NDi3

New Developments in
Immunology, Inflammation,
& Infection



Research Center Borstel

Leibniz Lung Center

Manor House

05.11.2026

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Welcome Note

Dear Colleagues and Friends,

It is with great excitement that we welcome you to the 48th New Developments in Immunology, Inflammation, and Infection (NDI_3) conference and present the collection of abstracts for this year. Organized by passionate second-year PhD candidates at the Research Center Borstel, NDI_3 continues to be a platform where groundbreaking research in immunology, inflammation, and infection comes to life.

This year's conference is designed to bring together curious and dedicated minds. With a rich program featuring renowned international keynote speakers, dynamic presentations, and interactive poster sessions, we aim to foster lively discussions, spark fresh ideas, and build lasting connections. The conference will conclude with a memorable networking evening, offering further opportunities to exchange knowledge and experiences.

We are confident that these abstracts will inspire and engage you, and we eagerly look forward to welcoming you in person to the Research Center Borstel for what promises to be an enriching and memorable event.

New Developments in Immunology, Inflammation, & Infection

Program & General Information

Program

08:30 – 09:00	*Arrival and Registration with coffee*
09:00 – 09:15	**Welcome Prof. Dr. Susanne Krauss-Etschmann**
	Session 1, chair: Dr. Marua Abu Risha
09:15 – 10:00	**Keynote lecture: Prof. Dr. Dunja Bruder,** *Helmholtz Centre for Infection Research, Germany* Beyond the Initial Insult: How Infection and Chronic Inflammation Reprogram the Pulmonary Immune Niche
10:00 – 10:45	**Sriram Kumar,** *Immunology of Respiratory Tract Infections- University Hospital Gießen and Marburg* TRIM28 S473 phosphorylation as a host-directed therapeutic target: Resolving lung hyperinflammation without compromising antiviral response during highly-pathogenic respiratory virus infection. **Arina Ozhiganova,** *Greifswald University* Protective effects of intravenous immunoglobulins against Streptococcus pneumoniae in a murine model
10:45 – 11:15	**Sponsor pitches**
11:15 – 11:45	*Coffee break*
	Session 2, chair: Dr. Lindsay Sonnenkalb
11:45 – 12:30	**Keynote lecture: Dr. Vivek Thacker,** *Heidelberg University Hospital, Germany* Imaging mycobacteria across scales: linking the ultrastructure of tuberculosis cords to host-directed therapy in microphysiological and animal models.
12:30 – 13:15	**Patricia M. Sánchez Carballo,** *Research Center Borstel, Germany* Early immunophenotypic heterogeneity is associated with treatment response in pulmonary tuberculosis.

Sambhasan Banerjee, *Centre for Structural Systems Biology, Deutsches Elektronen-Synchrotron DESY, Hamburg, Germany*
Structural and Functional Plasticity of the *Staphylococcus aureus* Virulence-Associated Amyloid Peptide PSMα1.

13:15 – 13:20 *Group picture in front of the manor house*

13:20 – 14:30 *Lunch*

Session 3, chair: Dr. Wasifa Nurieva

14:30 – 15:15 **Keynote lecture: Dr. Lorenz Adlung,** *University Medical Center Hamburg-Eppendorf, Germany*

From microbial sensing to mechanistic models: How innate immune receptors and mathematics decode intestinal barrier-tissue inflammation.

15:15 – 16:00 **Karosham Diren Reddy,** *Research Center Borstel, Germany*
Sex hormones modulate how early-life inflammatory responses prime long-term protective DNA methylation and transcriptional responses

Ayaho Yamamoto, *The University of Queensland, South Brisbane, Queensland, Australia*
Environmentally persistent free radicals enhance respiratory syncytial virus replication in respiratory epithelium

16:00 – 16:30 **Sponsor pitches**

16:30 – 17:00 *Coffee break*

Closing session

17:00 – 17:30 **Poster session 1** *(even poster numbers)*

17:30 – 18:00 **Poster session 2** *(odd poster numbers)*

18:00 – 18:30 **Awards and conclusion**

18:30 – 20:00 *Dinner*

General Information

VENUE

Research Center Borstel, Leibniz Lung Center
Parkallee 1 (Manor House)
D-23845 Borstel
WEBSITE: http://ndi3.fz-borstel.de/

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Take the B206 from Lübeck to Bad Segeberg, then continue on the B432 towards Hamburg. After approximately 15 km, turn left at the Borstel junction. The Research Center is on your right-hand side.

***via* Kiel**

Take the B404 from Kiel to Bad Segeberg, then follow the B432 towards Hamburg for about 15 km until you reach the Borstel turn-off. Turn left. The Research Center is located on the right-hand side.

***via* Bad Oldesloe**

From Bad Oldesloe, follow the main road towards Borstel via Grabau and Tönningstedt. The Research Center will be on your left-hand side.

Arrival by public transport

***via* Hamburg**

Start at Ochsenzoll station (U1). From there, take bus 7550 in the direction of Bad Segeberg and get off at “Borstel (Sülfeld), B432”. From the bus stop, it’s about a 15-minute walk to the venue. As the bus runs hourly, we suggest checking the schedule in advance.

***via* Bad Oldesloe**

Start at Bad Oldesloe train station. From there, take the bus 7141 in the direction of Henstedt-Ulzburg and get off at “Borstel (Sülfeld), am Ries”. From the bus stop, it’s about a 10-minute walk to the venue. As the bus runs hourly or less frequently, we suggest checking the schedule in advance.

Committee Members



This year's organization team: Lea Jäger, Lena Gonner, Emma Weilbeer, Jia-qi Wang, Shauni Belana Biedermann, Antonia Bohnhorst. Missing from the photo: Bastien Farcy, Lukas Kocher, Anna Nehrmann, Miriam Quentin.

We are all PhD candidates, highly motivated to host a conference in a productive, welcoming atmosphere where all participants can gain the most up-to-date information on progress across a broad spectrum within immunology, inflammation, and infection biology.

New Developments in Immunology, Inflammation, & Infection

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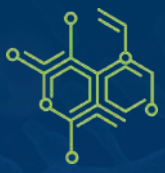
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- Fungal/Bacterial Whole Genome Sequencing -Illumina NovaSeq
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- Bacterial Complete Genome-PB Revio/ONT

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- ATAC-Sequencing-Illumina NovaSeq

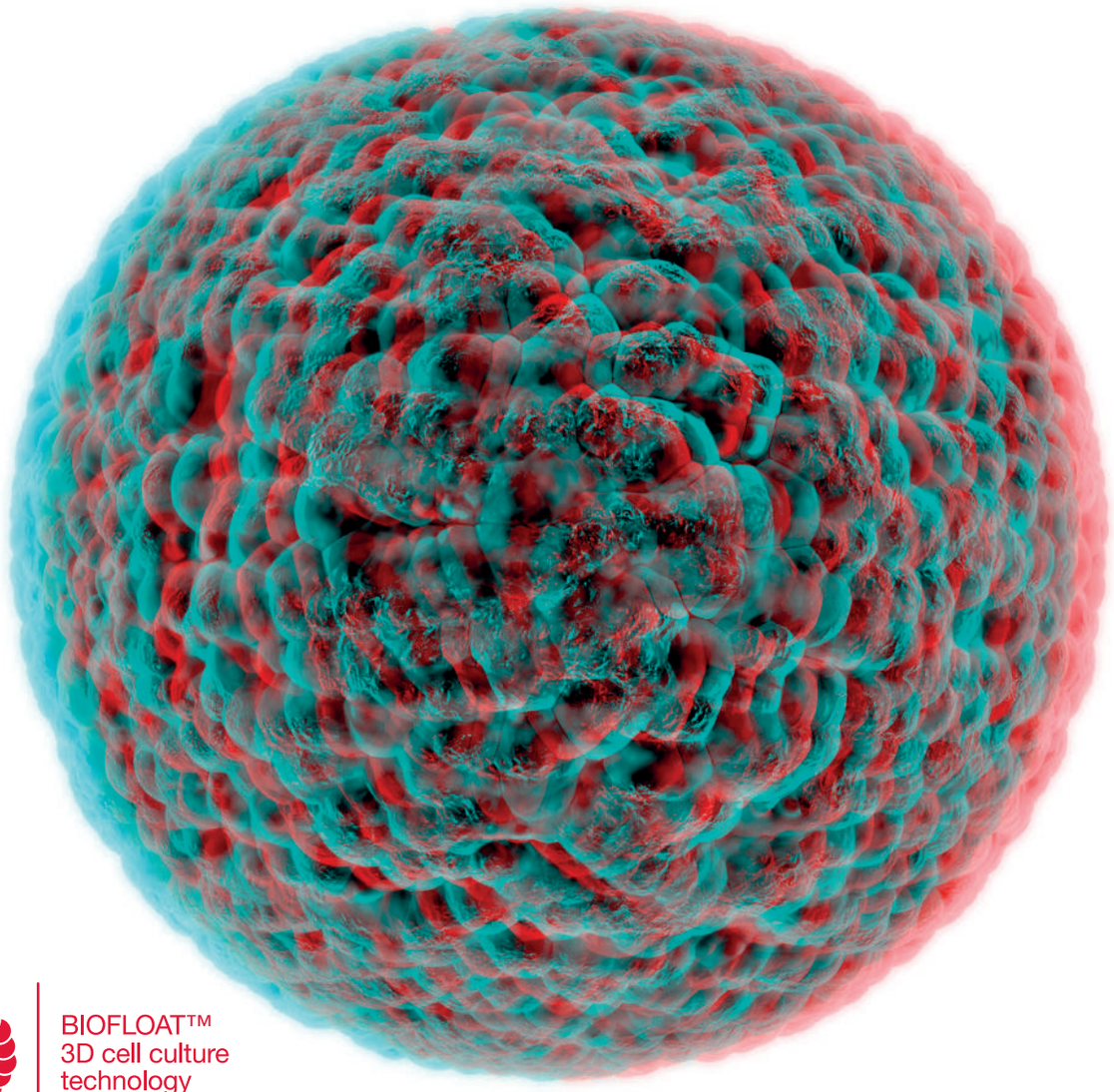
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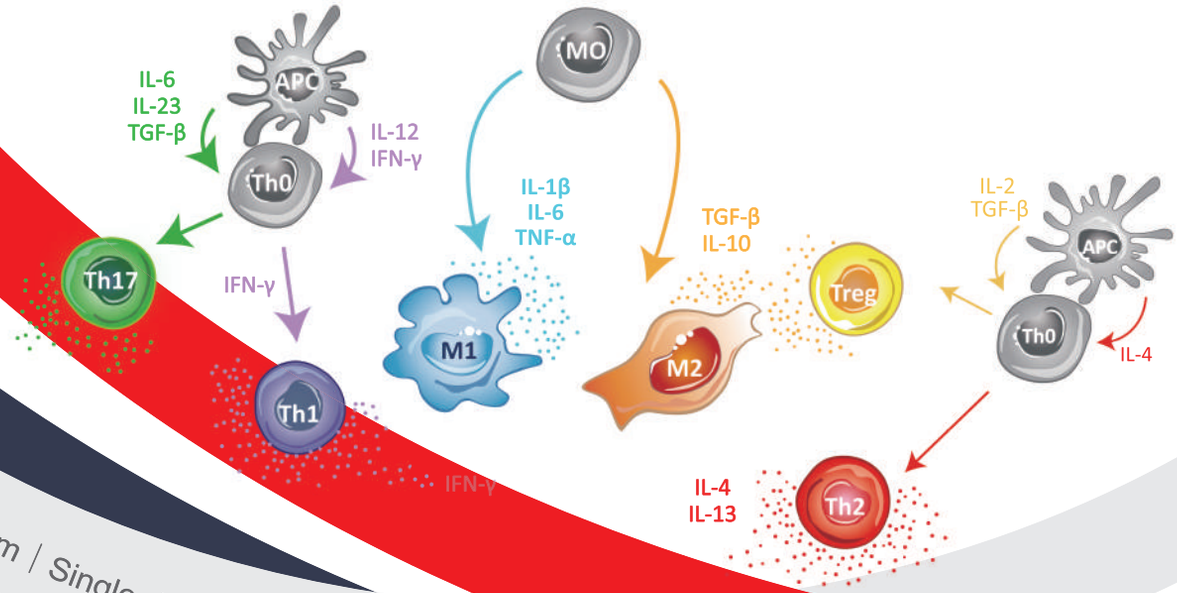
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New Developments in Immunology, Inflammation, & Infection

Keynote Speakers

Prof. Dr. Dunja Bruder, Helmholtz Centre for Infection Research, Germany

Beyond the Initial Insult: How Infection and Chronic Inflammation Reprogram the Pulmonary Immune Niche

The alveolar epithelium is increasingly recognized as a central regulator of pulmonary immunity, combining essential physiological functions with diverse immunological activities in host defense. In particular, alveolar type II epithelial cells (AECII) contribute to barrier integrity, tissue homeostasis, and the orchestration of antiviral and antibacterial responses in the distal lung, while also representing a major cellular target of influenza A virus (IAV) infection. This lecture will focus on the role of the alveolar epithelium in respiratory infection and on the ways in which prior viral exposure shapes subsequent antibacterial immunity.

