DECIDE

DECisions in Infectious Diseases

1st CRC DECIDE International Symposium

March 22-24, 2026

VENUE:

Hotel Melchior Park

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Julius-Maximilians-
UNIVERSITÄT
WÜRZBURG

DECIDE
DECisions in Infectious Diseases

DFG Deutsche Forschungsgemeinschaft

WELCOME TO WÜRZBURG

Welcome to the 1st CRC DECIDE International Symposium, an international event dedicated to host-directed therapies for human infectious diseases.

The worldwide rise of multidrug-resistant bacteria and pandemics, such as COVID-19, significantly affects our healthcare systems and societies. While pathogen-targeted therapies have been very effective in fighting infectious diseases, they drive the evolution of resistant microbes and facilitate unrelated diseases by disrupting the protective functions of the microbiota. Additionally, host immune responses can worsen the outcomes of many infectious diseases, like COVID-19. Therefore, solely targeting the pathogen is not always sufficient for successful treatment. Another strategy involves host-directed therapies, which focus on targeting host cellular processes vital for pathogen survival or adjusting immune responses to either strengthen immunity or lower immunopathology. However, we currently understand very little about possible host targets. In particular, the processes that lead from an initially harmless infection to a severe or chronic disease have not been systematically studied. There is a pressing need to identify molecular decision points in the host that control disease progression and outcome.

The three-day DECIDE Symposium includes talks by distinguished national and international speakers to provide a forum for discussing the opportunities and challenges of advancing host-directed therapies for a broader range of infectious diseases.

VENUE

As a center of science, research and home to one of **Germany's oldest and most renowned universities, Würzburg** has a long-established tradition of holding international meetings as well as conferences. Award winning museums, world-class wineries, and a wide range of cultural and music festivals allow for attractive social programs. Just an hour's drive from Frankfurt airport, Würzburg sits at the crossroads of numerous German traffic routes and is easily reached from everywhere.

Würzburg is full of intriguing contrasts: progress and tradition, a big-city buzz and countryside charm.

Surrounded by vineyards, the city exudes almost Mediterranean air. Würzburg's most outstanding architectural treasure, the Residence Palace, has been listed as a UNESCO World Heritage Site since 1981 and doubles as a MICE venue of exceptional character. With 4.700 hotel beds and a vibrant nightlife fuelled by the city's large student population, Würzburg is the ideal location for successful small to mid-sized meetings and events.

Scientific Programme

Sunday, March 22, 2026

From 13:00	**Arrival of delegates & Registration**
13:00 - 14:00	***Lunch***
14:00 – 16:05	**Session 1** Chairs: Mercedes Gomez & Fergal Hamrock
14:00 - 14:25	**Camilla Ciolli-Mattioli**, DECIDE *Image-enabled cell sorting coupled with transposon sequencing reveals bacterial determinants of intracellular replication heterogeneity*
14:25 - 14:50	**Lars Dölken**, DECIDE *Host cell Z-RNASs arising from viral disruption of transcription termination trigger ZBP1-mediated cell death during lytic HSV-1 and IAV infections*
14:50 - 15:05	**Alexandra Tietze**, DECIDE *Drug combination screen on Salmonella persisters in Macrophages identified synergistic drug interactions*
15:05 - 15:20	**Tobias Köhler**, DECIDE *IL-22 induced by C. albicans reduces the severity of invasive S. aureus pneumonia in mice*
15:20 - 15:35	**Helene Hemmer**, DECIDE *Heterologous viral infections induce long-lasting antiviral states in lung parenchymal cells*
15:35 - 15:50	**Anfei Huang**, DECIDE *Emergency dendritic cell-poiesis drives CD8 T cell exhaustion*
15:50 - 16:05	**Hao Wu**, DECIDE *GLUT3 promotes stemness and survival of activated CD8 T cells*
16:05 - 17:00	***Coffee Break & Get-together***
17:00 - 17:15	***Welcome note & Opening Remarks, Thomas Rudel***
17:15 - 18:00	***Keynote lecture, Andreas Wack, The Francis Crick Institute, UK*** *Endogenous and exogenous interferons in viral infections: benefits, risks, timings and intervention points*
18:30 - 20:00	***Dinner & Get-together***
20:00	***Poster Session***

Monday, March 23, 2026

08:30 - 10:10	**Session 2: Immunology** Chairs: Wolfgang Kastenmüller & Tobias Köhler
08:30 - 08:55	**Christian Münz**, University Zürich, Switzerland *Neuroinflammation as a result of non-neurotropic herpesvirus infection*
08:55 - 09:20	**Georg Gasteiger**, DECIDE *Different Guards, Same Grounds: Spatial Orchestration of Tissue Surveillance by Memory T Cells*
09:20 - 09:45	**Dorothee Viemann**, DECIDE *TBC*
09:45 - 10:10	**Susanne Herold**, UKGM Giessen, Germany *TBC*
10:10 - 10:45	***Coffee Break***
10:45 - 12:25	**Session 3: Pathogen & Tissue Interactions** Chairs: Martin Väth & Lisa Chiggiato
10:45 - 11:10	**Georg Häcker**, University Hospital Freiburg, Germany *Sub-lethal signals in the mitochondrial apoptosis pathway as contributors to infection*
11:10 - 11:35	**Katarzyna Jobin**, DECIDE *Host hyaluronan as a molecular decision point controlling bacterial spread from the urinary tract*
11:35 - 12:00	**Melanie Hamon**, Pasteur Institute, France *Bacteria mediated histone modification*
12:00 - 12:25	**Lena Pernas**, UCLA, USA *Social sensing of infection reprograms peripheral immunity in healthy mice*
12:30 - 14:00	***Lunch***
14:00 - 15:40	**Session 4: Advanced Infection Models & Technologies** Chairs: Alexander Westermann & Stefan Oberlin
14:00 - 14:25	**Alexander Mosig**, University Hospital Jena, Germany *Microphysiological Models of Gut and Lung to Study Host-Pathogen Interaction*
14:25 - 14:50	**Carmen Aguilar**, DECIDE *PPAP: a host molecular decision point for UPEC invasion into luminal prostate cells*
14:50 - 15:15	**Dominic Grün**, DECIDE *Decoding the cellular circuitry of organ tissues with spatial transcriptomics*
15:15 - 15:40	**Scott Younger**, Children's Mercy Research Institute Kansas, USA *Scalable approaches for modeling rare disease in patient-derived organoid systems*
16:00 - 18:00	***Poster Session & Networking***
18:00 - 20:00	***Dinner & Get-together***
20:30 - 22:00	***Night Watchman Tour of Würzburg Tour (Optional)***

Tuesday, March 24, 2026

09:00 - 10:40	**Session 5: Microbiome** Chairs: Franziska Faber & Nahyung Ko
09:00 - 09:25	**Jan Hendrick Niess,** Clarunis, Basel, Switzerland *Barrier Functions in the Colon and Esophagus*
09:25 - 09:50	**Jacob Zimmermann,** DECIDE *Induction, persistence, and function of gut microbe-specific memory TH cells*
09:50 - 10:15	**Irah King,** McGill University Montreal, USA *Innately understanding host-microbiome interactions in the early life intestine*
10:15 - 10:40	**Andreas Peschel,** University Tübingen, Germany *The perks of being a pathogen – an ecological perspective of staphylococcal infections*
10:40 - 11:15	***Coffee Break***
11:15 - 12:55	**Session 6: Host-directed Therapies & Anti-infectives** Chairs: Oliver Kurzai & Valentina Cosi
11:15 - 11:40	**Mariana Xavier Byndloss**, Vanderbilt University Medical, Nashville, USA *Can diet override genetic resistance to Salmonella infection*
11:40 - 12:05	**Stefan Ludwig**, University of Münster, Germany *Towards a novel host-targeted strategy against hyperinflammatory respiratory viral diseases*
12:05 - 12:30	**Thomas Pietschmann**, Twincore Hannover, Germany *Decoding the molecular basis of severe respiratory syncytial virus infection*
12:30 - 12:55	**Jörg Vogel**, DECIDE *TBC*
12:55 - 13:15	***Poster Prize & Concluding Remarks***
13:15	***Lunch & Departure***

ABSTRACTS – Speakers

(order according to programme)

Sunday 22. March – Session 1

Image-enabled cell sorting coupled with transposon sequencing reveals bacterial determinants of intracellular replication heterogeneity

Camilla Ciolli-Mattioli

Helmholtz Institute for RNA-based Infection Research, Helmholtz Centre for Infection Research, Würzburg, Germany, Institute for Molecular Infection Biology, University of Würzburg, Würzburg, Germany.

Intracellular pathogens such as *Salmonella enterica* serovar Typhimurium (*S.Tm*) display heterogeneous replication dynamics within macrophages, yet the bacterial genetic factors underlying this variability remain poorly understood. Here, we leveraged high-speed image-enabled cell sorting (ICS) to precisely enumerate and sort infected macrophages by intracellular bacterial load and coupled it with genome-wide transposon insertion site (TIS) sequencing to identify bacterial mutants influencing replication. This approach revealed both replication-defective and hyper-replicative candidates, including a *Δfre* mutant whose enhanced replication was attributed to desensitization to oxidative stress. Together, our findings establish ICS as a powerful tool for dissecting the bacterial genetic determinants of intracellular replication, enabling discovery of phenotypes typically overlooked by conventional screens.

Host cell Z-RNAS arising from viral disruption of transcription termination trigger ZBP1-mediated cell death during lytic HSV-1 and IAV infections

C. Yin[1*], A. Fedorov[2*], H. Guo[3*], C. Rousseau[4,5] , A. W. Whisnant[4,5], T. Hennig[4,5], M. Olguin[4,5], J. Rehwinkel[2] , A. Pichlmair[6], T. Zhang[7#] , A. Herbert[8#] , Lars Dölken[4,5#] , S. Balachandran[1#]

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joint corresponding authors

Herpes simplex virus 1 (HSV-1) and Influenza A virus (IAV) disrupt host transcription termination to suppress cellular gene expression and promote viral replication. We previously identified the HSV-1 protein ICP27 as a key mediator of this disruption by inhibiting the mRNA 3' processing factor CPSF. This results in widespread transcriptional read-through and the accumulation of unusually long aberrant host transcripts, termed long aberrant nuclear RNAs (lanRNAs). Here, we show that these lanRNAs are not merely by-products of viral host shutoff, but act as functional second messengers of innate immunity. Read-through transcription into endogenous retroelements generates Z-form double-stranded RNA structures that are sensed by the cytosolic receptor ZBP1, triggering programmed inflammatory cell death (necroptosis). Contrary to previous assumptions, the dominant ZBP1-activating ligands during HSV-1 and IAV infection are therefore host-encoded rather than viral RNAs. Viruses lacking ICP27 or NS1 (IAV) failed to induce lanRNA and Z-RNA accumulation and were strongly impaired in ZBP1 activation, whereas ectopic expression of these viral proteins or pharmacological inhibition of CPSF was sufficient to induce Z-RNAs, activate ZBP1 signaling, and trigger cell death. Together, these findings establish ZBP1-mediated cell death as a host response to viral interference with transcriptional termination. They illustrate how cells exploit prior viral invasions of their genomes to convert viral disruption of their gene expression machinery into an effective antiviral defense mechanism.

Drug combination screen on Salmonella persisters in Macrophages identified synergistic drug interactions

Alexandra Tietze[1,2] and A. R. Brochado[1,2,3]

[1]Department of Microbiology, Biocenter, University of Würzburg, Würzburg, Germany, [2] Interfaculty Institute of Microbiology & Infection Medicine Tübingen (IMIT), University of Tübingen, Tübingen, Germany, [3] Cluster of Excellence 'Controlling Microbes to Fight Infections' (CMFI), University of Tübingen, Tübingen, Germany.

Intracellular pathogens such as Salmonella pose a major challenge in the context of infectious diseases due to their ability to enter a persistent state, evading both host immunity and antibiotics. Understanding the interplay between pathogen survival strategies and innate immune responses, especially after pathogen internalization and establishment in the intracellular niche, is critical for developing new therapeutic approaches.

In this study, we developed a high-throughput screening approach to evaluate the effects of 3000 drug combinations on sustaining or aborting Salmonella Typhimurium infections within macrophages. We simultaneously target the bacterium and the macrophage using a combination of antibiotics and immunomodulatory drugs to identify conditions that interfere with host-pathogen interactions well after Salmonella's establishment in the intracellular niche, thereby altering its intracellular survival. We used recovery time after antibiotic removal to quantify bacterial viability and post-treatment-stress effects at scale.

We identified multiple drug combinations that effectively modulate the Salmonella's recovery time, primarily synergistic effects causing recovering times significantly longer than expected based on the effect of the drugs alone. Importantly, we found several compounds, which targets have been previously reported to hamper *Salmonella* intracellular survival (e.g. ROS production), thereby legitimating of our approach. In addition, we found synergistic interactions involving compounds targeting host pathways for which there is no previous evidence of relevance to *Salmonella* persistence. We are currently pursuing in-depth exploration of these leads, which will unveil the molecular mechanism of this synergy on the bacterial and host sides, and potentially reveal novel pathogen survival strategies.

In the long term, we aim to investigate how pathogen- or host-cell-specific our findings are, which will hint towards the need for broad-spectrum or targeted therapies. These results demonstrate the power of systems biology to uncover critical molecular interactions in innate immunity and provide actionable insights for combating persistent bacterial infections.

IL-22 induced by *C. albicans* reduces the severity of invasive *S. aureus* pneumonia in mice

Tobias Köhler[1], N.E. Nieuwenhuizen[1], S. Vielreicher[2], W. Böhnke[2], G. Braune[1], M. Batliner[1], A. Schöninger[1], I.D. Jacobsen[2], T. Hertlein[3], A.E. Saliba[4], Eslam Ibrahim[3], K. Ohlsen[3], O. Kurzai[1,2]

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Introduction: The commensal fungus *C. albicans* and the bacterium *S. aureus* frequently coexist in humans and can cause severe systemic infections in immunocompromised patients. Clinically, *S. aureus* commonly induces pneumonia and disseminates from the lung, whereas *C. albicans* almost never causes invasive pulmonary disease, even in mechanically ventilated patients with high fungal burdens.

Objectives: We aim to investigate the different behavior of *C. albicans* and *S. aureus* in the lung, and to assess how pulmonary pre-colonization with *C. albicans* influences subsequent *S. aureus* infection.

Methods: We established a lung pre-colonization/infection model using Balb/c mice. Mice were administered *C. albicans* intranasally at day 0 and infected intranasally with *S. aureus* on day 1. At 24, 48 and 96 hours post *S. aureus* infection, lung, liver and kidney bacterial/fungal burdens as well as lung immune responses were analyzed.

Results: Following intranasal administration, pulmonary loads of both pathogens declined over time; however, only *S. aureus* disseminated to liver and kidney, closely mirroring the clinical situation. Pulmonary pre-colonization with *C. albicans* markedly altered the lung immune landscape and resulted in enhanced recruitment of neutrophils and CD11b dendritic cells upon *S. aureus* infection. Cytokine profiling revealed a pronounced induction of IL-1β, IL-17, and IL-22 during fungal colonization followed by bacterial challenge. Importantly, *C. albicans* pre-colonization protected mice from lethal *S. aureus* lung infection. Neutralization of IL-22 abrogated this protective effect, identifying IL-22 as a critical mediator to *S. aureus* induced pneumonia. Early administration of recombinant IL-22 was sufficient to significantly enhance early survival during a lethal *S. aureus* pneumonia.

Conclusion: We established a physiologically relevant dual pathogen lung model and identified IL-22 as a key immunological switch determining the outcome of *S. aureus* lung infection.

Heterologous viral infections induce long-lasting antiviral states in lung parenchymal cells

Helene Hemmer

Würzburg Institute of Systems Immunology, Max Planck Research Group, Julius Maximilians Universität Würzburg, Würzburg, Germany.

Repeated pathogen exposure throughout life reshapes tissue immunity beyond classical immune memory. While circulating memory T cells are well characterized, far less is understood about how infection history durably alters the cellular landscape of barrier tissues including which (non-) hematopoietic cells retain transcriptional memory.
Using single-cell mRNA sequencing of CD45+ and CD45- lung cells from pathogen-experienced mice, sequentially infected with murine analogs of EBV, CMV, and IAV, we mapped long-lasting transcriptional changes across both hematopoietic and non-hematopoietic compartments, at resting memory and following respiratory viral rechallenge. We found that infection history durably reprograms gene expression in non-immune lung cells as well as innate immune cell populations. Specifically, interferon signaling emerged as a key pathway that was activated beyond the acute infection phase with IAV, suggesting that prior infection history fundamentally conditions how lung tissue responds to new respiratory threats.

