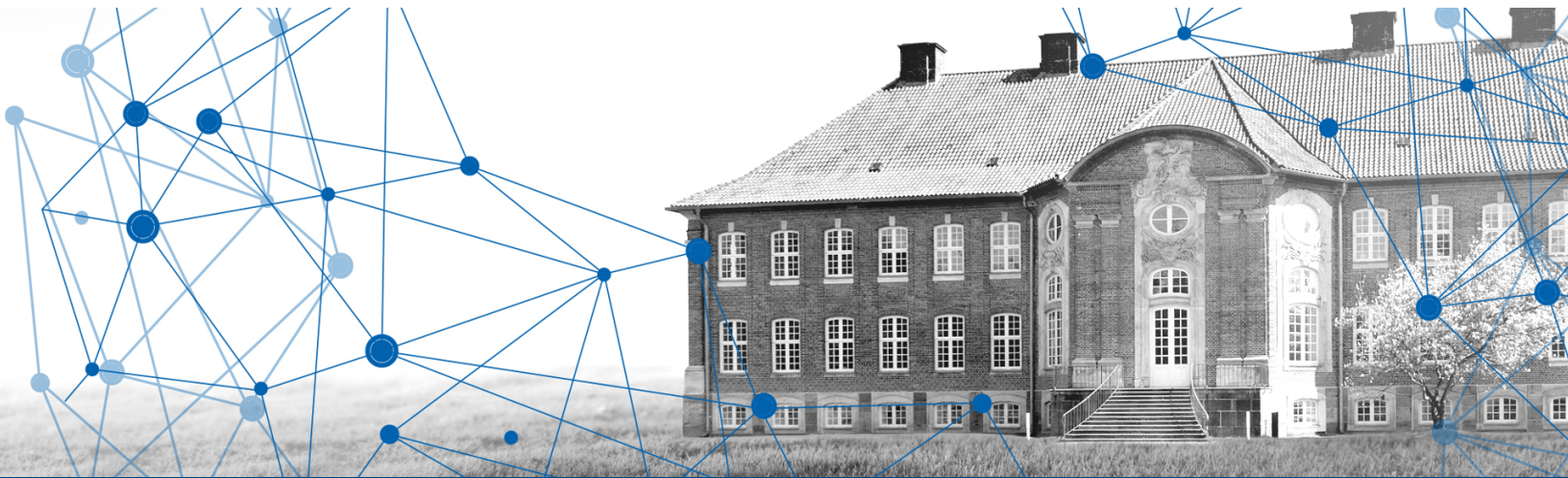


NDI₃



New Developments in Immunology,
Inflammation, and Infection



Research Center Borstel
Leibniz Lung Center

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Welcome note

The NDI₃ organizational team would like to gladly welcome all participants to the 43rd annual conference of New Developments in Immunology, Inflammation, and Infection. But this year's NDI₃ is not only a 43rd but also a 1st. Namely the 1st time the conference is hosted as an online event. This year the NDI₃ will focus on the topics of epidemiology, host-pathogen interaction, allergy & autoimmunity. We have an outstanding line-up of renowned, international keynote speakers and talks given by PhD students and postdoctoral research fellows from all across Germany. And despite the fact that we will not be able to see each other in person, we are still sure that we are going to have two days full of great discussions and scientific exchange.

<https://ndi3.my.webex.com/meet/NDI3-2021>

To ensure and provide the best experience for all presenters and participants, you can find some technical information regarding the sessions below.

Oral Sessions:

All participants start muted and without video. Speakers will be unmuted and allowed to have video by the host before the session starts. Once the Q&A starts, questions can be announced in the chat by typing "Q" and are moderated by the chairperson in order of precedence. Participants will be unmuted by the host and allowed to ask their questions via microphone. If no microphone is available, questions may also be typed in the chat along with the "Q". Typed questions will then be read by the chairing person. Once the Q&A is finished, the host will again mute the participants except for the presenter, and the chairperson will announce the next presentation.

Poster Session:

The poster session will be held in breakout rooms in parallel, one for each poster. The poster presenter will share their poster or slides via the file or screen share function. If the poster is shared via file share, you as the participant can freely navigate (zoom, scroll, etc.) by yourself. In case of screen sharing the presenter will navigate. All participants are encouraged to ask questions directly via microphone and presenters are asked to moderate the discussion as necessary.

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Program

Infection

Thursday, 11. November 2021

10:00 am: Welcome

10:15 am: 1st Keynote speaker

Prof. Dr. Luke O'Neill, Ireland

11:15 am: Break

11:20 am: Talk Session 1

O1. Cross-species host transcriptome signatures in *M. tuberculosis* infection

O2. *Mycobacterium tuberculosis* complex bacteria (MTBC) adaptation to antibiotics: Evolution informed tuberculosis

O3. Characterization of MEP pathway inhibitors in *Mycobacterium tuberculosis* as antituberculous

12:20 am: Lunch Break

1:20 pm: 2nd Keynote speaker

Prof. Dr. Karsten Hiller, Germany

2:20 pm: Break

2:25pm: Talk Session 2

O4. Interrogation of immunometabolism in sex differences in immune response in TB vaccine mediated immunity

O5. Viral triggers of tuberculosis disease: a case for cytomegalovirus

O6. The antagonizing effects of the C-type lectin receptor MINCLE during a *Strongyloides ratti* infection