A particular emphasis will be placed on the long-term consequences of influenza-induced imprinting of the alveolar epithelium. Data from preclinical mouse models show that IAV infection induces persistent molecular and functional alterations in AECII that remain detectable after viral clearance. These epithelial changes are associated with altered chromatin accessibility and with maladapted inflammatory responses during secondary infection with Streptococcus pneumoniae. Notably, the magnitude and quality of this response differ between pneumococcal serotypes, with serotype 7F inducing a particularly strong inflammatory program characterized by enhanced type I and type II interferon signatures. These findings support the concept that the alveolar epithelium retains a form of inflammatory memory that shapes later responses to bacterial challenge.

Beyond infection-associated epithelial imprinting in the lung, the lecture will also place these findings into the broader context of mucosal immune regulation by addressing current work on how chronic inflammation at mucosal surfaces influences local immune responses to subsequent bacterial infection. Together, these topics highlight how prior inflammatory experience reprograms mucosal tissues and thereby affects susceptibility, immune pathology, and host defense in respiratory disease.

Dr. Vivek Thacker, Heidelberg University Hospital, Germany

Imaging mycobacteria across scales: linking the ultrastructure of tuberculosis cords to host-directed therapy in microphysiological and animal models

Single virulent mycobacteria grow into visually-striking serpentine cords. This defining phenomenological feature was reported decades ago, but we still understand very little about the need for such a highly-ordered structure and why cording is so closely associated with virulence. Using microphysiological lung-chip models [1, 2] and volumetric imaging of C3HeB/FeJ mouse model tissue, we recently identified mechanical resilience as a defining feature of cords – linking immunosuppression early in infection in susceptible cells, with impact on long-term lesion architecture by facilitating dissemination, tissue damage, and necrosis [3]. In my talk, I will describe recent results studying this process across scales, linking biophysics at the bacterial scale to molecular mechanisms of pathogenesis. Our approaches use high-resolution imaging approaches to understand how such a tightly-packed architecture is maintained dynamically, even as the increasing numbers of constituent bacteria elongate and divide, and how mechanical resilience emerges. At the other end of the scale, we find that disrupting interbacterial interactions in lung-on-chip and animal models accelerates antibiotic clearance, demonstrating the relevance of mycobacterial aggregation as a promising target for further development as hostdirected therapy.

[1] Thacker et al., eLife, 2019. Doi: 10.7554/elife.59961.
[2] Garcia-Mouton et al., Doi : 10.64898/2025.12.18.695116v1
[3] Mishra & Hannebelle et al., Cell, 2023, 186(e28), 5135-5150

Dr. Lorenz Adlung, University Medical Center Hamburg-Eppendorf, Germany

From microbial sensing to mechanistic models: How innate immune receptors and mathematics decode intestinal barrier-tissue inflammation.

Innate immune receptors let barrier tissues read their microbial environment and tune inflammation accordingly, a principle that matters as much in the gut as in the lung. Using the murine intestinal crypt as a paradigm, we show that $Lgr5^{+}$ intestinal stem cells act as direct luminal sensors of the microbiota through apically localised Toll-like receptor 2 (TLR2), steering epithelial differentiation toward antimicrobial peptide (AMP) production in mature enterocytes and Paneth cells. Genetic knock-out of *Tlr2*, microbiome depletion and faecal microbiome transplantation establish causality between the microbiota and their epithelial sensors. Coupling single-cell flux-balance analysis with microbial metagenomics pinpoints shared metabolic axes that link microbiota, metabolites and antimicrobial output. TLR2 loss pushes epithelial cells toward a metabolic phenotype favouring lipid synthesis at the expense of antimicrobial defence. Transcriptional signatures from Wnt, BMP and Notch signalling inform a multi-scale model that describes how TLR2 loss reshapes epithelial composition and function. We are now testing whether the same framework predicts epithelial responses to infection, irradiation, resection and dysbiosis, using gene-expression signatures as informed priors for model parameters. I will close by sharing how I carried these findings to non-specialist audiences, and what science communication in the age of AI asks of early-career researchers.

New Developments in Immunology, Inflammation, & Infection

Oral Presentations

OP1: Sex hormones modulate how early-life inflammatory responses prime long-term protective DNA methylation and transcriptional responses

Karosham Diren Reddy [1,2,3], Abdulkadir Kalender [1,4], Lars Lunding [1,4], Xiaolin Liu [1,2], Henriette Tugiremariya [1,2], Susanne Krauss-Etschmann [5,6,7], Michael Wegmann [1,4] & Markus Weckmann [1,2,3]

[1]Airway Research Centre North, Member of the German Centre for Lung Research (DZL), Germany
[2]Department of Paediatric Pneumology & Allergology, University Clinics Schleswig-Holstein (UKSH), Lübeck, Germany
[3]Division of Epigenetics of Chronic Lung Diseases, Priority Area Chronic Lung Diseases, Research Centre Borstel - Leibniz Lung Centre, Borstel, Germany
[4]Division of Lung Immunology, Priority Area Chronic Lung Diseases, Research Centre Borstel - Leibniz Lung Centre, Borstel, Germany
[5]Early Origins of Chronic lung Disease, Priority Area Chronic Lung Diseases, Research Centre Borstel - Leibniz Lung Centre, Borstel, Germany
[6]DZL-Laboratory Experimental Microbiome Research, Research Centre Borstel - Leibniz Lung Centre, Borstel, Germany
[7]Institute of Experimental Asthma Research, Christian Albrecht University of Kiel

Sex hormones represent a critical early-life exposure shaping immune development and function. Estrogens promote pro-inflammatory and allergic responses, whilst androgens are immunosuppressive. These hormones govern networks contributing to sex-specific responses to inflammatory stressors, including viral infections. We investigated whether poly(I:C)-induced inflammation reprograms of sex-hormone signalling and alters DNA methylation (DNAm) and gene expression in a murine allergic asthma model.

BALB/6c mice were analysed at two stages: after initial poly(I:C) exposure (acute-model;n=8M/8F) and after completion of the poly(I:C)/HDM protocol (full-model;n=9M/7F). Allergic asthma induction was assessed by airway resistance following methacholine challenge and bronchoalveolar lavage (BAL) immune cell counts. Lung tissue was analysed by RNA-sequencing and DNAm profiling using the *MouseEPIC* array (n=4M/4F), processed in *Rv4.6.1*. Differentially expressed genes (DEGs;FDR<0.05,|logFC|>1.5) and differentially methylated sites (DMS;FDR<0.05,|logFC|>0.6) were identified, hormone pathway enrichment assessed using *fgsea*.

Acute poly(I:C) induced significant increases in neutrophils and lymphocytes in males only ($p<0.001$). poly(I:C) protected both sexes from an allergic-asthma, recognised by reduced eosinophils and airway resistance ($p<0.01$). HDM increased BAL lymphocytes in females, an effect reversed by poly(I:C) ($p<0.05$). Sex-stratified analysis identified 196 DEGs and 355 DMS, with only males indicating decreased androgen signalling after acute-poly(I:C), with this pattern maintained in the HDM-model (FDR<0.001). Integration of DNAm and gene expression demonstrated male-specific reprogramming of androgenic ($p<0.05$) and estrogenic ($p<0.01$) pathways, with no corresponding relationship in females.

Early-life poly(I:C) exposure induces male-specific molecular remodelling of the estrogenic and androgenic response pathways, suggesting differentially programming through epigenetic and transcriptional mechanisms that may contribute to sexually divergent pathways in allergic development.

OP2: TRIM28 S473 phosphorylation as a host-directed therapeutic target: Resolving lung hyperinflammation without compromising antiviral response during highly-pathogenic respiratory virus infection

S. Kumar [1], A. Faist [2], A. Mecate-Zambrano [3], K. Schughart [3], S. Ludwig [3], L. Brunotte [1]

[1]Immunology of Respiratory Tract Infections, Universitätsklinikum Gießen and Marburg, Gießen, Germany
[2]Institute for Anesthesiology, Surgical Care and Pain Therapy, Universitätsklinikum Münster, Münster, Germany
[3]Institute of Virology, Universitätsklinikum Münster, Münster, Germany

Background: Severe lung disease caused by highly pathogenic respiratory viruses such as H5N1 and SARS-CoV-2 is driven by excessive inflammation. We identified the transcriptional corepressor TRIM28, phosphorylated at serine 473 (S473) during infection, as a molecular switch amplifying cytokine expression, and targeted it from cells to mice.

Methods: Upstream signaling was mapped with PKR, p38, and MSK1 inhibitors; the immune gene network was defined using knockout cells and transcriptomics. Druggability was evaluated in HPAIV- or SARS-CoV-2-infected lung epithelial cells, patient-derived lung explants, and primary alveolar type II organoids, and in H5N1-infected mice treated orally with PH-797804.

Results: During HPAIV infection, cytosolic viral RNA sensing by PKR, independently of RIG-I and MyD88, activated a p38/MSK1 cascade phosphorylating TRIM28 S473, derepressing cytokine expression. Blockade at each tier (PKR: 2-AP, C16; p38: SB202190; MSK1: SB747651) abolished S473 phosphorylation and lowered IFN-beta, IL-6, and IL-8 without impairing viral replication. PKR knockout cells and TRIM28 S473 phosphomutants provided genetic support. In SARS-CoV-2-infected explants and organoids, clinically evaluated p38 inhibitors (PH-797804, VX-702) markedly reduced IL-6, CXCL8, CXCL10, and TNF-alpha while interferon induction, viral control, and barrier integrity were preserved, and synergized with remdesivir and molnupiravir. Transcriptomics defined the extensive gene network reprogrammed by TRIM28. In vivo, oral PH-797804 delayed symptom onset and improved survival after lethal H5N1 challenge.

Conclusions: Targeting TRIM28 S473 uncouples protective immunity from inflammatory lung damage, offering an actionable host-directed therapy; defining p38 activation kinetics and timing-optimized, biomarker-guided regimens will guide translation.

OP3: Protective effects of intravenous immunoglobulins against Streptococcus pneumoniae in a murine model

Arina Ozhiganova [1], Thomas Kohler [1], Franziska Voß [1] and Sven Hammerschmidt [1]

[1]Department of Molecular Genetics and Infection Biology, Greifswald Universtiy, Greifswald, Germany

Introduction: Streptococcus pneumoniae remains a leading cause of community-acquired pneumonia despite widespread vaccination and antibiotic use. Intravenous immunoglobulins (IVIG) contain high levels of IgG directed against pneumococcal surface proteins including the pore-forming toxin pneumolysin (Wiebe et al., 2022), suggesting that IVIG may provide therapeutic benefits in severe pneumococcal pneumonia.

