RTG-PRO
Proteases in pathogen and host: importance in inflammation and infection

IMPRS
for Infectious Diseases and Immunology
INTERNATIONAL MAX PLANCK RESEARCH SCHOOL

Joint Symposium Infection and Proteases

IMPRS-IDI meets RTG-PRO

Program and abstract booklet

October 25th 2024

Max Planck Institute for Infection Biology
Seminar Room 1+2

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Schedule

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Networking and Get-Together

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Venue

Registration starts at 9:30 in the Foyer of the Max Planck Institute for Infection Biology in Berlin

Approach with local trafic

From Berlin Hauptbahnhof: (approx. 10 Min.)

- Footway
- Bus 147 (direction: „S Ostbahnhof“) to „Schumannstraße“ 2

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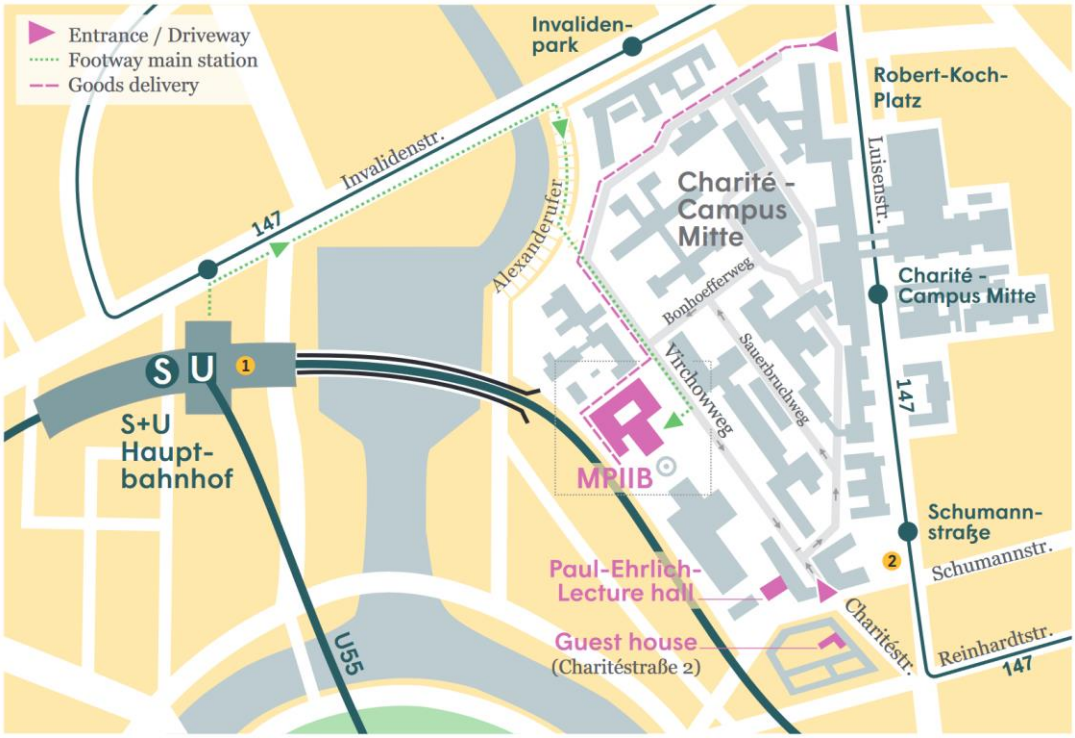

Speakers

Simone Reber
Quantitative Biology

Our goal is to elucidate the biochemical and biophysical principles that underlie the self-organization and scaling of subcellular organelles. Each cellular organelle carries out a distinct function, which is not only related to its molecular composition, but in many cases also to its size. The mitotic spindle for example must be large enough to span sufficient distance to physically separate chromosomes into two opposite halves of the cell.


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Olivia Majer
Innate Immune Regulation

Immune responses require tight regulation to provide effective protection against infectious threats, while at the same time avoiding overwhelming inflammatory reactions. How immune cells achieve this balance at the molecular level is the overall focus of our research. We are taking a cell biological approach to study how the spatial subcellular organization and trafficking of innate immune receptors contributes to immune homeostasis. We aim to understand how perturbations of these processes promote inflammation and autoimmunity. To address these questions, we are using a combination of advanced imaging techniques, genome-editing and state-of-the-art protein perturbations, biochemical assays, and *in vivo* models.

Talk Abstract

Alexandra Hrdina[1,2]
Shu Kondo[3,4], *Dagmar Frahm*[1], *Igor Iatsenko*[1]

[1] *Max-Planck-Institute for Infection Biology, Berlin, Germany*
[2] *Humboldt-Universtität zu Berlin, Berlin, Germany.*
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Immune role of *Drosophila melanogaster* putative Kazal-type serine protease inhibitor CG14933

Serine protease inhibitors (serpins) exhibit major regulatory functions in the proteolytic cascades of both arthropods and mammals, but despite their important function in *Drosophila melanogaster* immunity, few have been fully characterized and knowledge of their precise mechanism of action is still sparse. In this study, we investigate the immunological role of the previously uncharacterized gene *CG14933*. *CG14933* contains a Kazal domain, a protein domain that is often indicative of serpins. By using $CG14933^{SK1}$ flies, a mutant devoid of any *CG14933* expression, as well as RNAi knockdown and deficiency models, we show that flies lacking CG14933 exhibit increased susceptibility to infection with *Pseudomonas entomophila* and *Providencia alcalifaciens*, respectively. This suggests involvement of *CG14933* in *Drosophila* immunity, although *CG14933* itself is not induced by infection. We show that $CG14933^{SK1}$ larvae harbor increased numbers of crystal cells, specialized immune cells that store the key enzymes of the melanization cascade, the prophenoloxidases, and that adult $CG14933^{SK1}$ flies also have significantly increased levels of active phenoloxidases compared with wild type flies. We were able to rescue the sensitivity phenotype to *P. entomophila* infection by employing a double mutant devoid of both *CG14933* and *PPO1,* the prophenoloxidase activated in early stages of infection. We also observed higher levels of iron in the hemolymph of $CG14933^{SK1}$ flies, which may indicate a lower efficiency of the iron sequestration response, explaining the observed susceptibility to pathogens known to require iron for their virulence. Overall, we propose that CG14933 plays a role in negatively regulating the melanization response in *Drosophila*. Furthermore, we provide evidence of a possible link between excessive melanization and reduced iron sequestration that leads to increased susceptibility to *P. entomophila* and *P. alcalifaciens* infection.

Talk Abstract

Dora Cerina[1]
Matthieu Rousseau[2,3], *Carla Hart Olaiz*[1], *Molly Ingersoll*[2,3], *Arturo Zychlinsky*[1]

[1]*Max Planck Institute for Infection Biology, Charitéplatz 1, 10117, Berlin, Germany*
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[3]*Institut Pasteur, Paris 75015, France*

Neutrophil extracellular traps induce a novel regulatory system in uropathogenic *Escherichia coli*

Uropathogenic *Escherichia coli* (UPEC), the leading cause of urinary tract infections, must adapt to the hostile bladder environment to establish an infection. Thus, UPEC needs to evade the immune system, including infiltrating neutrophils and their antimicrobial neutrophil extracellular traps (NETs). To understand how UPEC interacts with the immune system, we analyzed its transcriptome after incubation with NETs. We show that NETs induce a novel regulatory system in UPEC, comprising of two genes encoded in a pathogenicity island $PAI_{UTI89}II$. Analysis of 10,000 *E. coli* genomes shows that 30% of clinical UPEC isolates possess this system. Upregulation of this system is driven primarily by NET-associated nucleosomes, rather than other NET components. This regulatory system in UPEC plays a role in reducing reactive oxygen production in neutrophils and NET formation *in vitro*. Deletion of this system leads to reduced bladder colonization in a mouse UTI model, highlighting its importance in UPEC pathogenesis and immune evasion. Understanding this interaction could pave the way for developing treatments for multidrug-resistant UPEC strains.