3:25 pm Closing remarks 1st day & Information for Friday

Asthma & Allergy

Friday, 12. November 2021

10:00 am: Welcome

10:15 am: 3rd Keynote speaker

Prof. Dr. John Holloway, United Kingdom

11:15 am: Break

11:20 am: Talk Session 3

O7. Functional studies to identify pathways underlying allergy-protective effects of cowshed microbiota

O8. C5a signalling axis may be a potential pharmacological target to regulate ILC2 development in allergic asthma

O9. Viral respiratory infection strengthens tumour-specific CD8+ T cell immunity and suppresses tumour growth

12:20 pm: Break

12:35 pm: Poster Session

1:35 pm: Prizes & Closing remarks

Keynote speakers

Prof. Luke O'Neill (Trinity Biomedical Sciences Institute, Trinity College, Dublin)

Luke O'Neill is Professor of Biochemistry in the School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute at Trinity College Dublin, Ireland. He is a world expert on innate immunity and inflammation. His main research interests include Toll-like receptors, Inflammasomes and Immunometabolism. He is listed by Thompson Reuters/Clarivate in the top 1% of immunologists in the world, based on citations per paper. Professor O'Neill is co-founder of Sitryx, which aims to develop new medicines for inflammatory diseases. Another company he co-founded, Inflazome was recently acquired by Roche.



He was awarded the Royal Dublin Society / Irish Times Boyle Medal for scientific excellence, the Royal Irish Academy Gold Medal for Life Sciences, The Society for Leukocyte Biology (SLB) Dolph O. Adams award, the European Federation of Immunology Societies Medal and in 2018 the Milstein Award of the International Cytokine and Interferon Society. He is a member of the Royal Irish Academy, EMBO (European Molecular Biology Organisation) and a Fellow of the Royal Society.

Luke also has a passion for communicating science to the public. He has a weekly radio slot on the Pat Kenny show on Newstalk. In 2018 he published with Gill the best-selling 'Humanology: a scientist's guide to our amazing existence' and in 2019 Gill published 'The Great Irish Science Book', a Science book for 10-12 year olds. His latest book, also published by Gill is called 'Never Mind the B#ll*cks Here's the Science'.

Prof. Karsten Hiller (Department of Biochemistry and Bioinformatics, Braunschweig Integrated Centre for Systems Biology, Braunschweig)

Professor Dr Karsten Hiller is the director of Biochemistry and Bioinformatics and head of the Immunometabolism Team at the University of Braunschweig. He obtained his diploma in Biology and Computer Science and performed his PhD in Bioinformatics and Microbiology at the University of Braunschweig, Germany and focused on the development of algorithms for the analysis of metabolomics data.

His team discovered that mammalian macrophages reprogram their metabolism to synthesize the antimicrobial and immunomodulatory metabolite itaconate. Moreover, Prof Hiller and his team investigate macrophage physiology during an infection with bacterial pathogens. They developed a strong expertise in stable-isotope assisted metabolomics and metabolic flux analysis both on a whole cell as well as on a mitochondrial sub-compartment level. The team takes advantage of its in-house bioinformatics background to develop tailored tools for the analysis of GC-MS based data obtained from stable-isotope labeling experiments.



In his talk, Prof. Hiller will talk about the metabolic crosstalk of *Clostridioides difficile* with mammalian epithelial cells in the intestine. *C. difficile* produces H_2S , which directly interferes with the electron transport chain complex, in particular the transmembrane protein cytochrome c oxidase. This in turn throws the entire cell metabolism into disarray.

Prof. Dr. John Holloway (University of Southampton)

Professor John Holloway is Associate Vice-President (Interdisciplinary Research) at the University of Southampton, UK and Professor of Allergy and Respiratory Genetics in the Faculty of Medicine. His research program focuses on genetics, epigenetics, and functional genomics of allergic and respiratory diseases such as asthma and COPD, with a particular focus on the mechanisms of transgenerational and prenatal programming of respiratory disease, epigenetic mechanisms underlying atopy and asthma susceptibility and gene-environment interactions in the early life origins of asthma and COPD.



He was appointed to a personal chair in the Faculty of Medicine in 2011. As well as his on-going research, he contributes to Molecular Cell Biology teaching as part of the Bachelor of Medicine program. He has held appointments on the Scientific Advisory boards of Asthma UK, and was member of Council for the British Society of Allergy and Clinical Immunology (2009-2012) and vice-chair of the COST:BM1201 Developmental Origins of Chronic Respiratory Disease network (2012-2016).

John is highly research active with extensive national and international collaborations with both academic and commercial research partners. He regularly is invited to present international meetings and has published over 150 papers field of allergy and respiratory genomics.

