition of silent and stimulatory flagellins

Miriam Haag[1], Michael E. W. Bell[1], John R. Weir[2], Ruth Ley[1]
[1]Max Planck Institute for Biology, Microbiome Science, Tübingen, Germany
[2]Friedrich Miescher Laboratory of the Max Planck Society, Tübingen, Germany

Toll-like receptor 5 (TLR5) is a human innate immune receptor which detects bacterial flagellins, monomeric subunits of flagella. Both commensal gut bacteria and pathogens express flagellins, raising the question of how epithelial TLR5 tolerates non-pathogenic flagellins. We have recently shown that many flagellins expressed by commensals bind TLR5 without triggering an immune response. These so-called "silent" interactions remain to be fully characterised. We theorised that silent flagellins dissociate faster from TLR5 than stimulatory flagellins, presumably preventing 2:2 signaling complex formation. To measure the binding kinetics of a truncated TLR5 (TLR5n14) and flagellins, we conducted Surface Plasmon Resonance (SPR). We employed different mutants of the stimulatory Salmonella typhimurium FliC and the silent Roseburia hominis FlaB to assess the influence of specific residues in driving differences in binding kinetics. Our results showed that FliC and FlaB exhibit similar binding strengths to TLR5n14, but arising from distinct kinetic features: FliC associates slower than FlaB, but forms a more stable complex. Mutations located in the primary interface (PIM) dramatically increased FliC's dissociation rate while FlaB PIM fails to bind. Analysing alphafold 3 predicted TLR5-flagellin complexes, we suggest that TLR5 activation depends on cooperative engagement of three interfaces. Our findings suggest flagellin association and fast dissociation at the TLR5-D1 primary interface may be partly responsible for the "silent" flagellin phenotype.

P 082

Understanding diversity in Toll-like receptor 5 and flagellins

Yi Han Tan[1], Ruth Ley[1]
[1]Max-Planck Institute for Biology Tuebingen, Department of Microbiome Science, Tübingen, Germany

Toll-like receptor 5 (TLR5) is a pattern recognition receptor (PRR) which senses bacterial flagellin. TLR5 plays an important role in regulation of the host microbiome and defence against pathogens. Mutations that modify the PRR-ligand interaction can significantly alter host immune responses. Interestingly, variants in both TLR5 and its ligand exist. Stimulatory flagellins are known to strongly bind and trigger a robust TLR5-dependent response, and the recently discovered class of silent flagellins bind TLR5 but do not engender TLR5 activity. Moreover, the functional TLR5 variants TLR5F616L and TLR5N592S are present in the human population at allelic frequencies greater than 10% and are associated with increased risk of inflammatory diseases such as colorectal cancer and Crohn"s disease.
The F616L and N592S single nucleotide polymorphisms (SNPs) are located in the ectodomain, a region of the TLR5 that is responsible for flagellin binding. Thus, these SNPs may affect modulation of flagellin binding and possibly downstream immune signalling. In this study, we investigate the effect of the F616L and N592S (SNPs) on interaction with flagellin variants and TLR5 signalling.
We have developed vectors for the transient expression of the dominant allele of TLR5 as well as the TLR5F616L and TLR5N592S variants in HEK293T cells. Our initial results corroborate published work, demonstrating that that TLR5F616L is attenuated in activity while TLR5N592S is more responsive than the dominant allele to a representative stimulatory and silent flagellin. Using a TLR5-flagellin pulldown assay, we observed that these differences in activity are independent of binding to the flagellin nD1 domain.
Future work will investigate whether differential binding of the flagellin D0 domain can explain these phenotypes and characterise differences in immune signalling downstream of the TLR5 variants. Given the nature of this study, our research could potentially inform therapeutic interventions.

