MAX DELBRÜCK CENTER

Berlin-wide Immunology & Inflammation Symposium 2024

DIGITAL BOOKLET

October 7, 2024

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WELCOME MESSAGE

We warmly welcome you to the 2024 Immunology & Inflammation (I&I) Berlin-wide symposium, a platform that harnesses the rich research landscape of the greater Berlin area. This year's I&I symposium is a unique opportunity to bring together researchers from various Berlin institutions, all with a shared interest in immunology, to foster scientific exchange, networking, and ignite new collaborative efforts, opening doors to exciting possibilities.

We have entered a new era of immunological research, positioning the immune system at the intersection of modern systems biology, pathologies, and transformative immunotherapies across diverse medical fields. The emergence of novel technologies has led to a rapidly expanding deeper understanding of our immune system. Pioneering immunology-based therapies have been approved and are successfully used in clinics and we are uncovering increasingly complex interactions between immune cells, organs, and tissues that offer previously unimaginable clinical possibilities.

With its high-profile research environment, Berlin is at the forefront of this immunology revolution. We hope this symposium strengthens scientific ties within Berlin's immunology community and fosters the city's status as an international hub of cutting-edge research.

Katja Simon & Simon Haas & The I&I Berlin Symposium 2024 organizing committee*

*** Inmaculada Martinez Reyes (MDC), Sebastian Hofer (MDC), Irene Mattiola (Charité), Maria Berruezo Llacuna (MDC), Somesh Sai (MDC), Alisa Iakupova (MDC), Tzu-Jiun Kuo (MDC), Thomas Krammertoens (Charité), Carolin Knappe (MDC), Caroline Holley (MPI)**

SCIENTIFIC AGENDA

08:00 – 08:50	**Registration**	*Centrum für Anatomie Wilhelm-von-Waldeyer-Haus Philippstraße 11, 10115 Berlin*
08:50 – 09:00	**Welcome** – *Simon Haas (BIH/MDC)*	

Session 1 - Innate Immunity

Chairs: Irene Mattiola & Caroline Holley

09:00 – 09:30	**Being in the Right Place - Mapping the TOLL Reads to Autoimmune Disease** - *Olivia Majer (MPI Infection Biology)*
09:30 – 09:45	Selected Short Talk – Abstract No. 3 Diet modulates colonic epithelial proinflammatory responses by influencing butyrate-producing microbiota – *Safak Bayram (Charité)*
09:45 – 10:15	**Innate Lymphoid Cells and Tissue Homeostasis** – *Andreas Diefenbach (Charité & DRFZ)*
10:15 – 10:30	Selected Short Talk – Abstract No. 29 Beyond the barrier: microbial signatures shape dermal innate lymphoid cell function and skin homeostasis – *Bruno Rocha (Charité)*
10:30 – 11:00	**NK cell memory and clonality** – *Chiara Romagnani (Charité & DRFZ)*
11:00 – 11:30	Coffee Break

Session 2 - Adaptive Immunity

Chairs: Inmaculada Martinez Reyes & Thomas Kammertoens

11:30 – 12:00	**How T-cells recognize antigens – insights from advanced imaging** – *Johannes Huppa (Charité)*
12:00 – 12:15	Selected Short Talk – Abstract No. 13 Autophagy and the autophagy-related kinase ULK1 contribute to T helper cell type 17 differentiation – *Elisabeth Gressler (MDC)*
12:15 – 12:45	**B cell memory, EBV infection and gene editing** **–** *Klaus Rajewsky (MDC)*
12:45 – 13:00	Selected Short Talk – Abstract No. 7 Towards the molecular mechanism of LMP1 induced B cell lymphomagenesis – *Yue Zong (MDC)*
13:00 – 14:20	Lunch Break
14:20 – 14:30	Group Photo

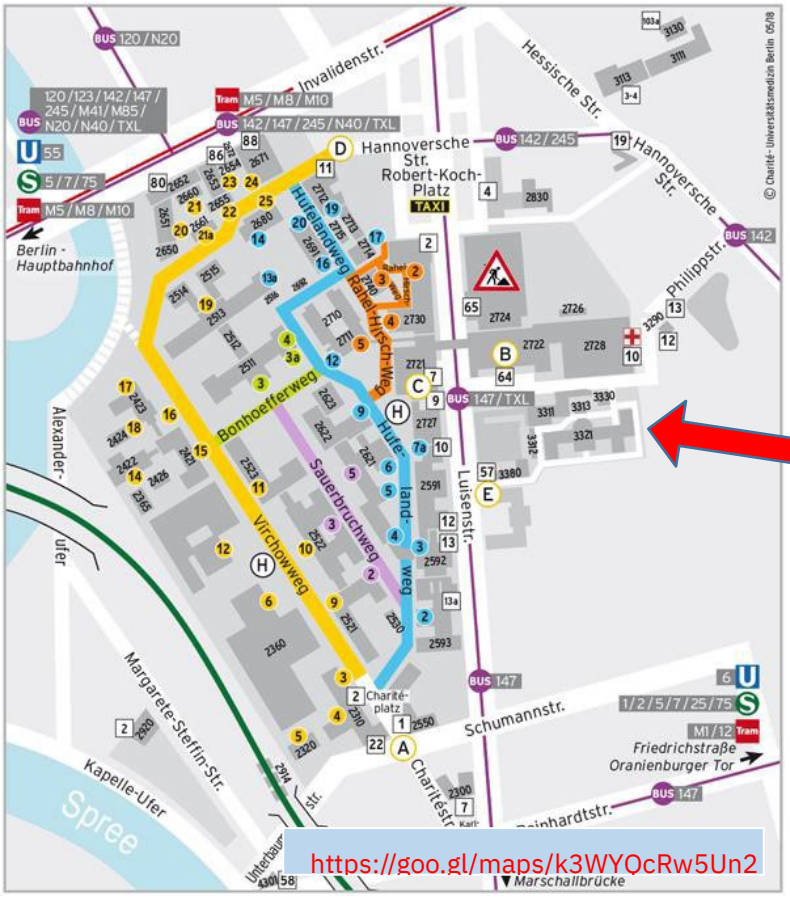
Session 3 - Translational Immunology	*Chairs: Carolin Knappe & Alisa Iakupova*
14:30 – 15:00	**Metabolic checkpoints controlling onset and resolution of inflammation** - *Gerhard Krönke (Charité & DRFZ)*
15:00 – 15:15	Selected Short Talk – Abstract No. 18 Understanding the Link Between Macrophage Metabolism and Age-Associated Diseases - *Maria Dzamukova (Charité)*
15:15 – 15:45	**Mechanisms driving hyper inflammatory T cell responses** – *Birgit Sawitzki (BIH)*
15:45 – 16:00	Selected Short Talk – Abstract No. 24 Autoimmune organ damage by an ILC1-monocyte-derived macrophage module – *Katerina Stergioula (DRFZ)*
16:00 – 16:30	**Cell death and inflammation** – *Eicke Latz (DRFZ)*
16:30 – 17:00	Coffee Break

17:00 – 17:45	Flash Talks	*Chairs: Maria Berruezo Llacuna & Sebastian Hofer*

1. Abstract No. 16 - The role of extracellular RNA on mediating the innate neuroinflammatory response following subarachnoid hemorrhage - *Monika Svecla (Charité)*
2. Abstract No. 19 - Sequential mutagenesis through a dual-recombinase allele: FOXO1 re-expression rescues class switching in germinal center B cells - *Carlota Farre i Diaz (MDC)*
3. Abstract No. 20 - Neutrophil extracellular traps induce a novel regulatory system in uropathogenic Escherichia coli - *Dora Čerina (MPI Infection Biology)*
4. Abstract No. 21 - Elucidating the anti-fungal role of kidney-resident macrophages in invasive candidiasis - *Karolin Hublitz (Charité)*
5. Abstract No. 30 - Inheritance of old mitochondria controls early T cell fate commitment and is regulated by autophagy - *Mariana Borsa (University of Oxford)*

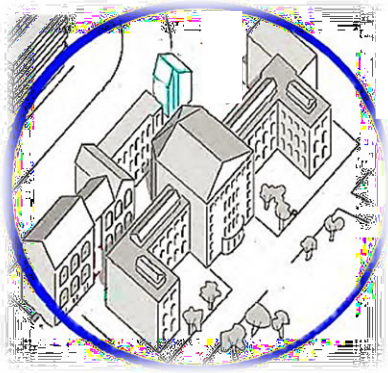
17:45 – 18:00	**Closing Words** – *Katja Simon (MDC)*
18:00 – 19:15	Posters
19:15 – 19:30	Awards for Best Short Talk, Best Flash Talk & Best Poster
19:30 – 21:00	Get-Together & Networking with Food & Drinks

SYMPOSIUM LOCATION



Charité Universitätsmedizin Berlin

Campus Charité Mitte

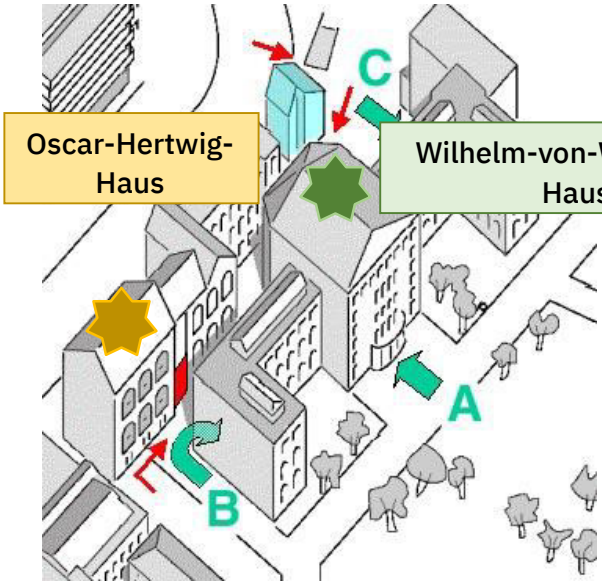


Centrum für Anatomie Wilhelm-von-Waldeyer-Haus Philippstraße 11, 10115 Berlin

Zugang über Philippstraße 10 (Zentrale Rettungsstelle Charite) bzw. Luisenstraße 57 + 64

CHARITÉ
UNIVERSITÄTSMEDIZIN BERLIN

BeST
BERLINER SIMULATIONS- & TRAININGSZENTRUM



A: Haupteingang,
Zugang zentrales Treppenhaus,
Treppe 3+4

SPONSORS

We gratefully acknowledge the financial support from the organizations below.

BIOSKETCHES

Olivia Majer

Max Planck Institute for Infection Biology

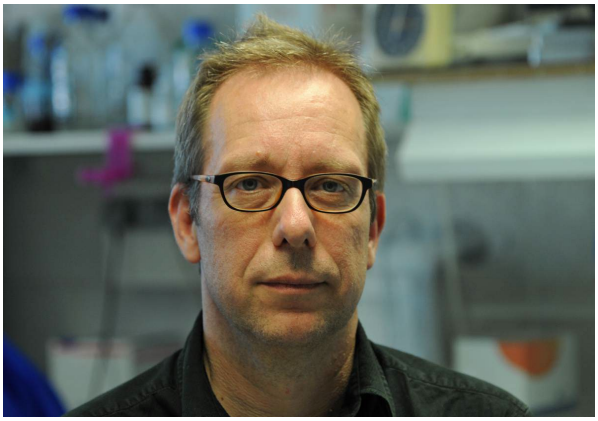
Olivia Majer is an Independent Max Planck Research Group Leader at the Max Planck Institute for Infection Biology in Berlin since 2020. Her research focuses on the detection of nucleic acids by the innate immune system, a crucial mechanism that distinguishes between microbial and host-derived nucleic acids. Olivia's work explores the trafficking pathways that deliver Toll-like receptors (TLRs) to their signaling compartments, crucial for proper immune function, and investigates the sorting defects that result in hyperactive responses towards self-ligands.