Abstracts of oral presentations

O1. Cross-species host transcriptome signatures in *M. tuberculosis* infection

Hawanot Olaitan Tijani

Cellular Microbiology, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

htijani@fz-borstel.de

In *Mycobacterium tuberculosis* (Mtb) infection, the genetic basis of controlled vs. exacerbated pathogenesis remains poorly characterized. We study host-pathogen interactions in a comparative manner by analysing host response signatures in lung specimen from tuberculosis (TB) patients and genetically defined murine models of experimental TB. Besides, characterization of immune histopathology, cytokine analysis and global gene expression profiling, we employed systems biology approaches, such as network analyses, to predict beneficial treatments. In our study, we aim to identify molecular signatures, especially focus on candidate genes, which suggest association with disease susceptibility, characteristics, and degrees of pathology, such as fibro-cavernous and non-fibro-cavernous tuberculoma. Moreover, we will validate our findings in line with published data available on databases for cross species lung transcriptome signatures in clinical and experimental TB. The results will provide not only novel insights into molecular mechanisms underlying TB pathogenesis but are also an important step towards improved diagnostics and prognostics of e.g. the degree of lung damage beyond successful therapy. Moreover, the study paves the way for the development of novel host-directed therapies adjunct to antibiotic treatment to extend the current therapeutic options especially for drug resistant TB, currently one of the top global health challenges associated with antibiotic resistance. Taken together, our study is the key for a better understanding of molecular mechanisms underlying TB etiopathology, which is crucial to improve the treatment of TB patients.

O2. *Mycobacterium tuberculosis* complex bacteria (MTBC) adaptation to antibiotics: Evolution informed tuberculosis

Teresa Walz

Molecular and Experimental Mycobacteriology, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

twalz@fz-borstel.de

Despite the objective of global health authorities to end tuberculosis (TB) within the next decades, *Mycobacterium tuberculosis* (Mtb) besides SARS-CoV-2, is still the leading cause of death by a single pathogen, globally. Moreover, 4 % of new cases reveal a multidrug resistant (MDR) strain. MDR-TB is defined as resistant to the two most effective first-line antibiotics, isoniazid (INH) and rifampicin (RIF). The rate of antimicrobial drug development is cumbersome. In order to circumvent the escalating resistance crisis, resistance evolution should be anticipated as an target for alternative treatments strategies. In this project, we investigated the effect of short-term drug switching in treating *Mycobacterium tuberculosis* strains, which has already been shown to be effective in *P. aeruginosa* and other pathogens. The presumed underlying concept is “cellular hysteresis” in which rapid drug switching enhances the susceptibility to the second antibiotic and circumvents resistance evolution. In our in vitro study we examine fast drug switching, using ethambutol (EMB) and rifampicin (RIF), in sub-inhibitory drug concentrations. We observed during two weeks’ time-kill assay, a trend towards negative hysteresis, enhancing RIF main-treatment. This effect appears to be strong drug order dependent for EMB on RIF. We hope this study provides insights to enhance new and innovative treatment strategies, to combat the rise of drug resistant tuberculosis, especially in complicated and highly-resistant cases.

O3. Characterization of MEP pathway inhibitors in *Mycobacterium tuberculosis* as antituberculotics

Vidhisha Sonawane

Microbial Interface Biology, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

vsonawane@fz-borstel.de

Tuberculosis (TB) is a communicable disease that is a major cause of ill health, one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent (ranking above HIV/AIDS). To tackle this epidemic, it is of utmost necessity to shorten the TB therapy and to find new drug targets. One of the promising drug targets identified is the 2C-Methyl-D-erythritol 4-phosphate (MEP) pathway. The MEP pathway is the sole producer of isoprenoid synthesis in *Mycobacterium tuberculosis* (MTB) which is involved in essential processes such as cell wall formation, protein transportation, etc. The main objective of this project is to evaluate the antimycobacterial activity of newly synthesized inhibitors of enzymes of the MEP pathway using *M. tuberculosis* in different systems. A batch of eighteen compounds were initially screened against fluorescent m-Cherry expressing MTB in concentrations ranging from 64µM to 1µM. Six drugs showing at least 60% inhibition were selected for further detection for cytotoxicity and activity inside macrophages. To validate the gene target of the selected compounds, conditional knock-down mutants of the target genes are generated using cloning. A comparison of the antibacterial activity of these selected compounds against conditional knockdown mutants and the wild-type strain will validate the target of the compounds. Testing the selected compounds in various systems is still in process. Furthermore, apart from the 18 compounds, testing of more compounds in the above systems will soon begin.

O4. Interrogation of immunometabolism in sex differences in immune response in TB vaccine mediated immunity

Gishnu Harikumar Parvathy

Coinfection, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

ghparvathy@fz-borstel.de

Tuberculosis (TB) is a disease that has evolved with humankind for millennia, and it remains with us even today, causing approximately 1.4 million deaths worldwide per annum. Although increased male affliction for TB and other infections were known from an epidemiological perspective, our mechanistic understanding of the underlying immunological divergences is relatively recent. As such, there is very little understanding regarding the sexually dimorphic nature of immune response to vaccines, particularly the TB vaccine. In the light of recent research pointing to the contributory role of sex hormones in divergent immune responses between females and males, it is conceivable that part of such effects of sex hormones is mediated by alterations in the immunometabolic landscape. Although yet to be fully appreciated for their role in immunity, sex hormones influence the metabolic landscape of the biological organism profoundly, as has been known for decades. In this context, my project aims to understand the potential differences in immunometabolism, influenced by the sex hormones, that contribute to differences in immunity between biological females and males in particular its influence in TB vaccination and protection against disease.

O5. Viral triggers of tuberculosis disease: a case for cytomegalovirus?