Poster session

P 084

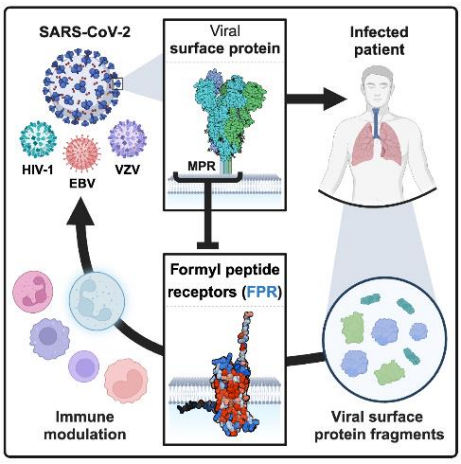
Viral surface proteins modulate innate immune responses via formyl peptide receptors

Bernd Bufe[1], Heiko Heilmann[1], Lukas Busch[1], Celine Buchmann[1], Islam Mohamed[2], Adrian Theiß[1], Sabryna Junker[1], Stefan Lohse[3]

[1]University of Applied Sciences Kaiserslautern, Department of Informatic and Microsystems Technology, Zweibrücken, Germany
[2]Saarland University Medical Center, Institute of Virology, Homburg, Germany
[3]Leibniz-Institute for New Materials (INM), , Saarbrücken, Germany

Formyl peptide receptors (FPRs) are pattern recognition receptors that are expressed in various parts of the body, where they help to regulate a considerable number of important immune functions. Our data suggest that human FPRs may play a general role in the innate immune response for many different viral infections.
We identified several viral activators and a conserved inhibitory motif for FPRs that are present on the surface proteins of various viral pathogens. Peptide fragments containing these motifs interact with all three members of the FPR family and can modulate various important immune functions in innate immune cells. In COVID-19 patients, viral degradation products containing these motifs were found in the spike protein. They can trigger various signal transduction pathways that modulate important immune responses. The physiochemical properties of FPR1 activators correlate with the occurrence of protein aggregation hotspots. Remarkably, similar hotspots are present on different surface proteins of unrelated viruses that can also activate FPRs. Furthermore, we can show that these reactions can be strongly attenuated by a conserved peptide motif in the membrane proximal region of SARS-CoV-2, which is also present in a number of completely different virus types. Peptides from these regions can therefore be used for targeted modulation of various immune functions. Finally, we identified a surprisingly large number of FPR3 activators, suggesting a previously unrecognized role for FPR3 in viral infections. Given the prevalence of FPRs in various important cell types in the body and their diverse roles in immune signalling, the newly discovered viral FPR ligands could influence numerous infection-relevant physiological processes.

Fig 1.



Poster session

P 085

Macrophages regulate T cell production and thymic size via TLR4-primed efferocytosis

Andri Lemarquis[1], Daniel Ghazarian[1], Marcel van den Brink[1]
[1]City of Hope (COH), Los Angeles, CA, United States

Despite its critical role in adaptive immunity, the genetic regulation of thymic size remains poorly understood. Using multiple mouse strains, we identified strain-specific differences, with C57BL/6J exhibiting the largest and C3H/HEJ the smallest thymus size throughout life and impaired regeneration post-injury. Genetic and knockout studies pinpointed a TLR4-dependent defect via TRIF, independent of MYD88, responsible for reduced thymic size. Macrophages showed highest thymic TLR4 expression, and macrophage-specific TLR4 deletion impaired thymus size. Phosphoflow and western blotting demonstrated impaired TLR4 signaling with overexpression of p65/NF-κB in TLR4 deficient macrophages. To determine whether altered p65 activity affected gene regulation, we performed CUT&RUN to map the genome-wide binding sites of p65. In TLR4-deficient HEJ macrophages, p65 exhibited reduced binding to key phagocytosis-related genes, including MERTK and AKT1, compared to FEJ macrophages. Consistently, bulk RNA-seq analysis of BMDMs revealed that C3H/FEJ macrophages exhibited higher expression of MERTK, along with lower levels of IKKγ, whereas the opposite pattern was observed in C3H/HEJ macrophages. This led to functional defects in efferocytosis, resulting in extracellular debris accumulation and undigested nuclei in the thymus with and defective degradation of phagocytosed material and increased IL1α and TNFα inhibiting thymic epithelial cell function. Therapeutically, TLR4 competent hosts were observed to have improved thymic reconstitution, Masilinic acid treatment targeting p65 in TLR4 deficient mice led to improved thymic cellularity and TLR4 activation using the nontoxic TLR4 agonist MPLA improved thymic size in wild type mice by increasing CX3CR1+ macrophages and improved thymocyte clearance. Collectively, we have identified a TLR4-dependent mechanism regulating thymic size and function via macrophage activation and efficient apoptotic thymocyte clearance.

Fig 1.

