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NEUE WEGE. SEIT 1607.

JUSTUS-LIEBIG-
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GIESSEN

1st GGL Digital Conference on Life Sciences

29th & 30th September

"The important thing is to never stop questioning"

Albert Einstein

GGL
International Giessen
Graduate Centre for the Life Sciences

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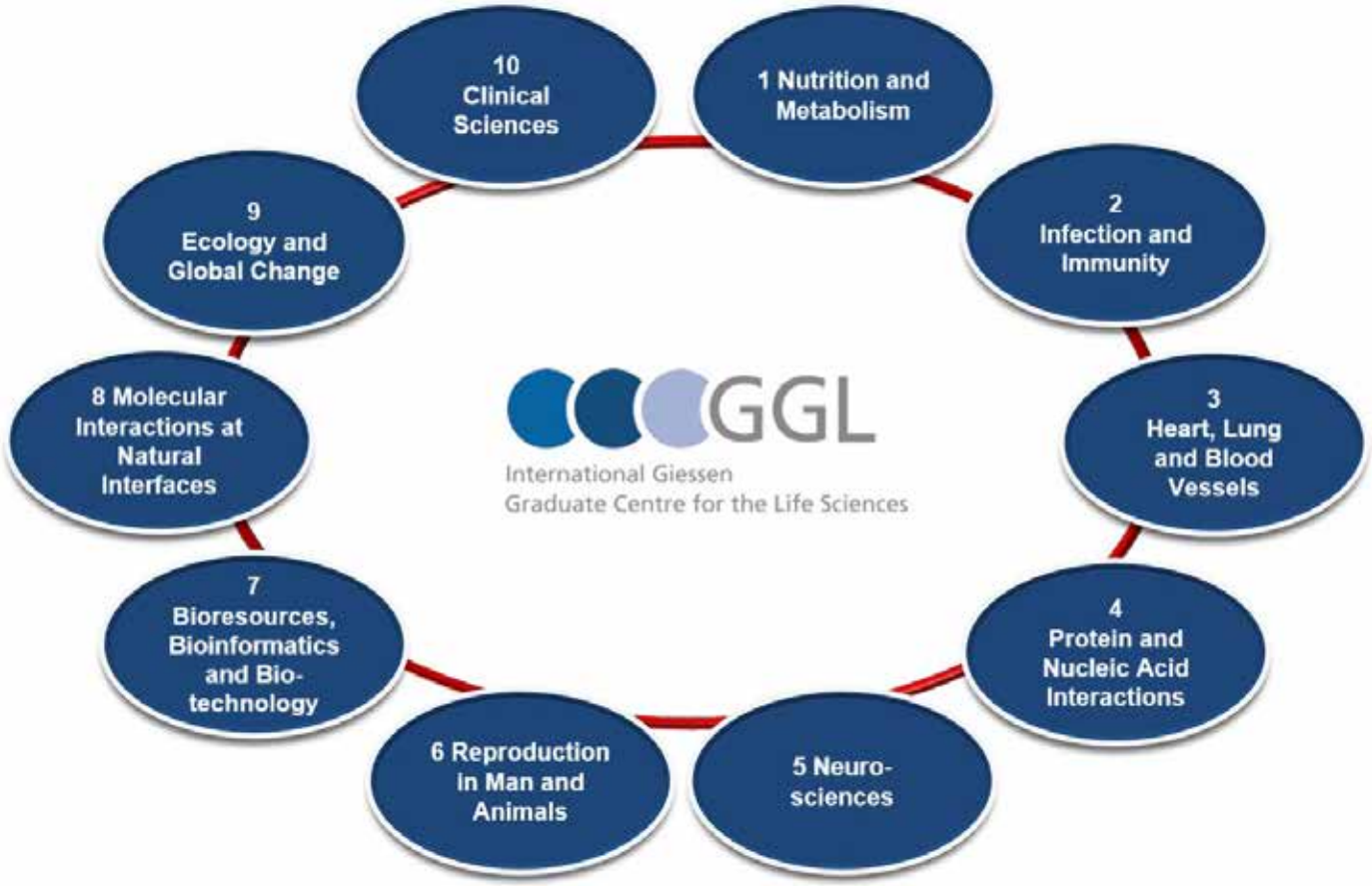

Justus Liebig University

Giessen - Germany

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Contents

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Programme

Tuesday, September 29th, 2020

Time slot	Programme	Speaker	Chairperson
09:00 - 09:15	**Opening Remarks**	**Prof. Dr. Eveline Baumgart-Vogt**	
Talks of guest speakers			
09:15 - 09:45	**Keynote Section 9**	**Prof. Feike Dijkstra, University of Sydney, Australia** Carbon and nutrient cycling in a changing world: role of plant-soil interactions	**Wiebke Hansen**
09:45 - 10:00	**Break**		
10:00 - 10:30	**Keynote Section 4**	**Dr. Maximilian Reuter, Imperial College London, UK** Towards the mechanism of replicative helicase activation in eukaryotes: A functional Analysis	**Johanna Seidler**
10:30 - 10:45	**Break**		
10:45 - 11:15	**Keynote Section 6**	**Dr. Jennifer Schön, Leibnitz-Institut für Nutztierbiologie Dummerstorf** Application of compartmentalized in vitro models to explore maternal interactions with gametes and early embryos	**Beatrix Stadler**
11:15 - 11:30	**Break**		

Talks of doctoral candidates (for titles and abstracts go on to the chapters)**:**

time	virtual room 1	virtual room 2	virtual room 3	virtual room 4
	Section 2	**Section 4**	**Section 6**	**Section 9**
Chairs	**Camilo Larrazabal**		**Hiba Hasan**	**Wiebke Hansen**
11:30 - 11:40	Lisa Fischer	Meike Schwan		Marcel Pierre Simon
11:40 - 11:50	Benadict Vincent Albert	Nicole Schmid	Hassan Kabesh	Kai Jansen
11:50 - 12:00	M.SAMER Shaban	Daniel Edelmann	Sèyi Fridaïus U. S. Vanvanhossou	Philipp Koellmann
12:00 - 12:10	Lu Liu	Corinna Ulshöfer	Yalong Yang	Wiebke Hansen
12:10 - 12:20	Nora Goldmann	Saina Azarderakhsh	Wei Peng	Alexander Konrad
12:20 - 12:30	Isabell Berneburg			Melanie Schindler

	Section 2	Section 4	Section 6	Section 9
Chairs	**Mudassar Mughal**		**Beatrix Stadler**	**Ferdinando Binacchi**
13:30 - 13:40	Anton Müller	Anna Didio	Rashidul Islam	Catarina Martins
13:40 - 13:50	Philipp Wolf	Olha Storozhuk	Raouda Sgaier	Marvin Rades
13:50 - 14:00	Vishnu Thottakkattumana Parameswaran	Vladislav Kunetki	Shanjid Ahmed Shiplu	Olivia Metz
14:00 - 14:10	Svenja Hartung	Marie Mosbach	Agnes Njoki Mwaura	Ferdinando Binacchi
14:10 - 14:20	Daniela Grob		Jane Maoga	
14:20 - 14:30	Parviz Ghezellou			
14:30 - 14:45	**Break**			

	Section 2	Section 4	Section 6	Section 3
Chairs	**Isabell Berneburg**		**Shanjid Shiplu**	**Xuran Chu**
14:45 - 14:55	Melissa Dillenberger	Maria Weller	Hang Yan	Wafaa Mahmoud
14:55 - 15:05	Yukino Kobayashi	Janek Boerner	Magdalena Anastazja Kuchta	Reshma Jamal
15:05 - 15:15	Kim Heimsch	Jacqueline Böhme	Hiba Hasan	Marija Gredic
15:15 - 15:25	Melanie Moser	Fatimah Alabudeeb	Shashika Kothalawala	Shirisha Bagari
15:25 - 15:35	Eric Springer	Timo Schlemmer		Julie Antoine
15:35 - 15:45	Hicham Houhou			
15:45 - 16:00	**Break**			

	Section 2	Section 4	Section 1	Section 3
Chairs	**Nora Goldmann**		**Bernhard Hellmann**	**Hafiza Idrees**
16:00 - 16:10	Lisa Segeritz		Julia Lisa-Marie Beranek	Arun Kumar Reddy Lingampally
16:10 - 16:20	Oliver Puckelwaldt		Alina Lucia Struff	Kathrin Malkmus
16:20 - 16:30	Camilo Larrazabal		Stefan Baumanns	Edibe Avci
16:30 - 16:40	Juan Velez		Bernhard Hellmann	Mohammad Rashedul Alam
16:40 - 16.50	Dordia Anindita Rotinsulu			
16:50 - 17.00				

Talks of guest speakers			
Time slot	**Programme**	**Speaker**	**Chairperson**
17:15 - 17:45	**Keynote Section 2**	**Prof. Jude Przyborski, Department of Biochemistry and Molecularbiology, JLU Giessen (17:15-17:30)** **Prof. Franco H. Falcone, Institute of Parasitology, JLU Giessen (17:30-17:45)** Worm vs. toxin hypothesis - the evolution of IgE-mediated	**Prof. Christoph G. Grevelding**
17:45 - 18:00	**Break**		
18:00 - 18:30	**Keynote Section 1**	**Prof. Lars Bode, University of California San Diego, USA** Human Milk Oligosaccharides – The Magic Sugars in Mom's Breastmilk	**Bernhard Hellmann**

Wednesday, September 30th, 2020

Time slot	Programme	Speaker	Chairperson
Talks of guest speakers			
09:00 - 09:30	**Keynote Section 3**	**Prof. Felix B.Engel, University of Erlangen** Heart regeneration: hopes and pitfalls	**Leili Jafari**
09:30 - 09:45	**Break**		
09:45 - 10:15	**Keynote Section 7**	**Prof. Samina Mehnaz, Forman Christina College, Lahore, Pakistan** Pseudomonas aurantiaca – a bacterium 'extraordinaire'	**Syeda Azka Jaffri**
10:15 - 10:30	**Break**		
10:30 - 11:00	**Keynote Section 5**	**Dr. Jean Christophe Delpech, University of Bordeaux, France** Role of microglia in neurodevelopmental disorders	**Osama Elyamany**
11:00 - 11:15	**Break**		

Talks of doctoral candidates (for titles and abstracts go on to the chapters):

time	virtual room 1	virtual room 2	virtual room 3	virtual room 4
	Section 2	**Section 7**	**Section 5**	**Section 3**
Chairs	**Hicham Houhou**	**Wendell Albuquerque**	**Dominic Osei**	**Reshma Jamal**
11:15 - 11:25	Max Möscheid	Fabian Jannik Tann	Ann-Kathrin Onkels	Fabienne Knapp
11:25 - 11:35	Xuesong Li	Patrick Blumenkamp	Vinothkumar Rajendran	Hafiza Idrees
11:35 - 11:45	Mandy Beutler	Patrick Barth	Stephan Leisengang	Paniz Adibi
11:45 - 11:55	Mudassar Niaz Mughal	Markus Weigel	Jessica Hernandez	Sebastian Werner
11:55 - 12:05	Pia Franziska Marie Naujack	Nadine Sella	Maanvee Mirakhur	Nicole Molenda
12:05 - 12:15	Jasmin Bazant	Elvis Katche	Benedicta Mensah	
12:15 - 13:15	**Lunch Break**	Wendell Albuquerque (until 12:30)		

	Section 8	**Section 10**	**Section 5**	**Section 3**
Chairs	**Simon Becher**	**Veronika Lehner**	**Benedicta Quaye**	**Mohammad Rashedul Alam**
13:15 - 13:25	Julia Büttner	Wenjie Sheng	Sara Shabani	Neslihan Sevinc
13:25 - 13:35	Nils Holger Anschütz	Chaoyu Zhang	Osama Elyamany	Leili Jafari
13:35 - 13:45	Katja Rebecca Wiedemann	Michaela Melzer	Rebecca Claßen	Dima Hamarsheh
13:45 - 13:55	David Lüke	Alina Hagen	Franz Nürnberger	Xuran Chu
13:55 - 14:05	Felix Marcel Graf			Zeki Ilker Kanbagl
14:05 - 14:15				
14:15 - 14:30	**Break**			

	Section 8	Section 10	Section 5	
Chairs	**Michael Waletzko**	**Rebecca Hasseli**	**Osama Elyamany**	
14:30 - 14:40	Svenja Sommer	Carla Doll	Dominic Osei	
14:40 - 14:50	Azar Rezaei	Jiawen Yong	Julia Diago Perez	
14:50 - 15:00	Parab-Jainal Haque	Reem Jamous	Aya Alserw	
15:00 - 15:10	Simon Becher	Veronika Lehner	Muyao Tang	
15:10 - 15:20	Darya Dudko		Daniela Daume	
15:20 - 15:30			Melina Kahl	
15:30 - 15:45	**Break**			

	Section 8	Section 10		
Chairs	**Azar Rezaei**	**Reem Jamous**		
15:45 - 15:55	Katrin Wiltschka	Rebecca Hasseli		
15:55 - 16:05	Julian Schneemann	Jessica Zilli		
16:05 - 16:15	Michael Waletzko	Anca-Laura Amati		
16:15 - 16:25	Domenic Dreisbach	Ruth Charlotte Dartsch		
16:25 - 16:35		Michael John Cekay		
16:35 - 16:45				
16:45 -17.00	**Break**			

Talks of guest speakers

Time slot	Programme	Speaker	Chairperson
17:00 - 17:30	**Keynote Section 10**	**Dr. Herbert Schiller, Helmholtz-Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt** Single cell analysis as a basis for a better understanding of lung injury and repair	Veronika Lehner
17:30 - 17:45	**Break**		
17:45 - 18:15	**Keynote Section 8**	**Dr. Jens Riedel, Sanofi -Aventis GmbH Frankfurt** Spatial metabolomics of cardiac glycoside sequestration in Danaus plexippus and Euploea core using high-resolution MALDI mass spectrometry imaging	Simon Becher
18:15 - 18:45	**Closing Ceremony**	Closing Remarks and Award Serving Prof. Dr. Eveline Baumgart-Vogt / Dr. Lorna Lück GGL Gießen	

Section 1 - Nutrition and Metabolism

Schedule of Section 1
Tuesday, 29 September 2020

	Chairperson: Bernhard Hellmann
16:00 - 16:10	**Julia Lisa-Marie Beranek**
	Impact of glyphosate and its formulation Roundup® on proliferation, differentiation and metabolism of equine adipose tissue derived mesenchymal stem cells
16:10 - 16:20	**Alina Lucia Struff**
	Impact of Perfluorooctane Sulfonate (PFOS) and Perfluorobutane Sulfonic Acid (PFBS) on proliferation, differentiation and the metabolism of equine adipose tissue derived mesenchymal stem cells
16:20 - 16:30	**Stefan Baumanns**
	Octanoic acid attenuates amyloid-β induced toxicity in an Alzheimer´s disease model of the nematode Caenorhabditis elegans
16:30 - 16:40	**Bernhard Hellmann**
	Effects of Hericium erinaceus extracts and fungal secondary metabolites on mitochondrial parameters in SH-SY5Y cells
Keynote Section 1	**Chairperson: Bernhard Hellmann**
18:00 - 18:30	**Prof. Lars Bode, University of California San Diego, USA** Human Milk Oligosaccharides – The Magic Sugars in Mom's Breastmilk

Octanoic acid attenuates amyloid-β induced toxicity in an Alzheimer´s disease model of the nematode Caenorhabditis elegans

Baumanns S.[1], Wenzel U.[1]

[1]Molecular Nutrition Research, Interdisciplinary Research Center, Justus Liebig University Giessen, Heinrich-Buff-Ring 26-32, D-35392 Giessen, Germany

Alzheimer's disease (AD) is a neurodegenerative disorder and the most common form of dementia. The pathogenesis is a complex process, in which the proteotoxicity of amyloid-β (Aβ) was identified as a significant factor. Mitochondrial dysfunctions frequently occur at an early stage of the pathogenesis, preceeding the irreversible loss of neurons. Octanoic acid, also known as caprylic acid, is a medium-chain fatty acid, which could contribute to maintain normal mitochondrial function through several pathways. Our aim was to investigate the effects of octanoic acid on Aβ-induced toxicity and the underlying mechanisms using the nematode Caenorhabditis elegans.

Computer-based analysis of motility was used as a measure of Aβ-toxicity in the Aβ overexpressing strain GMC101. For the investigation of molecular mechanisms, gene knockdowns were induced through RNA-interference by feeding E. coli expressing dsRNA derived from specific C. elegans gene fragments. Mitochondrial function was determined by measurement of adenosine triphosphate (ATP). Morphology of the mitochondrial network, including fission and fusion, and mitophagy were assessed by crossing GMC101 with fluorescent reporter strains, expressing either a stable fluorescent marker protein in mitochondria (strain SJ4103) or a fluorescent marker protein, that changes its fluorescent properties upon lowered pH to indicate the fusion of mitochondria with lysosomes (strain IR2539). Octanoic acid was able to improve the motility impaired by Aβ and therefore to attenuate Aβ-toxicity in GMC101. The effect of octanoic acid was lost under RNA-interference versus the NRF2 ortholog skn-1, which encodes a master regulator of stress response. In conclusion, this study provides evidence, that octanoic acid could be a potential therapeutic agent for AD. While further mechanisms need to be elucidated, the positive effects are, at least in part, mediated through NRF2.

Impact of glyphosate and its formulation Roundup® on proliferation, differentiation and metabolism of equine adipose tissue derived mesenchymal stem cells

Beranek J.[1], Failing K.[2], Arnhold S.[3], Mazurek S.[1]

[1]Institute of Veterinary Physiology and Biochemistry, Justus Liebig University Giessen
[2]Unit for Biomathematics and Data Processing, Veterinary Faculty, Justus Liebig University Giessen [3]Institute of Veterinary Anatomy, Histology and Embryology, Justus Liebig University Giessen

Glyphosate is a globally used broad-spectrum herbicide in agricultural practice. Since its market launch in the 1970s a broad variety of glyphosate-based formulations have been established. The best-known glyphosate formulation is Roundup®, produced by Bayer (formerly Monsanto).

Numerous publications in literature deal with the carcinogenic effects of glyphosate, whereas very few studies so far address the impact of glyphosate on proliferation and differentiation of stem cells. In our project, we investigate the impact of glyphosate as pure substance compared to its Roundup® formulation on proliferation, migration, differentiation as well as on the metabolism of equine adipose tissue derived mesenchymal stem cells (ADSC).

Dose finding studies with undifferentiated proliferating ADSC as well as adipogenic-differentiated stem cells revealed higher IC_{50}-values for the pure substance glyphosate in comparison to the Roundup® formulation. Metabolic turnover rate determinations in the cell culture supernatants showed a significant increase in glucose consumption and lactate production as well as alanine, serine and glutamate release after Roundup® supplementation. In contrast, the same concentration of pure glyphosate exhibited no effect on glycolysis, amino acid metabolism and adipogenic differentiation when compared to mock treated controls.

If supplemented to the medium during the entire 14-day period of adipogenic differentiation, Roundup® induced an increase in triglyceride synthesis and storage. At the same concentration, pure glyphosate had no effect on adipogenic differentiation.

Together these results imply a not inconsiderable influence of solvent-based ingredients in the Roundup® formulation on the cell division rate, glycolysis, amino acid metabolism and the adipogenic differentiation of ADSC.

In recently started experiments, we investigate the influence of Roundup® compared to pure glyphosate on osteogenic differentiation based on the expression rates of osteogenic marker genes such as Runx2, OPN and BMP-2.

Effects of Hericium erinaceus extracts and fungal secondary metabolites on mitochondrial parameters in SH-SY5Y cells

Hellmann B.[1], Zorn H.[2], Eckert G.P. [1]

[1]Department of Nutrition in Prevention & Therapy (Justus Liebig University Giessen, Biomedical Research Center (BFS, Schubertstrasse 81, 35392 Giessen)
[2]Institute of Food Chemistry and Food Biotechnology (Justus Liebig University Giessen, Institute of Food Chemistry and Food Biotechnology, Heinrich-Buff-Ring 17-19, 35392 Giessen)

Mitochondria are the power plants of the cell. They have the ability to adapt to cellular bioenergetic changes. A loss of this adaptive response has the potential to compromise cellular function and increases the risk for neuro-degenerative diseases such as Alzheimer's disease. Hericium erinaceus is an edible medicinal fungi used in Asia as a remedy against cognitive impairments. We aimed to test the effect of a commercial, a self-produced Hericium erinaceus extract and fungal low weight molecules on the effects on mitochondrial parameters.

Therefore, we tested for the mitochondrial membrane potential and ATP levels in a cellular neuronal Alzheimer's disease model. Furthermore, we were interested in the cell viability and neuroplasticity.

MMP levels were elevated for commercial and self-produced Hericium erinaceus extract in SH-SY5Y-MOCK and SH-SY5Y-APP cells. For both groups Rotenon impairments were elevated, whereas our self-produced extract showed stronger responses. For ATP-levels, no concrete results could be detetced. Looking at secondary metabolites commonly associated with fungal extracts, we found Pyrogallol, Syringic acid and Syringinaldehyd ameliorated MMP and ATP levels. We could show that our self-produced fungi extract showed better results on mitochondrial parameters than a comparable commercial product. We furthermore suggest positive effect on neuroplasticity and on cell-viability.

Lithocholic acid as a potential modulator of age-associated inflammatory changes in the intestine of Drosophila melanogaster

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In humans, bile acids play an important role in the digestion and absorption of diet-derived lipids. About 95 % of primary bile acids, being produced from cholesterol, undergo enterohepatic circulation whereas the remaining 5 % are converted by colonic bacteria to the secondary bile acids lithocholic acid (LCA), deoxycholic acid (DCA), and ursodeoxycholic acid (UDCA). These secondary bile acids have recently been recognized as signalling molecules, e.g., mediating changes in glucose homeostasis as well as in energy and lipid metabolism, and activating nuclear receptors such as farnesoid X receptor (FXR), pregnane X receptor (PXR), and vitamin D receptor (VDR).

LCA has been shown to exert anti-proliferative and pro-apoptotic abilities in cancer cells, and a lifespan-prolonging effect in Caenorhabditis elegans as well as in the fruit fly Drosophila melanogaster. However, the underlying mechanisms for these lifespan-prolonging effects are still unclear. Since annihilation of intestinal bacteria in the fruit fly reversed the lifespan-prolonging effect of LCA, a link to the intestinal microbiome has been suggested. Previous experiments showed that a dysbiosis of the intestinal microbiome is linked to an impaired intestinal barrier function and inflammation, often observed during aging. As also epigenetic effects have been discussed to be centrally involved in the process of aging, we hypothesize that LCA regulates the microbial balance in the intestine and modulates inflammatory processes via epigenetic mechanisms, thus improving the barrier function and subsequently leading to lifespan prolongation.

Impact of Perfluorooctane Sulfonate (PFOS) and Perfluorobutane Sulfonic Acid (PFBS) on proliferation, differentiation and the metabolism of equine adipose tissue derived mesenchymal stem cells

Struff A.[1], Failing K.[2], Arnhold S.[3], Mazurek S.[1]

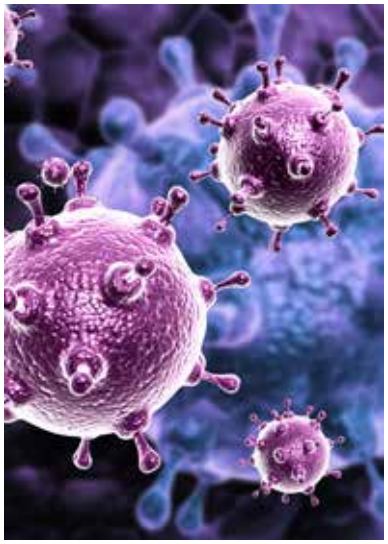
[1]Institute for Veterinary Physiology and Biochemistry, Justus Liebig University, Frankfurterstrasse 100, 35392 Giessen
[2]Unit for Biomathematics, Justus Liebig University, Frankfurterstrasse 95, 35392 Giessen
[3]Institute for Veterinary Anatomy, Histology and Embryology, Justus Liebig University, Frankfurterstrasse 94, 35392 Giessen

Perfluorooctane sulfonate (PFOS) and perfluorobutane sulfonic acid (PFBS) chemically belong to the group of perfluorinated alkyl substances (PFAS) used for the industrial production of dirt- and water-repellent surfaces of daily-use products, such as food packaging and outdoor clothing. PFAS are composed of a fluorinated carbon chain with different length (C_4-C_{20}) and a hydrophilic head group. The head group of PFOS and PFBS is sulfonic acid. PFOS consists of eight, PFBS of four carbon atoms. Today the use of PFOS is severely restricted by law due to their persistent, bioaccumulative and toxic characteristics. As a result, manufacturers replaced PFOS with short chain PFAS such as PFBS.

A dose finding study with undifferentiated equine adipose tissue derived mesenchymal stem cells (ADSC) revealed an about six times higher IC_{50} value for PFBS (1400µM) in comparison to PFOS (240µM). This result shows that PFOS has a much stronger inhibiting effect on the division activity of the ADSC compared to the short chain PFBS.

The IC_{50} concentrations of PFOS and PFBS were supplemented into the medium during the entire period of adipogenic (14 days) and osteogenic (21 days) differentiation. During adipogenic differentiation, both PFOS and PFBS induced an about 40 to 50 percent reduction of the cell number/well. Quantification of Oil-Red-O staining revealed an about two-fold increase of lipid droplets in both PFOS and PFBS treated ADSC in comparison to mock-treated adipocytes which points to a stimulation of adipogenic differentiation by both substances. Alizarin-Red staining of the osteogenic differentiation indicates a more inhibitory effect of PFOS on the calcification of

Section 2 - Infection and Immunity



Schedule of Section 2
Tuesday, 29th September 2020

Part 1	**Chairperson: Camilo Larrazabal**
11:30 - 11:40	**Lisa Fischer**
	Feline Coronaviruses - Identifying genome areas responsible for the development of feline infectious peritonitis
11:40 - 11:50	**Benadict Vincent Albert**
	The interplay of human coronavirus HCoV-229E induced host cell factors in virus-host response
11:50 - 12:00	**M. Samer Shaban**
	Characterizing the crosstalk of endoplasmic reticulum (ER) stress pathways with NF-κBB, JNK, P38 in Coronavirus infected cells
12:00 - 12:10	**Lu Liu**
	Functional impact of a specific M1 protein phosphorylation site on influenza A virus propagation
12:10 - 12:20	**Nora Goldmann**
	Replication of a novel animal hepatitis D-like virus in human cells: a new hepatotropic zoonotic virus transmissible to humans?
12:20 - 12:30	**Isabell Berneburg**
	NADPH-dependent Metabolic Pathways as Targets for new Antiinfective Agents

Part 2	**Chairperson: Mudassar Mughal**
13:30 - 13:40	**Anton Müller**
	The Alternative Animal Model Manduca sexta reveals a putative role of DUOX in the Etiology of Inflammatory Bowel
13.40 - 13:50	**Philipp Wolf**
	Cholinergic regulation of ATP-induced release of the pro-inflammatory cytokines interleukin-1β and interleukin-18 in inflammatory bowel disease (IBD) patients
13:50 - 14:00	**Vishnu Thottakkattumana Parameswaran**
	Nuclear Liver X Receptors in IL-1ß induced Phospholipid Release from Synoviocytes into Osteoarthritic Synovial Fluid
14:00 - 14:10	**Svenja Hartung**
	Investigations on the chemosensitivity of a feline large granular lymphocyte (LGL)-cell line
14:10 - 14:20	**Daniela Grob**
	Angiostrongylus vasorum L3-stages induce canine NETosis and primary canine endothelial cell activation
14:20 - 14:30	**Parviz Ghezellou**
	Mass spectrometry-based molecular profiling of scorpion, Hottentotta saulcyi, venom and venom gland: proteomics, lipidomics, and mass spectrometry imaging
Part 3	**Chairperson: Isabell Berneburg**
14:45 - 14:55	**Melissa Dillenberger**
	Posttranslational redox modifications as regulators of Plasmodium falciparum glycolysis
14:55 - 15:05	**Yukino Kobayashi**
	Contribution of unique regions of Alp1 and Alp2b in Plasmodium transmission
15:05 - 15:15	**Kim Heimsch**
	Redoxregulation of kinases in the malaria parasite Plasmodium falciparum
15:15 - 15:25	**Melanie Moser**
	Generation of Transgenic Plasmodium falciparum lines for Functional Characterization of Genes putatively involved in Sexual Differentiation
15:25 - 15:35	**Eric Springer**
	Real-time measurement of ATP dynamics in Plasmodium falciparum using genetically encoded fluorescent probes
15:35 - 15:45	**Hicham Houhou**
	Identification of aldehyde dehydrogenase as novel anthelmintic target in Fasciola hepatica

Part 4	**Chairperson: Nora Goldmann**
16:00 - 16:10	**Lisa Segeritz**
	Autochthonous Angiostrongylus cantonensis, Angiostrongylus vasorum and Aelurostrongylus abstrusus infections in terrestrial gastropods from Macaronesian Archipelagos of Spain
16:10 - 16:20	**Oliver Puckelwaldt**
	Single-Cell and Spatial Transcriptomics to Create a Cell Atlas of the Liver Fluke Fasciola Hepatica for Unraveling Developmental Processes
16:20 - 16:30	**Camilo Larrazabal**
	Ezetimibe blocks coccidian parasite replication in primary bovine endothelial cells
16:30 - 16:40	**Juan Velez**
	Cryptosporidium parvum: metabolic impact on host cells and metabolism-related parasite inhibition under physioxic/hyperoxic conditions
16:40 - 16:50	**Dordia Anindita Rotinsulu**
	Genetic diversity and antimicrobial susceptibility of Streptococcus equi ssp. equi isolates from horses
Keynote Section 2	**Chairperson: Prof. Christoph G. Grevelding**
17:15 - 17:45	**Prof. Jude Przyborski, Department of Biochemistry and Molecularbiology, JLU Giessen (17:15-17:30)** Raising the dead: how malaria parasites reboot their human host cell **Prof. Franco H. Falcone, Institute of Parasitology, JLU Giessen (17:30-17:45)** Worm vs. toxin hypothesis - the evolution of IgE-mediated

Schedule of Section 2
Wednesday, 30th September 2020

Part 5	**Chairperson: Hicham Houhou**
11:15 - 11:25	**Max Möscheid**
	Role of ovary-preferentially expressed nuclear receptors in egg and larval development of Schistosoma mansoni
11:25 - 11:35	**Xuesong Li**
	Identification of GPCR-neuropeptide interaction and their functional analysis in Schistosoma mansoni
11:35 - 11:45	**Mandy Beutler**
	Characterization of an aldehyde dehydrogenase as a potential drug target in Schistosoma mansoni
11:45 - 11:55	**Mudassar Niaz Mughal**
	Involvement of the nery in Reproductive Biology of Schistosoma mansoni
11:55 - 12:05	**Pia Franziska Marie Naujack**
	Mechanism of the cellular uptake of the Schistosoma mansoni infiltrin IPSE/alpha-1 in host cells - Infiltrins as novel paradigm in parasite-host-interaction
12:05 - 12:15	**Jasmin Bazant**
	Impact of hydrogen peroxide on pneumolysin

The interplay of human coronavirus HCoV-229E induced host cell factors in virus-host response

Albert B.V.[1], Shaban M.S.[1], Meier-Soelch J.[1], Mayr-Buro C.[1], Werner S.[1], Weiser H.[1], Kracht M.[1]

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Human coronavirus 229E is one among the four human coronaviruses associated with mild upper respiratory tract infections. HCoV-229E belongs to plus-strand RNA viruses that are characterized by a large genome of about 26-32kb. The virus replicates in the host cell cytoplasm and triggers immunomodulatory and ER-associated stress responses by poorly characterized mechanisms. HCoV-229E infection in Huh7 cells upregulated 1,073 genes at the genome-wide level which include transcription factors (ANKRD1, EIF2AK3, ATF4, BHLHE40/41, ZNF165, and KLF6) and metabolic factors (CHAC1, FICD, EDEM1, FUT1, CTH, and ERO1LB), whose role for the virus-host response is unknown. To obtain more insight into the functional relevance of coronavirus (CoV) induced genes in viral replication; we used CRISPR-CAS9 mediated loss-of-function approaches to suppress these host cell factors in Huh7 cells. So far, we have successfully depleted ANKRD1, EDEM1, FUT1, KLF6, and FICD. Genomic editing and knockout/knockdowns were confirmed by Sanger sequencing of PCR-amplified genomic regions and by immunoblotting of cell extracts of stable cell lines. The roles of these host cell factors will be systematically assessed for virus replication by measuring viral titers, viral transcripts and viral protein expression by qRT-PCR and western blotting. We will also assess the viral life cycle by high resolution imaging approaches and analyze key parameters of the host response (signaling, mRNA expression). These experiments will reveal the function of the most strongly regulated host cell factors, the underlying mechanisms involved and how they may form a CoV-regulated signaling network that controls (positively or negatively) HCoV-229E replication or the expression of host cell genes.

