How to get there:

Directions to: Hörsaalzentrum, Allgemeines Krankenhaus, Währinger Gurtel 18 – 20, 1090 Vienna, Austria.

Guests arriving from the Wien Schwechat airport can reach the Hörsaalzentrum by:

1. Taxi: Taxis are available right outside the airport terminal. 20 - 25 mins to the conference from the airport, fares ranging from 40 – 45 € (higher during night time). Contact: +43 676 7408005, email: http://www.123taxi.at/de/.

2. City Airport Train (CAT): 16 min to Wien-Mitte Landstraße every 30 mins (from 05:36 to 23:36). 12 € per ticket (with return 19€) available in ticket vending machines at the airport. From Wien-Mitte Landstraße, take U4 green metro line to Spittelau (towards Heiligenstadt). Then take the U6 brown metro line to Michelbeuern AKH (towards Siebenhirten). Metro tickets can be bought at vending machines in the metro station.

3. Schnellbahn (S – Bahn) S7: S – Bahn S7 trains have the same route as that of CAT trains with multiple stops running once in every 15 – 30 mins. Time to Wien-Mitte Landstraße is 25 mins and a single ticket costs 4,40 €. From Wien Mitte, follow the same directions as given above for the CAT. Tickets can be bought at ÖBB vending machines / counters.
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In order to offer cutting-edge patient treatment, providers of medical care must not only act in the wards but also in the laboratories. At the Medical University of Vienna, which also runs the Vienna General Hospital, we believe that by schooling students in medicine they will learn to provide effective relief to patients, but by additionally training them in science, they might move on to discover cures. To this end, we study the underlying causes for disease by focusing on how cells exchange vital information. Our doctoral training program “Cell Communication in Health and Disease” (CCHD) provides students with challenging research projects that range from basic biomedical sciences to translation into clinical application. CCHD gives students the opportunity to acquire skills that can be employed in highly divergent areas, by exposing them - within a single multidisciplinary framework - to four research themes that deal with organ-independent ubiquitous regulatory systems: neurobiology; vascular biology; immunology; and inflammation research.

Ten years ago, the first students joined CCHD, and we are proud and grateful to be able to celebrate the 10th anniversary of our program. Since 2007, 84 students started their thesis projects, 41 graduated, and all together our student fellows published over 150 papers. After several symposia that were held together with other excellent Austrian PhD programs, our in tenth International Workshop of Cell Communication in Health and Disease is organized by the current student members. In accordance with scientific multidisciplinarity as the major asset of CCHD, this workshop brings together experts in the four CCHD research topics with the aim of bridging the gap. The highly interesting program spans from infection biology to emotions and behaviour. I would like to thank the students for putting together this exciting program and our guests for accepting the students’ invitations in order to share their results and expertise with us. I look forward to illuminative presentations and lively discussions and I do hope that not only the CCHD students, but also all other participants will keep this CCHD workshop in mind as unforgettable event.
On behalf of the CCHD committee, we would like to welcome you to the 10th CCHD Bridging the Gap Symposium. As CCHD stands for ‘Cell Communication in Health and Disease’ we will follow in the footsteps of previous meetings by putting together a program focusing on cell signaling and translational research at the frontiers, along with the challenges and questions that remain unanswered in the fields of immunology, vascular biology, neurobiology and inflammation. We will discuss emerging technologies in these disciplines and attempt to bring about new scientific ideas leading the research to the cutting edge of science. Our goal is to present and learn about state of the art discoveries, as well as to promote and encourage communication, scientific interactions and collaborations that would facilitate the progress of basic and translational research.

The symposium consists of plenary talks by renowned international scientists in their respective fields, along with talks of new faculty members of CCHD. Student poster sessions will take place on both days of the symposium.

We would like to thank our sponsors from the pharmaceutical industry for kindly supporting the event. Our deep gratitude goes also to all students and staff who contributed to the organization of this symposium. But most importantly, we would like to thank you, the attendees, for making it all worthwhile and we hope that all of you will enjoy the symposium and we are looking forward to engaging talks, stimulating discussions and a memorable social event.

Sincerely,

The organizing committee:

Martina and Stella
# Program
## Day 1 - Tuesday, April 18th 2017

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<td><strong>Membrane dynamics of Zap70 kinase control T cell responses</strong> Björn Lillemeier, Salk Institute for Biological Studies, USA</td>
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Program
Day 2 - Wednesday April 19th 2017

09:30-10:00  Registration and Poster Mounting

10:00 - 11:30  Oral Session 1
chaired by Stefan Böhm, Thomas Klausberger

10:00-10:45  Perinatal programing and depression in later life
Daniela D. Pollak, Center for Physiology and Pharmacology, Medical University of Vienna, Austria

10:45-11:30  Moving by the Engram: from the neuronal encoding to the behavioural translation of a hippocampal memory trace
David Dupret, MRC Brain Network Dynamics Unit University of Oxford, Oxford, UK

11:30-11:55  Coffee Break

11:55-13:40  Oral Session 2
chaired by Hannes Stockinger

11:55-12:40  Communication at the Immunological Synapse
Gillian M Griffiths, Cambridge Institute for Medical Research, UK

12:40-13:40  Lunch + Poster session

13:40 - 14:25  Oral Session 3
chaired by Christine Mannhalter, Johannes Berger

13:40-14:25  Roles of platelets and platelet-derived extracellular vesicles in inflammation
Eric Boilard, Centre Hospitalier de l’Université Laval (CHUL), Quebec, Canada

14:25-14:40  Closing notes
Stefan Böhm, Center for Physiology and Pharmacology, Vienna, Austria
Peter Jonas studied medicine at the University of Giessen, Germany. He obtained his M.D. in physiology in the group of Werner Vogel. After a short time as a post-doc, he moved to the Max Planck Institute for Medical Research, Heidelberg, Germany as a research assistant, working with the Nobel laureate Bert Sakmann from 1990–1994. In 1994, he took over a position as Associate Professor at the Technical University of Munich, Germany. In 1995, he accepted a position as full Professor and head of department of physiology at the University of Freiburg, Germany. In November 2010, he moved to IST Austria as a full professor and founder of the Neuroscience research cluster at the Institute. Peter Jonas received several research prizes, including the Leibniz award of the German research Council, the highest research price in Germany, the Fick award, the highest award of the German Physiological Society, two ERC Advanced Grants, and the Wittgenstein Award of the FWF. He is also member of the German Academy of Sciences Leopoldina and the Academia Europaea.
Fast signaling in parvalbumin+ GABAergic interneurons: From molecular design to microcircuit function