Aim: to investigate of protective effects of IVIG in a murine model of acute pneumococcal pneumonia.

Methods: C57BL/6N mice were pretreated with either vehicle control or IVIG. After 24 hours, animals were intranasally infected with S. pneumoniae strain TIGR4. Sampling was conducted at 12, 24, and 36 h post-infection (h p.i.). Bacterial burden was assessed by colony-forming unit counts, leukocyte composition was analysed using flow cytometry, IVIG concentration was measured by ELISA and total protein concentration in bronchoalveolar lavage fluid(BALF) was measured using the BCA assay.

Results: Plasma IVIG concentrations remained comparable across time points. At 24 h p.i., IVIG significantly reduced bacterial burden in BALF and systemic circulation animals ($p < 0.05$) compared with vehicle-treated infected animals. BALF protein concentrations were also lower following IVIG treatment. Blood leukocyte composition was comparable to that of healthy controls, while leukocyte recruitment to the lungs was unaffected by IVIG.

Conclusion: In this murine model, IVIG decreased pneumococcal dissemination to the lungs and bloodstream and limited pulmonary barrier damage. The transient nature of the protective effect of IVIG requires further investigation.

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OP4: Early immunophenotypic heterogeneity is associated with treatment response in pulmonary tuberculosis

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Background: Tuberculosis (TB) is characterized by a complex inflammatory response in which excessive inflammation drives tissue pathology, whereas insufficient immune activation may impair pathogen clearance. Our goal to achieve immunophenotypic stratification of patients may therefore inform host-directed therapeutic strategies.

Methods: In this study, longitudinal whole-blood immunophenotyping was performed in adult pulmonary TB patients. For this purpose, flow cytometry analysis of whole blood cell surface markers, multiplex immunoassay of cytokines in cell plasma and microarray analysis of whole blood transcriptomics were performed on a clinically well characterized cohort.

Results: Temporal changes in T-cell and antigen-presenting-cell populations stratified patients and controls into distinct cell-based clusters. Early during treatment, one cluster exhibited a dysregulated activation-suppression phenotype characterized by increased frequencies of PD-L1^{+} myeloid cells, terminally differentiated T-cells, elevated inflammatory cytokines, and altered transcriptomic profiles consistent with simultaneous immune activation and regulatory exhaustion. Early-treatment cluster membership was associated with a high sputum mycobacterial burden, and persistence of the dysregulated phenotype after 14 days of therapy was linked to a longer time to sputum culture conversion.

Conclusions: These findings reveal substantial immunophenotypic heterogeneity associated with TB treatment response and support the development of personalized host-directed therapy approaches.

OP5: Structural and Functional Plasticity of the *Staphylococcus aureus* Virulence-Associated Amyloid Peptide PSMα1

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Phenol-soluble modulin α1 (PSMα1) is a cytolytic peptide secreted by *Staphylococcus aureus* that contributes to host-cell damage and biofilm stability, yet how its assembly landscape relates to function remains incompletely understood. We combined live-cell imaging, cytotoxicity assays, NMR, cryo-EM, AFM, X-ray fibre diffraction and vibrational spectroscopy to define PSMα1 structural and functional plasticity. PSMα1 accumulated at the plasma membrane before detectable permeabilization. Epigallocatechin gallate (EGCG) markedly reduced membrane-association and cytotoxicity despite only weak, distributed interactions with soluble PSMα1. Cryo-EM revealed two canonical cross-β fibril polymorphs with distinct protofilament arrangements but a conserved hydrophobic core and interface. pH redirected PSMα1 into different supramolecular states: extreme acidic and alkaline conditions favoured cross-β amyloid fibrils, whereas pH 3.9-7.9 stabilized heterogeneous α-helical nanotubes with cross-α-like packing. At neutral pH, nanotubes remained stable after heating to 90 °C, while under acidic conditions they appeared as transient intermediates preceding cross-β fibrils. Thus, PSMα1 is an environmentally responsive assembly system rather than a structurally fixed amyloid, linking physicochemical conditions to supramolecular architecture, membrane interaction and cytotoxic activity.

OP6: Environmentally persistent free radicals enhance respiratory syncytial virus replication in respiratory epithelium

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Introduction: Epidemiological evidence linking exposure to poor air quality with respiratory infections is substantial. Exposure to combustion-related air pollution, particularly that associated with traffic and bushfires, is associated with an increased risk of severe respiratory infections, including respiratory syncytial virus (RSV), reduced lung function, wheezing illnesses, incident asthma, and asthma exacerbations. Environmentally persistent free radicals (EPFRs) are oxidant components of air pollution and are present in substantial numbers on particulate matter (PM). EPFRs have half-lives of days to years resulting in prolonged biological effects. However, the precise mechanism linking pollution exposure to adverse respiratory outcomes remains unclear.

Aims: To determine whether EPFR exposure increases the severity of RSV infection of the nasal epithelium and the utility of antioxidants.

Methods: Primary human nasal epithelial cells from healthy adults were grown and differentiated at an air-liquid interface and pretreated with 10 μM astaxanthin. The well-differentiated epithelia were exposed to EPFRs for 3 days using a VITROCELL® Cloud 12 system, followed by RSV infection (MOI: 0.1). Outcomes included epithelial integrity, cytokine secretion, mRNA and protein expression.

Results: EPFR exposure prior to infection increased RSV replication by 1.6-fold, whereas astaxanthin prevented this increase. EPFR exposure reduced tight junction ZO-1 expression with a significantly greater decline in combination of RSV infection. EPFR exposure and RSV infection also decreased the antioxidant enzymes, CAT, SOD1 and TRX, while astaxanthin prevented these reductions.

Discussion: Our results demonstrate that EPFR exposure increases the severity of RSV infection by disrupting tight junction integrity and oxidative stress defence in respiratory epithelium.

New Developments in Immunology, Inflammation, & Infection

Poster Presentations

P1: Poster Hidden in the pipes: high prevalence of non-tuberculous mycobacteria in household water in Northern Germany

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Infections with non-tuberculous mycobacteria (NTM) are an increasing public health concern, particularly among individuals with pre-existing chronic lung disease. Despite their ubiquity in the environment, the routes of NTM persistence and transmission through water systems remain poorly understood. We investigated NTM prevalence in household water samples collected by master's students and teachers participating in an Infection Biology course in Schleswig-Holstein, Germany. A total of 15 samples (7 tap and 8 shower water) were cultured at 30°C and 37°C using MGIT, NTM Elite agar, and 7H10 media, and analysed by qPCR. Participants also completed a questionnaire on household characteristics. NTM were detected in 7/15 samples (47%) from 6/14 households. Identified species included Mycobacterium chimaera, M. chelonae, M. gordonae, M. gilvum, and a potential novel species. Culture-based detection was more sensitive than qPCR, while no clear association was found with building age or boiler temperature. To investigate potential sources, one positive household and neighboring households with the same construction date were resampled. The same NTM species were detected, showing closely related genetic profiles. However, subsequent sampling of the upstream water-source distributor was negative. These findings suggest that NTM may become established within downstream sections of the water distribution system. In particular, underground pipes and service (lateral) lines connecting individual properties to the main water network may represent important niches for NTM biofilm formation, persistence, and local dissemination. Overall, household water systems may represent relevant reservoirs for NTM exposure, highlighting the potential role of distribution infrastructure in NTM persistence and transmission.

P2: Dissecting immunoproteasome function in lung regeneration

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Immunoproteasomes (IP) are specialized proteasomes that play critical roles in multiple immune responses. Their three proteolytic subunits (PSMB8-10) are expressed at low levels in non-immune cells, such as alveolar epithelial cells, but are induced upon virus infection or stimulation with interferons (IFNs) thereby contributing to altered cellular protein degradation. As prolonged signalling of IFNs impairs proliferation of AT2 cells, we hypothesized that this effect is mediated by the induction of the immunoproteasome bestowing a role in lung regeneration.

Single cell RNA sequencing data analysis revealed that the IP is dynamically regulated upon AT2 to AT1 differentiation in mouse and human organoids with a transient increase in the AT0 stage. We confirmed this transient upregulation of the IP in a feeder-free mouse alveolar organoid system. Treatment of WT organoids with IFNg strongly stimulated IP expression and impaired organoid formation. Organoid formation, however,

was similar in IFNg treated organoids derived from AT2 cells that had been isolated from IP triple KO mice. These data suggest that the IP is not mediating the anti-regenerative effects of IFNg. In contrast, we observed improved AT1 differentiation in IP deficient AT2 cells at baseline as revealed by increased AT1-marker gene expression. Gene expression analysis showed that IP deleted AT2 cells have slightly higher expression of the stress responsive genes ATF4, Ddit3 and Nrf2 compared to WT cells suggesting that the loss of IP induces a mild cellular stress response, which has previously been shown to be required for AT1 differentiation. We are currently investigating the signals leading to the transient induction of the IP in AT0 cells to better understand the role of the IP in lung regeneration.

P3: Eco-friendly Manufacturing of Al_2O_3 Nanoparticles by Urtica dioica: Structural Insights and Biomedical Applications

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Stinging nettle (*Urtica dioica*) aqueous extract was employed as a natural reducing and stabilizing agent for the environmentally friendly synthesis of aluminum oxide (Al_2O_3) nanoparticles. The biosynthesized nanoparticles were characterized using UV–visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), and energy-dispersive X-ray spectroscopy (EDX). UV–visible analysis confirmed nanoparticle formation through a characteristic absorption peak at approximately 230 nm. FTIR analysis revealed Al–O bonds and phytochemical functional groups involved in the reduction and stabilization processes. XRD patterns confirmed the crystalline nature of the nanoparticles, with an average crystallite size of approximately 20 nm. Diffraction peaks at 2θ values of 37°, 45°, 60°, and 67° were assigned to the (311), (400), (511), and (440) planes of Al_2O_3, respectively. SEM analysis demonstrated nanoscale morphology, with particle size distribution influenced by precursor molarity. The biological activity of the synthesized nanoparticles was evaluated against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The nanoparticles exhibited strong antibacterial activity, with inhibition zones and minimum inhibitory concentration (MIC) values ranging from 100 to 250 µg/mL, while minimum bactericidal concentration (MBC) results confirmed bactericidal effects. No antifungal activity was observed against *C. albicans*. Hemolysis assays indicated good hemocompatibility and no significant harmful effects at the tested concentrations. The DPPH assay showed strong dose-dependent antioxidant activity for the plant extract, whereas Al_2O_3 nanoparticles exhibited comparatively weaker activity. Overall, this study demonstrates a sustainable phytochemical-assisted approach for producing crystalline Al_2O_3 nanoparticles with promising antibacterial activity, antioxidant potential, and acceptable blood compatibility for biomedical applications.

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P4: Quantitative Assessment of Proteasome and Immunoproteasome Activity in Human THP-1 Cells Using Activity-Based Probes

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The 20S proteasome is a central regulator of protein homeostasis and antigen processing in immune cells. In response to inflammatory stimuli such as IFN-γ, the constitutive proteasome is replaced by the immunoproteasome that contains catalytic subunits β1i, β2i, and β5i enhancing the generation of MHC class

I–presented peptides. However, assessing the functional activity of these protein complexes remains challenging due to methodological limitations in particular under treatment with proteasome inhibiting agents. Therefore, we validated existing quantitative, and functional assays to monitor both constitutive and immunoproteasome activity in human monocytic THP-1 cells using new and established activity-based probes (ABPs). THP1 cells were lysed and incubated with covalently binding (immuno)proteasome inhibitors directed against specific active β-subunits. Subsequently, cells were treated with a set of subunit-specific ABPs targeting the same catalytically active threonine residue in the respective subunits as the inhibitors. Remaining relative activity of proteasome and immunoproteasome subunits under inhibitor treatment was quantified by an in-gel fluorescence assay. This way subunit specificity of various proteasome inhibitors at different concentrations was quantitatively analysed. In conclusion, activity-based probes enable direct, quantitative, and functional profiling of both proteasome and immunoproteasome activity in human THP-1 cells. This approach provides a powerful tool to evaluate site- and pan-specific proteasome inhibitors as immunomodulatory drugs, and investigate the role of proteasome dynamics in inflammatory and autoimmune disease.