Impact of hydrogen peroxide on pneumolysin

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Streptococcus pneumoniae (Spn) is the main cause of bacterial pneumonia and induces a severe course of the disease. Spn releases two virulence factors, hydrogen peroxide (H_2O_2) and pneumolysin (PLY), a pore-forming cholesterol-dependent cytolysin (CDC), that damage lung tissue. H_2O_2 is capable to induce sulfenylation, a post-translational modification of cysteine residues via thiol oxidation. Sulfenylation is an indicator for oxidative stress and plays a critical role in several biological processes including transcriptional regulation, apoptosis and cytokine signaling. Since PLY has a single highly conserved cysteine residue (amino acid nr. 428) localized in the undecapeptide sequence, which is required for membrane binding, it is a good candidate for sulfenylation.
We applied a specific anti-sulfenic acid modified cysteine antibody, which detects level of sulfenic acid modified cysteine, to unveil H_2O_2 triggered sulfenylation in purified PLY. Using Charge-based immunoblot (ProteinSimple) we detected relative changes in protein sulfenylation between H_2O_2 treated PLY as compared to untreated control samples. Additionally, our preliminary data show declined haemolytic activities of PLY against sheep red blood cells when H_2O_2 is added to PLY Further investigations are ongoing to reveal the mechanisms, especially sulfenylation, involved in H_2O_2 triggered regulation of PLY.

NADPH-dependent Metabolic Pathways as Targets for new Antiinfective Agents

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The pentose phosphate pathway is a key metabolic pathway, where glucose is utilized within two branches, an oxidative and a non-oxidative branch. The oxidative unidirectional branch provides the main source of NADPH catalyzed by the two enzymes glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (6PGD). NADPH as an electron donor is essential for redox

homeostasis, parasite survival and infectivity, which is why both enzymes are considered as promising drug targets, as e.g. proven for the protozoan species Plasmodium falciparum and Trypanosoma brucei.

On this basis, we started to validate G6PD and 6PGD as drug targets in parasites causing neglected tropical diseases, including Leishmania donovani, Leishmania major and Schistosoma mansoni, the causative agents of visceral and cutaneous Leishmaniasis and intestinal Schistosomiasis. First knockout studies, using the CRISPR/Cas9 system indicated G6PD to be essential in Leishmania major. Final target validation of the remaining enzymes is in progress. Furthermore, we recombinantly produced the respective enzymes in E. coli, purified them and characterized them biochemically and kinetically. Our most promising Plasmodium falciparum G6PD inhibitor SBI-750, identified in a high-throughput screening of 400,000 small molecule compounds and following structure-activity relationship studies, revealed no significant effect in Leishmania and Schistosoma. Therefore, our aim is to identify new potent compounds using structure based docking studies followed by a high-throughput screening. Systematic crystallization trials with Leishmania donovani G6PD revealed crystals, co-crystallized with G6P and NADP+, diffracted to a resolution of 1.6 Å. The homodimer adopts the canonical G6PD fold, except an N-terminal α-helical domain, which is likely to be unique in Leishmania.

Characterization of an aldehyde dehydrogenase as a potential drug target in Schistosoma mansoni

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Schistosomiasis is one important neglected disease spread worldwide with increased incidence in poverty-related areas. For treatment against all adult schistosome species there is only one drug (praziquantel) available. Since there is the fear for emerging resistance, there is the need for identifying new targets and drugs.

One target enzyme might be aldehyde dehydrogenase (ALDH). In human, ALDH metabolizes alcohol. Disulfiram is used an approved drug to treat alcoholism by inhibiting ALDH activity, which leads to the typical hangover symptoms. Orthologs of ALDH have also been found in schistosomes. A proteomic approach suggested a localization of SmALDH in the tegument. In situ hybridisation against SmALDH1 (Smp_312440) and SmALDH2 (Smp_022960) revealed transcripts in tegument, parenchyma, gastrodermis and gonads. Treatment of adult worms with disulfiram in vitro showed effects on the integrity of the tegument and on egg production. Since disulfiram shows some liver toxicity, attempts have been performed to synthesize derivatives with lowered toxicity. In treated adults, these derivatives led to reduced gut peristalsis and the death of adult worms in vitro. We succeeded to express enzymatically active SmALDH1 using E. coli. This protein has been used to establish an enzyme activity assay. The recombinantly expressed protein is currently used for biochemical characterization and to screen for further inhibiting substances. Results on these attempts will be presented.

Posttranslational redox modifications as regulators of Plasmodium falciparum glycolysis

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Throughout the life cycle of the malaria parasite Plasmodium falciparum its survival and propagation is challenged by fluctuating levels of reactive oxygen and nitrogen species (ROS/RNS). Both, ROS and RNS can react with protein cysteine residues resulting in posttranslational modifications (PTMs). By modulating structure, function, and interaction of target proteins redox PTMs can contribute to the regulation of signaling pathways and metabolism. In P. falciparum trophozoites, we identified 493 tar-

gets of protein S glutathionylation and 319 targets of S nitrosation with affinity-purification based proteomic approaches. Recently, we were able to determine 109 targets of protein S sulfenylation. Among different central metabolic pathways, glycolysis revealed to be a major target for PTMs. Since glycolysis is essential for the ATP production of asexual blood stage parasites, we decided to study the redox regulation of enzymes related to the glycolytic pathway in more detail. In this study, we investigated the effect of S-glutathionylation and S-nitrosation on the catalysis of the three rate-limiting glycolytic enzymes: pyruvate kinase, hexokinase and phosphofructokinase. To identify catalytically relevant cysteines potentially affected by redox PTMs, we used enzymatic assays, site-directed mutagenesis, mass spectrometry and protein crystallization.

Feline Coronaviruses - Identifying genome areas responsible for the development of feline infectious peritonitis

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Feline coronaviruses (FCoVs) occur in two fundamentally different biotypes: Feline enteric coronaviruses (FECVs) induce persistent (but generally asymptomatic) intestinal infections, whereas feline infectious peritonitis viruses (FIPVs) cause the most important fatal infectious disease of cats called feline infectious peritonitis (FIP). According to the generally accepted internal mutation theory, 5-10% of the FECVs evolve into FIPVs by accumulating specific mutations in their genomes in the course of persistent infections. For none of the mutations suggested in previous publications, experimental evidence for a specific role in the FECV-FIPV biotype switch has been provided. To provide conclusive evidence for their critical role in pathogenesis, the generation of well-defined viruses by reverse genetics in combination with animal experiments is required. A major obstacle for studies on the molecular pathogenesis of FIP is the lack of cell culture systems suitable to propagate serotype I field FECVs/FIPVs in vitro. Recently, we were able to overcome this major obstacle and established a reverse genetics system for a serotype I FECV that allowed the production of recombinant FECV field viruses that were shown to cause productive infections in cats after experimental inoculation.
In this study, we used this system to identify the genomic regions responsible for the biotype switch. We generated a panel of recombinant viruses in which FECV genome regions of varying size were replaced with homologous genome regions from an FIPV isolate. Infection of cats with the chimeric viruses revealed that a virus containing the 3'-proximal part of the FIPV genome was capable of causing FIP. In a second experiment, we could narrow down the essential genomic regions to the structural genes. The irrelevance of the accessory proteins is an unexpected and interesting outcome. Our experiments demonstrate, for the first time, the critical importance of the structural genes for the FECV-to-FIPV pathotype conversion.

Mass spectrometry-based molecular profiling of scorpion, Hottentotta saulcyi, venom and venom gland: proteomics, lipidomics, and mass spectrometry imaging

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Scorpions represent a venomous group of arachnids commonly recognized by the pair of grasping pedipalps out front, and a long segmented tail that arched upward over the back. They have been successful inhabitants of our planet for over 400 million years. The capacity to produce and deliver venom is a critical evolutionary innovation that helped them in this success. The venom system is located in the last segment of the body called the telson. Production and storage of venom are implemented within the symmetrical pair of venom glands. The venom is delivered via the

needle-like curved stinger (aculeus) in proper condition for subduing prey and/or defense against predators. Scorpion venom is a complex secretory mixture that contains water, salts, lipids, amino acids, mucopolysaccharides, nucleotides, peptides, and proteins. The biological activities of peptides make up the toxic properties of scorpion venoms that target membrane-bound protein channels and receptors specifically. In this study, as a first step, we employed LC-MS/MS-based proteomics and lipidomics approaches of the venom of H. saulcyi which remained unstudied so far. Moreover, as a second step, the venom glands were subjected to LC-MS/MS lipidomics and high-resolution mass spectrometry imaging to investigate endogenous biomolecular distributions in scorpion venom gland tissue. Overall, our combined mass spectrometry-based approach aimed for progress in understanding the scorpion venom system, both, in the context of venom components and of venom gland biology.

Replication of a novel animal hepatitis D-like virus in human cells: a new hepatotropic zoonotic virus transmissible to humans?

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Hepatitis delta virus (HDV) is a single stranded RNA virus with negativ polarity. It is the smallest known human pathogenic RNA virus that depends on hepatitis B virus (HBV) surface proteins (HBsAg) for its assembly, secretion and infectivity. While a chronic HBV infection usually has a mild clinical course, it can worsen with HDV superinfection. For many years, HDV was the sole member of the genus Deltavirus. Recently, various non-mammalian deltaviruses have been detected, but not in any mammal besides humans. Here we present the first mammalian deltavirus found in the neotropical rodent Proechimys semispinosus. To analyze whether this novel HDV-like virus is a self-replicating agent, we cloned a tandem cDNA sequence of the rodent deltavirus (RDeV) genome into a mammalian expression vector, transfected human hepatocytes and performed viral RNA and protein analysis. Through clonal expansion of transfected cells and Northern blot we demonstrated that the novel RDeV replicates in human cells. A key feature of HDV is the expression of two viral proteins from a single open reading frame (ORF) by cellular RNA editing. During replication, the small delta antigen (S-HDAg) is translated first, at a later stage, the corresponding stop codon of the antigen ORF is edited and a 19 amino acid longer large delta antigen (L-HDAg) is translated for interaction with HBsAg. Since we also observed at the genomic level an extension of the RDeV antigen (RDeAg) by 19 amino acids, we investigated whether rodent deltavirus RNA is also edited during replication. But we could neither detect a large delta antigen by Western blot nor an edited RNA by NGS. Furthermore, we found no evidence of hepadnavirus coinfection in deltavirus-positive tested animals. Here we present a non-human mammalian deltavirus that has an autonomous replication nature, can replicate in human cells and does not require hepadnaviral co-infection.

Angiostrongylus vasorum L3-stages induce canine NETosis and primary canine endothelial cell activation

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The metastrongyloid nematode Angiostrongylus vasorum is a neglected parasitic nematode and causal agent of cardiopulmonary disorders in domestic and wild canids. The life cycle includes terrestrial gastropods (slugs/snails) as intermediate hosts in which first-stage larvae (L1) develop into infectious third-stage larvae (L3). After ingestion of A. vasorum L3-infected gastropods, larvae migrate through the intestinal wall to blood and lymph vessels to reach pulmonary arteries and the right heart in vivo. Referring to this localization of A. vasorum-L3 and adults in vivo, interactions with both, circulating leukocytes (e. g. polymorphonuclear neutrophils, PMN) and blood vessel-derived endothelial cells are very likely. We studied for the first time early host innate immune reactions by analyzing primary canine endothelial aortic cell (CAEC) - and PMN-derived responses against A. vasorum L3 stages. Canine PMN-derived effector mechanisms included studies on L3-triggered NETosis and PMN adhesion on A. vasorum antigen-stimulated CAEC layers under physiological flow conditions. Gene transcription profiles of adhesion molecules (i.

e. ICAM-1, VCAM-1, P-selectin, E-selectin) in A. vasorum antigen-treated CAEC were analyzed.

Canine PMN were isolated from fresh blood samples and exposed to A. vasorum L3 stages. Scanning electron (SEM) and immunofluorescence microscopy were performed to visualize and quantify L3-triggered NETosis and to evaluate the presence of different NET phenotypes [spread NETs (sprNETs), diffuse NETs (diffNETs), aggregated NETs (aggNETs)]. Primary CAEC were isolated from canine aorta and used to characterize early endothelial cell reactions against soluble A. vasorum antigens. Adhesion molecule (ICAM-1, VCAM-1, P-selectin, E-selectin) gene transcription and protein expression was quantified via qPCR and Western Blot analyses, respectively.

Microscopic analyses demonstrated that canine PMN reacted against motile A. vasorum L3 stages by extruding NETs. sprNETs were the most abundant. Individual donor variations of CAEC-derived immune reactions for adhesion molecule gene and protein expression were observed, corresponding to diverse canine immunopathological reactions in vivo.

Investigations on the chemosensitivity of a feline large granular lymphocyte (LGL)-cell line

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Large granular lymphocytes are a small and heterogenous group of peripheral blood leukocytes which comprise cytotoxic T cells, natural killer T cells, and natural killer cells. Lymphoma is the most common neoplastic disease in cats, making up about one third of all feline neoplasia. LGL-lymphoma is comparatively rare and described to be extremely aggressive with a bad prognosis and short median survival times even if treated with chemotherapy. The aims of the study are the assessment and characterization of the susceptibility of the cell line „S87" to chemotherapeutic agents and the establishment of IC40-values (inhibitory concentration in which 40 % of the cells survive the treatment) for subsequent studies on resistant sublines. The permanent cell line S87 was originally isolated from the abdominal effusion of a European Shorthair cat with the diagnosis of an LGL lymphoma and characterized in previous studies as non-MHC-restricted cytotoxic T cell line [1]. In this study it was tested on its in vitro chemosensitivity for different chemotherapeutic agents (vincristine, doxorubicin, L-asparaginase, prednisolone, methotrexate, hydroperoxycyclophosphamide) commonly used in cats. The viability of the cells was determined and the IC40 was calculated if possible. For prednisolone, an IC40 could not be defined due to the low sensitivity of the cells to prednisolone. The IC40 should be used for establishment of resistant sublines and for further investigations on resistance mechanisms.

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Redoxregulation of kinases in the malaria parasite Plasmodium falciparum

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Malaria is still one of the most life-threatening disease in the world. Due to the increased incidence of drug resistances, the need for new antimalarial drugs is high. Therefore, new potential drug targets have to be identified und mechanisms of drug action and resistance need to be understood.

With a number of 65 eukaryotic protein kinases, the malaria parasite Plasmodium falciparum has a relatively small kinome. Due to knockout (KO) studies, it was shown that kinases are most interesting drug targets and undergo redox-modifications, such as S-glutathionylation. Studying redox metabolism is of great interest for the understanding of mechanisms of drug action and resistance, as many currently employed antimalarial drugs mediate their effects, at least partially, through increasing the concentration of reactive oxygen species in the parasite.

To further investigate redox regulation of kinases in malaria parasites, we analyze 24 kinase KO lines, which are likely dispensable for the asexual erythrocytic lifecycle, to determine potential differences in their redox metabolism compared to wild-type parasites. As a first examination, we performed EC50 determinations to explore the effect of common antimalarial drugs as well as compounds effecting redox metabolism. Based on this results eight KO lines were identi-

fied which showed a significant difference in EC50 compared to wild-type parasites. Those KO lines will be further investigated using a genetically encoded GFP based redox sensors, which is a powerful tool for determining the glutathione-dependent redox potential in living parasites as well as detecting oxidizing effects in real-time. To allow a more precise and significant measurement we use an improved redox sensor with enhanced fluorescence intensity and increased dynamic range, as well as using flow cytometry as detection method rather than microscopy or plate reader detection.

Identification of aldehyde dehydrogenase as novel anthelmintic target in Fasciola hepatica

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The liver fluke Fasciola hepatica is a threat for both animal and human health. Fasciolosis causes economic losses in cattle and sheep farming worldwide, and it has been listed as a neglected tropical disease of humans with >2.4 million people being infected worldwide. Several reports showed the spread of resistance against the main effective drug, triclabendazole. Therefore, finding alternative treatment options is highly demanded.
Aldehyde dehydrogenases (ALDHs) are involved in cellular detoxification of reactive aldehydes. Real-time qPCR analyses showed that ALDH orthologues are expressed in various life stages of F. hepatica. Among others, ALDH transcripts were detected in gonadal and gastrodermal tissues using in situ hybridization. We therefore assessed possible anti-fasciolid effects of the known ALDH inhibitor disulfiram, an approved drug for use in humans. Newly excysted juveniles (NEJs), immature, and adult flukes were exposed for three days to disulfiram or chemically optimized derivatives with lower cytotoxicity in vitro. Disulfiram in a concentration of 20 µM led to severe effects on fluke motility and tegument integrity. Overall efficiency was enhanced when a novel disulfiram derivative was used, which showed severe effects on NEJs already at 2 µM. This derivative exhibited improved efficacy also against immature and adult flukes, which was comparable to that of triclabendazole in our in vitro-culture system. Electron microscopy demonstrated severe tegument damage after treatment with the derivative. RNA-interference experiments on in vitro grown juveniles finally showed a significant reduction of proliferating cells upon knock-down of both ALDH orthologues.
Our findings suggest that ALDH may represent a potential target and disulfiram a promising basis for the design of novel anti-fasciolid compounds. The novel compounds might as well be effective against other parasite species. Indeed, first evidence for fatal effects of disulfiram and its derivatives was obtained for schistosomes in vitro.

Contribution of unique regions of Alp1 and Alp2b in Plasmodium transmission

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Malaria is one of the deadliest diseases and kills more than 400,000 people every year. The causative parasitic agent of malaria, Plasmodium, is spread by the bite of female Anopheles mosquitoes. Effective malaria control therefore requires a blocking of the parasite transmission to suppress the number of infection cases. The Actin family of proteins, including actin-like proteins (Alps), is heavily involved in transmission to and through mosquitoes. While classical actins are known to regulate motility and propagation of the parasite, Alps are only recently discovered and their functions are still largely unknown. Moreover, these Alps are present only in the parasitic phylum of Apicomplexa, with some of them even being specific to Plasmodium. Our preliminary research revealed that removing Alp1 and Alp2b in Plasmodium resulted in

the parasites with severely impaired motility or inability to undergo sexual maturation during their transmission stages to the mosquito. Interestingly, these Alps contain several additional regions, which are highly divergent from the core structure of classical actins. This research aims to identify the role of these unique regions by 1) characterising the function of Alp1 and Alp2b through in vitro biochemical activity assays and crystallography and 2) fluorescence-based monitoring of their in vivo localisations in Plasmodium. Based on the structural and functional studies in 1) and 2), we will 3) generate mutants of Alp1 and Alp2b to observe their impacts on the parasite activities during a transmission between female mosquito and mouse. Bringing these aspects together, we will fully evaluate the role and potential of the unique regions of Alps as drug targets to stop Plasmodium transmission.

Ezetimibe blocks coccidian parasite replication in primary bovine endothelial cells

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Coccidian parasites (i. e. Toxplasma gondii, Neospora caninum, Besnoitia besnoiti) represent a large group of obligate intracellular parasites which affect both, humans and animals. In fast replicating species, parasite proliferation only takes 24-48 h and ends with lysis of host cells and release of parasitic offspring (tachyzoites). In this context, successful parasite proliferation requires host cellular nutrients and metabolites. Given that coccidian parasites are considered as defective in cholesterol synthesis, they need to scavenge this molecule from host cells, especially for offspring membrane biosynthesis. There are two main routes of cholesterol acquisition: cells may either enhance endogenous de novo biosynthesis or increase cholesterol uptake from extracellular sources. Several pharmacological strategies were described to interfere with cholesterol uptake in cells. In this context, Ezetimibe, which has successfully been used as hypolipidemic agent, seems suitable candidate as anti-coccidian drug. So far, this compound is the only drug capable of reducing intestinal absorption of cholesterol by interacting with its target molecule, Niemann-Pick C-1 like-1 protein (NPC1L1), a transmembrane protein highly expressed in enterocytes. Despite proven clinical effects of this drug in hypercholesterolemia, little data are available on potential effects of Ezetimibe on replication of T. gondii-, N. caninum- and B. besnoiti-tachyzoites in vitro.

Our data shows that treatments with Ezetimibe did not affect tachyzoite host cell invasion, but effectively blocked intracellular T. gondii-, B. besnoiti- and N. caninum-proliferation suggesting that Ezetimibe acts directly or indirectly on tachyzoite replication process. However, upon withdrawal of the drug, all parasite species recovered and restarted proliferation, suggesting Ezetimibe-derived inhibitory effects as reversible. Finally, treatments with Ezetimibe-glucuronide, which specifically blocks NPCL1-mediated effects, did not affect intracellular parasite proliferation. Consequently, here observed anti-parasitic effects of Ezetimibe may be rather NPC1L1-independent.

Identification of GPCR-neuropeptide interaction and their functional analysis in Schistosoma mansoni

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Schistosomiasis is a neglected tropical disease, caused by blood flukes (schistosomes) of the genus Schistosoma. Despite its profound global impact, only a single drug (praziquantel) is available to treat this disease. Neuropeptides are important messenger molecules that act via G protein-coupled receptors (GPCRs) as neurotransmitters, neuromodulators or hormones in the nervous system. Due to GPCR's pharmacological importance, and proven drug ability, GPCRs are promising targets for new ant-helmintics. Comparative transcriptomics of paired and unpaired worms and their gonads revealed 59 differentially regulated GPCR genes putatively involved in Schistosoma mansoni neuronal processes. According to the current analysis, 23 of 27 listed S. mansoni neuropeptide precursor (Sm_npp) genes expressed in adult S. mansoni show higher transcript levels in the head part of males (paired or unpaired) and unpaired females, which possibly indicates a function in the nervous system. However, our knowledge of S. mansoni GPCRs and their ligands are still fragmentary. Thus, a deeper understanding of GPCR signalling in schistosome biology is essential, which could be used in the treatment of schistosomiasis.

Goal of this study is to confirm Sm_npp-GPCR interactions by biochemical methods and to characterize the appropriate partners at the molecular level including functional analysis.
To this end, we analyzed the function of a presumptive allatostatin receptor (Smp_203500), which is male-preferentially expressed, and its candidate ligand (Smp_154970) by double-stranded RNA (dsRNA)-mediated RNA interference (RNAi) and subsequent phenotype studies. The results showed a significant knockdown of gene transcripts by real-time PCR but no detectable difference observed in the pairing stability of the dsRNA-treated worm pairs. However, there was a substantial decline in egg production compared with the untreated control group. These results indicate that the allatostatin GPCR Smp_203500 and its putative neuropeptide ligand Smp_154970 are possibly involved in influencing egg production in S. mansoni.

Functional impact of a specific M1 protein phosphorylation site on influenza A virus propagation

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Phosphorylation is a post-translational modification known to regulate the functions of proteins including proteins encoded by influenza A viruses (IAV). We previously compared the Ser/Thr and Tyr phospho-proteomes from the mouse-adapted SC35M virus or its non-adapted SC35 counterpart at early and late time points after infection of murine lung epithelial cells. The results identified 12 novel phosphorylation sites and confirmed 8 previously identified sites on several viral proteins. We then analyzed all published phosphorylation sites described to date, and comprehensively collected their structural context. From this analysis we selected several highly conserved phosphorylation sites with a functional implication. Using reverse genetics, subcellular fractionation and confocal laser-scanning microscopy we here provide experimental evidence that the phosphorylation site M1-T108 of SC35M affects intra-cellular M1 transportation. Compared to wild type M1 the phosphorylation-deficient M1-T108A mutant affects the cytoplasmic and nuclear accumulation of M1 and NP, thereby leading to the failure of viral rescue. To further analyze the effects of phosphorylation M1-T108 on protein/protein interactions an antibody-based affinity purification coupled to mass spectrometry is ongoing. Overall, our data reveals a functional impact of a specific M1 phosphorylation site on IAV replication by regulating intracellular localization and trafficking of IAV proteins.

Role of ovary-preferentially expressed nuclear receptors in egg and larval development of Schistosoma mansoni

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Schistosoma mansoni, a parasite of humans and animals, is the cause of the widespread infectious disease schistosomiasis. Schistosomes have a complex life cycle, which is typical for endoparasitic trematodes. However, the molecular biological processes of their larval development are still not fully understood. Previous work of our group showed that in paired schistosomes, receptors such as protein kinase receptors serve as essential signal transduction molecules controlling reproduction processes and tissue integrity. Comparative transcriptome studies of adult schistosomes and their (isolated) gonads have uncovered that genes coding for potential nuclear receptors are regulated in a pairing-dependent manner (Lu et al. 2016). Some identified, yet uncharacterized nuclear receptors comprise various potential retinoid and thyroid hormone receptors, which are preferentially or specifically transcribed in the ovaries of paired females. Receptors of the nuclear receptor superfamily are ligand-activated transcription factors that play diverse roles in cell differentiation and development, cell proliferation, and cell metabolism, emphasizing their biological importance. The binding of the li-

gand to its corresponding nuclear receptor results in transactivation of specific genes within a target tissue, which needs to be characterized in more detail. To obtain a better understanding of the role of these receptors in developmental processes, this project aims at functionally characterizing selected receptors. To this end we will perform RNAi knockdown experiments, inhibitor and interaction studies. First results of this new project will be presented.

References:

Lu, Zhigang; Sessler, Florian; Holroyd, Nancy; Hahnel, Steffen; Quack, Thomas; Berriman, Matthew; Grevelding, Christoph G. (2016): Schistosome sex matters: a deep view into gonad-specific and pairing-dependent transcriptomes reveals a complex gender interplay. Sci Rep 6, S. 31150.

Generation of Transgenic Plasmodium falciparum lines for Functional Characterization of Genes putatively involved in Sexual Differentiation

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Although many effective efforts in prevention, diagnosis and therapy reduced the incidence of malaria, the disease continues to be a major burden on humanity. The estimated 228 million clinical cases and 405.000 deaths per year worldwide remain a key health problem.
Regarding the discovery of new antimalarial drugs, the developmental switch between asexual and sexual differentiation is of particular interest. Counteracting this switch suppresses the transmission, can reduce morbidity and mortality of the disease and in long term helps limiting the spread of drug resistance. The aim of this project is to validate and functionally characterize target genes, which seems to be of importance for sexual differentiation in Plasmodium falciparum. Based on a list of genes, which are upregulated during sexual differentiation, we generated transgenic parasite lines using reverse genetics to apply a knockdown approach. The FK binding protein destabilization domain (ddFKBP)-system employed allows the regulation of the amount of the gene of interest (GOI)-specific protein in a ligand-dependent manner. The phenotypic characterization of the sexual development in the knockdown cell lines can show which of the GOIs are important/essential for sexual differentiation. Genetically modified parasites were generated for a subset of 23 GOIs. For our ongoing studies, we focused on two of these cell lines: Pfsex2_DD and Pfsex3_DD. After Validation of the knockdown approach by a comparative analysis of the amount of the GOI-specific protein under knockdown and wild type condition, the phenotypic investigation will show, which of the GOIs is crucial for sexual differentiation. The established methods can then be transferred to the other GOIs.

Involvement of the Autophagy Machinery in Reproductive Biology of Schistosoma mansoni

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Schistosomiasis is a disease of global importance caused by parasitic flatworms, schistosomes, which cause pathogenicity through eggs laid by the female worm inside the blood vessels of their host. Parasite reproduction requires constant pairing of a female with its male partner, which induces female sexual maturation as a prerequisite for egg production. We hypothesize a role for autophagy in this process. Here, for the first time, we obtained evidence for the participation of the autophagy machinery in pairing-dependent processes and reproduction of Schistosoma mansoni. We identified autophagy genes by in-silico analyses and analyzed the influence of in vitro culture on the transcriptional profiles of these genes in male and female worms by qRT-PCR. Among these genes were Beclin, Ambra1, Vps34, DRAM, DAP1, and LC3. Transcriptional analyses showed the sex-dependent expression of DAP1, which was significantly higher expressed in females compared to males. For DRAM, we found the opposite direction of gene regulation. Next, worms were treated with an autophagy inducer (rapamycin) or inhibitors (bafilomycin A1, wortmannin and spautin-1) to evaluate effects on autophagy protein expression, worm vitality, and reproduction. As shown by western blot analyses, the conversion of the key autophagy protein LC3, a marker for autophagic activity, was found to be increased by rapamycin and blocked by bafilomycin. All inhibitors affected worm fitness as well as egg production, and they negatively affected the morphology of gonads and intestine. In summary, typical players of the autophagy machinery exist in S. mansoni of which some interesting sex-dependent expression patterns.

Manipulation of autophagy in S. mansoni by inhibitors induced detrimental effects, which encourages subsequent studies to unreavel the underlying principles also against the background of identifying potential antischistosomal targets.