Emergency dendritic cell-poiesis drives CD8 T cell exhaustion

Anfei Huang[1,4], M. Ugur[1], A. Wirsching[1], X. Yang[1], A. V. Goncalves[1], D. Seetharama[1], R. Doucet-Ladeveze[1], Y. Ouyang[1], T. Kaisho[2], M. Vaeth[1], Y. Liang[3], G. Gasteiger[1] and W. Kastenmüller[1, #,*]

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Upon infection, emergency myelopoiesis (EM) profoundly remodels the production of neutrophils and monocytes thereby amplifying innate immune defense mechanisms. Here, we investigated how EM impacts on the development and function of dendritic cells (DCs) and, thus on adaptive antiviral immunity. Five days after infection, DC arising from EM predominated in lymphoid organs, yet were refractory and responded poorly to pathogen-associated molecular patterns. Functionally, refractory DC that presented viral antigens induced CD8 T cell exhaustion and consequently provided essential protection against fatal immunopathology. Mechanistically, we identified DUSP-1 as a key regulator that mediates the refractory state of DC in mice, and found it upregulated in DCs from convalescent COVID-19 patients, suggesting a conserved role in humans. Together our study proposes that CD8 T cell exhaustion is actively orchestrated by the immune system through a systemic negative feedback loop, thus providing new angles for immunotherapeutic intervention in chronic viral infection and cancer.

GLUT3 promotes stemness and survival of activated CD8 T cells

Hao Wu[1], M. Campillo Prados[1], M. Eckstein[1], S. M. Hochrein[1], W. Schmitz[2], M. Eilers[2], F. Imdahl[3], T. Krammer[3], L. Gehrke[4], M. Krämer[4], G. Mattavelli[4], H. Shaikh[5], A. Beilhack[5], A. E. Saliba[3], W. Kastenmüller[1], A. Riedel[4], and M. Vaeth[1]

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The maturation and differentiation of lymphocytes are tightly coupled to nutrient uptake and intermediary metabolism. Upon antigen receptor engagement, T cells upregulate a range of nutrient transporters to coordinate metabolite influx and efflux, thereby supporting activation and effector function. However, within sites of infection and the tumor microenvironment, lymphocytes are exposed to metabolically hostile conditions caused by limited vascular supply and the high metabolic activity of pathogens or cancer cells. These constraints result in hypoxia, nutrient deprivation and accumulation of toxic byproducts, ultimately driving T cell dysfunction, exhaustion, and apoptosis. Although nutrient transporters are critical regulators of T cell biology, their precise mechanisms of action and therapeutic potential remain incompletely defined. Here, we investigated the role of the glucose transporter GLUT3 in cytotoxic $CD8^+$ T cells during antiviral and antitumor immune responses. Unexpectedly, genetic ablation of GLUT3 did not impair $CD8^+$ T cell activation or proliferation. Instead, GLUT3-deficient $CD8^+$ T cells displayed accelerated exhaustion and increased apoptosis following antigen receptor stimulation. Integrated transcriptomic, metabolomic and functional analyses revealed that GLUT3 was dispensable for glucose uptake and central carbon metabolism but was essential for maintaining redox homeostasis in activated T cells through ascorbate recycling. Loss of GLUT3 led to excessive accumulation of reactive oxygen species, promoting T cell exhaustion, cell death, and impaired immune control. Conversely, enforced GLUT3 expression enhanced the persistence and effector function of antigen-specific T cells in models of chronic viral infection and cancer, supporting improved redox balance and upregulation of stemness-associated programs.

Sunday 22. March - Keynote Lecture

Endogenous and exogenous interferons in viral infections: benefits, risks, timings and intervention points

Andreas Wack

The Francis Crick Institute, London, UK.

Interferons (IFNs) are the quintessential antiviral cytokines. However, easy and linear rationales for using or targeting them are hampered by the complexity surrounding them: IFNs are pleiotropic; there are numerous members in three different IFN families signalling through distinct receptors that show different cell distribution; IFN expression is highly dynamic throughout the first days of equally dynamic virus presence in the infected organism. This makes IFNs a very interesting study object to understand the biology of host responses to viral infection, and equally a very difficult target for host-directed therapy. I will discuss our growing understanding of IFN actions and impact on infection severity, and along this journey, will discuss questions regarding the options and risks of IFN-targeted intervention.

Monday 23rd March – Session 2: Immunology

Neuroinflammation as a result of non-neurotropic herpesvirus infection

Christian Münz

Institute of Experimental Immunology at the University of Zürich, Switzerland.

The Epstein Barr virus (EBV) establishes persistent infection in nearly all human adults. In a small subset of these, EBV is associated with lymphomas and carcinomas, as well as some autoimmune diseases. Primarily, EBV has been suggested to initiate the prodromal phase of multiple sclerosis (MS), a demyelinating autoimmune disease of the central nervous system (CNS). Our studies could show that EBV infection expands T-bet$^+$CXCR3$^+$ memory B cells that then home to the CNS in humanized mice. These B cell subsets recruit and restimulate inflammatory lymphocyte infiltrates at submeningeal and perivascular locations in the brain. These infiltrates contain characteristics of human B and T cell subpopulations that are reminiscent of lymphocytes that can be found in post-mortem MS brains, and associated with increased neurofilament light chain (NfL) release as a prodromal marker of CNS autoimmunity.

Different Guards, Same Grounds: Spatial Orchestration of Tissue Surveillance by Memory T Cells

Georg Gasteiger

Würzburg Institute of Systems Immunology, Max Planck Research Group, Julius-Maximilians-University of Würzburg, Würzburg, Germany.

Memory T cells surveil tissues to detect and restrain infection. This task is shared between resident memory T cells that populate and adapt to specific tissue environments, and recirculating memory T cells that continuously travel through the blood, enter peripheral tissues, and return via the lymphatic system. Despite their critical role, pathogen-specific memory T cells are exceedingly rare and vastly outnumbered by the tissue cells that represent major targets of infection. A key question, therefore, is how these cells efficiently survey tissues and rapidly detect and respond to infection. In this talk, I will present a framework in which the spatial positioning of antiviral memory T cells and their interactions with antigen-presenting cells enable integrated local and systemic immune surveillance.

Monday 23rd March – Session 3: Pathogen – Tissue Interactions

Sub-lethal signals in the mitochondrial apoptosis pathway as contributors to infection

Georg Häcker

Institut für Medizinische Mikrobiologie und Hygiene, Universitätsklinikum Freiburg, Freiburg, Germany.

Mitochondrial apoptosis makes a contribution to decisions in infection: viruses, bacteria and parasites all can trigger or inhibit apoptosis, sometimes both. Cells may die or may be kept alive, which may affect the outcome of an infection. Recent research shows that, contrarily to a long-standing view, the mitochondrial apoptosis system can also provide 'sub-lethal' signals: low-level activity can be measured but the cell is not killed. All infections tested to date initiate sub-lethal signals, and a number of downstream effects can be measured. I will illustrate how this system is triggered in infection, demonstrating the molecular steps at mitochondria as well as the consequences for infection identified so far. Mitochondrial apoptosis can make life-death decisions. It turns out that sub-lethal signals can also affect decisions in infection.

Host hyaluronan as a molecular decision point controlling bacterial spread from the urinary tract

Katarzyna Jobin

Department of Microbiology and Institute of Systems Immunology, University of Würzburg.

Urinary tract infections (UTIs) are among the most frequent bacterial infections worldwide and remain a major cause of morbidity. Most are caused by uropathogenic *Escherichia coli* (UPEC) whose rising antibiotic resistance poses a growing health concern. This highlights the urgent need for alternative, host-directed therapeutic strategies.

Urinary tract employs multiple layers of defense to fight the invading bacteria. These include a hyaluronan-enriched extracellular matrix and a network of resident and infiltrating immune cells. Hence, most pathogens remain confined to the lower urinary tract, but a subset progresses upwards to the kidneys and can disseminate systemically. Yet, the molecular decision points that prevent bacteria from breaching into the circulation remain poorly understood.

Our data show that ascending UPEC accumulate in the renal pelvis and—upon dissemination—enrich within renal veins. These veins are encapsulated by macrophages embedded in a hyaluronan-rich matrix. Interestingly, when we locally degraded hyaluronan in a mouse model of UPEC pyelonephritis, we observed a striking increase in bacteremia.

This is notable because hyaluronan degradation is known to occur during various infections, but UPEC itself lacks hyaluronidase activity and cannot directly degrade host hyaluronan. We therefore hypothesize that infection-induced ECM remodeling may result from reactive oxygen species and/or hyaluronan-degrading enzymes produced by macrophages and other immune cells—or from co-infecting bacteria such as *Enterococcus faecalis* or *Staphylococcus aureus*, both relevant in urosepsis. Supporting this, co-culture of UPEC with *E. faecalis* renal pelvis isolate markedly increased hyaluronidase expression by the latter.

Together, these findings highlight hyaluronan as a critical determinant in controlling bacterial dissemination that governs whether infection remains localized or progresses to systemic disease. Its interplay with immune cells and co-infecting bacteria requires further investigation.

Bacteria mediated histone modifications

Melanie Hamon

Institut Pasteur, Paris, France.

Epithelial cells are the first point of contact for bacteria entering the respiratory tract. *Streptococcus pneumoniae* is an obligate human pathobiont of the nasal mucosa, carried asymptomatically but also the cause of severe pneumonia. The role of the epithelium in maintaining homeostatic interactions or mounting an inflammatory response to invasive *S. pneumoniae* is currently poorly understood. Our work focuses on understanding how chromatin modifications, at the histone level, induced by bacteria interfere with the host transcriptional program. We have shown that *S. pneumoniae* actively induces histone modifications to promote an efficient infection, but also to maintain asymptomatic colonization. Furthermore, we have recently revealed that *S. pneumoniae* leaves a lasting histone mark, which remain after bacterial clearance and is transmitted through cell division. In fact, infection establishes a unique epigenetic program affecting the transcriptional response of epithelial cells, rendering them more permissive upon secondary infection. In my presentation, I will present our current findings on the molecular mechanisms of *S. pneumoniae* induced histone modifications.

Social sensing of infection reprograms peripheral immunity in healthy mice

Lena Pernas

Dept. of Microbiology, Immunology and Molecular Genetics, UCLA, USA.

In plants and insects, social immunity enables individuals to detect infection in neighbors and mount protective, community-level responses. Whether mammals possess analogous mechanisms remains unknown. Here, we asked how the presence of sick cage-mates influences the physiology of uninfected neighbors. We found that healthy mice co-housed with conspecifics infected with the non-communicable murine pathogen *Toxoplasma gondii* undergo a shift in peripheral immune responses that establishes a primed immune state. This exposure-induced priming conferred physiological resilience to a sublethal lipopolysaccharide (LPS)-inflammatory challenge and was mediated by increased myeloid-derived IL-10 production. Blocking IL-10 signaling abrogated exposure-induced protection against a subsequent immune challenge. Thus, our findings show that immune state in healthy mammals can be shaped by exposure to infected conspecifics, hinting at social immunity-based protective mechanisms in mammals.

Monday 23rd March – Session 4: Advanced Infection Models and Technologies

Microphysiological Models of Gut and Lung to Study Host-Pathogen Interaction

Alexander S. Mosig

Institute of Biochemistry II, Jena University Hospital, Jena, Germany.

Understanding the complex interplay between microbial pathogens and the human host requires experimental platforms that balance the need to faithfully recapitulate tissue-specific physiology with the ability to enable controlled mechanistic investigation. Organ-on-chip (OoC) models of the intestinal and pulmonary mucosa offer a compelling approach to bridge the gap between oversimplified two-dimensional cell cultures and animal models that are inherently limited by species-specific differences in microbiota composition, immune responses, and host-pathogen tropism.

Despite their reductionist design, OoC systems can reconstitute key features of human barrier tissues, including polarised epithelial architecture, mucus production, and continuous perfusion, thereby establishing physiologically relevant organotypic microenvironments for studying infections with bacteria, fungi, and viruses while considering relevant biochemical and biophysical cues. Importantly, integrating human immune cells enables the dissection of human-specific immune mechanisms during host-pathogen interactions, providing insights difficult to obtain in conventional model systems.

A major advantage of microfluidic OoC platforms lies in their capacity for real-time monitoring of infection dynamics, for instance, through live-cell microscopy, and in the ability to perform targeted interventions during experiments. Continuous perfusion extends the experimental window considerably, enabling the establishment and investigation of microbial communities under homeostatic conditions, a prerequisite for studying commensal-pathogen interactions and dysbiosis-associated pathologies. Furthermore, the incorporation of human stem cell-derived tissue models opens avenues for donor-specific and personalised testing, enabling the assessment of individual susceptibility to infection and host response variability within the same experimental framework.

This presentation will highlight recent advances in establishing gut- and lung-on-chip infection models and how these microphysiological systems can be leveraged as human-relevant platforms for deciphering mechanisms of host-pathogen interactions.

PPAP: a host molecular decision point for UPEC invasion into luminal prostate cells

Carmen Aguilar

Host Pathways in Urinary Tract Infections Group, Institute of Molecular Infection Biology, University of Würzburg, Würzburg, Germany.

Bacterial prostatitis affects millions of men worldwide, with uropathogenic *Escherichia coli* (UPEC) as the most common cause. Acute infections can progress to chronic disease, leading to persistent urinary symptoms, sexual dysfunction, and in severe cases sepsis. Despite its high prevalence and clinical burden, the mechanisms underlying UPEC pathogenesis in the prostate remain poorly understood. To address this gap, we first developed an organoid-based model of the prostate epithelium that closely recapitulates the epithelial compartment of the in vivo tissue. Single cell RNA sequencing confirmed that the model reproduces the cellular composition and transcriptional profiles of the native prostate epithelium. The model also displayed a robust barrier function, with high transepithelial electrical resistance and well-organized tight junctions, indicating preserved epithelial integrity. Using this model, we show that UPEC can actively adhere to, invade, and replicate within prostate epithelial cells. Notably, bacteria preferentially infect differentiated luminal cells rather than basal stem-like cells. Mechanistically, we demonstrate that UPEC uses the adhesin FimH to bind and invade luminal prostate cells. Pull down experiments with a recombinant FimH protein identified the host protein PPAP as the receptor mediating this interaction. Genetic deletion of PPAP in prostate organoid cells using CRISPR/Cas9 strongly reduced bacterial adhesion and invasion. Similarly, blocking FimH binding with D mannose or mutating the N-glycosylation sites in the receptor produced comparable inhibitory effects. These findings indicate that PPAP functions as a molecular decision point that determines whether UPEC can successfully invade prostate epithelial cells. In addition to advancing our understanding of early infection events in bacterial prostatitis, this work also establishes a physiologically relevant model to study other urogenital infections and highlights the FimH-PPAP interaction as a potential therapeutic target.

Decoding the cellular circuitry of organ tissues with spatial transcriptomics

Dominic Grün

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Mathematical modeling of gene networks in systems biology has uncovered key design principles underlying network motifs that regulate transcriptional responses. Comparable engineering principles are thought to control the interactions of molecular response programs among neighboring cells within local tissue environments. These cellular circuits play a critical role in governing tissue development, maintaining homeostasis, and mediating responses to disease. Recent advances in spatial transcriptomics now enable precise measurement of cellular states within tissues; however, existing computational methods are largely descriptive and lack the capacity to predict these circuits or their downstream effects on cellular behavior. We introduce latent variable modeling approaches to integrate single-cell RNA-sequencing and high-resolution imaging-based spatial transcriptomics data for the inference of cellular circuitry. Focusing on bone marrow regeneration after CAR-T cell therapy of multiple myeloma patients, this modeling paradigm reveals a cellular niche network of immune cells and stromal cells, that sustains an inflamed bone marrow microenvironment and impedes the reestablishment of healthy hematopoiesis, leading to prolonged cytopenia – a severe side effect of CAR-T cell therapy.

Scalable approaches for modeling rare disease in patient-derived organoid systems

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Personalized antisense oligonucleotides (ASOs) have achieved positive results in the treatment of rare genetic disease. As clinical sequencing technologies continue to advance, the ability to identify rare disease patients harboring pathogenic genetic variants that may be amenable to this therapeutic strategy is likely to improve. To support this expanded patient population, we have established a platform to facilitate the rapid characterization of preclinical ASO leads. We developed a highly efficient and scalable pipeline for the derivation of iPSCs. Our noninvasive iPSC reprogramming method requires as few as 50K PBMCs (commonly available from prior genetic testing) and is typically complete within 2-3 weeks. A parallelized format enables the simultaneous reprogramming of dozens of patient samples, allowing the straightforward generation of nearly 300 patient-derived iPSC lines in under 6 months with >93% reprogramming success rates. Pairing our iPSC reprogramming pipeline with optimized organoid differentiation protocols further enables the functional profiling of patient-specific disease phenotypes at scale. As proof of principle, we designed personalized ASOs targeting a splice-disrupting intronic variant in the dystrophin gene of a Duchenne muscular dystrophy (DMD) patient and confirmed the reversal of contractile dysfunction in patient-derived cardiac organoids. We then selected a cohort of undiagnosed rare disease patients with complex neurological phenotypes, prioritized predicted splice-disrupting intronic variants based on genome sequencing data, designed patient-specific ASOs targeting candidate variants for each patient, and identified several ASOs that resulted in abatement of seizure-associated neuronal hyperactivity in patient-derived brain organoids. Our platform provides the foundation for an expedited path towards the design and preclinical evaluation of personalized ASO therapeutics for a broad range of rare diseases. Importantly, this platform is not limited to the characterization of ASOs and can be used to evaluate patient-specific responses to alternative therapeutic modalities including small molecules and biologics.