Fast-spiking, parvalbumin-expressing (PV+) interneurons contribute to several higher neuronal network functions, such as feedback and feedforward inhibition, oscillatory activity, and pattern separation. For all of these functions, the rapid signaling properties of PV+ cells play an important role. However, the mechanisms of rapid signaling in this important class of GABAergic interneuron remain incompletely understood. We addressed the signaling properties of PV+ cells at the molecular, subcellular and cellular, and microcircuit level. Combining paired recordings with genetic elimination and viral rescue approaches, we identified synaptotagmin 2 (Syt2) is the main Ca2+ sensor of exocytosis at output synapses of PV+ interneurons in cerebellum, and further demonstrated that the specific properties of Syt2 contributed to rapid signaling. Using subcellular patch-clamp recording, we examined the properties of voltage-gated Na+ channels in the axon of PV+ interneurons in the dentate gyrus. We found that fast Na+ channel gating, especially fast Na+ channel inactivation, contributed to both the brief time course and the high energy efficiency of the action potential. Finally, combining in vivo whole-cell recording and field potential recording in awake, behaving animals, we found that PV+ interneurons make a major contribution to the synaptic conductances underlying sharp wave-ripple oscillations in the hippocampal CA1 region. In conclusion, our results indicate that several molecular mechanisms contribute to rapid and energy-efficient signaling in PV+ interneurons, and further suggest that fast interneuron signaling plays a key role in the generation of high-frequency network oscillations in cortical circuits.

Gero Miesenböck studied medicine at the University of Innsbruck in his native Austria and did postdoctoral research at Memorial Sloan-Kettering Cancer Center in New York. He was on the faculty of Memorial Sloan-Kettering Cancer Center and Yale University before coming to Oxford in 2007. Gero is the founding director of the CNCB.

Gero has invented many of the optogenetic techniques used for visualizing and controlling nerve cells with light. He has study neural circuits.

Optogenetics: Lighting Up the Brain

Optogenetic methods enable an experimental dialogue with biological systems composed of many different cell types—in particular, with neural circuits in the brain. These methods are called “optogenetic” because they use light-responsive proteins (“opto-“) encoded in DNA (“-genetic”). Optogenetic devices can be introduced into tissues or whole organisms by genetic manipulation and be expressed in anatomically or functionally defined groups of cells. In a decade and a half, optogenetic control of neuronal activity has developed from a far-fetched idea to a widely used technique. My talk will recount how this happened, drawing on the earliest and latest results from my lab. To illustrate what is now possible, I will present recent work on the homeostatic regulation of sleep. Optogenetics has allowed us to pinpoint a homeostatic sleep switch in the brain, map its synaptic connections, and test mechanistic ideas of how the switch works.
Professor Gillian Griffiths FMedSci, FRS obtained her PhD at the MRC Laboratory of Molecular Biology in 1984, studying affinity maturation of the immune response with Cesar Milstein. After a post-doctoral fellowship with Irv Weissman at Stanford, she started her own lab at the Basel Institute for Immunology. She returned to the UK as a Wellcome Trust Senior Fellow, first at UCL and then the Dunn School of Pathology in Oxford. She moved to the Cambridge Institute for Medical Research (CIMR) as a Wellcome Trust Principal Research Fellow in 2007 and has been Director of the CIMR since December 2012. Gillian’s research interests are focused on understanding the cell biology of polarised secretion from lymphocytes, using insights gained from genetic disease to identify the molecular mechanisms underlying this process. Her work has identified a novel role for the centrosome in directing polarised secretion from cytotoxic T lymphocytes, and novel links with Hedgehog signalling. Together, these studies have highlighted the possible origin of the immunological synapse as a modified cilium.

**Communication at the Immunological Synapse**

Cytotoxic T lymphocytes (CTL) use polarized secretion of specialized lysosomes from the endocytic pathway to destroy virally infected and tumor cells with great specificity. Secretion is focused at an immunological synapse formed between killer and target, so named because of its similarities to neuronal synapses. This structure provides a remarkable example of cell polarity, with membrane receptors, cell cytoskeleton and secretory organelles focused towards the point of interaction. In order to understand how CTL provide such focused and efficient killing, we use high-resolution multi-colour imaging in 4D and 3D electron tomography revealing the mechanisms controlling secretion from CTL. Using CTL derived from patients with genetic defects that disrupt secretion we have been able to identify many of the mechanisms that lead to secretion. Surprisingly we also found that similarities also exist between the immunological synapse and primary cilia. In both cases the centrosome polarizes to the plasma membrane creating a region of the plasma membrane that becomes specialized in signaling, endocytosis and exocytosis. Insights from ciliogenesis now provide another way of identifying the molecular mechanisms controlling secretion.
Johannes Huppa studied biochemistry at the Freie Universität Berlin. For his diploma and doctoral thesis he joined Hidde Ploegh’s lab at the Massachusetts Institute of Technology and Harvard Medical School, where he analyzed the biogenesis and ER-quality control of the TCR-CD3 complex. As a postdoc Dr. Huppa worked with Mark Davis at Stanford University to devise live cell and single molecule imaging approaches as a means to study T-cell antigen recognition both on a cellular and molecular level. He showed that T-cells, when in contact with APCs, signal through their TCRs over many hours and that such prolonged signaling maintains the integrity of the immunological synapse and promotes the full effector T-cell potential. He then developed a Förster Resonance Energy Transfer (FRET)-based microscopy approach to visualize and quantitate TCR-antigen interactions within the immunological synapse. Dr. Huppa started his own lab in 2012 at the Medical University of Vienna, where he combines innovative (single molecule) microscopy methods (total internal reflection microscopy, FRET, particle tracking, super resolution), bioengineering and state-of-the-art immunological methods to identify and quantitate cell-specific parameters, which modulate TCR-ligand binding, TCR-crosstalk with accessory factors and downstream signaling.
How T-cells Recognize Antigens - an Imaging Approach

T-cells are remarkably sensitive towards antigen; they can detect the presence of even a single antigenic peptide/MHC complex among thousands of non-stimulatory peptide/MHC complexes on the surface of antigen-presenting cells (APCs). Of note, TCR-peptide/MHC interactions are only of moderate strength when measured in solution and 2-3 orders of magnitudes weaker than typical antibody-antigen interactions. How can we then explain the phenomenal degree of T-cell antigen sensitivity, a hallmark of the adaptive branch of immunity?