Talk Abstract

***Niranjan A. Srikanth*[1,2]**
Rafael Deliz-Aguirre[1, 3], Deepika Kumari Gola[1], Margaux Bilay[1], Elke Ziska[1] and Marcus J. Taylor[1]

[1] Max Planck Institute for Infection Biology, Chariteplatz 1, Berlin D-10117, Germany
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IRAK4 phosphorylation controls inflammatory signaling by controlling IRAK1/2/3 oligomerization

Inflammatory pathways utilize oligomerization of signaling proteins to regulate the transduction of signaling. For example, the IL-1 pathway utilizes this mechanism by forming an oligomeric complex called the myddosome, consisting of three distinct proteins: MyD88, IRAK4, and lastly IRAK1/2/3. The formation of this complex is accomplished by the oligomerization of the death domains (DD) of these proteins. However, the IRAKs are a family of kinases and possess a kinase domain in addition to their death domain. The role of the IRAK kinase activity in regulating myddosome formation and inflammatory signaling has been heavily debated, particularly the role of IRAK4 kinase activity. As IRAK4 is the only non-redundant IRAK involved in myddosome formation, its possible role as a crucial regulator of inflammatory signaling makes it an interesting therapeutic target.
To resolve the role of IRAK4 kinase activity in myddosome formation, we used a combination of gene-edited cell lines, lentiviral reconstitution, and pharmacological inhibition combined with live-cell TIRF microscopy to elucidate the mechanism involved. We found that the phosphorylation of IRAK4 is crucial for the recruitment of both human and mouse variants of IRAK1/2/3. This regulation is achieved as IRAK1/2/3 kinase domains inhibit their DD from complexing with IRAK4 DD. This inhibition is released when the kinase domains of IRAK4 interact with the kinase domains of IRAK1/2/3. Our data shows that multimerization of DDs can be controlled by biochemical processes, such as post-translational modification, beyond the specific interactions between these domains, pointing to a hierarchical interplay between macromolecular assembly and enzymatic activity.

Talk Abstract

***Sergio Gonzales San Miguel*[1]**
Arturo Zychlinsky[1]

[1]*Max Planck Institute for Infection Biology, Berlin, Germany.*

From Hematopoietic Stem Cells to CRISPR-edited neutrophils

Human neutrophils are the most abundant immune cell in circulation and the first line of defense against infections. The neutrophil's nucleus consists of three to five lobes connected by thin chromatin strands – a unique shape with unknown function. Neutrophils are terminally differentiated, non- proliferative cells with a very short-life span and alternative myeloid cell lines lack lobulation.
During granulopoiesis, neutrophils upregulate Lamin B Receptor (LBR) expression. The N-terminus binds Lamin B and heterochromatin while the C-terminus anchors LBR in the inner nuclear membrane. The first regulates gene silencing while the second synthesizes cholesterol. In humans, LBR mutations in Pelger-Huët Anomaly and Greenberg skeletal dysplasia result in poorly or non-lobulated neutrophils, while LBR overexpression or cholesterol accumulation leads to hypersegmentation.
Here, I established a protocol to obtain functional Hematopoietic Stem Cell (HSC)-derived neutrophils. This allowed me to generate LBR mutants using CRISPR/Cas system. Edited cells loss the lobulation and preliminary results indicate defects in Neutrophil Extracellular Traps (NETs). In summary, these protocols and findings will contribute to our understanding of neutrophil biology and may have implications for the development of novel therapeutic strategies targeting neutrophil-related disorders.

Talk Abstract

Abhishek K. Singh[1,2], Sophie Möller[1,3], Ole Schmöker[1,4]

[1] *Research Training Group - Proteases in pathogen and host: importance in inflammation and infection, Greifswald, Germany*
[2] *Friedrich-Loeffler-Institute for Medical Microbiology, University Medicine Greifswald*
[3] *Institute for Medical Biochemistry and Molecular Biology, University Medicine Greifswald*
[4] *Institute of Biochemistry, Dep. Synthetic and Structural Biochemistry, University Greifswald*

The ubiquitin system as a linchpin in infection and inflammation

The covalent attachment of the small protein ubiquitin (Ub) to other proteins resembles an essential mechanism to delicately orchestrate various cellular processes ranging from proteostasis, cellular trafficking, immune response and more. Achieving these key regulatory tasks requires a dynamic and tightly controlled machinery which holds (de-)ubiquitination in a constant cycle. This makes the ubiquitin system a bonafide target for internal and external challenges. We demonstrate, how the ubiquitin system can act as a focal point in both infection and inflammation. E3 ubiquitin ligases are crucial for mediating a proper host response to bacterial pathogens, as they are directly involved in antigen presentation via MHC class I/II. The E3 ligase Skp2, known for its involvement in cell cycle regulation, plays a critical role in *S. aureus* infection. Modulation of the host ubiquitin machinery is therefore important to counteract bacterial/viral pathogens. On the other hand, by encoding their own set of deubiquitinating enzymes (DUBs), especially Gram-negative pathogens actively remove ubiquitin species, in order to evade the host ubiquitin system and ensure their proliferation. These type of virulence factors come in many different forms and target various pathways, disturbing the cellular integrity. Ultimately imbalance of the ubiquitin-proteasome system (UPS) caused by genetic variants in proteasomal subunits and assembly factors results in severe disorders, called proteasomopathies, which encompass two distinct clinical phenotypes: i) PRAAS (proteasome-associated autoinflammatory diseases) and ii) NDD (neurodevelopmental disorders). Studies of patient cells show protein accumulation which further triggers activation of the unfolded protein response, the integrative stress response as well as type I interferon signaling. With the complexity of the human ubiquitin system and its many target points, our understanding of this fine-tuned machinery is crucial for our knowledge of host-pathogen interactions and clinical phenotypes, which in the future hopefully contributes to the development of novel diagnostics and therapeutics.

Talk Abstract

Larissa M. Busch[1,2], Supradipta De[1,3], Celina Würner[1,4]

[1] *Research Training Group - Proteases in pathogen and host: importance in inflammation and infection, Greifswald, Germany*
[2] *University Medicine Greifswald, Interfaculty Institute of Genetics and Functional Genomics, Department of Functional Genomics, Greifswald, Germany*
[3] *University of Greifswald, Interfaculty Institute of Genetics and Functional Genomics, Department of Molecular Genetics and Infection Biology, Greifswald, Germany*
[4] *University Medicine Greifswald, Department of Internal Medicine B, Molecular Cardiology, Greifswald, Germany*