Prior to leading her research group, Olivia completed a postdoctoral fellowship in Helge Ewers' lab at the Free University Berlin, where she utilized super-resolution imaging to study the trafficking of Toll-like receptors. From 2013 to 2019, she was a postdoctoral fellow in Greg Barton's lab at the University of California, Berkeley, focusing on the molecular mechanisms of self/non-self-discrimination by nucleic acid-sensing Toll-like receptors. Her foundational research training began as a PhD candidate in Karl Kuchler's lab at the Max Perutz Labs, Vienna, Austria, where she investigated the physiological role of type I interferons in disseminated fungal infections.

Her research has broad implications for understanding autoimmune diseases and improving therapies by elucidating the molecular underpinnings of innate immune system regulation.

Andreas Diefenbach

Institute of Microbiology, Infectious Diseases and Immunology, Charité – Universitätsmedizin Berlin
Deutsches Rheuma-Forschungszentrum (DRFZ)



Andreas Diefenbach studied Medicine at the University of Erlangen. After obtaining his doctoral degree in Microbiology and Immunology, he received postdoctoral training at the Department of Molecular and Cellular Biology, University of California Berkeley. He held faculty positions at the Skirball Institute of Biomolecular Medicine, New York University, at the University of Freiburg and at the Johannes-Gutenberg-University Mainz. Since 2016, Andreas Diefenbach is Professor and Chair of the Institute of Microbiology, Infectious Diseases and Immunology at Charité and the Founding Director of the Berlin Centre for the Biology of Health, a joint research institute of Charité and Free University Berlin devoted to understanding the molecular mechanisms that maintain health. He also is a Senior Group Leader at the German Rheumatism Research Center, A Leibniz Institute. His lab studies development and function of the innate immune system and is particularly interested in understanding how the innate immune system coordinates adaptation of multicellular organisms to their environments (e.g., microbiota, nutrients). Research in the Diefenbach laboratory is supported by the European Research Council (ERC-StG-2012; ERC-AdG-2021) and Deutsche Forschungsgemeinschaft (DFG). He is the coordinator of the DFG Priority Program 1937 (“Innate Lymphoid Cells”). Andreas is an elected member of the Berlin-Brandenburg Academy of Sciences.

Chiara Romagnani

Institute for Medical Immunology, Charité – Universitätsmedizin Berlin
Deutsches Rheuma-Forschungszentrum (DRFZ)

Chiara Romagnani pursued her medical studies at the University of Florence, Italy, before specializing as an Oncologist at the National Cancer Institute in Genova. Following the completion of her PhD in Immunology at the University of Genova, under the guidance of Lorenzo Moretta, she was granted an EMBO fellowship to train as a postdoctoral researcher at the German Rheumatism Center (DRFZ) in Berlin, Germany. She established there her research focus in innate immunity and inflammation, first as a group leader and later as a DFG-Heisenberg Professor. Her significant contributions include the identification of signals responsible for the differentiation and activation of Innate Lymphoid Cells (ILCs) and the discovery of human innate lymphocyte clonality and memory. Presently, she holds the position of Berlin University Alliance Joint Full Professor at the Charité University and Free University Berlin and serves as the Chair of the Institute of Medical Immunology at Charité–Universitätsmedizin Berlin. Additionally, C. Romagnani holds the role of Chief Editor at the European Journal of Immunology and was recently awarded an ERC Advanced Grant.

Johannes Huppa

Institute for Immunology, Charité – Universitätsmedizin Berlin

Johannes Huppa is a molecular immunologist with a profound interest in the biophysical and cell biological mechanisms underlying sensitized T-cell antigen recognition in health and disease. He arrived last month in Berlin to head the Institute of Tumor Immunology at Charité, located at the MDC.

After graduating in biochemistry from Freie Universität Berlin, Dr. Huppa conducted his PhD research at MIT and Harvard Medical School, where he focused on the molecular assembly and quality control of the TCR-CD3 complex in the endoplasmic reticulum. As a postdoctoral fellow at Stanford University School of Medicine and later as a principal investigator at Medizinische Universität Wien, he developed advanced live-cell imaging modalities to study T-cell antigen recognition. He demonstrated that continual TCR engagement is critical for maintaining immune synapse integrity and the T cells' full effector potential. His team discovered, using synthetic biology, superresolution, and single-molecule microscopy, that TCRs and pMHCs act within the immunological synapse as monomeric rather than higher-order entities, contrary to previous perceptions. Highly sensitized antigen detection was shown to be promoted through serial short-lived pMHC-TCR engagement under the influence of synaptic piconewton-scale mechanical forces acting on ligand-bound TCRs. The extent to which such forces drive T-cell antigen discrimination and TCR-downstream signaling is currently a subject of intense research activities.

Dr. Huppa's most recent studies focus on human T-cell antigen recognition in settings of viral infection, autoimmunity, non-HLA allorecognition, and cancer. In Berlin, he aims to add bioinformatics and structural biology to his imaging- and bioengineering-driven experimental repertoire to delineate the molecular, cell biological, and immunological parameters defining the anti-tumor T-cell response in cancers patients. Dr. Huppa's underlying ambition is to engage in basic science to accelerate much needed therapy development for cancer patients. Insights gained and technologies applied may also benefit other immunology-related fields such as rheumatology, transplantation medicine, allergology, and vaccine development.

Klaus Rajewsky

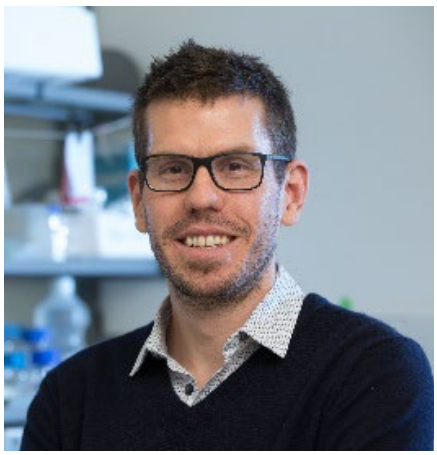
Max-Delbrück Center for Molecular Medicine (MDC)

Klaus Rajewsky and collaborators developed a general method of targeted mutagenesis in mouse embryonic stem cells by introducing bacteriophage- and yeast-derived recombination systems, opening the way to conditional gene targeting. Using this and other methods in their immunological work, they developed, together with N. A. Mitchison and N. K. Jerne, the antigen-bridge model of T-B cell cooperation, identified germinal centers as the sites of antibody somatic hypermutation, the B cell antigen receptor as a survival determinant of B cells, and the germinal center as a major site of human B cell lymphomagenesis, including Hodgkin lymphoma. Over the last years the work of his group has focused on mechanisms of microRNA control, targeted mutagenesis and gene repair in hematopoietic cells including mouse and human hematopoietic stem and progenitor cells, differentiation and subset determination in B lymphocytes, and the development of mouse models of human B and T cell pathologies.

Klaus Rajewsky obtained his medical degree at the University of Frankfurt. After postdoctoral work at the Institut Pasteur in Paris he built, from 1964, an immunology department at the Institute for Genetics, University of Cologne, where he stayed for 38 years, was the founding Program Coordinator of the EMBL Mouse Biology Program at Monterotondo near Rome from 1996-2001, worked for 10 years at Harvard Medical School in Boston from 2001, and is since 2012 Senior Group Leader at the Max-Delbrück-Center for Molecular Medicine in Berlin, Germany. He won numerous scientific awards and is a member of several learned societies including the National Academy of Sciences of the USA and the American Academy of Arts and Sciences. Many trainees from his laboratory have embarked on distinguished careers in academia and industry all over the world.

Gerhard Krönke

Department of Rheumatology and Clinical Immunology, Charité – Universitätsmedizin Berlin
Deutsches Rheuma-Forschungszentrum (DRFZ)



After graduating from the Medical University of Vienna in 2002, Gerhard Krönke worked as postdoctoral researcher at the Medical University of Vienna (2002-2004) and the University of Virginia, Charlottesville (2004-2006). From 2006-2015 he conducted his clinical training in Internal Medicine and subsequently in Rheumatology at the University Hospital Erlangen, where he worked as Senior Physician at the Department of Internal Medicine 3 from 2012-2023. In 2016, he was appointed Professor of Translational Immunology at the University of Erlangen-Nürnberg. In 2023 he moved to Berlin where he is now serving as director of the Medical Department of Rheumatology and Clinical immunology at the Charité University Hospital Berlin. His research focuses on cellular, molecular and metabolic pathways involved in the maintenance and break of immunological self-tolerance as well as the onset and resolution of inflammation. In particular, he is trying to understand the mechanisms and events leading to the development of inflammatory autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematodes and to develop novel strategies for their diagnosis and treatment. In 2014 he received an ERC Starting grant in 2014 as well as an ERC consolidator grant in 2020 and is currently acting as the spokesperson of the DFG research unit FOR2886 “PANDORA” (Pathways triggering AutoimmuNity and Defining Onset of early Rheumatoid Arthritis).

Birgit Sawitzki

Center of Immunomics, Berlin Institute of Health @ Charité (BIH)

Birgit Sawitzki is Head of the Center of Immunomics and professor for Translational Immunology at the Berlin Institute of Health at Charité (BIH).

Birgit Sawitzki studied biochemistry at the Humboldt-Universität zu Berlin and completed her doctorate at Charité's Institute of Immunology. She performed a postdoc at the Nuffield Department of Surgery, University of Oxford and was granted a Wellcome Trust Fellowship. Afterwards she returned to the Charité to lead the group for Transplantation Tolerance at the Institute of Medical Immunology. In 2009 she was appointed professor for immune tolerance at the Charité – Universitätsmedizin Berlin and in 2021 became full professor for Translational Immunology at the BIH. Birgit Sawitzki is actively engaged on activities of the German Society of Immunolgy (DGfI), European Society of Organ Transplantation (ESOT) and Transplantation Society. For the DGfI she serves as a member of the "Consultant Immunologist" subcommittee.

Scientifically the work of Birgit Sawitzki focusses on dissecting mechanisms driving immune pathologies operational in solid organ transplant rejections, acute and post-acute infections. To this end, she combines explorative state-of-the-art multi-omics technologies such as multi-plex cytometry, multi-plex immunohistochemistry as well as single cell RNA sequencing with mechanistic in vivo and ex vivo studies.

To ensure the generality of their findings, the group generates patient cohort data and develops methods that guarantee reproducibility across laboratories. For instance, Prof. Sawitzki coordinated comparative investigations between world-leading laboratories as part of EU-funded projects such as the 'ONE Study' and 'BIO-DrIM.' Her group has developed standardized flow cytometry panels ready for use in clinical trials, which are now being used worldwide by many consortia, even beyond transplantation medicine (Streitz et al. 2013, Kverneland et al. 2016, Ivison JCI Insight 2018). Using this approach, Prof. Sawitzki's group was able to elucidate the mode of action of modulating anti-CD4 antibodies and regulatory T cells for transplant tolerance induction (Sawitzki JEM 2005, Siepert Am J Transplant 2013, Lancet 2020). The group also uncovered both negative and positive control mechanisms of

hyperinflammatory T cell responses, with the co-inhibitory receptor CD96 serving as a negative regulator and the complement split product C5a as a positive regulator (Stanko PNAS 2018, Georg Cell 2022). Recent findings suggest that alterations in cytokine receptor signaling contribute to dysregulated hyperinflammatory immune responses during aging (Petrov in revision).

Eicke Latz

Deutsches Rheuma-Forschungszentrum (DRFZ)

Prof. Dr. (med.) Eicke Latz studied medicine at the Georg-August University in Göttingen and the Freie Universität Berlin, following which, he worked as an intensive care physician at the Charité Universitätsmedizin. In 2001, he moved to the USA, working as a postdoctoral researcher at Boston University, then at UMass Chan Medical School, where he held his first professorship. In 2010, he returned to Germany and founded the Institute for Innate Immunity at the University Hospital Bonn. He became Scientific Director of the German Rheumatism Research Centre Berlin, a Leibniz Institute, and Professor of Experimental Rheumatology at the Charité Universitätsmedizin Berlin in 2023. His research interests concern how the innate immune system maintains health and under what circumstances it can promote disease. In particular, he investigates the molecular mechanisms that lead to activation or inhibition of the immune system and how these influence the inflammatory reactions in various diseases, such as rheumatic diseases, arteriosclerosis or Alzheimer's disease.