TCR-peptide/MHC binding takes place, provides at least in part the answer. Binding parameters are severely influenced because receptors and ligands are pre-oriented, to some extent clustered and moreover subjected to cellular forces. To account for these nonlinear properties of the contacting cells, we have devised a non-invasive ultrasensitive live-cell imaging approach, in which synaptic TCR-pMHC binding events are directly detected and quantified in situ. We find TCR-binding indeed significantly increased within the synapse with both an accelerated association and, due to cellular forces, an accelerated dissociation. Moreover, TCR affinities vary substantially within different synaptic regions and also between different cells. These observations imply that TCR-peptide/MHC binding and the entire process of antigen recognition are controlled through not well-understood cell-biological parameters, which might also be subject to regulation. Identifying these parameters and quantifying their effects on the efficacy of T-cell antigen recognition stands in the forefront of our research.
Björn F. Lillemeier obtained his Ph.D. in Biochemistry at Cancer Research UK in London (UK) in 2001. For his postdoctoral training in Immunology he joined the laboratory of Mark M. Davis at Stanford University (USA) (2002-2009). During his postdoctoral research, he found that plasma membrane proteins are distributed in nanometer scale membrane domains, namely protein islands. He further showed that these membrane domains play a crucial role in T cell activation through the T cell receptor signaling pathway. In 2009, he was recruited to the Salk Institute for Biological Studies (USA) where he studies the molecular assembly and spatial organization of signaling pathways at the T cell plasma membrane. Using multidisciplinary strategies, he has discovered novel mechanisms that control T cell sensitivity and specificity. He is currently an associate professor and holds the prestigious NIH Director’s New Innovator Award.

Membrane dynamics of Zap70 kinase control T cell responses.

T cell activation is controlled by a multitude of actions that have to be orchestrated in space and time. This includes the segregation and co-localization of signaling molecules into nanometer sized membrane domains, the assembly of multi-protein complexes and the regulation of catalytic activities through conformational changes and post-translational modifications (PTMs). Using a multidisciplinary approach, we have found that T cell activation is controlled by the constantly changing interaction dynamics of Zap70 kinase with the T cell receptor (TCR) and the plasma membrane. Initially, the TCR-dwell times of Zap70 control T cell quiescence and activation thresholds. During early T cell activation, a tightly controlled cycle of Zap70 recruitment, activation, and release from the TCR turns the receptor into a “catalytic unit” that amplifies antigenic stimuli and generates a large number of active Zap70. Released Zap70 remains active and plasma membrane associated, and physically transfers TCR signals to spatially distinct membrane domains. T cell activation is maintained over longer periods of time by continuous signaling of stable TCR-Zap70 complexes. These findings suggest that constantly changing conformations and protein-protein interactions control how, when and where proteins fulfill specific functions. Because universally utilized protein domains, PTMs and membrane organizations control these mechanisms they are most likely applicable to many plasma membrane processes.
Daniela D. Pollak obtained her PhD in Medical Neurosciences at the Medical University of Vienna in 2005. She then did a three years postdoctoral research training in the laboratory of Nobel Prize Laureate Prof. Eric Kandel at Columbia University, New York, USA (2006-2008). In 2009 she returned to Vienna and is since then holding a position at the Centre for Physiology and Pharmacology, first at University Assistant, then as Assistant Professor before being promoted as Associate Professor in 2013. As of October 2016 Daniela D. Pollak has been appointed Professor of Behavioural Biology at the Medical University of Vienna where she is leading a research group focusing on the exploration of the neural underpinnings of psychiatric disorders in experimental animal model systems.

Perinatal programing and depression in later life

Early-life experience of positive and negative environmental influences has life-long consequences for neurodevelopment, brain function and behavior. Epidemiological studies in people as well as observations in animal models have demonstrated the detrimental consequences of adverse intrauterine and postnatal environments, such as those resulting from stress exposure during pregnancy and parental neglect and abuse. We have developed specific paradigms to study in laboratory mice two concrete, clinically and socioeconomically relevant conditions, namely gestational infection and prenatal iron deficiency and their long-term impact on the development of depression-like behavior later in life. Using a combination of behavioral, neuroimaging, cellular and molecular approaches, we are working on characterizing the principles mediating this biological embedding. Recently we have focused on the epigenetic basis and the transgenerational effects accounting for the persistency of the “perinatal programming” of emotionality and are elucidating the role of maternal care as central mediator in this context. Enhancing our understanding of the nature of very early-life imprinting of mental health and elucidating its neural underpinnings may contribute to the development of alternative preventive and therapeutic approaches for the treatment of some of the most prevalent and excruciating psychiatric disorders.
Thomas Marichal graduated in 2007 as a Doctor in Veterinary Medicine from the University of Liège (BE). He obtained a PhD in Immunology in June 2011 (Laboratory of Cellular and Molecular Immunology, GIGA-Research Institute, University of Liege, BE) and was awarded a Marie Curie International Outgoing Fellowship from the European Commission for a postdoctoral training at Stanford University (California, USA), with Prof Stephen J. Galli as a mentor. Since 2014, he is back as a junior principal investigator at the GIGA-Research Institute in Liege (BE), thanks to a “Chargé de recherches” fellowship of the F.R.S.-FNRS. In 2016, he has obtained a tenure-track position as a Research Associate of the F.R.S.-FNRS., and he is also a Lecturer at the Faculty of Veterinary Medicine at the University of Liege.

Dr. Marichal’s current research activity is mainly focusing on the pathophysiology of allergic disorders, with a special focus on the role of innate danger signals and myeloid cells in regulating homeostasis and type 2 allergic immune responses in the lung.
Innate danger signals influencing allergic asthma development: focus on bacterial and host DNA

Type 2 immune responses contribute to host defense against helminths and venoms, but can also be deleterious in the context of allergic disorders. In the lung environment, how allergen-induced type 2 responses are prevented in homeostasis and triggered in allergic asthma remain incompletely understood.

It has long been known that exposure to a microbe-rich environment and to unmethylated CpG-DNA from bacteria (CpG-DNA) is associated with a reduced risk of developing allergic asthma. However, how CpG-DNA confers protection remains elusive. Our group has shown that exposure to CpG-DNA protects from allergic asthma by expanding regulatory lung interstitial macrophages (IMs) from monocytes infiltrating the lung or mobilized from the spleen. Such innate mechanism may underlie the reduced risk of asthma associated with a microbe-rich environment, as well as the protective effects of synthetic CpG-DNA.

Respiratory viral infections represent the most common triggers of type 2 mediated-allergic asthma exacerbations, but how virus infection boosts type 2 responses during exacerbation is poorly understood. I will present you experimental evidence demonstrating that host double-stranded DNA (dsDNA) released by NETosis can act as a potent trigger of rhinovirus-induced type 2 allergic asthma exacerbation. We show that rhinovirus infection triggers neutrophil extracellular traps (NETs) formation and host dsDNA release in mice and humans. We further demonstrate that inhibiting NETosis by blocking neutrophil elastase or degrading NETs with DNase protects mice from type 2 allergic asthma exacerbations. Thus, NETosis and host dsDNA contribute to exacerbation pathogenesis and may represent potential targets for novel treatments of rhinovirus-induced asthma exacerbations.