Insights in infection models of Gram-positive bacteria

No life without infections and no life without proteases. Proteases are at the center of regulation of the main players of life, the proteins. They are performing precision proteolysis, which is equally important for the protein homeostasis of pathogen and host, and for the relationship between the two, as well as acting as virulence- and immune evasion factors such as the extracellular serine proteases of Gram-positive bacteria.
In Gram-positive pathogens, e.g. *Staphylococcus aureus*, protein homeostasis is largely mediated by the Clp system consisting of the ClpP peptidase, the unfoldases such as ClpX and ClpC. For investigation of the effect of an impaired protein homeostasis, *clpX* mutant strains were tracked during infection processes demonstrating a less virulent phenotype in *Galleria* and *in-vitro* cell culture infections compared to a protein homeostasis proficient strains. To gain insights into the underlying mechanisms, the effect of ClpX deficiency under infection-relevant conditions was recorded by proteomics revealing specific ClpX-dependent adaptation of bacterial fitness under infection relevant stresses. Extracellular serine proteases of Gram-positive organisms such as *S. pneumoniae* serine proteases PrtA, HtrA, SFP and CbpG, shape the bacterial exoproteome and might act at host extra cellular matrix proteins. To shed light onto the role of each individual serine protease on the proteome under infection-mimicking conditions, all extracellular serine proteases were knocked out one by one. In addition, for selected proteases activity assays with different extra cellular matrix proteins, such as vitronectin, Fibronectin and thrombospondin-1, were performed and suggested specific cleavage of the host proteins during survival and initiation of infections. The effect of the proteases was further tested in *Galleria* and *ex-vivo* lung slice infections. Gram-positive infections also prime the host immune system and induce inflammatory responses, e.g. *S. pneumoniae* infection causes priming and activation of the NLRP3 inflammasome in the heart in mice. Severe pneumonia, especially caused by *S. pneumoniae* is associated with cardiac complications such as heart failure, arrhythmias and myocardial infarctions, due to their ability to exacerbate to bacteremia and sepsis, which allows bacteria to interact directly with the heart. Activation of the NLRP3 inflammasome in the heart during pneumococcal pneumonia leads to protein degradation in cardiomyocytes contributing to cardiac dysfunction.
Thus, proteases shape host-pathogen-interactions in a multitude of aspects and precisely understanding of protease target spectra and activity conditions of proteases might be beneficial for future pathogen combating strategies.

Talk Abstract

Christopher Saade[1,2], Shruthi S. Peringathara[1,2], Hannes Wolfgramm[1,3]
Jessica von Fournier[1,2], Felix L. Glinka[1,4]

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[4] University of Greifswald, Institute of Biochemistry, Dept. of Biotechnology & Enzyme Catalysis, Greifswald, Germany

Hunting for substrates: The physiological role of *S. aureus* serine protease-like proteins

The virulence of *Staphylococcus aureus* is shaped by a range of tightly regulated virulence factors, including several proteases. While some of the twelve secreted extracellular *S. aureus* proteases are well-investigated, the serine protease-like proteins (SplA-F) remain poorly understood. The predicted cleavage sites suggest a distinct substrate spectrum, potentially adapted to specific hosts. A notable example of this host-specificity is Jep, a newly identified serine protease highly homologous to the Spls, predominantly found in mouse-adapted *S. aureus* strains.
The current projects aim to identify the substrates of the extracellular proteases SplB and Jep to elucidate their roles in host-pathogen interactions. We first characterized the newly identified protease Jep, both genetically and enzymatically. Following, we treated various physiological substrate ranges, including bacterial culture supernatants, plasma, and epithelial cells, with an active and an enzymatically inactive form of the proteases.
The results provide insights into the potential roles of SplB and Jep during infection. On one hand, these serine proteases seem to reduce the pathogen's own virulence. On the other hand, some identified targets and observations in a wound assay suggest a role in breaking down host tissue integrity. Interestingly, we also identified several targets of typically intracellular location, suggesting the importance of Spls and Jep during the intracellular phase of *S. aureus* infections.

Poster Abstract 1

Marko Rubinic[1]
David F. Duneau[2], *Igor Iatsenko*[1]

[1]*Max Planck Institute for Infection Biology, Germany*
[2]*Institute of Ecology and Evolution, Ashworth Laboratories, United Kingdom*

Sexual dimorphism in *Drosophila melanogaster* susceptibility to gut infection

Although sex differences in gut physiology and morphology have been previously described, whether and how these differences contribute to intestinal immunocompetence has not yet been fully understood. Here, we used the fruit fly Drosophila melanogaster and its enteric natural pathogen Pseudomonas entomophila to decipher the mechanisms underpinning the generally higher susceptibility of female flies to gut infection. We showed that *P. entomophila* causes gut transit blockage in a sexually dimorphic manner. *P. entomophila* induces instant gut transit blockage in female hosts coupled with increased oxidative stress, causing female flies to die. Contrariwise, male bias in levels of NADP+- reducing enzymes contributes to the higher gut antioxidant capacity needed to minimize the *P. entomophila* effect on gut transit blockage and overall lethality. Additionally, we performed genome-wide association analyses using the *Drosophila melanogaster* Genetic Reference Panel (DGRP) lines exposed to *P. entomophila* to identify novel candidate genes associated with sexual dimorphism in *Drosophila* gut immunity. Using Drosophila to understand how one sex suffers from infectious disease symptoms while the other does not will broaden our global understanding of variation in susceptibility to infectious diseases among the population. Given the conserved physiology and function of intestinal track among taxa, we anticipate that these findings will have significant implications for understanding sexual dimorphism in vertebrate immunity.

2 Poster Abstract

***Afonso Vieira*[1]**

Mastura Neyazi[2], Pilar Samperio Ventayol[1], Kirsten Glaser[3], Nicolas Schlegel[4], Sina Bartfeld[1]

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[2] *Institute for Molecular Infection Biology, Julius Maximilians Universität Würzburg, Würzburg, Germany.*

[3] *Department of General, Visceral, Transplant, Vascular and Pediatric Surgery, University Hospital Würzburg, Würzburg, Germany*

[4] *Division of Neonatology, Department of Women's and Children's Health, University of Leipzig Medical Center, Leipzig, Germany.*

Infant-derived intestinal organoids as a tool to study enteric infection

Infection and inflammation can be life-threatening conditions in newborns and young children. Two examples are infection with Enteropathogenic *E. coli* (EPEC) and necrotizing enterocolitis (NEC). The reason why these diseases have a high mortality rate in the early stages of life while not affecting or being easily resolved in adults is still unknown, partly due to a lack of model systems. To improve our understanding of the cell and molecular processes responsible for gut homeostasis and defense in the early human life, we use patient-derived human intestinal organoids.

We have generated intestinal organoids from adult and infant (pre-term or term born) donors, giving us the chance to characterize the developing human epithelium. Proliferation and differentiation capacity of organoids of adult and infant organoids was studied using organoid-seeding efficiency together with immunofluorescence, qPCR and RNA sequencing (RNAseq). Results show that infant-derived organoids proliferate more than adults. RNAseq analysis reveals differences between pre-term and term organoids, which may point to molecular mechanisms underlying the development of NEC, because pre-term birth is a major risk factor for NEC. Moreover, using organoid-derived monolayers and fluorescent-activated cell sorting (FACS), we were able to show that, upon infection with EPEC, bacterial attachment is higher in infant-derived organoids compared to adult-derived organoids.

Further investigation is essential to gain a deeper understanding of the molecular mechanisms underlying the vulnerability of infants to NEC and EPEC infections. Such research could help identify epithelial cell-intrinsic factors that are critical in strengthening and maturing the intestinal epithelial barrier. We hope that our findings will contribute to reducing the impact of these diseases in children worldwide.

