Digital

PostDoc Innovation Fund Symposium
February 26\textsuperscript{th}, 2021

Awardees of the PostDoc Innovation Fund will present their scientific results.

Register here: https://bit.ly/3bNd20e
# Program

**Online Symposium “Postdoc Innovation Funds”**  
**February 26th, 2021**

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| 9:10  | Block 1            | Tracking tumour cell dynamics during T cell immunotherapy using secreted luciferases and advanced stochastic modelling  
  *(Maike Effern)*  
|       |                    | Lineage tracing of Natural Killer cell plasticity  
  *(Dillon Corvino)*  
|       |                    | Characterization and quantification of regulatory B cell subsets in patients with aortic valve stenosis  
  *(Eva Steffen)*  
|       |                    | Distinct Roles of IgA During the Human Immune Response Against SARS-CoV-2  
  *(Bianca Schulte)*  |
| 9:40  | **Breathe through break** |                                                                                          |
| 9:45  | Block 2            | Immunomodulation of nucleic acid sensing by platelets  
  *(Lucas Secchim Ribeiro)*  
|       |                    | Role of Coronavirus G-quadruplexes in viral replication and host cell control  
  *(Philipp Schult)*  
|       |                    | SOCS1 and SOCS3 inhibit RIG-I and STING mediated type I IFN production by targeting IRF3 and IRF5  
  *(Chunfeng Yu)*  
|       |                    | SARS-CoV-2 virus-like particles as a system for mRNA delivery  
  *(Thomas Zillinger)*  |
| 10:15 | **Break: UKB Fitness** |                                                                                          |
| 10:30 | Block 3            | Multi-channel analysis of auto-immune nephritic kidneys  
  *(Alexander Böhner)*  
|       |                    | Generation and characterisation of iPSC-derived microglia to study genetic depression risk  
  *(Eva Beins)*  
|       |                    | Single immune cell analysis for patients with different stages of idiopathic pulmonary fibrosis  
  *(Jiangyan Yu)*  
|       |                    | The impact of postoperative inflammation on enteric neurons  
  *(Reiner Schneider)*  |
<p>| 11:00 | <strong>Breathe through break</strong> |                                                                                          |</p>
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| 11:15 | Eosinophil ETosis and the inflammasome  
  *Alexandra Ehrens*  
  NLRP3 is a sensor of endosomal dysfunction  
  *Matthew Mangan*  
  Uncovering the molecular basis of NLRP1 inhibition by DPP9  
  *Jonas Möcking*  
  Can NLRP3 control epigenetic reprogramming of microglia in Alzheimer’s disease  
  *Roisin McManus*  |
| 11:45 | **Break: UKB Fitness** |
| 12:00 | HMGB1-and histone expression on stress-induced extracellular vesicles and their impact on the immune response  
  *Katrin Reiners*  
  Functional Screening for Activating Nanobodies-towards Molecular Control of Interferon Induction  
  *Jennifer Wuerth*  
  Design and assessment of new HMGB1-targeting nanobodies  
  *Damien Bertheloot*  
  Cell intrinsic Antioxidant system is indispensable for ILC2-driven immune responses  
  *Jayagopi Surendar*  
  Immunomodulatory roles of cell cycle regulatory proteins or the effect of anticancer treatment on bacterial infection  
  *Clivia Lisowski*  |
| 12:40 | **Networking** (Break out sessions with the speaker) |
| 13:10 | **Final Remarks** |
Generation and characterisation of iPSC-derived microglia to study genetic depressionris

Eva Beins
Institute of Human Genetics/Division of Genomics

Microglia, the resident immune cells of the brain, have been implicated in the pathology of psychiatric disorders, including depression. These findings are mostly based on animal models, while results from humans are limited and mixed, likely due to methodological limitations and etiological heterogeneity of depression. The aim of this project is therefore to establish the differentiation of human induced pluripotent stem cells (iPSC) into microglia (iMG) to study the influence of genetic risk for depression on microglial function. Preliminary results of one iPSC line suggest successful differentiation into iMG after 40 days, indicated by the expression of macrophage/microglial surface markers and microglia-like morphology in vitro. Prospectively, we plan to obtain iPSC lines of healthy subjects, selected based on their polygenic risk score for depression, and analyse inflammatory responses of derived iMG in relation to their genetic background.