Prof. Dr. Latz is co-spokesperson of the Cluster of Excellence "ImmunoSensation2" and spokesperson of the Collaborative Research Centre "Metaflammation and Cellular Programming" (SFB 1454). He has also co-founded several biotech companies, including IFM Therapeutics (2017), Dioscure Therapeutics (2020), a 'Stealth' biotech' (2020), and Odyssey Therapeutics (2021), which translate his discoveries into novel therapeutics and preventive approaches. He has been a highly cited scientist in immunology since 2014 having published more than 300 publications. Prof. Dr. Latz was elected as a member of the German National Academy of Sciences (Leopoldina) in 2016 and has received a number of prestigious awards, including the Gottfried Wilhelm Leibniz Prize in 2018.sagittis vestibulum.

ABSTRACTS

Abstract No. 1

Gain-of-function variants in CLCN7 cause hypopigmentation and lysosomal storage disease

Tanushka Rana[1, 2, *], Maya M. Polovitskaya[1], Kurt Ullrich[3], Simona Murko[3], Tatjana Bierhals[3], Guido Vogt[1], Tobias Stauber[4], Christian Kubisch[3], René Santer[3], and Thomas J. Jentsch[1, 5]

[1]Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Berlin
[2]Humboldt-Universität zu Berlin, Germany
[3]University Medical Center Hamburg-Eppendorf (UKE), Hamburg
[4]Medical School Hamburg (MSH), Hamburg
[5]Charité Universitätsmedizin, Berlin
*Presenting author

ClC-7 together with its β-subunit OSTM1 forms the lysosomal 2Cl–/H+ exchanger. Pathogenic CLCN7 variants cause lysosome-related pathologies, including osteopetrosis, lysosomal storage, and pigmentation defects. Loss of ClC-7 results in recessive osteopetrosis and mostly neuronal lysosomal storage, while some missense variants cause benign osteopetrosis without CNS involvement. A recently reported de novo CLCN7 mutation, p.Tyr715Cys, causes widespread severe lysosome pathology characterized by hypopigmentation, organomegaly, and developmental and myelination delay (HOD), but no osteopetrosis.

We now describe two subjects with the previously described p.Tyr715Cys and a novel p.Lys285Thr mutation, respectively. Both mutations decreased ClC-7 inhibition by PI(3,5)P2, and shifted voltage- dependent gating to less positive potentials, an effect partially conferred to WT subunits in WT/mutant heteromers. Overexpressing either mutant induced large lysosome-related vacuoles. Fibroblasts from the p.Y715C patient displayed giant vacuoles. Lysosomes in p.K285T fibroblasts were only modestly enlarged- probably due to residual PI(3,5)P2 sensitivity. The gain-of-function caused by the shifted voltage-dependence likely is the main cause of their pathogenicity. Their loss of PI(3,5)P2 inhibition will further increase currents, but may not be a necessary feature of HOD.

Abstract No. 2

Inflammation-educated macrophages drive exacerbated re-injury patterns via innate immune memory

Yuting Wang[1, *], Paul Horn[1, 2], Tianjiao Zhang[1], Frank Tacke[1], Moritz Peiseler[1, 2], and Felix Heymann[1]

[1]Charité Universitätsmedizin Berlin, Campus Virchow Klinikum and Campus Charité Mitte, Berlin, Germany
[2]Berlin Institute of Health (BIH), Berlin, Germany
*Presenting author

Chronic liver injury leads to pronounced immune alterations, but the persistence of these changes and the impact of the immunological reprogramming on the liver's response to re-injury remains uncertain. Patients with chronic liver diseases suffer from phases of high disease activity followed by months of injury regression. Here we used a mouse model of chronic toxicity (CCl4) and regression of liver injury and simulated re-injury by administering a single dose of CCl4 after regression. We found that while liver architecture and damage returned to normal levels during regression, rechallenge injury resulted in significantly more severe liver damage compared to long-term chronic injury and acute injury on an otherwise healthy liver. Through the utilization of fate-mapping tools, intravital imaging, and multiplex flow cytometry, we show that chronic injury resulted in a significant influx of monocytes into the liver, with infiltration of monocyte-derived macrophages, which persisted into the regression phase. These monocyte-derived macrophages displayed a proinflammatory profile and engaged in frequent and prolonged interactions with circulating neutrophils. Furthermore, they were able to rapidly secrete cytokines such as TNF-α and IL-1β upon re-stimulation, indicating an increased pro-inflammatory potential. Our study reveals reprogramming of liver macrophages during the regression of chronic liver injury, resulting in a hyperirritable immune state of the liver and a heightened inflammatory response upon re-injury.

Abstract No. 3

Diet modulates colonic epithelial proinflammatory responses by influencing butyrate- producing microbiota

Safak Bayram[1, 2, *], Kimberly Hartl[1, 2], Hilmar Berger[1], Marie Florence Kiefer[1], Michael Schupp[1], and Michael Sigal[1, 3]

[1]Charité
[2]BIMSB
[3]Berlin Institute for Medical Systems Biology (BIMSB)
[*]Presenting author

The increasing prevalence of obesity poses a significant challenge to modern medicine and is intricately linked to a multitude of diseases, encompassing metabolic, inflammatory, and malignant conditions. Murine models of high-fat diet (HFD) have been established as a reliable tool to mimic obesity-associated-diseases. HFD has been shown to induce alterations in gut microbiota, potentially leading to dysbiosis and contributing to the pathophysiology of obesity-related ailments. However, the precise mechanisms underlying HFD-driven microbiota alterations and their impact on disease development remain incompletely elucidated. We hypothesized that HFD-driven changes of the microbiota alter the gut mucosal immune responses, leading to heightened intestinal inflammation, which in turn may be associated with increased systemic inflammatory responses and elevated susceptibility to inflammatory and malignant conditions. HFD-fed-mice exhibited a substantial weight gain of 67% over a 13-week-period. Examination of colonic tissue via immunofluorescence indicated immune cell infiltration, particularly macrophages, corroborated by the marker Iba1. 16S-sequence analysis of the microbiota of HFD-exposed-mice revealed notable shifts in composition and diversity, particularly a reduction in butyrate-producing-bacteria. Butyrate is a SCFA that serves as a nutrient to colonocytes and has been proposed to influence various physiological functions in the gut. Assessment of stool samples via gas-chromatography coupled to mass-spectrometry revealed a significant reduction in butyrate levels in HFD-exposed-mice. Exposure of epithelial cells in organoids to LPS causes a massive proinflammatory response by increased levels of the chemokines Cxcl1 and Cxcl2 that causes the recruitment of immune cells. Pretreatment of epithelial cells with butyrate attenuated the proinflammatory response when exposed to LPS. These findings underscore the potential role of butyrate in mediating tolerance to the microbiota and highlight how its depletion, induced by dietary intervention, can lead to heightened proinflammatory responses in the epithelium. Subsequent investigations are underway to discern the precise impact of butyrate on mucosal immunity and human health.

Abstract No. 4

Helicobacter pylori infection causes gastric mucosal reprogramming into a proinflammatory fetal-like alert state

Giulia Beccaceci[1, 2, *], Manqiang Lin[1, 2], Stefanie Müllerke[1, 2], Hans-Joachim Mollenkopf[3], Hilmar Berger[1], and Michael Sigal[1, 2]

[1]Charité Universitätsmedizin
[2]BIMSB, Max Delbrück Center for Molecular Medicine
[3]Max Plank Institute for Infection Biology
[*]Presenting author

Helicobacter pylori (H.pylori) infection is the main risk factor for gastric cancer. Infection leads to chronic inflammation and gastric gland hyperplasia, causing premalignant pathology. We found that infection induces alterations in the mesenchymal niche, such as loss of BMP ligands and increased expression of BMP inhibitors, and we now investigate how the downregulation of BMP signaling affects mucosal homeostasis and inflammatory responses. Spatial analysis of antral gastric tissue performed via immunolabeling shows that, upon H.pylori infection, the influx of immune cells coincides with the downregulation of BMP signaling and hyperplasia. In uninfected conditions, epithelial BMP loss (epi-Bmpr1aKO) in vivo leads not only to gland hyperplasia but also to strongly enhanced inflammation specifically at the top of the gland, where it is absent in mice with functional BMP signaling. Exploiting gene expression analysis of 3D organoids, we demonstrate that epithelial cells exhibit a strong pro-inflammatory ability, which is inhibited upon BMP signaling activation. We explore the consequences of increased inflammation upon epi-Bmpr1aKO, demonstrating via whole transcriptome analysis that this is linked to enhanced antimicrobial tissue responses and that epi-Bmpr1aKO mice show reduced H.pylori colonization. Moreover, we discover that the influx of Il1beta-producing cells observed upon Bmp signaling downregulation has an impact on mesenchymal stromal cells, which in turn produce pro-regenerative factors causing epithelial reprogramming into a highly proliferative fetal-like regenerative state. We demonstrate that mes-Il1r1KO mice, where stromal cells lack the ability to respond to Il1beta upon infection, do not show fetal-like reprogramming and are more colonized by H.pylori. In conclusion, chronic H.pylori infection causes tissue reprogramming into a fetal-like state via a crosstalk between epithelial, stromal and immune cells. This “alert state” is linked to an enhanced pro-inflammatory response, increased proliferation and boosters antimicrobial defense. We propose that long-term activation of this state may enhance the risk for malignant transformation.

Abstract No. 5

Loss of stromal BMP activity drives gastric pathology in mice

Jonas Wizenty[1, *], and Michael Sigal[1, 2]

[1]Charité - Universitätsmedizin Berlin
[2]Berlin Institute for Medical Systems Biology (BIMSB)
[*]Presenting author

Introduction: The gastric epithelial gland homeostasis is driven by insufficiently characterized sub- epithelial stromal signals. Gastric stem cells in the gland base are fueled by a Wnt/Rspohigh niche where Bmp activity is suppressed, while terminally differentiated mucous pit cells are maintained by a BMPhigh niche, that suppresses Wnt/Rspo activity. Infection with Helicobacter pylori reduces stromal and epithelial Bmp activity driving stem cell expansion. In line with this, genetic knockout of BMP activity in the epithelium leads to epithelial hyperplasia. The consequences of loss of BMP activity in the stroma on epithelial homeostasis and proliferative, regenerative, and inflammatory pathways in the interplay between stroma and epithelium are unknown.

Aim & Methods: The objective of this study was to analyze the epithelial-stromal interplay in the context of loss of BMP activity in the gastric stroma by using an inducible knockout of the BMP type 1 receptor in the stroma in mice.

Results: After two months of knockout of the BMP receptor in the gastric stroma, mice display drastic hyperplasia via stem cell expansion in the antrum. Loss of stromal BMP signaling causes the occurrence of a bulky stroma with proliferating and pro-inflammatory fibroblasts and aberrant glands within the pit area, that resemble the gland base with expression of stem cell factors, high proliferation, enhanced epithelial inflammation (marked by NF-κB p65) and expression of metaplasia markers (CD44v9, Aqp5). Using scRNAseq we now explore the mechanisms by which loss of stromal BMP affects epithelial homeostasis and characterize proliferating and pro-inflammatory fibroblast subgroups in the stomach in detail.

Conclusion: Collectively, our data demonstrate that alterations in stromal BMP activity can promote premalignant lesions. This model will allow us to study the underlying principles of stromal-epithelial interplay in the stomach and explore mechanistically the consequences of stromal responses observed upon H. pylori infection in detail.