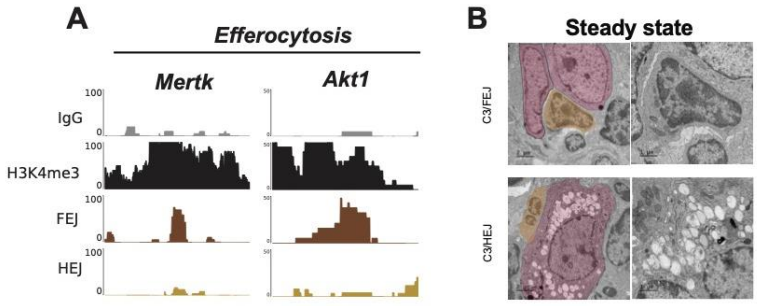


Figure 1: **(A)** CUT and Run tracks for p65 in C3H/HEJ and C3H/FEJ BMDM **(B)** Electron microscopy images of myeloid cells engulfing apoptotic thymocytes in steady-state C3H/FEJ and C3H/HEJ thymic samples. Engulfed apoptotic cells are pseudocolored orange and macropahges pink.

Poster session

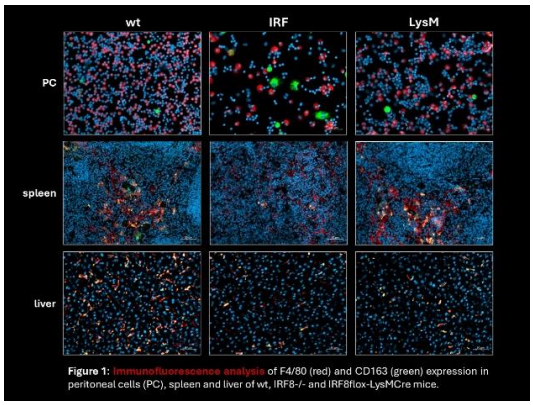
P 086

The role of IRF8 in development and function of CD163-positive macrophage subpopulations

Till Jordan[1], Johannes Roth[1], Katarzyna Barczyk-Kahlert[1]
[1]University of Münster, Institute of Immunology, Münster, Germany

CD163 is a scavenger receptor, expressed exclusively by monocytes/macrophages and a widely accepted marker for anti-inflammatory macrophages. CD163 was shown to dampen inflammatory responses, while enhancing the antimicrobial activity of monocytes/macrophages. The development of CD163-positive macrophages depends on the transcription factor interferon regulatory factor 8 (IRF8). IRF8-/- mice lack certain monocyte/macrophage subsets and have elevated serum levels of S100A8/A9 proteins. Using conventional (IRF8-/-) and myeloid lineage-specific conditional IRF8-knockout (IRF8flox-LysMCre) mouse models, we investigated the influence of IRF8 on the expression of CD163 in different macrophage subpopulations by flow cytometry, immunofluorescence microscopy and Western blot analysis. The results obtained demonstrated significant alterations in immune cell populations in the bone marrow and peritoneal cavity of IRF8-/- mice, compared to wt and IRF8flox-LysMCre mice. While the proportion of CD163-positive macrophage subpopulations was significantly decreased or absent in the bone marrow, spleen and liver of IRF8-/- mice, deletion of IRF8 led to an increase in CD163-positive macrophage subpopulations in the peritoneal cavity. This coincided with an altered immune response of peritoneal macrophages to LPS stimulation in vitro. These findings indicate that the development and function of CD163-positive macrophage subpopulations is not solely dependent on the expression of IRF8 but also appears to be influenced by the environmental milieu.
Figure 1: Immunofluorescence analysis of F4/80 (red) and CD163 (green) expression in peritoneal cells (PC), spleen and liver of wt, IRF8-/- and IRF8flox-LysMCre mice.

Fig 1.