Jaqueline Marschner

Coinfection, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

jmarschner@fz-borstel.de

Tuberculosis (TB) is the most prevalent bacterial infection in humans. With 10.4 million new cases and 1.7 million deaths per year TB remains a major global health problem. Several risk factors for developing active TB have been identified, with HIV co-infection being the most important one in high-burden settings. Viral infections other than HIV have been suggested to play a role in the aetiology of TB. Recent epidemiological evidence supports an association between cytomegalovirus (CMV) and TB disease progression. HCMV is a ubiquitous herpesvirus which is able to establish lifelong persistence in the host after primary infection. It can induce profound alterations in immune cell populations crucially involved in the control of *Mycobacterium tuberculosis* (Mtb) infection. As recent epidemiological studies have suggested a link between HCMV seropositivity and active TB disease, my project addresses whether a CMV infection will drive TB disease progression. To test this hypothesis, we will establish a coinfection mouse model using C57BL/6j mice, and analyze the outcome of Mtb infection and Mtb-specific immune responses in the context of an acute or latent murine CMV (MCMV) coinfection. While acute MCMV infection may trigger inflammatory responses that could exacerbate Mtb infection in the lung, latent infection may interfere with protective CD4+ and CD8+ Mtb-specific T cell responses which are indispensable for long-term control of Mtb during chronic infection.

O6. The antagonizing effects of the C-type lectin receptor MINCLE during a *Strongyloides ratti* infection

Timm Ramcke

Helminth Immunology, Molecular Biology and Immunology, Bernhard Nocht Institute for Tropical Medicine, Bernhard-Nocht-Straße 74, 20359 Hamburg, Germany

timm.ramcke@bnitm.de

Helminths are multicellular parasites that infect more than a quarter of the human population. The mammalian hosts control infections with parasitic helminths with a Th2 immune response. In response, helminths have evolved the ability to downregulate the host's immune system. *Strongyloides ratti* is a rodent-specific parasitic nematode that displays tissue-migrating and intestinal life stages. Our group uses *S. ratti* infection in mice to study the protective immune response against helminth parasites as well as helminth-induced immune evasion. Here we aim to investigate the role of the C-type lectin receptor (CLR) MINCLE in shaping the *S. ratti*-specific immune response. CLRs are a conserved group of pattern recognition receptors that sense pathogen and damage associated molecular patterns. MINCLE ligands show either agonistic traits, improving the immune response, or antagonistic traits, dampening the immune response dependent on the source of the MINCLE ligand. In a pilot study MINCLE ligand(s) were identified in the lysate of *S. ratti* L3 larvae. Additionally, MINCLE-deficient mice showed reduced parasite numbers in the small intestine and an increased production of Th2 cytokines, compared to their wildtype littermates. To analyse the effects of the putative *S. ratti* derived MINCLE ligand(s) on the host immune system, I compare bone marrow derived macrophages (bmMO) and dendritic cells (bmDC) of wildtype and MINCLE-deficient mice in vitro. Stimulation with *S. ratti* lysate did not result in a MINCLE-dependent IL-6 or TNF- α cytokine production. Stimulation with the agonistic MINCLE ligand TDB triggered production of IL-6 and TNF- α in wildtype bmMO and bmDC. Interestingly, addition of *S. ratti* lysate to TDB-stimulated bmDC and bmMO, decreased the TDB-induced TNF- α dose dependent. This points towards an antagonistic function of the putative MINCLE ligand(s) present in the *S. ratti* lysate, helping the parasite to escape the host's immune response.

O7. Functional studies to identify pathways underlying allergy-protective effects of cowshed microbiota

Johann Sieverding

Innate Immunity, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

jsieverding@fz-borstel.de

Background: Early childhood exposure to a diverse microbial environment is inversely related to the development of asthma and allergies, strongly implying a causal role for microbiota typically associated with farm-like environments. However, little is known about the mechanistic interactions between microbiome and human host underpinning healthy development. Objective: The objective of this study is to identify and differentiate mechanisms by which archaeal (*M. stadtmanae*, *M. smithii*) and bacterial (*L. lactis*) microbiota are respectively recognized by innate immune cells and modulate cellular signaling pathways, conferring protection from development of asthma and allergies.

Methods/Concept: Peripheral blood mononuclear cells (PBMCs) from healthy donors, and a co-culture of primary CD4 T-cells with autologous monocyte-derived dendritic cells (moDCs) are profiled by cytokine ELISA. To further elucidate the downstream mechanisms, stable and specific knockouts of genes coding for signaling molecules downstream of the main receptors involved are generated by using CRISPR/Cas9 on the monocyte-like cell line BLaER1. Supporting analysis is done via qPCR, Western Blot, and flow cytometry.

Results: Stimulation of dendritic cells with *M. stadtmanae* drives Th1 polarisation in naïve T-cells, and elicits a strong Th1 and IL-10 effector response from PBMCs, as shown by cytokine profiling. Initial studies of the generated KO cells indicate a shared signaling pathway via TLR8, IRAK4, IRF5 for bacterial and archaeal RNA.