David Dupret is a French-born behavioural neurophysiologist working at the MRC Brain Network Dynamics Unit, University of Oxford. David completed his Ph.D. in Neuroscience at the Institute François Magendie, for which he received the French Neuroscience Association’s 2007 Ph.D. prize of the year. After graduating, David joined the group of Prof. Jozsef Csicsvari at the MRC Anatomical Neuropharmacology Unit, funded by the ‘Institute of France’ (Foundation Louis D. Research Fellowship 2007) and the International Brain Research Organisation (IBRO Research Fellowship 2008). In 2009, David was appointed as a MRC Investigator Scientist, and joined St Edmund Hall College, Oxford. David was appointed to a MRC Senior Scientist position in 2011, and promoted to tenured MRC Programme Leader in 2015. In 2016 David was elected a scholar of the FENS-Kavli network. The general aim of his programme is to investigate neuronal dynamics in the hippocampus and related circuits during the formation of spatio-contextual memories and the expression of adaptive behaviours.

Moving by the Engram: from the neuronal encoding to the behavioural translation of a hippocampal memory trace

How does the brain support the emergence of internal representations of the external world and what are the mechanisms underlying the persistence of a limited subset of these representations for the purpose of memory-guided behaviour? In this talk I will present a series of experiments that address these questions by monitoring and manipulating neuronal dynamics in the mouse hippocampus. First, I will show how the neuronal encoding of a hippocampal representation of space, and its artificial recoding, supports the behavioural expression of a drug-place memory. I will then provide experimental evidence for a central role of off-line replay in the subsequent awake retrieval of newly-acquired place representations. Finally, I will present ongoing work identifying a neuronal gateway that turns a memory representation into its behavioural fingerprint. Altogether, these findings highlight how short-timescale neuronal dynamics can support the expression of an internal representation of space and its translation into a behaviour.
Eric Boilard obtained his PhD in Immunology at Universite Laval, in Quebec City, Canada, in 2003. Then he endeavoured a postdoctoral training at Universite de Nice Sophia-Antipolis in France, in molecular pharmacology (2003-2006), and a second postdoctoral training in rheumatology and immunology at Harvard Medical School in Boston, USA (2006-2010). During his trainings, he found that platelets and their microparticles could contribute to the inflammatory process in rheumatoid arthritis (Boilard, Science 2010). In 2010, he was recruited at CHU de Quebec and Universite Laval, where he is conducting his research on platelet roles in inflammatory conditions, such as rheumatic diseases and platelet transfusion adverse reactions. He is currently associate professor of medicine, he holds the prestigious Canadian Institutes of Health Research investigator award, and his research program is supported by a Canadian Institutes of Health Research Foundation grant.

**Roles of platelets and platelet-derived extracellular vesicles in inflammation**

On activation or apoptosis, platelets shed submicron vesicles known as microparticles, also called microvesicles. As any other cellular lineages may also produce extracellular vesicles, microparticles are heterogeneous on the basis of their cellular origin. Recent studies further demonstrate that microparticles derived from platelets harbour different surface markers, and that some of them can also convey functional organelles, such as mitochondria. Extracellular mitochondria, which are recognized damage-associated molecular patterns, were suggested to contribute to inflammation. Interestingly, several studies confirmed extrusion of mitochondria during platelet isolation, storage, and treatment with pathogen inactivation systems. Moreover, extracellular mitochondria are also observed in chronic diseases, such as rheumatic diseases. Microparticles and extracellular mitochondria can interact with cells in transfused recipients and in patients suffering of rheumatic diseases. Their accurate detection may reveal distinct roles for the subtypes of microparticles in transfusion medicine and rheumatic diseases.
Johannes Berger graduated from University of Vienna for biology/genetics in 1989. He performed his PhD at the Sandoz Research Institute, Department of Antiretroviral Therapy or training in molecular biology and biochemistry. He then joint the Institute of Neurology, University of Vienna. He improved his technical skills at the Kennedy Krieger Institute in Baltimore, received his habilitation for Molecular Biology and become associated professor in 1999 at the Medical University of Vienna. In 2003 he habilitated for Biochemistry at the University of Vienna. Since 2007 he is full professor at the Medical University of Vienna and head of the department for Pathobiology of the Nervous System at the Center for Brain Research. He coordinated a EU-project concerning the development of novel therapeutic strategies for X-linked adrenoleukodystrophy (5 Million €) as well as a EU-project to decipher the biological functions of peroxisomes in health and disease (8 Million €). Since 2006 he is coordinator of the PhD program neuroscience at the Medical University Vienna. He received several awards and honours among them the Kardinal-Innitzer Award in 2001 and the Otto Loewi Award in 2003 awarded by Austrian Neuroscience Association. In 2005 he was elected as Researcher of the Month by the Medical University of Vienna. His current research activities concentrate on the role of peroxiosmes in the nervous system for health and disease.
X-linked Adrenoleukodystrophy: From Basic Research to Possible Therapeutic Strategies

X-linked adrenoleukodystrophy (X-ALD) is the most common peroxisomal disorder. The default manifestation of X-ALD is a slowly progressive dying-back axonopathy affecting the spinal cord. About 60% of male patients, either in childhood or adulthood, develop rapidly progressive cerebral inflammation and demyelination (CALD) leading to premature death. X-ALD is caused by inherited mutations in the ABCD1 gene. The encoded ABCD1 protein, a member of the peroxisomal ABC transporter family transports CoA-activated very long-chain fatty acids (VLCFA) into the peroxisome for degradation by β-oxidation. Overexpression of ABCD2, the closest homologue of ABCD1, can compensate for ABCD1 deficiency in vitro and in vivo. Currently, the only curative treatment for CALD, allogenic hematopoietic stem cell transplantation, is only effective when performed at an early stage. We investigated the extent of the metabolic defect in the main immune cells of X-ALD patients: in T and B cells, which show high and intermediate ABCD2 expression, respectively, accumulation as well as peroxisomal β-oxidation of VLCFA (C26:0) was indistinguishable from that of healthy controls. In contrast, monocytes, which lack compensatory VLCFA transport by ABCD2, displayed a severely impaired VLCFA metabolism. Based on these results, we propose that in CALD, the halt of inflammation after allogeneic hematopoietic stem cell transplantation, relies particularly on the replacement of the monocyte/macrophage lineage. In experiments applying knockout mouse models, Abcd2 constitutes a strong modifier of the metabolic impairments in peritoneal macrophages of Abcd1-deficient mice. These, investigations provide the first hints of the level of ABCD2 expression required to obtain a therapeutic effect on the VLCFA metabolism in monocytes/macrophages. Analyses of the ABCD2 promoter in human monocytes demonstrate the feasibility of pharmacological induction of ABCD2. In summary, our results provide support for upregulation of ABCD2 in the monocyte lineage as a therapeutic strategy to halt the devastating inflammatory demyelinating in X-ALD.
18.04.2017 POSTER SESSION

Neurobiology

P1 Sex differences in nociceptive transmission

Viktoria Hadschieff, Ruth Drdla-Schutting, Jürgen Sandkühler

Center for Brain Research - Medical University of Vienna, Vienna, Austria

Background: A growing body of evidence suggests that glial cells play a major role in the processing of nociceptive information at the spinal level. A recent, however controversially discussed study suggested that this holds true only for male mice, whereas in female mice T-cells might play a crucial role. Long-term potentiation (LTP) at spinal C-fiber synapses is a well-established cellular model for pain amplification. However, research was so far exclusively performed on male rodents. Here, we tested whether there are sex-differences in LTP in male versus female rats. Additionally, we analysed the role of glial cells in this mechanism in both sexes.