Design and assessment of new HMGB1-targeting nanobodies

Damien Bertheloot
Institute of Innate of Immunity, University Hospital, Bonn, Germany

The study of HMGB1 has focused on its extracellular functions from alarmin, chemokine to tolerogenic mediator. Interestingly, post-translational modifications of HMGB1 precede its release and thus dictate its extracellular functions. The passive release of HMGB1 follows tissue damage (e.g. trauma), while its active secretion requires the activation of pro-inflammatory myeloid cells (e.g. inflammasome activation). Thus, HMGB1 is a promising and relevant target for clinical diagnostics and therapeutic applications. In our proposal, we planned to produce and test new nanobodies targeting specific post-translational forms of HMGB1 to gain valuable tools for use in the laboratory and to develop affordable assays for clinical detection of HMGB1 as alternative to expensive reagents available on the market.

Multi-channel analysis of auto-immune nephritic kidneys

Alexander Böhner
Institute for Experimental Immunology

Many renal diseases involve the immune system or are even caused by it. This is the case for crescentic glomerulonephritis, in which dendritic cells and macrophages infiltrate the glomeruli (corner stones of the kidney’s functional units) leading to the destruction of the glomerular filter. This manifests in humans and mice in the loss of protein via the urinary tract (proteinuria) as one hallmark of nephritic kidney disease. However, it remained unclear whether the extent of glomerular infiltration correlates with the proteinuria on a single-glomerulus level. We used large-scale 3D-Microscopy and novel bioinformatics to identify dendritic cells and macrophages as driving forces of renal impairment during crescentic glomerulonephritis.

Lineage tracing of Natural Killer cell plasticity

Corvino Dillon
Institute for Experimental Oncology

In addition to direct killing of tumor cells, Natural Killer (NK) cells are also integral in the coordination of innate and adaptive immune responses within the tumour microenvironment (TME). Recently, we described a process whereby NK cells within the TME undergo phenotype switching to a tumor-promoting innate lymphoid cell (ILC1)-like phenotype. Using barcode-based lineage tracing technologies we will investigate NK cell-ILC1 plasticity.

Tracking tumour cell dynamics during T cell immunotherapy using secreted luciferases and advanced stochastic modelling

Maike Effern
Institute of Experimental Oncology

Tumour cells that lose antigen expression during the course of immunotherapy pose a key challenge to successful therapy outcome. Using a transdisciplinary approach that combines life sciences (ImmunoSensation®) and mathematics (Hausdorff Center for Mathematics), we aimed to understand the interplay between melanoma cells and different immune cell populations during the course of adoptive cell transfer immunotherapy. We developed an
experimental and a stochastic model that allows us to monitor dynamics of antigen-competent melanoma cells and antigen-loss variants during the course of tumour growth and immunotherapy by secreted luciferases. The detailed investigation of tumour cell dynamics will help deepen our understanding of how individual melanoma cell populations contribute to resistance mechanisms and this knowledge could help to improve current immunotherapy treatment regimens.

Eosinophil ETosis and the inflammasome
Alexandra Ehrens
Institute for Medical Microbiology, Immunology and Parasitology

Eosinophils are a hallmark of parasitic helminth infections and recent work from our group demonstrated that eosinophil extracellular DNA trap cell death (EETosis) is contributing to protective immune responses against the microfilariae (MF) stage, the progeny of parasitic filarial nematodes. In this project, we analyzed the impact of the inflammasome on MF-induced EETosis. Using specific inhibitors and knock-out mice, it was shown that the MF- and PMA-induced EETosis is caspase-1 and partly ASC- and NLRP-3 dependent. Fluorescence microscopy and flow cytometry demonstrated an upregulation of caspase-1 but not caspase-3 in DNA releasing eosinophils, while the inhibition of caspase-3 had no impact on MF-induced EETosis. Thus, our results indicate that the NLRP-3 inflammasome and the activation of caspase-1 are important for eosinophil ETosis indicating a link between the cell death pathways ETosis and pyroptosis.