Tuesday 24th March – Session 5: Microbiome

Barrier Functions in the Colon and Esophagus

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Humans with chronic inflammatory diseases, such as inflammatory bowel disease or eosinophilic esophagitis, have an altered microbial, diet- and host-derived metabolome that regulates immune responses during inflammation. Whether these alterations initiate inflammation by penetrating substrates across epithelial barriers, or whether **the disruption of the metabolome results secondarily from inflammation, are possibilities to consider.** To address these questions, we employ a range of complementary experimental approaches to dissect the molecular pathways underlying the sensing and processing of endogenous and exogenous signals in the esophagus and gut. Our lab has identified a novel epithelial pathway that links environmental and endogenous chemical sensing to metabolic regulation in the colon. Central to this pathway is the aryl hydrocarbon receptor (AHR), which detects diverse chemical signals and induces expression of the extrahepatic enzyme Cyp2s1 in colonic epithelial cells. Manipulating Cyp2s1 expression in vivo revealed significant effects on host metabolic profiles and microbial community composition. These findings uncover a novel function of Cyp2s1 in IBD and highlight its potential as a therapeutic target for regulating gut metabolism and inflammatory responses. Conversely, inflammation fundamentally alters the metabolome. We discovered that tryptophan derivatives shift from quinoline end products to xanthenurate derivatives, which are recognized by the G protein-coupled receptor GPR35, in active eosinophilic esophagitis. Activation of GPR35, in turn, induces IL-18 production by macrophages, thereby further impairing the esophageal barrier. Considering that food- and aeroallergen-driven eosinophilic esophagitis can be treated with a six-food elimination diet that avoids tryptophan-rich foods such as milk, wheat, egg, soy, nuts, and fish, these examples highlight the complex interplay between the mucosal immune system and environmental, diet-derived, and endogenous substrates to alter the mucosal immune system in inflammatory conditions.

Induction, persistence, and function of gut microbe-specific memory T_H cells

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The human intestine is continuously exposed to a vast array of antigens derived from both the commensal microbiota and enteric pathogens, which stimulate mucosal T cell responses throughout life. To investigate how intermittent intestinal bacterial exposures drive antigen-specific CD4 T cell resident memory, we have genetically engineered mutant strains of microbiota symbionts such as *Lactobacillus reuteri* and *E. coli* but also pathogens such as *Salmonella typhimurium* that only transiently colonize the gut of germ-free mice. We have combined this system that uncouples live microbial exposure from permanent colonization with the tracing of antigen-specific T cells by custom adoptive transfer and peptide:MHC tools to address the induction, persistence, and function of gut microbe-directed CD4 T cell responses. We find that gut bacteria, including benign symbionts, elicit long-lived, antigen-independent, tissue-resident memory CD4 T cells that facilitate sensitised barrier-protective responses with significant implications for new therapeutic approaches against gastrointestinal infections.

Innately understanding host-microbiome interactions in the early life intestine

Irah King

Research Institute of the McGill University Health Centre, Montreal, Canada

Early postnatal life is a highly dynamic period in which the developing intestine is inundated with billions of microbes that not only battle for colonization, but also strongly influence immune system development and long-term function. While this rapidly changing environment provides a window of opportunity to imprint resilience against disease, how specific members of the gut microbiota influence intestinal immune activity during early life is only beginning to be understood. By performing a kinetic analysis of immune cell activation and microbiome composition in the colon of mice across the lifespan, we identified transient activation of a type 3 immune response during the weaning period. This response is characterized by selective production of IL-17 by fetal-derived gd T cells that occurred in an IL-1-dependent, microbiota-specific manner. Microbial gain-of-function approaches determined that asymptomatic colonization by the pathobiont C. difficile drives colonic gd T cell activation. Although a transient type 3 immune response enhanced intestinal barrier integrity in response to the presence of C. difficile, the subsequent differentiation of IL-10 producing T regulatory cells prevented protracted IL-17 production and immunopathology. Collectively, our results reveal how an agile multicellular immune network meets the demands of a maturing microbiota to support organ function.

The perks of being a pathogen – an ecological perspective on staphylococcal infections

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The incidence of antibiotic-resistant bacterial infections is increasing, and development of new antibiotics has been deprioritized by the pharmaceutical industry. Interdisciplinary research approaches, based on the evolutionary and ecological principles of bacterial fitness, competition, and transmission, could open new avenues to combat antibiotic-resistant infections. Many facultative bacterial pathogens including Staphylococcus aureus use human mucosal surfaces as their major reservoirs and induce infectious diseases to aid their lateral transmission to new host organisms. Beneficial bacterial commensals can outcompete specific pathogens, thereby lowering the capacity of the pathogens to spread and cause serious infections. Despite the clinical relevance, however, the understanding of commensal-pathogen interactions in their natural habitats remains poor. Research on the interactions between bacterial pathogens and commensals in the context of human microbiomes and host biology can lead to the development of innovative and sustainable ways of preventing and treating infectious diseases. This talk is part of the Kiel Symposium.

Tuesday 24th March – Session 6: Host-directed Therapies and Anti-Infectives

Towards a novel host-targeted strategy against hyperinflammatory respiratory viral diseases

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Institute of Virology (IVM), University of Muenster, Germany.

Severe influenza, COVID-19 and other hyperinflammatory viral diseases progress in at least two distinct stages. The initial stage is primarily characterized by tissue damage directly caused by viral replication, whereas the subsequent, more severe stage is driven by excessive cytokine induction. Consequently, direct-acting antivirals (DAAs) are often ineffective during the later stages of these infections. Therefore, novel therapeutic strategies are required to effectively treat severe viral infections associated with hyperinflammation. Previously, we have unraveled a strong dependence of influenza viruses replication on the cellular Raf/MEK/ERK kinase pathway and also obtained evidence that the pathway acts immunomodulatory. Furthermore, we could show that also other viruses, such as RSV, BDV, or Hantaviruses are sensitive to MEK inhibition, suggesting that MEK inhibitors could act as novel broadly active antivirals with a dual benefit: directly, via impairing virus replication and indirectly, by re-balancing overshooting immune responses. Accordingly, we demonstrated that the MEK inhibitor Zapnometinib results in a sustained inhibition of SARS-CoV-2 propagation and also leads to reduced expression of virus-induced proinflammatory cytokines while the antiviral type I interferon response appears not to be affected. The compound also acts synergistic with licensed DAA and moreover displays a high barrier towards emergence of resistance. The drug has been demonstrated to be safe and well tolerated in humans in a Phase I clinical trial (NCT04385420). Results of a phase II clinical trial employing Zapnometinib in hospitalized COVID-19 patients (RESPIRE, NCT04776044) indicate clinically relevant efficacy with a very favorable safety profile. Zapnometinib resulted in both, reduced virus titers and dampening of the cytokine response in patients. Thus, the concept of MEK inhibition as a novel anti-infective strategy is a clinically feasible and promising approach.

Decoding the Molecular Basis of Severe Respiratory Syncytial Virus Infection

Thomas Pietschmann

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Respiratory syncytial virus (RSV) remains the leading cause of severe lower respiratory tract infections in infants. However, the host determinants that govern viral control and disease severity are not fully understood, and there are limited treatment options for RSV.

To address these challenges, we conducted genetic analyses on a cohort of children with severe RSV infections (the IRIS cohort) to identify risk gene candidates, alongside functional systems virology approaches. These approaches included single-cell profiling of acute transcriptional responses to RSV infection in primary human lung cells, genome-wide CRISPR/Cas9-based knockout screening, and drug repurposing screening. By focusing our analysis and follow-up strategy on potential risk genes and variants enriched in patients with severe RSV disease and/or on potentially druggable pathways regulated during acute RSV infection, we reduced the search space to biologically meaningful pathways and candidates, including, for instance, host factors that modulate the ER stress response and apoptosis and IFN signalling.

Time-resolved single-cell RNA sequencing of infected primary airway epithelia revealed viral load-dependent suppression of ciliogenesis, antigen presentation and innate sensing pathways in infected cells, while bystander cells exhibited robust IFN-stimulated gene responses. Notably, IRF1 escaped viral suppression and its ectopic expression reduced viral replication, identifying it as a potential therapeutic target.

Screening 12,000 drug-(candidates) identified lonafarnib as a potent RSV fusion inhibitor, which was validated structurally and in vivo. Finally, the sequencing of 101 previously healthy infants with severe RSV bronchiolitis revealed 94 variants in 79 candidate risk genes. TMEM259 polymorphisms modulated RSV replication through ER stress and apoptosis in an allele-dependent manner.

Together, these convergent approaches define key antiviral pathways, identify actionable targets and reveal genetic risk factors. This paves the way for mechanism-based risk stratification and the development of tailored interventions against RSV.

ABSTRACTS – Posters

Poster 1

DECIDE/CRC 1583 Project A01

Establishing Editable Human Airway Platform for Advanced Infection Biology

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Mechanistic studies of respiratory infections require human airway models that are both physiologically relevant and genetically tractable. Primary airway tissue remains limited in scalability and editability, constraining precise interrogation of host determinants of infection. We are developing a CRISPR-engineerable hiPSC-derived airway epithelial model designed as a next-generation infection platform.

Using stage-specific modulation of developmental signaling adapted from McCauley et al. (2018), we generate NKX2-1$^+$ lung progenitors (LPs) and basal-like intermediates as a foundation for differentiation at air–liquid interface (ALI). However, NKX2-1$^+$ yield remains variable. CPM-based enrichment of NKX2-1$^+$ cells improves separation of NKX2-1$^+$ and NKX2-1$^-$ populations on flow cytometry gate, yet overall NKX2-1 purity remains ~30–50%, highlighting the need for further optimization. Interestingly, this CPM-based enrichment eliminates fully EpCAM$^-$ and enriches EpCAM$^+$ populations significantly.

In parallel, we are establishing CRISPR–Cas9 editing directly in hiPSCs to generate isogenic knockout lines of our gene of interest prior to airway differentiation. Plasmid-based lipofection (Lipofectamine Stem) produced low GFP expression without detectable editing. Notably, nucleofection of the identical plasmid dose that yielded low GFP in lipofection caused complete cell death across five independent experiments, whereas the same nucleofection conditions robustly expressed pmaxGFP control plasmid, confirming instrument and protocol integrity. Reducing plasmid concentration prevented cytotoxicity but abolished detectable GFP signal, suggesting a narrow or incompatible transfection window in hiPSCs.

We are currently expanding sgRNA designs and optimizing delivery strategies to overcome this bottleneck.

Our objective is to establish a scalable, gene-editable human airway epithelium enabling controlled dissection of host genes regulating epithelial differentiation, barrier integrity, and susceptibility to respiratory viral infection. We welcome expert input on improving progenitor enrichment and hiPSC genome editing efficiency to accelerate realization of this advanced infection model.

Poster 2

DECIDE/CRC 1583 Project A01

Age-dependent Influenza A virus disease severity is driven by epithelial resilience and inflammatory reprogramming

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Infants and young children often experience less severe influenza A virus (IAV) infections than adults, suggesting that disease severity is determined by age-dependent molecular mechanisms. In this study, we compared local epithelial responses and systemic innate monocyte responses in neonates and adults to explore differential host responses to IAV infections.

Transcriptomic and metabolic profiling revealed that glycolysis drives the response of blood monocytes to IAV with adult monocytes exhibiting high baseline glycolytic activity accompanied by strong IAV-induced inflammatory and antiviral responses. In contrast, neonatal monocytes were characterized by higher OXPHOS and constrained IAV-induced inflammatory responses. Glycolysis inhibition in adult monocytes strongly suppressed IAV-induced cytokine secretion.

As in monocytes, mini-tissues from term neonatal and adult airway epithelial cell (AEC) donors showed comparable IAV infectivity. Transcriptional differences after infection were largely driven by baseline variation. However, induction of antiviral interferon responses was similar in both groups. At baseline and after infection, adult epithelia exhibited higher expression of inflammatory stress/apoptosis genes and antigen-presentation genes, while neonatal epithelia showed higher expression of mitosis and epithelial–mesenchymal transition genes. The most pronounced age-dependent difference after infection was the neonatal-specific induction of epithelial morphogenesis and OXPHOS. Functional profiling confirmed higher OXPHOS activity and total energy metabolism in neonatal AECs, whereas glycolysis was comparable. Kinetic studies revealed significantly higher IAV-induced cell death, increasing viral titres, and epithelial barrier disruption in adult cultures, whereas neonatal AECs demonstrated recovery capacity, with declining viral titres and restored integrity over time. OXPHOS inhibition severely compromised barrier maintenance in both groups, while glycolysis inhibition had

minimal effects. Thus, the functional coupling of OXPHOS and epithelial morphogenesis was identified as key determinant of IAV resilience.

Collectively, the capacity for epithelial barrier maintenance and regeneration promoted by OXPHOS, combined with glycolysis-dependent inflammatory monocyte responses may explain the age-related variation in IAV disease severity.

Poster 3

Defining the roles for the Influenza A matrix proteins, the nuclear export protein and the small virus-derived RNAs for the regulation of the viral RNA polymerase

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Influenza A virus (IAV) possesses a segmented, negative-stranded RNA genome which is transcribed and replicated in the host cell nucleus by the viral RNA-dependent RNA polymerase (RdRp), composed of the viral proteins PB1, PB2 and PA. Each genomic segment forms a viral ribonucleoprotein complex (vRNP) with one RdRp. While most segments encode a single protein, segments 7 and 8 produce two proteins each via mRNA splicing (M1/M2 and NS1/NEP). Despite our extensive understanding of IAV biology, the exact mechanism by which the virus coordinates the conflicting activities of transcription and replication remains unclear. Encoding eight viral RNAs (vRNAs) of negative polarity that serve as templates for both mRNA and complementary genomic RNA (cRNA) synthesis, the virus must manage the activity of its polymerase to ensure a stoichiometric balance of these products. While the composition of the trimeric RdRp is not believed to change during these distinct processes it has been speculated that non-RdRp components such as NEP may contribute to the transition from transcriptase to replicase. Furthermore, recent studies have implicated small virus-derived non-coding RNAs (svRNAs) and a product of the M segment that may contribute to this process.

To gain insight into whether and how NEP, M1/M2, or svRNAs regulate the viral RdRp, we will take advantage of IAV reverse genetics to generate viruses expressing FLAG-tagged NEP, M1/M2, or polymerase proteins, to identify interactions during real infection. We also intend to use the auxin-inducible degron (AID) system, in which AID-tagged proteins are directed to proteasomal degradation by the plant E3 ligase TIR1 in the presence of the plant hormone auxin, by generating viruses expressing AID-tagged M1, M2, or NEP. Moreover, we will determine the association of svRNAs with the RdRp.

These studies will significantly enhance our understanding of the functions of svRNAs, NEP, and M1/M2 during IAV infection.

Poster 4

Preclinical evaluation of S100a8 supplementation demonstrates potent and safe protection against neonatal sepsis

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Following birth, the neonatal immune system must rapidly adapt to a complex extrauterine environment, integrating microbial and environmental signals to establish early immune homeostasis. The alarmins S100a8 and S100a9 have emerged as central host-derived molecular decision points, determining whether early microbial encounters are tolerated and promote immune maturation or elicit exceeding inflammatory responses. Produced primarily by myeloid cells and additionally delivered via breast milk, S100a8/a9 induces TLR4-dependent microbial tolerance in innate immune cells, thereby preventing excessive inflammation.

In a recent study, we demonstrated that S100a8/a9 shapes intestinal homeostasis in neonates by inducing a regulatory phenotype in lamina propria macrophages, strengthening epithelial barrier function, and promoting gut eubiosis. These effects reduced neonatal susceptibility to enteric infections. A previous murine study further showed that a single postnatal nutritional supplementation of S100a8 prevented a fatal course of subsequent *Staphylococcus aureus*-mediated sepsis.

In the present preclinical study, we evaluated the sepsis-protective efficacy and safety of nutritional S100a8 and S100a8/a9 supplementation in wild-type and *S100a9*$^{-/-}$ neonates using both gram-positive (*S. aureus*) and gram-negative (*Escherichia coli*) neonatal sepsis models.

We confirmed that S100 deficiency drives fatal *S. aureus* sepsis in neonates and demonstrated, for the first time, comparable vulnerability in neonatal *E. coli* sepsis. Nutritional S100 supplementation limited inflammatory overshooting, improved survival, and enhanced bacterial clearance in S100-deficient neonates and tendentiously also in S100-competent neonates. Thereby, S100a8 outperformed S100a8/a9, especially in gram-negative sepsis. Safety assessment in neonatal mice revealed no toxic or adverse effects even under established sepsis, underscoring its strong translational and therapeutic potential.

These findings establish S100a8 as a host-directed therapeutic strategy targeting a molecular decision point that balances protective immunity and immunopathology. S100a8 supplementation represents a promising approach for preventing and treating neonatal hyperinflammatory diseases such as sepsis, supporting targeted host modulation in line with antibiotic stewardship principles.

Poster 5

DECIDE/CRC 1583 – Project A02

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

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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.

Poster 6

DECIDE/CRC 1583 – Project A02

A primary cell-based model reveals novel insights into *Campylobacter jejuni* infection of the human intestine

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Campylobacter jejuni is a leading cause of bacterial foodborne gastroenteritis[1]. *C. jejuni* resides primarily extracellular in the gut lumen, but internalization of gut epithelial cells correlates with in disease progression[2,3]. Despite its prevalence, little is known about how *C. jejuni* causes diseases, intestinal cell type tropism by the bacteria, or underlying molecular mechanisms of pathogenesis. This is in part due to the limitations of currently employed *in-vitro*, cell line-based infection models that lack *in-vivo* characteristics of the human intestinal tract, such as cell type diversity.