The Alternative Animal Model Manduca sexta reveals a putative role of DUOX in the Etiology of Inflammatory Bowel

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Bacteria heavily colonize animal epithelia. The dual oxidase (DUOX) is part of the first-line defense in gut epithelia and attacks pathogenic bacteria via synthesis of HOCl. Because human DUOX and its immunological context are similar to MsDUOX, we established M. sexta as a model to understand the role of DUOX in gut inflammation. In this study, we tested if the activation of DUOX can cause gut inflammation in M. sexta.
The occurrence of HOCl in the gut of uracil fed but not in control larvae was demonstrated. HOCl production in larvae fed with uracil and DPI showed a significant reduction of HOCl production compared to uracil only fed larvae (Fig 2). Induction of MsDUOX after uracil treatment was demonstrated with western blot and in situ hybridization. Furthermore, survival kinetics of larvae fed with uracil showed a significantly lower survival ($p < 0.0001$) and a significantly lower weight at L2d4 ($p = 0.0208$). We detected a significant higher CT and MR contrast-enhanced gut wall thickness ($p < 0.0001$, $p = 0.0146$), CT and MR gut wall signal enhancement ($p < 0.0001$, $p = 0.004$) and FDG uptake ($p = 0.039$) in larvae fed with uracil compared to the uracil-negative control group. Inflammation was further confirmed using scanning electron microscopy. Larvae fed with uracil and DPI, or uracil and NAC, showed significantly reduced production of HOCl (DPI) and a significant difference in CT, MR, and PET imaging compared to animals fed with uracil only. We can present strong indications that M. sexta DUOX is activated via uracil, and catalyzes the production of ROS, which causes gut inflammation. This new aspect should be considered and may lead to new strategies for understanding inflammatory bowel diseases.

Mechanism of the cellular uptake of the Schistosoma mansoni infiltrin IPSE/alpha-1 in host cells - Infiltrins as novel paradigm in parasite-host-interaction

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The parasitic blood trematode Schistosoma mansoni is the most prevalent cause of schistosomiasis in humans, a disease which, untreated, may lead to the development of severe pathology. With the only effective drug being praziquantel, the treatment options against schistosomiasis are limited. Therefore, it is of great importance to investigate in alternative treatments, including a better understanding of the interaction between the parasite and the human host. During the infection, adult worms settle in human mesenterial vessels, where they release up to 300 eggs a day. These then transit through different tissues into the intestine to finally be released with the excretes. To facilitate their migration, eggs secrete various antigens, which interact with human cells, such as the infiltrin IPSE/alpha-1 (IL-4 inducing principle of S. mansoni eggs), a dimeric glycoprotein of the γ-crystallin-like family. After its secretion, IPSE is taken up by the host cell, through a still unknown mechanism. With the help of its nuclear localization sequence, IPSE/alpha-1 translocates and enters the cell nucleus to bind and interact with host DNA. Once inside the nucleus, it acts as an immunomodulator that triggers IL-4 production in human basophils, by being activated via cross-linking receptor-bound IgE, supporting Falcone the switch of Th-immunity. Since the cellular uptake mechanism of IPSE/alpha-1 by the host epithelial cells still remains unknown, the aim of this study is to investigate this aspect. Hereby, the focus will be set on whether IPSE is harboring cell penetrating peptide (CPP) sequences or alternatively whether it enters the cell via endocytosis or receptor-mediated processes.
For that purpose, IPSE/alpha-1 will be expressed by using recombinant gene expression in bacterial E. coli and human HEK 293 6E cells, which will be then used to penetrate human CaCo-2 cells

in combination with endocytosis-inhibitors. Additionally, potential CPP-sequences of IPSE will be synthesized to conduct a penetration-assay.

Single-Cell and Spatial Transcriptomics to Create a Cell Atlas of the Liver Fluke Fasciola Hepatica for Unraveling Developmental Processes

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The liver fluke Fasciola hepatica is a parasitic flatworm found in many countries of the world including Germany, and the cause of the widespread infectious disease fascioliasis. Infection of sheep and cattle cause huge economic losses, also human infections are common. Treatment relies on a limited number of compounds, with resistances reported for all, including the main drug triclabendazole. With a fading number of treatment options, a better understanding of this organism is urgent to facilitate the development of new alternative treatment approaches.

This project aims to promote a better understanding of the development and survival of liver flukes within their mammalian host, in which they undergo a fascinating development. Juvenile worms excyst from infective cysts and penetrate via the intestinal wall into the body cavity, from where they migrate to the liver. After a developmental phase, the adult flukes reside and reproduce in the bile ducts where they can persist for several years. During its development, the fluke grows from 200 µm length to 2-3 cm while adapting to the different environments in the host.

We will use "Single cell RNAseq" to create single cell atlases for the different intra-mammalian stages. This approach will allow a detailed view on cell type-specific gene expression including putative drug target genes. One major question on how stem cell populations change in the course of development will be addressed by this approach. As a second focus, fluke tissue will be analyzed with "Spatial Transcriptomics", a technique which allows transcript recovery in a spatial context. The latter will facilitate the understanding of tissues and local gene expression patterns. Together, the datasets will help understanding liver fluke development and adaptation to the host environment.

Genetic diversity and antimicrobial susceptibility of Streptococcus equi ssp. equi isolates from horses

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Strangles is a highly contagious disease of equines worldwide. The disease is caused by a host-adapted bacterial pathogen, Streptococcus equi ssp. equi (S.ee). The objectives of our study were to enlighten the genetic diversity and antimicrobial susceptibility of S.ee in order to direct future research towards the development of better controlling strategies.
Putative S.ee isolates from equines in Germany, Denmark, the Netherlands, and Indonesia (2001-2020) were investigated. All isolates were re-assessed by MALDI-TOF MS for their taxonomic assignment. Additionally, all isolates were examined by PCR for the presence of the sodA gene of Sc. equi, and subspecies-specific loci ICESe2 (S.ee) and ICESz1 (specific for S. equi subsp. zooepidemicus, S.ez). Antimicrobial susceptibility of S.ee isolates was assessed according to CLSI standards by using the Micronaut System. Selected S.ee isolates were subjected to multilocus sequence typing (MLST) and seM typing using whole genome sequencing (WGS) data and the PubMLST database (https://pubmlst.org/szooepidemicus/). MALDI-TOF MS analysis confirmed all iso-

lates (n = 216) as S.ee. All these isolates encoded the sodA gene while none carried the ICESz1 locus. The ICESe2 locus was present in 215 (99.5 %) S.ee isolates. All S.ee isolates of this study proved sensitive to each of the five beta-lactam antibiotics investigated (including penicillin G) but resistant to enrofloxacin. MLST revealed only two and highly related sequence types in the 71 S.ee isolates selected for this analysis, namely ST151 (60.6 %) and ST179 (39.4 %). Furthermore, 20 different seM types were identified in these isolates, including 7 novel seM types.
Our results suggest that phylogenetic diversity of S.ee is low but seM typing appears suitable for identifying and tracking epidemics of strangles. In those cases of strangles where antimicrobial chemotherapy is clinically indicated, penicillin G can continue to be the first choice for treatment.

Autochthonous *Angiostrongylus cantonesis*, *Angiostrongylus vasorum* and *Aelurostrongylus abstrusus* infections in terrestrial gastropods from Macaronesian Archipelago of Spain

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Zoonotic relevant *Angiostrongylus cantonensis* infections, causing eosinophilic meningitis in humans, were recently reported in rat final hosts and gastropod intermediate hosts in Tenerife. The nematodes *Angiostrongylus vasorum* and *Aelurostrongylus abstrusus* are of veterinary concern, affecting canine and feline respiratory and cardiovascular system. Currently these parasites are considered as spreading in Europe and as emerging in previously non-endemic areas. Therefore, aim of this work was to conduct an epidemiological survey on metastrongyloid nematodes in native slug and snail intermediate hosts for islands of Macaronesia, Spain. Overall, 131 terrestrial slug/snail species (*Plutonia lamarckii*, *Cornu aspersum*, *Theba pisana*, *Rumina decollata*) were collected in 27 selected locations of Tenerife, Gran Canaria, El Hierro, Lanzarote, La Palma and Fuerteventura. Gastropod samples were examined microscopically via artificial digestion for the presence of metastrongyoid lungworm larvae.
Current study revealed a total prevalence of 0.8% for *A. cantonensis*, 4.6% for *A. vasorum* and 3.8% for *A. abstrusus* in Macaronesian gastropods. In Tenerife, all three lungworm species were found thereby reconfirming endemicity of *A. cantonensis* for this island. Snails originating from El Hierro were positive for *A. abstrusus* and *A. vasorum* larvae with prevalences of 5% and 10%, respectively, showing rather high larval burdens of up to 290 larvae per specimen.
This epidemiological survey expands geographic distribution of human, canine and feline lungworms in Macaronesia. The presence of these neglected lungworm parasites in Spain, particularly of anthropozoonotic *A. cantonesis*, calls for future large-scale monitoring on metastrongyloids in obligate intermediate hosts, paratenic hosts as well as final hosts.

Characterizing the crosstalk of endoplasmic reticulum (ER) stress pathways with NF-κBB, JNK, P38 in Coronavirus infected cells

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Coronaviruses (CoV) are a group of very large plus-strand RNA viruses whose life cycle is strictly confined to the host cell cytoplasm. CoV replication triggers alterations in the pattern of gene expression and the activating phosphorylation of several protein kinases in the infected cell. Among the pathways altered by CoV infection, several are related to the endoplasmic reticulum (ER) stress response, the NF-κB pathway and the mitogen-activated protein (MAP) kinases P38 and JNK. To understand the role of these pathways in the life cycle of CoV and to characterize a possible cross talk among them and how this affects or fine-tunes the outcome of the infection, we first treated HuH7 cells infected with human CoV 229E with small-molecule inhibitors to independently inhibit; PERK , an ER stress sensor, P38 or JNK. Inhibition of PERK with GSK2606414 and inhibition of JNK with SP600125 resulted in pronounced reduction in the level of viral nucleocapsid (N)N-protein as assessed by western blotting. Both inhibitors also affected the phosphorylation levels of PERK, eIF2α, P38, JNK and the total protein level of IκBα, the major negative regulator of NF-κB. Interestingly, the JNK inhibitor also caused reduction in the total protein levels of PERK, P38 and JNK. In contrast, inhibiting 38 with SB203580 did not result in any significant effect on viral N-protein synthesis nor on other pathways investigated in this study. Next, we generated PERK–deficient cell lines using CRISPR-CAS9 to further characterize the role of this kinase in viral life cycle. Contrary to the results obtained by PERK inhibitor, preliminary data indicated no reduction in the level of N-Protein. However, the PERK knockdown

was functional as it caused a reduction in phosphorylation of the PERK substrate eIF2α (a major event in inhibition of protein translation) upon CoV infection. Collectively, the obtained data until now reveal an interconnected role of several of these kinases in the life cycle of the virus. Their cross-talk possibly serves to fine–tune the outcome of the infection. Further characterization and investigation of the host response at the level of virus-induced signaling complexes and gene expression are, however, required to gain in-depth understanding of the mechanisms involved.

Real-time measurement of ATP dynamics in Plasmodium falciparum using genetically encoded fluorescent probes

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Malaria is a serious infectious disease and remains one of the greatest public health burdens for humankind. In 2018, an estimated 228 million cases occurred worldwide. Of these, around 405,000 people died, with the majority of victims being children under five. Of all malaria-causing Plasmodium (P.) species, P. falciparum has the highest impact on global health causing most infections as well as the deadliest form of the disease. With resistance formation on the rise, basic research for development of new drugs remains critical for global health.

Because of its pivotal role, the energy metabolism of P. falciparum is an interesting target for drug design and its central metabolite adenosine triphosphate (ATP) a most interesting factor to analyse. So far, only cell disruptive or intensiometric ATP assays were developed for P. falciparum with different drawbacks and partly inconsistent results. Therefore, we stably integrated fluorescent probes, based on förster resonance energy transfer (FRET) and known as Adenosine 5-Triphosphate indicator based on Epsilon subunit for Analytical Measurements (ATeam), into the genome of P. falciparum. ATeams are capable of measuring ATP levels in a ratiometric manner, thereby, facilitating in vivo measurements of ATP dynamics in real-time using fluorescence microscopy, plate reader and flow cytometry, while overcoming many obstacles of established ATP analysing methods. We already verified stable integration of ATeams into the P. falciparum genome. In a next step, we will analyse the proof of principle by comparison of in vitro measurements of recombinant ATeam protein with in vivo calibrated transgenic parasites. Besides measuring ATP levels in the cytosol, we will also target the sensor to different subcellular compartments of the parasite, as well as to the erythrocyte host cell. In this way, we hope to uncover so far unknown mechanisms of action of selected antiparasitic compounds and get new insights into parasite-host interactions.

Nuclear Liver X Receptors in IL-1ß induced Phospholipid Release from Synoviocytes into Osteoarthritic Synovial Fluid

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Previously, we reported that the phospholipids (PLs) of synovial fluid (SF) increase substantially during osteoarthritis (OA), however, the exact mechanism governing this change is largely unknown. Endogenous agonists of the nuclear liver X receptors (LXR) are oxysterols generated e.g. by cholesterol 25-hydroxylase (CH25H) and cytochrome P450 family 7 subfamily B1 (CYP7B1). Here, we test the hypothesis that IL-1 enhanced the PL release from fibroblasts-like synoviocytes (FLS) by stimulating the expression of both cholesterol hydroxylases respectively oxysterols and, thus subsequently of LXR and of ABC-lipid transporters.
Cultured FLS isolated from knee synovial tissues of OA patients undergoing knee replacement surgery were treated with IL-1β, and the LXRα agonists TO901317 or GW3965 for 24 and 48 hours (n=6). Our analysis revealed that IL-1β significantly elevated the expression of both cholesterol hydroxylases (CH25H, CYP7B1) already after 24 hours whereas T0901317 displayed no effect. Furthermore, IL-1β and TO901317 significantly increased the release of [3H]-labelled PLs from FLS into media during 48 h. The effect of TO901317 could be reproduced with another LXR-α agonist GW3965. RT-qPCR analysis demonstrated that the key elements required for the release of PLs from FLS, namely the ABC transporters ABCA1, ABCG1 and the apolipoprotein E (APOE) were elevated after 24 h treatment with TO901317 but not with IL-1β. However, our western blot analysis revealed that the ABCA1 protein was elevated after 48 h in both TO901317 and IL-1β treated samples. Taken together, our data suggest an indirect and thus delayed effect of IL-1β on ABC-transporters and APOE involved in PL release from FLS. In conclusion, our results indicate that IL-1β follow the novel discovered IL-1β-CH25H-CYP7B1 pathway in inducing LXRα which in turn activates the PL release from FLS

into the SF during OA by an enhanced expression of ABC-transporters.

Cryptosporidium parvum: metabolic impact on host cells and metabolism-related parasite inhibition under physioxic/hyperoxic conditions

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Cryptosporidium parvum is an important zoonotic protozoan parasite causing severe enteritis in young calves, children and immunocompromised humans. Epicellular replication of this parasite requires energy and cell building blocks, however, C. parvum owns only minimal metabolic capacities. Consequently, C. parvum highly depends on host cell metabolism. Therefore, we here analyzed metabolic signatures of C. parvum-infected host cells under physioxic (5% O_2) and hyperoxic (21% O_2) conditions and examined effects of targeted inhibition of selected metabolic pathways on C. parvum infection.
For C. parvum detection, a Vicia villosa-based assay was used. Based on data of conversion rates of key metabolites in supernatants of C. parvum-infected HCT-8, targeted inhibition studies were performed using following metabolic blockers: galloflavin (lactate dehydrogenase blocker), lonidamine (hexokinase blocker), syrosingopine (MCT1/MCT4 blocker) and compound 968 (C968, glutaminase 1 blocker). In addition, glycolytic responses of C. parvum-infected HCT 8 cells were analyzed by Seahorse® and luminescence technology. Moreover, in order to establish a more realistic C. parvum in vitro culture, a recently reported air-liquid system is actually being adapted to bovine small intestinal epithelial spheroids.
Overall, lonidamine, galloflavin, syrosingopine and compound 968 treatments all efficiently reduced C. parvum infection rates under both oxygen conditions. In line, glycolytic responses were found up-regulated in C. parvum-infected HCT-8 at 21% O_2 (24 h p. i.) as detected by Seahorse® instrumentation. Likewise, analyses on glycolytic ATP generation confirmed a parasite-triggered up-regulation of this energy-related pathway in HCT-8 cells. The establishment of an air-liquid system based on primary bovine stem cells is currently ongoing and will deliver an improved cell culture system for C. parvum being closer to the in vivo situation.

Cholinergic regulation of ATP-induced release of the pro-inflammatory cytokines interleukin-1β and interleukin-18 in inflammatory bowel disease (IBD) patients

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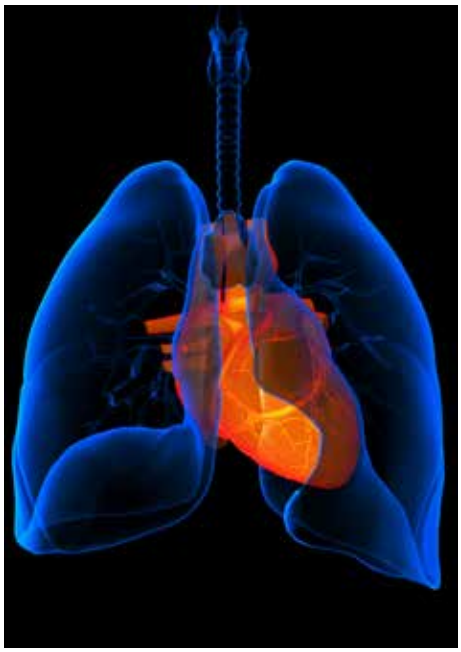
Patients diagnosed with inflammatory bowel diseases (IBD) like Crohn´s disease (CD) and ulcerative colitis (UC) suffer from a broad spectrum of symptoms e.g. diarrhea, abdominal pain or vomiting, resulting in a reduced quality of life. The natural history of IBD is one of periods of remission and flares.The pro-inflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18) are known to play a crucial role in the pathogenesis of IBD. Natural sources of IL-1β and IL-18 are monocytes, macrophages and epithelial cells. In preliminary experiments, we have identified a panel of novel, unconventional nicotinic acetylcholine receptor (nAChR) agonists that efficiently inhibit the adenosine triphosphate (ATP)-dependent release of IL-1β from monocytes of healthy human donors. The aim of this project is to test if this cholinergic mechanism is active in IBD patients as it was observed that cigarette smoke is a risk factor for disease onset and progression in CD but appears to be protective in UC. Our preliminary data show, that the cholinergic mechanism is functional in CD patients. In addition, we were able to show for the first time, that the cholinergic mechanism inhibits the ATP-dependent release of IL-18 from primary monocytic cells. ATP-independent mechanisms, however, remain unimpaired. Further experiments on monocytes from UC patient are needed. The next goal of this project is to clarify the underlying cholinergic signaling pathways. Therefore, we will perform basic research on monocytic cell lines, differentiated macrophages and ex vivo experiments on freshly isolated monocytes from healthy donors as well as IBD patients. In the presence or the absence of nAChR agonists, the ATP-induced release of pro-inflammatory cytokines (IL-1β, IL-18) from cells primed with lipopolysaccharide (LPS), will be analyzed in cell culture supernatant via ELISA and Western blot. Cellular cytokine expression will be analyzed via real-time RT-PCR and Western blot.

Section 3 - Heart, Lung and Blood Vessels



Schedule of Section 3
Tuesday, 29th September 2020

Part 1	**Chairperson: Xuran Chu**
14:45 – 14:55	**Wafaa Mahmoud**
	CXCL13 defines a neuroendocrine cell phenotype in the murine trachea and lung
14:55 – 15:05	**Reshma Jamal**
	Can the lungs develop outside the body? Unfolding the in-vitro isolated lung model in the postnatal alveolarization period
15:05 – 15:15	**Marija Gredic**
	Myeloid cell-specific inducible nitric oxide synthase drives pulmonary hypertension in chronic obstructive pulmonary disease
15:15 – 15:25	Shirisha Bagari
	Role of miR-142 in Idiopathic Pulmonary Fibrosis
15:25 – 15:35	Julie Antoine
	Role of the ER-stress-associated factors C/EBP homologous protein (CHOP) and Apoptosis signal-regulating kinase 1 (ASK1) in the development of pulmonary fibrosis

Part 2	Chairperson: Hafiza Idrees
16:00 – 16:10	**Arun Kumar Reddy Lingampally**
	Characterization of Tg(Etv4-GFP) and Etv5RFP Reporter Lines in the Context of Fibroblast Growth Factor 10 Signaling During Mouse Embryonic Lung Development
16:10 – 16:20	**Kathrin Malkmus**
	Classical transient receptor potential proteins 1, 3 and 6 play a role in chronic hypoxia-induced pulmonary hypertension
16:20 – 16:30	**Edibe Avci**
	The role of HDAC9 deficiency during age-related chronic inflammation in
16:30 – 16:40	**Mohammad Rashedul Alam**
	Effects on the peroxisomal compartment and associated gene transcription during differentiation of T7 alveolar epithelial cells type II (AEC II)

Schedule of Section 3
Wednesday, 30th September 2020

Keynote Section 3	Chairperson: Leili Jafari
09:00 - 09:30	**Prof. Felix B. Engel, University of Erlangen** Heart regeneration: hopes and pitfalls
Part 3	Chairperson: Reshma Jamal
11:15 - 11:25	**Fabienne Knapp**
	Differential effects of right and left heart failure on skeletal muscle in rats
11:25 - 11:35	**Hafiza Idrees**
	Role of sAC-dependent cAMP pool in cardiac cell autophagy: relevance to cardiovascular pathology
11:35 - 11:45	**Paniz Adibi**
	Identification of growth factors and regulatory cytokines during postnatal cell cycle exit in cardiomyocytes
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	Signal-mediated chromatin changes in right heart failure
11:55 - 12:05	**Nicole Molenda**
	The ubiquitin E3 ligase SIAH2 contributes to right ventricular hypertrophy

Identification of growth factors and regulatory cytokines during postnatal cell cycle exit in cardiomyocytes

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Cardiac growth during intrauterine development is primarily mediated by cardiomyocyte proliferation. Murine cardiac myocytes rapidly lose their proliferative ability such that adult cardiomyocytes are almost unable to divide. If the cell cycle arrest in cardiomyocytes is initiated immediately after birth, cardiomyocyte numbers in humans born preterm might be reduced compared to term births, which may elevate the risk for cardiovascular disease. Therefore, understanding how the exposure to an extrauterine environment induces cardiomyocyte cell cycle withdrawal has important implications for preterm birth. Using immunofluorescence staining for proliferation markers, directly after birth, we noticed a drop in Ki67 positive cardiac cells and phospho-histone-H3 positive cardiomyocytes. Moreover, our western blot results showed that within several hours after exposure to the extrauterine environment the activity of mTOR and MAP-kinase pathways was reduced, in mouse ventricular myocardium. These signaling pathways are at least partially regulated by growth factors. Consequently, we hypothesize that the birth-mediated cardiomyocyte proliferation reduction is caused by altered growth factor and cytokine availability in postnatal compared to intrauterine environment. This study's aim is to identify growth factors and cytokines in perinatal hearts with a potential role in cardiomyocyte proliferation. Indeed, our in silico screen in mice and humans revealed that among 161 studied growth factors and cytokines, 68% exhibited variation in their RNA expression patterns upon exposure to extracellular environment. In upcoming analyses, proteomic tools will be used to quantify growth factors and cytokines in mouse perinatal hearts by antibody-array screening. By comparing in silico and proteomic results, candidate growth factors and cytokines will be selected for in vitro studies to induce proliferation in isolated neonatal mouse cardiomyocytes. Taken together, this study could reveal new mechanisms of cardiomyocyte cell cycle regulation in the immediate perinatal period relevant for preterm birth.

Effects on the peroxisomal compartment and associated gene transcription during differentiation of T7 alveolar epithelial cells type II (AEC II)

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Among the 40 different cell types of the lung, only alveolar epithelial cells type II (AECII) and club cells contain substantial amounts of peroxisomes (*Karnati and Baumgart-Vogt, 2008, 2009). However, only hypothetic information is available in the literature on the functions of these cell organelles (e.g. surfactant metabolism) in AECII after treatment with peroxisome proliferating drugs (Fringes and Reith, 1988). Since AECII are difficult to isolate and cannot be maintained in a differentiated state in primary cell culture over long time periods for functional experimental studies, better cell culture models in which AECII exhibit a high differentiation state are needed. Therefore, for further functional studies on the relationship of AECII-differentiation on the peroxisomal compartment, we established a T7 AECII cell culture system. The T7 cell line, derived from isolated AECII from the H-2kb-tsA58 transgenic mouse ("immortomouse") can be cultured under proliferation (permissive) or differentiation (non-permissive) conditions and aspect of differentiation on the peroxisomal compartment can be nicely studied. The results of our study reveal a much higher abundance of peroxisomes in differentiated T7 AECII in comparison to cells in the proliferation state. Furthermore, differentiated T7 AECII showed a specific protein composition and gene expression pattern, similar to the ones observed in Type II alveolar cells in morphological studies on lung tissue sections in situ. The differentiation process was confirmed by the increase of the AECII specific cell marker protein SP-C and the other surfactant proteins (SP-A, SP-B and SP-D). Immunofluorescence, qRT PCR, and Western blotting experiments showed a marked elevation of the mRNAs and proteins of the peroxisomal biogenesis proteins (PEX13p, PEX14p), peroxisomal β-oxidation enzymes (MFP2 and

thiolase), and ether lipid synthesizing enzymes (AGPS and GNPAT) in differentiated T7 AECII in comparison to lower levels in proliferating T7 AECII. Interestingly, the mRNA and protein for the peroxisome proliferator-activated receptor PPAR-beta increased very strongly, suggesting its involvement in the maturation of the peroxisomal compartment in dfferentiating T7 AECII.

Role of the ER-stress-associated factors C/EBP homologous protein (CHOP) and Apoptosis signal-regulating kinase 1 (ASK1) in the development of pulmonary fibrosis

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Idiopathic pulmonary fibrosis (IPF) is an age-related interstitial lung disease with fatal outcome, for which no curative treatment exists to date. The main characteristic of IPF is Endoplasmic Reticulum (ER)-stress and apoptosis in type-II alveolar epithelial cells (AECII), which consequently cause chronic epithelial injury and progressive lung fibrosis. Induction and upregulation of the proapoptotic ER-stress factor CHOP in AECII of IPF-patients is an eminent observation. We developed double-transgenic mice with AECII-specific overexpression of Chop by using the Tetracycline-On-system (SP-C rtTA/tetO7-Chop). However, 3 months old Chop transgenic mice did not develop lung fibrosis in response to 28 days doxycycline-feeding, despite nuclear Chop-overexpression in AECII and apoptosis-induction. We thus suggested that the extent of AECII-apoptosis in the 'Chop-mice' was not sufficient to cause lung fibrosis, and that 'second hits' such as the age may be required for 'high-level' AECII-ER-stress and apoptosis. We therefore exposed 14 and 27 months old 'Chop-mice' to doxycycline-feeding, and currently, we are phenotyping these mice. Furthermore, immunoblot-experiments indicated that ASK1 and its downstream effector kinases JNK1/2 and p38MAPK are hyperactivated in IPF-lungs as compared to normal donor-lungs. Immunohistochemical analyses revealed that ASK1-signalling is upregulated in both the apoptotic AECII as well as in the apoptosis-resistant fibroblast-foci of IPF-lungs, thus revealing a dual function in pulmonary fibrosis. Because ASK1 becomes activated by oxidative- and ER-stress, we hypothesize that in lung fibrosis including IPF, the ASK1-signalling pathway induces through the ASK1-p38 axis as well as the ASK1-JNK axis apoptosis in AECII, while in lung fibroblasts, both ASK1-signalling pathways mediate survival-related and profibrotic responses. We therefore aim to evaluate the therapeutic blockade of ER-stress-signalling on development of lung fibrosis in vivo by using Ask1(-/-)-knockout mice or the small-molecule Ask1-inhibitor selonsertib in the bleomycin-mouse model of lung fibrosis. We conclude that chronic ER-stress mediated by ASK1 plays a major role in IPF-pathogenesis.

The role of HDAC9 deficiency during age-related chronic inflammation in lung

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Aging is known to be associated with altered inflammatory markers, influenced by epigenetic modifications. Aging immune system has persistent low-grade pro-inflammatory state. Histone deacetylase 9 (HDAC9), a member of class IIa, catalyzes the removal of acetyl groups from lysine residues in both histone and non-histone proteins. HDAC9 is shown to control the target genes in tumor formation, inflammation, atherosclerosis, and metabol-

ic diseases. The aim of study is to reveal the role of HDAC9 deficiency leading to chronic inflammation in response to differential signaling mechanisms in aged lung. The study is performed in 3 different age groups (Group 1= 8-12 weeks old (young), Group 2= 30-34 weeks old (middle aged), Group 3= ≥ 48 week old (aged)). Interestingly, aged HDAC9 -/- mice showed reduced survival and decreased body weight. Hematoxylin and eosin stainings revealed that aged HDAC9 -/- mice have severe chronic inflammation in the lungs when compared to young and middle aged mice as well as wild type (WT) mice of each group. Importantly, CD45+/CD68+ monocytes and CD45+/CD3+ T-cells were accumulated in inflamed sites of HDAC9 -/- aged mouse lungs. Furthermore, isolation and differentiation of bone marrow derived monocytes to macrophages from aged WT and HDAC9 -/- mice as well as cytokine profiling from human HDAC9 depleted M1 macrophages demonstrated that HDAC9 depletion creates dysbalance in anti- and pro- inflammatory status of macrophages. Importantly, HDAC9 depletion also decreases pro-SPC and E-cadherin expression and increases Vimentin expression in alveolar type II epithelial cells and in lung organoid cultures generated from WT and HDAC9 -/- mice. These results indicate that HDAC9 deficiency is involved in chronic inflammation and age-related functional and structural changes of the lungs. The findings of the study will provide important insights to reveal the role of HDAC9 in the regulation of inflammaging-related lung remodeling.

Role of miR-142 in Idiopathic Pulmonary Fibrosis

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Idiopathic Pulmonary Fibrosis (IPF) is a highly lethal, chronic lung disease that usually gets worse over time and leads to mortality in 2-3 years after diagnosis. MicroRNAs are non-coding RNAs of 21-22 nucleotides in length that mostly act by repressing the expression of protein coding genes at the post-transcriptional level via binding to 3' untranslated region (UTR) of targeted mRNAs. MicroRNAs regulate various physiological processes including cell proliferation, cell differentiation and apoptosis. In this study, we focused on miR-142, an emerging key regulator of the Wnt signaling pathway, already demonstrated having a vital role in IPF. The maturation of hairpin transcript of miR-142 gives rise to miR-142-3p (guide strand) and miR-142-5p (passenger strand). It has been revealed that miR-142-3p contributes to maintain proper proliferation of mesenchymal progenitors by physically binding to Adenomatous polyposis coli (Apc) mRNA, a negative regulator of Wnt signaling, regulating its mRNA level. RT-qPCR analysis and Immunofluorescence imaging of lung samples from patients with IPF as well as lungs from mice exposed to bleomycin (murine lung fibrosis model), revealed that hyper proliferative foci with enhanced Wnt signaling are strongly associated with high miR-142-3p expression.