Methods: C-fiber-evoked field potentials were recorded in the superficial laminae of the dorsal horn in deeply anesthetised male and female rats. LTP was induced by high electrical frequency stimulation (HFS) of the sciatic nerve or by abrupt morphine-withdrawal. The glial-cell blockers minocycline as well as fluoroacetate was applied to assess the contribution of glial cells on the induction of synaptic LTP.

Results: HFS as well as morphine withdrawal significantly potentiated C-fiber-evoked field potentials in both male and female rats. Systemic application of minocycline or spinal application of fluoroacetate completely prevented HFS-induced LTP induction in rats of both sexes.

Conclusion: Thus, our data obtained so far from rats do not support the sex differences reported in mice. Planned experiments will address if species differences exist or if pain models are relevant.

P2 Correcting misfolded dopamine transporter using pharmacological chaperones

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Background: Neurotransmitter transporters are transmembrane proteins belonging to the solute carrier 6 gene family. These proteins harbor a cytosolic C terminus that contains a conserved RI/RL motif. The RI motif has been shown to interact with the endoplasmic reticulum (ER)-export machinery, particularly the COPII component SEC24; of which 4 isoforms exist, SEC24A-D. Mutation of conserved RI / RL to AA results in impaired ER-export which leads to transporter concentrating in the somatodendritic compartment of cultured raphe neurons. It remains interesting to understand if the conserved R is sufficient enough for the recruitment of SEC24 isoforms. Current research addresses if the conserved R607 of human serotonin transporter (SERT) is sufficient enough for recruitment of SEC24C and if this can be phenocopied in Drosophila melanogaster.

Methods: Uptake and binding assays were performed on HEK293 cells, which were transiently transfected with tagged hSERT and hSERT-R607A. Microinjection were performed in Drosophila embryos for obtaining transgenic flies stably expressing dSERT-R599A and dSERT. Various genetic tools were used to visualize protein trafficking in fly brain.
Results: The SERT R607A mutant eventually reaches to plasma membrane in SEC24C independent manner. In raphe neuronal culture, the SERT R607A mutant remained confined to somatodendritic compartment. The distribution of SERT R607A mutant varies from wild-type SERT in Drosophila melanogaster.

Conclusion: Advanced genetic tools are currently being used to study the trafficking of SERT R607A mutant in single cell clones in Drosophila brain. The SERT R607A mutant will be expressed in SERT null background to study the functional importance of somatodendritically located SERT.

**P3 Firing Pattern of Distinct Types of Excitatory Neuron in Rat Prefrontal Cortex During Working Memory and Cognitive Flexibility**

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Background: Prefrontal cortex plays role in goal-predictive decision making, spatial working memory, and cognitive flexibility. The cerebral cortex in general, and the PFC in particular comprises distinct types of neuron which orchestrate network activity differentially. The contribution of each cell type to this coordination may depend on dendritic and axonal arborization, input and output connectivity, molecular expression pattern and physiological properties. However, it remains elusive what role distinct types of excitatory neuron play in driving a behavior.

Methods: We carried out in vivo extracellular recordings and juxtacellular labelling of single cells in rats performing a strategy switching task, we investigated different contributions of distinct excitatory cells in medial prefrontal cortex comprehensively. Using moveable tetrode and silicon probes, we recorded neural activities and local field potentials across all cortical layers to explore population coding underlying working memory and cognitive flexibility.

Conclusion: The diverse changes of firing patterns upon rule switch might be related to the existence of distinct types of pyramidal cell in the prefrontal cortex. By recording and juxtacellularly labelling these cells during the rule switching task, we test the hypothesis that distinct types of pyramidal cell, differentiated by their axonal projections and molecular expression, contribute differentially to cognitive flexibility and representation of changing.

**Immunology & Inflammation**

**P4 Probing the immuno-regulatory function of Lipocalin 2 in pulmonary diseases**

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Background: Influenza is one of the most important viral pathogens and well known for its ability to cause pandemic infections that are associated with high mortality and severe immunopathology. Recent work shows that the commensal microbiota composition plays a critical role in the regulation of immune responses to viral infections. Lipocalin 2 (Lcn2)
is a small protein, which limits iron acquisition of siderophore-dependent bacteria. This effect is important for host defense against infections, but can also influence commensal communities during homeostasis. Furthermore, Lcn2 is involved in immuno-modulatory mechanisms as it deactivates macrophages and impairs bacterial clearance in pneumonia caused by Streptococcus pneumoniae (a siderophore independent pathogen). The role of Lcn2 in antiviral immunity has not been studied.

**Methods:** We investigate the influence of Lcn2 on antiviral immune responses and immunopathology during influenza infection using Lcn2-deficient mice and a mouse adapted influenza strain.

**Results:** We found that pulmonary Lcn2 was induced in WT mice following influenza virus infection. Lcn2-deficient mice displayed increased weight loss and enlarged mediastinal lymph nodes after infection, even though there were no differences in viral load. FACS analysis of lungs revealed increased CD8+ T-cell and T helper 1 (Th1) cell counts in Lcn2-deficient mice, while virus specific antibody responses were decreased. Cohousing of Lcn2-deficient mice with WT controls could rescue the increased weight-loss and inflammatory response.

**Conclusions:** Our results indicate that Lcn2 modulates antiviral immune responses by affecting the microbiome. Ongoing studies are focused on elucidating the effect of Lcn2 on commensals and on the potential therapeutic benefit of these mechanisms.

**P5 A potential role of osteopontin in NAFLD-induced hepatocellular carcinoma development**

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**Background:** Hepatocellular carcinoma (HCC) is the cause of approximately one million deaths yearly. Nutrition-related diseases significantly contribute to the increasing prevalence of HCC. In Obesity, osteopontin (OPN, gene Spp1) is upregulated in liver and leads to insulin resistance, type 2 diabetes and NASH.

**Methods:** Using a recently established mouse model, which faithfully reproduces human NAFLD-induced HCC development by treating newborn mice with streptozotocin (STZ) and feeding them a high fat diet (HFD) (STAM mice), we investigated metabolic and immunological parameters throughout the entire disease evolution.