P5: NTHi-induced exacerbation in a COPD mouse model with comorbid obesity

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Background: COPD is frequently linked to obesity, a comorbidity associated with higher airway disease severity. Infection-induced exacerbations drive disease progression, with non-typeable *Haemophilus influenzae* (NTHi) being the most common pathogen. However, markers that indicate an imminent exacerbation are currently lacking. We therefore investigated these mechanisms using a mouse model of smoke-induced COPD with comorbid obesity and NTHi infection.

Methods: Female C57BL/6J wildtype mice were fed either a high-fat diet (HFD) or control diet (CD) ad libitum; in parallel, exposed to cigarette smoke (CS) or room air (RA) for 6 months, followed by infection with 1×10^6 CFU of NTHi isolate (strain H-189).

Results: Longitudinal plethysmography revealed reduced breathing frequency and airflow limitation in CS mice. Invasive lung function showed increased hyperresponsiveness in non-infected HFD mice, while CS+HFD promoted emphysema. Consistent with these functional findings, lung histological assessment demonstrated emphysema-like structural changes. Following NTHi infection, histological inflammatory scoring revealed increased pulmonary inflammation in CS-NTHi mice, independent of HFD. At the cellular level, bronchoalveolar

lavage (BAL) cytology also revealed significantly higher neutrophil levels in CS-NTHi mice at 48h post-infection (pif), which was not observed in RA-NTHi controls.

Conclusion: Smoking and obesity act additively on the COPD phenotype with a faster progression of lung function decline and emphysema-like changes. However, the altered inflammatory response to NTHi appeared to be associated with CS exposure and independent of HFD. Ongoing RNASeq analyses aim to identify early markers of recurrent exacerbation events for early intervention and prevention of irreversible lung function loss in these patients.

P6: Bioactive Green-Synthesized ZnO Nanoparticles: Antimicrobial, Antioxidant, and Cytotoxicity Profiling

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The scalable and eco-friendly synthesis of nanoparticles with potent antibacterial and antioxidant properties, while ensuring biocompatibility and minimizing cytotoxicity, is crucial for their safe and effective application in biomedical fields. In this study, zinc oxide nanoparticles (ZnO NPs) were successfully synthesized under mild and environmentally friendly conditions using an aqueous extract of ribwort plantain (*Plantago lanceolata*) leaves. The synthesized nanoparticles were designated as ZR. Their structural and morphological properties were characterized using conventional spectroscopic and microscopic techniques. The average particle size of the synthesized ZR nanoparticles was approximately 25 ± 5 nm.

The synthesized ZR NPs exhibited significant antimicrobial activity against the tested microorganisms, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. ZR demonstrated higher antimicrobial activity against these microorganisms than the standard antibiotics azithromycin and amoxicillin. Notably, ZR exhibited greater antimicrobial efficacy against the fungal strain *C. albicans* than against the tested bacterial strains.

The minimum bactericidal concentration (MBC) values were 100, 25, and 100 µg/mL for *C. albicans*, *E. coli*, and *S. aureus*, respectively. The minimum inhibitory concentration (MIC) values were reported as 12.5, 10.5, and 12.5 µg/mL, respectively. These findings indicate that ZR possesses considerable antimicrobial activity against the tested microorganisms.

The hemolysis assay revealed that ZR caused minimal damage to red blood cells, with a hemolysis rate of only 3%, indicating a low hemolytic effect and favorable blood compatibility. Microscopic examination of blood smears before and after treatment with ZR showed no detectable morphological changes in red blood cells. In addition, the biocompatibility assay, with sodium dodecyl sulfate (SDS) and normal saline used as controls, demonstrated that ZR did not cause significant toxicity at concentrations up to 100 µg/mL. The observed lysis rate was only 1.9%, further supporting the favorable biocompatibility of the synthesized nanoparticles.

Regarding antioxidant activity, ZR exhibited only a slight antioxidant effect. Therefore, its biological activity appears to be more pronounced against the tested microbial strains than in the antioxidant assay.

Overall, the findings demonstrate that the biogenic synthesis of ZnO nanoparticles using ribwort plantain leaf extract provides an environmentally friendly and mild approach for producing nanoparticles with promising antimicrobial activity and favorable biocompatibility. The low hemolytic and lysis rates, together with the absence of observable morphological alterations in red blood cells, suggest that ZR has good biocompatibility under the tested conditions. These characteristics make ZR promising candidates for future biological and biomedical applications, particularly in antimicrobial applications.

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P7: Molecular Mechanisms Underlying the Uptake and Intracellular Fate of Methanogenic Archaea in Human Innate Immune Cells

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The human microbiome comprises not only bacteria but also archaea, whose interactions with the host immune system remain poorly understood. Unlike bacteria, archaea lack several classical bacterial pathogen-associated molecular patterns, including lipopolysaccharide and peptidoglycan, raising questions about the mechanisms underlying their recognition and uptake by innate immune cells. My PhD project investigates the host-cell receptors and signaling pathways involved in the interaction of human innate immune cells with the methanogenic archaea Methanosphaera stadtmanae and Methanobrevibacter smithii. A βestradiol-induced transdifferentiation model based on CEBRA cells has been established to investigate these interactions, and confocal microscopy has been used to examine the intracellular localization of fluorescently labelled M. stadtmanae. To identify host-cell receptors involved in archaeal uptake, the project combines targeted approaches, including siRNA-mediated gene silencing and receptor-blocking antibodies, with the development of a lentiviral CRISPR/Cas9-based screening strategy. Candidate receptors are being investigated through gene expression analysis, protein-expression assessment, and functional uptake assays in cell lines and primary human monocytes. By elucidating the molecular mechanisms governing archaeal recognition and internalization, this work aims to advance our understanding of innate immune responses to methanogenic archaea and their potential roles in immune homeostasis and inflammatory processes.

P8: Label-free identification and sorting of *Mycobacterium tuberculosis*-infected human monocyte-derived macrophages using autofluorescence and imaging-derived parameters

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Background: Conventional identification of *Mycobacterium tuberculosis* (Mtb)-infected human monocyte-derived macrophages (MDMs) by flow cytometry typically relies on fluorescent probes or genetically modified bacteria, potentially limiting downstream applications. We investigated whether intrinsic autofluorescence and infection-induced morphological changes could enable label-free identification and sorting of infected cells.

Methods: MDMs from three healthy donors were infected for 72 h with wild-type Mtb (H37Ra) or a Scarlet-I3-expressing strain as positive control. Cells were analyzed on the BD FACSDiscover™ S8 Cell Sorter combining spectral fluorescence with single-cell imaging. Concatenated data were visualized in FlowJo™ using t-SNE, followed by Phenograph clustering based on image-derived parameters, excluding Scarlet-I3-associated channels. BD CellView™ Lens visualized representative clusters. HyperFinder identified discriminative imaging features and generated a simplified gating strategy for sorting infected populations. Sorted cells were validated by confocal microscopy.

Results: Infected and uninfected MDMs were discriminated label-free using intrinsic autofluorescence and imaging-derived morphological features. Clusters identified in Scarlet-I3-infected macrophages, without using Scarlet-I3 fluorescence, closely mirrored wild-type infected populations and were distinct from uninfected cells. HyperFinder translated these signatures into a simplified 2D gating strategy enabling reproducible sorting of both infected populations. Confocal microscopy confirmed Mtb presence and showed increasing bacterial load with enrichment of infected-cell cluster signatures.

Conclusion: Spectral flow cytometry integrated with real-time imaging enables label-free identification and sorting of Mtb-infected macrophages using computational and simplified gating strategies. This workflow supports downstream transcriptomic and proteomic applications, expanding the utility of cytometric sorting in infectious disease research.

P9: Immunophenotyping of gut-colonised mice to study the gut-lung axis in lung health

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Development of chronic respiratory diseases such as asthma is influenced by multiple factors, including the microbiome and its circulating metabolites. With increasing evidence linking pathobionts such as *Haemophilus influenza* to the development of childhood asthma, efforts have been intensified to understand the underlying reasons. The gut microbiota can influence distant organs, including the lungs, potentially affecting lung health. However, experimental models to study microbiota-related effects on lung health via the gut-lung axis are largely lacking. Gnotobiotic mice are a valuable tool to study microbe-microbe and microbe-host interactions *in vivo*.

We immunophenotyped gnotobiotic germ-free (GF) and Oligo-mouse microbiota (OMM12), and compared them to conventionally colonized specified pathogen free (SPF) mice at different ages, to study microbiota-

related influences on the developing immune system, with focus on asthma-related cells. Flow cytometric analyses revealed a strong influence of the gut microbiota on lung cell types such as eosinophils, ILCs and CD8+ T cells, all implicated in asthma development, whiles other cell-types were significantly influenced by both the microbiota and age of mice. Using hydrolysis probe-based qPCR, we observed that the lungs of the gnotobiotic GF and OMM12-colonised mice are completely sterile. This finding provides an important basis for future investigations whether *Haemophilus influenzae* plays a causal or consequential role in asthma development. Next, we will investigate which microbiota-derived metabolites may play a role in influencing the immune cell development by analysing serum samples. Additionally, lung tissues will be sequenced to further confirm its sterility.

P10: Age dependent sex differences in DNA methylation Age in asthma

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Asthma exhibits age associated sex differences, being more severe in boys during childhood and in females after puberty. Underlying mechanisms remain incompletely understood. Interleukin (IL)-6 is a key factor driving accelerated biological aging (DNAge) and asthma severity. The relationship between IL-6, DNAge and sex differences in asthma remains unclear.
We aim to investigate DNAge differences between asthmatic males and females, focusing on IL-6 DNA methylation (IL6m).
Nasal brushings from the ALLIANCE cohort (Pediatric:n=60F/92M; asthma (A)=74, healthy (H)=43, wheeze=35; avg_{age} =10yr, IQR=4–14yr; Adult:n=117F/97M; A=172, H=42; avg_{age} =55yr, IQR=42–65yr) were collected. DNA methylation (DNAm) analysed by Illumina EPIC 850K array using *R* (v4.5.1). Using skinHorvath clock, DNAge acceleration was calculated relative to chronological age. Based on symptoms reported over the previous 4 weeks, patients were classified as controlled, partially controlled, or uncontrolled. IL6m was assessed via Ward hierarchical clustering to stratify patients into high and low IL6m clusters. Patient distribution between IL6m status and DNAge association was assessed by Chi-square testing.
Chronological age correlated strongly with DNAge (pediatric R=0.92; adult R=0.81). Pediatric, uncontrolled females showed higher DNAge than males (0.99yr;$p<0.05$) further increased post-puberty (1.52yr;$p<0.05$). High IL6m was associated with decelerated DNAge in female children ($p<0.05$) but not in males. In adults, this association was observed regardless of sex ($p<0.001$).
HS pediatric asthmatic females exhibited higher DNAge than males specifically post-puberty. IL6m and DNAge demonstrated sex and age specific associations. Further research is needed to determine how the relationship between asthma, sex and DNAge contributes to asthma heterogeneity.

P11: Targeting IL-13 signalling pathway as a potential therapeutic approach in experimental models of pulmonary arterial hypertension.