P 087

Deciphering neutrophil heterogeneity in chronic inflammation using classical and imaging flow cytometry

Julia Cygan[1], Juri Habicht[1], Amelie Bauerdick[1], Guido Wabnitz[1]
[1]Institute of Immunology Heidelberg, Heidelberg, Germany

Neutrophils are key players in innate immunity, rapidly responding to inflammatory cues and shaping immune responses. However, their heterogeneity and functional specialization remain underexplored, particularly in chronic inflammation. To address this, we leveraged imaging flow cytometry, high-dimensional flow cytometry, and MorphoMapping, a novel computational approach, to visualize and classify neutrophil subpopulations in patients with chronic inflammatory diseases and healthy controls.
Using a 24-antibody panel, we identified distinct neutrophil subpopulations with specialized functions: pro-NETotic ($CCR5^+$) neutrophils, implicated in tissue damage; NETosing (MPO^+) neutrophils, involved in pathogen defense and autoimmunity; $CD80^+$ $HLA\text{-}DR^+$ neutrophils, suggesting antigen presentation; immature ($CD33^+$ CD10low) neutrophils, indicative of granulopoiesis; and neutrophil-platelet aggregates (NPAs, $CD36^+$), which promote thromboinflammation and exhibit NETosis.
To further quantify and visualize NPAs and NETosis, we applied MorphoMapping, integrating cell morphology with high-dimensional marker expression to define neutrophil activation states. This allowed us to distinguish NETosis-prone NPAs from classical NETosing neutrophils, providing unique visualization of their structural transformations. Imaging flow cytometry enabled single-cell analysis, capturing mature circulating neutrophils (CD16high CD62Lhigh), activated immunoregulatory neutrophils (CD16high CD62Llow), and reverse-migrating neutrophils (CD54high CD11bhigh CXCR2low), which contribute to unresolved inflammation and tissue remodeling.
By combining imaging flow cytometry, MorphoMapping, and advanced data visualization, we provide an in-depth view of neutrophil diversity, NPAs, and NETosis, linking phenotype to function in chronic inflammation. This approach enhances our understanding of innate immune cell plasticity and holds promise for identifying disease-specific biomarkers and therapeutic targets.

Poster session

P 088

Anti-citrullinated histone antibody CIT-013 blocks neutrophil extracellular trap release and prevents their inflammatory consequences – a promising therapeutic for immune-mediated inflammatory diseases

Kelsy Waaijenberg[1], Annemarie Kip[1], Eline Zwiers[1], Stephanie van Dalen[1], Sangeeta Kumari[1], Tirza Bruurmijn[1], Josephine Stein[1], Martyn Foster[2], Renato Chirivi[1], Eric Meldrum[1], Maarten van der Linden[1]
[1]Citryll, Bio-analytics, Oss, Netherlands
[2]Experimental Pathology Consultancy, Benfleet, United Kingdom

Neutrophil extracellular traps (NETs) contribute to the pathophysiology of multiple immune mediated inflammatory diseases (IMIDs), including Rheumatoid Arthritis (RA). While different NET-targeting approaches have preclinically shown beneficial effects, none are yet in clinical use. CIT-013, a first-in-class monoclonal antibody, binds citrullinated histones H2A and H4 in NETs and has demonstrated anti-inflammatory effects in various mouse models. The aim of this study is to determine CIT-013"s mechanism of action (MoA) and outline CIT-013"s potential as therapeutic for RA. To assess CIT-013"s MoA, confocal microscopy was used to visualize NET release and macrophage-mediated NET phagocytosis. Cytokine release by macrophages was measured in response to CIT-013 opsonized NETs. SPECT/CT visualized radiolabelled mouse CIT-013 (mCIT-013) NET-targeting capability in a collagen-induced arthritis (CIA) mouse model. In a separate CIA model, the therapeutic effects of mCIT-013 on inflammation-induced bone resorption were investigated. Furthermore, the presence of CIT-013"s epitope was detected in serum and synovial tissue from RA patients. CIT-013"s dual MoA encompasses inhibition of NET release and enhancement of Fc-domain dependent clearance of NETs and netting neutrophils by macrophages. In addition, in vitro exposure to CIT-013 opsonized NETs skews macrophages towards an anti-inflammatory phenotype. To prove CIT-013"s therapeutic potential in RA, we show that mCIT-013 specifically distributes to the inflamed joints, prevents NET-mediated tissue damage and disease progression in CIA mice. Finally, we demonstrated that CIT-013"s epitope levels are elevated in serum and tissue of RA patients.
CIT-013 has a unique dual NET-targeting MoA, eliminating the pathological consequences of NETs. This approach reinforces the promising therapeutic potential of CIT-013 for treatment of RA, and other IMIDs. A phase 2a clinical study in RA patients, CITY DREAM, is due to commence in 2025.