**Results:** Compared to HFD-fed-only (HFD-o) mice, STAM mice showed comparable increased hepatic expression levels of metabolic genes. The expression of pro-inflammatory markers was, however, significantly higher in the STAM animals, indicating the induction of inflammation to further develop fibrosis and HCC. Between 15 and 19 weeks of age, STAM mice but not HFD-o and STZ-injected-only (STZ-o) mice developed liver tumors. In contrast to HFD, STZ alone recruited comparable levels of liver macrophages (LM) as observed in STAM mice, but the expression of M1-activation marker Cd11c and notably of Spp1 was significantly lower. Furthermore, Spp1 and other main immunological cytokines look like to be fine-tuned between the 15th and 19th week in order to elicit first a M2-like (implicated in tumor expansion and metastasis) and later a M1-like LM polarization.

**Conclusions:** Our preliminary data confirm the necessity of inflammation for the establishment of HCC and point for the first time to a potential role of Opn in NAFLD-induced liver cancer.

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P6 Can acute stress dampen allergic reactions? Epinephrine induces a regulatory M2b-like macrophage phenotype, which attenuates cord blood-derived mast cell (CBMC) IgE-mediated degranulation in a human model of allergic inflammation

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Background: The M2a macrophage subtype is a notorious promoter of the Th2 environment during allergic inflammation. Catecholamines on the other hand, are emerging immune modulators and data from mouse studies suggest that these stress hormones may induce immunoregulatory macrophages. We hypothesized that treating M2a macrophages with catecholamines will influence their cross talk to mast cells, thereby dampening allergic inflammation.

Methods: Primary monocytes from healthy PBMCs were first differentiated into M2a macrophages using M-CSF in the presence of IL-4 and IL-13 cytokines. After overnight incubation with epinephrine, supernatants were collected and analyzed by ELISA, whereas cell surface markers were evaluated using flow cytometry. Subsequently, both M2a and epinephrine-treated M2a supernatants were transferred onto cord blood-derived mast cells (CBMCs) for further overnight incubation, after which IgE-mediated degranulation was assessed by the β-hexosaminidase release assay.

Results: After overnight epinephrine treatment, M2a macrophages showed an increase in IL-10, TNF and IL-6 production, but no IFN-γ and IL-12 expression was observed. Epinephrine treatment also downregulated surface markers CD206 and CD163 and upregulated CD86. When supernatants from epinephrine-treated M2a macrophages were added to CBMC cultures, IgE-mediated degranulation was impaired compared to CBMCs treated with supernatants of unstimulated M2a macrophages. Taken together, epinephrine promoted a phenotypic shift of M2a polarized human macrophages toward an M2b-like regulatory phenotype that was able to reduce the IgE-mediated degranulation of CBMCs.

Conclusion: We conclude that prolonged acute stress exposure in allergic patients may attenuate symptoms of acute allergy by directing macrophages towards an immunosuppressive phenotype, which can further dampen mast cell degranulation.

Vascular Biology

P7 Mitochondrial activity is a major contributor to the pro-inflammatory capacity of microvesicles

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Background: Microvesicles (MVs) are recognized as promoters of inflammation and increased numbers of MVs have been described in cardiovascular diseases. Because mitochondrial content has been shown to be present in MVs and promotes pro-inflammatory responses, we investigated whether MVs released by LPS-stimulated monocytes are enriched in mitochondrial content and if this content contributes to their ability to activate endothelial cells.

Methods: MVs were isolated from conditioned media of THP-1 monocytes by differential cen-
and their ability to activate human umbilical vein endothelial cells (HUVECs) was tested.

Results: MVs from LPS-stimulated monocytes that had been labelled with a mitochondrial and cytoplasmic dye were enriched in mitochondrial dye, mitochondrial RNA, and stained positively the mitochondrial outer membrane protein TOM22. These MVs induced IL-8 production and VCAM and ICAM mRNA level in HUVECs. Blocking of IL-1beta, which has been previously reported to be present in MVs, -matory potential of MVs was dramatically reduced when MVs were derived from cells, i) depleted for mitochondrial DNA, and ii) cultivated in the presence of pyruvate, which alters mitochondrial activity. Moreover, only mitochondria isolated from LPS-stimulated monocytic cells were able to activate ECs.

Conclusions: Thus, MVs released by LPS-stimulated monocytes are enriched in mitochondrial content. Furthermore, mitochondrial stress and activity rather than simply mitochondrial LPS-stimulated monocytes to induce an EC response.

P8 B cells in thrombus resolution and Chronic Thromboembolic Pulmonary Hypertension

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Background: Chronic thromboembolic pulmonary hypertension (CTEPH) is a rare consequence of venous thromboembolism. Mechanisms underlying thrombus persistence are unclear. Since splenectomy is a risk factor for CTEPH and the spleen is important for B cell maturation, we investigated the role of B cells in thrombus resolution and CTEPH.

Methods: We studied thrombus resolution mice by subjecting Balb/c mice to partial ligation of the vena cava 4 weeks after splenectomy. We injected isolated B cells i.p and monitored thrombus resolution for 28 days by ultrasound. Furthermore, we analyzed 9 CTEPH patients by mass cytometry and compared them to 9 idiopathic pulmonary arterial hypertension (IPAH) patients and 11 healthy subjects. We stained PBMCs, at baseline and after cell stimulation, with a panel of antibodies against 21 surface markers, 11 phosphoproteins and 7 cytokines. Results: Treating splenectomized mice with isolated B cells resulted in smaller thrombi at days 1 and 3 after ligation. Mass cytometry revealed decreased total B cells in CTEPH compared to IPAH and controls. This did not affect B1 cells, resulting in increased B1 cell frequency relative to total B cells in CTEPH. CTEPH and IPAH B cells showed increased production of IL-6 after stimulation and increased baseline Stat1 phosphorylation.

Conclusions: Our data suggest an important role for B cells in thrombus resolution. B cells promoted thrombus resolution in mice, and individuals with CTEPH were characterized by decreased circulating B cells. CTEPH B cells had an activated phenotype, which may reflect spontaneous germinal center formation and increased (auto-)antibody production.

P9 Hematopoetic Complement Factor H deficiency reduces atherosclerosis in LDLR deficient mice

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Background: Complement factor H (CFH) is the major regulator of alternative complement activation. CFH deficiency is known to induce uncontrolled C3 cleavage leading to complement-triggered tissue damage, which is the primary cause of certain nephropathies and has also been implicated in atherogenesis. Although CFH is primarily secreted by hepatocytes, macrophages can also be sources of CFH. Therefore we investigated the role of hematopoietic CFH in atherosclerosis.

Methods: The expression of CFH by murine and human macrophages was measured by qPCR and ELISA. To study the effect of hematopoietic CFH on lesion formation, 26 10-week-old female Ldlr-/- mice were transplanted with bone marrow from wildtype or Cfh-/- donors and fed an atherogenic diet for 12 weeks. Plasma lipid levels were determined and atherosclerosis was assessed in the entire aorta and the aortic origin.