To overcome these limitations in studying *C. jejuni* pathogenesis, we utilized human intestinal organoid models[4,5]. Our infection model is derived from jejunum primary cells and includes the major gut epithelial cell types, such as proliferative and differentiated secretory and absorptive cells. Compared to infection of immortalized cell lines, our primary cell model showed a strongly decreased bacterial burden and lower numbers of infected cells. Furthermore, primary cells are less uniformly infected suggesting a potential cell type tropism by *C. jejuni*.

To better understand the basis of these infection patterns, we performed single-cell RNA sequencing of infected and bystander cells. Here, we found proportionally higher infections of proliferative cells and decreased infection of absorptive enterocytes. By additionally employing dual RNA-seq to address the transcriptomic changes of host and pathogen, we could corroborate this host cell tropism. Our RNA-seq data indicate that numerous flagellar genes are upregulated in bacteria following infection, which supports earlier research identifying many flagellar components as infection-relevant factors[6]. Moreover, these data will help us identify host molecular decisions points influencing *C. jejuni* pathogenesis as well as bacterial virulence determinants employed by the pathogen.

Poster 7

DECIDE/CRC 1583 Project A03

Maternal microbial metabolites shape skin development by influencing embryonic cell fate decisions and differentiation

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The microenvironmental cues guiding skin progenitor cell differentiation during embryonic development, critical for skin barrier formation and wound healing, remain largely unknown. Our previous research demonstrated that maternal microbial metabolites enhance neonatal intestinal barrier function. Using a murine model of gestational colonization, we show that these metabolites regulate keratinocyte differentiation by modulating transcriptional regulators and cellular metabolism necessary for the transition from proliferation to differentiation. In their absence, skin barrier function is compromised at homeostasis and post-injury, though permeability recovers by two weeks of age. This recovery is not accompanied by improved epidermal structure but increased lipid content and adipogenic cells. This altered environment lacks key fibroblast subsets required for hair follicle development, tissue regeneration, and antimicrobial peptide production. Our aim is to uncover how maternal microbiota influence keratinocytes differentiation and fibroblast fate to coordinate the cellular behaviours essential for proper skin development and regenerative responses.

Poster 8

DECIDE/CRC 1583 Project A03

Molecular determinants of *Staphylococcus epidermidis* virulence.

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Preterm infants frequently experience early skin colonisation by pathobiomes and exhibit higher rates of late-onset sepsis due to an underdeveloped epidermal barrier, delayed microbiota establishment, immature immunity, and environmental influences. *Staphylococcus epidermidis*—normally a benign skin commensal—has increasingly emerged as a drug-resistant, device-associated cause of neonatal sepsis. The absence of paradigmatic virulence factors in *S. epidermidis* complicates the distinguish between commensal and pathogenic strains compared to archetypal *S. aureus,* thereby delaying diagnosis. Here, we analysed the dynamics of the skin *S. epidermidis* community and its contribution to neonatal sepsis in a longitudinal cohort of preterm infants. *S. epidermidis* isolates from neonatal skin swabs were classified into 30 clonotypes based on genes associated with multidrug resistance, biofilm formation, colonisation, and quorum sensing. The most prevalent clonotype carried mobile genetic elements conferring multidrug resistance and biofilm regulation, suggesting selection under environmental pressure. Blood culture-positive sepsis isolates matched this pathogenic clonotype, highlighting the risk of the skin microbiota acting as reservoir for pathobiomes in preterm infants. To investigate functional differences between commensal and pathogenic lifestyles, human and murine 3D-epidermal models, as well as gnotobiotic mouse models were colonised with very low-biomass bacteria, reproducing the natural colonisation schema. Commensal-like strains, which do not form biofilms, expanded rapidly, colonised deeper skin layers, and triggered innate immune activation. In contrast, pathogenic-like strains failed to expand and formed immune-silent biofilms on the epidermal surface. Pathogenic-like strains preferentially colonise immature epidermis, characteristic of premature infants, whereas commensal-like strains favoured mature epidermis. Colonisation of immature epidermis by *S. epidermidis* resulted in deregulation of over fivefold more genes compared with mature epidermis, with substantial overlap between commensal and pathogenic strains, including genes involved in bacterial recognition and inflammatory pathways. Notably, only commensal strains enhanced epidermal differentiation pathways in the immature epidermis, resembling responses observed in the mature skin. Together, these findings provide new insights into risk and benefits of *S. epidermidis* colonisation of immature preterm skin with and establish a foundation for the development of targeted therapeutic interventions to prevent infection while preserving the beneficial early-life skin microbiota.

Poster 9

DECIDE/CRC 1583 Project A04

Mucus-associated *Bacteroides thetaiotaomicron* modulates the epithelial responses to *Clostridium difficile*

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Bacteroides thetaiotaomicron is a prominent gut commensal that contributes to the digestion of diverse carbohydrates and maintenance of intestinal homeostasis, whereas *Clostridium difficile,* a low-abundance member of the microbiota, can thrive and produce toxins under disrupted gut conditions. Our initial study of *B. thetaiotaomicron and C. difficile* co-colonization in a hypoxic epithelial transwell model revealed elevated pathogen burden, toxin production, and spore formation as compared to *C. difficile* mono-infection. Additionally, combined transcriptomics and metabolomics indicated that *B. thetaiotaomicron* altered its metabolism to consume host mucus-derived glycans instead of simple sugars, in presence of *C. difficile*. *Bacteroides* mucus grazing may facilitate toxin diffusion toward the epithelial layer*,* indicating a shift in *B. thetaiotaomicron*'s role from protective to pro-invasive. Our present study aims to characterize how *B. thetaiotaomicron* activities shape the mucosal niche and render the epithelium more susceptible to *C. difficile* infection. To address this question, we screened *B. thetaiotaomicron* transposon mutants of individual polysaccharide utilization loci (PULs) and identified a set of genes involved in mucus digestion that support the growth of the bacteria in mucin media. Moreover, we examined the spatial organization and molecular dynamics of *B. thetaiotaomicron* and *C. difficile* co-colonization within a human gut-like environment. To this end, we established hybridization chain reaction-fluorescence *in situ* hybridization and combined it with immunohistochemistry within the above transwell model and a newly developed primary colonoid model (see the poster of Sara Giddens). This approach enabled the simultaneous visualization of the two bacterial species in the context of the host mucous layer. Altogether, our work establishes an imaging framework for anaerobic members of the mucus associated, colonic microbiota, advancing our understanding of microbiota-pathogen-host interactions.

Poster 10

DECIDE/CRC 1583 Project A04

In vitro models of healthy vs dysbiotic colon epithelium to investigate host responses to a toxin-producing pathogen

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The primary risk factor for *Clostridioides difficile (Cd)* infections (CDI) is antibiotic-induced gut microbial dysbiosis. This dysbiosis not only creates an environment favoring *Cd* spore germination, vegetative growth, and toxin production, but also disturbs gut epithelial physiology; e.g., by depleting butyrate-producing commensals, antibiotics disrupt butyrate sensor PPAR-γ signaling, whose pathway stabilizes epithelial barrier function[1] and promotes mucus[2] by maintaining physiological hypoxia and stabilizing TF HIF-1α[3]. Hence, restoring gut homeostasis presents a promising strategy to prevent *Cd* pathologies. We have established human mucus-producing colon models based on the HT29 cell line or colon organoids that support mono- and co-colonization with *B. thetaiotaomicron (Bt)* and *Cd*, enabling deeper analyses of host-commensal-pathogen responses in an accessible format.
Integrated metabolomics and transcriptomics of the HT29 model demonstrate cross-feeding between *Bt* and *Cd* and reveal that fructose depletion during co-colonization is a trigger for increased *Cd* spore production. Host transcriptomics propose that interdependent NFκB and PPAR-γ signaling, as well as mucus production, are modulated by *Bt*, leading to a dampened immune response, thereby identifying them as therapeutic intervention candidates.
Both colon models can be differentiated in the presence of butyrate to compare epithelial responses in a homeostatic vs. antibiotic-disrupted gut environment in future work.

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Poster 11

DECIDE/CRC 1583 Project A05

Long-lasting reprogramming of the lung immune-landscape following repeated pathogen exposure

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Life-long exposure to pathogens alters immune responses and affects gene expression across various organs, raising concerns about the translational relevance of “clean” specific pathogen-free (SPF) laboratory mice for modeling human tissue immunity. SPF mice lack differentiated and mucosally distributed memory T cells, in contrast to pathogen-experienced (“dirty”) mice, which display immune cell populations and gene signatures more closely resembling those of adult humans.

To address this gap, we established a sequential infection model (SIM) to generate pathogen-experienced mice under BSL2 conditions, using sequential exposure to murine γ-Herpes virus (MHV-68), mouse Cytomegalovirus (MCMV), and Influenza strain WSN— which serve as experimental models of prevalent human pathogens EBV, CMV, and IAV.

Flow cytometric analyses of lungs from pathogen-experienced mice revealed significant increases and alterations in both circulating and tissue-resident immune cell populations. Single-cell mRNA sequencing of CD45+ and CD45- lung cells, both at resting memory and following respiratory viral challenge, demonstrated long-lasting changes in immune-related gene expression patterns in both, the hematopoietic and non-hematopoietic compartments. In addition, mice exposed to MCMV and MHV-68 were protected from respiratory viral challenge.

Thus, our SIM approach enables the study of the mechanisms of tolerance and resistance to infection in a potentially more physiologically relevant context and provides a platform for investigating the mechanisms by which life-long pathogen exposure shapes immune function.

Poster 12

DECIDE/CRC 1583 Project A05

Differential requirements for the activation of tissue-resident NK cells during primary versus secondary infections

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Recently, we found that acute resolving viral and bacterial infections of the skin triggered the recruitment of conventional NK (cNK) cells and their differentiation into a newly identified population of long-lived Tcf1^{hi}CD69^{hi} tissue-resident NK (trNK) cells. Interestingly, trNK cells were rapidly activated and mediated accelerated effector response to secondary challenges (Torcellan et al. 2024).

In our follow-up study, we investigated the signals that regulate activation of NK cells in the tissue during primary and secondary infection. Interestingly, we found that NK cells require different signals if activated in the context of an innate primary or adaptive recall response.

Therefore, we hypothesized that trNK cells might engage in crosstalk with other tissue-resident populations and structural stromal cells. Our preliminary findings indicate that trNK cells interact with tissue-resident T cells during secondary infection. The accelerated effector response of trNK cells is partially dependent on T cells, as it is reduced in genetic mouse models that lack T cells and upon depletion of T cells during the established memory phase.

We will discuss the signals activating NK cells during primary versus secondary infection and the cell types that may contribute to their interaction with T cells.

Poster 13

DECIDE/CRC 1583 Project A06

Project A06 - Analyzing defined human Macrophage *in vitro* models to identify decision points in early immune response to *Aspergillus fumigatus*

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Aspergillus fumigatus (AF) forms 2-3 um small conidia, which can reach the lung alveoli by inhalation, where they embed in the surfactant layer covering the airway epithelium, allowing them to swell and germinate. As part of the immune system, alveolar macrophages (AM) are known to efficiently kill conidia and prevent fungal persistence and disease development. While immunocompromised patients can develop life-threatening invasive aspergillosis, healthy individuals maintain lung homeostasis. Details about whether this airborne lung pathogen will be contained or invades the alveolar epithelium to cause infection remains elusive.

We aim to investigate early key factors in AM upon initial AF encounter by employing various human macrophage (MΦ) populations.

We differentiated CD14+ monocytes into Alveolar-like (ALM) or GM-CSF MΦs (GM) and compared them to primary AM (pAM) isolated from human lung biopsies. Evaluations comprised phenotypic differences observed by microscopy and flow cytometry, where we visualized AM marker expression on all three populations as well as higher fluorescence intensity of ALM and pAM regarding CD68 and the MHC II compared to GM. We tested all MΦ types employing the FLARE model to evaluate phagocytic and killing capacity. ALM and pAM showed fast phagocytosis of AF after 2h in contrast to GM. Furthermore, we characterized ALM and GM genotypically employing dual RNA-sequencing, where we observed steady-state of uninfected ALM and a fast upregulation of proinflammatory genes after AF encounter. CXCL10 showed significant upregulation in the ALM population during AF challenge and will be evaluated as a decision point by employing various inhibitors and challenge with other lung pathogens.

We successfully established the ALM model for use in early AF infection. Genotypic comparison between both *in vitro* cell models visualized targeted induction of NFkB- and TNFa-pathways as well as chemokine expression by ALM to recruit other cells during early AF challenge.

Poster 14

DECIDE/CRC 1583 Project A06

CSF-1R as an alveolar macrophage decision point in pulmonary aspergillosis

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Invasive pulmonary aspergillosis causes high mortality in immunocompromised patients, including allogeneic hematopoietic cell transplantation (allo-HCT) recipients, whereas immunocompetent hosts are largely protected. The innate immune decision points determining fungal clearance versus lethal disease remain poorly defined.

We investigated how CSF-1R signaling shapes alveolar macrophage (AM) function using super-resolution and dynamic confocal microscopy in vitro, and murine allo-HCT (8 Gy TBI, C57BL/6 → BALB/c) and intratracheal *Aspergillus fumigatus* infection models in vivo. Survival and lung immune responses were analyzed by flow cytometry and three-dimensional light-sheet microscopy.

AMs emerged as dominant regulators of pulmonary antifungal defense. In immunocompromised mice, AMs protected against lethal aspergillosis at day 6 but not day 4 post-allo-HCT. Compared with neutrophils and monocytes, AMs showed greater abundance, proliferation, and phagocytic activity, and depletion caused uncontrolled infection. CSF-1R activation by M-CSF, but not IL-34, enhanced AM

migration, phagolysosomal function, and fungal killing in murine and human tissue-resident AMs. Local M-CSF expanded AMs 1.5-fold, preserved lung integrity, reduced systemic inflammation, and protected 90% of mice from lethal infection at day 4 post-HCT; protection was lost upon AM depletion.

These data identify CSF-1R–dependent AM programming as a decisive checkpoint controlling susceptibility to invasive aspergillosis.

Poster 15

DECIDE/CRC 1583 Project A06

Pulmonary *Aspergillus* infection outcome determined by an early alveolar macrophages decision point

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Invasive aspergillosis, primarily caused by *Aspergillus fumigatus*, poses a major global health threat. Although humans inhale several hundred *A. fumigatus* conidia daily, most infections are aborted in the alveoli, where alveolar macrophages (AMs) act as firs-line defenders. How early host–fungus interactions determine clearance versus progression to invasive disease remains poorly defined.

Using intratracheal *A. fumigatus* infection in immunocompetent BALB/c mice, we show that AMs mount a rapid and decisive antifungal response, achieving fungal clearance within 10 hours, prior to recruitment of neutrophils and monocytes. This early phase was characterized by AM activation, elevated pro-inflammatory cytokines (TNF-α, IL-1α, IL-6), reduced IL-10 and chemokine profiles consistent with antifungal immunity. Concomitantly, AMs upregulated adhesion molecules (ICAM-1, CD29) and downregulation of the inhibitory receptor SIRP1α/CD172a, coinciding with enhanced intracellular killing capacity marked by increased Cathepsin B activity.

Conclusively, these findings identify AMs as key arbiters of an early immunological decision point that determines whether *A. fumigatus* is cleared at the alveolar surface or progresses toward invasive disease. Targeting this initial macrophage-mediated checkpoint may provide new opportunities to reinforce early host defense against invasive aspergillosis.

Poster 16

DECIDE/CRC 1583 Project A06

Targeting TNFR2 to Enhance Innate Immunity Against Pulmonary Aspergillus Infection

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Tumor necrosis factor (TNF) is a key mediator of inflammation and homeostasis, exerting its effects via two receptors, TNFR1 and TNFR2[1,2]. While TNFR1 can induce cell death and inflammation, TNFR2 primarily promotes immune regulatory functions that often result in anti-inflammatory effects and modulated immune responses[1,2]. Macrophages express both receptors and are a major source of TNF in pathological conditions[3].

Recent studies highlighted the therapeutic potential of TNFR2 agonists in modulating immune responses in cancer and autoimmune diseases[4]. Here, we examined how TNFR2 targeting shapes pulmonary myeloid cells and antifungal immunity during *Aspergillus fumigatus* infection, focusing on alveolar macrophages (AM), key sentinels of the lung's first defense against inhaled pathogens.

In vitro, AM treated with the murine TNFR2 agonist NewSTAR2[5] showed enhanced phagocytosis, increased cathepsin B activity, and greater lysosomal acidity after *A. fumigatus* infection. *In vivo*, systemic NewSTAR2 administration, increased AM, interstitial macrophages, neutrophils, monocytes and regulatory T cells frequencies. In a murine transplantation model, prophylactic NewSTAR2 reduced weight loss and improved clinical outcome after *A. fumigatus* infection.

Overall, these findings support TNFR2 targeting as a promising approach to strengthen antifungal immunity without compromising immune regulation in the lung.