Immunomodulatory roles of cell cycle regulatory proteins or the effect of anticancer treatment on bacterial infection
Clivia Lisowski
Institute of Experimental Immunology

The cell cycle plays an essential role for the development and function of (immune) cells. It is tightly regulated by a set of well-studied proteins. Interestingly, non-canonical functions of these proteins were discovered and suggest an immunomodulatory role. Previous (unpublished) data suggest that application of cell cycle inhibitors like CDK4/6i, which are widely used in cancer therapies, negatively affect the outcome of infection with Salmonella Typhimurium. In the current project, the effect of cell cycle inhibitors on the outcome of S. aureus infection is investigated.

NLRP3 is a sensor of endosomal dysfunction
Matthew Mangan
Institute of Innate Immunity

NLRP3 is a cytosolic immune sensor that forms an inflammasome complex in response to diverse range of cellular stresses, resulting in release of pro-inflammatory cytokines and inflammatory cell death. However, the cellular events required for NLRP3 activation in response to cellular stress are still unclear. We propose that NLRP3 is senses dysfunction of the endo-lysosomal system, detecting stalled vesicle trafficking or breakdown. Our preliminary results suggest that disruption of the endosomal trafficking system differentially controls NLRP3 activation dependent on the NLRP3 activator used. We have begun to investigate this further through in vitro lipid binding assays to determine whether NLRP3 can associate with different lipid species directly. Additionally, we have developed optogenetic tools that enable specific manipulation of phospholipid content, which will be used to investigate the role of lipid composition of NLRP3 in a cellular context.

Can NLRP3 control epigenetic reprogramming of microglia in Alzheimer’s disease?
Roisin McManus
DZNE

Unpublished results from our lab suggest that the NLRP3 inflammasome influences glutamate-related metabolism in microglia resulting in changes to the inflammatory state of the cell. The aim of this proposal was to uncover the mechanism in detail, and investigate whether epigenetic changes are responsible for this switch in activity. We prepared wildtype, NLRP3-/- and NLRP3-inhibitor treated microglia for an Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq) to unveil regions of open chromatin enabling gene transcription. We examined the oxygen consumption rate in microglia in the absence of NLRP3-inflammasome signaling and tested which intermediates of the glutamate metabolic pathway impacted anti-inflammatory activity including phagocytosis. Together the data indicates that the NLRP3 inflammasome can control important cellular functions such as metabolic activity, and in the absence of NLRP3 signaling there is enhanced utilization of glutamate leading to increased phagocytosis of Alzheimer’s disease-related peptides.
Uncovering the molecular basis of NLRP1 inhibition by DPP9
Jonas Möcking
Institute of Structural Biology

In recent years, dipeptidyl peptidase DPP9 was identified to have a central role in keeping the inflammasomal sensor protein NLRP1 in an inhibited conformation. To understand the molecular basis of this inhibitory interaction, we established purification protocols for recombinant NLRP1 and DPP9 proteins as both separate subunits and as a complex. Using surface plasmon resonance, a high-sensitivity and label-free technique for characterizing biomolecular interactions, we can measure direct interaction of NLRP1 and DPP9. By size exclusion chromatography and multi-angle light scattering we characterize the composition and stoichiometry of the protein complex. Additionally, crystallographic trials are running to generate structural information of the interaction site. With this combined approach we aim to get a more detailed understanding of the mechanism of NLRP1 inhibition by DPP9, potentially exposing therapeutic target sites for the treatment of NLRP1-associated autoinflammatory diseases.