Poster Abstract 3

Shah Faisal[1]
Ulrike Zedler[1], *Gabriele Stooß*[1], *Björn Corleis*[1], *Gang Pei*[1], *Elke Krüger*[3], *Anca Dorhoi*[1,2]

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[3] *Institute of Medical Biochemistry and Molecular Biology, University Medicine Greifswald, Germany*

Hypoxia reshapes lysosomal landscape and elicits BCG-induced inflammation

Hypoxia and inflammation are intimately linked. Inflammatory events, ranging from acute immune cell infiltration to tissue remodeling, cause transient or persistent tissue hypoxia. Oxygen limitation alters cellular metabolism and organelle dynamics. Since immunometabolism and endo-lysosomal features influence macrophage responses to intracellular pathogens, we investigated the effects of hypoxia in mycobacteria-infected human macrophages. RNA sequencing revealed an enrichment for endosomal processes in hypoxic cells, and high content imaging confirmed a reduction in the activity of lysosomal proteases under hypoxia. Despite reduced proteolytic activity, cysteine cathepsins were essential for limiting Bacillus Calmette-Guérin (BCG) replication within alkalized phagosomes. Inactive cathepsins, enlarged lysosomes localized to the periphery of hypoxic macrophages and showed alterations in their membrane permeability. Hypoxia induced type I interferon production, but left IL-1β and TNF-α levels unchanged in macrophages infected with mycobacteria lacking membrane-disrupting virulence factors such as BCG. A remodeling of the lysosomal landscape was validated for artiodactyl macrophages, indicating a species-conserved response of mycobacteria-infected macrophages to oxygen restriction. The lysosomal changes and pro-inflammatory responses induced by mycobacteria in hypoxic macrophages are relevant for tuberculosis vaccination and the pathophysiology of mycobacterial diseases.

4 Poster Abstract

***Saeedeh Asgarbeik*[1]**
Ulrich Weiss[1], *Matthias Sendler*[1]

[1]*Department of Internal Medicine A, University Medicine Greifswald, Germany*

Endogenous inhibition of Cathepsin B regulates intracellular protease activation during acute pancreatitis

Acute pancreatitis (AP) is an inflammatory disorder that occurs as a result of protease activation and subsequent auto-digestion of the pancreas. The trypsin as a serine protease is a key player in this process. Normally it gets activated by enterokinase in the duodenum, Cathepsin B can act similarly to enterokinase and activate trypsinogen. The Cathepsin B activity can be inhibited by cystatin B when it translocates to the cytoplasm. Cathepsin B plays a crucial role in apoptosis by cleaving Bid and triggering the release of cytochrome C from mitochondria. In addition to its inhibitory effect on Cathepsin B, cystatin B protects the mitochondria by mitigating reactive oxygen species (ROS) production. Thus, cystatin B serves a dual protective role: it shields the cytoplasm from premature protease activation and safeguards mitochondrial function. This study aimed to investigate the role of cystatin B on Cathepsin B activity in pancreatitis. The proteome analysis was conducted to investigate the protease inhibitors at subcellular fractions. AP was induced by hourly repetitive injections of caerulein (50µg/kg/body weight) in C57Bl/6 and Cystatin B-/- mice. Disease severity was determined by lipase and amylase activity measurements in serum and by pancreas histology. Protease activity was measured using fluorogenic substrates in pancreatic homogenate, while apoptotic cell quantification was determined by tunnel assay. The mitochondrial membrane potential was assessed using the TMRM assay. Furthermore, protease activation and cellular damage were evaluated in isolated acinar cells. Disease severity increased significantly in CysB-/- mice compared to the control. In the absence of the inhibitor, the activity of trypsin and also serum amylase and lipase elevated dramatically. In addition, higher cell death was observed in knock-out mice. The absence of cystatin B might be responsible for potential imbalances in the mitochondrial membrane. Further exploration of this pathway may unveil potential therapeutic targets for the treatment of pancreatitis. The Cathepsin B activity can be regulated by cystatin B. The cytosolic cystatin b can protect the cell against Cathepsin B activity and its role in the induction of apoptosis at the later time point of pancreatitis. Furthermore, Cystatin B helps in protecting cells against mitochondrial ROS production while maintaining mitochondrial membrane potential.

Poster Abstract 5

Larissa M. Busch[1]
Hannes Wolfgramm[1], Supradipta De[2], Christian Hentschker[1], Manuela Gesell Salazar[1], Celina Hopp[1], Meike Kröber[1], Gina Wockenfuß[1], Alexander Ganske[1], Ulrike Mäder[1], Sven Hammerschmidt[2], Ulf Gerth[3], Stephan Michalik[1], Alexander Reder[1], Kristin Surmann[1], Uwe Völker[1]

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[2] *University of Greifswald, Interfaculty Institute of Genetics and Functional Genomics, Department of Molecular Genetics and Infection Biology, Greifswald, Germany*
[3] *University of Greifswald, Department for Microbial Physiology and Molecular Biology, Greifswald, Germany*

A proteomic model to investigate the effect of ClpX deficiency in *Staphylococcus aureus* in infection processes

Host-mediated stressors affect bacterial pathogens during infection processes. In response to those stressors, bacteria adapt their gene expression as well as ultimately proteome profile and activity. In *Staphylococcus aureus* protein homeostasis is largely mediated by the Clp system consisting of the ClpP peptidase, the unfoldases ClpX and ClpC, and the chaperons ClpB and ClpL. The proteases ClpXP and ClpCP are crucial for general and targeted proteolysis, which rely on the unfoldases interacting with specific targets. ClpX is particularly involved in overall regulation of bacterial virulence and fitness and it is the most conserved Clp unfoldase. However, the majority of *S. aureus* ClpX targets remains elusive.
For investigation of the effect of ClpX deficiency the *S. aureus* mutant strain HG001 Δ*clpX*::*km* and the complemented strain HG001 Δ*clpX*::*km* pTripleTREP_clpX were constructed using the newly developed plasmid systems pSauSE (allele exchange plasmid) and pTripleTREP (controllable expression plasmid). When tracking infection processes, the *S. aureus* mutant strain HG001 Δ*clpX* revealed a less virulent phenotype in a *Galleria* model compared to the HG001 wild type and drastically low intracellular replication in *in-vitro* HBE cell culture infection experiments. The effect of ClpX deficiency under infection-relevant conditions was recorded after cultivating HG001 wild type, the HG001 Δ*clpX* mutant and the complemented strain HG001 Δ*clpX* pTripleTREP_clpX under iron limitation and microaerobic conditions by mass spectrometry in data independent mode. To address differences in proteome composition caused by the strain background and growth conditions, an external standard based normalization strategy was employed. The standard consists of ^{15}N-labeled *Bacillus subtilis* proteins enabling reliable distinction between standard and sample proteins and robust relative quantification of sample proteins. The proteomic profiles revealed specific ClpX- and condition-dependent changes as well as entanglement of those. An iModulon-inspired analysis resulted in 37 independent components, unravelling the different influences. Known ClpX-targets such as Spx and Sle1 were confirmed as robust targets and new targets such as NikA and SAOUHSC_00671 were suggested. Out of the two investigated infection-relevant conditions, iron limitation generally induced the Fur regulon, and under microaerobic condition the Rex regulon was induced. Moreover, ClpX-dependent condition-specific effects, such as a subregulon of the known Fur regulon, which is less responsive to the provoked iron limitation in the Δ*clpX* mutant, were also observed. With our proteomics data, we provide a global insight into ClpX-dependent adaptation of *S. aureus* physiology under infection-relevant conditions.