Abstract No. 6

Differential immune induction by high- and low-pathogenic genotypes of Dobrava-Belgrade virus

Linah Chibrac-Ahad[1, *], Daniel Bourquain[1], Tobias Pfeiffer[1], Steffanie Schürer[1], Andreas Nitsche[1], and Lars Schaade[1]

[1]Robert Koch-Intitute
[*]Presenting author

Dobrava-Belgrade virus (DOBV) is a rodent-borne European hantavirus prevalent in different Apodemus species. DOBV causes mild to severe hemorrhagic fever with renal syndrome (HFRS) in humans, depending on the causative DOBV genotype. The focus of the study is to investigate if high- and low-virulent DOBV genotypes differ in their capability to evade innate immunity in human cells. Therefore, we analyzed the MAVS-dependent induction of interferon (IFN) responses by DOBV genotypes in human A549 epithelial cells and podocytes and investigated the antiviral function of IFN-induced transmembrane proteins (IFITMs) against DOBV.

We could show, that the low pathogenic genotype DOBV-Aa induces a strong IFN response in A549 cells and podocytes, which leads to an effective inhibition of viral replication. This IFN response was MAVS-dependent and resulted in an increased expression of the IFN-induced MX1 protein. In contrast, the highly pathogenic genotypes DOBV-Af and DOBV-Ap triggered only a weak and delayed IFN response, which was insufficient to inhibit viral replication. Interestingly, both genotypes induced a strong pro-inflammatory response independent of MAVS-signaling, which was not observed after DOBV-Aa infection. Notably, the highly pathogenic DOBV genotypes did not only evade IFN induction but were also less sensitive towards the antiviral effects of IFN-induced IFITM-proteins. Via overexpression of IFITMs in A549-ΔMAVS knockout cells, we could show that DOBV-Aa is efficiently inhibited by particularly IFITM3, while DOBV-Af and especially DOBV-Ap show a remarkable resistance to these proteins.

This leads to the conclusion that highly pathogenic genotypes of DOBV are able to avoid the innate immune defense of the host. These resistance mechanisms may likely contribute to the higher pathogenicity of DOBV-Af and -Ap genotypes in humans. The results provide insights into the interactions between hantaviruses and the innate immune response and into the mechanisms that define hantavirus pathogenicity in humans.

Abstract No. 7

Towards the molecular mechanism of LMP1 induced B cell lymphomagenesis

Yue Zong[1,*], Max Ruwolt[2], Raku Sun[3], Yasuhiro Murakawa[3], and Klaus Rajewsky[1]

[1]Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany
[2]Leibniz-Forschungsinstitut für Molekulare Pharmakologie (FMP), Berlin, Germany
[3]RIKEN Center for Integrative Medical Sciences, Yokohama, Japan
[*]Presenting author

EBV is associated with B cell malignancies while usually kept in check by immune surveillance. During lifelong EBV latent infection, once immune surveillance fails, rare infected B cells can be activated and spread the virus to other B cells which, as a result, undergo proliferation and ultimately malignant transformation. Previous research of our group has shown that the expression of a single EBV oncogene, LMP1, in B cells of T cell-deficient mice is sufficient to induce rapid, fatal lymphoproliferation and lymphomagenesis. We have now analyzed mouse B cells early after LMP1 induction and found that LMP1 induces B cell activation within 12 and proliferation between 36 and 42 hours. By applying cap analysis of gene expression on LMP1 activated B cells at different time points, we found that LMP1 induces significant transcriptional reprogramming within 12 hours, with a large number of enhancers being involved. At the same time, LMP1 induces the formation of liquid- liquid phase separation condensates of transcription coactivators (MED1 and BRD4) whose number and volume increase along with LMP1 expression. It has been proposed that the MED1 and BRD4 condensates might contribute to gene regulation at super-enhancers. We plan to identify the transcription factors and super-enhancers recruited to the transcription coactivator condensates in LMP1 activated B cells. To further understand LMP1 signaling, we investigated the B cell specific interactome of LMP1 via BioID2 pull-down. Since LMP1 is considered to functionally mimic CD40, we included CD40 activation in all assays as a control, trying to explore the CD40-independent function of LMP1. Initial results of these analyses will be presented. This study aims at uncovering the mechanism of LMP1 induced B cell lymphomagenesis.

Abstract No. 8

Neoantigen-specific T cell receptors from tumor-bearing hosts provide optimal functionality

Leonie Rosenberger[1, *], Leo Hansmann[1, 2], Kimberley Drosch[1], Christina Möwes[1], Vasiliki Anastasopoulou[1, 2], Steven Wolf[3, 3], Xinyi Feng[3], Guoshuai Cao[3], Jun Huang[3], Erlend Strønen[4], Taigo Cato[5], Poh Yin Yew[3], Yusuke Nakamura[6], Johanna Olweus[7, 8], Gerald Willimsky[1, 2], Thomas Blankenstein[1, 2], Hans Schreiber[3, 3], and Matthias Leisegang[1, 3]

[1]Charité Universitätsmedizin Berlin
[2]German Cancer Consortium (DKTK)
[3]The University of Chicago
[4]Department of Cancer Immunology, Institute for Cancer Research
[5]Osaka University Graduate School of Medicine
[6]Japanese Foundation for Cancer Research
[7]Oslo University Hospital Radiumhospitalet
[8]University of Oslo
[*]Presenting author

The treatment of cancer using T cell receptor (TCR)-engineered T cells (TCR-Ts) relies on high-affinity TCRs that are highly specific for tumor antigens. When tumors are infiltrated by neoantigen-specific T cells the question whether these contain high quality TCRs is of relevance for the design of optimal TCR-Ts for therapy. Since tumor infiltrating lymphocytes (TILs) in progressively growing tumors are unable to control tumor growth and are chronically exposed to high levels of tumor antigens, high- avidity T cells may be deleted in tumors. To understand whether the quality of neoantigen-specific TCRs in TILs is inferior to TCRs obtained from antigen-negative donors, we used two model antigens and for each of them several TCRs derived from different backgrounds. We tested human mutated Cyclin Dependent Kinase 4 (CDK4) specific TCRs that were derived either from TILs, from healthy donors after in vitro stimulation, immunized mice with human TCR locus or a vaccinated and recovered patient. On the other hand, we sequenced mouse TCRs specific for mutated p68 (mp68), that were obtained either from TILs or from spleens of immunized mice and compared them to a published TCR that was isolated from an immunized mouse. To obtain the TCRs from TILs, we used doxycycline-inducible antigen induction on day 21 after tumor transplant to prevent mp68-specific T cell priming during tumor induction. We transduced these TCRs into donor T cells and evaluated their capacity for cytokine secretion after co-culture with target cells as well as their potential to kill tumor cells in vitro and induce tumor shrinkage in vivo. We found for both antigens that TIL-derived TCRs were of high quality, comparable to TCRs derived from healthy donors. Therefore, our experiments support the use of TIL-derived TCRs in adoptive T cell transfer targeting neoantigens.

Abstract No. 9

The neurotoxic effects of vanadium (a ubiquitous environmental pollutant) across different age groups in laboratory rodents

Olumayowa Igado[1, 2, *], James Olopade[1], Idris Azeez[3, 4], Oluwaseun Mustapha[5, 1], Oluwabusayo Folarin[6], Funmi Olopade[7], Anna Andrioli[4], and Marina Bentovoglio[4]

[1]University of Ibadan, Ibadan, Nigeria
[2]Charité Universitätsmedizin Berlin and Max Delbrück Center for Molecular Medicine, Berlin, Germany
[3]University of Jos, Plateau State, Nigeria
[4]University of Verona, Italy
[5]Federal University of Agriculture, Abeokuta, Nigeria
[6]College of Medicine, University of Ibadan, Nigeria
[7]College of Medicine, University of Ibadan, Ibadan, Nigeria
[*]Presenting author

Vanadium, a transition metal released during some industrial activities, induces oxidative stress and lipid peroxidation. In all experiments, vanadium (3mg/kg) was administered as sodium metavanadate. Exposure of neonatal mice to vanadium via lactation for 15-22 days caused reduced daily weight gain. Behavioural tests showed reduced locomotor activity and increased scores for negative geotaxis tests. Brain immunohistochemistry showed astrocytic activation and demyelination. Intraperitoneal administration, 14-day-old mice for 14 days caused reduced weight gain and locomotory deficits, Purkinje cell layer degeneration, hippocampal (CA1) neurodegeneration, generalised demyelination, mostly in corpus callosum, retrosplenial and somatosensory cortices, with microglia and astrocytic activation. Immunohistochemistry revealed oligodendrocyte progenitor cells increased NG2-immunolabelling with hypertrophy and bushy, ramified appearance of processes, with depletion of GST-π positive mature oligodendrocytes. Neonatal mice dosed for 3 months had reduced body weight gain and locomotor impairment. Astrocytes, microglia, and nonphosphorylated neurofilaments revealed regional heterogeneity. Myelin damage involved the midline corpus callosum, cortical gray matter fibers, hippocampus, and diencephalon, with axonal damage. Astrocyte and microglial activation were identified in the same regions and internal capsule. Double-immunofluorescence revealed induction of high tumor necrosis factor (TNF) immunoreactivity in reactive astrocytes. Western blotting analysis showed significant induction of TNF and interleukin-1β expression. Ten-week-old male albino rats dosed for 5 days, the cerebellar cortex revealed generalised cellular vacuolation, cerebellum showed Purkinje layer degeneration and a marked reduction in myelin tracts. Biochemical assays showed increase in thiobarbituric acid–reactive substances (TBARS) in cerebrum and hippocampus, with decreased superoxide dismutase activity in cerebrum and cerebellum, relative to the control groups. In addition, there was increased lipid peroxidation in cerebrum,

cerebellum and the hippocampus. Intraperitoneal administration, 4-week-old mice (thrice weekly, 18 months), sacrificed 3rd-monthly, showed increasing pathology. Reversal of some of the pathologies was possible with withdrawal and also reduction of detectable vanadium in the brain, using laser-ablation-inductively coupled plasma- mass spectrometry.

Abstract No. 10

Viral and cellular determinants of HIV-1-induced innate immune activation

Nicolas D. Arnow[1, *], Michelle Stuck[2], Norbert Bannert[1], and Oya Cingöz[3, 1]

[1]Robert Koch Institute
[2]Heidelberg University
[3]Charité Medical University
[*]Presenting author

Despite advances in combined antiretroviral therapy (cART), HIV-1 remains incurable, and efforts to effectively manage the infection must continue. While cART suppresses viral titers and halts the progression of AIDS, HIV-induced inflammation persists due to activation of the innate immune system by cellular and viral determinants. Macrophages, which play a central role in HIV-1 pathogenesis, are instrumental in the establishment of the virus and the initiation of inflammation. However, the exact cellular sensor that control the recognition of the viral determinants responsible for inflammation in monocyte-derived macrophages (MDMs) are still not clear. In this study, two stages of immune recognition in MDMs during HIV-1 infection are revealed. The first recognition stage precedes reverse transcription and is influenced by the stability of the viral capsid, while the later stage, characterized by a robust response, is triggered by a short peptide sequence encoded by the N-terminus of Gag. The presence of viral RNA in the cytoplasm is critical for translation of the Gag protein, but its N myristoylation site is critical for innate recognition in MDMs. Significant differences were observed in the responses of primary cells compared to differentiated THP-1 or U937 cells.

These results enable new research targets in regard to find the cellular sensor, which induces HIV-1 chronic inflammation in MDMs and highlight the importance of research in primary cell systems.