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Systemic sclerosis (SSc) is a severe autoimmune disease characterized by inflammation, fibrosis, and vasculopathy. Pulmonary arterial hypertension (PAH), one of its most fatal complications, is driven by progressive pulmonary vascular remodeling and vascular occlusion. Current therapies improve symptoms but do not adequately prevent disease progression because the molecular mechanisms underlying vascular remodeling remain incompletely understood. Based on our previous findings, we propose a Two-Hit Hypothesis, in which autoimmune activation provides the initial inflammatory response, while IL-13 overexpression acts as a second hit driving pulmonary vascular remodeling. This study aimed to define the molecular mechanisms underlying IL-13-induced pulmonary vascular remodeling and to establish ex-vivo experimental models for therapeutic evaluation. Our previous in vivo studies demonstrated that IL-13 transgenic (IL-13tg) mice develop severe occlusive pulmonary vasculopathy independent of AT1R immunization, characterized by pulmonary arterial smooth muscle cell (PASMC) hyperplasia. Building on these findings, we investigated the signaling pathway mediating IL-13-induced proliferation in human pulmonary arterial smooth muscle cells (HPASMCs). IL-13 induced dose-dependent HPASMC proliferation, which was significantly inhibited by IL-4Rα blockade, whereas IL-13Rα2 blockade had minimal effect, identifying the IL-13Rα1/IL-4Rα receptor complex as the predominant signaling pathway mediating this response. To complement these studies, we established a long-term mouse precision-cut lung slice (PCLS) model by optimizing culture conditions to maintain tissue viability for more than 14 days. Following IL-13 stimulation, H&E and α-smooth muscle actin (α-SMA) staining demonstrated smooth muscle cell hyperplasia and vascular occlusion in small pulmonary arteries, partially recapitulating the phenotype observed in IL-13tg mice. However, these findings require further validation. Overall, this multi-system approach identifies the IL-13 signaling pathway as a key driver of pulmonary vascular remodeling, provides a translational platform for testing targeted inhibitors, and highlights IL-13 signaling as a promising therapeutic target in SSc-associated PAH.

P12: Atomic Force Microscopy Reveals Cytoadhesion Mechanisms of Plasmodium Falciparum Infected Red Blood Cells

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Malaria remains among the deadliest infectious diseases worldwide, with most fatal cases caused by *Plasmodium falciparum*. Severe disease results from parasite–host interactions, most notably the cytoadhesion of infected red blood cells (iRBCs) to the vascular endothelium of vital organs. This process is mediated by the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) family, large transmembrane proteins concentrated in membrane protrusions termed knobs. Although cytoadhesion is central to malaria pathogenesis, the biophysical mechanisms linking PfEMP1 binding properties, knob architecture, endothelial cell receptor (ECR) profiles, and disease severity remain poorly understood.

In this project, the molecular binding properties between iRBCs and ECRs will be quantified using single-cell force spectroscopy (SCFS), including measurements of binding events, rupture forces, bond lifetimes, and dissociation kinetics. Second, the influence of PfEMP1 on iRBC morphology and mechanics will be characterized, focusing on knob size and elasticity. PfEMP1 distribution will be analyzed by molecular recognition imaging with different binding partners. Third, synthetic peptides derived from ECR binding epitopes will be iteratively optimized and tested as potential inhibitors of cytoadhesion.
Preliminary observations revealed distinct adhesion behaviors, with rolling interactions via CD36 and stable adhesion to other ECRs such as ICAM-1. SCFS demonstrated receptor-specific differences in binding behavior, indicating distinct force regimes and bond stabilities. These findings support the hypothesis that PfEMP1 variant, endothelial cell origin, and physical conditions determine cytoadhesion and influence disease severity. This project provide insight into how cytoadhesion contribute to malaria pathogenesis at molecular level, and aims to generate therapeutically applicable active substances against malaria.

P13: Smoke and Adiposity: Transgenerational Consequences for Respiratory and Systemic Health

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Lifestyle factors such as high-calorie diets and cigarette smoke are major drivers of metabolic and physiological dysfunction, contributing to chronic diseases including respiratory disorders. Yet, their combined impact on organismal physiology and tissue homeostasis remains poorly understood. To address this, we use the fruit fly *Drosophila* as a model to investigate how these lifestyle-related exposures affect systemic physiology and airway homeostasis.
In intragenerational experiments, flies were exposed to a high-calorie diet and cigarette smoke either individually or in combination. Both interventions significantly shortened lifespan ($p<0.0001$), with the combination producing the strongest effect ($p<0.0001$). At the tissue level, both interventions increased intestinal stem cell proliferation and compromised gut integrity ($p<0.0001$). We also observed structural abnormalities in the intestinal tracheal network, suggesting that lifestyle-related stressors disrupt local airway organization at the gut–trachea interface. Notably, flies exposed to both stressors showed increased susceptibility to airborne challenges ($p=0.0002$), indicating reduced respiratory resilience.
Building on these findings, we are currently analyzing how these exposures affect airway structure and airway progenitor cells, as well as physiology and metabolism of offsprings: maternal exposure reduced offspring's lifespan ($p<0.0001$). Parental detrimental lifestyle significantly impaired tracheal coverage of the intestinal epithelium and affected lipids, and protein metabolism. Interestingly, effects on body composition extended to the subsequent 'grand-children'-generation, suggesting long-lasting effects of parental lifestyle.

P14: Deciphering the Metabolic Potential of CF Sputum Commensals: An LC-HRMS Guided Approach to Novel Biofunctional Metabolites.

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Background: The respiratory microbiome plays a vital role in preventing opportunistic infections. *Pseudomonas aeruginosa* remains a major priority pathogen causing chronic lung infection and high mortality in cystic fibrosis (CF) patients. While certain resident commensal bacteria exhibit antagonistic interactions against *P. aeruginosa*, identifying and retrieving the specific underlying chemical drivers remains a major natural products bottleneck due to low physiological expression levels and complex matrix masking effects.

Methods: In this study, 20 distinct commensal bacterial strains isolated from sputum samples of CF patient were evaluated. Whole-genome sequencing paired with antiSMASH genomic mining was employed to map biosynthetic gene clusters (BGCs). Untargeted metabolomics was executed via cell-free culture supernatant extraction followed by Liquid Chromatography-High Resolution Mass Spectrometry (LC-HRMS). Structural annotations of bacterial metabolites were resolved utilizing mass spectral libraries and database of chemical structures.

Results: Genomic mining revealed rich BGC topographies primarily dominated by Ribosomally Synthesized and Post-translationally Modified Peptides (RiPPs) alongside Non-Ribosomal Peptide Synthetase (NRPS) assemblies. While crude cell-free supernatant extracts tested up to 5 mg/mL did not display dominant standalone antimicrobial activity against *P. aeruginosa*, LC-HRMS profiling successfully dereplicated 31 known metabolites and resolved 8 abundant, highly promising unknown candidate compounds predicted as novel peptides.

Conclusion and Outlook: To circumvent matrix dilution effects, we aimed to fractionate the crude extracts, isolate candidate metabolites, and subsequently verify targeted bioactivity. Detected candidate compounds were computationally annotated as novel peptides with high structural prediction scores. This workflow underscores a high-resolution, metabolomic pipeline to understand the mechanism of *Pseudomonas* inhibition in CF patients.

P15: *Haemophilus Influenza*-derived Metabolite May Activate Rhinovirus Induced Trained Immunity in Respiratory Epithelial Cells.

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Background: Childhood asthma exacerbations are mainly caused by human rhinovirus (HRV) infection and linked to airway microbiome changes. Repeated HRV infections induce epigenetic training in bronchial epithelial cells (Beas-2B), reduce DNA methylation (DNAm) of *IL6*, which is linked to persistent asthma phenotype. Early-life colonisation with non-typeable *Haemophilus influenza* (NTHi) increases asthma risk, with an abundant inflammation-related NTHi metabolite hypoxanthine. However, interactions between respiratory infections, DNAm, and hypoxanthine in asthma remain unknown.

Objective: Investigation of whether NTHi-derived hypoxanthine upregulates *IL*6 gene expression and potentially activates trained immunity induced by prior viral infection.

Methods: To model recurrent viral infections, Beas-2B cells were infected five times with HRV and DNAm was detected by Illumina 450k arrays. Differential methylation was analysed in R (version 4.5.1, *limma*). Nasal brushings from ALLIANCE children (asthmatics=34/wheezers=19) were analysed using Illumina 850k arrays. Patients were stratified by IL6 DNAm status using Ward hierarchical clustering, followed by trajectory analysis of exacerbations and medication use. To investigate inflammatory effects of hypoxanthine, Beas-2B cells were treated with hypoxanthine followed by detecting inflammatory markers by RT-qPCR.

Results: In Beas-2B cells, 33,826 CpGs (FDR p<0.01) changed with five recurrent HRV infections. A persistent asthma phenotype revealed overlapping methylation sites among its differentially methylated 10,140 CpGs (FDR p<0.01). Hypoxanthine(25 μM) induced a 2.5-fold increase in *IL6* expression after 3 hours versus control (adjusted p-value < 0.05, n=3).

Conclusion: These findings suggest that recurrent viral exposure and NTHi-derived hypoxanthine promote persistent inflammatory responses in respiratory cells, requiring further study of the underlying interplay of viral and hypoxanthine exposure.

P16: Immunoproteasomes in feto-maternal immune responses and immune priming

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The immunoproteasome is a specialized type of proteasome constitutively expressed in immune and medullary thymic epithelial cells but inducible in non-immune cells by interferon signalling. It plays a crucial role in establishing self-tolerance, autoimmunity, and infection responses by degrading intracellular, including viral, proteins into MHC class I antigens for CD8+ T cell activation. We propose that the immunoproteasome will be regulated by maternal immune activation (MIA) in response to viral infections. The focus will be on delineating the role of the immunoproteasome in anti-viral immune responses in the pregnant host. We will investigate this by comprehensive analysis of immunoproteasome activation, MHC class I antigen presentation, CD8+ T cell activation in influenza-infected allogenically pregnant mice with wildtype (WT) and constitutive and cell-specific immunoproteasome knockout (KO) background. Moreover, as the immunoproteasome is also crucial for T cell selection in the thymus, we speculate that MIA during pregnancy impacts immune priming and development of tolerance in the offspring by affecting thymic selection processes. The effects of MIA on the offspring will be determined by analysis of the thymus focusing on the regulation of the thymoproteasome and its consequences on CD8+ T cell selection and a/b TCR repertoire. The project positions the immunoproteasome at the crossroads of viral infection and autoimmune responses and provides a potential biomarker to identify pregnant women at risk of severe viral infections.

P17: Association of systemic sclerosis-related interstitial lung disease with tumor-associated antigens and their autoantibodies

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Background: Interstitial lung disease (ILD) is the leading cause of systemic sclerosis (SSc) patients' morbidity and mortality. The role of tumor-associated antigens (TAAs) in SSc-ILD remains unclear.

Methods: A total of 106 SSc patients were enrolled at the First Affiliated Hospital of Xiamen University, China. Clinical and serological data, including serum levels of TAAs, were retrospectively collected from medical records. Single-cell RNA sequencing (scRNA-seq) data were obtained from the public GEO database (GSE128169) to evaluate the expression of TAAs in SSc-Fibrosing-ILD (SSc-F-ILD) patients lung tissue. Additionally, immunohistochemical staining was performed to validate carcinoembryonic antigen (CEA) protein expression in SSc-F-ILD patients lung tissue.