6 Poster Abstract

Hager Elsheikh[1]
Juliane Glaubitz[1], *Frank U. Weiß*[1], *Matthias Sendler*[1]

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The IL4/IL13 Macrophage polarization by STAT6 in acute and chronic pancreatitis

Pancreatitis is a condition characterized by inflammation in the pancreas, which can lead to serious complications if untreated. Both acute and chronic pancreatitis are associated with immune reactions and fibrosis, which further damage the pancreas. The type 2 immune response, primarily driven by alternative activated macrophages (AAMs), plays a significant role in the development of fibrosis. The IL-4/STAT6 pathway is a crucial signaling pathway for the activation of M2 macrophages. Pancreatic fibrosis is induced by dysregulated inflammatory responses and can result in the autodigestion and necrosis of pancreatic acinar cells. The aim of this study is to investigate the impact of STAT6, a crucial molecule in the IL-4/STAT6 pathway, on the severity and development of fibrosis during acute and chronic pancreatitis. The research also aims to understand the influence of the JAK/STAT6 signaling pathway on the balance between fibrosis and regeneration in the presence of different macrophage populations. The research utilizes murine models of acute and chronic pancreatitis induced by cerulean injection. Animal models will be employed to study the effect of STAT6 knockout on disease severity and fibrosis. Isolation of acinar cells and cell culture techniques will be used to assess the impact of different macrophage populations on wound healing and regeneration. Various techniques such as PCR, histology, immunofluorescence, and transcriptomics will be employed to analyze the tissues and cells. The research aims to provide insights into the mechanisms underlying tissue fibrosis and wound healing during acute and chronic pancreatitis. By investigating the influence of the JAK/STAT6 signaling pathway and different macrophage populations, the study aims to understand their impact on tissue fibrosis, disease severity, and pancreatic regeneration. This research contributes to our understanding of the role of specific signaling pathways, macrophage polarization, and the type 2 immune response in pancreatitis. It provides insights into the molecular mechanisms underlying tissue fibrosis and the potential for targeted therapies. Data will be collected through the use of murine models, isolation and culture of acinar cells, and various experimental techniques such as PCR, histology, immunofluorescence, and transcriptomics. Data will be analyzed using appropriate statistical methods and techniques, and the findings will be interpreted in the context of the research objectives. What is the role of STAT6 in the severity and development of fibrosis during acute and chronic pancreatitis? How does the JAK/STAT6 signaling pathway influence the balance between fibrosis and regeneration? How do different macrophage populations regulated by the type 2 immune response contribute to tissue fibrosis and disease severity in pancreatitis? By investigating the mechanisms of tissue fibrosis and wound healing during acute and chronic pancreatitis, this research aims to enhance our understanding of the disease progression and potential therapeutic targets. The findings have theoretical importance in expanding our knowledge of pancreatic fibrosis and the role of macrophage polarization in the context of the type 2 immune response.

Poster Abstract

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IL-1α promotes fibroblast activation and regulates tissue fibrosis during chronic pancreatitis

Chronic pancreatitis (CP) is a progressive fibro-inflammatory condition characterized by the irreversible destruction of the exocrine and endocrine pancreatic parenchyma, which is subsequently replaced by fibrotic tissue. IL-1α is a pro-inflammatory cytokine released by acinar cells and pancreatic macrophages. In this study, we explored the role of IL-1α in fibroblast activation and fibrogenesis using a mouse model of CP. CP was induced in C57Bl/6 and IL-1α deficient mice through repeated injections of caerulein (50 µg/kg body weight) over a period of 4 weeks. The IL-1 receptor pathway was blocked by intraperitoneal injections of Anakinra (100 µg/mouse). Pancreatic fibrosis was assessed by azan blue staining, and immunofluorescent labeling of CD206, IL-1R1, FGFR, αSMA, collagen-1, FAP, CD3, and Ki67 was conducted. Serum IL-1α levels were measured using LegendPlex. Fibroblasts and macrophages were isolated from bone marrow or pancreatic tissue and treated with 10 ng/ml IL-1α. The transcriptional profiles of these cells were analyzed using Affymetrix Chip Arrays and qRT-PCR. Elevated serum IL-1α levels were observed in mice with CP. Histological analysis revealed a significant reduction in pancreatic fibrosis in IL-1α deficient mice compared to wild- type controls. The absence of IL-1α led to a decreased number of FGFR+, αSMA+, and FAP+ fibroblasts, while the number of CD206+ alternatively activated macrophages remained unchanged. Specific labeling indicated the expression of the IL-1 receptor (IL-1R1) on the surface of αSMA+ fibroblasts. IL-1α stimulation of pancreatic fibroblasts triggered a transcriptional shift towards a pro-inflammatory phenotype. In vivo therapeutic inhibition of IL-1R1 by Anakinra reduced tissue fibrosis in CP mice. Our study demonstrates a pivotal role for IL-1α derived from macrophages and acinar cells in the development of pancreatic fibrosis. IL-1α directly activates fibroblasts via IL-1R1, promoting tissue fibrosis in a mouse model of CP. These findings establish a direct connection between acinar cell damage, macrophage activation, and fibrogenic tissue replacement. Inhibiting this pathway with Anakinra may offer a potential therapeutic strategy to mitigate fibrogenesis in CP.

8 Poster Abstract

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Investigations of the Protease Inhibitor Cystatin C in Acute Pancreatitis

Acute pancreatitis (AP) is one of the leading causes of hospitalization among gastrointestinal diseases. The disease is characterized by self-digestion of the pancreas by its own proteases. The pathophysiological initiating event during AP occurs inside pancreatic acinar cells. Under physiological conditions, digestive enzymes are stored as inactive precursors in zymogen granules and become activated after secretion within the duodenum. However, during AP trypsinogen becomes prematurely activated within the secretory compartment of acinar cells. This premature activation of proteases is mediated by the lysosomal protease cathepsin B and results in autodigestive injury, acinar cell death, and the initiation of a pro-inflammatory immune response. Cathepsin L, another lysosomal protease, acts as a counterpart to cathepsin B and is able to degrade active trypsin. In this project, the functions of these relevant proteases cathepsin B and L, and the endogenous cysteine protease inhibitor cystatin C are studied. For this, cathepsin B and L as well as cystatin C were recombinantly expressed and purified. We could show that cystatin C regulates the balance between the activation and inhibition of the cathepsins B and L. Furthermore, we could demonstrate that the dimerization state of cystatin C plays a central role in the interaction with these two proteases. We identified that dimerized cystatin C is able to enhance cathepsin B activity due to binding to an allosteric binding pocket, while it has no effect on the activity of cathepsin L. The presence of this allosteric pocket of cathepsin B was verified by mutagenesis of the most important residues of the binding pocket. Moreover, we could show that cleavage of cystatin C by trypsin results in a less effective inhibitory capacity for cathepsin B, while cathepsin L inhibition is just slightly reduced. These results suggest that depending on the monomer and dimer ratio of cystatin C and the digestion rate by trypsin, cathepsin L activity is still inhibited by monomeric cystatin C while dimeric cystatin C enhances cathepsin B activity and therefore fuels the protease activation cascade

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Elucidation of molecular patterns in two clinically distinct subgroups of proteasomopathies

The ubiquitin-proteasome system is a major cellular protein degradation system capable of maintaining proteostasis but also cell fate determination through its involvement in various cellular processes by degrading ubiquitin-modified proteins. In recent years, our understanding of the proteasome as a molecular machine has been enhanced by the identification of genetic variants in proteasomal subunits or their assembly factors that cause two rare phenotypically distinct syndromes: (i) systemic autoinflammation with interferonopathy in proteasome-associated autoinflammatory syndromes (PRAAS) or (ii) neurodevelopmental disorders (NDD) with cognitive impairments [1-5]. Regardless of the clinical phenotype, there are typical molecular signatures of cells from patients, including accumulation of ubiquitin conjugates and protein aggregates, activation of the unfolded protein and integrated stress responses, and dysregulation of type I interferon (IFN) signaling [1-5]. Using expanded T cells from PRAAS and NDD patients, we applied proteomic and transcriptomic approaches to identify molecular patterns that are distinct for each clinical phenotype. Functional results from pathway analyses indicate that the proteasome variants have severe effects on mitochondrial function as well as lipid and amino acid metabolism [1,2,4]. For certain variants, we have generated surrogate cell lines to study the effect of the mutation in more detail. First results on assembly and maturation defects as well as structural analyses will be discussed.