Origin and fate of smooth muscle cells during pulmonary vascular remodeling and reverse remodeling

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During pulmonary hypertension (PH), pulmonary vascular remodeling (VR) is triggered, which happens primarily in pulmonary arterioles. There are three types of vascular remodeling. First, the appearance of smooth muscle actin positive cells in distal pulmonary arterioles, which is not muscularized in normal conditions. Second, the thickening of the tunica media of the proximal arterioles is caused by an increased number of smooth muscle cells. Last, the thickening of tunica adventitia, a layer of connective tissue in vessels, is due to the appearance of smooth muscle cells. Recent studies done by Sheikh et al. (Cell Rep. 2014), have shown that Sma-positive cells in the tunica media and ectopic Sma-positive cells in the non-muscularized distal arterioles are derived from pre-existing Sma-positive cells, namely PH-associated

SMCs. However, we think that other mesenchymal cells that transiently express Acta2 contribute to SMC formation during the remodeling process. GLI1+ cells, located very close to blood vessels and which probably represent a subpopulation of pericytes, maybe the other source of SMCs. In this study, we are going to use in vivo lineage tracing tools to study the origin of PH-associated SMCs by using hypoxia-induced and tobacco smoke-induced PH mice models. Gli1CreERT2/+ targeting the progenitors of SMCs has been crossed with red fluorescent protein reporter tdTomatoflox mice to generate Gli1CreERT2/+; tdTomatoflox mice. This line allows us to permanently label the GLI1+ cells, upon tamoxifen-based Cre activation, 2 weeks prior to hypoxia or smoke treatment.

Myeloid cell-specific inducible nitric oxide synthase drives pulmonary hypertension in chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is a progressive and incurable disease, predicted to become the third leading cause of death worldwide by 2030. COPD is characterized by persistent airflow limitation, often described with terms "chronic bronchitis" and "emphysema". Additionally, most of the COPD patients have mild to moderate pulmonary hypertension (PH), which is associated with increased mortality and morbidity. Intriguingly, pulmonary vascular alterations precede alveolar destruction and have been suggested to drive emphysema development.

Our previous study identified inducible nitric oxide synthase (iNOS) as a key player in the development and reversal of smoke-induced PH and emphysema in mice. Moreover, we demonstrated that iNOS expression in bone-marrow-derived cells drives pulmonary vascular remodelling and consequent PH, but not emphysema.

In this study, we aimed to identify bone-marrow-derived cell type driving smoke-induced PH and decipher pro-proliferative pathways involved in this process.

To achieve these goals, we 1) chronically exposed myeloid cell-specific iNOS knockout mice to smoke to assess the development of emphysema and PH in vivo, and 2) used co-cultures of macrophages and pulmonary artery smooth muscle cells (PASMC), to decipher pro-proliferative pathways in vitro.

Myeloid cell-specific iNOS knockout mice were protected against smoke-induced PH but developed emphysema. Interestingly, iNOS deletion in myeloid cells ameliorated smoke-induced changes in the phenotype of lung inflammatory cells, including the increase in expression of CD206, a marker of M2 polarization, on interstitial macrophages. In co-cultures of M2 macrophages with PASMC, smoke-induced pro-proliferative signalling was abolished by inhibition of iNOS in phagocytic cells. Importantly, numerous iNOS-positive and CD206-positive macrophages accumulated in the proximity of remodelled vessels in lungs of COPD patients, as shown by immunohistochemistry.

Overall, our results demonstrate that deletion of iNOS in myeloid cells prevents the development of smoke-induced PH and suggest that iNOS-dependent communication of M2 macrophages and PASMC drives underlying vascular remodelling.

Sensory epithelial cells in the respiratory tract of mice, pigs and man

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Specialized sensory epithelial cells sensing noxious chemicals and initiating protective reflex are found along the mammalian respiratory tract. Studies in rodents suggested the presence of at least three populations: 1) neuroendocrine cells with basal dense core vesicles, 2) solitary cholinergic chemosensory cells, 3) cells expressing villin with no further known characteristics. Still, even mice and rats differ considerably in some aspects. This study aims to provide a positional and sensory signalling pathway focused inventory of such potentially sensory cells in the respiratory tract of a larger mammal (pig) in comparison to mice, the most common model organism.
Specimens of human taste buds (positive control) and respiratory tract were obtained from anatomy body donors, pig specimens were obtained from the butchery, mouse tissues were taken from TRPM5-eGFP reporter and C57BL6/J mice. Single- and multiple-labelling immunofluorescence was conducted on paraffin and fixed frozen sections with relevant marker antibodies. In mice, neuroendocrine cells (PGP9.5+, CGRP+) extend from the trachea deep into lung until final branching of bronchioles but are missing in alveoli. Chemosensory cells (TRPM5+, GNAT3+, PLCβ2+) were found in the trachea, extrapulmonary bronchi and only exceptionally in intrapulmonary bronchioles. In pig, PGP9.5+, CGRP+, villin+, advillin+ and ChAT+ cells were found in the trachea, main bronchus and in the intrapulmonary bronchioles. In human, solitary PGP9.5+ epithelial cells were found at a density of (1.0-6.8) cells/mm basement membrane in the trachea (N=10). Additional DCLK1+ and PLCβ2+ cells were indicated in only very few experiments, and no immunolabeling for GNAT3, TRPM5, and Pou2f3 was observed.

These data highlight marked species differences in spatial distribution and equipment with signalling pathways. In particular, data from the most common model organism, the mouse, can hardly be transferred to humans.

Role of sAC-dependent cAMP pool in cardiac cell autophagy: relevance to cardiovascular pathology

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Autophagy is an evolutionary conserved cellular regulatory pathway that works as a house-keeper during normal physiological conditions and can be a source of survival during stress conditions. During stress, autophagy clears the damaged and non-functional molecules as well as organelles from the cell. The removal of damaged mitochondria by autophagy is termed as mitophagy and is an important cellular mechanism that facilitates cell survival by preventing disturbance of redox and metabolic homeostasis. Therefore, recycling of intracellular components by autophagy in cardiomyocytes plays crucial role during cardiac stress. A wide range of cardiac disease have been associated with altered autophagy such as hypertrophy, ischemic- and pressure-overload heart failure. Within several signaling pathways controlling autophagy, increasing evidence suggests a role of cAMP signaling. cAMP is an important secondary messenger in cell signaling and is generated by the activation of membrane-bound and soluble form of adenylyl cyclases. In present study we have hypothesized that metabolic and redox imbalance due to dysregulation of sAC dependent cAMP is the underlying mechanism of cardiomyopathy. The objective of this study is to investigate the causal role of sAC downregulation in disturbance of autophagy/mitophagy flux and find out the underlying molecular mechanisms in cardiac cells. The study is planned to be performed by using H9C2 myoblasts, adult rat cardiomyocytes and endothelial cells (HMEC1 cell line, HUVEC). The main methodology will include cloning, cell culturing, siRNA/shRNA-mediated downregulation, lentiviral-mediated over expression of sAC, western blotting, RT-PCR, live-cell imaging, and cell death assays etc.

Comparison of the transcriptional landscape of human right ventricle in chronic thromboembolic pulmonary arterial hypertension before and after pulmonary endarterectomy

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Chronic thromboembolic pulmonary hypertension (CTEPH), a subclassification of pulmonary hypertension (PH), is a rare disease affecting the pulmonary vasculature. CTEPH is characterized by thromboemboli, vascular remodelling, and chronic obstruction in the pulmonary arteries. The increase in pulmonary artery pressures causes right ventricular (RV) hypertrophy and dilatation and finally leads to right heart failure and death. Surgical removal of thromboembolic clots by pulmonary endarterectomy (PEA) in CTEPH patients provides an opportunity for relief, as it restores RV function to normal and leads to significant clinical improvements in clinical parameters as documented by cardiac magnetic resonance. Using transcriptomic profiling, this study aimed to identify the potential biomarkers, master regulators, and signalling pathways that are specifically involved in the effect of PEA on the RV of CTEPH patients.
RNA -sequencing (RNA-seq) performed on RV biopsies obtained from CTEPH patients before PEA surgery, and the results were compared with those from RV biopsies obtained during follow-up evaluation at 12 months post-PEA. Bioinformatic analysis of RNA-seq data showed 2799 genes (n=14; Log2 fold change ≥0.585; FDR≤0.05) differentially regulated between pre and post PEA sample groups. This substantial number of differentially expressed genes (DEGs) indicates a major change in the transcriptional landscape of the RV in these patients. To further investigate the potential biomarker candidates from the large pool of 2799 DEGs, extensive bioinformatic analysis of different data sets shortlisted 250 DEGs that were functionally associated with cardiovascular development or disease. The findings of this study reveal prominent transcriptional changes that occur in response to PEA. Pathway analysis and gene ontology enrichment confirmed altered regulation of signaling involving hypoxia-inducible factor 1 (HIF-1) signalling, mitogen-activated protein kinase (MAPK) signalling, advanced glycation end products and their receptors (AGE-RAGE), hippo signalling, the Janus kinase/ signal transducers and activators of transcription (Jak-STATs) signalling pathway, and proteoglycans after PEA compared with before PEA.
Transcriptomic profiling of RV biopsies from CTEPH patients, revealed a major change in the transcriptional landscape of these patients occurs following reduction the pressure overload of the RV by PEA.

Establishment of CRISPR/Cas9 mediated gene editing for the treatment of COPD

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COPD is a severe progressive disease with poor prognosis. The pathogenesis of COPD is not completely elucidated and the treatment is not available. Recently, we have identified that tobacco smoke increased the expression of NADPH oxidase organizer-1 (Noxo1) and inducible nitric oxide synthase (Nos2, iNOS) in humans and with experimental animals leading to the development of emphysema and pulmonary hypertension via excessive peroxynitrite formation. Thus, the current study aimed to establish CRISPR/Cas9 mediated knockout of Nos2 and Noxo1 genes as a new potential treatment of COPD. CRISPR/Cas9 mediated knockout of Nos2 and Noxo1 decreased mRNA expression of these genes and inhibited the cigarette smoke extract (CSE)-induced decrease of cellular viability of mouse lung epithelial (MLE12) as well as murine alveogenic lung carcinoma (CMT167) cells. Moreover, CRISPR/Cas9 mediated knockout of Noxo1 increased cell viability

and decreased apoptosis after exposure to CSE in precise cut lung slices (PCLS). CRISPR/Cas9 mediated knockout of Nos2 and Nox01 may be a propitious technique to target smoke-induced COPD.

Differential effects of right and left heart failure on skeletal muscle in rats

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Exercise intolerance is a cardinal symptom in right (RV) and left ventricular (LV) failure. The underlying skeletal muscle contributes to increased morbidity in patients. Here, we compared skeletal muscle sarcopenia in a novel two-stage model of RV failure to an established model of LV failure. Pulmonary artery banding (PAB) or aortic banding (AOB) was performed in weanling rats, inducing a transition from compensated cardiac hypertrophy (7 weeks) to heart failure (22-26 weeks). Cardiac function was characterized by echocardiography. Skeletal muscle metabolism was analysed by histological and biochemical methods, qPCR, and Western Blot. Two clearly distinguishable stages of left or right heart disease with a comparable severity were reached. However, skeletal muscle impairment was significantly more pronounced in LV failure. While the compensatory stage resulted only in minor changes, soleus and gastrocnemius muscle of AOB rats at the decompensated stage demonstrated reduced weight and fibre diameter, higher proteasome activity and expression of muscle-specific ubiquitin E3 ligases, increased expression of atrophy markers, increased autophagy activation and impaired mitochondrial function and respiratory chain gene expression. Skeletal muscles of PAB rats did not show significant changes in muscle weight, proteasome or autophagy activation but mitochondrial function was mildly impaired as well. Plasma Interleukin (IL)-6 and angiotensin II were strongly increased at the decompensated stage (AOB>>PAB). Soleus and gastrocnemius muscle itself demonstrated an increase in IL-6 expression independent from blood-derived cytokines only in AOB animals. In vitro experiments in rat skeletal muscle cells suggested a direct impact of IL-6 and Ang II on distinctive atrophic changes. Manifold skeletal muscle alterations are more pronounced in LV failure compared to RV failure despite a similar ventricular impairment. Most of the catabolic changes were observed in soleus or gastrocnemius muscle. Mitochondrial dysfunction and upregulation of atrophy markers were identified as the earliest signs of skeletal muscle impairment.

Characterization of Tg(Etv4-GFP) and Etv5RFP Reporter Lines in the Context of Fibroblast Growth Factor 10 Signaling During Mouse Embryonic Lung Development

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Members of the PEA3 transcription factors are emerging as bone fide targets for fibroblast growth factor (FGF) signaling. Among them, ETV4 and ETV5 appear to mediate FGF10 signaling during early embryonic lung development. In this paper, recently obtained Tg(Etv4-GFP) and Etv5CreERT2–RFP fluorescent reporter lines were generally characterized during early embryonic development and in the context of FGF10 signaling, in particular. We found that both Tg(Etv4-GFP) and Etv5CreERT2–RFP were primarily expressed in the epithelium of the lung during em-

bryonic development. However, the expression of Etv5CreERT2–RFP was much higher than that of Tg(Etv4-GFP), and continued to increase during development, whereas Tg(Etv4-GFP) decreased. The expression patterns of the surrogate fluorescent protein GFP and RFP for ETV4 and ETV5, respectively, agreed with known regions of FGF10 signaling in various developing organs, including the lung, where ETV4-GFP was seen primarily in the distal epithelium and to a lesser extent in the surrounding mesenchyme. As expected, ETV5-RFP was restricted to the lung epithelium, showing a decreasing expression pattern from distal buds to proximal conducting airways. FGF10 inhibition experiments confirmed that both Etv4 and Etv5 are downstream of FGF10 signaling. Finally, we also validated that both fluorescent reporters responded to FGF10 inhibition in vitro. In conclusion, these two reporter lines appear to be promising tools to monitor FGF10/FGFR2b signaling in early lung development. These tools will have to be further validated at later stages and in other organs of interest.

CXCL13 defines a neuroendocrine cell phenotype in the murine trachea and lung

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The conducting airways are lined by different cell types, comprising basal, secretory, ciliated, and rare cells, including ionocytes, solitary cholinergic chemosensory cells, solitary neuroendocrine cells and neuroepithelial bodies. The cellular composition and structure of the epithelium varies from proximal to the distal airways. The airway neuroendocrine cells in the mouse have been implicated with different functions including mechanosensation, chemosensation, regeneration and development of small cell lung cancer, in addition to their role in the regulation of type 2 immune responses through neuropeptide calcitonin gene-related peptide (CGRP). We assessed here chemokine (CXCL13, B-cell attracting chemokine) expression by these cells in C57BL/6J mice by immunohistochemistry, RT-PCR and immunoelectron microscopy. In addition, in silico analysis of publicly available data sets was performed. In silico-analysis of published sequencing data of murine tracheal epithelium revealed CXCL13 mRNA in 68% (36/53) of neuroendocrine cells whilst expression in other cell types was negligible. Immunolabeling also defined 2 phenotypes of tracheal neuroendocrine cells (n = 5), solitary PGP9.5+/CXCL13+ (69%, 1561/2254 cells) and solitary PGP9.5+/CXCL13- (31%, 693/2254 cells). In the lung (n = 5), 4 phenotypes were identified: solitary neuroendocrine cells (5%, 73/1548 cells) in which 7% of them were CXCL13+ and 93% were CXCL13-, and neuroepithelial bodies (95%, 1475/1548 cells) in which 5.5% of cells were CXCL13+ and 94.5% were CXCL13-. Ultrastructural immunohistochemistry validated CXCL13+ and CGRP+ immunoreactive cells as neuroendocrine cells by the presence of numerous cytoplasmic secretory granules. In conclusion, we identify a phenotype of neuroendocrine cells producing the chemokine CXCL13 in the naïve mouse. CXCL13 is predominantly expressed in tracheal solitary neuroendocrine cells and to a lesser extent in solitary cells and neuroepithelial bodies of the bronchial epithelium. Our observation demonstrates phenotypic heterogeneity in airway neuroendocrine cells and points towards a potential immunoregulatory role in bronchial-associated lymphoid tissue formation and B cell homeostasis.

Classical transient receptor potential proteins 1, 3 and 6 play a role in chronic hypoxia-induced pulmonary hypertension

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Chronic hypoxia-induced pulmonary hypertension (CHPH) is a life threatening disease, which is accompanied by a rise in mean pulmonary arterial pressure (mPAP) above 25 mmHg. In addition, it is characterized by an increased right ventricular systolic pressure (RVSP) due to pulmonary vascular remodeling. Latter results in increased muscularisation of small pulmonary arteries and pulmonary vascular resistance leading to right heart hypertrophy and ultimately RV failure. The main cell type involved in vascular remodeling is the precapillary pulmonary arterial smooth muscle cell (PASMC). High level of intracellular calcium concentration is associated with abnormal proliferation and migration of PASMC, which is supposed to be the reason for the development of PH. Although CHPH has a high mortality, the underlying pathomechanism is still barely understood. Classical transient receptor potential channels (TRPC) are non-specific cation channels especially gating calcium and sodium ions and are able to build homomeric and heteromeric channels, which is why we hypothesize that their composition effects their activatability. We suggest that TRPC channels are crucial channels in the pathogenesis of CHPH and that a loss of TRPC proteins 1, 3 and 6 improves the symptoms of CHPH. In fact, the results demonstrated that RVSP and right ventricular wall thickness of TRPC1/3/6 deficient mice (TRPC1/3/6-/-) are ameliorated when compared to wildtype controls whereas the muscularisation of small pulmonary vessels is unaltered. However, in normoxic and hypoxic TRPC1/3/6-/- left ventricular systolic pressure (LVSP) and left ventricular wall thickness are increased. Therefore, we assume that loss of TRPC1, 3 and 6 proteins affects left heart morphology. Along this finding, our further aim is to elucidate the effect of TRPC1/3/6 knockout on vascular remodeling and right heart as well as left heart function in CHPH.

The ubiquitin E3 ligase SIAH2 contributes to right ventricular hypertrophy

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An increased afterload of the right ventricle (RV) can be experimentally induced by chronic hypoxia or mechanic obstruction of the RV outflow, resulting RV remodeling. One of the proteins regulating the hypoxic response is the ubiquitin E3 ligase SIAH2 (seven in absentia 2), but its impact on RV remodeling is unknown. The role of SIAH2 was investigated in Siah2-/- mice in two different models of RV hypertrophy (RVH), induced by hypoxia or by hypoxia-independent pulmonary artery banding (PAB). Cardiac remodeling was characterized by echocardiography, hemodynamic measurements and histology. Hypoxic response was monitored by gene arrays and fluorescence molecular tomography (FMT). Isolated cardiomyocytes or fibroblasts were utilized in functional studies. Siah2-/- mice are largely protected from RV hypertrophy and fibrosis induced by PAB or hypoxia. Reduced fibrosis in Siah2-deficient animals is due to increased anti-fibrotic Apelin signaling and its crosstalk with decreased TGF-β1 signaling pathways. While SIAH2-mediated protection from PAB-induced RVH is also due to elevated levels of the cardioprotective heme oxygenase 1 enzyme in RV cardiomyocytes of Siah2-/- animals, protection from hypoxia-induced maladaptive hypertrophy also involves changes in hypoxia-regulated gene expression. Gene arrays revealed that Siah2-deficient cardiac fibroblasts show an incomplete induction of hypoxia-induced gene expression and a complete lack of hypoxia-mediated gene repression. Siah2 acts as a negative cardiac fibrosis and hypertrophy regulator in response to RV overload induced by hypoxia or PAB. Understanding the precise role of SIAH2 may provide novel therapeutic targets directed against the development of cor pulmonale.

Can the lungs develop outside the body? Unfolding the in-vitro isolated lung model in the postnatal alveolarization period

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Lung development has been studied extensively in recent years, generating new cognizance into the origins of different cell types that exist in the lung as well as molecular pathways that regulate structural changes. Using transgenic mice often is limited due to a global lethal phenotype. This led to the development of tools such as organoids formation or precision cut lung sections. However, such tools described had a major missing component, such as breathing movement inducing stretch forces. Including these forces to mimic the in-vivo situation requested to develop an in-vitro isolated, perfused ventilated lung model.

This project involves the establishment and validation of in-vitro isolated ventilated and perfused lung model. Neonatal mouse lungs are ventilated and perfused in-vitro for periods of 4 until 12 hours at the age of one day after birth until postnatal day 14. Structural and cellular changes are compared with structural features of in-vivo grown respective neonatal mouse lungs. Optimizing ventilation and perfusion parameters in order to reach conditions of neonatal mouse physiology represents the first milestone of the project. Thus, prevention of ventilation induced lung damage and analyses of possible structural and cellular changes of isolated perfused and ventilated lungs versus structural features of in-vivo grown neonatal mouse lungs shall be achieved.

Establishment of an in-vitro method including all physiological aspects is needed to characterize and modulate alveolarization using lineage tracing, cell depletion, pharmacological interventions and gene editing. Thus, cellular and molecular targets for the development of new therapeutic concepts for pulmonary structural diseases can be identified.

The role of the guanine-nucleotide exchange factors in the regulation of endothelial barrier function

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The integrity of Adherens junctions (AJs) is important in the regulation of endothelial barrier stability. Endothelial AJs and the barrier are supported by Rac1, a member of the Rho family of GTPases. In contrast to that, Rac1-mediated activation of NADPH oxidases leads to the overproduction of reactive oxygen species (ROS) and disruption of the endothelial barrier. The activity of Rac1 is regulated by guanine nucleotide exchange factors (GEFs), which switch the GTPase from inactive GDP-bound form to active GTP-bound form. In endothelial cells (ECs), several regulators of Rac1 has been identified. We hypothesized that differential effects of Rac1 activation on endothelial barrier function are mediated through activation of Rac1 by different regulators depending on the type of a signal. More specifically, activation of Rac1 by P-Rex2 protein causes activation of Rac1/NADPH dependent ROS production. The study is performed in human umbilical vein endothelial cells (HUVEC) and a human endothelial cell line. Cells were infected with lentiviral vectors for ectopic overexpression of P-Rex2. In vitro permeability assay revealed that overexpression of P-Rex2 increases the basal activity of Rac1 and causes barrier disruption. Furthermore, TNF-α stimulation amplified permeability in P-Rex2 overexpressing ECs. TNF-α also increased the expression of cell adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1), as well as pro-inflammatory cytokines such as interleukin 6 (IL-6) and interleukin 8 (IL-8). Overexpression of P-Rex2 also enhanced the migration of ECs and potentiated both VEGF and TNF-α mediated EC angiogenesis. These results indicate that P-Rex2 protein is involved in EC barrier disruption. The findings of the study will provide important insights to reveal the role of Rac1 in the regulation of endothelial barrier integrity. Down-regulation of P-Rex2 might be a therapeutic approach to maintain barrier stabilization.

Signal-mediated chromatin changes in right heart failure

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Heart failure is a progressive pathology, developing in response to structural or stress induced damage to cardiomyocytes. It is characterized by morphological and molecular changes to compensate for the additional load in the hypertrophic compensatory phase, followed by rapid degradation at the onset of heart failure. The molecular background of this decompensation and its possible differences in right and left heart failure are incompletely understood. To address these questions a collaborating group established PAB and AOB models in rats. Hearts from these models were on a large scale analyzed on a transcriptomic level to establish differences between the progressions of both pathologies in right and left ventricle as well as septum. These transcriptomic data were subjected to extensive downstream analysis to discover regulatory pathways and interaction networks implicated in left and right heart failure. Specific expression patterns differentiating left ventricular from right ventricular failure were discovered. To further understand the enhancer and chromatin landscape and its relation to the transcriptomic landscapes in pathological remodeling in the left and right heart in response to different stress factors and to identify possible targets for intervention a large scale ChIPseq experiment has been performed in the same animal model. These datasets were also subjected to extensive downstream bioinformatics analysis to correlate these with the expression patterns from the transcriptomic experiment.

Section 4 - Protein and Nucleic Acid Interactions

Schedule of Section 4
Tuesday, 29th September 2020

Part 1	**Chairperson:**
11:30 - 11:40	**Meike Schwan**
	How do bacteria restrict the assembly of a single polar flagellum?
11:40 - 11:50	**Nicole Schmid**
	The influence of bacteriophages on biofilm formation
11:50 - 12:00	**Daniel Edelmann**
	Molecular mechanism of a highly persistent Escherichia coli strain
12:00 - 12:10	**Corinna Ulshöfer**
	CircRNAs as a tool for protein inhibition
12:10 - 12:20	**Saina Azarderakhsh**
	The small proteome of the plant symbiont Sinorhizobium meliloti

Part 2	**Chairperson: Christina Pfafenrot**
13:30 - 13:40	**Anna Didio**
	Circular RNA sponges as splicing modulators
13.40 - 13:50	**Olha Storozhuk**
	MutH recruitment depends on conformational changes after MutS/MutL ternary complex formation on DNA
13:50 - 14:00	**Vladislav Kunetki**
	Covalent trapping mismatch activated long-lived signaling clamp state of MutS
14:00 - 14:10	**Marie Mosbach**
	Sequence- and size-dependent release of EV-associated RNAs
Part 3	
14:45 - 14:55	**Maria Weller**
	Pig Retinal Explants as Model System for Gene Therapy of the Eye
14:55 - 15:05	**Janek Boerner**
	Characterization of an RNase III mutant strain of Rhodobacter sphaeroides
15:05 - 15:15	**Jacqueline Böhme**
	Remodelling of messenger ribonucleoprotein complexes in Schizosaccharomyces pombe
15:15 - 15:25	**Fatimah Alabudeeb**
	Characterization of DNA repair mechanisms and genome editing efficiency in differentiated neurons
15:25 - 15:35	**Timo Schlemmer**
	Elucidating the role of extracellular vesicles in the Barley-Fusarium interaction
Keynote Section 4	**Chairperson: Johanna Seidler**
10:00 - 10:30	**Dr. Maximilian Reuter, Imperial College London, UK** Towards the mechanism of replicative helicase activation in eukaryotes: A functional Analysis

Characterization of DNA repair mechanisms and genome editing efficiency in differentiated neurons.

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Inherited retinal dystrophies is a group of heterogeneous disorders, which vary in severity and progression and have an estimated incidence of 1:3.000 people. There are more than 270 genes that have been associated with these diseases, most of the being expressed in photoreceptors. The anatomic and physiologic advantages of the eye, including its immuno-privileged properties, easy visibility and accessibility, make it an ideal target for gene therapy applications using viral vectors as transfer vehicles. However, the transfer of large genes such as the ABCA4 gene into the retina is hampered due to the existing size limitations for the most important viral vector delivery methods. The genome editing field, in which the CRISPR-Cas9 technology is used to introduce DNA double strand breaks (DSB) that are subsequently repaired by the cells own repair systems, has gathered much attention in recent years and is currently the new focus of research. The major pathways for DSB repair are non-homologous end-joing (NHEJ) and homology directed repair (HDR). The main obstacle for its application in the retina is the lack of adequate information of DNA repair efficacy in postmitotic neurons, such as photoreceptors. The goal of this project is to identify and to study DNA repair and to improve genome editing efficacy in mature neurons. We standardized cell culture conditions for iNGN cells, which are inducible pluripotent stem cells that differentiate to mature neurons upon activation of the TET ON system by adding doxycycline to the culture media. In addition, we will compare the efficacy of DNA repair in iNGN vs iNGN-Tet-3KO to determine the effect of the Tet3 protein, a master regulator of neuron cell type specific gene expression. We will transfect the iNGN cell line with the traffic light reporter (TLR)-3 system, which allows to study NHEJ and HDR efficiency simultaneously, using a CRISPR-Cas version that is inducible with 4-hydroxy tamoxifen prior to differentiation. As a control, we successfully transfected HEK293T cells applying the inducible system. In addition, our second approach is lentiviral transduction of the differentiated iNGN cells. In conclusion, we aim to study the efficacy of the different genome editing approaches in mature neurons and to analyze the impact of Tet3 on the DNA repair system.

The small proteome of the plant symbiont Sinorhizobium meliloti

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Our model organism Sinorhizobium meliloti is a soil-living plant symbiont belonging to the class of the alphaproteobacteria. It plays an important role as nitrogen-fixing natural fertilizer. To identify small proteins (max. 50 aa) in S. meliloti, a 6-frames protein data base was created and used in mass spectrometry analyses, particularly after solid phase enrichment (SPE). This approach led to the detection of 61 small protein candidates, 17 of them being even smaller than 30 aa. Clear differences in the small proteome were observed under different growth conditions. In minimal medium (MM), 14 small protein candidates were identified in (3 after SPE). In contrast, 22 candidates (19 after SPE) were detected in rich medium (TY). Surprisingly, 10 min exposure to tetracycline used at a subinhibitory concentration in TY medium led to the detection of 34 small protein candidates (28 after SPE). One of the SPE-detected, tetracycline-induced small proteins was peTrpL, which plays a role in multiresistance. A small ribosomal protein (rpmJ) was detectable under all culture conditions without SPE enrichment and therefore can be seen as an internal control. Analyses of the start codon characteristics in general reveal that ~40% of the small open reading frames (sORFs) up to 50 aa start with ATG, while the other start codons are almost equally distributed between GTG, CTG and TTG (each ~20%). When only proteins up to 30 aa were taken into account, the distribution between all four start codon was similar.

CTG was rarely found as a start codon under MM conditions. In a bioinformatic prediction, only sORFs preceded by a Shine-Dalgarno sequence and starting with ATG were considered. The 12 predicted sORFs were in range of 41 to 47 codons, and 8 of them were verified by mass spectrometry. For validation of the sORF candidates identified by bioinformatics prediction and mass spectrometry, and for detection of new sORFs, we aim to use ribosomal profiling. Most recent results will be presented.

Characterization of an RNase III mutant strain of *Rhodobacter sphaeroides*

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In this work an RNase III deletion mutant of the facultative phototrophic α-proteobacterium *Rhodobacter sphaeroides* was characterized. The aim of this study was to identify differences in phenotype and RNA physiology between wild type and mutant, which can be explained by a loss of RNAse III activity. We analyzed the growth behaviour of the mutant and the wild type under different growth conditions, including microaerobic and phototrophic mode of growth. As *Rhodobacter sphaeroides* is known for its metabolic versatility, we were also interested in regulation of the transcriptome. The results suggests that the deletion of the RNase III coding gene (rnc) strongly impacts phenotype and transcriptome of *Rhodobacter sphaeroides*.