O8. C5a signalling axis may be a potential pharmacological target to regulate ILC2 development in allergic asthma

Rabia Ülkü Korkmaz

Allergic Asthma, Institute of Systemic Inflammation, Ratzeburger Allee 160, 23562 Lübeck, Germany

rabialkue.korkmaz@uksh.de

Worldwide more than 300 million people are affected by asthma, an inflammatory disorder characterized by a dysregulated immune response to allergen. Innate Lymphoid cells type 2 (ILC2) are thought to play a key role during allergen sensitization. Further, the soluble factors anaphylatoxins C3a and C5a have been recognized as potent regulators of both development and severity of the disease via their binding to their cognate receptors C3aR, C5aR1, and C5aR2. Interestingly, while C3a/C3aR and dendritic cells have been recognized as important regulators of ILC2 functions in patients with allergic rhinitis, the role of C5a in an ILC2-myeloid cell crosstalk remains poorly understood. Here, using bone marrow (BM)-derived or pulmonary primary cell coculture models, we investigated the impact of the C5a-induced signaling on myeloid cells for the development and functions of ILC2. Using BM-derived cells, we observed a significant development of ILC2 in the presence of both BM-derived macrophage/dendritic cells (BM-MDCs) and C5a, via the phosphorylation of STAT3, 4 and 6, and the expression of gata3 and Killer cell lectin-like receptor subfamily G member-1 (KLRG-1). The employment of sorted pulmonary MHC II+ cells reinforced these observations, except for the phosphorylation of STAT4. Mechanistically, C5a triggered the expression of gata3, KLRG-1, and Interleukin IL-5 in the ILC2. Finally, to delineate the nature of the MHCII+ cells involved in this C5a-driven regulation, we investigated sorted cells. While the pulmonary DCs improved the ILC2 development in a C5a/C5aR1 dependent manner, the alveolar macrophages that improved ILC2 function relied on C5a/C5aR2 signaling. Furthermore, while AMs derived IL1 α regulate the ILC2 via C5a, DCs required cell to cell contact. Overall, our data show that C5a contributes to the development of the ILC2 via its two cognate receptors. This suggests that the C5a signalling axis may be a potential pharmacological target to regulate ILC2 development in allergic asthma.

O9. Viral respiratory infection strengthens tumour-specific CD8+ T cell immunity and suppresses tumour growth

Philine Steinbach

Institute of Medical Microbiology, Essen University Hospital, Hufelandstraße 55, 45147 Essen, Germany

philine.steinbach@uk-essen.de

Influenza virus infection is a worldwide threat that despite intensive research still leads to a high prevalence of hospitalizations and lethal outcomes. As parallelly, the number of cancer patients steadily increases, it is important to study the interactions between infectious diseases and the anti-cancer immunity. Here we show that respiratory influenza virus infection strengthens the tumour-specific CD8+ T cell response. Surprisingly, growth of subcutaneously transplanted B16 or CT26 tumours was significantly decreased in mice with influenza infection compared to uninfected mice, suggesting that the infection modulates the anti-tumour response. Indeed, the depletion of CD8+ T cells but not macrophages or NK cells reversed this effect. This supports the importance of CD8+ T cells in this model. Analysis of immune cells in the tumour indicated that CD8+ T cells show an enhanced activation and proliferation upon additional infection. In line with these findings, an adoptive cell transfer of congenically marked CD8+ T cells from tumour bearing mice into coinfecting recipient tumour bearing mice demonstrated both, the migration of tumour derived CD8+ T cells into the tumour as well as into the infected lung where the CD8+ T cells are potentially modulated by the inflammatory environment. Thus, our results demonstrate an important crosstalk between respiratory viral infection and CD8+ T cell cancer immunity and will advance our understanding of the underlying mechanisms how infections modulate the anti-cancer defence.

Abstracts of posters presentations

P1. Pathogenesis of Yellow Fever Virus in Human Immune System Mice

Anneke Pfister

Molecular Biology and Immunology, Bernhard Nocht Institute for Tropical Medicine,
Bernhard-Nocht-Straße 74, 20359 Hamburg, Germany

Anneke.Pfister@t-online.de

Yellow Fever Virus (YFV) is a mosquito-transmitted RNA virus that causes Yellow Fever Disease (YFD) in humans and non-human primates. YFV usually causes a mild infection; however, in 15% of the cases, it can develop into severe disease (toxic phase) that is characterized by recurring fever, liver failure and hemorrhagic disease. Yellow Fever severe disease can show up to 50% lethality. Even though there is a very efficient live-attenuated vaccine against YFV, no effective therapeutic drugs are available to date. This is in part due to the lack of knowledge about the pathogenesis of YFD in the toxic phase and the lack of suitable in vivo models for YFV infection. Mice transplanted with human immune cells, also called humanized mice, have become an important tool for investigating infectious diseases (Shultz et al., 2017. Annu Rev Pathol.). In the past years, our lab has established human immune system mice to study the pathogenesis of human Adenovirus and Ebola virus infection (Rodriguez et al., 2017. JID; Lüdtke et al., 2015. JVI). By using a NOD *Scid* IL2Rg $^{-/-}$ K α Db $^{-/-}$ mouse strain that expresses human HLA-A2 (NSG-A2-KDnull mice) we were able to reconstitute a human immune system, including HLA-A2-restricted mature T cells. Here, we present preliminary data about the establishment of a YFV in vivo model of pathogenesis in this mouse strain.