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Poster 17

DECIDE/CRC 1583 Project A07

Deciphering HSV-1 host shut-off in human ectocervix models

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Recurrent genital herpes, caused by *Herpes simplex virus 1 and 2* (HSV-1 & HSV-2), relies on viral proteins vhs and ICP27 to induce host shut-off, suppressing immune responses and redirecting cellular resources. In this study, we established HSV-1 infection in a human ectocervix mucosa model to investigate how host shut-off shapes epithelial immunity.

The models were first characterized by single-cell RNA sequencing (scRNA-seq), revealing distinct cell types and differentiation states. HSV-1 infection was then established using wild-type (WT) and mutant viruses, including a host shut-off-deficient mutant, HSV-1dVHS. Infection spread was monitored by confocal and electron microscopy, viral titers, and cytokine secretion assays. WT HSV-1 caused widespread infection and destruction of the models within 72–120 hours post-infection (hpi), depending on multiplicity of infection (MOI). In contrast, HSV-1dVHS replicated more slowly, producing smaller infected areas and significantly reduced viral titers.

Consistent with impaired host shut-off, HSV-1dVHS induced increased secretion of IL-1β and CXCL10, which were largely absent during WT infection. Furthermore, scSLAM-seq analysis revealed downregulation of numerous immune genes, including inflammasome-related genes, specifically in WT infection but not in the mutant.

These findings provide insights into how HSV-1-mediated host shut-off shapes epithelial immune responses in a physiologically relevant model of the female reproductive tract.

Poster 18

DECIDE/CRC 1583 Project A09

Investigating molecular decision points that determine colorectal cancer colonization by *Fusobacterium nucleatum*

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The anaerobic oral microbe *Fusobacterium nucleatum* has recently gained attention for its ability to colonize tumors distal from its primary niche. Its presence in the tumor tissue is linked to tumor growth, metastasis, and resistance to chemotherapy. While bacterial removal reduces tumor burden, sustained systemic antibiotic treatment has severe side effects. To interfere with tumor colonization in a more selective manner, it is essential to understand the molecular host factors that enable fusobacterial colonization of the tumor environment.

In order to address this question, we established a hypoxic colonization protocol using three different colon cancer cell lines (Caco-2, HT-29, and HCT116) and GFP-expressing fusobacteria in physiological 1 % O_2 conditions. Interestingly, we observed different colonization rates for each cell line. This might be due to distinct cellular properties such as observed differences in cytokine profiles that could affect the colonization of fusobacteria. To further dissect host and bacterial gene expression changes upon colonization, we separated bystander from colonized cells using fluorescence-activated cell sorting and performed dual RNA-seq. This method allows monitoring gene expression changes of both organisms in parallel as separation of host and bacterial transcripts is done *in silico*, which increases the sensitivity for detecting bacterial-induced responses in colonized cells. We will be focusing further analysis on differentially expressed genes to determine key factors that facilitate successful fusobacteria colonization, with the ultimate goal of defining new targets for specific intervention.

Poster 19

DECIDE/CRC 1583 Project A09

Intracellular *Acinetobacter baumannii* acts as a reservoir in lung infection *via* a 'persist and resist' strategy

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Although traditionally considered an extracellular pathogen, *Acinetobacter baumannii* can survive and replicate within macrophages in vitro. Intracellular bacteria are often shielded from host immunity and antibiotic exposure, potentially contributing to chronic or recurrent infections. To examine the fate of intracellular *A. baumannii* during infection, we transferred bronchoalveolar lavage fluid (BALF) from infected mice, containing an intracellular bacterial population, into naïve immunocompromised mice. This BALF transfer resulted in lung infection, indicating that intracellular bacteria may function as a transient reservoir during pulmonary infection. Using dual-proteomics, we characterized the interactions between *A. baumannii* and macrophages. Infected macrophages displayed an inflammatory and type I interferon response, including elevated Acod1 (IRG1) protein levels. Intracellular *A. baumannii* upregulated proteins associated with evasion of nutritional immunity, stress tolerance, surface modification, and metabolic adaptation. Together, these findings suggest that *A. baumannii* employs a multifactorial strategy to persist and replicate within macrophages, potentially shaping infection dynamics *in vivo* and reducing treatment efficacy.

Poster 20

Programmable Gene Silencing in *Enterococcus faecalis*: From Genetic Toolkit to Next-Generation ASObiotics

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Enterococcus faecalis remains a challenging organism for genetic manipulation, creating a bottleneck in our understanding of its pathogenicity and antibiotic resistance mechanisms, particularly in Vancomycin-Resistant Enterococci (VRE). To overcome this barrier, we have developed a transcript-targeting platform using Peptide Nucleic Acid (PNA) antisense oligomers (ASOs). This approach bypasses the need for complex genetic engineering, providing a rapid method for phenotypic analysis.

We report the successful delivery and target engagement of PNA-ASOs in *E. faecalis*. Building on this validation, we are deploying the platform for two critical applications. First, we target essential metabolic pathways to induce growth arrest, validating the PNA-ASOs as a novel class of antimicrobial agent. Second, we utilize the sequence specificity of ASOs to selectively inhibit resistance genes, effectively breaking VRE resistance and resensitizing the pathogen to vancomycin. This work expands the genetic toolkit available for *E. faecalis* and demonstrates the translational potential of antisense technology in reshaping the treatment landscape for multidrug-resistant infections.

Poster 21

Antisense oligomers mitigate the genotoxic activity of colibactin-producing *Escherichia coli*

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The development of bacterial antisense oligomers (ASOs) has primarily been focused on antimicrobial strategies targeting essential bacterial genes to kill bacterial pathogens. However, their potential to selectively suppress harmful traits in commensal bacteria without eradicating them remains largely unexplored. Here, we investigate the use of ASOs to inhibit the production of the genotoxin colibactin in a clinical *Escherichia coli* isolate.

Colibactin is a secondary metabolite produced by several Enterobacteriaceae. Its genotoxic activity has been linked to the development and progression of colorectal cancer. Colibactin is encoded by a gene cluster, referred to as *pks* (polyketide synthase) island, that comprises 19 *clb* genes (*clbA*-*clbS*). We designed peptide nucleic acid (PNA)- based ASOs targeting different mRNAs from the *pks* island. These ASOs effectively blocked target protein synthesis in the colorectal cancer isolate *E. coli* CCR20 *pks*$^{+}$. Among them, lead compounds markedly reduced colibactin-induced DNA damage in infected HeLa cells.

Together, our findings demonstrate targeted inhibition of colibactin biosynthesis and associated genotoxicity via antisense knockdown. Our approach highlights the potential of ASOs as a strategy to selectively modulate unwanted gene expression in otherwise benign members of the human microbiome, extending their application beyond antimicrobial use.

Poster 22

DECIDE/CRC 1583 Project B01

Temporal dynamics of macrophage heterogeneity during *Chlamydia* infection associated with reproductive tract pathology.

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Ascending *Chlamydia trachomatis* infection of the female reproductive tract (FRT) is a major cause of pelvic inflammatory disease and infertility. However, the innate immune mechanisms regulating pathogen dissemination and infection-associated tissue pathology remain incompletely understood. Macrophages are among the earliest immune populations recruited to the infected FRT, yet how macrophage heterogeneity evolves over the course of infection and contributes to disease progression is poorly defined.

Our previous studies demonstrated that ISG15, secreted by infected epithelial cells, promotes macrophage polarization toward an M2-like phenotype *in vitro*. Consistent with this, ISG15-deficient mice exhibit impaired resolution of inflammation during infection, suggesting a role for ISG15 in regulating macrophage fate *in vivo*.

In this study, we further investigated the temporal dynamics of macrophage polarization during *Chlamydia* infection of the FRT. Using flow cytometric analyses, we quantified pro-inflammatory (M1-like) and anti-inflammatory (M2-like) macrophage populations across multiple stages of infection and compared responses between wild-type and ISG15-deficient mice. We observed a staged and non-linear shift in macrophage polarization during early and later phases of infection. Notably, ISG15 deficiency altered the timing and magnitude of macrophage subset transitions.

Together, these findings demonstrate that macrophage polarization is temporally programmed during ascending *Chlamydia* infection and suggest that host factors regulating macrophage fate may influence bacterial dissemination and FRT pathology. Ongoing studies aim to define the functional roles of distinct macrophage subsets in disease progression.

Poster 23

DECIDE/CRC 1583 Project B01

Impact of Herpes Simplex Virus on Chlamydia trachomatis Persistence

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Chlamydia trachomatis and Herpes simplex virus represent the two most prevalent sexually transmitted pathogens worldwide. Exposure to Herpes simplex virus type 1 (HSV-1) has been shown to trigger *C. trachomatis* (Ctr) persistence, which is morphologically characterized by formation of non-dividing aberrant bodies and make Ctr resistant to current therapeutic interventions. In this study, we aimed to elucidate the mechanisms underlying HSV-1–induced Ctr persistence. Co-infection was established in HeLa 229 cells and in an air–liquid interface (ALI) model of primary human ectocervical cells using fluorescently labeled HSV-1 (ICP0-GFP) and Ctr (L2 mCherry).

Distinct infection patterns were observed for HSV-1 and Ctr in both 2D and 3D models, with preferential targeting of different cellular subsets. Ultrastructural analysis by transmission electron microscopy demonstrated that HSV-1–induced Ctr persistence is characterized by unique morphological and intracellular features that differ from IFN-γ–induced persistence. Analysis using a Ctr TIMERbac strain revealed a marked reduction in green fluorescence, indicating decreased metabolic activity and persistence induction during HSV-1 co-infection. Notably, co-infection with an HSV-1 mutant lacking host shut-off activity (ΔVHS) resulted in robust Ctr persistence, comparable to that induced by IFN-γ, in both experimental models. These findings suggest that the HSV-1 virion host shut-off factor may play a modulatory role in the induction of Ctr persistence.

Poster 24

Chlamydia trachomatis Prolongs Neutrophil Survival to Evade Immunity and Disseminate

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Neutrophils are rapidly recruited to infection sites, where they combat pathogens through phagocytosis and the formation of neutrophil extracellular traps. During transcervical infection of mice with *Chlamydia trachomatis*, neutrophils are drawn to the infection site, triggering inflammation by releasing cytokines such as IL-6, TNFα, and IFNγ while attempting to control the pathogen. Depletion of neutrophils disrupts this inflammatory response and promotes the expansion of anti-inflammatory macrophage subsets. Despite their defensive role, *C. trachomatis* can persist within neutrophils by stabilizing the anti-apoptotic protein Mcl-1, prolonging the survival of both mouse and human neutrophils beyond 96 hours. This survival is driven by NF-κB activation, which is critical for Mcl-1 stabilization. Prolonged neutrophil survival provides *C. trachomatis* with a protective niche, as infected neutrophils resist TNFα-induced cell death. Neutrophils exhibit a dynamic phenotypic shift during *C. trachomatis* infection. Throughout infection, neutrophils undergo a marked phenotypic shift, evolving from $CXCR4^{low}$ $CD62L^{high}$ $CD47^{low}$ cells to a $CXCR4^{high}$ $CD62L^{low}$ $CD47^{high}$ profile distinct from uninfected counterparts. These changes reflect the adaptive response of neutrophils to infection, influencing their function and lifespan. Moreover, *C. trachomatis* promotes efferocytosis, enabling infected neutrophils to be preferentially engulfed by macrophages. These macrophages develop an anti-inflammatory phenotype ($CD206^{high}$, $CD163^{high}$, $CD86^{low}$), supporting chlamydial survival. Infected neutrophils are capable of functioning as antigen-presenting cells, as evidenced by their ability to induce T cell proliferation in co-culture assays. Notably, these long-lived neutrophils ($CCR7^{+}$) migrated to lymph nodes, potentially acting as "Trojan horses" for *C. trachomatis*, facilitating its dissemination and contributing to lymph node pathology.

Collectively, these findings reveal that while neutrophils are critical early responders in controlling infection, *C. trachomatis* subverts their survival and function to promote immune evasion and macrophage polarization, and dissemination through lymphatic tissues.

Keywords: *C. trachomatis*; Neutrophil survival; efferocytosis; macrophage polarization; Immune evasion

Poster 25

Interplay of a chlamydial deubiquitinase and host cell ubiquitinating enzymes during *Chlamydia trachomatis* infection

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As intracellular bacteria, *Chlamydia trachomatis* express various proteins to interfere with host cell trafficking and signaling pathways to maintain their replicative niche, a membrane-bound vacuole called inclusion. Ubiquitination is a posttranslational modification (PTM) that is involved in various cellular processes, such as host-defense, mediated, for example, through the recruitment of the autophagy machinery to ubiquitinated intracellular pathogens. Said pathogens have in turn evolved various ways to interfere with ubiquitin-based threats to their intracellular survival and replication. Here we present the interaction of the deubiquitinating enzyme Chlamydial deubiquitinase 1 (Cdu1) and the host ubiquitin machinery. Cdu1 is a secreted chlamydial effector protein localized in the inclusion membrane with its catalytically active site facing the host cell cytosol [1]. Inactivation of Cdu1 affects chlamydial growth in cell culture [1, 2] and chlamydial survival in mouse infection [1]. At the cellular level, Cdu1 contributes to a functional preservation of the inclusion surface. It protects chlamydial inclusions from ubiquitination by the host cell and the resulting recruitment of the autophagy machinery but also influences the redistribution of the Golgi around the inclusion [2]. We aim to identify, interaction partners of Cdu1 which become deubiquitinated during infection and enable successful replication, as well as host cell ubiquitinating enzymes mediating the recognition and ubiquitination of substrates at inclusions of Cdu1-deficient *Chlamydia*.

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Poster 26

Investigating the role of *Chlamydia trachomatis* infection in cancer development

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Chlamydia trachomatis (*Ctr*) is an obligate intracellular pathogen and the leading cause of sexually transmitted bacterial infections worldwide[1,2]. It has been associated with the development of ovarian cancer, but the causality and underlying mechanisms are not yet fully understood. During its biphasic lifecycle, *Ctr* manipulates host signaling to acquire nutrients and evade apoptosis[2,3]. Crucially, *Ctr* promotes DNA damage and proliferation while suppressing the p53 tumor suppressor, which are key hallmarks of carcinogenesis[4,5]. The nuclear effector (NUE) of *Ctr* is a type III secretion system target shown to translocate into the nucleus in bacteria-free overexpression experiments[6]. *In vitro*, NUE possesses histone methyltransferase activity towards host histones, suggesting a role in the epigenetic manipulation of the host cell during infection[6].
To investigate the role of NUE during *Ctr* infection, we created different *Ctr* strains, that allow us to conditionally overexpress and knock-down NUE. Using these strains, we showed that a dysregulation of NUE-expression impairs *Ctr* development. Further, we were able to validate that NUE is secreted into the host cell during infection. Upon overexpression of NUE, we also saw an increase of the DNA-damage marker γH2AX. Additionally, we established an oviduct organoid infection model that can harbour stable *Ctr* infection for more than 5 weeks that will allow us to study the long-term effects of *Ctr* infection and NUE expression.

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Poster 27

DECIDE/CRC 1583 Project B03

Mechanisms driving persistence of *Yersinia pseudotuberculosis* in lymphoid tissues of the intestinal tract

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Yersinia pseudotuberculosis is a Gram negative bacterium, transmitted through ingestion of contaminated food, which results in invasive disease in human hosts accompanied by an acute inflammatory response. The cytotoxic necrotizing factor-Y (CNF_Y) functions through the constitutive activation of the small GTPase RhoA, resulting in improved Yop (Yersinia outer protein) translocation and enhanced inflammation. Previous work from our group revealed that loss of CNF_Y-induced inflammation drives *Y. pseudotuberculosis* into persistence (Heine *et al.,* 2018). In order to determine molecular decision points that trigger the switch from acute to persistent infection in *Y. pseudotuberculosis*, we employed an ad-libitum oral feeding model of BALB/c mice infected with a sub-lethal dose (10^6) of *Y. pseudotuberculosis* wildtype YPIII or a Δ*cnfY* mutant. A dynamic time course experiment with a multi-organ panel was conducted spanning the gut and secondary lymphoid organs over the acute phase of infection. Our data revealed that loss of CNF_Y results in a higher bacterial tropism in the cecum with diminished recruitment of innate and adaptive immune cells as early as day 1 post infection compared to the wildtype strain. Furthermore, cecum cytokine expression profiles revealed a switch from an IL-6 mediated inflammatory response in the wildtype YPIII infection to an IL-1 dominant response in the Δ*cnfY* mutant driven infection. Taken together, our data aims to unravel a global picture of *Y. pseudotuberculosis* persistent infection across the gut-immune axis and secondary organs while determining a molecular trigger for persistence.

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DECIDE/CRC 1583 Project B03

Understanding immune cell dynamics and host-pathogen interactions in gut-associated lymphoid tissue during Yersinia pseudotuberculosis infections

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Neutrophils are the key players of host-pathogen interactions that work synergistically to neutralize the pathogen. However, several bacteria including human pathogenic Yersiniae have developed strategies to counteract host immune responses, and are hence able to colonize at the site of infection, and later translocate to distant organs. Previously it has been shown that neutrophil specific transcripts can be found in tissue dual RNAseq of Yersinia infected Peyer's patches during an acute infection in mice. However, the dynamic role of neutrophils in combating Yersinia infection is still not known. We are currently investigating how Yersinia infection results in generating an immune response in gut-associated lymphoid tissue. Our data indicates that following an oral infection, high bacterial burdens can be isolated from gut and its associated lymphoid tissues, with massive inflammation in the cecum. We also observe that bacterial colonization is followed by neutrophil infiltration to the Peyer's patches as early as day 2 post infection. Furthermore, our spectral flow cytometry and single-cell RNA sequencing data indicates, heterogeneity within neutrophil subpopulations in different infected tissues in an acute infection. We are currently investigating the role key Yersinia virulence factors in modulating this heterogeneity in neutrophils both in vivo and in vitro.