Remodelling of messenger ribonucleoprotein complexes in Schizosaccharomyces pombe

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Eukaryotic gene expression is a fundamental and highly controlled process to ensure the functionality of the organism. The mRNA plays an elementary role in this process, since after its transcription from the DNA in the cell nucleus it is exported into the cytoplasm where it is needed for translation and therefore the synthesis of proteins. Already co-transcriptionally, various proteins bind the mRNA and package it into so-called messenger ribonucleoprotein complexes (mRNPs). Those proteins do not only regulate mRNA processing, but also other mechanisms such as nuclear export and mRNA localization, translation and degradation.
These proteins include the essential helicases Dbp2 and Dbp5, which have crucial roles in mRNA processing[1] and the export of mRNA from the cell nucleus into the cytoplasm[2], respectively. Tho2, Tho5, Mlo3 and Uap56 are important to connect transcription and mRNA export[3]. Like Tho2, Paf1 is responsible for the elongation of transcription[4]. The poly(A)-binding protein Pab2 is involved in metabolic processes[5]. However, these different functions only exemplify the different regulatory mechanisms of a few proteins during mRNP remodelling.
For this reason, the protein composition of these new mRNPs as well as the early mRNP remodelling steps are of special interest with regard to the role of the aforementioned proteins. For this purpose, RNA-protein crosslinking and time-resolved comparative proteome analysis are performed in the model organism Schizosaccharomyces pombe.

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Circular RNA sponges as splicing modulators

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Circular RNA (circRNA) is a newly described class

of stable RNAs, generated by alternative splicing from pre-mRNAs by circularization of certain exons. Depending on the cell type, circRNAs are currently estimated to be expressed from up to 22% of active genes. Most circRNAs are present in low levels in comparison to the linear forms, as the process of backsplicing is not efficient. However, the resistance to exonucleases allows some circRNA to accumulate in significant quantities. The most widely described circRNA functions are sponging of proteins and miRNA, regulation of RNA expression and protein translation. Two naturally expressed circRNAs, CDR1as/ciRS-7 and SRY, have been shown to contain conserved microRNA (miRNA)-binding sites and act as miRNA sponges, suppressing miRNA activity and resulting in increased RNA expression. Due to their elevated stability compared to linear RNA, circRNAs may be an interesting tool in molecular biology. Our study focuses on circular RNA production from different vectors, including permuted intron-exon (PIE) splicing strategy for the generation of circRNAs with protein sponge function. Here we clone C/A reach elements, which are known to bind a global regulator of alternative splicing hnRNP L. In addition to that, we develop artificial circRNAs comprising multiple repeats of SELEX sequence, which was shown to bind HnRNP L with high affinity in our previous studies. As an alternative, we use Tornado expression system for designer sponge production. In human cell lines, this system ensures high circRNA expression rate, comparable with the housekeeping RNAs. After introducing these circRNAs in mammalian cell cultures, we assay alternative splicing of hnRNP L target genes by RT-PCR. Therefore we are aiming to establish artificial designer circRNA affecting mRNA splicing by protein sponging. In prospect this strategy can become a promising tool in molecular medicine and biology.

Molecular mechanism of a highly persistent Escherichia coli strain

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Bacteria are frequently exposed to harmful conditions, even while dwelling in their natural habitats. Hence, versatile survival strategies are required to endure inhospitable environments. Persister cells can endure several different stress conditions, including antibiotic treatments. The molecular basis leading to persistence remains enigmatic until today. However, persistence is often linked to a reduced cell growth and altered metabolism.

The type I toxin-antitoxin system TisB/IstR-1 was linked to bacterial persistence due to its mode of action. In the case of DNA damage, the small hydrophobic toxin TisB is expressed, conferring cellular depolarization and ATP depletion to its host. Translation of TisB is tightly regulated by its RNA antitoxin IstR-1 and a complex RNA structure formed by the first 41 nucleotides of the 5'-UTR of the tisB mRNA. Previous investigations showed a highly persistent phenotype in an E. coli strain, in which both regulatory RNA elements were deleted (strain ΔistR Δ1-41), but the molecular basis remained elusive. Flow cytometry analysis of highly persistent strain ΔistR Δ1-41 revealed the presence of a growth-inactive subpopulation, which is absent in E. coli wild-type cultures. Cell sorting experiments determined that the non-growing population contributes to the high persister frequency. Northern blot analysis showed accumulation of tisB mRNA during stationary phase. Western blot experiments confirmed TisB expression independently of any DNA damage. We suggest that TisB expression during stationary phase impedes growth resumption and thereby contributes to persister formation. We provide a detailed description of the physiology and molecular mechanism of TisB-dependent persisters in strain ΔistR Δ1-41. Hence, we suggest strain ΔistR Δ1-41 as a valuable new model system for fundamental research of bacterial persistence.

Covalent trapping mismatch activated long-lived signaling clamp state of MutS

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The DNA mismatch repair system is a key process in the correction of replication errors in all three kingdoms of life. The process in Escherichia coli starts with recognition of mismatches by the ABC-ATPase MutS. After conformational changes caused by mismatch binding and ATP, another ATPase, MutL binds MutS to form a ternary complex leading to activation of enzymes required for strand discrimination, error removal. Despite years of research, the structure of the protein-protein complexes have remained elusive, and the MMR mechanism raises many questions. One reason for these problems lies in the highly dynamic nature of the complexes involved in the MMR-pathway. We focused on the first step of DNA mismatch repair, namely on the conformational changes of MutS and its functions. Using the site-specific cross-linking with a single-cysteine MutS variant, we trapped a transient active state of MutS on DNA. Combining this approach with the Förster Resonance Energy Transfer (FRET method and biochemical assays, we show that this transient state is important for the next steps in the DNA repair pathway, namely in the MutL recruitment and DNA nicking by MutH and likely resembles the mismatch-active signaling clamp state of MutS on DNA. To investigate an intermediate conformation between the mismatch-bound and sliding clamp states, we used chemically modified oligonucleotide with a sulfur reactive crosslinker and show mismatch and ATP dependent movement of connector domain.

Sequence- and size-dependent release of EV-associated RNAs

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Within the last decade, extracellular vesicles (EVs) turned more and more into the focus, due to their important role in intercellular communication. In addition to cells which undergo programmed cell death and thereby secrete vesicles (apoptotic bodies), healthy cells also massively release vesicles. These EVs are important factors for cell-cell communication, acting as vehicles of biological material between parental and recipient cells. EVs contain all types of biomolecules such as different classes of RNAs, like mRNA, miRNAs and circular RNAs. Recently it was shown that RNAs are selectively exported into vesicles [1-3]. However, the factors and mechanisms that contribute to this specificity remain mainly elusive. For example, a so-called Exo-motif has been described for miRNAs, which, however, cannot be transferred to all RNA classes. In addition, for circular RNAs a size-dependent export was suggested [1]. Moreover, only a few putative protein factors involved in packaging have been described so far [2]. To explore potential RNA-length preferences for export into EVs, we designed five individual constructs, which produce RNAs with different length (80, 120, 200, 360 and 680 nts). These constructs were transfected and expressed in HEK293 cells. The expression of all five constructs is based on an RNA polymerase III promoter and a U6 terminator.

In addition, we designed a modified in vivo SELEX approach (Systematic Evolution of Ligands by Exponential Enrichment) to identify putative RNA sequence elements acting as export signals for the selective release of certain RNAs species into EVs. Therefore we generated a random sequence pool (N40), which was transfected and expressed in HEK293 cells. Moreover, several expression constructs were used, which consist of either an RNA Pol II or a Pol III promoter to analyze possible modification effects at the 5'-end of the RNA. Similarly, we introduced transcription terminators at the 3'-end to prevent polyadenylation. EVs were isolated from transfected cells, followed by RNA isolation, library preparation, RNA-seq analysis and bioinformatic identification of enriched RNA motifs. Sequence and motif enrichment analyses are now in progress. This unbiased method should contribute to our understanding of how RNAs are specifically packaged.

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Elucidating the role of extracellular vesicles in the Barley-Fusarium interaction

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Fusarium graminearum (Fg) is a necrotrophic fungal pathogen that causes devastating diseases on its crop hosts barley and wheat. Recently, small RNAs (sRNAs) were identified as mobile communication signals between eukaryotes and their pathogens, symbionts or parasites. It has been shown that pathogens secrete sRNAs as effectors to suppress plant immunity and plants use endogenous sRNAs to resist infection, a phenomenon termed cross-kingdom RNAi; ckRNAi. However, little is known about the transport of fungus- or plant produced sRNAs to silence genes that contribute to immunity. Extracellular vesicles (EVs) are predicted playing a key role in the bidirectional transfer of sRNAs that mediate ckRNAi. To address this knowledge gap, we investigated the effects of EVs isolated from barley and Fg on their counterparts during plant-fungal interaction. Towards this, we developed a protocol for the isolation of EVs from Fg liquid cultures and assessed how Fg EVs contribute to fungal pathogenesis in barley using infiltration assays. To test the interdependence of EVs during Barley-Fg interaction, we treated Fg cultures with barley EVs. We found that infiltration of Fg EVs caused host specific phytotoxic effects in barley and barley EVs impaired Fg growth.
Of note, Fg cultures showed an increase in purple pigmentation upon inoculation with barley EVs, suggesting a stress-induced premature formation of fruiting bodies. Together, our results demonstrate that EVs contribute to the Barley-Fg interaction, however, further studies are needed to unravel the nature of EV cargoes (e.g. protein and/or sRNA) responsible for affecting its plant/fungus counterpart.

The influence of bacteriophages on biofilm formation

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There are two different main bacterial lifestyles that can be distinguished: a planktonic and a sessile lifestyle. The sessile lifestyle is commonly called biofilm. In biofilms, cells are embedded in an extracellular matrix which consists of various substances such as polysaccharides and DNA. Cells in biofilms are more protected against several stressors such as antibiotics. Hence, biofilm formation is a considerably issue in medical and industrial settings. The potential usage of viruses (bacteriophages) that infect and lyse bacteria to prevent biofilm formation has been discussed for several years.
To study the influence of bacteriophages (phages) on biofilm formation, we used *Shewanella oneidensis* MR-1 as a model organism and isolated natural phages that can infect cells of this species. Among others, the isolated phages Thanatos and Phonos were characterized. Thanatos and Phonos are both lytic phages with completely different properties. While Thanatos uses specific sugar residues of LPS (lipopolysaccharide) as an adsorption receptor, Phonos attaches to the major pilin from the MSHA pilus, which is an important factor for initial biofilm formation. Also the lysis behaviour of both phages differs. Cultures treated with Thanatos are quickly lysed while lysis due to Phonos can be barely observed. Surprisingly, first biofilm experiments with Thanatos or Phonos showed that biofilm formation is increased when phages are added at an initial phase. In contrast, a treatment with either of both phages leads to reduced biofilm biomass in later stages of biofilm formation. Taken together, these results show that the social biology of phages is extremely complex and not only based on simple killing the host.

How do bacteria restrict the assembly of a single polar flagellum?

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Flagella are organelles of locomotion and multiprotein complexes whose positioning, number and assembly requires complex spatiotemporal control. In many bacterial species, the MinD-type ATPase FlhG is central to the numerical control of flagella. The deletion of flhG in polarly flagellated bacteria leads to polar hyperflagellation. Here, we have studied the molecular mechanism of this numerical control of flagella in Shewanella putrefaciens CN 32 as a general model organism. To limit the number of flagella to a single one, FlhG must reach the cell pole by passive transport with the flagellar switch complex protein FliM to the nascent flagellum. Preventing FlhG-FliM interaction, results in a flhG deletion phenotype, a polar hyperflagellation. Besides the switch complex protein FliM, FlhG also interacts with the flagellar transcriptional master regulator FlrA. While FlrA and FliM share the same binding site on FlhG, their binding depends on the ATP-dependent dimerization state of FlhG. FliM interacts with the ATP-independent FlhG monomer and FlrA interacts only with the ATP-dependent FlhG dimer. Interaction of FlrA and FlhG inhibits the FlrA transcriptional activity and therefore the production of flagellar components. An overproduction of FlhG means that the cells are no longer able to form a flagellum and an overproduction of FlrA results in a hyperflagellation like an flhG deletion. Thus, FlhG partner switching between the flagellar switch complex protein FliM and flagellar master regulator FlrA underlies the mechanism of flagella numerical restriction, in which the transcriptional activity of FlrA is down-regulated through a negative feedback loop. Not only the cellular copy number of FlhG but also its subcellular localization is critical for its function in the numerical regulation of flagella. This study demonstrates another level of regulatory complexity underlying the spatio-numerical regulation of flagellar biogenesis, and implies that flagellar assembly transcriptionally regulates the production of initial building blocks.

MutH recruitment depends on conformational changes after MutS/MutL ternary complex formation on DNA

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Mismatch repair is an evolutionary conserved system that maintains genome stability by identification and correction polymerase misincorporation errors. In *Escherichia coli* the process starts with mismatch recognition by MutS followed by ATP-dependent formation of sliding-clamp and complex formation with MutL leading to nicking at the hemi-methylated GATC site by MutH, which provides an entry point for the subsequent unwinding/excision step to remove the mismatch.
Limited information is available on the timing of MutH recruitment and activation but it has been shown to require the ATP-binding function of MutL. We developed an ensemble FRET-based assay allowing us to monitor protein recruitment to long circular DNA containing a single-mismatch in a sequence and position independent manner. DNA was stained non-covalently with donor dyes and single-cysteine variants of MutS, MutL or MutH were labeled with acceptor fluorophores. MutH binding to DNA revealed complex kinetics with a prominent lag phase. Order of addition experiments suggested that MutS sliding clamp formation and ternary complex formation with MutL were not sufficient for MutH recruitment. We identified an additional slow step after ternary complex formation that controlled MutH recruitment, probably, involving a conformational change in MutL. Moreover, the assay allowed us to observe multiple loading of MutH onto DNA which was dependent on MutS and MutL concentrations but independent on number of GATC sites further supporting the multiple loading/sliding clamp model of MMR.

CircRNAs as a tool for protein inhibition

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Circular RNAs (circRNAs) form a new class of non-coding RNAs in eukaryotes. They are generated by back-splicing of pre-mRNAs, i.e. an upstream splice acceptor is joined to a downstream splice donor. This covalently linked circular junction makes them resistant to exonucleases and thus results in long half-lives and accumulation in the cytoplasm. Although the functions of endogenous circRNAs are largely uncharacterized, there are several lines of evidence suggesting diverse regulatory roles within the cell, e.g. sponging of microRNAs or RNA-binding proteins (RBPs). In addition, the unusually high stability of circRNAs makes them a useful tool for new strategies to treat human diseases.
In this project, synthetic circRNAs are generated to bind and thus inhibit the RBP IMP3. IMP3 is a well-known tumour marker and its upregulation in tumour tissue is associated with poor prognosis for the patient. Thus, inhibition of this protein is expected to lead to a positive effect on patient survival. The binding of synthetic designer circRNAs was first tested in vitro and is in a second step tested in vivo. In vitro binding was tested by incubation of radioactively labelled circRNA with purified recombinant IMP3. In vivo binding is examined by transient transfection of circRNA into human cell lines followed by immunoprecipitation and qRT-PCR. Once verified, the inhibition of IMP3 can further be tested by functional assays, e.g. by assessing the effect of circRNA sponging on the stability of IMP3 targets.

Pig Retinal Explants as Model System for Gene Therapy of the Eye

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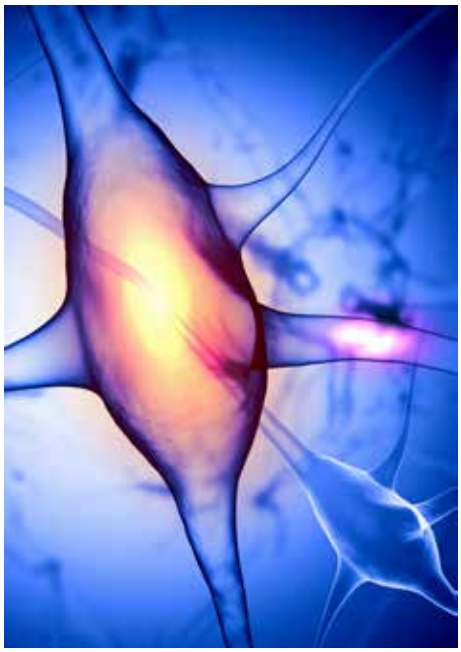
Genome Editing is one of the most exciting developments in medical research over the last years. Especially the CRISPR/Cas9 approach is easily implementable in almost every research lab and seems to be a straightforward approach for treating genetic disorders. But there is a long way until an idea can actually be transferred into a treatment option for human patients. After a few molecular cloning steps, the desired guide-RNA has to be tested for efficacy in bringing the Cas9 endonuclease to its target in the genome. At first these tests take place in vitro after transfecting a cell culture with the respective plasmids. The next step after finding the most active guide-RNA it would normally be transferred into an in vivo model such as a knockout mouse. In most cases though, this proves difficult because of the differences in species and handling. A promising intermediate ex vivo approach for further tests especially regarding dosage and safety issues could be the organotypic retinal explant culture. In the present study porcine retinal explants were obtained from eyes of healthy adult pigs and cultured on a semipermeable membrane for up to 20 days. Different medium compositions were tested with regard to tissue integrity. Haematoxylin-Eosin (HE) staining on frozen retinal explant sections was used to detect the quality of tissue preservation. HE staining revealed changing morphology of the retinal explants over time. Therefore, the various media compositions used had different effects on the cultured explants. Foetal calf serum (FCS) appears to have a negative influence on the retinal integrity. Especially, cultures without FCS but supplemented with insulin instead showed little to no degeneration of retinal layers. Additional experiments have to prove if these culture parameters can ensure retinal tissue integrity over time without compromising a future genome editing approach.

Section 5 - Neurosciences



Schedule of Section 5
Wednesday, 30th September 2020

Part 1	**Chairperson: Dominic Osei**
11:15 - 11:25	**Ann-Kathrin Onkels**
	Adipokine Treatment of Microglia in an altered TNF-System
11:25 - 11:35	**Vinothkumar Rajendran**
	FGFR inhibitor BGJ398 protects against inflammation and demyelination in EAE through ERK/Akt phosphorylation
11:35 - 11:45	**Stephan Leisengang**
	Mesenchymal stem cells inhibit the inflammatory response of spinal dorsal horn cells in a new model of co-cultivation
11:45 - 11:55	**Jessica Hernandez**
	The effects of neutropenia on the generation and expression of a sickness response during systemic inflammation.
11:55 - 12:05	**Maanvee Mirakhur**
	Effect of protease signalling on myenteric neurons for regulation of gastrointestinal motility
12:05 - 12:15	**Benedicta Mensah**
	The ligamentum arteriosum: Smooth muscle with idiosyncratic innervation.

Part 2	**Chairperson:**
13:15 - 13:25	**Sara Shabani**
	The Role of Oxytocin in Sexual Sensation Seeking: A Candidate Gene Study Looking at rs3796863 and rs53576
13:25 - 13:35	**Osama Elyamany**
	Top-Down Modulation of a Dichotic Listening Task with simultaneous Electroencephalography (EEG) Recording
13:35 - 13:45	**Rebecca Claßen**
	Functionalised nanoparticles binding to muscarinic and adrenergic receptors
13:45 - 13:55	**Franz Nürnberger**
	Lipopolysaccharide Induced Tolerance: Effects On Rat Primary Cultures Of The Afferent Nociceptive System
Part 3	**Chairperson: Osama Elyamany**
14:30 - 14:40	**Dominic Osei**
	Numerical abundance of PEX14- and Catalase-positive peroxisomes in different brain cell types of 42-day-old wild-type, TNF-α-transgenic, TNFR1- and TNFR2-Knockout mice
14:40 - 14:50	**Julia Diago Perez**
	HYPOXIA-INDUCED EPIGENETIC REPROGRAMMING IN TUMOR DEVELOPMENT
14:50 - 15:00	**Aya Alserw**
	The role of α-ketoglutarate homeostasis in tumour invasion and metastasis
15:00 - 15:10	**Muyao Tang**
	Analysis of P2X7 Receptor and Panx1 Expression of Healthy and Osteoporotic Donors
15:10 - 15:20	**Daniela Daume**
	Lateral and medial projection neurons target different regions in the olfactory cortex of larval Xenopus laevis
15:20 - 15:30	**Melina Kahl**
	Olfactory nerve transection leads to transient activation of olfactory ensheathing cells in larval Xenopus laevis
Keynote Section 5	**Chairperson: Osama Elyamany**
10:30 - 11:00	**Dr. Jean Christophe Delpech, University of Bordeaux, France** Role of microglia in neurodevelopmental disorders

The role of α-ketoglutarate homeostasis in tumour invasion and metastasis

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[3]These authors contributed equally to this work

Metabolic reprogramming, a recognized hallmark of cancer, has been associated with tumour invasion and metastasis. Initiation of metastasis requires epithelial-mesenchymal transition (EMT), a dynamic change in cellular phenotype and motility. EMT is regulated by several transcription factors, including Snail. α-ketoglutarate (α-KG) is a metabolite whose intracellular level depends on the activity of Isocitrate dehydrogenase 1 (IDH1), as well as a complicated network of transporters and enzymes. It contributes to many physiological processes besides cellular metabolism, most importantly, it acts as a co-substrate for α-KG-dependent dioxygenases. This enzyme superfamily modulates different biological functions such as hypoxic response, DNA, and histone demethylation, RNA modification and collagen synthesis, which are all implicated in metastasis.
The aim of this study is to identify the key factors that control α-KG levels in glioblastoma, breast and lung cancer models, and to delineate the homeostatic function of α-KG in the regulation of EMT and tumour progression.
Here we show that α-KG controls tumour cell invasion and impacts EMT regulators and markers by using Boyden chamber, western blot, and RT-qPCR assays. Our results specify Snail as one of the main α-KG-regulated EMT factor. We also demonstrate that IDH1 is a key enzyme in the control of intracellular α-KG levels in our model systems, and show how its expression is regulated by microenvironmental cues that enhance tumour invasiveness. Finally, we show that depletion of IDH1 induces EMT and invasiveness.
Possible mechanistic pathways involved in Snail regulation by α-KG are currently being investigated. We are also aiming to identify other α-KG key regulating enzymes and transporters using CRISPR/Cas9 screen as a future perspective. Collectively, our findings emphasize the interplay between metabolism and metastasis and reveal novel mechanistic insights into the regulation and role of intracellular α-KG levels on the invasive capacity of tumour cells.

Functionalised nanoparticles binding to muscarinic and adrenergic receptors

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Nanoparticles are defined as particles with a size between 1 and 100 nm. By targeting ligands to their surface, the nanoparticles can be functionalised and used for therapeutic or diagnostic application. The dense multivalent presentation of agonists on the surface of functionalised nanoparticles leads to a tremendous potentiation of these agonists at their respective receptors. For this project, spherical gold nanoparticles with different sizes, pH values and ligands are synthesised. Carbachol, a muscarinic agonist, induces a contraction of the intestinal smooth muscle cells and an enhanced intestinal chloride secretion. Nanoparticles functionalised with carbachol might be an alternative treatment of postoperative ileus. On the other hand, atropine is a competitive muscarinic receptor antagonist reducing the intestinal motility. Therefore, nanoparticles functionalised with atropine could be a therapy option of spasms in the gastrointestinal tract. In organ bath experiments, they inhibited the contraction of the jejunum caused by carbachol. Additionally, atropine-nanoparticles induced a size-dependent inhibition of the carbachol-induced chloride secretion in the rat jejunum. Adrenergic agonists, e.g. adrenaline, lead to a relaxation of the respiratory smooth muscle cells via stimulation of the beta2-adrenergic receptor. Though, nanoparticles functionalised with these agonists did not relax respiratory smooth musculature in the organ bath, but led to an increased potassium secretion of the large intestine in the Ussing chamber. Hence, the functionalised nanoparticles do apparently not bind to the beta2-adrenergic receptor in the respiratory tract but stimulate other adrenergic receptors. To test whether the beta1-adrenergic receptor is responsible for the observed effect in the intestine, adrenaline-functionalised nanoparticles will be tested on cardiomyocytes. For the treatment of intestinal disorders in the future, the nanoparticles have to be absorbed by the intestinal mucosa. Future experiments will be performed to investigate how the nanoparticles are resorbed by the intestinal epithelium by using transmission electron microscopy.

Lateral and medial projection neurons target different regions in the olfactory cortex of larval Xenopus laevis

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The neuronal network of the olfactory system is highly conserved in vertebrates. Sensory neurons from the nose are connected with second order neurons in the olfactory bulb. How second order neurons from the olfactory bulb project to higher brain centers varies in different species. While in mice two types of neurons project in a different manner to separate brain regions, in the zebrafish only one cell type and projection pattern is known. Here we show the secondary olfactory pathway, form the olfactory bulb to higher brain centers, in larval Xenopus laevis. Different colored dye injections as well as electroporation of single olfactory bulb projection neurons revealed axonal projections in regions like the medial and lateral amygdala, the dorsal pallium and the preoptic area. Thereby we found a separation in two olfactory tracts targeting different regions in the brain. Functional Calcium Imaging with a GCaMP6 transgenic Xenopus line made it possible to visualize a functional connection to these regions. The population of projection neurons in the olfactory bulb as well as higher cortical regions were described via immunohistochemical stainings.

Hypoxia-Induced Epigenetic Reprogramming in Tumor Development

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One of the crucial questions in tumour biology is to understand how tumour cells can hijack the pro-tumorigenic functions of hypoxia and overcome the growth inhibitory and stress signals to fully activate hallmarks of cancer. Considering that along the metastatic process cancer cells undergo periods of hypoxia and re-oxygenation, we generated an in vitro model by using lung, breast and glioma cancer cells exposed to repeated hypoxia-reoxygenation cycles, termed intermittent (IM) hypoxia.
We aim to elucidate the molecular mechanisms that lead to a more invasive phenotype of tumour cells under such conditions, and focus primarily on potentially stable epigenetic alterations. We hypothesize that hypoxia-inducible transcription factors (HIFs) may promote the induction and establishment of an adapted invasive phenotype as they are strongly induced by IM hypoxia. Since the establishment of the invasive phenotype possibly requires their concerted action with epigenetic regulatory enzymes, we will investigate the involvement of the DNA demethylase enzyme TET1, which is upregulated under IM hypoxia. Furthermore, the crosstalk with histone methylation, especially with histone lysine demethylases (KDMs) will be explored. Various KDMs were recently described as cellular O_2 sensors and thus have the potential to orchestrate the regulation of tumour progression with HIFs and TET1 upon diverse hypoxic conditions. To address these questions, we employ the combination of targeted and genome-wide analyses of transcriptome and methylome changes with functional studies, and monitor the acquisition of the invasive tumour phenotype under IM hypoxia at molecular and cellular level.
Results from our laboratory suggest a crosstalk between HIF2α, TET1 and Snail, a transcription factor that controls tumour cell invasiveness. Therefore, we currently utilize the CRISPR/Cas9 technology to knock out the aforementioned factors and elucidate their function in detail in the establishment and maintenance of the IM-hypoxia-induced phenotype and adaptive epigenome changes.

Top-Down Modulation of a Dichotic Listening Task with simultaneous Electroencephalography (EEG) Recording

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A dichotic listening task (DLT) involves the presentation of two different sounds simultaneously to both ears (one to the right ear and the other to the left ear). Participants with left-hemispheric dominance report more sounds from the right ear (showing a right-ear advantage). During the left-ear report, the auditory information must be transferred from the right to the left (dominant) hemisphere. Accordingly, using Electroen-

cephalography (EEG), functional connectivity between both auditory cortices increased during left-ear reports. In this study, we aimed to apply a top-down modulation of DLT asking the participants to deliberately attend to either the right or the left ear. Such attention instructions may induce more reports of the left or the right ear sounds, respectively. Therefore, 32 right-handed participants (17 females and 15 males) performed three blocks of DLT with different attention instructions during simultaneous EEG recording. While the first block was always without attention instructions, the second and the third blocks were randomised between left- and right-ear attention conditions. Twenty-three participants exhibited a right-ear advantage in the first block. As hypothesised, the left-attention condition significantly decreased right-ear reports while the right-attention condition increased them. The left-attention condition showed a significant reduction of right ear reports only in participants who received the left-attention instructions in the second block, but not the third block. In conclusion, top-down modulation of DLT affects the behavioural outcome by increasing the reports from the ear attended to. The order of instructions (left or right first) dictates how the left-ear condition can decrease the right-ear reports. It may refer to the difficulty of the attention resources to switch to the left-ear attention from the right-ear attention condition which is already boosted by the built-in left hemispheric dominance (the right-ear advantage).

The effects of neutropenia on the generation and expression of a sickness response during systemic inflammation

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Central nervous system-induced sickness responses, such as fever, lethargy, and adipsia, are known to occur during systemic inflammation. The innate immune system plays a pivotal role in shaping acute inflammatory responses through immune-to-brain signaling. Interestingly, previous studies have shown that leukopenia can alter sickness responses leading to a prolonged fever response. Moreover, neutropenic fever is a severe clinical status of unknown origin. Thus, here, we aimed to specifically investigate the effects of neutropenia on the sickness response and immune-to-brain signaling during acute systemic inflammation in mice. To induce neutropenia, mice received an intraperitoneal injection of anti-polymorphonuclear serum (PMN, 1 ml/kg), which reduced circulating neutrophils by approximately 20% compared to control mice that received normal rabbit serum (NRS) in dose-response experiments. Systemic inflammation was induced 24 or 48 hours after receiving PMN or NRS via an intraperitoneal injection of a high dose of the inflammatory component lipopolysaccharide (LPS, 2.5 mg/kg). Brains, peripheral tissue, and serum were collected 24 hours after LPS-stimulation. Initial experiments revealed that neutropenia inhibited recruitment of neutrophil granulocytes (NG) to the brain and was associated with inhibited LPS-induced expression of the anti-inflammatory cytokine interleukin 10 and the NG specific chemokine CXCL1 (48 h) suggesting some potential anti-inflammatory role for NG. To investigate the physiological significance of neutropenia, we then continuously recorded locomotor activity, core body temperature, food, and water intake using a telemetric system in a second set of experiments. Here, we saw 24h PMN-pretreatment exacerbated LPS-induced hypothermia compared to NRS controls while adipsia anorexia and loss in body weight was not affected by neutropenia. Further analyses revealed that LPS-induced increase in corticosterone serum levels were higher in PMN mice when compared to NRS counterparts. Together, our ongoing experiments suggest a potential anti-inflammatory role of NG with neutropenia exacerbating sickness and immune responses during systemic inflammation.