P2. Regulatory functions of oxidative/antioxidative processes during host-pathogen interactions in tuberculosis

Benthe Beu

Cellular Microbiology, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

bbeu@fz-borstel.de

Infection with *Mycobacterium tuberculosis* results in neutrophils undergoing necrosis instead of apoptosis. This leads to uncontrolled proliferation of the pathogen after it has been taken up by macrophages and to increased tissue damage in the host. Signaling pathways underlying the increased necrosis rate in neutrophils remain unclear. Preliminary data generated by using the so-called OxRAC method indicates increased or decreased oxidation of neutrophil and macrophage proteins after infection with *M. tuberculosis*. The oxidative burst in neutrophils and macrophages leads to oxidation of various cell molecules. The function of oxidated enzymes may be altered and the oxidative status of signaling molecules or antigens may influence downstream pathways. Therefore, goal of this work is to uncover essential thiol modifications in the proteome of infected neutrophils and macrophages to identify a set of suitable candidate proteins for later experiments. The influence of these proteins and their oxidative status will be investigated later in the project using various knock-out approaches both in vivo and in vitro. The resulting knowledge could give us a better understanding of how *M. tuberculosis* modifies its host's immune system and where targets for host-directed therapies might be found.

P3. The Experimental Probiotic “ACLS” in Mouse Models of Antibiotic-Induced Dysbiosis and Respiratory Infection

Celina G. Prosch

Cellular Microbiology, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

cprosch@fz-borstel.de

Gastrointestinal and respiratory tract infections (RTIs) can be associated with dysbiotic microbiomes. However, it remains unclear whether an altered microbiome influences the immune response or vice versa. Our preliminary data revealed that antibiotic-treated mice (Vancomycin, Metronidazole, Neomycin, Ampicillin (VMNA) recovered more quickly from gut dysbiosis when treated with murine lung bacterial isolates (*Actinobacillus*, *Corynebacterium*, *Lactobacillus*, *Staphylococcus* (ACLS)). Published data shows that mice infected with *Klebsiella* (*K.*) *pneumoniae* develop a stronger disease course when they had been pre-treated with VMNA (Brown, Sequeira and Clarke, 2017). Here we outline our strategy to determine whether immunological mechanisms contribute to the microbiota resilience, and whether this ameliorates immunological control over *K. pneumoniae* infection. Therefore, we will investigate whether resilience depends on administration of live bacteria, or if inactivated ones have a similar effect. Further, we will analyze the *K. pneumoniae* disease course in mice that are rendered dysbiotic with VMNA and where recovery is initiated with ACLS. A better understanding of the reciprocal interaction between microbiota and the immune system of the intestine and lungs could lead to improved probiotic therapies, which in turn offer new treatment options for RTI.

P4. Proteomic comparison of highly and moderate pathogenic African swine fever virus isolates

Elisabeth Wöhnke

Laboratory for Biochemistry and Protein Analysis, Institute of Molecular Virology and Cell Biology, Friedrich-Loeffler-Institut, Südufer 10, 17493 Greifswald, Germany

elisabeth.woehnke@fli.de

Against the highly pathogenic viral disease of swine African swine fever (ASF) no vaccine or treatment is available. Genomic variations alter the virulence of ASFV, but the molecular backgrounds are mainly unknown. We performed proteome analysis of primary macrophages infected with two closely related ASFV isolates of different pathogenicity.

P5. Susceptible windows of microbial colonization for shaping innate immunity and asthma risk

Draginja Kovacevic

DZL Laboratory for Experimental Microbiome Research, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

dkovacevic@fz-borstel.de

Background: The host microbiome has important functions for the maintenance of human health. Its composition changes dynamically from birth to childhood, until a more diverse and stable microbiome is developed. Microbe-immunity crosstalk during this period is considered as a critical window of opportunity for shaping the postnatal development of the immune system. Further, infants with *Haemophilus influenzae* (Hi) colonisation have an increased risk of developing asthma later in life. Yet, whether there is in fact a cause-effect relationship between both events is currently unclear.

Methods: To address this question, we used gnotobiotic mice that are colonised with a defined oligo-mouse-microbiota (OMM-12) at birth vs. adulthood. Germ-free mice were used as a control. In the first step, as a baseline comparison of the groups, innate lymphoid cells (ILCs) in the lungs and bone marrow were characterized via flow cytometry analysis.

Results: Preliminary results showed that the germ-free control group had lower levels of CD45+CD90.2+Lin- ILC-like cells in the lungs compared to the OMM-12 colonized groups. The majority of detected ILC-like cells were CD25+ST2+ which defines them as ILC2 cell population. Also, ILC2 cells in the lungs differed based on colonization status. Among this cell population, germ-free mice showed lower expression of CD38 marker and slightly higher expression of CD73. Additionally, c-kit+ ILC2 cells were less abundant in the lungs of mice colonized by birth. In the bone marrow, ILC-like and ILC2 cells showed different results to the populations detected in lungs, with lower levels in the adulthood-colonized group.

Conclusion: The first pilot data indicated differences in ILC-like and ILC2 populations based on their colonization status. In the next step, immune screening between the groups will be made after intranasal inoculation with Hi, and asthma phenotype will be tested upon IL-33 application.