[1] Brehm et al., J Clinic Invest. 125(11). (2015)
[2] Ebstein et al., Science translational medicine. 15(698), eabo3189. (2023)
[3] Isidor et al., Genetics in medicine 24(1). (2022)
[4] Küry et al., medRxiv. (2024)
[5] Papendorf et al., Frontiers in immunology 14. (2023)

10 Poster Abstract

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The deubiquitinase OTUB1 affects heart tissue homeostasis potentially through regulation of the DNA- and RNA-binding protein YB-1

The physiological function of the deubiquitinase OTUB1 is to regulate lung development and whole-body energy metabolism. In addition, OTUB1 was reported to regulate immune responses both against tumors and infections. Interestingly, we recently found that mice with induced global as well as heart-specific Otub1 deletion demonstrate a cardiac phenotype with reduced cardiac output, increased left ventricular mass, extended isovolumetric relaxation and contraction time, and preserved ejection fraction. These observed cardiac alterations are comparable to human heart failure (HF) with preserved ejection fraction (HFpEF), which accounts for about 50% of all HF patients. The underlying mechanism of human HFpEF are largely unclear and no pharmacologic treatment exists to date. We therefore aimed to assess how Otub1 deletion affects heart tissue homeostasis and function, analyzing mouse embryonic fibroblasts (MEFs) and rat myoblast (H9c2) cells with homozygous Otub1 deletion (Otub1-/-). Sustained AKT activation has previously been reported to lead to pathological cardiac hypertrophy and heart failure. In Otub1-/- MEFs, we observed an increase in basal AKT phosphorylation (pAKT) as well as pAKT after FCS removal. However, in Otub1-/- H9C2 cells, pAKT was decreased which was not in agreement with the identified cardiac hypertrophy. Recently, it was reported that the phosphorylation of the DNA- and RNA-binding protein YB-1 is increased in the absence of OTUB1, affecting heart homeostasis in a murine model of diabetes. Interestingly, in Otub1-/- H9c2 cells, we also found an increased YB-1 phosphorylation. Therefore, Otub1 deletion potentially affects heart tissue homeostasis through regulation of YB-1 phosphorylation.

Poster Abstract 11

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Functional insights into Jep: A novel serine protease of the mouse-adapted *S. aureus* strain JSNZ

Staphylococcus aureus (*S. aureus*) exhibits a long evolutionary history and a broad host range. Recent studies have identified laboratory and wild mice as natural hosts for *S. aureus*. Murine strains have adapted to their hosts by acquiring novel virulence factors while losing genes encoding superantigens and human-specific immune evasion factors. Proteases are essential in *S. aureus* colonization, invasion, and immune evasion, and have been linked to host specificity. The dominant *S. aureus* lineage in laboratory mice worldwide is CC88. The CC88 prototype strain JSNZ was isolated from a C57BL/6 colony. Exoproteome analysis of *S. aureus* JSNZ revealed Jep, a novel, abundantly secreted serine protease. Jep shares sequence similarities with other *S. aureus* proteases and contains the conserved catalytic triad His-74, Asp-113, and Ser-189. Recombinant Jep and an enzymatically inactive mutant protein were expressed to investigate Jep's biochemical properties and its role in barrier disruption. Biochemical analysis revealed Jep's stability across a broad pH range (3-11) and its exceptional thermal resilience (85-100°C), maintaining residual activity post-heat-inactivation. In a scratch wound assay, wild-type and mutant Jep protein promoted wound closure, indicating a role in cell migration independent of its proteolytic activity. Wild-type Jep protein, but not the mutant, induced vascular endothelial damage in a murine vascular leakage model. These findings suggest that Jep plays a dual role in host interactions, contributing to cell migration independent of its proteolytic function while promoting vascular leakage through proteolytic mechanisms. Overall, these results highlight Jep's critical role in *S. aureus* tissue invasion, and disruption of barrier integrity.

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Exploring Substrates of *Staphylococcus aureus* Serine Proteases-Like Protein (SplB) in Human Serum

Bacterial proteases, such as those secreted by *Staphylococcus aureus*, can interfere with the host's homeostasis and promote disease development. While *S. aureus* produces 12 extracellular proteases, the serine protease-like proteins (SplA-F) are poorly understood. This study aimed to identify the substrates of the extracellular protease SplB in human serum to clarify its function during infection. Human serum was treated under three conditions: untreated negative control, 10 µg/mL SplB WT/mut, and 20 µg/mL SplB WT/mut, after paraformaldehyde crosslinking, Spl-bound proteins were isolated using Magstrep beads. Proteins were identified *via* mass spectrometry, and cleavage sites were mapped using the HUNTER technique. *In vitro*, assays were performed to validate candidate substrates by incubating recombinantly expressed proteins with SplB to confirm direct cleavage. Normalized mass spectrometry data identified several candidate substrates, including demin, alpha-enolase, and heat shock proteins, all of which were also proven to be cleaved by SplB *in vitro*. Cleavage sites in Desmin were pinpointed at positions 100E and 259V/261V/263M. Two other tested proteins, Serpina 12 and Elongation Factor-1-alpha-1, showed no cleavage by SplB. Identifying desmin, alpha-enolase, and heat shock proteins as substrates of SplB suggests a targeted strategy by *S. aureus* to interfere with key host cellular processes. Desmin, a cytoskeletal protein essential for maintaining muscle integrity, may represent a significant host target, with its cleavage potentially contributing to tissue damage during infection. This finding aligns with other reports that bacterial proteases disrupt host cytoskeletal components, facilitating bacterial spread. Cleavage of alpha-enolase and heat shock proteins further suggests that SplB may disrupt the host's metabolic pathways and stress response mechanisms. These proteins play critical roles in cellular energy production and protein folding, respectively, and their degradation may impair host defenses and support bacterial survival. One major factor connecting the identified substrates—desmin, alpha-enolase, and heat shock proteins—is their intracellular localization. This suggests an important role for SplB during *S. aureus'* intracellular phase of infection.

Poster Abstract 13

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Elucidating structure and function of CE-clan protease-related bacterial pathogenicity factors with dual ubiquitin-protease and acetyltransferase activity

Regulation of protein function by ubiquitination and acetylation has been investigated extensively and is known to be exploited by pathogens to hijack their host. Especially gram-negative bacteria (e.g. *Yersinia* sp., *Vibrio* sp., *Chlamydia* sp.) encode for effector proteins that are injected into host cells in order to promote infection and ensure bacterial survival. According to the MEROPS database, many of these bacterial virulence factors belong to the CE-clan, an enzyme family mainly comprised of cysteine proteases with deubiquitinase (DUB) or ubiquitin-like protease (ULP) activity, but also acetyltransferases (AcT). Some effectors even exhibit a dual activity as DUB/ULP and AcT. However, the underlying mechanisms regulating their enzymatic activity on a molecular level are often not completely understood and their exact role within the infection process remains elusive. By using tools from structural biology, biochemistry and cell biology we elucidate the structure-function relationship of a bacterial CE-clan effector in context of host cell infection. The *Chlamydia*-like pathogen *Simkania negevensis* encodes for a CE-clan member called SnCE1. Besides its protease activity as a deubiquitinase and deSUMOylase, we report strong auto-acetylation for SnCE1, suggesting a dual specificity similar to *Chlamydia* effector ChlaDUB1. ChlaDUB1 has been reported to carry out both, AcT and DUB activity, by the same active site. Despite structural homology between these two bacterial effectors, X-ray crystallographic analysis reveals differences in variable regions, therefore hinting at alternative mechanistics in SnCE1. Modelling of SnCE1 in complex with acetyl group donor Coenzyme A (CoA) identifies key residues for CoA-binding, supported by mutational studies and mass-spectrometric data. To further elucidate the physiological role of SnCE1 we set up infection studies in cell culture. Upon infection of HEK293T cells with *Simkania*, SnCE1 localizes to the ER and also mitochondria. The exact pathways targeted by SnCE1 still have to be identified. If there is a potential cross-talk between acetylation and ubiquitination/SUMOylation, as well as an impact of the acetylation status on infection, remains question of our research. Our studies contribute to a comprehensive understanding of these type of bacterial virulence factors and their role in infection, as it is crucial for our understanding of host-pathogen interactions