Olfactory nerve transection leads to transient activation of olfactory ensheathing cells in larval Xenopus laevis

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The olfactory system of vertebrates has the remarkable capability to regenerate after injury to main-

tain the sense of smell. Axons of olfactory receptor neurons, projecting from the olfactory epithelium to glomerular targets in the olfactory bulb, are closely associated with a specific type of glia, the olfactory ensheathing cells (OECs). OECs support the continuous regeneration of olfactory receptor neurons and exhibit growth promoting and axon targeting properties. However, the underlying molecular and cellular mechanisms, as well as their participation in regeneration processes are not yet fully understood. In this study, we identified OECs in the olfactory system of Xenopus laevis larvae, characterized their morphology, and monitored their response after olfactory nerve transection. Morphologically distinct subpopulations, including Schwann cell-like and astrocyte-like OECs, were labelled in the olfactory nerve via single cell electroporation. Immunohistochemical staining of phosphorylated ribosomal protein S6 (rpS6), an indicator for activation of the mammalian target of rapamycin complex 1 signalling, was used as a proxy for cellular responses after olfactory nerve transection and during regenerative processes. We observed that a population of fusiform cells in the olfactory nerve showed an increased phosphorylated rpS6 staining as early as one hour after olfactory nerve transection. The increased staining lasted for approximately 48 hours, and after 72 hours it was no longer detectable. The morphology of these cells, their location in the olfactory nerve, and their increased activity immediately after nerve injury, strongly suggests that the described cells are a subpopulation of OECs. Nerve injury might stimulate the phagocytic function of these cells, thus supporting the removal of apoptotic olfactory receptor neuron debris and the innate immune system in general. Together, these results of the present study led us to the assumption that phosphorylation of rpS6 in OECs is an important molecular mechanism that supports regenerative processes in the olfactory system.

Mesenchymal stem cells inhibit the inflammatory response of spinal dorsal horn cells in a new model of co-cultivation

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Nociceptive and thermoreceptive information is transmitted by afferent axons of dorsal root ganglia to neurons in the dorsal part of the spinal cord (laminae I and II, termed substantia gelatinosa). Inflammatory stimulation results in an increased sensation of pain due to nociceptive stimulation (hyperalgesia) or even normally non-painful stimuli (allodynia). Mesenchymal stem cells have been shown to have analgesic and anti-inflammatory effects in animal models of inflammatory and neuropathic pain. However, the underlying cellular mechanisms are still poorly understood. We recently established and characterized a neuro-glial primary culture of the rat superficial dorsal horn (SDH) and tested responses of this structure to inflammatory stimulation with lipopolysaccharide (LPS). Therefore, we measured LPS-induced release of tumour necrosis factor-α (TNFα) and interleukin-6 (IL-6) into the supernatants and investigated the activation of inflammatory transcription factors NF-kB, NF-IL6 and pCREB. Inflammatory stimulation lead to significantly increased concentrations of TNFα and IL-6 in supernatants of LPS-exposed SDH cell cultures. We further showed that nuclear translocation of NF-kB, NF-IL6, and pCREB was increased in microglial cells due to LPS-stimulation. To investigate possible anti-inflammatory effects of adipose tissue derived stem cells (ADSC's) we established a model of co-cultivation with both cell cultures. After 24 hours of co-cultivation we performed an inflammatory stimulation with LPS for 2 hours. Supernatants were collected for measurements of cytokine release (TNFα, IL-6) and cells were used for immunocytochemical detection of transcription factor activation. LPS-induced increases of TNFα and IL-6 release were significantly reduced when SDH cells were cultured in presence of ADSC's. LPS treatment resulted in enhanced nuclear NF-kB staining intensities in microglial cells. This increased nuclear translocation was significantly blunted in presence of ADSC's.

Thus, we assume anti-inflammatory capacities of ADSC's on LPS-exposed SDH primary cultures. In vivo treatment with ADSC's might thus serve as a tool to reduce inflammatory pain.

The ligamentum arteriosum: Smooth muscle with idiosyncratic innervation

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The ligamentum arteriosum (LA), considered a fibrous remnant of the embryonic bypass (ductus arteriosus) from the pulmonary circulation to the aortic arch, obliterates soon after childbirth. Our experiments set out to explicate the morphology, innervation and neurochemistry of this structure. The mediastinum of reporter mouse strains (chrna3-eGFP, n=4; chat-eGFP, n=4), 10 human and 9 pig ligaments were subjected to routine histology and single- and double-labeling immunofluorescence, with antibody cocktails labelling smooth muscle cells and subtypes of nerve fibers. Ligaments of 6 wild-type mice and 5 pigs were processed for transmission electron microscopy (TEM); ligaments from 30 pigs were subjected to pharmacological and electrical field stimulation (EFS) in organ bath experiments. Histology, immunofluorescence and TEM revealed the canonical arterial wall structure with intimal layer (though mostly obliterated), muscular media and surrounding adventitia. Single- and double immunofluorescence reported the presence of a dense innervation which was mainly sympathetic (tyrosine hydroxylase, i.e. noradrenergic, and neuropeptide Y-positive) and to a lesser extent sensory (substance P/calcitonin gene-related peptide-positive). TEM showed nerve terminals with vesicle accumulations innervating smooth muscle cells and in the connective tissue. In organ bath experiments, LA contracted in response to both EFS and noradrenaline (0.1 µM – 1 mM). Noradrenaline-induced contraction, but not that evoked by EFS, was sensitive to the alpha1-adrenergic receptor blockers prazosin (0.01 µM – 50 µM) and tamsulosin (1 µM – 1 mM), respectively. In conclusion, the LA retains muscular structure and function. It is a smooth muscle with noradrenergic innervation, contracting in response to stimulation of alpha1-adrenergic receptors. Contraction of the LA, though neurogenic, may not be only noradrenergic. This may ultimately play a role in adjusting distensibility of the vascular segments to which it is attached, thereby influencing the compliance of the pulmonary trunk at its bifurcation with potential impact on prognosis in pulmonary hypertension.

Effect of protease signalling on myenteric neurons for regulation of gastrointestinal motility

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Various studies have suggested that stimulation of proteinase-activated, G protein-coupled receptors (PARs) influence gastrointestinal function. Depending on the cellular environment, PARs can contribute to both inflammation and tissue repair. Proteolytic cleaving of the extracellular N-terminal domain of proteinase-activated receptors leads to formation of new NH_2-terminus, which binds and activates the receptor itself. To understand the regulation of gastrointestinal motility, our project aims to study the effect of protease signalling on myenteric neurons as PARs have been found to be highly expressed throughout the gastrointestinal tract and previous studies have shown that PAR-2 plays an important role in calcium ion mobilization in jejunal and pancreatic tissues. During inflammation, proteinases such as thrombin (agonists of PAR-1, PAR-3 and PAR-4) and trypsin (agonist of PAR-2) are released in the gastrointestinal tract. By understanding this mechanism, there is possibility to develop therapeutic solutions by which the control of protease signalling in gastrointestinal tract will help with neuro-inflammation.

In the current project, the myenteric plexus were extracted from 4 day old rats. The neurons were cultured for up to 4 days for imaging experiments. To develop the initial understanding of the reaction of PARs on neurons, the agonist thrombin and trypsin were used. Using imaging technique with the Ca^{2+}-sensitive fluorescent dye fura-2, our data showed that administration of thrombin caused firing of myenteric neurons. We studied the pathophysiological response to PAR1 and PAR2 in the gastrointestinal muscles prepared from adult rats using organ bath and found that the PAR1 agonists caused contraction, while the PAR2 agonists caused relaxation. Ongoing experiments are designed to characterize the underlying signal mechanisms and the receptor distribution in myenteric neurons and intestinal smooth muscle cells to clarify their role in the control of gastrointestinal motility.

Lipopolysaccharide Induced Tolerance: Effects On Rat Primary Cultures Of The Afferent Nociceptive System

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Dorsal root ganglia (DRG) and the substantia gelatino-

sa (SG) of the spinal cord represent the principle transfer stations for nociceptive signals from the periphery into the brain. One maladaptive consequence of inflammatory stimulation with lipopolysaccharide (LPS) in both structures is the manifestation of neuropathic pain. However, treatment with a moderate dose of LPS induces a state of LPS tolerance. In this refractory state the production of inflammatory mediators is diminished or absent when exposed to LPS again. In the present study we investigated the influence of LPS tolerance with regard on the inflammatory gene expression, the release of inflammatory mediators into the supernatants and the translocation of inflammatory transcription factors into the nuclei of different cells (macrophages/microglia, astrocytes, neurons) in primary cultures of DRG and SG. In LPS tolerance DRG primary cultures showed a decrease in TNF-α, but not IL-6 expression and an increased ratio of IL-10/TNF-α. The TNF-α and IL-6 release was not affected by LPS-tolerance. In DRG macrophages the translocation of NFκB and NFIL6, as well as STAT3 translocation in DRG neurons is reduced in the state of LPS tolerance. In SG primary cultures LPS tolerance resulted in a reduced TNF-α, but not IL-6 expression and an increased ratio of IL-10/TNFα. In addition the release of TNF-α and IL-6 was reduced in LPS tolerance. Furthermore the translocation of STAT3 in SG astrocytes and translocation of NFκB and NFIL6 in SG microglia was decreased in the state of LPS tolerance. In summary we can say that in the state of LPS tolerance pro-inflammatory mediators were down-regulated and anti-inflammatory mediators were up-regulated. Still there was a clear difference between DRG and SG cells, as well as the different inflammatory mediators investigated showed different reaction patterns. Our data show a possible effect of glial cells (macrophages/microglia and astrocytes) in the manifestation of LPS tolerance in DRG as well as in SG primary cultures.

Adipokine Treatment of Microglia in an altered TNF-System

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Obesity is a great problem in the western world, leading to numerous other diseases. Adipokines belong to the substances generated in fatty tissue and have in impact on different organs, including the brain, promoting or impeding inflammatory processes and sickness in obese humans or animals. Tumor-Necrosis-Factor (TNF) influences adipokine effects in different ways. For instance, TNF upregulates leptin, a pro-inflammatory adipokine, and in case of adiponectin, an anti-inflammatory adipokine, secretion is impeded by TNF-treatment. Microglia are a main source of TNF in the brain and are also activated by TNF so that a link between fat tissue and brain status is given. Moreover, TNF exerts pro- and anticonvulsant effects, depending TNF-receptor 1 (TNFR1) or TNFR2 signalling.

In this study, the interaction of adipokine treatment on microglia with an altered TNF-system was investigated. Microglia cultures of wild type, neuronal TNF-transgenic (overexpressing), TNF-Receptor 1-KO (TNFR1-KO) and TNFR2-KO-mice were treated with 3 mg/ml leptin and 3 mg/ml adiponectin for 24 h. For cytokine-profiling enzyme-linked immunosorbent assays (ELISAs) were carried out for TNF, Interleukin-6 (IL-6), IL-10 and monocyte chemotactic protein 1 (MCP-1). Additionally, immunofluorescence (IF) was done for Iba1 expression, characterizing the microglial morphology after the different treatments. Mock treatment was used as control. Overall, TNF, IL-6 and IL-10 levels increase with treatment of adipokines, regardless using leptin or adiponectin and regardless of the transgenic status. Treatment seems to have no impact on MCP-1 levels. Exceptions were TNFR2-KO microglia, which do not continuously react with an increase of IL-6 by leptin treatment and TNFR1-KO microglia, which have slightly increased levels of MCP-1 by adiponectin treatment. Morphology changes of microglia reflect their activation by adipokine treatment of all different groups in IF. In conclusion, in vitro, microglia react quite in the same way on leptin and adiponectin treatment, mostly regardless of their genotype.

Numerical abundance of PEX14- and Catalase-positive peroxisomes in different brain cell types of 42-day-old wild-type, TNF-α transgenic, TNFR1- and TNFR2-Knockout mice

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Borna disease virus (BoDV-1) can elicit inflammation and neurodegeneration; and both processes involve profound morphological and functional abnormalities of neuronal and glial cells of the central nervous system. To understand the mechanisms of Borna disease virus-induced neuroinflammation, the role of peroxisomes in tandem with that of the dynamic pro-inflammatory cytokine, tumor necrosis factor-alpha (TNF-α) needs to be investigated. Using indirect immunofluorescence staining, quantitative analyses of the peroxisomal biogenesis protein PEX14 (best marker to quantify this organelle) and the peroxisomal matrix enzyme catalase (marker for the redox status) were performed in three different brain regions (hippocampal formation, cerebellum and cerebral cortex) of 42-day-old non-infected wild-type mice in comparison to TNF-α transgenic mice (TNFTg; TNF-α is overproduced mainly in the hippocampus, cerebral cortex, striatum and thalamus), TNF receptor (TNFR) 1- and TNFR2- knockout mice. Analyses of the numerical abundances of PEX14- and catalase-positive peroxisomes involved distinct neuronal cell types, that is: i) granule neurons of dentate gyrus and pyramidal cells of CA bands, all part of hippocampal formation; ii) pyramidal cells of cerebral cortex; iii) granule and Purkinje cells of the cerebellum. We identified no significant difference in the numerical abundance of PEX14-positive peroxisomes in almost all neuronal cell types between the three genetically modified strains compared to wild-type mice. However, PEX14-positive peroxisomes were 1.5-fold more abundant in Purkinje cells of TNFR2Ko mice than the wild-type (p=0.0414). Moreover, there is the tendency in all the brain regions of TNFR2Ko mice to contain the highest numbers of PEX14-positive peroxisomes amongst the four cohorts. This indicates that the anti-apoptotic TNFR2 pathway seemed to inhibit peroxisomal proliferation or conversely, the TNFR1 has an inductive role on peroxisomal biogenesis. Catalase abundance was significantly lowest in all neuronal cell types analyzed in the TNF-α overproducing (TNFTg) hippocampal regions in contrast to TNFR1ko-, TNFR2ko- and wild-type mice [Granule cells of the dentate gyrus: Wt vs TNFTg (2.6-fold; p=0.0004), TNFR2Ko vs TNFTg (2fold; p=0.0110); and also in pyramidal cells of CA band, Wt vs TNFTg (2-fold; p=0.0168), TNFR2Ko vs TNFTg (2-fold; p=0.0038)]. We speculate that, prolonged exposure of neurons to elevated levels of TNF-α may deplete catalase, and thus, could weaken neuronal ability to detoxify reactive oxygen species, subsequently becoming more prone to oxidative injury. We shall perform further analyses in BoDV-1 infected cohorts to understand the relevance of persistent infections on different neuronal cell types from similar genetically modified strains.

1H-MRS hypothalamic Glutamine-glutamate metabolites' concentration differences and their correlations with pro-inflammatory cytokines in subjects with depression: A case-control study

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The hypothalamus is involved in the regulation of many physical and psychological processes, but is also associated with inflammation. There are numerous studies that indicate an inflammatory component in depression in favor of overstimulation of the HPA axis. An important metabolite and neurotransmitter for arousal, activity and metabolism is glutamate (Glu) and its precursor glutamine (Gln). The aim of this study was therefore to determine the concentration differences between hypothalamic glutamine-glutamate metabolites in depression and to investigate the relationship between these metabolites and the pro-inflammatory cytokines IL-6, TNF-α, IFN-γ and IL-1β. 24 participants with previously diagnosed depression (DE) and 25 healthy community control persons (HC) took part in the study. The glutamate-glutamine metabolites (Gln, Glu and glutamine-glutamate = Glx) were recorded using 1H-MRS with total creatinine (tCr) as the reference metabolite. Peripheral plasma concentrations were assessed using an ELISA for cytokine assessment. Significant differences were found between subjects with depression and healthy controls, especially at Gln concentrations ($U = 196.50$; $p = 0.02$; $\eta 2 = 0.09$) and Glx concentrations ($U = 216.00$ $p = 0.047$; $\eta 2 = 0.06$). In the rank-correlation analysis, a positive correlation between TNF-α and Glx concentrations ($\rho = 0.54$, $p = 0.004$, CI95 [0.24; 1]) was found in the group of participants with depression. The results of this study suggest that the proportion of Glx concentration in patients with depression is increased due to higher Gln concentration values. A positive correlation between TNF-α and Glx concentrations, but not with Gln or Glu concentrations, reveals a synergistic effect of both glutamine-glutamate concentrations compared to the cytokine TNF-α. In summary, the results suggest that people diagnosed with depression

have increased activity on the HPA axis. Inflammation then stimulates the activity of the HPA axis and the glutamate and glutamine (Glx) levels rise.

FGFR inhibitor BGJ398 protects against inflammation and demyelination in EAE through ERK/Akt phosphorylation

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Fibroblast growth factors and its receptors (FGF/FGFR) play an important role in proliferation, differentiation and survival of oligodendrocytes, neurons and astrocytes in the central nervous system. Previously our group demonstrated that conditional deletion of oligodendroglial FGFR 1 and FGFR 2 leads to milder experimental autoimmune encephalomyelitis (EAE) symptoms, less inflammation, less myelin loss and increased axonal density. In the present study, we investigated the preventive effect of BGJ398 an orally bioavailable, selective pan-FGFR kinase inhibitor in EAE mouse model of multiple sclerosis.

EAE was induced with MOG35-55 peptide in 8-week-old C57BL/6 female mice. BGJ398 (30 mg/kg/daily) and placebo were administered orally from day 0 to 9 post EAE induction. The EAE disease course was monitored until day 41, using a standard EAE scale 0-5. The spinal cord was analyzed for inflammation and demyelination. FGFR signalling proteins and myelin proteins were analyzed by Western blot.

BGJ398 treated mice showed a milder EAE disease course from day 9 to 41 ($p \leq 0.05$), reduced inflammation ($p \leq 0.05$), B cell and T cell -infiltration ($p \leq 0.001$), macrophage infiltration ($p \leq 0.001$) and demyelination ($p \leq 0.01$). Protein expression of FGFR1, FGFR2, and FGF2 were less; phosphorylation of ERK, Akt and TrkB, CNPase, myelin basic protein (MBP), proteolipid protein (PLP) were increased in BGJ398 treated mice ($p \leq 0.05$).

Our results suggest that inhibition of FGFR by BGJ398 reduces EAE disease severity. The molecular mechanisms underlying the decreased symptoms in BGJ398 treated mice are due to an alteration of downstream signalling of ERK, Akt and TrkB enhancing CNPase, MBP and PLP production. These data suggest that preventive application of BGJ398 has beneficial effects in a mouse model of MS.

The Role of Oxytocin in Sexual Sensation Seeking: A Candidate Gene Study Looking at rs3796863 and rs53576

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Researchers have shown the involvement of oxytocin in sexual relationships and in intimacy and bonding with partners. However, some people with high sexual sensation seeking (SSS) mostly prefer to have casual sex and change partners frequently. Thus, it would be interesting to investigate the potential relationship between oxytocin and SSS levels. Participants (N=274) provided saliva samples and completed the Sexual Sensation Seeking Scale (SSSS) and the Personal Assessment of Intimacy in Relationship (PAIR). The saliva samples were analyzed to genotype the two SNPs of rs3796863 (CD38-functionality) and rs53576 (oxytocin receptor (OXTR) sensitivity) by PCR. Data analysis indicated that the carriers of homozygous A-alleles of rs3796863 had significantly lower scores in SSSS in comparison to carriers of homozygous and heterozygous C-alleles. While, none of the different genotypes of rs53576 SNP showed any significant difference in the levels of SSS scores, neither did the interaction between these two SNPs. Moreover, the carriers of homozygote A-alleles of rs3796863 SNP had significantly lower levels of sexual intimacy than the carriers of homozygous and heterozygous C-alleles had. Although SSS scores were not significantly associated with any type of intimacy in relationships, high sexual sensation seekers (HSSS) were significantly more involved in stable relationships than low sexual sensation seekers (LSSS). In conclusion, only the polymorphisms of rs3796863 SNP could predict the level of SSS, not the rs53576, nor the interaction of these two SNPs. In addition, rs3796863 SNP could predict the level of intellectual intimacy. It is surprising that

HSSS and LSSS do not differ in intimacy in relationships; therefore, investigating the intimacy in long-term relationships of HSSS becomes motivating.

Keywords: CD38, Oxytocin Receptor Gene, Sexual Sensation Seeking, Intimacy in Relationship.

Analysis of P2X7 Receptor and Panx1 Expression of Healthy and Osteoporotic Donors

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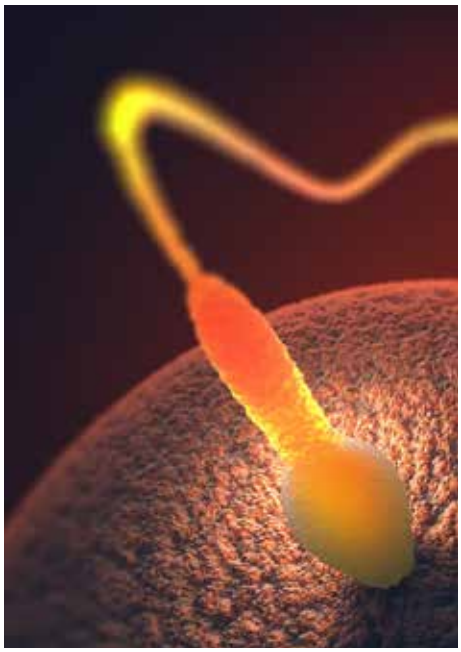
Osteoporosis, characterized by bone mineral density decrease, leads to a high risk of bone fracture. It is now widely recognized that purinergic signaling plays an important role in the regulation of bone remodeling. One receptor subtype, P2X receptor is considered to be important in relation to osteoporosis. Activated Pannexin1 channels provide a pathway for activation of P2X receptors. Mesenchymal stem cells were isolated from samples of 10 female patients, among which 5 were osteoporotic. As a second model we used 45-48 week old female rats with an induction of osteoporosis by ovariectomy and malnutrition. The 28 rats were put into 4 groups: sham, ovariectomy (OVX), diet and ovariectomy with diet (OVXD). Finally, an implant associated fracture model of genetically osteoporotic mice (KO, n=12) and their corresponding wildtypes (WT, n=12) was used. RNA was isolated and the expression of P2X7 receptor and Panx1 was investigated by real-time RT-PCR. Of human groups, there were no significant changes. In rat osteoporosis model, in P2X7, there was a significant down regulation in the Diet group (P=0.005) and OVXD group (P=0.02) compared to Sham group. There was also a down regulation in the OVXD group compared to OVX (P=0.045). In Panx1, there was a significant down regulation in the OVXD group compared to OVX (P=0.002). In the mouse osteoporosis fracture model, in P2X7, there was a significant up regulation in the KO group compared to the WT (P=0.002). And in Panx1, there was a significant down regulation in the KO group compared to the WT (P=0.026). These results indicate that P2X7 receptor and Panx1 contribute to osteoporosis in the animal models. The increase of P2X7 and Panx1 in rat might help to establish new therapeutic strategies for osteoporosis. In humans, it is necessary to increase the number of samples because of the high donor variance.

Section 6 - Reproduction an Man and Animals



Schedule of Section 6
Tuesday, 29th September 2020

Keynote Section 6	**Chairperson: Beatrix Stadler**
10:45 - 11:15	**Dr. Jennifer Schön, Leibnitz-Institut für Nutztierbiologie Dummerstorf** Application of compartmentalized in vitro models to explore maternal interactions with gametes and early embryos
Part 1	**Chairperson: Hiba Hasan**
11:40 - 11:50	**Hassan Kabesh**
	Formation of the Testicular Immunological Barrier Through Immune Modulation by Somatic Cells
11:50 - 12:00	**Sèyi Fridaïus U. S. Vanvanhossou**
	Genetic characteristics of African breeds: an example of multi-breed GWAS for morphometric traits in Beninese indigenous cattle
12:00 - 12:10	**Yalong Yang**
	Crosstalk between metabolism and inflammation in testis□25HC and IRF7 play critical roles with M-CSF in maintaining TM profiles
12:10 - 12:20	**Wei Peng**
	Loss of CCR2 inhibits the development of testicular fibrosis - possible implications for activin A
12:20 - 12:30	**Jane Maoga**
	The role of Tissue Inhibitor of Metalloproteinase 3 and Membrane-type Metalloproteinases in the Pathophysiology of Endometriosis

Part 2	**Chairperson: Beatrix Stadler**
13:30 - 13:40	**Rashidul Islam**
	Tumour infiltrating T lymphocytes in human testis cancer – identification and functional analysis
13.40 - 13:50	**Raouda Sgaier**
	Proteomic biomarkers in seminal plasma and testicular interstitial fluid as predictors of reproductive potential in azoospermia men
13:50 - 14:00	**Shanjid Ahmed Shiplu**
	Ten-eleven-translocation enzyme 1 (TET1) and polycomb repressive complex 2 (PRC2): Roles in spermatogenesis and male fertility
14:00 - 14:10	**Agnes Njoki Mwaura**
	The role of soluble betagycan in TGF- β signalling in human endometrial an endometriotic cells
Part 3	**Chairperson: Shanjid Shiplu**
14:45 - 14:55	**Hang Yan**
	Epigenetic Dysregulation of Tumor Suppressor Genes in CP/CPPS: studies on liquid biopsies for biomarker development
14:55 - 15:05	**Magdalena Anastazja Kuchta**
	C-type natriuretic peptide and analogues are potential drugs for BPH treatment
15:05 - 15:15	**Hiba Hasan**
	The role of activins and chemokines in the development of inflammatory responses during chronic testicular inflammation in mice
15:15 - 15:25	**Shashika Kothalawala**
	Sperm morphology and motility in mice and men: Investigations of defective male germ cell differentiation

The role of activins and chemokines in the development of inflammatory responses during chronic testicular inflammation in mice

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Infection and inflammation affecting the male reproductive tract are significant causes of male infertility. Experimental autoimmune orchitis (EAO) serves as a model of autoimmune-based chronic testicular inflammation and it shares many common features with clinical male infertility. It is characterized by the production of auto-antibodies against testicular antigens, disruption of testicular immune privilege as well as severe immune cell infiltration into the interstitium. In the EAO model, there is an increase in the production of pro-inflammatory cytokines and chemokines including activin A, TNF, IL-6 and CCL2. Moreover, an increase in CD4+CD8+ double positive T cells in EAO testis has been documented.
Elevated levels of activin A and CCL2, as the most prominent inflammatory mediators during testicular inflammation in EAO mice, are hypothesized to act as pro-inflammatory and pro-fibrotic agents leading to testicular damage. However, the mechanisms of their possible influence on testicular immune response is unclear. Therefore, the influence of activin A and chemokines (mainly CCL2) on the generation and functional properties of immune cells will be investigated. Moreover, the expression of chemokines and chemokine receptors in EAO testis from wild type and CCR2 (a receptor for CCL2) deficient (CCR2-/-) mice will also be analyzed to identify potential therapeutic targets.
Preliminary results demonstrate that CCR2-/- mice are protected from EAO damage as seen by histological staining. Moreover, activin A (50ng/ml) decreases the mRNA expression of CCL4 and CCL7 in bone marrow derived macrophages – a surrogate for testicular macrophages. Our preliminary analysis by flow cytometry has revealed that stimulation of murine splenocytes by activin A (50ng/ml) for 4 days led to increase in CD3+ T cell numbers. Within the population of T cells, we observed a decrease in the subpopulation of CD4+ T cells and an increase in CD8+ and CD25+ T cells.
Our initial observations point to a role of activin A in altering immune cell subpopulations and in regulating the expression of CCL4 and CCL7. Moreover, CCL2 plays a crucial role in exacerbating testicular inflammation.

Tumour infiltrating T lymphocytes in human testis cancer – identification and functional analysis

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T cells represent a major component of tumour infiltrating lymphocytes (TIL) population in different tumours, including human testicular germ cell tumours (TGCTs), and play an important role in tumour development and immune surveillance. Different subtypes of CD4+ helper T cells, including regulatory T cells (Treg) and T follicular helper cells (Tfh) are often present in tumour microenvironment and strongly associated with poor prognosis of different cancers, such as in breast cancer, melanoma, renal cell carcinoma, etc. However, the functional involvement of Treg and Tfh cells in TGCTs remains to be unveiled. Therefore, we sought to identify and functionally characterise Treg and Tfh cells within TGCTs, specifically in germ cell neoplasia in situ (GCNIS) and seminoma (SE), compared to testes with normal spermatogenesis (NSP) and intact spermatogenesis associated with focal immune cell infiltrates. To achieve our goals, human testis samples obtained immediately following surgery were categorised into NSP, hypospermatogenesis (HYP) with lymphocytic infiltrates (LY), GCNIS with and without LY, and SE, and processed for immunohistochemistry (IHC), immunofluorescence (IF) and RT-qPCR. Flow cytometry was performed from fresh TGCTs specimens using a panel of immune cell

markers CD_3, CD_4, CD8, CD20cy, CD68, CD_{25}, $FOXP_3$, $CXCR_5$ and BCL6. Our preliminary data (n=5) show that Treg and Tfh cells are mainly found in SE compared to the other groups, suggesting that Treg and Tfh cells are important in TGCT biology. Functional analyses including cyto- and chemokine detection are pending. Next, gene expression profiling using Fluidigm Microfluidics Dynamic Arrays as well as RNA-seq from sorted CD4+/CD8+ T cells will be performed. These experiments will provide the first molecular and functional characterisation of gene and protein expression in Treg and Tfh cells within TGCTs. Such insights may identify their roles in TGCT development and progression, and thereby indicate improvements for TGCT management.

Formation of the Testicular Immunological Barrier Through Immune Modulation by Somatic Cells

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The blood-testis-barrier (BTB), which is based upon Sertoli cells (SCs), divides the seminiferous epithelium into basal and adluminal compartments. The main role of the BTB is to form an immunological barrier in order to preserve the meiotic and post-meiotic stages of the germ cells from the immune system. The BTB is composed of a number of tight junction proteins, mainly the claudins family. Disorder of the BTB´s integrity caused by any internal or external factors might result in infertility in males. Even if the SCs are absent, only few immune cells enter the seminiferous tubules when peritubular cells (PTC) are present. This suggests that other cell types or factors contribute to the testicular immunological barrier (TIB).
Our study is aimed at elucidating the role of different cell combinations (mainly SCs and PTC) on the BTB integrity and to elucidate the contribution of each cell type to the TIB. Furthermore, we aimed to treat rat primary SCs with particular cytokines to address their effects on the BTB integrity and on the TJ proteins.
Our experiments showed that SCs are the main constituent of the BTB. Co-culturing of both SCs and PTC on Matrigel only had a negligible effect on the BTB integrity. Treatment of 93RS2 rat SCs with bone morphogenetic protein2 (BMP2) demonstrated a negative effect on the BTB integrity. This effect was reversed by pretreatment with a BMPR1 inhibitor. Our results also showed that the gene expression of the junctional adhesion molecule-3 (JAM-3) was upregulated after treatment of primary SCs with testosterone via the classical or non-classical androgen pathway. Further research is needed to understand the physiological implications of the results obtained.