P6. Repetitive rhinovirus infection primes for amplified IL-13 induced airway epithelial responses

Huan Ma

DZL Laboratory for Experimental Microbiome Research, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

hma@fz-borstel.de

Background: Infection with human rhinoviruses (HRVs) is common and has been identified as risk factor for asthma development and exacerbation. We hypothesized that recurrent HRV infection increases susceptibility to asthma by inducing an aberrantly amplified innate immune response in airway epithelial cells.

Method: Primary normal human bronchial epithelial (NHBE) cells were cultured at the air-liquid interface and infected with HRV-16 on days 14, 17, 20 post air-lift, IL-13 was added from day 23 to day 26.

Results: The intracellular HRV-16 viral load peaked 24 hours after virus inoculation then gradually decreased until the next infection. Repeated HRV-16 infection followed by IL-13 lead to an increase in the expression of MDA-5, IL-8, CXCL-10 and IL-4 as compared to single treatment with virus or IL-13 alone.

Conclusion: The results indicate that recurring infection of airway epithelial cells with HRV-16 infection could pave the way to exacerbate IL-13 induced airway inflammation, but it warrants further functional studies.

P7. The Stem Cell Niche of *Mycobacterium tuberculosis*

Pit Engling

Cellular Microbiology, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

pengling@fz-borstel.de

The inability of our immune system to ultimately clear a *Mycobacterium tuberculosis* infection is one of the main reasons why 1/4 of the world population is considered latently infected and thus, at risk to develop active tuberculosis. One putative key-element responsible was initially reported by *Tornack et al.* in 2017. They observed that *M. tuberculosis* (Mtb) not only infects mature innate immune cells of myeloid origin but also the ultimate immune cell source: hematopoietic stem cells (HSC). However, cellular and molecular interactions between Mtb and HSCs and their implications for downstream immunity and pathogenesis remain obscure.

To analyse Mtb-HSC interactions in vitro, protocols to achieve sufficient numbers of both, murine and human HSC infected with Mtb have been successfully established. Intracellular localization of Mtb in both murine and human HSC in vitro was confirmed by confocal microscopy. To characterize the impact of Mtb on the functionality and the downstream fate of HSC and their successors, we further designed a protocol to isolate a homogenous population of live and infected HSC. With these methods established, we are now finally able to perform functional assays on pure infected HSC. In addition, we characterised mechanisms turning otherwise uninfected cells to those susceptible to Mtb. We could show that HSC get infected in an active and actin-dependent process that seems to be triggered during the interaction with Mtb. To differentiate whether acquisition of susceptibility to Mtb is a mere reaction to mycobacterial PAMPs or requires metabolically active bacteria, HSC cultures pre-treated with or without Mtb-lysates were analysed. Pre-treatment of HSC with Mtb lysate did only increase infection rates of very few cells. These results indicate that to become permissive for Mtb HSC require triggering signals generated by metabolically active mycobacteria such as microbial metabolites. Connecting the established methods for HSC purification and tracking with the available in vitro systems each comprising different unique traits will allow us to more detailly understand the interactions between HSCs and Mtb.

P8. The impact of MBL on the outcome of infection with representative mycobacterial strains

Mario Alejandro Duque Villegas

Infection Immunology, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

aduquevillegas@fz-borstel.de

Tuberculosis (TB) is the most prevalent bacterial infectious disease in humans and is caused by bacteria of the *Mycobacterium tuberculosis* complex (MTBC), which are composed of three different human-adapted pathogens *Mycobacterium tuberculosis* (Mtb), *Mycobacterium africanum*, and *Mycobacterium canettii*. Mannose-binding lectin (MBL) recognizes specific cell surface carbohydrates such as mannosylated lipoarabinomannan, one of the main cell wall components of slow-growing mycobacteria. MBL can act as an opsonin or activate the lectin pathway of the complement cascade and thereby modulate the innate and adaptive immune response. Host protection against Mtb infection critically depends on the development of a robust T cell response. Human epidemiological studies investigating an association between genetic variants of mannose-binding lectin (MBL) and susceptibility to TB have yielded inconsistent results. We could previously demonstrate that strains of the ancient lineage *Mycobacterium africanum* bind MBL to a higher extend than modern stains and that a specific MBL2 variant confers protection against TB caused by *Mycobacterium africanum*. The current project aims to comprehensively investigate the MBL-mediated modulation of host-pathogen interaction during infection with selected lineages of the MTBC. To this end, different lineages were chosen by comparative sequence analysis of genes involved in the synthesis of various carbohydrates. Subsequently, the binding of MBL to different MTBC isolates are compared by flow cytometry. Since macrophages take up mycobacteria after infection, which is a critical step for establishing infection, in the next step, macrophages are infected with preselected MTBC isolates in the presence or absence MBL and the uptake, intracellular replication and production of proinflammatory cytokines are compared. Furthermore, by exploiting MBL-null and Wild-type mice, host-pathogen interactions which are influenced by MBL will be elucidated in detail.