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DECIDE/CRC 1583 Project B04

Drug combination screen on Salmonella persisters in Macrophages identified synergistic drug interactions

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Intracellular pathogens such as Salmonella pose a major challenge in the context of infectious diseases due to their ability to enter a persistent state, evading both host immunity and antibiotics. Understanding the interplay between pathogen survival strategies and innate immune responses, especially after pathogen internalization and establishment in the intracellular niche, is critical for developing new therapeutic approaches.

In this study, we developed a high-throughput screening approach to evaluate the effects of 3000 drug combinations on sustaining or aborting Salmonella Typhimurium infections within macrophages. We simultaneously target the bacterium and the macrophage using a combination of antibiotics and immunomodulatory drugs to identify conditions that interfere with host-pathogen interactions well after Salmonella's establishment in the intracellular niche, thereby altering its intracellular survival. We used recovery time after antibiotic removal to quantify bacterial viability and post-treatment-stress effects at scale.

We identified multiple drug combinations that effectively modulate the Salmonella's recovery time, primarily synergistic effects causing recovering times significantly longer than expected based on the effect of the drugs alone. Importantly, we found several compounds, which targets have been previously reported to hamper *Salmonella* intracellular survival (e.g. ROS production), thereby legitimating of our approach. In addition, we found synergistic interactions involving compounds targeting host pathways for which there is no previous evidence of relevance to *Salmonella* persistence. We are currently pursuing in-depth exploration of these leads, which will unveil the molecular mechanism of this synergy on the bacterial and host sides, and potentially reveal novel pathogen survival strategies.

In the long term, we aim to investigate how pathogen- or host-cell-specific our findings are, which will hint towards the need for broad-spectrum or targeted therapies. These results demonstrate the power of systems biology to uncover critical molecular interactions in innate immunity and provide actionable insights for combating persistent bacterial infections.

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DECIDE/CRC 1583 Project B05

Uncovering Decision Points Underlying the Vacuolar-to-Cytosol Bacterial Transition

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Salmonella enterica serovar Typhimurium (henceforth *Salmonella*) is a common cause of gastroenteritis in humans. After invading epithelial cells, *Salmonella* either remain in the *Salmonella*-containing vacuole (SCV) or escape into the nutrient-rich cytosol, where they start to hyper-replicate (> 50 bacteria per infected cell). The host decision points underlying the transition remain poorly understood. Previous research on the two distinct populations has mostly been focusing on the bacterial effectors that influence the decision. Here, for the first time, we aim to identify the host-cell factors that drive *Salmonella* to either remain in the vacuole or escape from the SCV at the single-cell level.

To study the dynamics of the infection, we have implemented a fluorescence reporter system to distinguish the bacterial populations for FACS sorting in combination with a high-throughput plate-based single-cell RNA-seq method (so-called FLASH-seq) and RNA metabolic labeling. We revised the infection model of HeLa cells and demonstrated an unexpectedly strong difference between different genetic backgrounds allowing us to reveal host decision points. We have curated the transcriptomes of three different HeLa cell lines, in the context of *Salmonella* infection we identified 12 host factors we are systematically targeting with siRNA to decipher their influence on the bacterial lifestyle. Notably, we focused on key pathways of autophagy and vesicle trafficking. Altogether, our multi-dimensional single-cell analysis is revealing the host factors that underlie the fate decision of bacterial divergent lifestyles.

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DECIDE/CRC 1583 Project B05

Spatial and temporal dynamics of host responses to *Yersinia pseudotuberculosis* in the cecum

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Infections deeply reshape tissue anatomy and cellular organization, yet the spatial patterns associated with disease progression decision points are very poorly understood. Here, we used *Yersinia pseudotuberculosis* (herein *Yersinia*), a Gram-negative extracellular pathogen that triggers gastrointestinal inflammation and forms granuloma-like structures. Because the cecum is a major site of *Yersinia* colonization, persistence, and lesion formation, we focused our analysis on this tissue to examine the structural and molecular dynamics of infection foci. Histopathological analysis of infected tissues revealed a sharp transition in cecal architecture between day 2 and day 3 post-infection, with pronounced tissue disorganization, epithelial damage, and increased infiltration of innate immune cells.

To capture the molecular changes at the tissue level across space and time, we combined a multi-pronged spatial transcriptomics approach with single-cell resolution (10x Genomics Visium HD) along with *Yersinia*-targeted RNA probes and immunostaining. Using tissue microarrays covering multiple infection time points, this strategy enabled systematic mapping of host–pathogen interactions and their comparison throughout disease progression.

Overall, this multi-timepoint spatial framework is unveiling new insights into how *Yersinia pseudotuberculosis* infection alters the cecal microenvironment and shapes host immune and tissue responses, building on previous work identifying the cecum as a key site for bacterial colonization and persistence.

Poster 32

Dissecting the Regulatory Circuitry of Macrophage Reprogramming During *Salmonella* Infection

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Macrophages are equipped to eliminate invading pathogens, yet several intracellular bacteria exploit them as replicative niches. Salmonella enterica serovar Typhimurium subverts host immunity by injecting effector proteins that remodel macrophage function. While macrophages typically induce a pro-inflammatory program upon bacterial invasion, Salmonella can redirect them toward an anti-inflammatory and replication-permissive state. The host regulatory mechanisms underlying this reprogramming remain uncharted.

Here, we used a multipronged approach combining bacterial reporter, temporal single-cell RNA-seq with RNA metabolic labeling, transcription factor (TF) footprinting, and single-cell CRISPR perturbations to chart macrophage polarization dynamics during early infection. We identified a critical bifurcation at 6 hours post-infection, where a subset of macrophages transitioned toward an anti-inflammatory phenotype. This shift involved the dampening of the initial NF-κB–driven inflammatory program, induction of specific transcriptional modules, and activation of Salmonella pathogenicity island 2 (SPI2). TF footprinting revealed additional host candidate regulators of the reprogramming trajectory, including SPI1 and AP-1 family members, which were tested by single-cell CRISPR to map the underlying gene network.

Together, our study uncovers host decision points in macrophage polarization circuitry and reveals a vulnerability exploited by Salmonella to modulate host immunity.

Poster 33

DECIDE/CRC 1583 Project C01

From residents to recruits: Effector UTCs sustaining tissue immunity

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Unconventional T cells (UTCs), including γδ T cells, NKT cells, and MAIT cells, bridge innate and adaptive immunity and populate barrier tissues early in life to provide rapid protection. Although parabiosis studies have suggested that UTCs are largely tissue-resident, our previous work (Ataide et al., 2022) identified a population of tissue-derived effector UTCs (eUTCs) that continuously migrate from peripheral tissues to draining lymph nodes (LNs). However, the fate of these cells and the functional logic underlying this migration remained unclear.

Using a Staphylococcus aureus subcutaneous infection model, we show that tissue-resident eUTCs, particularly γδ T cells, initiate local immune responses but require the draining LN as a site for TCR-dependent proliferation. By combining an eUTC reporter system, in vivo photoconversion of tissues and LNs, and single-cell RNA sequencing of eUTCs from LNs and blood, we define a migratory circuit in which eUTCs exit tissues, expand in draining LNs, enter the circulation, and subsequently return to their tissue of origin. This establishes the existence of a recirculating pool of transiently tissue-resident effector UTCs.

Functionally, we identify this process as a critical decision point in host–pathogen defense. While resident eUTCs are sufficient to initiate inflammation, sustained immunity depends on further recruitment of eUTCs from the blood. Using S. aureus infection and sterile wound-healing models coupled to photoconversion, we show that circulating eUTCs act as a rapidly deployable reservoir that is preferentially recruited to inflamed tissues, where they display heightened effector cytokine production and sustain neutrophil recruitment.

Together, these findings challenge the view of UTCs as strictly tissue-resident and reveal a dynamic migratory strategy that governs the decision to amplify and sustain early antimicrobial immunity. Ongoing studies disrupting eUTC trafficking aim to define how this circuit shapes protective versus pathological tissue responses.

Poster 34

DECIDE/CRC 1583 Project C03

IL-22 induced by *C. albicans* reduces the severity of invasive *S. aureus* pneumonia in mice

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Introduction: The commensal fungus *C. albicans* and the bacterium *S. aureus* frequently coexist in humans and can cause severe systemic infections in immunocompromised patients. Clinically, *S. aureus* commonly induces pneumonia and disseminates from the lung, whereas *C. albicans* almost never causes invasive pulmonary disease, even in mechanically ventilated patients with high fungal burdens.

Objectives: We aim to investigate the different behavior of *C. albicans* and *S. aureus* in the lung, and to assess how pulmonary pre-colonization with *C. albicans* influences subsequent *S. aureus* infection.

Methods: We established a lung pre-colonization/infection model using Balb/c mice. Mice were administered *C. albicans* intranasally at day 0 and infected intranasally with *S. aureus* on day 1. At 24, 48 and 96 hours post *S. aureus* infection, lung, liver and kidney bacterial/fungal burdens as well as lung immune responses were analyzed.

Results: Following intranasal administration, pulmonary loads of both pathogens declined over time; however, only *S. aureus* disseminated to liver and kidney, closely mirroring the clinical situation. Pulmonary pre-colonization with *C. albicans* markedly altered the lung immune landscape and resulted in enhanced recruitment of neutrophils and CD11b dendritic cells upon *S. aureus* infection. Cytokine profiling revealed a pronounced induction of IL-1β, IL-17, and IL-22 during fungal colonization followed by bacterial challenge. Importantly, *C. albicans* pre-colonization protected mice from lethal *S. aureus* lung infection. Neutralization of IL-22 abrogated this protective effect, identifying IL-22 as a critical mediator to *S. aureus* induced pneumonia. Early administration of recombinant IL-22 was sufficient to significantly enhance early survival during a lethal *S. aureus* pneumonia.

Conclusion: We established a physiologically relevant dual pathogen lung model and identified IL-22 as a key immunological switch determining the outcome of *S. aureus* lung infection.

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DECIDE/CRC 1583 Project C03

Myeloid TGF-beta signalling supports host resistance to *Mycobacterium tuberculosis* by regulating macrophage activation and metabolism

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Background: *Mycobacterium tuberculosis* (Mtb) is the leading cause of death by infectious disease, with approximately 11 million cases of tuberculosis (TB) and 1.2 million deaths annually. While TGF-beta is a known suppressor of adaptive T cell responses, its effects on myeloid cells, the primary host cells of *Mtb*, remain poorly defined during infection.

Objectives: We aimed to elucidate how TGF-beta signalling within myeloid cells modulates host resistance during pulmonary TB.

Methods: We generated a myeloid-specific knockout model by crossing LysM^{cre} mice with *Tgfbr2fl/fl* mice, resulting in the targeted deletion of TGF-beta receptor 2 (TGF□R2). WT, heterozygous (Het), and knockout (KO) mice were challenged with 200 CFU of Mtb H37Rv via aerosol. Flow cytometry, cell sorting, and transcriptomic profiling (RNA-seq) were carried out to evaluate pulmonary pathology and cell-specific immune signatures.

Results: Loss of myeloid TGF-beta signalling significantly impaired bacterial control. Both Het and KO mice exhibited significantly higher pulmonary Mtb burdens and inflammatory infiltrates compared to WT controls. KO mice displayed a marked influx of monocytes and infected neutrophils. Transcriptomic analysis identified alveolar macrophages (AMs) as the primary responders to TGF□R2 deficiency, exhibiting a complex, dysregulated activation profile. This was characterized by the upregulation of genes associated with cell recruitment and immunomodulation (e.g. *Mgl2*, *IL7r*, *Ccl7*, *C3ar1*, *CD93*, *Adam8*, *Nlrp10*). KO neutrophils further displayed elevated expression of *Mmp12*, a key driver of tissue remodelling. Mechanistically, pathway analysis identified a selective downregulation of p38 MAP kinase signalling and significant effects on sphingolipid metabolism and glycolysis in TGF□R2-deficient AMs.

Conclusion: Our findings reveal a paradoxical, protective role for TGF-beta signalling in TB. While typically viewed as immunosuppressive, myeloid TGF-beta signalling contributes to restricting Mtb replication and preventing pathological inflammation. These data suggest that TGF-beta fine-tunes macrophage homeostasis and metabolism to support an effective host response.

Poster 36

Differential effects of recombinant IL-17 and IL-22 on the response of A549 epithelial cells to *S. aureus* and *C. albicans* infection

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Introduction: The commensal fungus *C. albicans* and the Gram-positive bacterium *S. aureus* frequently coexist as part of the normal flora on human mucosal surfaces and skin. However, they can cause severe systemic infections, in immunocompromised patients. Unlike *S. aureus*, *C. albicans* rarely causes invasive pneumonia, but it often massively colonizes the lung during mechanical ventilation. Epithelial barrier integrity plays a central role in host defence against pathogen dissemination. Cytokines involved in mucosal immunity, including IL-22 and IL-17, modulate antimicrobial responses and barrier function.

Objectives: We aimed to investigate the effects of rIL-17 and rIL-22 on the adherence and invasion of *C. albicans* and *S. aureus* in A549 cells and to assess associated changes in epithelial barrier permeability upon infection.

Methods: An in vitro lung epithelial cell model was established using A549 cells. Pathogen adhesion and invasion were assessed by CFU plating and fluorescence microscopy. Changes in epithelial barrier integrity were evaluated by transepithelial electrical resistance (TEER) measurements using Transwell inserts. Cellular damage was quantified using an LDH cytotoxicity assay.

Results: Pretreatment with rIL-22 significantly reduced bacterial burdens following infection of A549 cells with *S. aureus* compared to the untreated control, whereas rIL-17 did not. In addition, rIL-22 showed a trend towards reducing cell damage in the LDH assay. Barrier integrity was enhanced by rIL-22 pre-treatment in the infected group, as reflected by higher TEER values compared to the other infected experimental groups.
In contrast, rIL-17 or rIL-22 pre-treatment did not affect fungal burdens, TEER values or LDH release upon *C. albicans* infection compared to the untreated control.

Conclusion: These findings indicate a pathogen-specific protective role for rIL-22 in *S. aureus* infection, characterized by reduced bacterial burden and preserved epithelial barrier integrity, whereas no protective effects were observed during *C. albicans* infection.

Poster 37

Monocyte-Mediated Immune Responses to Co-Infections with *Candida albicans* and *Staphylococcus aureus*

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Candida albicans and *Staphylococcus aureus* are common human commensals, but also opportunistic pathogens that can cause life-threatening systemic infections. Polymicrobial infections occur commonly but are under-researched and pose a particular challenge to innate immunity.

We investigate the responses to polymicrobial infections in human monocytes, central innate immune cells that help co-ordinate adaptive immune responses.

Human pan-monocytes were confronted in vitro to *C. albicans* and/or *S. aureus*, with and without opsonization. Flow cytometry resolved uptake kinetics and activation profiles across classical, intermediate, and non-classical monocytes, while live-cell fluorescence microscopy enabled real-time visualization of pathogen–host interaction dynamics. To dissect mechanistic determinants of phagocytosis, we employed *C. albicans* mannosylation-deficient mutants with defined alterations in cell-wall architecture.

Complement and SP-D increased pathogen uptake, with the strongest effects observed during co-infection. Classical monocytes exhibited the highest overall infection burden, whereas non-classical monocytes preferentially targeted *S. aureus*. Live *C. albicans* rapidly overgrew monocytes and triggered cell death within ~60–90 min, while yeast-locked and thimerosal-killed mutants induced substantially delayed cytotoxicity (≥8 h). Disrupted *Candida* mannosylation significantly reduced monocyte uptake and profoundly reshaped activation signatures.

Distinct subset-specific phagocytic dynamics and pathogen surface properties shape monocyte responses to fungal–bacterial co-infections. Ongoing work includes single-cell transcriptomics, extended co-culture (72 h) with T cells, and characterization of cell-death modalities to clarify impacts on adaptive priming and cytokine signatures.

Poster 38

DECIDE/CRC 1583 Project C04

Zone-specific hepatocytes orchestrate the early onset of host defence mechanisms during *Staphylococcus aureus* bloodstream infection

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Bloodstream infections (BSI) cause substantial morbidity and mortality, with *Staphylococcus aureus* among the most lethal pathogens. The liver plays a central role in host defence against blood-borne microbes, yet how its zonal organization shapes this response remains unclear. The established influence of liver zonation on immune cell function and the surrounding microenvironment strongly suggests that it may be a crucial factor in determining the course of infection, even though there is currently a lack of direct experimental evidence specifically connecting liver zonation to the immune response against *S. aureus* BSI. In this context, the present study aimed to describe the compartmentalization of the early immune response in the liver to blood-borne *S. aureus.* By integrating an intravenous infection model, we found that the liver captured ~90% of circulating *S. aureus* within 4 h and significantly reduced bacterial loads by 24 h, indicating rapid activation of intrahepatic immune defenses. Bulk RNA sequencing revealed strong induction of acute-phase and interferon-associated genes at 4h of infection, alongside elevated levels of IL-6, IL-1α/β, TNF-α, and IFN-γ. Single-cell transcriptomics identified hepatocytes as the principal responders, displaying pronounced zone-specific transcriptional programs across the hepatic lobule. Among the most highly upregulated genes was *Bmper*, encoding a regulator of bone morphogenetic proteins (BMPs) signaling not previously linked to bacterial infections. *Bmper* inductions was selective for periportal and midzonal hepatocytes. Chemokine production was likewise compartmentalized, with hepatocytes as the source of the neutrophil chemoattractant Cxcl1, whereas Kupffer cells and liver sinusoidal endothelial cells expressed monocyte-attracting CCL chemokines. An expansion of Kupffer cells was also observed in infected livers, probably caused at least in part by the local proliferation of resident Kupffer cells.