Results: Compared with healthy controls, patients with SSc showed significantly elevated serum levels of CEA, cytokeratin 19 fragment (CYFRA 21-1), neuron-specific enolase (NSE), carbohydrate antigen 125 (CA125) and carbohydrate antigen 153 (CA153). Among those SSc-associated TAAs, serum CEA, CYFRA 21-1 and CA153 levels were positively correlated with high-resolution computed tomography (HRCT)-derived alveolar and fibrosis scores. Patients with SSc-ILD showed significantly higher serum levels of CEA, CYFRA 21-1, CA153 and pro-gastrin-releasing peptide (proGRP) than those without ILD. Furthermore, patients with SSc-F-ILD exhibited significantly higher serum levels of CEA, NSE and CA153 than those with SSc-non-F-ILD. scRNA-seq analysis revealed that genes encoding CEA, CYFRA 21-1 and CA153 were predominantly expressed in lung epithelial cells and were significantly upregulated in multiple epithelial subtypes in patients with SSc-F-ILD. Immunohistochemical analysis further confirmed predominant CEA protein expression in lung epithelial cells and increased CEA protein levels in lung tissues from patients with SSc-F-ILD compared with healthy donors.

Conclusions: Serum levels of multiple TAAs, particularly CEA, CYFRA 21-1 and CA153, are elevated in SSc and associated with pulmonary involvement, particularly fibrosing ILD, with lung epithelial cells representing a plausible pulmonary source of these TAAs.

P18: Pangenome Graph-Based Genotyping Identifies Structural Variants Linked to Asthma

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Structural variants (SVs), genomic alterations larger than 50 bp, can substantially affect gene regulation and disease risk. In contrast to single nucleotide polymorphisms (SNPs), which have been extensively studied, SVs

have been underexplored. Recent advances in long-read sequencing enable haplotype-resolved genome assemblies capturing variations from SNPs to complex SVs. In parallel, pangenome graphs integrate multiple genomes into a single reference, reducing bias inherent to linear references. The Human Pangenome Reference Consortium (HPRC) has released a high-quality pangenome graph resource based on diverse human genomes.

We used PanGenie, a graph-based genotyping tool, together with the HPRC pangenome graph to genotype SNPs and common SVs from short-read sequencing data of 99 European-ancestry genomes from the 1000 Genomes Project. We then screened for SVs in linkage disequilibrium (LD) with SNPs associated with asthma and reported at least five times in the genome-wide association studies (GWAS) catalog. Gene expression data and transcription factor binding site annotations were used to assess functional relevance.

We identified ten loci with common SV sites in strong LD with asthma-associated GWAS SNPs. Notably, a 68 bp insertion in high LD with rs12788104 was detected upstream of *MUC5AC*, a structurally variable gene encoding a mucin produced by goblet cells and known to be upregulated in asthma. Single-cell analysis of IL-13 challenged bronchial epithelial cells confirms strong *MUC5AC* upregulation in secretory cells, supporting a role in asthma pathology.

P19: Molecular Characterisation of Epithelioid Cells in Human Tuberculosis

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Infections with mycobacteria from the Mycobacterium tuberculosis complex are characterised by the formation of granulomas, organised immune structures that recruit macrophages, which undergo a distinct morphological transformation. Resembling epithelial cells with euchromatic nuclei and connections to neighbouring cells, these macrophages have been termed “epithelioid cells”. Animal models have shown that reprogramming events resembling the mesenchymal epithelial reprogramming of embryonic development occur in these cells. While this reprogramming has been observed in human tuberculosis, the spatial organisation within granulomas and possible differences across lesion types remain unclear. This study investigates and quantifies the magnitude of mesenchymal-epithelial transition (MET) of macrophage populations within different lesions of surgical samples from TB patients by multiplexed immunofluorescence (mIF) staining and downstream analysis of cellular neighbourhoods. The panel consisted of antibodies against CD206, the mannose receptor of mature macrophages, and CD68, a glycoprotein of monocytes and macrophages to detect macrophage populations. To investigate mesenchymal epithelial reprogramming, we included an antibody against E-cadherin, which is a membrane protein essential for epithelial cells. The fourth antibody in the panel was directed against ZO-1, which is a component of tight junctions. Preliminary results

showed quadruple-positive cells were significantly more abundant within granulomas than in adjacent non-granulomatous tissue or uninfected control regions suggesting increased numbers of activated macrophage populations that can perform an epithelial-like barrier function in granulomas. This indicates that the MET is significantly upregulated in granuloma-associated macrophages, with distinct spatial distribution across different granuloma types, suggesting MET as a driver of granuloma organisation and maturation.

P20: Spatial Profiling of Langhans Giant Cells and their Cellular Neighbourhoods in Human Tuberculosis

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Tuberculosis (TB) is characterized by the formation of granulomas, highly organized structures composed of diverse immune and stromal cell populations. Langhans giant cells (LGCs) are multinucleated macrophages observed in TB lesions and are thought to contribute to the organization and immune regulation in granulomas. There is little known about LGCs in TB pathology and their cellular neighborhoods remain poorly understood. To investigate the local immune environment of LGCs in TB, 46 formalin-fixed, paraffin-embedded human patient samples were analyzed using multiplex immunofluorescence targeting fibroblasts (FAP), V-ATPase (ATP6V1B2), T cells (CD3), macrophages (CD68) and B cells (CD20). LGCs within whole-slide images were identified based on their characteristic morphology and expression of CD68 and classified according to their specific localization within TB lesions as granuloma-associated and non-granuloma-associated. Cell-type-specific classifiers and the single-cell-resolved spatial microenvironment of LGCs were computed using QuPath and downstream analyses using R package imcRtools, focusing on the abundance and spatial distribution of T cells, B cells and fibroblasts near to LGCs and their V-ATPase expression, as a surrogate of lysosomal acidification. This study provides a thorough investigation of the cellular environment surrounding LGCs in human TB, while also providing insights into their overall spatial distribution in TB lesions. Comparing LGCs according to their association with granulomatous structures and their level of ATP6V1B2 expression may reveal differences in their local cellular microenvironment and provide further insights into the spatial organization and potential heterogeneity of LGCs in human TB.

P21: Transgenerational Consequences of Parental Lifestyle: Individual Effects of Parental Smoking and Obesity on Offspring Systemic Health

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Lifestyle in Western societies is characterized by a caloric dense diet with highly processed food containing large amounts of fat and sugar. Additionally, the consumption of stimulants, such as tobacco impacts respiratory and systemic health. However, the transgenerational consequences of such lifestyle habits remain poorly understood although they potentially comprise serious health issues for offsprings.
This project aims to disentangle the impacts of parental diet and smoke exposure on offspring health including the relative contributions of maternal and paternal lifestyle.
Using the model organism *Drosophila*, we exposed the parental generation either to a Western Diet or to Cigarette smoke. Parental diet was closely related to offsprings body composition and feeding behavior as lipid accumulation was significantly increased ($p<0.0001$) in offspring who had one parent on Western Diet. Additionally, also the bodyweight of offspring whose mother was on Western Diet showed a significant increase ($p<0.0001$).
Parental smoke exposure seriously comprised offsprings health as the lifespan was significantly reduced in male and female offsprings ($p<0.0001$). Besides, female offspring fecundity was impaired ($p<0.0001$) and bodyweight was increased ($p<0.0001$).
Furthermore, the human homolog to tracheoles named terminal trachea cells that cover the intestinal tissue were impaired by both stressors individually, highlighting the systemic consequences of both respiratory and intestinal stressors.

P22: Advanced Imaging and AI Analysis Reveal Sex-Specific B Cell Responses During Mycobacterium tuberculosis Infection

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Recent studies have highlighted the importance of B cells in tuberculosis (TB) immunity, challenging the traditional view that these cells are non-essential for TB defense. B cells accumulate at infection sites within organized structures called B cell follicles in the lungs of experimental animals and humans, where they are associated with protective immunity. However, the functional role of B cells in TB remains poorly defined. Beyond antibody production, B cells serve as antigen-presenting cells and secrete cytokines that profoundly shape the local immune environment. Our previous work revealed a sex bias in pulmonary B cell follicle formation, with males developing significantly smaller follicles. This finding unveils a previously unrecognized structural basis for sex-biased TB susceptibility. To analyze these complex structures and elucidate the role of B cell follicles and B cells during *Mtb* infection, advanced analytical techniques are required. We employed light sheet microscopy, multiplex immunohistochemistry, and artificial intelligence-based flow data analysis to investigate the role of B cells in TB. These techniques provide unprecedented spatial and phenotypic

resolution, enabling detailed characterization of cellular interactions and immune responses within the pulmonary microenvironment. Importantly, these approaches are not limited to animal models but can be applied to patient samples, offering translational potential. Our findings contribute to understanding the immunological mechanisms underlying sex-specific differences in TB pathogenesis and may inform the development of targeted therapeutic strategies. By revealing how B cells and their organizational structures influence TB outcomes differently between sexes, this work opens new avenues for personalized approaches to TB prevention and treatment.

P23: Establishment of a human bronchial epithelial barrier model to study mucosal immune responses

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Mucosal-associated invariant T (MAIT) cells are multifunctional immune cells contributing to cytotoxic, inflammatory and regulatory responses, wound healing and antiviral defense. Their canonical activation relies on antigen presentation by the MHC-I-related molecule MR1 and recognition via their invariant T cell receptor within an immunological synapse, requiring cell-cell contact. In contrast, this project focuses on perception at the respiratory epithelium of stimuli with contact-independent signaling. We use a Normal human bronchial epithelial (NHBE) cell air-liquid interface (ALI) model to study how apically applied pathogen-associated stimuli are perceived by primary human MAIT cells placed basally. The distance between epithelial barrier and MAIT cells excludes direct contact and thereby isolates contact-independent signaling. Preliminary tests (stimulation with the MR1-ligand 5-OP-RU) have already shown that, despite an increased surface expression of MR1 on NHBE cells, MAIT cell activation (CD69 surface expression) was not observed. First, we define value ranges for quality and physiological relevance of NHBE barrier cultures, combining software-assisted light microscopy analysis (confluence / cell density), TEER measurements and H&E histology with flow cytometry and fluorescence microscopy of differentiation markers (EpCAM, acTub, MUC5AC, CD271, CD66c). Suitable cultures are then stimulated apically and responses are analyzed by mass spectrometry-based proteomics and flow cytometry of activation markers (MR1, CD274, CD279, CD69). Next to pathogen-like stimuli (LPS, Poly(I:C), LTA, MALP-2), also more physiologically relevant models (Staphylococcus aureus, GFP-tagged Herpes simplex) will follow up.

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P24: The Drosophila mirtrons miR-1006 and miR-1011 regulate tracheal development and organismal fitness

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Background: Tobacco-smoke exposure disrupts airway epithelial homeostasis and is a major risk factor for chronic lung diseases, including COPD, yet the post-transcriptional regulators involved remain poorly defined. In smoke-exposed Drosophila airways, transcriptomics revealed dysregulated EGFR, WNT and Notch signaling pathways. TargetScan predicted 25 microRNAs targeting all three pathways, and a tracheal screen prioritized two uncharacterized Drosophila-specific mirtrons, miR-1006 and miR-1011. [1].

Methods: Using the btl-GAL4/UAS system, miR-1006 and miR-1011 were inhibited by miRNA sponges or overexpressed specifically in the airway system. Developmental timing, body size and weight, locomotor activity, lifespan, intestinal barrier integrity, hypoxia tolerance and tracheoblast phenotypes were assessed in both sexes. Whole-trachea and tracheoblast transcriptomes were analyzed by RNA sequencing (RNAseq).

Results: Airway-specific inhibition or overexpression of miR-1006 and miR-1011 delayed development and produced distinct, sex-dependent changes in body size, locomotor activity, lifespan, intestinal barrier integrity and hypoxia tolerance. Impairment of barrier integrity and hypoxia tolerance was more pronounced in females. Overexpression reduced tracheoblast area, while miR-1006 overexpression increased apoptosis specifically in females. Whole-trachea RNAseq revealed recurrent dysregulation of ion transport (ppk13) and extracellular matrix-related genes. miR-1011 tracheoblast RNAseq further identified altered metabolic, immune and epithelial migration pathways.