P9. Investigation of the role of IL-6 in a mouse model of systemic sclerosis

Liang Zhang

Pulmonary Immune Diseases, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

lzhang@fz-borstel.de

Systemic sclerosis (SSc) is one kind of fibrosis rheumatic multi-systemic disease, not only is the fibrosis on skin always considered as a common clinical manifestation, but the disease also involves the lesions of internal organs such as lung. In general, the incidence of SSc is female-biased (sex ratio from 3/1 to 8/1), the prevalence varies ranges from 30 to 240 per millions. Recently, SSc is caused by the dysregulation on immune system is widely accepted, but the pathogenesis of SSc is fully complex and only partially understood. Considering the adverse effects of SSc on human, the investigation of its pathogenesis is critical, which can contribute to the clinical treatment of SSc. In order to make a further research on SSc, we established a mouse model called humanized mouse model which can induce SSc disease in mice. Accumulating evidences indicated that SSc is relevant with cytokines, which may be regarded as a new target for SSc treatment. IL-6, a special cytokine, which has both pro- and anti-inflammatory properties. In past two decades, IL-6 had been proved to be related to autoimmune diseases, which means IL-6 maybe also can influence the pathogenesis of SSc. So, in my project, I would try to investigate the role of IL-6 in SSc disease via the humanized mouse model.

P10. The role of small GTPases in neutrophil transmigration, adhesion, and proteolytic tissue damage in COPD

Adeela Sana

Pulmonary Immune Diseases, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

adeelasana86@gmail.com

Chronic obstructive pulmonary disease (COPD) is characterized by chronic inflammation, obstruction of airways, airway remodelling and hyper-responsiveness. Neutrophils contribute significantly to pathogenesis of COPD. In addition, several studies shown that neutrophil-derived exosomes might exert pro-inflammatory response. Exosomes are 30-100 nm vesicles that are involved in cell-cell communication and transfer nucleic acids, proteins, and metabolites to recipient cells that contribute in physiological and pathological processes. Recognition of adhesion molecules and small GTPases Cdc42 and Rab1b represent the critical step in neutrophil-mediated tissue damage. In this study, I will investigate whether the transmigration of neutrophils or neutrophil-derived exosomes are responsible for proteolytic tissue damage in chronic lung disease. Major hypothesis are: 1) Transmigration of neutrophils through epithelial cells is controlled by different small GTPases which is further mediated by recognition of adhesion molecules specifically expressed on epithelial cells. 2) Transfer of neutrophil-derived exosomes to epithelial cells might change their miRNA and protein expression profile which could contribute to pathogenesis of COPD. The objectives will be to study the direct and indirect effects of neutrophils on epithelial cells. Direct effects of neutrophils on epithelial cells will be analyzed by expression of adhesion molecules, junctional proteins, small GTPases on epithelial cells and inflammatory markers. Moreover, effects of neutrophil-derived exosomes will be investigated by differential miRNA profiling of epithelial cells, small GTPases and inflammatory markers. For this purpose, inverted and non-inverted cell culture systems consisting of human and murine epithelial cells have been established in our group. Epithelial barriers were exposed to neutrophils and their directed transmigration toward different stimuli was examined. According to first results, neutrophils migrate through the epithelium in basolateral-to-apical direction that depicts polarity of epithelial cells. After transmigration of neutrophils, gene expression of adhesion and junctional proteins was studied. In initial experiments to observe the indirect effect of neutrophils, exosomes were isolated from human neutrophils. In the next approach, I will identify further adhesion molecules on epithelial cells and analyze functional and genetic changes in epithelial cells after neutrophil transmigration and uptake of neutrophil-derived exosomes.

P11. Establishment of an Whole Blood Assay to investigate clinical TB

Annemarie Hintz-Rüther

Cellular Microbiology, Research Center Borstel, Parkallee 1-40, 23845 Borstel, Germany

aruether@fz-borstel.de

Tuberculosis (TB) is an infectious lung disease caused by *Mycobacterium tuberculosis* (*Mtb*), the top infectious killer worldwide with 10 Mio cases in 2019 (WHO Global Tuberculosis Report 2020).

Eum *et.al.* showed that neutrophils are the primary infected cell in the lower airways of TB patients. Our group revealed in *in vitro* experiments, that *Mtb* induces a ROS dependent necrotic cell death in neutrophils leading to enhanced mycobacterial growth after phagocytosis of *Mtb*-containing necrotic neutrophil cell bodies by macrophages (Dallenga et al, 2017).

We hypothesize, that I) neutrophils play an important role for disease progression, severity and long-term outcome in TB and II) neutrophil effectors can be employed as biomarkers and targets for host-directed therapy (HDT) of TB.

To investigate neutrophil and monocyte functions in acute TB patients, we established a whole blood infection assay w/ and w/o addition of HDT drugs followed by flow cytometry based comparison of surface expression of activation and effector markers.

Preliminary data shows differential ROS-production and expression of CD66b, CD15, CD62L, CD11b.

To screen ROS inhibitors as potential HDT compounds for personalized treatment approach in active TB patients, we established a multimode analysis using Cytation1. CD45+ Leucocytes isolated by magnetic cell sorting and treated with HDT drug candidates were analyzed for extracellular ROS production using Luminol and Lucigenin as well as necrosis and NETosis by detection of extracellular RNA using Sytox green. Treatment with ABAH and Amiloride significantly reduced both, ROS production and necrotic cell death. To analyse infection rate and necrotic cell death, we established a high throughput microscopy assay.

In the next step, we will analyze responses of leucocytes derived from TB patients with different severities of disease and during antibiotic treatment for their susceptibility to HDT drugs to develop a biomarker stratified host-directed therapy approach.

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