Sperm morphology and motility in mice and men: Investigations of defective male germ cell differentiation

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Approximately 30 - 40% of male infertility cases are caused by genetic abnormalities such as chromosomal defects or gene mutation, leading to decreased germ cell production or function. Disturbances of sperm number, morphology and motility can occur, leading to azoospermia, asthenozoospermia and/or teratozoospermia. So far, only few clinically relevant gene mutations/polymorphisms or novel genes have been identified. This study aims to identify novel genes, splicing variants or single nucleotide polymorphisms related to sperm morphology and motility by next generation sequencing (NGS).
For this, biopsies indicated by azoospermia were taken, histologically evaluated and categorized in: normal spermatogenesis (NSP, n=3), spermatid arrest (SDA, n=4) and Sertoli cell only syndrome (SCO, n=3). RNA was extracted from adjacent cryopreserved biopsies followed by RNA-Seq (Illumina NextSeq 500 sequencer). Differentially expressed genes (DEGs, filter for significance: base mean >= 5, -0.585 <= log2fc >= 0.585, FDR <= 0.05) were as follows: NSP Vs SCO -

10,253, NSP Vs SDA – 1,873, SDA Vs SCO - 4,017. DEGs were filtered based on Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway terms related to spermatogenesis – calcium pathway – cAMP pathway – flagella proteins and highly down-regulated DEGs between NSP Vs SDA. By this, 11 genes potentially involved in sperm morphology and motility have been identified: CFAP47, PDE4A, ZP1, SLC9C1, SLC8B1, SPATA31E1, ORAI1, CACNB2, TNC, TEKT3, and TMEM37. To validate RNA-Seq results, primers were designed for RT-PCR. All genes were detected in NSP; in contrast, gene expression in SDA samples varied. Gene TEKT3 was detected only in NSP and SDA samples while CFAP47 was detected mostly in NSP. Immunohistochemistry experiments are ongoing to localize ORAI1 and SPATA31E1 genes on protein level, in histological sections. Further ongoing experiments, quantitative RT-PCR and immunohistochemistry using different pathologies will narrow down the list of potential genes involved in defective male germ cell differentiation.

C-type natriuretic peptide and analogues are potential drugs for BPH treatment

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While the exact mechanisms for the development of benign prostatic hyperplasia (BPH) still remain unknown, it is suggested that hormonal regulation plays a criti-cal role. BPH occurs in the transition zone of the prostate and affects predomi-nantly smooth muscle cells in which it comes to an increase of muscle tone and cell proliferation. Cyclic GMP (cGMP) signaling pathways regulate a variety of physiological functions, such as relaxation of smooth muscle cells or cell prolifera-tion. An activator of this pathway is C-type natriuretic peptide (CNP) that is ex-pressed in the male reproductive system. A high concentration was found in the prostate (1). In addition, high levels of CNP were also detected in porcine seminal plasma (2). The expression of the CNP receptor guanylyl cyclase B (GC-B) in hu-man prostate samples was shown at mRNA level (3). However, the function of CNP in prostatic smooth muscle cells is not clear. For this, the hormonal influence of CNP on the cGMP signaling pathway and its significance for the development of BPH needs to be investigated. First of all, we showed that GC-B is expressed in cultured primary human prostatic smooth muscle cells. By cGMP-ELISAs we found that CNP, more than ANP, activates the cGMP production in primary pros-tatic smooth muscle cell cultures in a dose-dependent manner. The effects of CNP and the synthetic chimeric natriuretic peptide Vasonatrin (VNP) on sponta-neous contractility of prostate glands were investigated by Live-Imaging. Both peptides could significantly decrease the contraction frequency and had the de-sired rapid effect. Long-term effects of CNP and VNP were analysed in primary smooth muscle cells. We found, that the cell number was reduced after CNP or VNP treatment for 3 days. Thus, natriuretic peptides affecting muscle tone and proliferation might be promising potential drugs for BPH treatment.

The role of Tissue Inhibitor of Metalloproteinase 3 and Membrane-type Metalloproteinases in the Pathophysiology of Endometriosis

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Approximately 2-10% women of the reproductive age suffer from endometriosis, a disease characterized by the presence of endometrial tissue outside the uterus. Matrix metalloproteinases (MMPs) are proteases involved in extracellular matrix remodeling. Dysregulation of TIMPs and MMPs has been previously reported in a number of cancers and thus we postulate that this trend might be replicated in endometriosis, since the two conditions share similar developmental stages.
This study is aimed at elucidating the role of tissue inhibitor of metalloproteinase (TIMP3) and membrane-type metalloproteinases; MMP14, MMP15 and MMP16, in the pathophysiology of endometriosis and as potential non-invasive biomarkers. The localization of these proteins in eutopic and ectopic endometrium was determined by immunohistochemistry. We found a preferential localization of all proteins in the glandular epithelial cells and stromal cells of

both endometriotic and non-endometriotic patients except that MMP14 was not observed in the stromal cells of both cases. Although MMP15 expression was highly similar between endometriotic and non-endometriotic patients in the eutopic uterus, significantly higher MMP15 expression was found in lesions of adenomyotic patients compared to endometriotic and non-endometriotic patients. Additionally, MMP15 expression was significantly higher in lesions of adenomyotic patients compared to ovarian, peritoneal and deep infiltrating tissue. When uterine samples from the endometrium of both endometriotic and non-endometriotic samples were pooled together, MMP15 expression in lesions of adenomyotic patients was higher compared to eutopic endometrium of both endometriotic and non-endometriotic patients. The enhanced expression MMP-15 in adenomyotic lesions suggests a possible role of MMP15 in the development of adenomyosis. Reduced MMP15 expressions in ovarian, peritoneal and deep infiltrating endometriosis suggest a possible reduction in the invasive nature of the endometrial tissue after implantation. Presence of these proteins in the uterine suggests possible roles in uterine functions that may perhaps be related to endometriosis and thus further experiments are necessary.

THE ROLE OF SOLUBLE BETAGLYCAN IN TGF-β SIGNALING IN HUMAN ENDOMETRIAL AND ENDOMETRIOTIC CELLS

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Endometriosis is a chronic gynaecological condition characterized by the presence of endometrial-like tissue outside the uterine cavity. The TGF-β superfamily, consisting of TGF-βs, activins and inhibins, is expressed in the endometrium and is putatively associated with endometriosis. Betaglycan (BG) is an important membrane-bound co-receptor and modulator of the TGF-β superfamily ligands. It undergoes cleavage to release soluble betaglycan (sBG) which is implicated in several physiological and pathological processes. The present study aimed to investigate the probable role of BG shedding in endometrial and endometriotic cells which is currently unknown. Endometriotic epithelial cells (12Z) and endometrial stromal cells (THESC) were treated with increasing concentrations of TGF-β1/β2 (1-10 ng/ml), activin A (5-50 ng/ml) or inhibin A (5-50 ng/ml). The levels of sBG, matrix metalloproteinase (MMP) -2 and -3 were subsequently evaluated using ELISAs after 24, 48 and 72 hours of stimulation. Inhibition of BG shedding was analyzed using a pan-MMP inhibitor (10 μM, GM6001) and a MEK inhibitor (50 μM, UO126). TGF-β1/β2 along with activin A and inhibin A stimulation of 12Z cells and TGF-β1/2 and activin A stimulation of THESC cells resulted in a significant time- and dose-dependent reduction in BG shedding. In contrast, inhibition of the non-canonical MAPK/ERK pathway using the UO126 inhibitor did not affect TGF-β-mediated shedding of BG, suggesting involvement of the canonical Smad-dependent pathway. Partial inhibition (~40%) of BG shedding was observed using the pan-MMP GM6001 inhibitor signifying the involvement of MMPs in the shedding of BG. Interestingly, a time- and dose-dependent increase in secretion of MMP-2 and MMP-3 was observed with TGF-β1/2 and activin A, but not with inhibin A. Collectively, these data demonstrate that sBG might be involved in modulating TGF-β1/β2, activin A and inhibin A signaling in endometriotic and endometrial cells. The mechanisms involved and possible roles in endometriosis merit further investigation.

Loss of CCR2 inhibits the development of testicular fibrosis - possible implications for activin A

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Fibrosis is one of the significant features of testicular inflammation that lead to infertility. Experimental autoimmune orchitis (EAO) is a mouse model of chronic testicular inflammation to study the pathology observed in the human testis. Pro-inflammatory and profibrotic mediators, such as TNF, monocyte chemoat-

tractant protein-1 (MCP-1) and activin A, are increased during EAO followed by the infiltration of leukocytes into the interstitium as well as fibrotic remodeling. Sertoli cells upregulate activin A secretion upon stimulation with inflammatory factors, particularly TNF. It is known that fibrocytes characterized by the co-expression of hematopoietic markers and extracellular matrix proteins as well as dysregulated matrix metalloproteases (MMPs) contribute to the fibrotic responses in many organs. Therefore, in this study the influence of the MCP-1/CCR2 axis and activin A on the development of testicular fibrosis was investigated. EAO was induced in WT and transgenic mice deficient for CCR2 (a MCP-1 receptor) and the percentage of fibrocytes in the testis was determined by flow cytometry. The results indicate that the percentage of CD45+collagen I+ fibrocytes was reduced in CCR2-/- EAO testis compared to WT EAO testis. Moreover, the influence of activin A on the production of extracellular matrix proteins and the enzymatic activity of MMPs produced by bone marrow derived macrophages (BMDMs) alone or in a co-culture with Sertoli cell conditioned medium (SCCM) was analyzed by qRT-PCR and gelatine-zymography. Preliminary results show that TNF stimulated SCCM induced the mRNA expression of fibronectin. In BMDMs activin A increased the mRNA expression and enzymatic activity of MMP-2 with a concomitant decrease of MMP-9. Taken together, our initial data demonstrate that the MCP-1/CCR2 axis and activin A could be involved in the fibrotic remodeling during testicular inflammation in mice.

Proteomic biomarkers in seminal plasma and testicular interstitial fluid as predictors of reproductive potential in azoospermia men

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Azoospermia, characterized by the absence of sperm in the ejaculate, is responsible for 5-20% of male infertility cases. With the advent of Assisted Reproductive Technology, an exact diagnosis of male factor infertility is of prime importance. This study aimed to discover biomarkers for non-invasive differential diagnosis of azoospermia. Using label-free LC-MS/MS, we compared proteomic profiles of seminal plasma and testicular interstitial fluid from fertile men (HC, n=8) and men diagnosed with three different forms of azoospermia: obstructive (OA, n=7), mixed atrophy (MA, n=8) and Sertoli cell-only syndrome (SCO, n=7). Proteins significantly down-regulated in SCO seminal plasma compared to the control group included testis-specific LDHC (FC= 25.11, p= 9,89E-05) and DPEP3 (FC=11, p=0.01) and testis-enriched heat-shock proteins HSPA2 (FC= 7.31, p=0.02) and HSPA4L(FC= 3.25, p=0.02). This marked decrease is caused by the near-complete depletion of germ cells in the SCO testis. Immunolocalization of LDHC, HSPA2, and HSPA4L on testicular histological sections presenting normal spermatogenesis (OA), hypospermatogenesis (MA), and germ-cell aplasia (SCO) confirmed their distinct expression levels between the three types of Azoospermia, and their enrichment in post-meiotic germ cells. Validation of mass spectrometry findings by Western blot showed a significantly lower abundance of LDHC and HSPA2 in SCO seminal plasma compared to the control. HSPA2 concentration was significantly higher in MA compared to SCO, both in seminal plasma and testicular interstitial fluid. These results affirm that certain testis-specific proteins, when measured in seminal plasma, can serve as indicators of the presence of sperm in the testis and predict the success of sperm retrieval. Used in conjunction with conventional clinical tests, these proteomic biomarkers can be great tools for accurate, non-invasive diagnosis of idiopathic male infertility. Supported by DFG IRTG "Molecular Pathogenesis of Male Reproductive Disorders" GRK 1871/4.

Ten-eleven-translocation enzyme 1 (TET1) and polycomb repressive complex 2 (PRC2): Roles in spermatogenesis and male fertility

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TET1 and PRC2 regulate DNA and histone methylation (H3K27me3), respectively. In mice, TET1 and PRC2 dysregulations are associated with male infertility. According to our previous study, TET1 expression level was significantly downregulated in infertile patients. We hypothesize, TET1 interacts with PRC2 and promotes DNA demethylation at H3K27me3-enriched genes. We aim to clarify, where TET1 and PRC2 co-express during spermatogenesis, and co-localize in the mature sperm genome and how this interaction is changed in the sperm of subfertile men and Tet1-KO mice.

Immunohistochemical analyses (IHC) have been performed for TET1, H3K27me3, core PRC2 components (EED, EZH2, SUZ12) on testis sections from men with obstructive azoospermia and mice (wild-type wt and Tet1-KO). Western blot (WB) analyses of core PRC2 components and H3K27me3 have also been done on human sperm.

In humans, IHC results showed that TET1, H3K27me3, and EZH2 co-express in the spermatogonia B, leptotene spermatocytes and round spermatids. Compared to wt, in Tet1 homozygous KO mice, Tet1 showed no expression in germ cells. H3K27me3 expression had shifted from stage V-VI spermatogonia B to stage VII-VIII pre-leptotene spermatocytes, and from stage IX-XI elongating spermatid to stage X-XI elongating spermatids. EZH2 had gained expression in stage VII-VIII pre-leptotene spermatocytes, in stage VI-X pachytene spermatocytes whereas SUZ12 had shown new expression pattern in the late elongating spermatids and elongated spermatids. EED lost expression completely. However, WB analyses have shown that PRC2 components were not preserved in the sperm except H3K27me3.

So, knocking out Tet1 has potential impacts on Suz12 and Eed. Eed mutant mice are lethal at gastrulation and Tet1 might be a potential regulator which leads to infertility. Analysis of putative TET1-binding sites and their methylation status will be performed for selected genes (e.g. HAND1, ESX1L) to determine if there is a link between male infertility and the interaction of TET1 and PRC2.

Genetic characteristics of African breeds: an example of multi-breed GWAS for morphometric traits in Beninese indigenous cattle

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In the African livestock breeding context, routine data recording systems for performance traits especially body weight rarely exist. In contrast, morphometric traits are more easily measurable and often correlated to performance traits. They are therefore potential indicator traits for novel breeding and conservation strategies in the large but poorly studied animal genetic resources in Africa. The aim of the present study was to estimate genetic parameters and to identify functional loci underlying the variability of six morphometric traits in four indigenous breeds (Borgou, Pabli, Lagune, Somba) kept in small herds in Benin. In total, 449 cattle with morphometric records for height at withers (HAW), sacrum height (SH), heart girth (HG), hip width (HW), body length (BL) and ear length (EL), were genotyped for 36,720 SNP. This dataset was used to estimate heritabilities, genetic and phenotypic correlations, and to perform a multi-breed GWAS. Estimated SNP-based heritabilities for the six morphometric traits ranged from 0.46±0.14 (HG) to 0.74±0.13 (HW). The genetic and phenotypic correlations ranged from 0.14±0.10 (HW-BL) to 0.85±0.02 (HAW-SH) and 0.25±0.05 (HW-BL) to 0.89±0.01 (HAW-SH), respectively. The multi-breed GWAS detected two genome-wide and 25 chromosome-wide significant SNP associated with the morphometric traits. The identified SNP were located near (±25kb) or within a total of 15 genes among which 11 genes were related to morphological, growth and carcass traits like body weight and fat deposition in cattle as well as in other livestock species and humans. The genes were additionally involved in biological processes and pathways such as hemostasis and metabolism (PIK3R6, PIK3R1), immunity or inflammatory responses (PTAFR, LYPD8), chromatin organization (PBRM1), DNA repair (EYA3), DNA binding (SSH2), and regulation of hepatocyte growth factor receptor signaling pathway (ADAMTS12). The association between all identified genes either with feed efficiency, stress or immune response suggests that adaptability to scarce food resources, disease and extensive managements plays an important role in the phenotypic variability of the indigenous

breeds. With regard to moderate to high heritabilities and the functional annotation for the associated SNP, the studied morphometric traits are suitable indicator trait candidates to develop breeding schemes for genetic improvements of the indigenous breeds.

Epigenetic Dysregulation of Tumor Suppressor Genes in CP/CPPS: studies on liquid biopsies for biomarker development

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Chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) is diagnosed in 90% of prostatitis patients. CP/CPPS may potentially lead to prostate cancer (PCa) in elder age, but the molecular background still remains unclear. Tumor suppressor genes (TSGs) prevent neoplastic transformation and epigenetic dysregulation of TSGs often participates in carcinogenesis. TSG dysregulations could be vital in the development of CP/CPPS to PCa. Therefore, the goals of our project are to identify epigenetic modifications in TSGs using liquid biopsies of CP/CPPS patients and to evaluate the most promising sources for biomarker development.
Somatic cells from ejaculates (ESCs) and the cell pellet of exprimate urine (ExU, urine after prostate massage) were isolated by density gradient centrifugation and normal centrifugation, respectively. Immunocytofluorescent staining (ICF) was conducted for calculating the proportion of epithelial cells and leucocytes in ESCs and ExU. Afterward, DNA and RNA of ESCs samples (50 patients vs. 30 healthy controls) and ExU samples (41 patients vs. 27 healthy controls) were extracted. Promoter methylation analysis was performed on PCa-associated TSGs (BMP4, EDNRB, BMP7, PTGS2, PITX2, and CDKN2A) by using pyrosequencing after bisulfite treatment of DNA samples. The RNA samples were used for expression analysis of selected TSGs by RT-qPCR.
The ICF analysis among ESCs and ExU cells showed that leucocytes comprise the majority (53.3% - 80.8%). CP/CPPS-patients' ESCs the gene promoters of BMP4, CDKN2A, EDNRB, BMP7, PTGS2 are significantly higher methylated than in healthy controls, whereas only CDKN2A and EDNRB showed high methylation in ExU. Expression of CDKN2A and PTGS2 was significantly downregulated in both patients' ESCs and ExU, but there are no differences for other TSGs.

Our results show that promoter methylation of TSGs can be frequently detected in liquid biopsies of CP/CPPS patients and that expression level of gene CDKN2A is correlated to the methylation level in both ESCs and ExU.

Crosstalk between metabolism and inflammation in testis25: HC and IRF7 play critical roles with M-CSF in maintaining TM profiles

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Testicular macrophage (TM) is the majority immune cell population in the testis, and displays critical functions in spermatogenesis with Leydig cells: hormone production from Leydig cells is TM-dependent; sperms with neo-antigens are protected with immune privilege of TM from being attacked by immune system. Resident macrophages with specific profiles have been proved to be induced by special microenvirnmental factors in local tissue, such as microglia in the brain, is maintained by CSF1, IL-34 and TGF-β through the downstream transcription factors, SMADs. 25HC (25-hydrocholesterol) and IFN-β can be produced by macrophages and highly expressed in the testis, and both could regulate the inflammatory responses. IRF7, the master transcription factor to induce IFN-β, is also proved to balance the macrophage functions and is highly expressed in TM. Our study found that 25HC displayed double-sides of inflammatory regulations depend on the initial activation by M-CSF or GM-CSF, which was also regulated by IRF7. To our knowledge, we were the first study including all male reproductive organs for variety immune cell populations in Irf7-/- mouse, and found that the deficiency of Irf7 decreased macrophages only in the testis and enhanced MHC II expression in most organs. In conclusion, we related metabolism of cholesterol with inflammatory regulations in the male reproductive organs. And we found M-CSF in the testis was the initial factor to create a high level of 25HC to induce immunosuppressive profiles of TM, which was controlled by IRF7.

Section 7 - Bioresources, Bioinformatics and Biotechnology

Schedule of Section 7
Wednesday, 30th September 2020

Keynote Section 7	**Chairperson: Seyda Azka Jaffri**
09:45 - 10:15	**Prof. Samina Mehnaz, Forman Christina College, Lahore, Pakistan** Pseudomonas aurantiaca – a bacterium 'extraordinaire'
Part 1	**Chairperson: Wendell Albuquerque**
11:15 - 11:25	**Fabian Jannik Tann**
	Feature generation and selection for (antiviral) peptide prediction
11:25 - 11:35	**Patrick Blumenkamp**
	Advanced and reproducible bioinformatics approaches for high-throughput RNA-Seq data analyses
11:35 - 11:45	**Patrick Barth**
	Detection of novel structured RNAs by structure profiling experiments
11:45 - 11:55	**Markus Weigel**
	Airway microbiome profiling in mice with cystic fibrosis like symptoms
11:55 - 12:05	**Nadine Sella**
	Genome and transcriptome analysis of the medicinal mushroom Wolfiporia cocos
12:05 - 12:15	**Elvis Katche**
	Hybrid Speciation in Brassica
12:15 - 12:30	**Wendell Albuquerque**
	Can peptidases from the fruit fly Drosophila suzukii prevent wine haze?

Can peptidases from the fruit fly Drosophila suzukii prevent wine haze?

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Prevention of haze in white wines is a remaining challenge for winemakers. Peptidases are considered as an alternative to traditional fining methods, although an effective proteolysis under typical winemaking conditions (acidic pH and low temperature) is difficult to be obtained. The fruit fly Drosophila suzukii feeds on grapes and therefore is a potential source of proteolytic enzymes for the hydrolysis of wine haze proteins. Peptidases from D. suzukii (adult and larvae) have been extracted (by mortars under liquid nitrogen), purified using an FPLC system (anion exchange and size exclusion columns) and characterised using LC-MS/MS. The activities of the fractions against wine proteins were tested by agar diffusion plate assays (mixture of agar and wine protein solution) at different pH values (3, 5 and 7). The degradation of wine proteins was additionally confirmed by measuring the absorbances at 540 nm after a heat test at 75 °C. The analysis by SDS-PAGE showed that protein bands in the range below 20 kDa were generated after proteolysis by larval extracts at pH 5 and 7. No significant activity was detected for the extracts of adult flies. The mass spectrometric analysis of extracts showed proteolytic enzymes with similar sequences to the Pfpl peptidase from Zunongwangia profunda and to the venom serine peptidase from Lygus lineolaris.

Detection of novel structured RNAs by structure profiling experiments

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RNAs are important molecules that fulfill a diverse range of functions within a living cell. By carrying genetic information from the genome to the ribosome they perform an essential part in the biogenesis of proteins. Additionally, many RNAs can form complex structures that allow them to perform biological processes. By forming riboswitches, RNAs can adjust the gene expression to the presence of specific molecules. RNase P is an example of an RNA that can act as a protein-based enzyme. Its complex three-dimensional structure allows it to catalyze the cleaving of other RNA molecules.

Due to these properties, the detection and analysis of RNA secondary structures are essential for a better understanding of processes within a cell. To support the discovery of these structures several structure profiling methods have been developed. In 2016 a method called DMS-MaPseq was published that allows the generation of structure profile data in a high-throughput manner.

By treating samples in vivo or in vitro with dimethyl sulfate, unpaired adenines and cytosines within RNA molecules are methylated. These RNAs are then reverse transcribed by a specific reverse transcriptase which inserts a random nucleotide at methylated positions. After sequencing, the random nucleotides are detected as mismatches which can be used to calculate a DMS-value for each adenosine and cytosine. From these values, a structure profile can be created which contributes to the prediction of secondary structures. Besides its potential, the detection of structured RNAs still poses a challenge. To improve secondary structure detection, we plan to provide new methods and algorithms by evaluating the structure profile data of closely related species and comparing the results of similar methods.

Advanced and reproducible bioinformatics approaches for high-throughput RNA-Seq data analyses

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The yearly increasing citations of DESeq2, edgeR, and limma (an increase of 535 % from 2015 to 2018) show that differential gene expression (DGE) analyses are still on an emerging path. The vast amount of data generated by current

sequencing instruments underpins the need for automated and reproducible analysis pipelines. Thus, we developed a two-component software for analyzing and visualizing RNA-Seq data with a focus on DGE analyses. The first part is a modularized Snakemake pipeline generator consisting of quality control, preprocessing, mapping, and in-depth analysis modules, called Curare. The pipelines are built for high-throughput analyses and can be executed on local machines as well as on high-performance compute clusters. Each pipeline is entirely reproducible, and the existing collection of modules, which are customizable and extendable, increases the flexibility of the pipeline generation. The second component is a tool for visualizing DGE results. With the Gene Expression Visualizer (GenExVis), DGE results can be interactively analyzed, and numerous charts can be created. All charts can be saved in common image file formats for usage in presentations and publications. Both components combined create an environment that supports the full process of data analysis from the initial handling of RNA-seq raw data to the final DGE analyses and result visualization.

Hybrid Speciation in Brassica

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Interspecific hybridisation is an important path to generating evolutionary novelty. The genus Brassica is an important agricultural genus with a history of interspecific hybridisation. The allotetraploids B. juncea, Brassica carinata, and Brassica napus are hybrids formed by pairwise hybridisation of the diploid progenitors Brassica rapa, Brassica oleracea and Brassica nigra. Though crossing the allotetraploids is possible and has been carried in several studies either to study chromosome behavior or transfer useful traits, attempts to generate novel, stable and fertile synthetic hybrids through this method have not been reported. We generated interspecific hybrids (BBAC = F1 = 35) by crossing B. juncea (2n = AABB = 36) × B. carinata (2n = BBCC = 34) where A and C genomes lack homologous pairing partners and self-pollinated these hybrids for six generations by selecting for fertility. We intend to answer the question if in the absence of homologous pairing partners, the A and C genomes can pair, restructure and stabilise to form viable and fertile off springs. Analysis of later generation hybrids show that recombination and genome restricting between the A and C genome can lead to the formation of new, stable and fertile hybrids with stable chromosome transmission across generations. This pathway can be useful for generating evolutionary novelty which can be transferred to other Brassica species and also to produce new useful crop types.

Phylotranscriptomics to unravel the origin of angiosperms

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Angiosperms are a group of land plants containing the majority of species in the kingdom of plantae. One of the important unifying traits within this group is the flower which often contains both of the sexual organs and is responsible for reproduction of the plant. Development of each of the different whorls in the flower is controlled by complex network of genetic, epigenetic, hormonal and metabolic interactions. The aim of this study is to clarify the transcription of genes during carpel development. Most important common genes regulating the development of carpel will be identified with a comparative transcriptome analysis. Transcriptomes have been generated from carpels of phylogenetically informative angiosperm species. In addition to the different species, carpel RNA was sequenced at different developmental timepoints to clarify the temporal regulation of genes over time.

An angiosperm model species, mouse-ear cress (*Arabidopsis thaliana*), early branching eudicot, California poppy (*Eschscholzia californica*) and a commercially important agricultural crop, tomato (*Lycopersicon esculentum*) were chosen as the target species for transcriptome sequencing. Amongst many other things transcriptome analysis of *A. thaliana* has revealed temporality in protein interactions and expression of important regulatory genes related to carpel development, hormonal response and epigenetic regulation.

Genome and transcriptome analysis of the medicinal mushroom Wolfiporia cocos

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The basidiomycete Wolfiporia cocos is a fungus that is traditionally used in Chinese medicine for its anti-inflammatory and antitumor activity. Apart from that, the fungus is able to produce a variety of aroma compounds amongst others volatile terpenoids, which are highly desired by cosmetics industry. To reveal biosynthetic pathways of the aroma compounds, a bioinformatics approach has been set up, which combines genome, transcriptome and metabolome analysis. Therefore, the volatile compounds produced by mycelium of W. cocos during growth on black current pomace have been analysed by means of gas chromatography mass spectrometry. Depending on the occurrence of the main aroma compounds, a transcriptomic study at the corresponding time points has been performed. To assemble and map the transcriptomic reads, a whole genome sequencing has been conducted resulting in a reference genome of W. cocos. Putative genes encoding for the mevalonate pathway, which is responsible for the backbone of terpene structures, have been identified. A first identification of so far unknown terpene synthases will be presented together with and outlook on the allocation of further genes to novel aroma biosynthetic pathways.

Feature generation and selection for (antiviral) peptide prediction

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Predictions with machine learning methods have established new insights over the last years in many fields. They are promising in reducing costs in wet-lab work by in silico preselection and can reveal new information.

Due to viral threats and little or no options in their treatment, classifiers for predicting antiviral peptides are trained to find new drug possibilities. Antiviral peptides occur naturally in many organisms. They can have additional effects like antibacterial properties, reducing the chance of secondary infections of the weakened host.

Choosing the right features for classification is crucial for the prediction outcome. Hence a great variety of properties are calculated, normalised, grouped and finally drafted through a weighted vote of different feature selection strategies. Automation shall boost performance of classifiers, resulting in higher productivity of researchers.

Airway microbiome profiling in mice with cystic fibrosis like symptoms

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Cystic fibrosis (CF) is caused by mutations of the cystic fibrosis transmembrane regulator (CFTR) gene and remains the most common fatal hereditary disorder in Caucasian populations. Although CF is a complex multi-organ disease, morbidity and mortality are mainly determined by chronic lung disease characterized by airway mucus plugging and chronic bacterial infection triggering an overwhelming inflammatory host response that drives progressive structural lung damage in patients with CF. Traditionally, based on culture-dependent diagnostics, chronic lung infection in CF has been viewed as mono infection with Staphylococcus aureus, Haemophilus influenza, Pseudomonas aeruginosa and Burkholderia cepacia. However, with the emergence of culture-inde-

pendent molecular techniques for the detection and quantification of bacteria that cannot be detected by standard culture techniques it has become evident that even the lungs of healthy individuals are not sterile, but harbour a unique steady-state microbiome and that airway microbiota are characteristically altered in patients with CF and other chronic lung diseases.
In this study we have established a 16S metagenomic and bioinformatics workflow for a more specific and unbiased approach of analysing microbial communities. For our experiments we utilized βENaC-Tg mice, which shares key features with CF in humans including early onset airway mucus plugging, chronic inflammation characterized by airway neutrophilia and macrophage activation, structural lung damage, increased susceptibility to CF pathogens. We focused our study on the very early stages of CF in three-day old mice. To this end we compared samples of bronchoalveolar lavage fluid, lung tissue and trachea tissue of βENaC-Tg mice and wild type mice.
Preliminary results indicate a dominance of the Firmicutes phylum in βENaC-Tg mice for all three sample types facilitated trough a strong presence of different Streptococcus species. Furthermore, culture and sequencing-based methods lead to the identification of a novel bacterial species which belongs to the order Lactobacillales.