Results: We confirmed that murine bone marrow-derived and peritoneal macrophages as well as THP1-derived macrophages produce CFH. Hematopoietic CFH deficiency caused no changes in plasma cholesterol and triglycerides upon exposure to atherogenic diet. Nevertheless, it led to an increase in plasma IgM levels and a significant decrease in lesion size and necrotic core formation despite no systemic effects on complement activation measured by plasma C3 levels. This was accompanied by elevated numbers of CD21+CD23- B cells in the spleen. Although splenic B cells do not express CFH, we found that they can bind CFH on their surface. Our current work is dedicated to explore the mechanistic link how hematopoietic CFH affects IgM secretion in atherosclerosis.

Conclusion: Our findings suggest that hematopoietic CFH aggravates atherosclerosis by suppressing the expansion of atheroprotective secreted IgM.

19.04.2017 POSTER SESSION

Neurobiology

P10 A Novel Extra-Dimensional Attentional Set-Shifting Task for Rodents to Explore Prefrontal Networks

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Background: Attention aids adaptive usage of the limited capacity of neural processing systems. An innate part of information processing is the attentional set, which facilitates selection of the relevant, and inhibits processing of distracting information. With assessing the capability of attentional set-shifting, it is possible to measure cognitive flexibility and executive functions. The most widely used neuropsychological task for the evaluation of these functions in humans is the Wisconsin Card Sorting Test, which requires the subject to alter the response strategy and use previously irrelevant information to solve a new set of problems. The test has proven clinical relevance, as poor performance has been reported for multiple neuropsychiatric conditions. However, similar tasks used for rodent models are limited because of their manual-based testing procedures.

Methods: Water-deprived and head-fixed C57BL/6 mice were placed in a virtual environment and exposed to a decision-making task to retrieve small water reward. In addition, silicon probe recordings were performed in the medial prefrontal cortex (mPFC) to address the underlying network mechanisms.

Results: In this novel behavioural task, animals learnt to discriminate two visual perceptual dimensions and they successfully switched their attention between them. We show that neuronal activity in the mPFC is modulated by dif-
different temporally structured task episodes, as well as reward delivery or reward omission. Furthermore, neuronal activity changes after the rule switch.

Conclusions: We demonstrate that our extra-dimensional set shift task for head-fixed mice is an effective tool to study the molecular and cellular mechanisms within neuronal networks underlying executive functions. The understanding of the role of prefrontal network operations in cognitive flexibility is invaluable for understanding numerous neuropsychiatric diseases, such as schizophrenia and depression.

**P11 Actions of hydrogen sulfide in the autonomic nervous system**

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Background: Hydrogen sulfide (H2S) is a toxic gas also produced in mammalian tissues where it can exert various functions as gasotransmitter, such as opening of smooth muscle K-ATP channels resulting in vasorelaxation. Recently H2S was found to be synthesized and released in sympathetic ganglia and to potentiate ganglionic transmission [1]. This project aims to elucidate the role of H2S in autonomic nervous system.

Methods: Primary cultures of rat superior cervical ganglion (SCG) were used to determine release of previously incorporated [3H] noradrenaline and to measure membrane potential and ion currents via the perforated patch clamp technique.

Results: In electrophysiological experiments, NaHS hyperpolarized the SCG membrane potential and reduced action potential firing probably via K-ATP channels. In addition, NaHS inhibited currents through Kv7 channels in a concentration-dependent manner. We studied the possible effect of NaHS on cholinergic miniature excitatory postsynaptic currents (mEPSC) in long-time cultured SCG neurons and found that NaHS increased their frequency, thus indicating an increase in the probability of acetylcholine release. Furthermore, in radiotracer release experiments, stimulation-triggered outflow was enhanced by 0.1 to 1 mM of the H2S donor NaHS in a concentration-dependent manner.

Conclusion: These results show that H2S hyperpolarizes SCG neurons and reduces membrane excitability. Since K-ATP blockers prevented the effects of H2S, we conclude that the effect on membrane excitability was caused by opening of K-ATP channels. In addition, NaHS increased the frequency but not the amplitude of cholinergic mEPSCs as well as stimulation-triggered neurotransmitter release. These excitatory actions most likely underlie the observed increase in ganglionic transmission.

**Immunology & Inflammation**

**P12 Molecular imaging of the antigen recognition dynamics in CD8+ cytotoxic T-cells**

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Background: Cytolytic T-cells (CTLs) can detect with their low affinity T-cell antigen receptors (TCRs) the presence of even a single antigenic peptide-loaded MHC molecule I (pMHC-I) among thousands of structurally related yet non-stimulatory pMHCs (Purbhoo et al. 2004). How they achieve this is not clear but appears to depend at least in part on the special binding conditions within the special constraints of the immunological synapse, the area of contact between a T-cell and an antigen presenting cell. Here receptors and their ligands are not only pre-oriented, but they are...
often enriched in specific membrane domains and also subjected to cellular forces. To relate these cell biological parameters to T-cell antigen sensitivity in a more comprehensive manner we are monitoring TCR-pMHC binding in nascent synapses with the use of molecular imaging modalities. Furthermore we also plan to assess the role of CD8 co-receptor engagement with the use of pMHCI mutants, which are deficient in CD8 binding. In this fashion we expect to gain novel insights into cell biological and molecular processes underlying the phenomenal sensitivity of CTLs towards antigen.

Methods: We confront TCR transgenic CTLs with a glass-supported lipid bilayer (SLB) functionalized with pMHCI, adhesion and co-stimulatory molecules. This allows us to conduct (single molecule) measurements in noise-attenuated Total Internal Reflection Fluorescence (TIRF) mode, to control for ligand composition and density to quantitate their specific influence on TCR-pMHCI binding and TCR-proximal downstream signaling.

Results: We have generated labeled wild-type and CD8-binding deficient pMHCI. Those pMHCI have been titrated on the lipid bilayer in order to investigate the influence of the CD8 co-receptor binding on antigen sensitivity. Functional evaluation of bilayer-resident pMHCI with regard to mobility, stimulatory potency and suitability as FRET-sensor has been achieved.

Conclusion: This system will allow us to study TCR-pMHCI binding characteristics of CD8+ cytotoxic T-cells with special focus on the influence of CD8 or adhesion molecules and relate initial binding events to downstream signaling.

P13 Identification of novel mutations in DGAT1 in congenital diarrhea with protein losing enteropathy

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Background: Chronic diarrhea during infancy can be a manifestation of a variety of underlying conditions, including early-onset inflammatory bowel disease (EO-IBD) or non-inflammatory diseases such as congenital chloride or sodium diarrhea. Some of these conditions can be attributed to single gene defects as evidenced recently with the identification of human IL10-receptor deficiency.

Methods: We used targeted sequencing, or combined homozygosity mapping and exome sequencing to identify underlying genetic aberration. Immunohistochemistry, western blotting, and RT-PCR was performed to assess mutations. Flow-cytometry based lipid droplet staining was used to assess protein function.