Conclusions: miR-1006 and miR-1011 are dosage-sensitive regulators of airway development and organismal fitness. Their recurrent association with DEG/ENaC family member ppk13 suggests a potential miRNA–ion-channel regulatory axis; the human counterpart ENaC governs airway liquid clearance and is implicated in cystic fibrosis and COPD. Ongoing genetic rescue experiments will test whether ppk13 inhibition rescues the developmental and functional defects.

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P25: From Host–Pathogen Interactions to Polymicrobial Lung Infection: Investigating *Stenotrophomonas maltophilia* Pathogenesis at the Polarized Airway Epithelium

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Stenotrophomonas maltophilia is an emerging opportunistic pathogen of increasing relevance in chronic respiratory diseases. Its high intrinsic antimicrobial resistance and capacity to form biofilms on different surfaces make it a considerable concern in clinical settings. Yet its interactions with the human airway epithelium remain poorly understood. Moreover, chronic airway infections often are shaped by complex microbial communities, highlighting the need to investigate the pathogenicity of *S. maltophilia* in a polymicrobial context. One particularly intriguing partner is the environmental fungus *Aspergillus fumigatus*: both opportunists can be found in the airways of patients with chronic respiratory conditions, yet little is known about how their interaction shapes infection.
Here, we use polarized Calu-3 airway epithelial cells grown at the air-liquid interface (ALI) to investigate host-pathogen interactions of the clinical *S. maltophilia* isolate Sm314. Microscopy revealed that Sm314 invades the intercellular space of the airway epithelium. This involves the putative proteases DegQ and DegP, which contribute to cleavage of the adherens junction protein E-cadherin. A *degQ/degP* double-knockout mutant failed to colonize the intercellular space, indicating their importance for epithelial invasion. In addition, electron and fluorescence microscopy demonstrated intracellular bacteria, with a subset localizing to LAMP1-positive compartments, suggesting entry into the endolysosomal pathway.
Building on these findings, we are extending the ALI model to investigate polymicrobial infection with *A. fumigatus*. Both microorganisms colonize diseased airways and are co-detected in chronic respiratory conditions, especially in cystic fibrosis. We aim to determine how bacterial-fungal interactions influence microbial growth and antimicrobial susceptibility, as well as epithelial integrity and host responses.

P26: Analysis of lipoteichoic acids and glycosyldiacylglycerols of *Streptococcus canis*

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Streptococcus canis is an opportunistic pathogen observed in animals, especially dogs and cats, but occasionally also in humans. Usually, this pathogen is classified into the Lancefield group G. Recently, two strains of *S. canis* belonging to Lancefield group C (IMT52870 and IMT56394) were identified. Whole genome sequencing analysis pointed towards a horizontal gene transfer between *S. canis* and *Streptococcus dysgalactiae*.[1]
Besides the Lancefield assignment, little is known about cell wall components of *S. canis* on the molecular level. The aim of this study is to close this gap of knowledge by structural analysis of the lipoteichoic acid (LTA)

and glycosyldiacylglycerols (GLs) from six selected *S. canis* strains: four group G strains (G361, IMT49926, AGF1074 and AGF1293) and the two group C strains. For this, we applied our expertise for the isolation and structural characterization of Gram-positive bacterial cell wall components by mass spectrometry and nuclear magnetic resonance spectroscopy.[2,3]

All examined strains express two GLs, α-D-glucose-3-(1,2-diacyl)-glycerol and α-D-glucose-(1→2)-α-D-glucose-3-(1,2-diacyl)-glycerol. They also produce a type I LTA with the diglucosyl-DG as GL anchor and poly-glycerolphosphate chains that are partially substituted with alanine. The conserved structure of GLs and LTAs in *S. canis* is shared with several other streptococci. Based on this, it was possible to predict the members of the biosynthetic pathway for LTA and GL synthesis in *S. canis* using BLAST analysis.

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P27: Optimization of the microfluidic platform SynerChip for analyzing synergies between antibiotics and antimicrobial peptides on giant lipid vesicles and bacteria.

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The combination of antimicrobial peptides (AMPs) with antibiotics (ABs) is a promising therapeutic strategy against multi-drug-resistant bacteria. These drug combinations can act synergistic, additively or antagonistically. To identify synergistic combinations, a fast and resource efficient method for screening of various combinations of concentration is therefore desired. For that, we developed the microfluidic Platform *SynerChip*, which allows us to generate and investigate various combinations of concentrations of two analytes, AMPs and ABs, simultaneously according to their membrane permeabilization efficiency. Taking advantage of microfluidic methods enables us to investigate these interactions resource-efficient and fast. Vesicles filled with fluorescent dye are produced microfluidically by octanol-assisted liposome assembly, hundreds trapped individually in the *SynerChip* and exposed to the specific titrated concentration-combinations. This allows us to draw a conclusion about the synergistic effects of analytes (AMP-AB) on the vesicle membrane, using a dye release assay.

This work focuses on developing the *SynerChip* by increasing the number of concentration-combinations, encapsulating bacteria in water-in-oil droplets, and evaluating different mixing strategies of analytes in microfluidic systems. Due to laminar flow in microfluidics, combined flows only mix by diffusion. At the flow rates needed this is not sufficient for ideal mixing of solutes, producing undesired concentration-gradients, notable at the investigated vesicle membranes – a mixing strategy is needed. To minimize the effect of undesired concentration-gradients, the mixing geometry of the microfluidic platform were iteratively optimized by computer-aided-design (CAD), chip fabrication using in-house lithography and experimental testing using fluorescent dyes. Tested mixing strategies are passive advection-based micromixers with intersecting channels.

P28: Airborne Microbiomes and Antimicrobial Resistance Genes in Agricultural Dust

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Background: Antimicrobial resistance (AMR) is one of the greatest global health threat, and has been recognized by the United Nations as a major One Health challenge. By 2050, the mortality associated with AMR is predicted to reach 10 Million deaths per year. Livestock production represents an important environmental reservoir, where antimicrobial use selects for resistant bacteria and antimicrobial resistance genes (ARGs). Airborne dust can serve as a transmission vehicle dispersing microbial communities and clinically relevant ARGs beyond farm boundaries.

Methods: This PhD-Project characterizes the microbiome and resistome of dust from pig farms in Germany and Denmark. Farm dust will be analysed using shotgun metagenomic sequencing and targeted qPCR. Taxonomic and resistome profiles will be determined using established bioinformatic tools, to identify farm-specific microbial and resistance signatures and establish a microbiome and resistome atlas of pig-farm dust.

Results: First dust specimens from a pig farm have been collected and processed. A workflow to extract DNA from settled dust samples has been established, and initial DNA samples are currently undergoing sequencing. In parallel, bioinformatic workflows are being established and optimized for subsequent analysis.

Conclusion: The project will provide insights into the airborne dissemination of AMR in livestock production systems an generate genomic resources for environmental AMR surveillance. By integrating environmental metagenomics with advanced resistome analyses, it will improve our understanding of AMR transmission at the human-animal-environment interface. As part of the AiRisk consortium, the project complements biophysical, epidemiological, and quantitative microbial risk assessment studies thereby strengthening interdisciplinary One Health approaches to mitigate the spread of AMR.

P29: Methanogenic Archaea in Human Alveolar Macrophage Models

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Background: Methanogenic archaea are well established in the human gut and have also been detected in respiratory samples, including bronchoalveolar lavage. However, their biological relevance in the lung remains unclear. Human alveolar macrophages (AMs) are specialized immune cells that maintain pulmonary homeostasis and respond to inhaled microorganisms. Although methanogenic archaea can modulate human

immune cells, it is unknown whether their effects depend on macrophage differentiation and lung-specific identity. We therefore investigated archaeal responses across macrophage models and primary human AMs.

Methods: Primary human AMs were exposed to *Methanobrevibacter smithii* or *Methanosphaera stadtmanae* and analysed by RNA-seq. In parallel, peripheral blood monocytes were differentiated into conventional monocyte-derived macrophages (MDMs) or alveolar macrophage-like (AML) cells. Cytokine responses to *M. stadtmanae*, *M. smithii*, and *Methanobrevibacter woesei* were assessed by ELISA. AML phenotype was evaluated by flow cytometry, with transcriptional validation ongoing.

Results: In primary AMs from one donor, *M. smithii* and *M. stadtmanae* induced distinct transcriptional responses, with upregulated genes linked to transcriptional regulation and chromatin-related functions rather than canonical inflammatory pathways. Across multiple donors, preliminary flow cytometry showed increased expression of selected alveolar-associated markers, including CD64, MARCO, and CD206, with donor-dependent variation. Preliminary ELISA analyses across five donors showed cytokine responses to methanogenic archaea in both MDM and AML models, with variation across donors and archaeal species.

Conclusions: These findings support further investigation of how macrophage differentiation and lung-specific identity shape responses to methanogenic archaea. The AML model is being further validated as a controlled system for studying archaeal interactions with lung macrophages.

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P30: Increasing the effectiveness of antimicrobial therapy and reducing resistance evolution in Mycobacterium tuberculosis through drug cycling

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Tuberculosis (TB) remains a global health crisis, particularly due to rifampicin- and isoniazid-resistant strains, which are classified as multidrug-resistant (MDR) TB. Current regimens such as BpaLM (bedaquiline, pretomanid, linezolid, and moxifloxacin) have markedly improved outcomes, yet their effectiveness is often limited by treatment-related toxicity. Adverse effects compromise patient adherence, lead to treatment interruptions, and ultimately reduce treatment success. Optimizing how existing drugs are administered, rather than developing new ones alone, offers a promising strategy to address this challenge. Drug cycling, i.e. the intermittent, time-limited administration of individual drugs within a combination regimen, could reduce cumulative toxicity without compromising antimicrobial efficacy. A successful cycling model is expected to lower the incidence of adverse effects, decrease treatment discontinuation, and enhance overall regimen performance. Ultimately, such optimized dosing strategies could improve the quality of care for patients with MDR-TB and contribute to global TB control efforts. This poster will examine existing research on the BPaLM regimen and discuss how its administration could be optimized to improve treatment outcomes in MDR-TB. Building on these findings, it will outline possible in vitro approaches to pilot such strategies.

P31: Soluble Antigen Arrays: Precision, Infection-Sparing Immunotherapy for Autoimmune Disease

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Autoimmune diseases like type 1 diabetes (T1D) and lupus are still managed with broadly immunosuppressive therapies that shut down the immune system indiscriminately, leaving patients vulnerable to infection. This review examines antigen-specific immunotherapy (ASIT) as a precision alternative and synthesizes recent evidence for soluble antigen arrays (SAgAs) as the platform now closing ASIT's long-standing efficacy gap. Unlike free peptides, which engage T cells weakly and carry anaphylaxis risk, SAgAs tether antigenic peptides multivalently to a hyaluronic acid backbone via hydrolysable or click-chemistry linkers. The result functions as an inverse vaccine, retraining only the disease-driving T cells toward tolerance rather than triggering an immune attack. Preclinical evidence in the non-obese diabetic mouse model shows that SAgA-delivered peptide combinations, but not the same peptides given free, arrest spontaneous T1D onset, sustain antigen-specific T-cell engagement, and upregulate tolerance markers including CD73, IL-10, and PD-1, while lowering anaphylaxis risk. Mechanistic follow-up work shows SAgAs steer antigen-specific T cells toward regulatory or anergic/exhausted states, in contrast to the effector phenotype driven by free peptide. Together, this literature positions SAgAs as a credible precision-immunotherapy platform capable of halting autoimmune disease while sparing the T-cell repertoire needed for infection defence. Extending this linker-tunable approach to human autoantigens relevant to T1D, lupus, and multiple sclerosis is the next translational step toward antigen-specific immunotherapy as a genuine alternative to blanket immunosuppression.