HMGB1-and histone expression on stress-induced extracellular vesicles and their impact on the immune response
Katrin Reiners
Institute of Clinical Chemistry and Clinical Pharmacology

Extracellular vesicles (EVs) are shed by almost all cell types under both, physiological and pathological conditions. They are known to have immune activating as well as immune inhibitory effects depending on their cargo, which is determined by the functional status of the originating cell. HMGB1 and cell free Histones are found in serum and plasma and have the potential to serve as biomarker in cancer patients. HMGB1 plays a vital role in cancer development and is described to promote tumor growth as well as antitumor immune responses. So far, studies have focused on soluble nucleosome or HMGB1 detection, but have rarely differentiated between soluble proteins and extracellular vesicle associated-proteins. In this project, we investigate the impact of stress-induction (e.g. heatshock or chemotherapeutic treatment) on the release of HMGB1 and histones as soluble or EV associated proteins in vitro and ex vivo in serum of glioblastoma patients.

Immunomodulation of nucleic acid sensing by platelets
Lucas Secchim Ribeiro
Institute of Innate Immunity

Platelets are known for their role in hemostasis and their importance in immunity is steadily growing. We recently showed that platelets amplify inflammasome activity of leukocytes but the potential for platelet-derived components in other immune pathways remains to be explored. Our preliminary findings show that platelets additionally dampen the production of type I interferons by immune cells, a finding corroborated by in vivo experiments. Since the recognition of self/exogenous nucleic acids is an underlying mechanism of several inflammatory and infectious diseases, our objective is to investigate the regulation of type I interferon by platelets during an inflammatory event.

The impact of postoperative inflammation on enteric neurons
Reiner Schneider
Department of Surgery

Neuroinflammation is a hallmark of various diseases in the enteric nervous system. Many cell types are involved in the ongoing inflammatory processes contributing to a complex system that can induce regenerative mechanisms or detrimental chronic complications. Recent studies began to focus on neuronal cells and indicated that neurons react to inflammatory mediators under disease conditions. Remarkably, besides numerous inflammatory diseases in the enteric nervous system, detailed investigations focusing on neurons have not yet been conducted. Therefore, this project is designed to specifically analyze the neuronal transcriptome during acute postoperative inflammation by applying a mouse model accessing mRNA from enteric neurons. The aim is to gain new insights into neuroimmune activation and discover novel targets for modulation of inflammatory gastrointestinal diseases.

Role of Coronavirus G-quadruplexes in viral replication and host cell control
Phillip Schult
Medical Clinic III

In this study, we aim to elucidate the presence and function of G-quadruplexes in the genome of SARS-CoV-2. To this end, we employed in silico prediction, as well as in vitro structural and functional analyses. This approach facilitated the discovery of a G-quadruplex in a functionally relevant region of the viral genome. In parallel, we are developing a plasmid-based reporter replicon to study the SARS-CoV-2 intracellular life cycle under S2 conditions. Once implemented, this system will allow investigation of targeted mutations in the identified G-quadruplexes and serve as a platform for general drug screening.
Distinct Roles of IgA During the Human Immune Response Against SARS-CoV-2

Bianca Schulte
Institute of Virology

tba

Characterization and quantification of regulatory B cell subsets in patients with aortic valve stenosis

Eva Steffen
Heart Center Bonn, Molecular Cardiology

Calcifying aortic valve stenosis is one of the most common valve diseases in the western world. The immune system has been identified as an important modulator of aortic valve calcification. Preliminary data of our workgroup indicate that B cells might be involved into the regulation of aortic valve stenosis in mice. Our study aimed to analyze the role of B cells and especially Interleukin 10 secreting Bregs as potential biomarker in human aortic valve stenosis in a flow cytometric approach.

Cell intrinsic Antioxidant system is indispensable for ILC2-driven immune responses

Jayagopi Surendar
Institute of Clinical Chemistry and Clinical Pharmacology

Activation of the immune system is a metabolically costly endeavour. Immune cells rapidly take up external nutrients to perform effector functions. Our group has shown that ILC2 acquire external glucose and fatty acids to drive proliferation and activation during allergen-driven airway inflammation. Increased mitochondrial activity driving ILC2 activation generates reactive oxygen species (ROS) that could potentially damage the cellular components leading to cell death. We found that ILC2 activated upon allergen exposure upregulated genes of the thioredoxin (Trx) pathway to balance ROS production. Assessing the functionality of this important pathway for ILC2 biology we found that Trx inhibition impairs activation and expansion of ILC2. In addition, we observed that Trx inhibition also decreased mitochondrial activity. This was probably mediated by increased production of reactive lipid species causing lipotoxicity. Furthermore, papain challenged mice treated with Trx inhibitor ablated the accumulation of ILC2 in the lungs of allergen challenged mice. This suggests that Trx blockade could be used as an efficient treatment of ILC2 driven airway inflammation.