Abstract No. 11

Unraveling the mystery of mast cell secretory granule biogenesis and maturation – a novel approach

Pia Lazki-Hagenbach[1,*], and Francesca Bottanelli[1]

[1]Freie Universität Berlin, Faculty of Biology, Chemistry and Pharmacy, Institute for Biochemistry, 14195 Berlin, Germany
[*]Presenting author

The role of mast cells beyond allergy, specifically in innate immunity, has become evident in the last decades. Accordingly, MCs respond to a variety of external triggers, both Immunoglobulin-E (IgE)- dependent and -independent, by degranulation, which describes the rapid release of inflammatory mediators. The latter are preformed and -stored in the specialized lysosomal-related organelles of the MCs – the so-called secretory granules (SGs). This heavily regulated process involves ligand binding to the plasma membrane (PM)-localized receptor, its subsequent endocytosis as well as the vesicular trafficking of SGs for fusion with the PM, therefore resulting in the controlled release of their content. Despite the central role of SG biogenesis and maturation knowledge on its intricate steps are only fragmentary, which in turn is needed to finally understand and be able to interfere with the process of degranulation at earlier steps. Until now the only therapeutic intervention is the use of antihistamine agents which only relieves a situation in which the MC has already responded and affects its environment. However, a connection between SG maturation and endocytosis of PM- residing receptors, such as G-protein coupled receptors (GPCRs), has been suggested. Lack of appropriate super-resolution live cell imaging techniques was so far limiting insights on this process. In order to be able to gain a better understanding of the relationship between endocytic and exocytic processes of GPCRs and SG biogenesis and maturation steps, we set out to develop a model system to be able to study the spatiotemporal dynamics of SG biogenesis and maturation by employing a combination of CRISPR/Cas9 gene editing together with dynamic confocal or stimulated emission depletion (STED) super-resolution microscopy. With this system we aim at shedding light on this long unanswered aspect of MC research.

Abstract No. 12

Natural killer cell specificity towards different human cytomegalovirus strains

Alexandra Forrai[1,*], Timo Rückert[1], Eva Borst[2], Martin Messerle[2], and Chiara Romagnani[3]

[1]Deutsches Rheuma-Forschungszentrum
[2]Institute of Virolgy, MHH, Hannover
[3]Institute of Medical Immunology, Charite, Berlin
[*]Presenting author

Natural killer cells are innate lymphocytes that can differentially recognize peptides presented onto the non-canonical class I molecule HLA-E. In different human cytomegalovirus (HCMV) strains, the viral gene UL40 encodes for nonameric peptides that are presented onto HLA-E yielding different levels of NK cell activation via the HLA-E-NKG2C axis. In a collaborative project aiming to characterize the HLA-E-binding peptide repertoire, a novel HCMV peptide was predicted to bind and signal through CD94/NKG2x. The novel HCMV UL120-encoded peptide strongly stabilized HLA-E onto RMA- S cells and activated NK cells via CD94/NKG2C upon co-culture. Could UL120-encoded peptides be targets of NKG2C-mediated recognition? To answer this question, we employ an in vitro model of HCMV infection of human umbilical vein endothelial cells in co-culture with NK cells to assess differential recognition and activation.

Abstract No. 13

Autophagy and the autophagy-related kinase ULK1 contribute to T helper cell type 17 differentiation

A. Elisabeth Gressler[1,*], and Anna Katharina Simon[1]

[1]Max-Delbrück-Center for Molecular Medicine
[*]Presenting author

T helper cells are critical for organizing the immune response against infectious diseases, but are also involved in the development of autoimmune diseases. Understanding the molecular factors contributing to their differentiation process is thus crucial to finding novel approaches on how to manipulate their phenotype as a therapeutic option. We focused on autophagy, a cellular recycling and nutrient management process, which is also known to decline with age in immune cells. T helper cell subsets were differentiated from naïve mouse T cells ex vivo and their autophagy levels measured. T helper cell type 17 (Th17) cells showed particularly high autophagic activity on day 2 and day 3 of their differentiation compared to other T helper cell subsets. Inhibition of autophagy through genetic knockout or inhibition of the kinase ULK1, which acts upstream of the autophagy pathway, lead to reduction of IL-17A production. Interestingly, expression of the Th17 lineage transcription factor RORγt was significantly reduced after inhibition of ULK1, but only mildly in autophagy knockout cells, arguing for an autophagy-independent role of ULK1 in Th17 differentiation. In addition, we observed production of TNFα in autophagy-deficient, but not autophagy-competent Th17 cells. Increased TNFα expression was also measured in other T helper cell subsets upon inhibition of autophagy. We hypothesize that this might contribute to inflamm- aging observed in aged individuals. Further experiments will deal with identifying a) the targets of ULK1, b) the cargo of autophagosomes upon Th17 differentiation, and c) elucidating the potential of autophagy deficiency to contribute to inflamm-aging through aberrant TNFα production by T helper cells.

Abstract No. 14

Impact of Age-Related Mitochondrial Dysfunction on the Functional Profile of Macrophages

Eugenia Diez[1, 2, *], Maria Dzamukova[1, 2], Nayar Duran[1, 2], Max Löhning[1, 2], and Gerhard Krönke[1, 2]

[1]Charité Universitätsmedizin Berlin
[2]Deutsches Rheuma-Forschungszentrum Berlin, Ein Institut der Leibniz-Gemeinschaft
[*]Presenting author

Aging comes along with a higher risk for many diseases, like dementia, osteoarthritis, respiratory infections, cancer and diabetes (WHO, 2022). Most of these diseases are accompanied by a chronic inflammatory state of the body termed “inflammaging”. This inflammatory polarization of the body’s immune system is due to the increasing accumulation of damage over time, and the decreasing ability of the immune system to cope with it (Li et al., 2023). Macrophages may play a central role in this process, since they are widespread in the body and are key in maintaining homeostasis (Oishi and Manabe, 2016). Among the various hallmarks of aging, mitochondrial dysfunction in macrophages seems to be an important factor driving inflammaging. (Seegren et al., 2023).

Our aim is to investigate weather mitochondrial dysfunction in resident macrophages and microglia is sufficient to cause inflammaging-related pathologies, and to study the mechanisms by which this is brought about. In-vivo models replicating the accumulation of defects in the mitochondria of resident macrophages were used and the resulting phenotype was assessed in multiple organs using different techniques, such as sequencing, flow cytometry and immunohistochemistry. We found inflammatory signatures in the examined organs, which resemble the ones found in aged animals. To better understand the molecular mechanism behind this phenotype, we use in vitro systems of differentiated macrophages with several types of mitochondria malfunction. We showed that introduced metabolic changes lead to alteration in phagocytic capacity, activation status and overall pro-inflammatory polarization of macrophages. In future experiments, we aim to identify and target the molecules responsible for the shift towards a pro-inflammatory phenotype.

Abstract No. 15

A multi-omic approach to characterize somatic structural variants in B lymphocytes in Systemic Lupus Erythematosus (SLE)

Marcella Emilia Franco[1, *]

[1]Max Delbrück Center (MDC)
[*]Presenting author

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by loss of tolerance to self-antigens and hyperactivity of autoreactive B lymphocytes. Despite significant progress, the precise mechanisms behind immune tolerance breakdown in SLE remain unclear. Genome-wide association studies (GWAS) have identified over 100 germline risk variants for SLE, primarily in regulatory elements for B cell activation. However, GWAS focus on single nucleotide polymorphisms (SNPs), leaving the impact of more complex genomic rearrangements, such as structural variants (SVs), unexplored. Besides genetic predisposition, environmental factors like UV radiation and viral infections significantly contribute to SLE onset and progression, and are known to induce somatic mutations. Whether these mutations contribute to SLE pathogenesis remains unclear. The rapid cell cycle of hyperactive B cells further increases their susceptibility to acquiring somatic mutations. Moreover, while somatic changes in most healthy tissues occur gradually, B lymphocytes have the ability to rapidly mutate their genome through V(D)J recombination and somatic hypermutation. These mechanisms, while targeted to specific loci, are error-prone, and whether they also induce mutations outside the immunoglobulin loci is yet unexplored. We hypothesize that somatic SVs accumulate in autoreactive B lymphocytes in lupus patients due to environmental exposure, recombination errors, and constant activity towards self-antigens, enabling cells to evade tolerance checkpoints and gain clonal advantage. To address this, we will measure somatic SVs in autoreactive and non-autoreactive B lymphocytes of lupus patients using Strand-seq, a single-cell DNA sequencing technology. To investigate how the identified somatic SVs contribute to the loss of immune tolerance, and determine whether they confer a clonal advantage and are associated with expanded BCR clones, we will integrate our Strand-seq data with scRNA-seq and scVDJ-seq. This study will reveal the somatic mutational landscape of autoreactive and non-autoreactive B cells and their functional consequences in the context of lupus.

Abstract No. 16

The role of extracellular RNA on mediating the innate neuroinflammatory response following subarachnoid hemorrhage

Monika Svecla[1,*], Mikayel Poghosyan[1], Anja Nitzsche[1], Pia Pötschner[1], Katharina Tielking[1], Aabi Okute[1], Susan Brandenburg[1], Peter Vajkoczy[1], and Ran Xu[1]

[1]Charité Universitätsmedizin Berlin
[*]Presenting author

Objective: Subarachnoid hemorrhage (SAH) is a life-threatening type of stroke caused by aneurysmal rupture and bleeding into the subarachnoid space (SAS). Secondary brain injury is caused by an accumulation of innate immune cells, microglia cells, and neuronal cell loss following SAH. Here, we investigate the ability of extracellular RNA (exRNA) to trigger this intra-parenchymal immune response following an extra-parenchymal injury.

Methods: In an animal model of SAH and a patient cohort with aneurysmal SAH, exRNA release and immune response were assessed. Animals underwent SAH surgery and RNase treatment1, and MRI, electron microscopy, immunofluorescence, and brain CD11b+ cells were analyzed. While in the cerebrospinal fluid (CSF) of SAH patients, RNase levels and immune-inflammatory proteomics profile were performed.

Results: As an acute change after SAH (day 1) in mice, we observed an 80-fold increase of exRNA in the SAS and a progressive spread in the parenchyma within the time course of SAH. Brain CD11b+ cells revealed an increase in gene expression in pathways (FDR<0.05) concerning inflammatory response, positive regulation of TNF production, cytokine-mediated signaling, as well as activation of microglia, neutrophil, and monocyte chemotaxis. RNase1 application reduced exRNA levels in both SAS (SAH vs. SAH+RNase1: 1013.9±223.9*102 µm² exRNA/mm² tissue vs. 40.6±32.7*102 µm² exRNA/mm² tissue) and parenchyma (SAH vs. SAH+RNase1: 30.9±7.4*102 µm² exRNA/mm² vs. 2.6±0.11 µm² exRNA/mm², p=0.002) and the proinflammatory signature in CD11b+ cells in the animal model. Furthermore, principal component analysis revealed a clear separation between SAH patients and the control in the overall increase in the proinflammatory profile in the proteome of SAH patients.

Conclusion: Our findings suggest that exRNA plays a critical role in exacerbating neuroinflammation and subsequent circulating proinflammatory profile post-SAH. RNase1 emerges as a potent therapeutic agent that can mitigate these effects by degrading exRNA, suggesting a promising approach for targeting the inflammatory response in SAH patients.

Abstract No. 17

Design of EBV gp350 variants with abrogated CD21 binding facilitates the discovery of receptor-binding site targeting antibodies

Emre Ipekoglu[1, 2, *], Mikhail Lebedin[2, 3], Sabrina Horn[3], Lisa Spatt[2], Gerald Willimsky[3, 4], and Kathrin de la Rosa[1, 2]

[1]Helmholtz Center for Infection Research (HZI), Braunschweig, Germany
[2]Max Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany
[3]Charité- Universitätsmedizin Berlin, Germany
[4]German Cancer Research Center (DKFZ), Heidelberg, Germany
[*]Presenting author

Epstein-Barr virus (EBV) is a DNA virus causing infectious mononucleosis and is associated with lymphoproliferative disorders and pathogenesis of multiple sclerosis through the infection and transformation of human B cells. B cell infection of EBV starts with the attachment of the virions to B cells through gp350/220-CD21 interaction. Monoclonal antibodies (mAbs) targeting this interaction could be employed as an effective prevention strategy. This interaction is, however, expected to obstruct the discovery of mAbs using membrane-bound BCR staining of B cells with antigen bait due to its high affinity towards CD21, which is coexpressed on B cells and enhances BCR signaling.