Results: We identified a total of 4 novel mutations in the gene diacylglycerol-acyltransferase-1 (DGAT1) in 6 patients from 4 families and hypoproteinemia with sometimes lethal course of disease. The corresponding protein product diacylglycerol-acyltransferase-1 is involved in the terminal step of triglyceride synthesis. 2 different mutations in DGAT1 has recently been reported to underlie a congenital diarrheal disorder, but the molecular mechanism is poorly understood. The mutations we identified resulted in a lack of protein product as shown by immunohistochemistry staining and western blot. Furthermore, patient-derived dermal fibroblasts show a lack of lipid drop-
let formation upon treatment with oleic acid, pointing to lack of DGAT1 function. We reconstituted patient derived fibroblasts with wild-type DGAT1 and rescued the lipid droplet formation phenotype.

Conclusion: Our findings show that early-onset conditions affecting the gut can be monogenic in nature, and that biallelic mutations in DGAT1 impair lipid metabolism leading to manifestations in the gastrointestinal tract.

**P14 Solute Carriers: Proteins at the Interface of Host Metabolism and Viral Life Cycle**

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Background. Host factor requirements for many classes of viruses are yet to be unraveled. Replication of the viral genome and synthesis of viral proteins inside the host cell are associated with altered, often enhanced cellular metabolism and increased demand in nutrients and specific molecules. With some 400 identified members in humans, the solute carriers (SLC) represent the largest family of trans-membrane proteins dedicated to the transport of small molecules, such as amino acids, sugars, nucleotides and ions. Herein we aim to characterize the role of host SLCs in viral replication as well as confirm their function as new regulatory group of proteins in the antiviral immune response.

Results: To obtain insights about the role of transporters in viral infection we performed unbiased screens with a custom-made lentiviral CRISPR library targeting all the SLC transporters. We both inactivated and overexpressed these genes in the several cell lines. A primary screen performed using the Influenza A/WSN/33, vesicular stomatitis and vaccinia viruses suggests that changes in expression levels of several of the SLC genes substantially affect the susceptibility of these cells to infection with either one or all viruses. We are currently carrying on further characterization of the most interesting SLC candidates in order to dissect their role in the viral life cycle.

Conclusions: Together, this “viral transportome” may offer new insights into possible strategies to pharmacologically interfere with viral infections.

**Vascular Biology**

**P15 Fibrin-derived Bβ–15-42 peptide is a gatekeeper of thrombus resolution**

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Background: Thrombus resolution is driven by leukocyte recruitment and thrombus angiogenesis. An effective inhibition of leukocyte transmigration in vitro is mediated by naturally occurring peptide Bβ–15-42, which is a competitive inhibitor of the interaction between N-terminus of the fibrin beta chain and vascular endothelial cell cadherin (VE-cadherin). Bβ–15-42 consists of 28 amino acids corresponding to the N-terminal sequence of the β-chain of fibrin. Fibrin clots from patients with chronic thromboembolic pulmonary hypertension (CTEPH) are resistant to fibrinolysis. Therefore, we explored the role of peptide Bβ–15-42, a plasmin-generated cleavage product of fibrin in thrombus resolution.

Methods: Study groups of 8-12 weeks old C57BL/6 mice were injected i.p. over various time periods with Bβ–15-42 or random peptide after thrombus had been induced by subtotal inferior vena cava (IVC) ligation. Thrombi from each animal were collected for further Immunohistochemistry (IHC) and RT-PCR
analysis. Pulmonary endarterectomy (PEA) specimens were collected during surgery from randomly selected patients with CTEPH. Anti-Bβ15-42 immunoblots of human red (erythrocyte-rich) CTEPH thrombi and white (collagenous) CTEPH thrombi were compared.

Results: Bβ15-42 delayed thrombus resolution after IVC ligation. Thrombi of all treated groups were larger than controls. We observed a significant decrease of thrombus macrophages and microvessel density. Measurements of Bβ15-42 in red clot of human cases of chronic thrombosis indicated higher concentrations compared with controls.

Conclusions: Our data suggest that excess thrombus resolution, presumably by inhibiting VE-cadherin mediated leukocyte migration.

P16 Bacteria Educated Platelets (BEPs)-negative and Gram positive bacteria in human platelets

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Background: Circulating blood platelets contain about 9500 different mRNAs, variable classes of non-coding RNAs and circa 16 kb mitochondrial DNA. These cells are unable of de novo transcription. However they can produce functional proteins, as they contain mRNA splicing and translating machinery.

Methods: Citrated blood of healthy donors (n=4) was used to isolate platelets by OptiPrep density gradient centrifugation. The washed platelets were incubated with three Gram negative (E.coli K12, E.coli O18, K.pneumoniae) and two Gram positive (S.aureus NWT, S.epidermis) bacteria strains. Platelet RNA was isolated by the Trizol method, and sequenced on the Hiseq 2500 Illumina platform. Using flow cytometry, CD41, P-selectin and CD63 platelet surface proteins were evaluated (BD FACSCalibur).

Results: We found 75 differentially expressed genes studying the intron-spanning reads (log counts per million > 3) of platelets after exposure to Gram positive bacteria. There was 24 genes affected in the Gram negative treated samples (P-value < 0.05), mainly with 2-8 logarithmic - fold concentration change. None of these genes were overlapping between the two groups. Based on the gene ontology analysis, bacteria treatment effects mRNA splicing, the Gram negative exposure was also involved in mitochondria related changes.

Conclusions: The certain Gram negative and Gram positive bacteria have strain specific effects on platelet transcriptome, these differ strongly between the two groups. Functional changes may happen, this requires further investigation.
Acknowledgements

All good things must come to an end and so our symposium is drawing to its end. We would like to thank everybody for attending, contributing and actively participating in the symposium. We hope to have fulfilled your expectations, that you experienced exciting and invigorating talks, witnessed fruitful discussions and that valuable connections have been established.

We would like to express our gratitude to the Medical University of Vienna and the CCHD PhD program, without which we would not be here at all, as well as FWF for providing the essential funds for organising this symposium. We would like to thank Stefan Böhm for having the faith in us to allow this event to be genuinely student planned. It is truly a challenging task, but the satisfaction after a successful outcome makes it all worthwhile.

Number of people were involved in the organisation and realisation of this event, and some of us spent endless hours and days on troubleshooting and perfecting every aspect of this event. Sincere thanks to Florian and Martin for managing the catering, Ray and Alex for negotiation with our sponsors, Rico for making the symposium come to life online, Markus for ensuring our speakers having a comfortable stay in Vienna, Viki for organising the social event and keeping us on track in many other aspects. We also appreciate the help of our youngest CCHD generation for tending to the smooth run of the symposium during the last two days. Finally, we are grateful to Stella and Martina for putting all our efforts together and for materialising this meeting, however, special thanks to Stella for filling in many gaps in all aspects of the organisation.

The success of this meeting is a result of a collaboration of many people, just like in research, and we hope to continue building bridges not only in research but also in everyday life.

THANK YOU!

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