P 089

SIRPα is an inhibitory receptor that regulates NK cell activation and function

Lamin B. Cham[1], Thamer A. Hamdan[2], Hilal Bhat[3], Khaled Saeed Tabbara[1], Eman Farid[1], Mohamed Ridha Barbouche[1], Tom Adomati[4]
[1]Arabian Gulf University, College of Medicine and Health Sciences, Department of Microbiology, Immunology and Infectious Diseases, Manama, Bahrain
[2]Al-Ahliyya Amman University, Department of Basic Dental Sciences, Faculty of Dentistry, Amman, Jordan
[3]Otto-von-Guericke University, Medical Faculty, Magdeburg, Germany
[4]University of Antwerp, Laboratory of Experimental Medicine and Pediatrics, Faculty of Medicine and Health sciences, Antwerp, Belgium

Signal regulatory protein alpha (SIRPα or CD172a) is an inhibitory receptor on macrophages and dendritic cells. Recent cancer research studies have reported evidence of upregulation of SIRPα on NK cells. Using SIRPα knockout mice (SIRPα–/–) and lymphocytic choriomeningitis virus (LCMV) as a prototypical viral infection model, we investigated the role of SIRPα in NK cells during viral infection. We found that SIRPα is expressed on activated NK cells during LCMV infection. The lack of SIRPα in (SIRPα–/–) mice resulted in increased NK cells proportion and activation. Moreover, absence of SIRPα led to a significantly increased expression of NK cell cytotoxic markers and NK cell mediated killing of target cells. Mechanistically, SIRPα exerts an intrinsic effect and lack of SIRPα was associated with the downregulation of Src homology region 2 containing protein tyrosine phosphatase-1 (SHP-1) in NK cells. Furthermore, knockout of SIRPα led to concomitant loss of CD8+ T cells and poor viral control. An in vivo killing assay indicated that the activated NK cells mediated killing of CD8+ T cells in the SIRPα–/– mice. Experimental depletion of NK cells in SIRPα-deficient mice partially restored T cell immunity, ameliorated immunopathology and enhanced virus clearance. Our results provide important insight that SIRPα is an essential immune checkpoint molecule with regulatory function on NK cells. The disruption of SIRPα signaling could be amendable as a potential target for improving NK cells function in cancer and infectious diseases. However, therapeutical targeting SIRPα need to put into account for a possible consequences of NK cell mediated killing of T cells.

Poster session

P 090

TLR4 signaling pathway in Metabolic Dysfunction Associated Steatotic Liver Disease and Parkinson"s disease networks

Christina Flourou[1], George Vavougios[2]
[1]Nicosia General Hospital, Internal Medicine Department, Nicosia, Cyprus
[2]Medical School University of Cyprus, Nicosia, Cyprus

Background and aim: Immune dysregulation is increasingly recognized as an important component of Parkinson"s disease pathogenesis. Furthermore, a growing body of studies have implicated shared immune pathways in the pathogenesis of MASLD and Parkinson"s disease. One such pathway is mediated by TLR4, an immune receptor implicated in immunosurveillance and associated with neuroinflammation and liver parenchyma inflammation and fibrinogenesis. The aim of our study was to explore TLR4 mediated immune networks and their contribution in the overlap between both diseases.Methods: We scrutinized disease-disease and gene-disease data from the DisGeNet database to identify TLR4 gene networks in both diseases. We performed gene set enrichment analyses to identify significantly enriched pathways involving TLR4, including gene-drug enrichments. Based on our findings, we conducted a scoping review of available studies that accounted for TLR4 mechanisms in either disease.Results: DisGeNet indicated 619 shared genes, and 35 SNPs shared between both diseases. TLRs including TLR4 as well as activation molecules such as CD14 were among these shared genes. TLR4 SNP rs4986791 was the unique shared SNP identified in both diseases. Gene Set Enrichment analysis revealed multiple biological processes associated with cytokine signaling, inflammation and fibrogenesis. Gene-drug enrichment identified statins and fibrates among other compounds enriched by TLR4-containing networks.Conclusion: Our results suggest that TLR4 dysfunction may account shared immune aspects of MASLD and iPD pathogenesis. This dysfunction results in aberrant regulation of inflammation, with tissue-specific effects further shaping the molecular pathology of both diseases. Targeting TLR4 signaling may be a promising strategy in developing or repurposing disease modifying therapeutics for both diseases.