To discover mAbs, we generated gp350 baits lacking CD21 binding. To retrieve the footprint of CD21 on gp350, we used the Alphafold2-multimer algorithm. Based on predicted footprint, we screened a library of gp350 single amino acid variants using deep mutational scanning (DMS) via mammalian cell display. Selected variants were used to probe serum antibody titers as well as memory B cells from human donors. The latter were used to capture paired V(D)J sequences via single cell immune profiling. A subset of antibodies was recombinantly expressed and tested for gp350 binding.

Our DMS results illustrate that the amino acid variants between 155-165 positions of gp350 are deleterious for CD21 binding. Of note, serum antibody reactivity against two variants lacking CD21 binding were similar to that of wild type gp350, suggesting that circulating antibodies were still able to bind to mutants. We isolated the gp350 specific antibody 20C1, which is sensitive to other mutations in the predicted CD21-gp350 interface indicating its recognition site as binding interface. In conclusion, we created gp350 mutants lacking CD21 binding to discover mAbs against EBV. This strategy could deliver superior neutralizing antibodies since it enables to capture whole human B cell repertoire targeting gp350 receptor binding site.

Abstract No. 18

Understanding the Link Between Macrophage Metabolism and Age-Associated Diseases

Maria Dzamukova[1, 2, *], Eugenia Diez[1, 2], Nayar Duran[1, 2], Hilal Garibagaoglu[1], Max Löhning[1, 2], and Gerhard Krönke[1, 2]

[1]Charité Universitätsmedizin Berlin
[2]Deutsches Rheuma-Forschungszentrum Berlin, Ein Institut der Leibniz-Gemeinschaft
[*]Presenting author

As populations continue to age, the prevalence of age-associated diseases rises, highlighting the need for a deeper understanding of their underlying mechanisms. Macrophages, as versatile immune cells, play diverse roles in tissue homeostasis and inflammation. Previous studies have shown that aging impairs mitochondrial function in tissue-resident macrophages (Seegren et al., 2023; Zhong et al., 2022; Plataki et al., 2019).

In this study, we investigate the potential link between macrophage metabolism and various organ- specific age-related conditions, such as osteoarthritis, Alzheimer's disease, or neuroinflammation. Specifically, we focus on how oxidative phosphorylation shapes the aging phenotype of macrophages and contributes to the pathogenesis of age-related diseases. To explore this, we use mouse models where mitochondrial aging is artificially accelerated in tissue resident CX3CR1+ macrophages. As a reference for natural age-associated changes, we analyze cells and organs from 2-year-old wild-type (WT) mice.

Our findings reveal that mitochondrial dysfunction in tissue-resident macrophages triggers an inflammatory phenotype in several organs, including the brain and knee joint, as early as 7 months of age. These alterations closely mirror those seen in 2-year-old WT mice. Microglial cells in mutant mice were highly activated, exhibiting a strong pro-inflammatory signature, though the blood-brain barrier remained intact. In the joint synovium, we observed activated fibroblasts, disruption of the macrophage lining, and the appearance of CD86+ pro-inflammatory macrophages. However, signs of osteoarthritis were not yet present at this stage. The exact molecular mechanisms driving these changes are currently being explored.

The insights gained from understanding the relationship between macrophage metabolism and age- related diseases offer potential for developing novel interventions aimed at promoting healthy aging and improving quality of life in older adults.

Abstract No. 19

Sequential mutagenesis through a dual-recombinase allele: FOXO1 re-expression rescues class switching in germinal center B cells

Carlota Farre Diaz[1, *], Eleni Kabrani[1], Wiebke Winkler[1], Claudia Salomon[1], F. Thomas Wunderlich[2], Martin Janz[3], and Klaus Rajewsky[1]

[1]MDC
[2]University of Cologne
[3]MDC & Charité
[*]Presenting author

The modeling of complex (patho)physiological processes by sequential mutagenesis in mice is limited by the lack of optimized genetic tools and complex breeding strategies. Here, we present a new Cre/DreERT2 dual-recombinase mouse strain in the context of germinal center (GC) B cells, with co- expression of the recombinases from a single allele. This enables highly efficient Cre and subsequent Dre-mediated recombination. As a proof-of-concept experiment, we sequentially targeted the endogenous Foxo1 locus during the GC reaction. Cre-mediated FOXO1 knockout perturbs GC physiology, with loss of the dark zone compartment and defects in class switch recombination. Time controlled, sequential Dre-mediated FOXO1 re-expression leads to a phenotypic and functional rescue in GC B cells, including the ability to class-switch. This novel approach of co-expressing two distinct recombinases from a single allele demonstrates suitability for precise targeted sequential mutagenesis in vivo, easily extendable to other cellular contexts.

Abstract No. 20

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

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

[1]Max Planck Institute for Infection Biology, Charitéplatz 1, 10117, Berlin, Germany
[2]Université Paris Cité, INSERM U1016, CNRS UMR 8104, Institut Cochin, Paris 75014, France
[3]Institut Pasteur, Paris 75015, France
[*]Presenting author

Uropathogenic Escherichia coli (UPEC), the leading cause of urinary tract infections, must adapt to the hostile bladder environment to establish an infection. Thus, UPEC needs to evade the immune system, including infiltrating neutrophils and their antimicrobial neutrophil extracellular traps (NETs). To understand how UPEC interacts with the immune system, we analyzed its transcriptome after incubation with NETs. We show that NETs induce a novel regulatory system in UPEC, comprising of two genes encoded in a pathogenicity island PAIUTI89II. Analysis of 10,000 E. coli genomes shows that 30% of clinical UPEC isolates possess this system. Upregulation of this system is driven primarily by NET-associated nucleosomes, rather than other NET components. This regulatory system in UPEC plays a role in reducing reactive oxygen production in neutrophils and NET formation in vitro.

Deletion of this system leads to reduced bladder colonization and higher pro-inflammatory cytokines in a mouse UTI model, highlighting its importance in UPEC pathogenesis and immune evasion.

Understanding this interaction could pave the way for developing treatments for emerging multidrug-resistant UPEC strains.

Abstract No. 21

Elucidating the anti-fungal role of kidney-resident macrophages in invasive candidiasis

Karolin W Hublitz[1, *], Iván Ballesteros[2], Andrey Kruglov[3], Bernhard Hube[4, 5], and Efstathios G Stamatiades[1]

[1]Institute of Microbiology, Infectious Diseases and Immunology (I-MIDI), Charité - University Medical Centre, Berlin
[2]Centro Nacional de Investigaciones Cardiovasculares Carlos III, Madrid, Spain
[3]Deutsches Rheuma-Forschungszentrum (DRFZ), an Institute of the Leibniz Association, Berlin
[4]Leibniz Institute for Natural Product Research and Infection Biology - Hans Knoell Institute, Jena
[5]Friedrich Schiller University, Jena
[*]Presenting author

Invasive candidiasis is the most common fungal infection among hospitalised patients, associated with high mortality and morbidity. In order to develop new, more specific treatments it is necessary to better understand the mechanistic underpinnings of the disease. The innate immune response to candida (namely monocytes and neutrophils) has been extensively studied. Surprisingly, the role of tissue-resident macrophages remains enigmatic. Herein we used the murine model of disseminated candidiasis coupled with state-of-the-art genetic tools to specifically target kidney-resident macrophages (krMΦs) and to unravel their role in infection. Fate mapping studies showed that krMΦs are not replaced by monocyte-derived macrophages (mdMΦs) in infected kidneys; both macrophage populations co-existed in situ, albeit in different niches. Flow cytometry, immunofluorescence microscopy, gene analysis and specific deletion of krMΦs revealed that krMΦs have a unique, non-redundant role in initiating the anti-candida immune response by promoting the early recruitment and activation of monocytes and neutrophils, which in turn exhibit candidacidal function. While monocyte/mdMΦs-derived Tnf was redundant for anti-candida immunity, specific deletion of krMΦs-derived Tnf revealed its instrumental role in promoting the activation/maturation of kidney-infiltrating myeloid cells to kill candida. Our results demonstrate that the in vivo role of krMΦs in disseminated candidiasis is far more complex than just 'bug-eaters'. krMΦs act as the conductors of an immune cell orchestra by orchestrating the recruitment, activation and candidacidal function of monocytes and neutrophils.

Abstract No. 22

Chasing Antigen-Specific T Cells with High Avidity (CATCH)

Kalliopi Zampeta[1, 2, *], Larissa Henze[1, 2], Andreas Thiel[1, 2], and Lucie Loyal[1, 2]

[1]Si-M / "Der Simulierte Mensch" a science framework of Technische Universität Berlin and Charité - Universitätsmedizin Berlin, Germany
[2]Berlin Institute of Health at Charité - Universitätsmedizin Berlin, Berlin, Germany
[*]Presenting author

Cellular immunity to a specific antigen can be detected and characterized by the analysis of the quantity or functions of specific T cells either isolated ex vivo from blood or tissues or from in vitro systems. T cells express a highly variable set of specific immune receptors (T cell receptors, TCR) to recognize various peptides mostly in the context of HLA molecules. The strength and duration of the interaction of TCR with peptide-HLA complexes determine initial developmental steps of T cells, but are also decisively pivotal for the activation, following differentiation steps and the maintenance of functionally competent T cells that react efficiently in secondary challenges. For immediate productive immune reactions immune receptors with high affinity for their targets are indispensable. We here introduce the CATCH assay, the cytometric assessment of the CD3/TCR complex downregulation, as a novel technology to easily characterize functional avidities of T cell receptors directly in a precise and fast manner (Loyal et al., Science, 2021). By combining flow cytometric activation induced marker (AIM) assays with the analysis of CD3 downregulation we demonstrate efficient analysis and isolation of high avidity pathogen specific T cells within 4 hours covering a broad antigen concentration range. The method can be directly combined with functional analytics, but also be implemented for TCR engineering in human and mice. CATCH enables the high throughput ex vivo characterization of an individual's or a cohorts' T cell population quality.

Abstract No. 23

Modulation of intestinal networks by neutrophils and group 3 innate lymphoid cells

Anna Fagundes[1, *], and Andreas Diefenbach[1, 2]

[1]Charité Institute of microbiology infectious diseases and immunology
[2]DRFZ
[*]Presenting author

Type 3 innate lymphoid cells (ILC3s) are integral for maintenance of intestinal health. They translate microbial and environmental signals into crucial modules for tissue repair and immune response.

CCR6+ ILC3s, alongside B cells and dendritic cells, assemble into clusters known as solitary intestinal lymphoid tissues (SILTs), whose entire range of components and functions requires further comprehension. This project seeks to elucidate the immunological networks occurring within SILTs and their contribution to maintenance of intestinal balance. Our preliminary findings indicate a dynamic interaction between ILC3s and neutrophils within SILTs, where ILC3s play a key role in neutrophil recruitment. Using a novel mouse model targeting such crosstalk, and single-cell RNA sequencing of intestinal neutrophils, our study explores the impact of this axis on tissue function. Our results highlight the intricate nature of intestinal homeostasis, wherein different cell types synergise to maintain a state of equilibrium. ILC3s and neutrophils engage in a previously unrecognised interplay that regulates intestinal health, and further research is imperative to unravel the complexity of SILT-mediated immune responses.