Poster 39

DECIDE/CRC 1583 Project C04

Exploring the impact of IκBNS on macrophage function in innate immune response

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IκBNS, encoded by the *Nfkbid* gene, is a member of the atypical IκB family of NF-κB inhibitors. In unstimulated cells, NF-κB is kept inactive in the cytoplasm by binding to classical IκB proteins. Upon stimulation by pathogen-associated components such as LPS, NF-κB becomes activated and translocates to the nucleus, where atypical IκB proteins further modulate its activity. Although the function of IκBNS has been extensively studied in adaptive immune cells, particularly in T and B cell development, its role in innate immune cells remains largely unexplored. This study aims to investigate how IκBNS modulates the effector functions of macrophages, which are the first line of defense against invading pathogens, in response to diverse Toll-like receptor (TLR) signals and bacterial stimuli. To this end, bone marrow-derived macrophages (BMDMs) were generated from mice with conditional IκBNS ablation in myeloid cells ($Nfkbid^{\Delta LysM}$) and stimulated with either LPS (a TLR4 agonist), Pam3CSK4 (a TLR1/2 agonist), or infected with *Staphylococcus aureus* (Gram-positive) or *Salmonella typhimurium (Gram-negative).* Macrophage responses were assessed by analyzing *cytokine production, phagocytosis, bacterial killing, polarization, and metabolic changes, together with transcriptomic profiling to uncover gene expression changes, functional states, and underlying regulatory pathways.*

Poster 40

DECIDE/CRC 1583 Project C04

Sequencing of Transposon Insertion sites to identify virulence and dissemination strategies of *Staphylococcus aureus*

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The Gram-positive bacterium *Staphylococcus aureus* is an opportunistic human pathogen that can shift from a harmless colonizer of 20-30 % of the human population to an invasive pathogen causing severe infections such as osteomyelitis, endocarditis, pneumonia, bacteraemia and sepsis.

Severe staphylococcal infections often involve the dissemination of the bacteria throughout the body via the bloodstream. Representing the first line of defence, the liver harbours specialised tissue-resident macrophages, so called Kupffer cells, that serve the purpose of eliminating bacteria from the blood. Interestingly, despite the Kupffer cells' strong bactericidal activity, a proportion of *S. aureus* seems to escape the immune mechanisms in the liver and subsequently can reach secondary organs like kidneys and bones.

By Transposon sequencing (TnSeq), we aim to identify bacterial factors that are decisive for the establishment of bacterial infections within the liver or the secondarily infected kidneys. Therefore, we generated transposon mutant libraries in the pathogenic *S. aureus* strains 6850 and JE2 using the mariner transposon Himar-1 that were sequenced on an Illumina NextSeq 2000 platform and analysed for their density and complexity. We currently employ these libraries in i) an *in vitro* infection screen in a human hepatocyte cell line to identify genes important for survival and phagosomal escape in this niche, as well as in *in vivo* screens in ii) a mouse model of metastatic bloodstream infection and iii) a humanized mouse model to investigate survival and dissemination related genes at a whole organism scale.

The identified genes will lead us to interacting host cell factors, shedding light on decision points that determine the fate of systemic *S. aureus* infections.

Poster 41

DECIDE/CRC 1583 INF Project

Integrative metabolomics and constraint-based modeling reveal a "healing metabolism" in *Leishmania mexicana*-infected mice following L-arginine treatment

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Cutaneous infections of C57BL/6 mice with the protozoan parasite *Leishmania mexicana* lead to chronic skin lesions, which are characterized by the depletion of L-arginine. Chronicity of disease was prevented or cured by the deletion of arginase (Arg) 1 or the oral supplementation of L-arginine, which increased lesional arginine levels and reduced parasite burden (Rai et al., BioRxiv 2025). Based on these findings, we hypothesize that L-arginine is a critical component of the immunomicrotope and causes metabolic reprogramming, which allows for healing of the disease. Here, we analyzed data from targeted LC–MS metabolomics of skin lesion lysates from (i) myeloid cell–specific arginase 1 KO mice (Arg1ΔCx3cr1) versus littermate controls at different timepoints of infection, and from (ii) wild-type mice receiving oral L-arginine or normal drinking water.

Differential abundance analysis revealed systematic shifts in amino-acid–centered pathways at the site of infection. To mechanistically connect the observed metabolic changes to pathway activity, we constructed an infection-relevant, constraint-based mouse core metabolic network derived from Mouse-GEM, prioritizing significantly enriched subsystems and including energy metabolism and transport. Condition-specific simulations (pFBA/FVA) of arginine-centered flux states identified differential flux patterns and directionality across key enzymes (NOS2 versus arginase axis) in Arg1 KO versus WT mice (90 dpi) and in L-arginine-treated vs versus control mice on normal water. Ongoing work integrates metabolite-informed subnetwork prioritization, targeted validation, and additional constraints (uptake/secretion, expression-informed bounds) to refine predictions, extend to host-pathogen networks, and guide future intervention hypotheses, but will also evaluate omics datasets of asymptomatic or symptomatic *Leishmania*-infected patients.

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DECIDE/CRC 1583 INF Project

Making the most of your host-pathogen data: Combining AI and omics data to reveal key decision steps in infection.

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Omics data are important for infection research, such as large-scale analysis of transcriptome data (bulkRNA; scRNAseq) including dual and triple RNAsequencing (host cell in contact with pathogens).

To understand these data better we present AI and bioinformatics approaches to decipher underlying key interactions, hub proteins, signalling pathways including receptors and key kinases. Regarding sample size, group separation (gene signatures) and type or quality of protein interactions (e.g in Chlamydia) AI provides a powerful boost.

To have manageable data quantities to handle and achieve clear results we focus on molecular decision points for specific infections representative of key decision steps in infection. We map the analysed transcriptome data to proteome and protein interactions, calculate hub and interacting molecules, and consider different time steps.

To understand containment versus active infection after initial contact we consider kRAS signaling in *Influenza A* virus infection and the kRAS-interaction network and PPARgamma signalling in *Bacteroides thetaiotaomicron* infection and how it changes in co-infection with *Clostridium difficile*

For Active/acute versus persistent/chronic infection, we look at *Chlamydia* infection, and the regulatory network interacting with the host, involving interferon gamma signalling and directing also sphingolipid metabolism.

Regarding localized infection versus systemic spread we look at IL22 as molecular decision point as well as IL17 on human lung epithelial cell responses to infection with *C.albicans* or *S.aureus*. IL22 pathways are considered and different gene expression data sets mapped to the network. A therapeutic application could be a protective inhalation therapy for the lung barrier function.

Poster 43

DECIDE/CRC 1583 Z02 Project

3D transcriptomics in infected tissues

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Single-cell and spatial transcriptomics have transformed our understanding of cellular identities, stimulus-induced responses, and spatial organization. However, tissue dissociation and two-dimensional spatial transcriptomics impose fundamental limitations on the study of intact tissue biology. In heterogeneous tissues, accurately estimating cell-type composition or immune infiltration can require dozens to hundreds of serial sections to obtain reliable measurements, a requirement that is rarely met in practice. Moreover, complex spatial features such as localized infection foci are difficult to reconstruct from 2D sections alone. To overcome these limitations, we established a three-dimensional transcriptomics approach for the characterization of infected tissues.

We are establishing CycleHCR [1] (Hybridization Chain Reaction), a spatial transcriptomics method that enables highly specific, multiplexed detection of RNA molecules at single-molecule resolution. CycleHCR combines sequential hybridization with robust signal amplification, making it well suited for imaging thick tissue samples without the need for complex image alignment or registration. The strong fluorescent signal allows flexible imaging conditions and supports deep tissue imaging in complex samples.

To further enhance imaging depth and spatial resolution, we combine CycleHCR with tissue clearing and physical tissue expansion. This integrated workflow enables imaging of thick organ sections up to 300 µm[2], allowing the visualization of large tissue volumes in three dimensions while maintaining single-molecule sensitivity. Tissue clearing improves optical transparency, while physical expansion separates molecular and cellular structures, enhancing probe accessibility and effective resolution beyond the diffraction limit.

We are applying this integrated approach to study host–pathogen interactions in intact tissue infection models. This workflow enables volumetric mapping of infection-associated gene expression changes and provides insight into localized cellular responses and pathogen distribution within native tissue microenvironments.

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Poster 44

Bladder Organoid Platform for Deciphering UPEC Infection Pathways

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Urinary tract infections caused by uropathogenic *Escherichia coli* (UPEC) are among the most common and recurrent infections worldwide. As the global antimicrobial resistance crisis worsens, there is an urgent need for antibiotic-sparing treatment strategies, particularly innovative host-directed therapies. However, developing and improving these strategies requires a deeper understanding of the host pathways involved in infection. This, in turn, demands physiologically relevant models of the bladder urothelium.

To address the current lack of relevant bladder urothelium models, we have developed an adult stem cell-derived organoid-based system that faithfully replicates the native tissue architecture and cell composition. Upon seeding organoid cells onto an ECM-coated 2D surface, cells proliferate and differentiate into a stratified urothelium-like structure with basal stem cell-like cells at the bottom, intermediate cells in the middle, and fully differentiated superficial cells on the top surface. Whereas standard medium used for 3D organoids mainly yields basal and intermediate cells in the organoid-based model, an adjusted composition of the medium promotes the robust terminal differentiation of superficial cells, positive for UPK3a. This model also shows a chondroitin sulfate-rich GAG layer on top. The higher TEER in this condition further indicates functional tight junctions that protect the underlying layers.

Infection of this model with the UPEC strain UTI89 revealed pronounced bacterial invasion and replication within the superficial cells. In contrast, bacteria remained largely quiescent in the lower basal layers. These results reflect the cell-type-specific infection heterogeneity patterns observed in mouse models and human tissue.

In summary, we have developed a model that accurately replicates the structure and cell composition of the bladder epithelial compartment and that resembles the infection dynamics observed in vivo. The model provides a physiologically relevant approach to studying UPEC infection and holds significant potential to advance our understanding of urogenital diseases and guide the development of innovative therapeutic solutions.

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Poster 45

Deciphering Host-pathogen Interactions in BKPyV Infection Using a Human Bladder Organoid Model

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BK Polyomavirus (BKPyV) represents a significant threat to immunocompromised individuals, particularly recipients of bone marrow and kidney transplants. Following primary infection, BKPyV establishes lifelong latency in the urogenital tract, with seroprevalence exceeding 80% in the adult population[1]. Loss of immune surveillance can lead to viral reactivation and severe clinical disease. In bone marrow transplant recipients, BKPyV infection of the bladder causes polyomavirus associated hemorrhagic cystitis, a frequent and debilitating complication for which effective therapeutic options are limited. In contrast to BKPyV kidney infection, the molecular mechanisms controlling BKPyV infection in the bladder remain poorly defined. To address this knowledge gap, we use human-derived bladder organoids and bladder cancer cell lines to investigate virus-host interactions and to identify host determinants of viral entry, replication, and persistence. Immunofluorescence analysis of early and late viral protein expression revealed infection-associated alterations in nuclear architecture. Comparative analysis of BKPyV infection efficiency showed heterogeneous susceptibility among bladder cancer cell lines, whereas the differentiated bladder organoid based model exhibited a high infection efficiency. Notably, the bladder organoid-based model supported productive BKPyV infection and released infectious viral progeny. Single-cell RNA sequencing of BKPyV-infected organoids revealed preferential infection of a highly proliferative subset of umbrella cells. Ongoing studies aim to define host factors and molecular decision points that shape BKPyV infection dynamics in the bladder epithelium. Together, these results validate the human bladder organoid-based model as a physiologically relevant model of BKPyV bladder infection and support their use for the identification of host pathways likely to be relevant to therapeutic intervention.

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Poster 46

The *Pseudomonas aeruginosa sirB2* gene is a fitness determinant of anaerobic growth and its inactivation affects virulence and rugose small colony variant

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Pseudomonas aeruginosa (*Pa*) chronic infections in patients with cystic fibrosis (pwCF) are challenging to eradicate. Infection success relies on *Pa*'s ability to adapt to the complex CF lung environment. Transcriptional analysis of *Pa* communities from sputum samples indicates that *Pa* growth in CF airways is associated with a distinct transcriptional profile. Most of the genes modulated *in vivo* remain poorly characterized.

In this study, we characterized the gene of unknown function PA14_RS04555 (*sirB2*), whose expression is particularly stimulated in the CF lung environment and shares homology with virulence determinants in *Salmonella enterica*. Our research indicates that *sirB2* is transcriptionally controlled by the virulence regulators Vfr and AmrZ. Its deletion enhances *Pa* pathogenicity, increasing virulence in *Galleria mellonella* larvae and promoting bacterial translocation and biofilm formation in a differentiated human airway epithelial infection model. In vitro, we confirmed that *sirB2* inactivation triggers biofilm formation only when oxygen access is restricted. Under these conditions, the *sirB2* mutant leads to an increased emergence of hypervirulent rugose small-colony variants (RSCV) through the accumulation of secondary mutations in the *wsp* operon, thereby increasing the second messenger c-di-GMP levels. Our data indicate that RSCV emergence is linked to an imbalance in the NAD+/NADH ratio under oxygen-limited conditions. Indeed, the absence of the *sirB2* gene reduces fitness under anaerobic growth conditions with nitrate as the sole electron acceptor, and this phenotype is independent of the ubiquinone pool, suggesting that the *sirB2* gene is an important determinant of survival in the lungs of pwCF.

Further studies are underway to decipher the mechanism of action of the *sirB2* gene.

Poster 47

Adaptation of UPEC to the prostate extracellular environment

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Urinary tract infections (UTIs) affect approximately 400 million people worldwide each year [1]. About 80% of cases are caused by uropathogenic *E. coli* (UPEC) [2]. Although, bladder is the most affected organ, in men, the bacteria can also migrate to the prostate and other organs of the male reproductive tract and establish infection [3]. These infections are considered as complicated UTIs as they are very challenging to treat. Treatment options rely solely on prolonged antibiotic treatment and pain reduction [4]. As in other bacterial infections, increasing antimicrobial resistance makes the development of alternative therapies essential. However, the pathophysiology of bacterial infection in the male reproductive tract remains poorly understood. During bladder infection, UPEC senses urine and adapts to it by regulating the expression of specific virulence genes, ensuring a successful infection [5]. However, whether UPEC adapts similarly to the male reproductive tract environment is still unknown. Here, we aim to study how UPEC adapts to seminal plasma (SP; the extracellular fluid covering the male reproductive tract epithelium) and how this affects bacterial infection. For this, we treat bacteria with SP from human donors and analyse multiple virulence factors. Bacteria treated with LB or urine are used as controls. Analysis of bacterial growth, biofilm formation, motility, and virulence gene expression showed a clear effect of SP on UPEC. We further found that SP increases the expression of the main UPEC adhesin, FimH, which is essential for establishing infection. Current work focuses on characterising how SP modulates these virulence factors and how these changes impact infection.

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Poster 48

Expression Levels of the Attachment Protein G differ Between Strains of a Murine Pneumovirus and Determine the Virulence

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Pneumonia virus of mice (PVM), the mouse homolog to respiratory syncytial virus (RSV), is increasingly used as surrogate model to study pneumovirus pathogenesis in a more natural pathogen-host context. Two major PVM strains, 15 and J3666, are currently used in laboratories, with preferences for either one the other based on the well-documented isolation history of strain 15 or the suggested higher virulence of strain J3666.

Using conventional and long-read sequencing, we found that the PVM J3666 represents two distinct virus populations, which are defined by sequence and structure of the G and SH genes encoding the attachment and small hydrophobic proteins, in addition to further nucleotide polymorphisms. Specifically, a nucleotide polymorphism A65U in the G gene results in either an upstream open reading frame (uORF) preceding the main ORF, or an extension of the major G ORF by 18 codons. The impact of the different J3666-G gene forms was examined by generating recombinant PVMs differing exclusively in the distinctive 5' portion of the respective gene. This revealed that the population expressing a G protein with an extended main G ORF was more virulent, whereas the presence of a uORF attenuated virulence. PVM virulence correlated with increased G levels, whereas attenuation was rather associated with downregulated G expression due to the presence of a uORF. Thus, modulation of G protein levels may be an important mechanism by which pneumoviruses modulate virulence. Furthermore, the organization of the G gene that we describe aligns with that of several newly identified pneumoviruses, i.e., canine and swine pneumoviruses.