Functional Screening for Activating Nanobodies-towards Molecular Control of Interferon Induction

Jennifer Wuerth
Institute for Innate Immunity

Nanobodies, or so-called variable domains of camelid heavy chain-only antibodies (VHHs), allow for highly specific detection and precise functional perturbation of target proteins in living cells. Of note, nanobodies can stabilize distinct protein conformations, making them attractive tools for controlled activation of innate immune sensing. However, candidate VHHs are usually selected from nanobody libraries based on their binding affinity, which yields functionally active nanobodies only by chance. Thus, we are creating a lentivirus-based functional screening platform for selective enrichment of activating nanobodies that drive interferon induction. On the long run, we anticipate that the nanobodies identified via this platform will be transformed into intracellular nanobody switches, thereby allowing manipulation of innate immune sensing with high temporal and spatial control.
SOCS1 and SOCS3 inhibit RIG-I and STING mediated type I IFN production by targeting IRF3 and IRF5

Chungfeng Yu
Department of Dermatology and Allergy

The activation of cytoplasm-located pathogen recognition receptors (PRRs) such as RIG-I and cGAS/STING triggers the production of Type I IFNs, which play essential roles in eliminating viral infections and mediating autoimmune diseases. The molecular mechanism of regulating Type I IFN production is vital for human innate immunity. In the study, activation of RIG-I or STING by its cytosolic PRR ligand promptly induced the production of suppressor of cytokine signaling protein1 (SOCS1) and SOCS3. Furthermore, the upregulated expression of SOCS1/3 then suppressed RIG-I or STING mediated type I IFN production through mediating proteasomal degradation of transcription factors IRF3 and IRF5. Together, identifying SOCS1 and SOCS3 as new negative regulators of the RIG-I/STING-IRF3/5-Type I IFNs signaling pathway in our study may provide intrigue therapeutic targets for the treatment of human viral infections and autoimmune diseases.

Single immune cell analysis for patients with different stages of idiopathic pulmonary fibrosis

Jiangyan Yu
Life and Medical Sciences Institute

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive lung disease and typically affects patients with an age over 60 years old. Due to the poor understanding of the pathophysiological mechanisms underlying IPF, diagnosis and treatment choices are often suboptimal. Recent studies implicate that immune cells play an important role in both promotion and suppression of IPF pathogenesis. In this study, we will perform the single cell RNA (scRNA) sequencing and high-dimensional flow cytometry (HFDC) analysis to characterize immune cells in bronchoalveolar lavage (BAL) samples of IPF patients. Simultaneously, the comprehensive transcriptomic profiling of BAL samples from patients with different stages of IPF will enable us to better understand the determinants of fibrosis progression, allowing better prediction of disease development and thus a personalized treatment and care for the patient.

SARS-CoV-2 virus-like particles as a system for mRNA delivery

Thomas Zillinger
Institute of Clinical Chemistry and Clinical Pharmacology

Coronaviruses are the single-stranded RNA virus family with the largest genome size, and thus harbor considerable potential as a genetic transfer vector for transient delivery of long messenger RNA. For SARS-CoV-2 specifically, high selectivity for its receptor Angiotensin Converting Enzyme 2 (ACE2) could allow a level of specificity for target cell types that is not easily achievable with other viral vectors or transfection methods. Here we investigated the requirements for a SARS-CoV-2-based Virus-like particle system to package a model messenger RNA and characterized the innate immune response to this formulation. This system could be used to target mRNA transfer to a defined ACE2-positive cell population, and also be used to encapsulate mRNA vaccines while mimicking SARS-CoV-2 viral infection and tropism.