Abstract No. 24

Autoimmune organ damage by an ILC1-monocyte-derived macrophage module

Katerina Stergioula[1, 2, *], Stylianos-Iason Biniaris-Georgallis[1, 2], Andreas Diefenbach[3, 2], Masatoshi Kanda[4, 5], and Antigoni Triantafyllopoulou[1, 2]

[1]Department of Rheumatology and Clinical Immunology, Charité-Universitätsmedizin Berlin, Campus Mitte, Berlin, Germany
[2]German Rheumatology Research Center, A Leibniz Institute, Berlin, Germany
[3]Institute of Microbiology, Infectious Diseases and Immunology (I-MIDI), Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin, Berlin, Germany
[4]Cardiovascular and Metabolic Sciences, Max Delbrück Center for Molecular Medicine in the Helmholtz Association (MDC), Berlin, Germany
[5]Department of Rheumatology and Clinical Immunology, Sapporo Medical University School of Medicine, Sapporo, Hokkaido, Japan
[*]Presenting author

Lupus nephritis results from immune dysregulation and contributes to significant morbidity and mortality in patients with systemic lupus erythematosus. The mechanistic underpinnings of severe kidney damage remain unclear. We hypothesized that tissue resident immune cells, such as innate lymphoid cells (ILCs) may act as amplifiers of inflammatory responses following the development of systemic autoimmunity (Biniaris-Georgallis SI*, Aschman T*, Stergioula K* et al. Nature 2024). To address this, we combined scRNA-seq analysis of kidney NKp46+ ILCs, leukocytes and whole kidney cells, with monoclonal antibody treatments and genetic targeting. Surprisingly, activation of tissue- resident NKp46+ ILCs was required for severe kidney injury. NKp46 signaling induced an unconventional ILC1 transcriptional program. NKp46-activated ILC1 via CSF2 led to a population expansion of pro-inflammatory, monocyte-derived, disease-associated macrophages, which amplified epithelial tissue damage. At the same time, NKp46 activation promoted the expansion of TREM2+ macrophages, which limited tissue injury. These findings uncover an ILC-macrophage-parenchymal cell module that controls the severity of autoimmune kidney damage, offering potential new avenues for therapeutic intervention to prevent kidney damage in lupus patients.

Abstract No. 25

The role of IL-22 signaling on Mammary Gland development

Diego Perez-Vazquez[1,*], Irene Mattiola[1], and Andreas Diefenbach[1]

[1]Charité Universitätsmedizin
[*]Presenting author

The Mammary Gland (MG) is a tissue that undergoes several structural and functional changes throughout life. Mammary Stem Cells (MaSC) sustain these adaptation cycles of epithelial differentiation, proliferation and apoptosis, occurring mainly during pregnancy and lactation. MaSC regenerative potential must be tightly regulated to prevent the development of neoplasms and cancer. Data from our lab showed that IL-22 production by Group 3 Innate Lymphoid Cells (ILC3) and gd T cells regulated the DNA damage response in intestinal Lgr5+ Stem Cells and prevented cancer. We therefore aim at better understanding the role of IL-22 in MG postnatal organogenesis and to evaluate its impact on MaSC, both in homeostatic and pathological conditions. Our preliminary data show that Lgr5+ MaSC express the IL-22 receptor and IL-22 deficiency affected MaSC numbers in homeostasis. Importantly, the frequencies and numbers of ILC3 populations in the MG increase during the reproductive cycle, particularly during late pregnancy and lactation, suggesting a potential implication in the regulation of MaSC homeostasis. To further test the functional role of IL-22 in MaSC biology we plan to delete IL-22 receptor from MaSC during pregnancy and lactation and evaluate changes in epithelial proliferation or differentiation.

Abstract No. 26

Exploring the therapeutic potential of non-pharmacological interventions in alzheimer's disease via targeting the pathological imprinting

Ceccon M[1,*], Rutsch A[1,5], Joseph N[5], Schaible P[4], Arslan-Ergül A[2], Salta E[3], Erny D[4], Al Nabhani Z[5]& Ronchi F[1,5]

[1]Institute of Microbiology, Infectious Diseases and Immunology, Charité – Universitätsmedizin Berlin, Germany;
[2] Molecular Biology and Genetics Department Zebrafish Facility, Bilkent University, Ankara, Turkey
[3]Laboratory of Neurogenesis and Neurodegeneration, Netherlands Institute for Neuroscience, Amsterdam, The Netherlands
[4]Institute of Neuropathology, Faculty of Medicine, University of Freiburg, Freiburg, Germany
[5]Department of Biomedical Research, University Clinic of Visceral Surgery and Medicine, Inselspital, University of Bern, Bern, Switzerland
[*]Presenting author

The gut microbiota influences the host immune and nervous system and may impact Alzheimer's disease (AD) development. Early-life disruptions in microbiota exposure can lead to long-term effects on both the immune and central nervous system, termed "pathological imprinting"1. This project aims to understand if non-pharmacological approaches in adult life could ameliorate AD pathological imprinting. To uncover the cellular and molecular pathways through which our chosen treatments, such as ketogenic diet (KD), exercise and cognitive training could act we will use ex-vivo and in vivo approaches to study microglia, immune cells and inflammatory pathways in 5xFAD mice at different stages of AD development. We will perform locomotor, cognitive and behavioral tests under different hygiene conditions (axenic and specific-pathogen free) to dissect the microbiota's role in the pathogenesis in our experimental conditions. Our preliminary data suggest that KD induces a significant up-regulation of genes associated with ribosome function, inhibition of Amyloid fibrils formation and oxidative phosphorylation pathways in adult mice colonised with a diverse microbiota but not in germ-free mice. We are investigating these mechanisms at the single-cell level in CNS cells. This research may improve strategies to prevent and treat AD in late adulthood.

Abstract No. 27

Engineering B cells through the integration of HIV-receptor exons

Ata Ul Wakeel Ahmad[1, 2, *], Christoph Ratswohl[1], Lisa Ispatt[1], Emre Mert Ipekoglu[1], Clara Vázquez García[1], Mikhail Lebedin[1], Casper Silvis[1], and Kathrin de la Rosa[3, 1]

[1]MDC Berlin
[2]Charite
[3]Zentrum für Individualisierte Infektionsmedizin Hannover
[*]Presenting author

Antibody diversification plays a key role in fighting infections through the production of specific antibodies of high affinity against pathogens. Rarely, B cells can diversify by incorporating pathogen receptors resulting from a genomic insertion in the switch region. Here, we aim to replicate this natural mechanism using AID and CRISPR-based integration of exon-inserts and their splicing into final antibody mRNAs.

To guide substrate design, we generated recombinant antibodies bearing HIV-specific Llama-VHHJ3 and human CD4 domains and confirmed their expression, breadth, and specificity. To enable optimal splicing, a GFP-based fluorescent screening system was developed to identify B cell-specific intronic- splice-enhancers (ISE). Utilizing a high-throughput sequencing approach, we identified and validated novel intronic splice enhancer sequences in B and T cells.

Building upon this work, we engineered primary human and murine B cells with VHHJ3 and CD4 substrates employing AID and CRISPR knock-in strategies. We show successful in vitro engineering of primary human and murine B cells with stable expression of VHHJ3 and CD4 respectively. On-target integration was confirmed in the genome, the RNA, and by secretion of insert-containing antibodies. Furthermore, an in vivo study with adoptive transfer of AID and CRISPR-edited B cells exhibited germinal center recruitment and production of CD4-positive antibodies as a result of heterologous immunization with HIV-BG505-gp140 and HIV-Bal-gp140.

Prospectively, AID-based editing of B cells by the addition of exons encoding receptor binding elements represents a promising alternative to the CRISPR-based replacement of V(D)J heavy and light chain genes, without affecting the fitness of a cell to take part in the immune response and reducing the chance of off-targets. This method represents a novel approach to engineering B cells, offering a promising strategy for enhancing immune responses against pathogens such as HIV.

Abstract No. 28

Identification of a gut microbiota signature in patients with drug-resistant epilepsy upon ketogenic diet treatment

Laura Díaz-Marugán[1,*], Angela Kaindl[2], and Francesca Ronchi[1]

[1]Institute of Microbiology, Infectious Diseases and Immunology (I-MIDI) Charité - Universitaetsmedizin Berlin, Berlin, Germany
[2]Department of Pediatric Neurology, Charité – Universitätsmedizin Berlin, Berlin, Germany
[*]Presenting author

Epilepsy is a neurological disease characterized by seizures that affects 50 million people world-wide. Around 30% of the patients do not respond to the current drugs. Ketogenic diet (KD), highly enriched in fats, is an established therapy for drug-resistant epilepsy (DRE) that shows a reduction of >50% of seizures frequency in ~50% of DRE patients. Interestingly, microbiota from DRE patients responsive to KD is different from KD-non-responder DRE patients and it is still unclear why some patients do not respond to KD. Our research project will address microbiota differences in DRE patients, responders and non-responders to KD, and identify strains of bacteria with potential anti-seizure effect. For this purpose, fecal samples will be collected from patients with DRE before and during treatment with KD up to 6 months together with clinical and dietary information. Stool microorganism composition will be analysed through shotgun metagenomic sequencing, stool and blood metabolites (including ketone bodies) will be measured through targeted and untargeted metabolomics, and biochemical and inflammatory parameters (e.g., C-reactive protein, procalcitonin, cytokines) from serum samples will be analyzed. We expect that >30-50% of the patients under KD will have >50% reduction of seizures, ~30% will experience <50% reduction of seizure frequency and

~5-10% will not show any improvement or eventually will get worse upon KD introduction. Healthy relatives and patients with DRE without KD will be analyzed as controls. The ultimate goal of this project is to describe which microbes are associated with KD-dependent epilepsy amelioration and to test their effect in vivo in preclinical animal models of epilepsy. Identification of those microbes could be used as probiotics for a potential treatment for KD non-responder DRE patients, as well as alternative to KD, which cannot be kept long-term. These results will shed light for diet-microbiota based treatments for other neurological diseases.

Abstract No. 29

Beyond the barrier: microbial signatures shape dermal innate lymphoid cell function and skin homeostasis

Bruno Rocha Cordeiro Costa[1, 2, *], Luke Houghton[1, 2], Johanna Ehl[1], Omer Shomrat[1, 2], and Andreas Diefenbach[1, 2]

[1]Charité-Universitätsmedizin Berlin
[2]German Rheumatology Research Center, A Leibniz Institute, Berlin, Germany
[*]Presenting author

The epithelial barrier of the skin is a vast interface with the environment that is constantly exposed to microbes, light, irradiation and physical or mechanical stress. Innate Lymphoid Cells (ILC) are a recently described subset of leukocytes and its subtypes (ILC1, ILC2 and ILC3) have been linked to important homeostatic and pathological mechanisms across different organs. Recent studies indicate that dermal ILC are in a transient-like state that can assume different identities and functions depending on the signals they receive. However, the mechanisms underlying this phenomenon remain elusive. We sought to understand the commensal microbiota as a pre-requisite to enhance function of dermal ILC and the role of its derived cytokines on skin structure.

Comparison between germ free (GF) and specific pathogen free (SPF) mice showed that an enriched microbiota leads to accumulation of dermal ILC, which produce higher levels of cytokines (e.g. IL-13, IL-17 and IL-22). We performed single cell RNA sequencing comparing GF and SPF mice, as well as wild type and IL-22 knock out mice. Our data underscore the tissue-specific phenotype of dermal ILC, intricately molded by microbiota, with IL-22 emerging as a pivotal mediator of production of insulin- like growth factor (IGF-1) by dermal fibroblasts and subsequent maintenance of mitochondria fitness in dermal cells. Our results indicate that the dermal microbiota enables ILC to regulate the proliferation and differentiation of keratinocytes, as well as maintain fibroblast populations and production of extracellular matrix. This process is crucial for epithelial resistance and maintenance of the skin barrier.

In summary, our data suggests a novel mechanism of cross talk amongst the microbiota, the immune system, and the tissue, which involves IL-22 and downstream production of IGF-1. This will open new avenues in our understanding of the transcriptional output of commensal bacterial communities that may in turn shape human health.