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Changes in the host ubiquitination machinery as a response to *S. aureus* infection

Staphylococcus aureus is a significant pathogen responsible for a range of clinical manifestations. Host cells recognize *S. aureus* through pattern recognition receptors, with ubiquitination playing a key regulatory role in this process. Ubiquitination is mediated by E3 ubiquitin ligases and reversed by deubiquitinating enzymes. While much research on ubiquitination has been focused on viral and Gram-negative bacterial infections, its role in Gram-positive bacteria like *S. aureus* is less understood. This study examined E3 ligases involved in the host response to *S. aureus* USA300, with a focus on Skp2, a key E3 ligase in cell cycle regulation. Skp2 inhibition is linked to enhanced autophagy and reduced viral infections (Gassen, 2019), and its deficiency increases NF-κB activation, leading to inflammation (Kunpeng Liu, 2018). To investigate host responses, THP-1 macrophage-like cells were infected with *S. aureus* USA300, and E3 ligase expression was analysed at the protein and RNA levels. A proteomics study and pathway enrichment analysis identified key biological pathways influenced by infection. The results indicated a reduction in ubiquitinating enzymes, along with the activation of EGFR signalling pathways. Furthermore, the stability of the E3 ligase Skp2 was enhanced during infection, potentially due to its post-infection acetylation and phosphorylation. Moreover, gene silencing of Skp2 affected the NF-κB pathway. In conclusion, Skp2 plays a critical role in *S. aureus* infection. Future studies should focus on Skp2's interactions and post-translational modifications to better understand its role in host-pathogen dynamics and therapeutic potential.

Poster Abstract 15

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Exploring the function of the secreted *Staphylococcus aureus* protease Jep by N-terminomics

The identification of protease substrates in complex samples, such as plasma or cell culture supernatants, can be challenging, particularly when the substrate is not degraded but cleaved at specific sites by proteolytic processing. In such cases, classical label-free bottom-up mass spectrometry reaches its limits in identifying proteins of interest, as the abundance of most peptides remains unchanged. However, N-terminomics can overcome this issue by enriching the N-termini prior to mass spectrometric analysis[1]. With this approach, the full potential of mass spectrometry can be focused on the peptides of particular interest. Moreover, in addition to identifying protease substrates, this method can provide information on protease-specific cleavage motifs. In our study, we focused on the novel serine protease Jep, which is found almost exclusively in mouse-adapted *Staphylococcus aureus* strains. In contrast to clinical *S. aureus* isolates, mouse-adapted strains were demonstrated to persistently colonise mice under physiological conditions. Consequently, these strains are crucial for improving current mouse models for investigating *S. aureus* colonization and infection[2], which is necessary for the development of new prophylactic and therapeutic strategies against this notorious hospital pathogen. To understand the role of Jep as a highly host-specific factor is a pivotal step in defining the model. We have shown that the deletion of *jep* in the mouse-adapted *S. aureus* strain JSNZ led to hypervirulence in a murine bacteraemia model. However, no substrates were known so far, and a classical label-free bottom-up proteomic approach did not reveal any differences in the proteome pattern comparing the deletion strain with the wild-type strain. Various potential substrate groups were subsequently screened for Jep substrates using N-terminomics. Tested samples include bacterial culture supernatants of JSNZ wild-type and a *jep* deletion mutant as well as mouse plasma and murine lung epithelial cells incubated with purified Jep protease. Most interestingly, this revealed alterations in the N-termini of a number of secreted bacterial proteins, including known virulence factors such as the subunits of LukAB. Our results suggest that the protease Jep influences virulence rather by targeted proteolytic processing of specific secreted virulence factors than by protein degradation. This example illustrates the power of N-terminomics in the investigation of proteases, to reveal effects that cannot be covered by classical label-free bottom-up proteomic approaches.

1.Kleifeld O, Doucet A, auf dem Keller U, et al. Isotopic labeling of terminal amines in complex samples identifies protein N-termini and protease cleavage products. Nat Biotechnol. 2010.
2.Trübe P, Hertlein T, Mrochen DM, et al. Bringing together what belongs together: Optimizing murine infection models by using mouse-adapted Staphylococcus aureus strains. Int J Med Microbiol. 2019.

16 Poster Abstract

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Streptococcus pneumoniae activates the inflammasome in the heart and induces heart stress in a pneumonia sepsis model

Severe pneumonia is associated with cardiac complications such as heart failure, arrhythmias and myocardial infarctions in up to 30% of patients. Especially *Streptococcus pneumonia* (pneumococci) are associated with cardiac complications, due to their ability to cause a high inflammatory host response and to exacerbate to bacteremia and sepsis, which allows bacteria to interact directly with the heart. How the *pneumococcus* damages the heart and causes cardiomyopathy is not well understood, but pro-inflammatory cytokines, such as interleukin 1β (IL-1β) are implicated. We have previously shown that activation of the NLRP3 inflammasome that is required for IL-1β activation and release contributes to the development of septic cardiomyopathy. If this plays a role in *S. pneumoniae* induced heart failure is not known. Here we developed a mouse model of *S. pneumoniae*-induced heart failure and tested the hypothesis that *S. pneumoniae* infection leads to an activation of the NLRP3 inflammasome in the heart. Female CD-1 mice were intranasally infected with *S pneumoniae* TIGR4lux (6×10^7 bacteria, n=16) or PBS (n=10) for 72h and progression of the infection was monitored every 12h using the IVIS® Spectrum Imaging System. Cardiac function was monitored by transthoracic echocardiography at 24h and 72h post infection. Changes in mRNA expression and protein contents of inflammasome components of the heart were quantitated by qRT-PCR and Western blot analysis, respectively. The course of infection showed a noticeable variability between individual animals ranging from only slight infection to severe sepsis. All *S. pneumoniae* infected animals showed a stress reaction in the heart with increased Nppa and Nppb expression and a switch to the fetal gene program with an increased Myh7/Myh6 ratio, which was independent of the infection status. *S. pneumoniae* infection caused an increase in the mRNA and protein expression of the NLRP3 inflammasome components IL-1β, NLRP3, Caspase-1 and GSDMD in the heart indicative for priming of the NLRP3 inflammasome. Accordingly, cleavage of Caspase-1 and GSDMD indicating NLRP3 inflammasome activation occurred in the hearts of infected mice. *S. pneumoniae* infection causes priming and activation of the NLRP3 inflammasome in the heart. Activation of the NLRP3 inflammasome in the heart during pneumococcal pneumonia may contribute to the development of cardiac complications. Further studies are needed to elucidate this pathway and to test if this can be used to prevent or treat cardiac complications during *S. pneumoniae* infection.

Poster Abstract 17

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Mass spectrometry-based biomarker discovery to stratify microbial (co-)infections in severe COVID-19 patients

During the late phase of the coronavirus pandemic, many patients continued to suffer from severe COVID-19 and the underlying reasons remained unclear. Initial research suggested that bacterial coinfections could be a potential factor intensifying the severity. In this project, we utilized a multi-omics approach to differentiate between monoinfections and coinfections in severe COVID-19 patients. By integrating proteomics, metabolomics adn clinical data, we identified a subset of distinct biomarkers associated with coinfections. One metabolite in particular demonstrated high classification scores. We developed a LC-MS based measurement for this metabolite and we are validating our findings using additional samples from other cohorts to ensure robustness and generalizability. This work represents a significant step forward in the identification and understanding of coinfections in severe patients, potentially leading to improved diagnostic and therapeutic strategies in managing the disease.