Poster 49

The evolutionary dominant 72-nucleotide duplication in the 2nd hypervariable region of RSV-G does not affect replication fitness in primary human airway models

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Human respiratory syncytial virus (HRSV) causes severe infections in children, elderly, and immunocompromised individuals. The attachment protein G is crucial for entering ciliated cells in the human airway epithelium via the CX_3CR1 receptor. Novel genotypes with G gene duplications that add 20 (RSV B BA) or 24 amino acids (RSV A ON1) to their ectodomains have rapidly become dominant, suggesting an evolutionary advantage.

To investigate the mechanisms behind this advantage, we generated a recombinant RSV based on strain A2 that mimics the 72-nt duplication in the ON1-G gene (rRSV A2 G_{+72nt}). A potential advantage in replication was addressed in HEp-2, Calu-3 cells, and primary human tracheo-bronchial epithelial cells (hTECs) differentiated at the air-liquid interface. We also compared low-passage clinical isolates from before and after ON1 emergence.

Overall, no significant difference in replication was observed between RSVs with or without the G nucleotide duplication. Both rRSVs replicated comparably in HEp-2, Calu-3, and hTECs. In hTECs, the replication of the rRSVs reached an average peak titer of $>10^6$ PFU/mL on day (D) 3, declining to 10^5 PFU/mL by D7. Clinical isolates exhibited similar replication kinetics, maintaining titers $>10^6$ PFU/mL from D3 to D7. Confocal laser-scanning microscopy revealed gradual epithelial disruption from D2 to D7 with MUC5B hypersecretion. By D7, we observed diffused acetylated-tubulin signals, which indicate ciliary destruction. Transmission electron microscopy confirmed these observations.

In conclusion, the 72-nt duplication in G ectodomain affects neither RSV replication kinetics nor histopathology. This suggests that evolutionary dominance results from immune-evasive mechanisms rather than enhanced replication.

Poster 50

Gut Immunity Modulated by Plasticity and Antigenic Context of T cell Activation

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The microbiota deeply influences gut immunity by inducing T cell responses, yet how the diverse array of bacterial antigens is prioritized remains unclear. Recent studies have identified cross-reactive T cells capable of recognizing epitopes from both commensals and pathogens. Such responses may be beneficial, with commensals boosting memory T cells and enhancing pathogen clearance, but they may also be detrimental by driving plastic TH17 cells towards a pathogenic TH17+1 phenotype. Different gut colonizers exert distinct effects on host T cells, with their phenotype being shaped by the antigenic context present during priming. However, it remains unknown how antigenic context during a later challenge influences the stability and plasticity of commensal-primed T cells. To probe how antigenic context shapes T-cell fate in vivo, we developed a transient colonization model in germ-free mice using engineered bacteria that uniquely enables modification of an antigen's bacterial chassis during subsequent reversible colonisations. Informed by this state-of-the-art system allied with adoptive T cell transfer, multiparametric flow cytometry, and single-cell RNA sequencing, we hypothesize that challenge with distinct bacteria ectopically expressing the priming antigen, will boost specific T cell numbers while modulating their phenotype. Specifically, we predict that the pathogenic Salmonella enterica serovar Typhimurium – known to drive TH1 responses will repolarize microbiota-primed TH17 cells toward a pro-inflammatory TH17+1 state. These insights will contribute to novel strategies that prevent waning immunity, while also illuminating the ontogeny of T cells that drive chronic pathological inflammation.

Poster 51

Uropathogenic *E. coli* infection induces G2 cell cycle arrest to promote intracellular replication

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Urinary tract infections caused by uropathogenic *Escherichia coli* are among the most common and recurrent bacterial infections worldwide. With antimicrobial resistance steadily increasing, host directed therapies are gaining attention as antibiotic sparing alternatives. Regulatory host RNAs such as microRNAs (miRNAs) are integral components of the host response to infection, where they modulate post transcriptional gene regulation in a context dependent manner. MiRNAs are particularly attractive candidates in infection biology due to their dual potential as biomarkers of disease and as therapeutic targets. Studying miRNA regulation during infection can also uncover key host pathways involved in infection progression. However, exploiting their full potential requires a detailed understanding of how miRNAs are regulated and function during infection. To study this in a more physiologically relevant model, we established an adult stem cell-derived organoid model of the human bladder that recapitulates urothelial heterogeneity, including differentiated umbrella cells and an intact glycosaminoglycan layer (for more details see abstract Guedes et al.). Upon infection of the model with UPEC, cells showed a strong upregulation of the miR-27a-5p expression. Analysis of *in silico* predicted targets with mRNA genes downregulated during the infection revealed a group of 22 genes associated with cell cycle control during G2 phase. Cell cycle analysis showed that indeed, a bigger proportion of infected cells were arrested in G2 phase compared to the bystander cells. Pharmacological arrest of urothelial cells in G1 (CINK 4) or G2 (RO-3306) phases confirmed that cells arrested in G2 contained more bacteria intracellularly compared to G1 arrested cells. Validation of direct interaction with cell-cycle related genes is currently undergoing. These findings suggest that UPEC may induce miR-27a-5p–mediated cell-cycle regulation to promote intracellular growth. Targeting miR-27a-5p or modulating G2-associated susceptibility may offer new host-directed strategies to prevent or mitigate UPEC bladder infections.

Poster 52

Super-resolution microscopy unravels bacterial organization and pathobiology at the nanoscale

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Bacteria are highly organized in space and time, enabling rapid reaction to the environment. Processes that mediate this adaption and ensure robust cell growth and proliferation occur on small spatial scales, down to the nanometer range. While this scale is inaccessible for conventional microscopy, super-resolution techniques allow to study target molecules at ~ 10 – 30 nm resolution and in multicolor [1]. Using single-molecule localization microscopy (SMLM) techniques such as PALM, dSTORM or (DNA)-PAINT, and STED microscopy, we study the nanoscale organization of gram-negative bacteria during fast growth and under virulence-promoting conditions [2, 3].

3D SMLM and STED imaging in fixed cells and live-cell imaging of vertically oriented cells reveals that the nucleoid of fast-growing Escherichia coli cells adapts an intriguing helical configuration close to the inner membrane. Antibiotic treatments indicate that active transcription and translation attach the nucleoid to the inner membrane, a mechanism that is known as transertion [2, 3]. We are currently studying this process in more detail by co-imaging the nucleoid together with the transertion machinery and the cytoskeleton. Translating this question to pathobiology, we started to investigate nucleoid organization in the pathogen *Yersinia enterocolitica* [4]. We find a strong reorganization of chromosomal and plasmid DNA under secreting conditions, correlating with effector proteins expression but not showing evidence for transertion of effector proteins.

Together, our super-resolution toolbox delivers a nanoscale view on bacterial organization and underlying mechanisms. In the future, we aim to extend our efforts to the host-pathogen interaction context [5] and to study the organization of intracellular pathogens.

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Poster 53

On thermal tolerance in fungi: *Candidozyma* (formerly *Candida*) *auris* as a harbinger

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Mammals are thought to be protected from fungal pathogens by two evolutionary pillars: adaptive immunity and elevated body temperature.
However, anthropogenic climate change and the decline of human basal body temperature are eroding this thermal barrier.
The emergence of *Candidozyma* (formerly *Candida*) *auris*, a fungal pathogen that emerged on five continents as genetically distinct clades, may exemplify the consequences of breaching this thermal sanctuary.
Unlike most fungi, *C. auris* thrives at 37°C and tolerates temperatures up to 47°C.
It exhibits concerning characteristics: persistent nosocomial transmission, resistance to antifungal drugs, robust biofilms, and 30-60% mortality in invasive infections.
Its independent, near-simultaneous global emergence suggests common selective pressures consistent with global change.
We review the evolutionary biology, ecology, and clinical significance of *C. auris* within the context of climate-driven pathogen emergence, then examine hypotheses for its origin and evaluate molecular mechanisms underlying its thermotolerance and virulence.
It may represent the first of many fungal species adapted to circumvent mammalian thermal defences.
Understanding evolutionary mechanisms driving *C. auris*' emergence is essential for anticipating future fungal diseases.
We must prioritize predictive models, enhanced surveillance, and novel antifungal strategies before thermal barrier erosion renders mammals as vulnerable to fungi as ectothermic vertebrates already are.

Poster 54

Cold atmospheric plasma and alveolar epithelial cells: Safety and efficacy for therapeutic use in bacterial lung infections

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Background

Ventilator-associated pneumonia (VAP) is a significant complication in intensive care units (ICUs), affecting 20-26 % of critically ill patients and exhibiting a high mortality rate. Therapeutic strategies depend heavily on broad-spectrum antibiotics, which have contributed to the rise in multidrug-resistant (MDR) pathogens.

The process of cold atmospheric plasma (CAP) formation is initiated by the partial ionization of gas particles, leading to the generation of a mixture of ions, electrons, reactive oxygen, and nitrogen species, in addition to ultraviolet radiation. CAP is known for its strong antimicrobial effects, making it a potential alternative to conventional antibiotic treatment.

Objective

Using physiologically relevant model we aim for a systematic evaluation of CAP's therapeutic potential in VAP, by identifying an effective and safe CAP application strategy for future clinical use.

Methods and Results

We employ Air-Liquid Interface (ALI) cultures of CI-huArlo cells as our model system to replicate the alveolar epithelium. These cultures are exposed to CAP in the P.R.I.T. ExpoCube.

This study examined the effects of CAP treatment on barrier integrity, inflammation and cellular toxicity. The effects of CAP are dose- and time-dependent. Low doses were tolerated, preserving barrier integrity with no relevant cytotoxicity. Our results show that short CAP exposure does not significantly affect the inflammatory response of the cells. Longer exposure at a higher concentration resulted in a slight pro-inflammatory response. Nevertheless, reactive species generated by CAP at 6% duty cycle are sufficient to eliminate relevant respiratory pathogens, such as *S. aureus*, *K. pneumoniae* and *P. aeruginosa*.

Conclusion

This study systematically examines the therapeutic potential of CAP for the treatment and prevention of VAP, emphasizing its promise as a novel therapeutic approach for respiratory infections. Future studies will focus on elucidating the effects of CAP on epithelial integrity and inflammatory responses.

Poster 55

Acid ceramidase deficiency enhances effector functions of CD4$^+$ and CD8$^+$ T cells

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Acid ceramidase (Ac) is a lysosomal enzyme that catalyses the conversion of ceramide to sphingosine. Although Ac inhibitors are currently being explored for clinical use in oncology, the role of Ac in immune responses, particularly antiviral immunity, remains incompletely understood. To investigate the impact of Ac on leukocyte populations, we generated a Tamoxifen-inducible global Ac knockout mouse model (iAc-KO). To study the consequences of Ac deficiency in a cell type-specific manner, we established *ex vivo* protocols using highly purified preparations of CD4$^+$ or CD8$^+$ T cells from iAc-KO mice. Successful recombination of the floxed *asah1* gene after addition of 4OH-Tamoxifen *in vitro* was confirmed for both subsets and strong reduction in enzymatic activity as well as the predictable shifts in sphingolipid composition indicated loss of Ac protein expression. Following CD3/CD28 costimulation, Ac deletion did not affect activation of CD4$^+$ or CD8$^+$ T cells, as read out by CD69 and CD25 expression, but resulted in enhanced IFNg release into culture supernatants of CD4$^+$ T cells. Mechanistic experiments revealed that higher concentrations of IFNg in culture supernatants were due to reduced 'consumption' of IFNg by Ac-deficient versus wild-type CD4$^+$ T cells through the IFNg receptor. Due to the tenfold higher secretion of IFNg by CD8$^+$ compared to CD4$^+$ T cells this mechanism did not crucially influence IFNg concentrations in the supernatants of CD8$^+$ T cells. Ac deficiency, however, clearly increased release of lytic granules and augmented cytotoxic activity of CD8$^+$ T cells compared to wild-type controls. Collectively, our findings indicate that Ac deficiency enhances effector functions of both CD4$^+$ and CD8$^+$ T cells. This study was supported by the DFG through GRK2581 ‘SphingoINF’.

Poster 56

Behavioral investigation of reactive and neoplastic lymphocytes in human lymph nodes in 4D

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Adaptive immunity critically depends on cell motility. Migration and cell–cell interactions enable pathogen recognition and elimination. However, cell motility in human tissue remains poorly characterized [1].
Here, we integrate tissue biology and bioinformatics to establish cell motility as a new layer of human tissue immunology. Using staining for CD20 (B cells) and CD35 (follicular dendritic cells), we analyzed B-cell behavior in germinal centers of reactive lymphoid tissue (20 cases (C), 58 movies (M)) and neoplastic lymph nodes diagnosed as marginal zone lymphoma (MZ, 4C, 16M), nodular lymphocyte predominant Hodgkin lymphoma (NLPHL, 3C, 19M), follicular lymphoma (FL, 3C, 6M), and diffuse large B-cell lymphoma (DLBCL, 2C, 10M). We identified three movement types: Low Motion (LM), Moving and Turning in place (MT), and Long Distance movement (LD). B cells were tracked using Imaris and a Random Forest classifier was trained for pattern identification.
B cells in reactive germinal centers showed a distribution with ~50% LM, ~40% MT, and ~10% LD. Interpreting these patterns in their spatial context linked LM to proliferating or mutating B cells, MT to interactive scanning, and LD to migration. In neoplastic tissue, two graded alterations were observed: NLPHL and MZ showed partial restriction of motility, whereas FL and DLBCL exhibited near-complete loss of B-cell movement. These results highlight lymphocyte motility as a new functional layer in human lymphoid tissue immunology.

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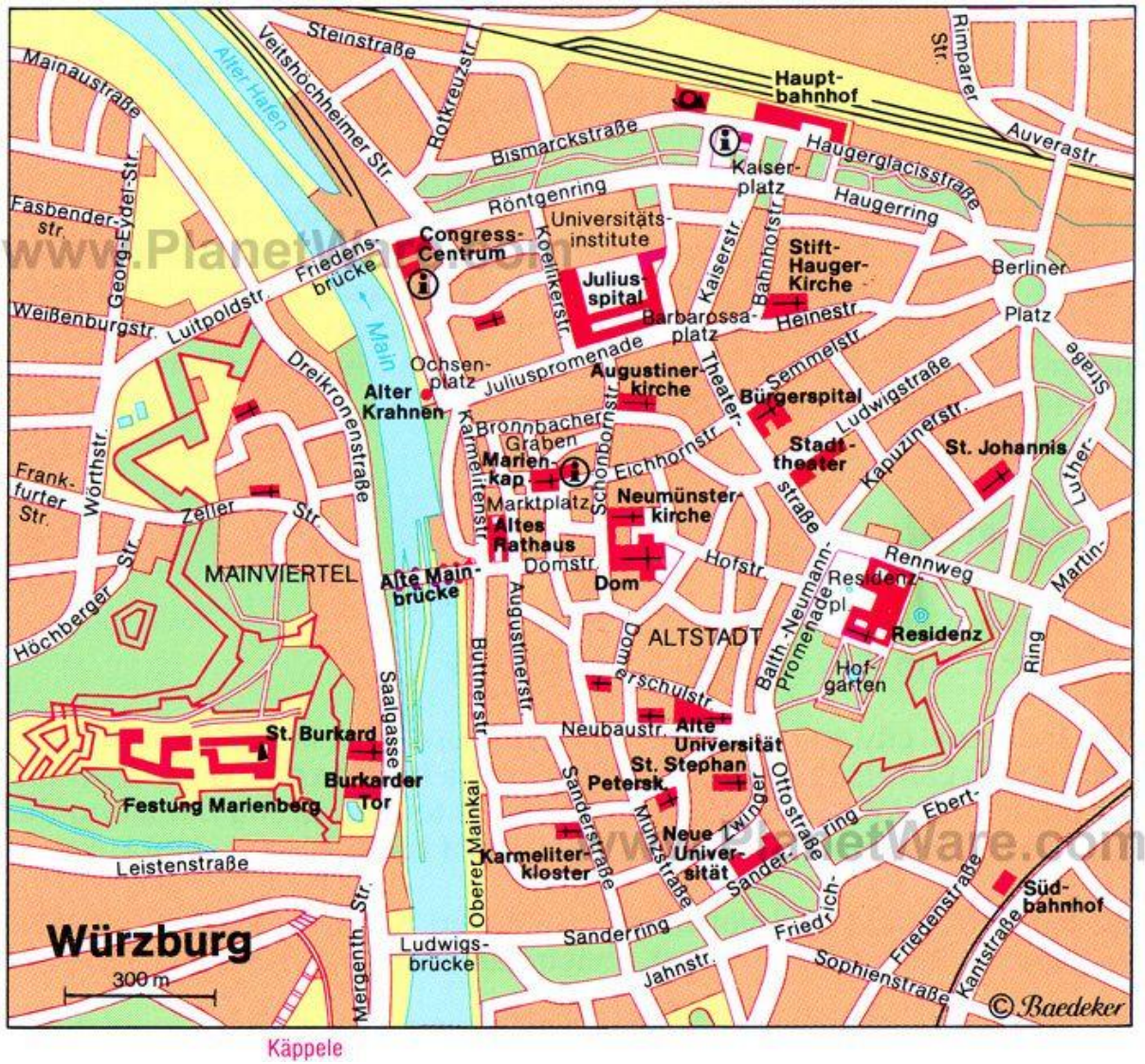
CITY MAP OF WÜRZBURG



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