Abstract No. 30

Inheritance of old mitochondria controls early T cell fate commitment and is regulated by autophagy

Mariana Borsa[1, *], Ana Victoria Lechuga-Vieco[2], Amir H. Kayvanjoo[3], Edward Jenkins[1], Yavuz Yazicioglu[1], Ewoud B. Compeer[1], Felix C. Richter[1], Simon Rapp[3], Robert Mitchell[1], Tom Youdale[4], Hien Bui[5], Emilia Kuuluvainen[5], Michael L. Dustin[1], Linda V. Sinclair[4], Pekka Katajisto[5, 6], and Katja Simon[3, 1]

[1]University of Oxford
[2]Institute for Research in Biomedicine Barcelona
[3]MDC Berlin
[4]University of Dundee
[5]University of Helsinki
[6]Karolinska Institute
[*]Presenting author

T cell immunity deteriorates during ageing, particularly in memory responses needed for efficient vaccination. Autophagy and asymmetric cell division (ACD) are cell biological mechanisms key to memory formation, which undergo a decline upon ageing. We aimed to decipher whether autophagy regulates the early-rise of asymmetric T cell fates and investigate whether there is a causal link between ACD and T cell fate decisions. By analysing the proteome of TCR-activated first-daughter CD8$^+$ T cells, we observed that mitochondrial proteins rely on autophagy for their asymmetric inheritance and that damaged mitochondria are polarized upon first division. We then evaluated the functional impact of unequal inheritance of different mitochondrial populations on T cell function.

We used a novel mouse model that allows sequential tagging of mitochondria, enabling *in vivo* analysis of CD8$^+$ T cell progenies based on the inheritance of a pre-mitotic cell cargo: old mitochondria. Autophagy-deficient CD8$^+$ T cells showed impaired clearance and symmetric inheritance of old mitochondria, suggesting that both segregation and degradation events are needed to generate cells devoid of old organelles. Daughter-cells inheriting old mitochondria are more proliferative, glycolytic and show poor survival in absence of TCR stimulation. Adoptive transfer of cells followed by antigen-specific infection revealed that progenies inheriting old organelles have reduced memory potential, whereas daughter cells that have not inherited old mitochondria from the mother cell are long-lived, able to re-expand upon cognate-antigen challenge and produce effector cytokines. Proteomic and single-cell transcriptomic analysis of cells inheriting aged mitochondria suggest that their early fate divergence relies on one carbon metabolism as a consequence of poor mitochondrial quality and function. These findings increase our understanding of how T cell diversity is early-imprinted and will help foster the development of strategies to modulate T cell function, which is particularly relevant in the context of immune rejuvenation and regenerative medicine.

Abstract No. 31

TNFSF receptors overexpression in exhausted T cell populations from cervical cancer patients

Jose Manuel Rojas-Diaz[1, *], Fabiola Solorzano-Ibarra[1], Nadia Tatiana Garcia-Barrientos[1], Ksenia Klimov-Kravtchenko[1], Pedro Ivan Urciaga-Gutierrez[1], Miriam Ruth Bueno-Topete[1], Jesse Haramati[2], and Susana del Toro-Arreola[1]

[1]Instituto de Investigación en Enfermedades Crónico, Universidad de GuadalajaraDegenerativas,
[2]Laboratorio de Inmunología, Universidad de Guadalajara
[*]Presenting author

Background: T cells play a critical role in the fight against cancer; however, these cells can become exhausted while fighting tumors, losing their capacity to kill, and increasing inhibitory receptors, such as PD-1 and TIGIT. Activation of T cells depends on a balance between activating and inhibitory signaling; TNFSF receptors such as 4-1BB and ICOS could be a promising target for reactivation of exhausted T cells in tumors, such as cervical cancer (CC), which continues to be the gynecological cancer with the highest mortality in low and middle-income countries. Nevertheless, the expression pattern of costimulatory receptors such as TNFSF in exhausted T cells from CC patients remains unknown.

Objectives: To identify the expression of the costimulatory receptors 4-1BB and OX-40 in exhausted peripheral and tumor-infiltrating T cells from CC patients.

Methods: PBMCs were obtained from CC patients and healthy donors (HD). Tumor biopsies were collected, and tumor-infiltrating cells were isolated by enzymatic disaggregation. Expression of the costimulatory and inhibitory receptors in T cell subpopulations (CD3+CD4+, CD3+CD8+, and CD3+CD56+) was analyzed by flow cytometry.

Results: We found an increased population of putatively exhausted (PD-1+TIGIT+) in CD3+CD4+, CD3+CD8+, and CD3+CD56+ peripheral T cells in CC patients, which overexpressed 4-1BB and OX-40. Additionally, both costimulatory receptors were selectively overexpressed in exhausted T cells (PD- 1+TIGIT+), compared to non-exhausted cells (PD-1-TIGIT-). In tumor-infiltrating T cell populations the exhausted cells were more abundant than in periphery and also overexpressed ICOS and 4-1BB. PD- 1high T cell populations significantly elevated in the tumor, with concomitant coexpression of the inhibitory receptor TIGIT.

Conclusion: There is an increase of putatively exhausted T cells (PD-1+TIGIT+) in cervical cancer patients, with a selectively elevated expression of the costimulatory receptors 4-1BB and OX-40 in both peripheral and tumor-infiltrating T cells. PD-1high T cells are particularly abundant in the tumor and are coexpressing TIGIT.

Abstract No. 32

Analyzing tissue niches of ILCs in health and disease using multiplexed microscopy & AI-based tools

Pendar Alirezazadeh[1, *], Sandy Kroh[1, 2, *], Anna Pascual-Reguant[1, 2], Artür Manukyan[3, 4], Ralf Uecker[2], Robert Günther[1], Ralf Köhler[1], Peggy Mex[1], Lars Philipsen[5], Markus Landthaler[3, 4], Raluca A. Niesner[1, 6], and Anja E. Hauser[1, 2]

[1]Deutsches Rheuma-Forschungszentrum (DRFZ)
[2]Charité - Universitätsmedizin Berlin
[3]Berlin Institute for Medical Systems Biology (BIMSB)
[4]Max Delbrück Center for Molecular Medicine
[5]Otto-von-Guericke University Magdeburg
[6]Freie Universität Berlin
[*]Presenting author

ILCs are rare immune cells owning various roles in processes controlling tissue homeostasis, barrier integrity, autoimmunity, and pathogen defense. Although being scarce in numbers, they are potent tissue sensors and cytokine secretors. The similarities to T cells and different subtypes with additional markers represent some of the challenges faced when studying ILCs in tissue context. Here, we aim to identify ILC subtypes in murine lung and SI tissue as well as changes in their microenvironment during inflammatory processes. Combining multi epitope ligand cartography (MELC), a Type 2 systemic inflammation model based on consecutive IL-33 injections with a semi-automated image analysis workflow was used for segmentation, and extracting single-cell information with spatial information MELC data in murine lung and SI. Dimensionality reduction, cluster and spatial co-enrichment analysis was adapted and optimized for the needs of MELC data. Additionally, we used our data set to train a DeepLabv3+ model for automated cell segmentation. We identified ILC subtypes and major cell types in both organs. Lung ILC2s clustered in tissue niches shared with lymphatics and myeloid cells, while NK cells/ILC1s rather localized in parenchymal tissue areas associated with blood endothelial cells in the lungs. We observed intestinal NK cells/ILC1s/ILC3s predominantly present in basal regions of villi and crypts under homeostasis shifting towards the luminal site of the villi after induction of inflammation. Our trained AI-model achieved mean Pixel Accuracy (mPA) of 93.06% highlighting the effectiveness of DeepLabv3+ in addressing the challenges of cell segmentation. Here, ILC subtypes were resolved for the first time in MELC data from murine lung and SI tissue and determined different predominant tissue niches of ILC subtypes in both organs representing the basis for further investigations linking spatial and phenotypical/functional behavior. Newly AI-based solutions customized to high-parameter microscopy data will help with exploiting the full potential of the data.

Abstract No. 33

Immunophenotyping in bullous pemphigoid to identify new therapeutic targets

E. Bodner[1, *], F. J. Hilke[1], K. Meier[1], F. Solimani[1], K. Ghoreschi[1]

[1]Department of Dermatology, Venereology and Allergology, Charité – Universitätsmedizin Berlin, Berlin, Germany
[*]Presenting author

Background: Bullous pemphigoid (BP) is a rare autoimmune, blistering skin disorder mostly affecting the elderly. Affected patients develop skin-targeting autoantibodies, produced by B cells with the help of T cells. Previous studies have described T cell involvement in other blistering skin diseases, but the exact role of T cell subsets in BP pathogenesis needs further investigation.

Objective: We aim to examine the distribution of Th and Tfh populations from peripheral blood mononuclear cells (PBMCs), their cytokine profiles and tissue cytokine gene expression in BP patients and non-BP controls in an age-dependent manner.

Methods: Multiparametric flow cytometry was utilized to study the frequencies of Th and Tfh cell subsets and their cytokine outputs. Real-time quantitative PCR was performed for transcriptome analyses of BP lesional versus healthy skin. Patient medical histories were examined to record comorbidities and autoantibody titers against BP antigens 180 and 230.

Results: There were 66 BP patients and 65 non-BP controls enrolled in this study. Results showed increased numbers of circulating Th and Th17.1 cells in the peripheral blood of patients with active disease versus those in remission. Controls also exhibited higher levels of circulating memory T cells compared to active and remittent BP patients. In skin, Th2-related cytokine gene expressions (IL-4, IL-5, IL-13, IL-31) were elevated in active BP patients in contrast with controls and were found to have strong positive associations with one another via a Spearman correlation analysis. In addition, IL-10 expression in skin was upregulated in active BP patients as opposed to controls.

Conclusion: Patients with active BP present with higher numbers of Th and Th17.1 cells in peripheral blood and increased Th2 and Treg-related cytokine gene expressions in lesional skin.

Abstract No. 34

The role of terminal fucosylation in pneumococcal pneumonia

Cengiz Goekeri[1,*], Sebastian Schickinger[1], Alina Nettesheim[1], Kerstin Linke[1], Leila Bechtella[2], Gael Vos[2], Elena Lopez-Rodriguez[1], Vladimir Gluhovic[1], Anne Voß[2], Sandra Kunder[2], Jade Bath[3], Katharina Ribbeck[3], Kevin Pagel[2], Achim Gruber[2], Matthias Ochs[1], Peter Seeberger[4], Martin Witzenrath[1], and Geraldine Nouailles[1]

[1]Charité - Universitätsmedizin Berlin, [2]Freie Universität Berlin, [3]Massachusetts Institute of Technology
[4]Max Planck Institute of Colloids and Interfaces

[*]Presenting author

Background: Mucosal tissue surfaces such as that of lungs are rich in N- and O-glycans containing terminal sugars such as fucose. Some individuals have mutations in the fucosyltransferase (FUT) genes (FUT1/FUT2), resulting in the inability to secrete α-(1,2)-fucosylated glycans. Such mutations render these individuals susceptible or resistant to infection by certain pathogens.

Aim: Investigation of how terminally fucosylated glycans influence Streptococcus pneumoniae (S.pn.) infection. Methods: We developed a murine model of pneumococcal pneumonia using mice systemically treated with 2-deoxy-D-galactose (2d-Gal), a biochemical inhibitor of fucosylation. We evaluated the murine glycome using liquid chromatography-tandem mass spectrometry. Lung immune cell recruitment was analyzed via flow cytometry. Pro-inflammatory cytokine concentrations and degree of lung permeability were determined through ELISA. Bacterial gene expression was analyzed following RNA sequencing of S.pn.

Findings: Fucose utilization genes are upregulated in S.pn. following incubation with natively purified gastric porcine MUC5AC in vitro. Fucosylated glycans are shed into the bronchoalveolar lavage (BAL) during S.pn. infection. Systemic treatment of mice with 2d-Gal resulted in incorporation of 2d-Gal into the murine glycome and inhibition of fucosylation in vivo. Mice treated with 2d-Gal displayed improved physiological parameters such as infection-induced body weight and temperature loss. Treatment with 2d-Gal led to reduced pulmonary bacterial burden in mice following S.pn. infection. Flow cytometry revealed that 2d-Gal treatment enabled quicker resolution of neutrophilic inflammation following infection. 2d-Gal treatment led to reduced lung permeability and lower IL-6 and TNF-α levels in BAL fluid of infected mice.

Conclusion: Our findings reveal that 2d-Gal treatment prevents the establishment of pneumococcal infection in mice. Inhibiting pulmonary fucosylation could limit S.pn. virulence and pneumonia pathogenesis.