18 Poster Abstract

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Deciphering the *P. Falciparum* Malaria Host Response by Plasma Proteomics

Malaria presents with a wide range of disease severities, from asymptomatic infection to multi organ failure and death. High-throughput plasma proteomics helps in understanding host responses to infectious diseases , identifying risk factors, and discovering novel biomarkers. This study aims to use the plasma proteome to identify disease severity and immunity signatures in malaria patients from endemic regions and non-immune travellers. We subjected plasma samples of 482 patients to MS-based measurement resulting in a dataset of over 330 high-abundant quantified proteins. Our cohort consists of patients with varying disease severities (asymptomatic, uncomplicated, severe), different immunity status and backgrounds (Germany, Gabon), and includes longitudinal measurements as well as complementing routine clinical diagnostics. We identified 146 proteins that were differentially regulated in infected patients compared to healthy controls. These proteins primarily reflected acute phase responses (e.g. AHSG, SAA1, CD14), immune reactions (e.g., immunoglobulins), and tissue reconstitution (e.g. GSN, EFEMP1). Employing machine learning classifiers, we could robustly distinguish between different severity levels of infection and predict clinically relevant outcomes. Longitudinal analysis of the plasma proteome revealed distinct patterns: some proteins, like acute-phase proteins, responded quickly with progressing disease, while others, involved in the adaptive immune system, showed slower response patterns. Integrating proteomic data with routine clinical data and machine learning techniques enhances the understanding of host responses to Plasmodium falciparum malaria in populations with varying immunity levels. This is crucial as effective malaria management reduces exposure, leading to waning semi-immunity in previously endemic regions.

Poster Abstract 19

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Disrupted degradative sorting of TLR7 is associated with human lupus

Recently, mutations occurring within TLR7 or its associated trafficking factor UNC93B1, have been reported as monogenic causes of human lupus. A common consequence of these mutations and a hallmark of many autoimmune diseases is observed to be hyperactive TLR7 signaling. TLR7 is a single-stranded RNA sensing transmembrane receptor, located intracellularly within late endosomes. The receptor, along with its trafficking chaperone, traverses through an intricate endosomal network to reach its signaling compartment. Transport and interactions within these endosomal compartments are tightly regulated in order to prevent inappropriate activation of the receptor. These endo-lysosomal compartments, in which the TLR resides, are highly dynamic organelles. They serve as a central platform to facilitate various cellular processes like signaling and degradation. Whether disruption of endocytic processes or proteins can result in hyperactive TLR7 signaling and trigger auto-immune or auto-inflammatory responses is unknown. We report dysregulation of endosomal compartment as a novel constituent of unrestricted TLR7 signaling and human lupus. The late endosomal BLOC-1- related protein complex (BORC) along with its small GTPase Arl8b controls homeostatic turnover of the TLR7 receptor. An interaction between Arl8b and Unc93b1 is required for TLR7 to be recognized as a cargo for degradation. Mutating the interaction sites in Unc93b1 or absence of BORC leads to ineffective degradation, accumulation of the receptor and thereby enhanced signaling. Finally, we also identified an amino acid insertion in UNC93B1 in a young female patient with childhood-onset lupus. This amino acid insertion reduces the interaction of Unc93b1 with the BORC-Arl8b complex and leads to endosomal TLR7 accumulation. Therefore, dysregulation of homeostatic degradation of TLR7 through the endocytic pathway can lead to a break in immune tolerance to nucleic acids in humans. Our work highlights the importance of an intact endomembrane system in prevention of autoimmune disease.

20 Poster Abstract

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Polypharmacy-induced drug-drug interactions affect antifungal susceptibility in *Candida albicans*

Candida albicans is a major fungal pathogen that poses a global health threat, especially due to its high prevalence and frequent treatment failures. Despite rising antifungal resistance, understanding of underlying mechanisms and potential modifiers remains limited. Individuals with severe comorbidities, often requiring polypharmacy, are particularly vulnerable to life-threatening *Candida albicans* infections. Therefore, we investigated the impact of polypharmacy on antifungal resistance in this study. We systematically analyzed 813 clinical guidelines to identify medical interventions and treatments that increase the risk of fungal infections as a comorbidity. Based on this analysis, we selected a total of 40 pathologies or medical interventions with an increased risk on fungal infections, assembled a library of 119 compounds used in the treatment of these conditions and tested their effects on the efficacy of standard antifungals against the *Candida albicans* reference strain SC5314 in liquid culture, on agar, and in infected *Galleria mellonella* larvae. We identified 34 drug-antifungal interactions, including 10 antagonistic interactions that increased antifungal resistance in *Candida albicans*, when the antifungal fluconazole was combined with drugs of the established library. Most of these drugs are permanently given to patients, and their antagonistic interactions with fluconazole have, to our knowledge, not previously been described. Further, examination in the *Galleria mellonella* model for invasive candidiasis suggested also an increased risk of treatment failure in vivo, when fluconazole treatment was combined with the fluconazole antagonists identified, suggesting follow-up investigations of fluconazole treatment efficacy in patients receiving concomitantly therapy of the antagonising drugs.

Poster Abstract 21

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Unraveling Ancient Defense Mechanisms: Death Domain Proteins Across the Domains of Life

Death-Fold Domain-containing proteins, pivotal in metazoan immune responses, have recently been identified in bacteria, suggesting an ancient origin. My research investigates these bacterial Death Domains (bDDs) to uncover their roles in anti-phage defense systems. I aim to determine whether bDDs share biochemical functions with their metazoan counterparts, particularly the ability to self-assemble into signaling complexes that drive immune responses. Using a combined wet and dry lab approach, I explore the evolutionary diversity and structural properties of bDDs. Preliminary data suggests that these bacterial proteins mimic the assembly-driven signaling seen in metazoans, potentially revealing a conserved mechanism that spans across the domains of life. This study not only illuminates bacterial immunity but also enhances our understanding of the evolution and function of immune signaling pathways.

22 Poster Abstract

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Investigating the effect of reactive oxygen species on the *Drosophila melanogaster* microbiota

An important feature of the microbiota is its resilience to the host immune reaction in response to infection. The innate immune reaction of *Drosophila melanogaster* includes antimicrobial peptides (AMPs) and reactive oxygen species (ROS). Resistance mechanisms to AMPs were previously described, while those to ROS remain incompletely understood. This study aims to elucidate bacterial mechanisms of resistance to ROS in the *Drosophila* microbiota. To assess the overall effect of ROS on the microbiota, ROS were induced both *in vivo* and *in vitro* and bacterial viability post-treatment was analysed. *In vivo*, a resistance of the *Drosophila* microbiota to paraquat was seen, while *in vitro* the sensitivity of commensals to ROS-inducers paraquat and hydrogen peroxide varied. To further investigate resistance mechanisms, paraquat-sensitive mutants of the *Drosophila* commensal *Lactiplantibacillus plantarum* were selected in a transposon screen. The mutant P26/H11 was chosen for its sensitivity to paraquat *in vitro*. Further experiments will use this mutant to elucidate mechanisms of resistance of *L. plantarum* to ROS. Taken together, the results of upcoming experiments should shed light on the importance of resistance to ROS in the gut microbiota in response to infection, and uncover mechanisms of resistance by commensal bacteria.

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