P32: Reprogramming of human macrophages in response to Mycobacterium tuberculosis complex (MTBC) infection

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Mycobacterium tuberculosis complex (MTBC) bacteria have evolved diverse strategies to persist within the human host, including potential adaptation to specific human populations. One such strategy involves epigenetic reprogramming of host cells, particularly cells of the innate immune system. DNA methylation, a key epigenetic mechanism, has been shown to be altered in individuals with TB.
We investigate the epigenetic response to MTBC infection using an early-stage human macrophage infection model. We also examine long-term epigenetic changes in TB patients from a Gambian cohort. Specifically, we explore how MTBC lineages L4 and L6 influence DNA methylation and gene expression patterns in host cells. These findings are then correlated with DNA methylation profiles of peripheral blood mononuclear cells (PBMCs) and clinical outcomes, including lung function.
By integrating in vitro and patient-derived data, we aim to identify lineage-specific host responses and epigenetic signatures associated with disease outcome. Our results can offer new insights into how MTBC modulates the host immune response through epigenetic mechanisms, advancing our understanding of TB pathophysiology and host-pathogen interactions.

P33: Cigarette Smoke and HRV-16 Infection Induce Lipocalin-2 Secretion from Airway Epithelium

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Background: Cigarette smoke (CS) is the main risk factor for COPD. Viral infections are more frequent and more severe in smokers. Our previous findings showed that lipocalin-2 (LCN2), a mediator of innate immune defense, was strongly upregulated in murine cell-free bronchoalveolar lavage fluid (BALF) upon short-term

smoke exposure. Here, we investigated whether airway epithelial cells secrete LCN2 in response to CS and viral infection.

Methods: Public scRNA-seq datasets from non-smokers, smokers, and COPD patients were analysed to assess LCN2 expression. Primary bronchial epithelial cells from healthy (NHBE) and COPD (CHBE) donors were cultured at air-liquid interface (ALI) and exposed to smoke (1R6F at 1 puff/min, ExpoCube) for 14 days, followed by infection with human rhinovirus (HRV-16) for 4 hours. Analyses were performed 24 hours post-infection.

Results: In silico LCN2 expression was significantly higher in ciliated and goblet cells of smokers and COPD patients than in non-smokers. Experimentally HRV-16 infection significantly induced LCN2 release in ALI cultures. Moreover, CS further exacerbated LCN2 levels from CHBE, compared with air-exposed controls. In contrast, no significant difference in LCN2 release was observed between CS-exposed and air-exposed NHBE cells after HRV-16 infection.

Conclusions: Together, our findings suggest that LCN2 is secreted by the airway epithelium in smokers and COPD patients. Furthermore, CS exacerbates LCN2 secretion in HRV-infected CHBE cells, potentially

P34: Comparative Analysis of Lipid Droplet Formation in Murine C3HeB/FeJ and C57BL/6 Macrophages During *Mycobacterium tuberculosis* Infection

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Mycobacterium tuberculosis (Mtb), the intracellular bacterium causing tuberculosis (TB), infects about one-fourth of the global population, with 5–10% progressing to active disease. Lung granulomas, a hallmark of TB, comprise epithelial, innate, and adaptive immune cells centred around infected macrophages. Macrophages serve as primary host cells for Mtb and often exhibit a foamy phenotype due to lipid droplet (LD) accumulation. LDs contain neutral lipids such as cholesterol esters and triacylglycerols. Although lipids are a key carbon source for Mtb, LD biogenesis, dynamics, and composition in primary macrophages remain poorly studied.
Animal models remain crucial for studying disease mechanisms. No model fully replicates human TB pathology, and mouse strains differ markedly in susceptibility and pulmonary responses. C57BL/6 mice are relatively resistant and develop pathology differing from that observed in humans, whereas C3HeB/FeJ mice are highly susceptible, showing increased bacterial numbers associated with necrotic granulomas, hypoxia, and cavity formation.
In this study, we compared primary macrophages from C3HeB/FeJ and C57BL/6 mice following Mtb infection. Early macrophage activation was closely linked to increased neutral lipid accumulation within one day, while prolonged infection at lower bacterial burdens also promoted LD accumulation. High-content imaging revealed that C3HeB/FeJ macrophages inherently contained more neutral lipids than C57BL/6 cells. Shotgun lipidomics further identified distinct global lipidomic profiles. Ongoing work focuses on phenotypic and metabolomic

characterization to define mechanisms underlying strain-specific differences in lipid metabolism and their potential impact on Mtb pathogenesis.

P35: Paternal preconception e-nicotine exposure alters offspring development and airway-associated phenotypes in *D. melanogaster*

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Background: Electronic cigarette use is increasing among adolescents, but its potential effects on their offspring remain unclear. Previous studies have focused mainly on maternal exposure during pregnancy, whereas the consequences of paternal exposure before conception are poorly understood. We therefore investigated whether paternal preconception e-nicotine exposure affects offspring development, airway morphology and physiological fitness in *Drosophila.*

Methods: Newly eclosed male Canton-S wild-type *Drosophila melanogaster* were exposed to e-nicotine for three days and then mated with 5-day-old unexposed virgin females. Survival was monitored in F0 males. F1 offspring were assessed for development, body size, locomotion, hypoxia-related phenotypes and survival. Larvae tracheal morphology and F1 airway transcriptomes were also analyzed.

Results: Paternal e-nicotine exposure reduced survival in F0 males and impaired airway development in F1 offspring, as indicated by reduced tracheal terminal cell length. F1 also exhibited reduced body length at the L1 stage, in male L3 larvae and in adults, together with sex- and stage-dependent alterations in larval crawling, adult locomotor activity and survival. Whole-trachea RNA-seq revealed sex-specific transcriptional changes in fatty acid metabolism and biosynthesis, while *FASN2* expression was increased in offspring of both sexes, suggesting *FASN2* as a potential contributor to the tracheal developmental defects.

Conclusion & Outlook: Paternal preconception e-nicotine exposure induces intergenerational alterations in offspring growth, behavior and airway-associated phenotypes in *Drosophila*. Airway transcriptomic analysis identifies altered expression of genes involved in lipid metabolism, particularly increased *FASN2* expression, providing potential molecular pathways to the observed tracheal phenotypes. Functional studies are required to determine the underlying mechanisms.

P36: Lineage-dependent Rifampicin tolerance in Mycobacterium tuberculosis complex strains

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Drug resistance in Mycobacterium tuberculosis complex (MTBC) threatens global TB (tuberculosis) control, with antibiotic tolerance— increased bacterial survival under antibiotic exposure—acting as a key precursor to resistance. Tolerant bacteria prolong treatment, contributing to the 6-month regimens that drive poor adherence and resistance emergence. Differences in resistance profiles between MTBC lineages 2 and 4 suggest potential variations in tolerance, highlighting the need for shorter, more effective therapies tailored to lineage-specific responses.

In this work, the tolerance capacity between clinical strains of L2 and L4 was compared. The strain panel was assessed by determining growth parameters and rifampicin (RIF) minimal inhibitory concentration (MIC), which confirmed homogeneous growth and RIF susceptibility. Tolerance was measured by time-kill assays following quantification by colony forming unit (CFU), a direct measure of bacterial viability. At 0.5 µg/mL RIF (15 × MIC), strains of L4 showed a significantly higher tolerance compared to strains of L2. To further elucidate the underlying cause for this difference the Transcriptomic profile of theses strains were investigated.

These results demonstrate a clear lineage-dependent difference in RIF tolerance. This study reveals a previously underappreciated potential for using tolerance profiling in precision medicine approaches to shorten TB treatment regimens. It highlights the critical role of antibiotic tolerance—not only as a precursor to resistance but also as a key factor in treatment failure across genetically diverse MTBC strains.

P37: Insights into mechanisms relevant to asthma: β-glucan-induced trained immunity in airway epithelial cells alters responses to house dust mite allergens

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Background: Asthma remains a complex disease, the mechanisms initiating its development are not fully understood. Evidence suggests that airway epithelium can acquire a persistent memory after microbial exposures. This may contribute to asthma progression through long-lasting alterations in barrier function and epigenetic regulation. β-glucan, a microbial cell wall component with immunomodulatory properties is widely used in trained immunity research and has been implicated in occupational asthma.

Objective: Investigating whether β-glucan shapes epithelial responses to later allergen exposure.

Method: Calu-3 airway epithelial cells were exposed to β-glucan for 24 h, rested for 5 days, and challenged with the recombinant house dust mite allergen rDer p 5. Transcriptional changes were assessed using RNA sequencing. The affected epigenetic changes upon β-glucan training were captured as a chronic effect (3 repeated β-glucan exposures), including both histone modifications and DNA methylation. Barrier integrity and cytokine release (IL-6 and IL-8) were also measured.

Results: Exposure to the allergen mainly induced significant transcriptional changes after the training. The training resulted in persistent transcriptional reprogramming. Trained cells exhibited an altered epigenetic profile (reduction of H3 modifications, increased H4 modifications), changes in epithelial barrier function and reduced IL-6 and IL-8 release following the allergen challenge.

Conclusion: Our results support the hypothesis that β-glucan acts as a priming agent, inducing epigenetic and functional changes in the airway epithelium. The modulation of allergen responsiveness is consistent with trained immunity and may support the hygiene hypothesis. However, our in vitro system does not directly test the clinical outcome nor prove.

P38: Paleogenomics reveals leprosy at the Late Antique burial site of Fallward in northern Germany

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Human mobility was a major factor which shaped cultural and societal transformations in Late Antiquity, driving the exchange of people, ideas and disease across Europe. Yet, despite this understanding, the genetic and pathogen landscape of many communities from this period remain uncharacterised. Here we present the first palaeogenomic investigation of inhabitants of the coastal settlement of Fallward (3rd–5th century AD) in present-day northwestern Germany. Genomic data from three individuals revealed heterogeneous ancestries: two aligned closely with northwestern European Iron Age populations, while one adult male exhibited affinities with Roman frontier groups from central-eastern Europe. Metagenomic screening further identified that this man was infected with *Mycobacterium leprae*, with the recovered genome belonging to an early lineage that may be associated with the Mediterranean region. Combined with the Roman-influenced grave goods accompanying his elaborate burial, these findings suggest he or his recent ancestors may have had close

connections to the populations living at the frontier of the Roman Empire. Overall, the results highlight how pathogens could spread across considerable distances in the Late Antiquity through complex mobility networks and cross-cultural interactions, reaching even small and remote communities such as Fallward.

P39: Keratinocyte-targeting functional autoantibodies in systemic sclerosis are associated with interstitial lung disease

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Introduction: Systemic sclerosis (SSc) is a chronic autoimmune connective tissue disease characterized by multiple organ involvement and heterogeneous clinical manifestations. Interstitial lung disease is a common complication and a major cause of SSc-related mortality. In addition to autoantibodies directed against nuclear antigens, SSc patients also produce autoantibodies targeting cell surface receptors, which may serve not only as biomarkers but also as mediators of disease pathogenesis. This study aimed to identify novel functional autoantibodies that contribute to the development of SSc.

Material and Methods: Serum samples from SSc patients and healthy controls were analyzed for IgG binding to the surface of normal human epidermal keratinocytes (NHEKs) using a cell-based immunofluorescence assay. Associations between anti-NHEK IgG levels, disease susceptibility, and clinical manifestations were evaluated using appropriate statistical analyses. The functional effects of these autoantibodies were assessed by cytokine array analysis.

Results: IgG autoantibodies against NHEKs were detected in human serum samples, with significantly higher levels in SSc patients compared with healthy controls. Notably, SSc patients with pulmonary fibrosis exhibited higher levels of these autoantibodies than those without, suggesting a potential link to fibrotic progression. Furthermore, stimulation of NHEKs with purified IgG isolated from anti-NHEK-positive SSc sera induced a marked increase in the release of pro-fibrotic cytokines, including PDGF-BB.

Conclusion: These findings support the presence of functional autoantibodies in SSc that target keratinocytes and promote the secretion of pro-fibrotic mediators. This mechanism may contribute to fibroblast activation, myofibroblast differentiation, and the development of fibrosis in SSc.