Section 8 - Chemical Design and Analysis of Molecular Systems

Schedule of Section 8
Wednesday, 30th September 2020

Part 1	**Chairperson: Simon Becher**
13:15 - 13:25	**Julia Büttner**
	Production of natural flavourings by biotransformation of agricultural side streams with fungi
13:25 - 13:35	**Nils Holger Anschütz**
	AP-SMALDI-MSI of Cryptosporidium parvum and Neospora caninum-infected cells and tissue
13:35 - 13:45	**Katja Rebecca Wiedemann**
	AP-SMALDI MSI of Besnoitia besnoiti cysts in cattle
13:45 - 13:55	**David Lüke**
	Deciphering glycolipids in parasites
13:55 - 14:05	**Felix Marcel Graf**
	Identification of Novel Yeasts for Potential Wine Aroma Improvement
Part 2	**Chairperson: Michael Waletzko**
14:30 - 14:40	**Svenja Sommer**
	Composition of flavours produced by the fungus Wolfiporia cocos grown on black currant side streams
14:40 - 14:50	**Azar Rezaei**
	Chemical topography of metal-associates allergens on non-planar everyday items

14:50 - 15:00	**Parab-Jainal Haque**
	High versatility of IPP methyltransferases enables synthesis of C6, C7 and C8 isoprenoid building blocks
15:00 - 15:10	**Simon Becher**
	Fragmentation Mechanisms of Metal-Lipid Complexes in the Gas Phase
15:10 - 15:20	**Darya Dudko**
	Investigation of the known producing strains and novel candidates on the ability of vitamin B12 biosynthesis
Part 3	**Chairperson: Azar Rezaei**
15:45 - 15:55	**Katrin Wiltschka**
	AntiPOP - Dechlorination of polychlorinated biphenyls in contaminated waters using palladium nanocatalysts
15:55 - 16:05	**Julian Schneemann**
	Low-temperature plasma (LTP) post-ionization of apolar analytes
16:05 - 16:15	**Michael Waletzko**
	Nanospray Desorption Electrospray Ionization: a Fast and Accurate Method for Ambient Mass Spectrometry Imaging of Lipids and Small Molecules
16:15 - 16:25	**Domenic Dreisbach**
	Spatial metabolomics of cardiac glycoside sequestration in Danaus plexippus and Euploea core using high-resolution MALDI mass spectrometry imaging
Keynote Section 8	**Chairperson: Simon Becher**
17:45 - 18:15	**Dr. Jens Riedel, Sanofi -Aventis GmbH Frankfurt** Spatial metabolomics of cardiac glycoside sequestration in Danaus plexippus and Euploea core using high-resolution MALDI mass spectrometry imaging

AP-SMALDI-MSI of Cryptosporidium parvum and Neospora caninum-infected cells and tissue

Anschütz N. Holger[1], Gerbig S.[1], Larrazabal C.[2], Muñoz J. Diego Velez[2], Silva L. M. R.[2], Hermosilla C.[2], Taubert A.[2], Spengler B.[1]

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Parasites and resulting diseases pose health and economic threats worldwide. In order to overcome these problems, the parasites need to be studied extensively. In this study, closely related but distinct obligate intracellular apicomplexan parasites, i.e. Neospora caninum, Eimeria bovis or Cryptosporidium parvum were studied, using mass spectrometry (MS) and MS imaging (MSI), combined with high- performance liquid chromatography (HPLC) or matrix-assisted laser desorption/ionisation (MALDI). The aim was to identify molecular biomarkers for parasitic infections of host cells and to clarify their function. With MALDI MS(I), infected and non-infected cell pellet samples were examined for possible markers. For this purpose, a Q ExactiveTM HF orbital trapping mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) in combination with a AP-SMALDI5 AF imaging ion source (TransMIT GmbH, Giessen, Germany) was used (Mass resolution R = 240,000 @ m/z 200; pixel size: ≥ 5 µm). The two software packages Mirion (TransMIT) and Perseus (MPI of Biochemistry, Martinsried, Germany) were used to find potential biomarkers, and HPLC-MS/MS (Dionex UltiMate 3000 RSLC-System, Thermo Fisher Scientific, Dreieich, Germany) experiments were performed for their structural identification. The software tool LipidMatch (SECIM, Gainesville, USA) was used for assignment. Highly immunoreactive primary bbovine umbilical vein endothelial cells (BUVEC) or bovine small intestine cells (BSIC) were used to be the closest to the in vivo situation as possible. Immortalized cell lines were used for comparison as a simplified infection model system. MSI of monolayers allowed for depicting marker compounds in parasite-infected single host cells in comparison to non-infected controls. In case of cryptosporidiosis, C. parvum-infected bovine intestinal biopsy samples were additionally examined by MALDI MSI, thereby mimicking the in vivo situation. It was found that the lipid class of phosphatidylcholines in particular was highly abundant. The current state of marker detection for N. caninum and C. parvum will be presented here.

Fragmentation Mechanisms of Metal-Lipid Complexes in the Gas Phase

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Glycerophospholipids (GPs) are essential physiological molecules. Cellular membranes, protein-protein interactions and molecular signaling are influenced by GPs. One phospholipid class, phosphatidylcholines (PCs), are the major components of most eukaryotic cell membranes and have been associated with multiple biochemical processes. Discrimination of the fatty acid (FA) identity and positions on the glycerol backbone is crucial to understand PC associated biochemical events. Mass spectrometry (MS) is arguably the bioanalytical method of choice to study PC structures. Sum formulae are derived from accurate mass measurements while tandem MS (MS2) is used to identify head groups and FA composition. Many researchers investigated PC fragmentation using collision-induced dissociation (CID) MS2 and deduced empirical fragmentation mechanisms that are consistent with experimental fragment ion masses. These previous studies showed that charge carriers influence PC fragment ion identities. Proposed mechanisms suggest that dioxolane or dioxane derivatives are formed upon headgroup loss, whereas five- and six-membered phosphodiester structures are discussed as a result of FA loss. Our study intends to identify precursor and fragment ion structures of PCs as a function of charge carrier in order to rationalize PC ion fragmentation mechanisms. Investigations of the PC-cation complexes were carried out using electrospray ionization MS, structurally probing gas-phase ions by infrared multiple photon dissociation (IRMPD) spectroscopy at the Free Electron Lasers for Infrared eXperiments (FELIX) facility. PC adducts with H+, Na+, K+ and corresponding fragment ions were formed upon CID prior to IRMPD. IR-photons were spectroscopically interrogated in the range of 1850 to 650 cm-1. Qualitative analysis of the IRMPD spectra was performed by comparing spectra of PCs with IRMPD results for synthesized authentic standards. By comparing experimental with theoretical results and IRMPD spectra of authentic standards, we were able to assign structures to most PC adducts and corresponding fragment ions. These structures are in line with proposed fragmentation pathways of gas-phase PC adducts and hint to predominate five-membered ring formation in investigated fragment ions.

Production of natural flavourings by biotransformation of agricultural side streams with fungi

Büttner J.[1], Sommer S.[1], Schlering C.[2], Fraatz M. A.[1], Rühl M.[1], Zorn H.[1]

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Natural flavour compounds are either produced by extraction from plants or by bioconversion of suitable natural precursors with microorganisms or enzymes derived thereof. [1] Agricultural side streams containing e.g. fibres and various secondary metabolites represent rich sources of potential aroma precursors. Those precursors can be transformed into aroma compounds by fungi due to their broad biochemical potential and enzymatic spectrum. Especially fungi of the division Basidiomycota are used in these biotransformations because many of them are edible mushrooms which simplifies the use of the generated products in food and cosmetics.

The aim of the current project (AROMAplus) was thus to convert various agricultural side streams such as blackcurrant pomace into attractive natural flavours. Therefore, different fungi were cultivated in surface culture on media containing the agricultural side stream as the sole nutrient source. The best substrate-fungus combinations were determined by sensory analysis of the agar plates. Subsequently, media optimisation of the interesting fermentations were performed to increase or further improve the formed aroma. The cultivation of Wolfiporia cocos, an edible fungus which is also used in traditional Chinese medicine, on blackcurrant pomace, led to the formation of a flowery and fruity flavour resembling that of wild strawberries. This aroma could be intensified by supplementation of sodium aspartate. The relevant aroma compounds of this flavour were identified as linalool, geraniol, benzaldehyde, and methyl anthranilate. Although methyl anthranilate is known as the character impact compound of wild strawberries [2], its biosynthetic pathway in fungi has not been elucidated yet. Thus, the elucidation of this pathway is the main aim of this work.

[1]R. G. Berger, Food Flavour Technology 2010, 89-126.
[2] J. Pillet, BMC Plant Biology 2017, 17:147.

Spatial metabolomics of cardiac glycoside sequestration in Danaus plexippus and Euploea core using high-resolution MALDI mass spectrometry imaging

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During 350 million years of coevolution, plants evolved a multitude of defensive traits against herbivorous insects, including a tremendous diversity of secondary plant metabolites. Strikingly, many insects are not only able to cope with plant toxins but also accumulate the toxic metabolites in their body (sequestration) to defend themselves against predators. The monarch butterfly (Danaus plexippus) that sequesters cardiac glycosides from its host plant milkweed (Asclepias spp.), represents an emerging model system in chemical ecology and evolutionary biology. Nevertheless, the physiological and molecular mechanisms underlying sequestration are still unknown. To understand the pharmacokinetics of cardiac glycosides in Danaus plexippus, we applied atmospheric-pressure matrix-assisted laser/desorption ionization mass spectrometry imaging to visualize the selective absorption, dissemination, and storage of cardiac glycosides in D plexippus and the closely related Euploea core. All experiments were carried out using an AP-SMALDI5-AF ion source coupled to a Q Exactive HF Orbitrap mass spectrometer. First, an optimized sample preparation protocol for 5th instar larvae was established, resulting into transversal and longitudinal sections with excellent morphological preservation. In D. plexippus, 7 out of 10 detected cardiac glycosides were taken up into the body tissues and finally stored in the larval integument, showing that sequestration is an active and selective process. The concentration of sequestered cardiac glycosides were found to decrease along the gut passage, suggesting that the uptake occurs primarily in the anterior region. In contrast, caterpillars of E. core did not sequester cardiac glycosides and only had low concentrations in the gut lumen, indicating efficient degradation. In conclusion our results suggest that high-resolution AP-SMALDI MSI is a promising technology for studying the pharmacokinetics of metabolites across insect tissues by demonstrating how two closely related milkweed butterfly species have evolved diverging strategies to cope with defensive plant compounds.

Investigation of the known producing strains and novel candidates on the ability of vitamin B12 biosynthesis

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Vegetarian and vegan diets have developed into modern nutritional trends in recent years and only in Germany the number of vegetarians has increased more than tenfold over the last 20 years. Nevertheless, vegans are at a particular risk of vitamin B12 deficiency since this vital nutrient is not present in the foods of plant origin. A lack of vitamin B12 can cause severe health problems, which is why there is an urgent need for vegan vitamin B12 sources. Since many bacteria are able to synthesize vitamin B12, microbial biosynthesis is nowadays applied for the commercial production of vitamin B12. Thus, we aim to search for known and novel vitamin B12 -producing strains and to analyze them for vitamin B12 production with different analytical techniques. For this purpose, microbiological assay, vitamin B12 riboswitch-based biosensor and LC-MS/MS were applied for the vitamin B12 analysis in this work. Since the microbiological assay and the riboswitch biosensor turned out to be sensitive to inactive B12-analogues and to have very high detection limit, respectively, we have chosen the LC-MS/MS analysis for the further investigation. In this study, we developed a sensitive LC-MS/MS-based method and, using this technique, we were able to identify and quantify vitamin B12 in the cell extracts of three producing strains. Moreover, we were able to select further eight possible candidates for vitamin B12 production and the LC-MS/MS analysis revealed vitamin B12 in the cell extracts of five of the selected candidates.
The developed LC-MS/MS-based method proved to be reliable for fast and sensitive determination and quantification of vitamin B12. Identification of vitamin B12 in the cell extracts of the candidate strains suggested the possibility to find even more vitamin B12-producing strains and, as a result, even more vegan vitamin B12 sources.

Identification of Novel Yeasts for Potential Wine Aroma Improvement

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High amounts of aroma compounds in fruits occur in form of glycosides. These glycosides are not directly accessible for the human aroma perception due to the high polarity (and thus low volatility) of the sugar residues. However, they can contribute to beverage aroma improvement after hydrolysis of the precursors during the processing or fermentation of fruit juices. This liberation is either a result of acidic hydrolysis or an enzymatic process catalyzed by glycosidases. While acidic hydrolysis can be accompanied by rearrangements of the molecules, enzymatic hydrolysis preserves the native structure of the compounds. Enzymes used commercially for wine aroma improvement are often limited in their enzymatic capacity due to the harsh fermentation parameters. Moreover, they can also mediate hydrolysis of unstable pigments and the release of unpleasant flavours. Therefore, yeast strains with selected hydrolytic activities for specific aroma glycosides can be a great opportunity to improve fruit juice and wine aroma complexity. 2100 non-Saccharomyces yeasts isolated from grapes and black currants at the Hochschule Geisenheim University were cultivated and screened for their β-glycosidic activities using the model substrate 4-methylumbelliferyl β-D-glucopyranoside. β-Glycosidase specificity was measured by gas chromatography/solid phase micro extraction (GC-SPME) in samples of glycoside extracts from grape must incubated with whole cells of promising strains. Several isolates with high β-glucosidase activities were identified in the collection of autochthonous non-Saccharomyces yeasts. Measurements under various conditions revealed differences in the β-glucosidase pH-optimum, while subsequent GC-SPME analyses indicated a high selectivity of some of the identified glycosidase activities for specific aroma compounds. The isolates identified in this screening are potential candidates for the optimization of fruit processing leading to the production of beverages with an improved aroma composition. Additionally, further investigations can help to understand the mechanisms of aroma liberation specificities of the selected yeasts.

High versatility of IPP methyltransferases enables synthesis of C6, C7 and C8 isoprenoid building blocks

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Terpenoids represent the largest and most diverse class of natural products with more than 80.000 compounds. Although the terpene biosynthesis pathways are highly modular and lead to immense structural diversity, their universal isoprenoid building blocks are almost exclusively the C5 units IPP (isopentenyl pyrophosphate) and DMAPP (dimethylallyl pyrophosphate). The structural diversity of terpenoids and thereby the biotechnological exploitation of terpenoid biosynthesis would be greatly expanded if modifications could be added to the canonical building blocks. Such a strategy would take advantage of the generally high substrate promiscuity of prenyltransferases and terpene synthases.
For this purpose, we screened bacterial genome sequences for uncharacterized prenyl pyrophosphate methyltransferases. We were not only able to identify S-adenosyl-L-methionine (SAM)-dependent IPP methyltransferases which catalyze mono- or dimethylation of IPP, but also discovered an IPP methyltransferase which enables sequential addition of three methyl groups to IPP. Our results present an overview about the diversity of IPP methyltransferase reactions, which will allow a strong expansion of the terpenoid chemical structure space.

Deciphering glycolipids in parasites

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Glycoconjugates are an important class of molecules, involved in several biological processes, for example cell-cell recognition. Glycosphingolipids in particular are considered to play a major role in parasite-host interactions. Therefore, the analysis of glycosphingolipids is important to obtain structural information enabling drug or vaccine development. Despite the importance of this compound class as diagnostic and drug targets, the challenge of glycosphingolipid analysis originates from their structural complexity, including the glycan moiety and ceramide backbone. Often targeting only the glycan head-group, enzymatic cleavage is performed, but important functional information is lost when neglecting the ceramide structure. Therefore, we aim to develop a sensitive hyphenated nano liquid chromatography mass spectrometry method for the untargeted analysis of intact glycosphingolipids in parasitic organisms. Sum compositions of glycolipids are readily available from high-performance mass spectrometry. Subsequent tandem mass spectrometry allows to infer structural motifs of glycan moieties by in-depth analysis of fragment ion identities. Moreover, tandem mass spectrometry of permethylated intact glycosphingolipids provides a clearer fragmentation pattern compared to underivatized species. In addition, Paternò-Büchi reactions were tested to pinpoint the double-bond position of ganglioside GM1 or ceramide C18. For that, various reactants were tested like acetone, benzophenone and acetylpyridine. Improving ionization efficiency by introducing a positive charge is also a major aspect for glycosphingolipid analysis, as tested with acetylpyridine. Nano liquid chromatography as a sensitive separation technique is also performed for upcoming complex sample mixtures. Either HILIC chromatography or reversed-phase chromatography is used for native glycosphingolipids or permethylated species, respectively. Taken together, preliminary experiments were conducted to establish a toolbox for the glycosphingolipid analysis of parasitic organisms. Additional experiments with matrix-assisted laser desorption/ionization mass spectrometry imaging will then provide spatial information about the glycosphingolipid distribution in tissue.

Chemical topography of metal-associates allergens on non-planar everyday items

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Metals are essential to almost every aspect of our life. They are commonly used in jewellery and coins. Trace amounts of metals and chemical compounds containing metal ions can affect human health, i.e., cause allergy or inflammation. The human skin covers most of the surface of the body. Therefore, it is a major entrance point for a wide range of metal-associated allergens. As a consequence, skin allergies caused by everyday items have appeared. The best-known example is allergic contact dermatitis upon contact with nickel-containing jewellery. When a metal-containing material comes into contact with the skin, the material surface is affected by sweat, present on the skin, potentially resulting in allergic hypersensitivity, a form of eczema. However, details of this metal-induced allergic reaction are not well understood. Complexes of nickel, copper and zinc are known to

be easily formed through contact of metal alloys with ubiquitously available organic compounds such as triglycerides, amino acids and fatty acids found in skin secretions. Thus, an analytical technique is required to characterize and identify metal complexes formed on metallic everyday products, ideally offering a chemically specific, laterally resolved view of allergy-causing processes. In order to characterize metal-organic compounds formed on the surface of jewellery or coins, we here present an analytical workflow and first results for compounds formed on everyday items, using 3D-surface autofocusing laser desorption ionization mass spectrometry imaging (3D-LDI-MSI). In first experiments, chemically cleaned everyday metal objects were investigated. These experiments allowed reconstructing 3D-topographic maps of jewellery and coins before contact with human skin. Subsequently, these objects were brought into contact with skin and analysed with 3D-LDI-MSI. Metal-containing ions, only found after contact with skin, were assigned based on accurate mass measurements and isotopic distributions. Future measurements will investigate how these metal compounds affect the skin.

Acknowledgement
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Keyword
LDI mass spectrometry imaging, skin allergy, metal allergy.

Low-temperature plasma (LTP) post-ionization of apolar analytes

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Apolar compounds including metabolites and lipids are challenging to detect using ionization techniques like matrix-assisted laser desorption/ionization (MALDI) and nano-electospray ionization (nanoESI), because they suffer from low ionization efficiencies. Efficient ionization techniques for apolar analytes are plasma-based ionization methods, such as low-temperature plasma (LTP), which, however, cannot efficiently desorb compounds from biological tissue. By combining the ionization techniques infrared (IR)-MALDI and nanoESI with a plasma-based post-ionization technique, it was possible to efficiently detect apolar analytes.

This study investigated the combination of IR-MALDI and nanoESI with LTP for post-ionization to detect different groups of apolar analytes. A homebuilt atmospheric pressure IR-MALDI source was modified. In the source the laser was focused through a centrally bored objective lens onto the sample surface. The LTP probe was integrated in a way that the active species from the plasma stream could interact with the desorbed analytes while minimizing the shading of the laser beam. Helium was used as a discharge gas. By removing the Peltier-cooled, moveable stage, a nanoESI source could be used with this setup.
By systematically optimizing parameters for LTP, the signal intensity was optimized. Different apolar analytes were measured with IR-MALDI or nanoESI, and the effect of LTP for post-ionization was investigated.
MALDI mass spectrometry imaging (MSI) in combination with LTP post-ionization was used to visualize cholesterol in different biological tissues with a spatial resolution down to 100 µm. No sample preparation steps, other than freezing the sample, were necessary.
The combination of IR-MALDI and LTP for post-ionization increased signal intensities up to 140-fold or made it possible to detect substances that were not possible to detect without post-ionization.
Here, we present a technique that makes apolar analytes accessible by IR-MALDI and nanoESI. In first proof-of-concept experiments the potential of this methodology for MALDI MSI was demonstrated.

Composition of flavours produced by the fungus Wolfiporia cocos grown on black currant side streams

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Natural flavours may be produced efficiently, cheaply and sustainably by fungal fermentation. Twenty-six species from the fungal phylum of Basidiomycota were cultivated on pomace and leaves of black currant, which are side streams of the juice production. The cultures were screened in regard to their growth rates and sensory impressions. The most promising combinations were analysed using stir bar sorptive extraction in combination with gas chromatography-mass spectrometry (GC-MS). The

changes of the respective volatilome over a period of fourteen days were investigated. The flavour profiles were analysed by liquid-liquid extraction (LLE) and dynamic headspace analysis (DHS). To elucidate the key aroma compounds, an aroma extract dilution analysis (AEDA) of the LLE as well as an aroma dilution analysis (ADA) by means of DHS were performed. ADA was validated using authentic standards. The fungus-substrate combination of Wolfiporia cocos with pomace and sodium aspartate showed the most interesting sensory impressions. A sensory panel (n=10) described the odour as fruity, flowery, sweetish, and reminiscent of wild strawberries [1]. After ten days of cultivation, the overall impression as well as the GC-MS data showed the highest flavour intensities. Linalool, geraniol, 2 aminobenzaldehyde and methyl anthranilate were identified as key aroma compounds. Furthermore, odour relevant as well as imperceptible linalool oxides were detected.

References:

1. Zorn, Sommer, Sella, Schlering, Rühl, Fraatz, Büttner: Biotechnologische Herstellung von Aromastoffen aus Johannisbeertrester am 15.05.2019 (EP19174737.7)

Nanospray Desorption Electrospray Ionization: a Fast and Accurate Method for Ambient Mass Spectrometry Imaging of Lipids and Small Molecules

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Nanospray desorption electrospray ionization (nano-DESI) is an ionization method that combines liquid extraction on the sample surface and electrospray ionization in the front of the MS inlet capillary. The defined liquid extraction using a constant droplet on the surface, allows imaging of metabolites and lipids directly from the sample surface with less or without further sample preparation, in contrast to other ambient ionization techniques. Nano-DESI is potentially soft and less destructive, which allows subsequent sample processing such as matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI MSI).
The nano-DESI ion source was combined with a high-resolution Q Exactive HF-X Orbitrap mass spectrometer for MSI experiments. The results were compared to MALDI results acquired on the same mass spectrometer. Cerebellum (Mus musculus) was used as a standard system for comparison due to its large variety of lipids, metabolites and structure.
The nano-DESI MSI data were acquired by scanning the sample surface line by line with a constant solvent bridge between the two capillaries and the sample surface, delivered by the primary capillary to the surface and led to the MS inlet capillary by the secondary capillary. The same continuous extraction of molecules was imitated by the MALDI laser, ablating sample material with a continuous scan mode.
The nano-DESI data were compared to MALDI MSI data with the same lateral resolution, to confirm that the local information of nano-DESI MSI is consistent and no spreading of molecules was observed. All data sets were acquired in positive ion mode.
Preliminary annotations were generated with METASPACE and Lipid Maps without further evaluation by tandem MS.
Nano-DESI ion images showed molecular distributions in agreement with reference data obtained with MALDI MSI. The achieved improvements establish nano-DESI as a reproducible extraction-based method for soft and less destructive, ambient MSI of large sample areas with reasonable lateral resolution.

AP-SMALDI MSI of *Besnoitia besnoiti* cysts in cattle

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Besnoitia besnoiti is an obligate intracellular, cyst-forming apicomplexan parasite which causes bovine besnoitiosis, an infectious disease that is considered as emerging in Europe. Bovine besnoitiosis leads to economic losses in the cattle industry and causes animal welfare impairment. During the chronic phase of the infection, tissue cysts containing bradyzoites are mainly formed in the skin. As such, chronic symptoms are immense skin thickening (elephant skin), swelling of joints and orchitis eventually leading to sterility of bulls. Currently, no treatment is available. MALDI MSI (matrix-assisted laser desorption/ionization mass spectrometry imaging) is a powerful tool to visualise the distribution of substances of interest.
Sections (20 µm thick) of infected skin tissue were prepared using a cryomicrotome (HM 525, Thermo Fisher Scientific, Bremen) and coated with 100 µL solution of DHB matrix (30 mg/mL in H2O/Acetone 1:1 v/v, 0.1%

TFA) for positive-ion mode, using a pneumatic sprayer (SMALDIPrep, TransMIT GmbH, Giessen). MS images were recorded using an AP-SMALDI5 AF ion source (TransMIT GmbH) coupled to a Q Exactive high-resolution orbital-trapping mass spectrometer (Thermo Fisher Scientific, Bremen) with 5 to 20 µm pixel size and a mass resolution of R = 240,000 at m/z 200.
By applying MALDI MSI, it was possible to identify markers for *B. besnoiti* infection in skin sections. Some markers were assigned to specific parasite structures within the tissue, such as cyst wall or cyst content. As a preliminary result, bradyzoite-related marker signals were partially identified by a database search. Further molecular identification via LC-MS/MS and optimization of sample preparation for measurements in negative-ion mode will be performed.
Cholesterol is important for many metabolic pathways and parasite replication. *B. besnoiti* is auxotrophic for cholesterol, therefore it scavenges this molecule from the host cell. As a preliminary result, cholesterol presence in parasitized skin was analysed, showing cholesterol accumulation around the cysts.

AntiPOP - Dechlorination of polychlorinated biphenyls in contaminated waters using palladium nanocatalysts

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Polychlorinated biphenyls (PCB) belong to the group of persistent organic pollutants (POPs), which are characterised by high persistency and low biodegradability in the environment, relatively high mobility and partially high toxicity. Until their ban in 2004 by the Stockholm Convention on POPs, PCB were manufactured worldwide in large quantities due to their thermal and chemical stability and were used as industrial chemicals. Although they have not been produced in relevant amounts over the past years, they are still ubiquitous in the environment. Relevant sources of present contamination are wastewater from scrap yards or pumped-out mine water. To protect the environment, regulations exist e.g. to limit PCB concentrations in surface waters. Filter systems can reduce the amount of discharged PCB, but relevant limitations of the technique remain, such as an inadequate efficiency and the necessity to dispose of filter residues. A promising new approach is to catalytically dechlorinate PCB with nanoparticles. In the AntiPOP project, funded by The German Federal Environmental Foundation (Deutsche Bundesstiftung Umwelt, DBU) the dechlorination of PCB by palladium nanoparticles will be performed both on a laboratory level and on-site at discharged mine water. To prevent nanoparticles from getting into the environment and causing additional problems, the nanoparticles should be incorporated into a coating. This allows the nanoparticles to be used over a long period of time, which makes the process very resource-efficient. At the end of the project, it is planned to build a model flow system in order to test the newly acquired knowledge under real conditions. The project intends to improve the environmental conditions by reducing PCB loads from discharges of polluted sites and to pave the way for further environmentally friendly technologies.

Section 9 - Ecology and Global Change

Schedule of Section 9
Tuesday, 29th September 2020

Keynote Section 9	**Chairperson: Wiebke Hansen**
09:15 - 09:45	**Prof. Feike Dijkstra, University of Sydney, Australia** Carbon and nutrient cycling in a changing world: role of plant-soil interactions
Part 1	**Chairperson: Wiebke Hansen**
11:30 – 11:40	**Marcel Pierre Simon**
	Reconstructing agriculture and industrial activity with lake sediments
11:40 – 11:50	**Kai Jansen**
	Quaternary ammonium compounds in soil - the role of microaggregation
11:50 – 12:00	Philipp Koellmann
	Impact of farming practice on segetal species composition in central Hessen/ Germany
12:00 – 12:10	Wiebke Hansen
	Invasive legume affects species and functional composition of mountain meadow plant communities
12:10 – 12:20	Alexander Konrad
	Forest soil nanoparticles and colloids enhance delivery of phosphorus into sinks mimicking plant
12:20 – 12:30	Melanie Schindler
	Differential effects of a recurrent flooding on the flooding tolerance of eight hardwood floodplain forest saplings

Part 2	**Chairperson: Ferdinando Binacchi**
13:30 – 13:40	**Catarina Martins**
	Effects of ocean acidification on key physiological processes of the reef-building coral Porites cylindrica
13:40 – 13:50	**Marvin Rades**
	Reef-building corals may not develop mechanisms to prevent microplastic uptake
13:50 – 14:00	**Olivia Metz**
	High microplastic turnover rates in the freshwater mussel Dreissena sp.
14:00 – 14:10	**Ferdinando Binacchi**
	Roots is the way: legumes´ below ground biomass enhances soil organic carbon storage

Roots is the way: legumes´ below ground biomass enhances soil organic carbon storage

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Assessing below ground biomass in legume-wheat crop rotations has often been overlooked, leading to underestimations in calculating carbon and nitrogen inputs into the soil. We report how 6 different legume varieties grown on two contrasting soil types, may contribute to improving soil fertility under organic cropping systems. We also assessed plant´s 15N natural abundance to measure the contributions of biologically fixed N to field scale nutrient balances. The study will shed light on integrating legume crops which maximize N returns for subsequent companion crops.

Invasive legume affects species and functional composition of mountain meadow plant communities

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Plant invasions are among the key drivers of global biodiversity and ecosystem change. They often cause reductions in native species richness and overall biodiversity. Nitrogen-fixing plants are problematic as they affect soil nutrient availability and outcompete species of nutrient-poor sites.
Here we assessed the impacts of the legume Lupinus polyphyllus on species and functional diversity of mountain meadow communities in the UNESCO Biosphere Reserve Rhön.
We compared species diversity (richness, evenness and effective species number), functional diversity (functional richness, evenness, divergence and dispersion) and similarity of plots in three characteristic vegetation types (Nardus grassland, mesic and wet mountain hay meadows) between different lupine cover classes. We calculated community weighted means (CWMs) of single plant traits and plotted them against lupine cover classes.
The invasion of L. polyphyllus homogenizes vegetation composition since the similarity among plots of the different vegetation types increased with increasing lupine cover. It significantly affected species diversity in terms of richness and effective species number and the functional divergence of the vegetation. The trait set of species occurring together with lupine was shifted towards more competitive trait values. We demonstrate strongly negative impacts of L. polyphyllus on different mountain meadow vegetation types since L. polyphyllus, fosters the growth of competitive species and leads to overall more productive plant communities.

Quaternary ammonium compounds in soil - the role of microaggregation

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Quaternary alkylammonium compounds (QAACs) are cationic organic substances with amphiphilic properties that are widely used as surfactants and disinfectants in industry, households and agriculture. Due to their high production volumes, they are ubiquitously found in aquatic systems and soils. In soils, QAACs mainly bind to negatively charged constituents, such as bacterial cell walls, organic matter and clay minerals. Several studies suggest that exposure to QAACs may not only lead to QAAC-resistance in microorganisms, but also promote resistance against different antibiotics. Binding to the interlayers of clay minerals can lead to inaccessibility of QAACs to microorganisms, thus reducing their acute toxicity. In previous experiments, it has been observed that clay minerals flocculate after the addition of QAACs. An influence on aggregate stability appears likely. Aggregates govern the retention and sequestration of contaminants in soils, due to their structure and properties. Microaggregates, which are heterogeneous, compound soil structures consisting of mineral and organic materials, are of particular importance here because of their size, large surface area and porosities. Being smaller than 250 µm, microaggregates are expected to play a role in the accessibility of QAACs, the buffering of acute toxicity for microorganisms and in increasing the

probability of resistance gene formation. Therefore, the aim of this project is to address the role of soil microaggregates regarding the fate of QAACs. By means of soil physical, chemical and microbiological methods paired with imaging techniques, the influence of microaggregates on QAACs will be investigated. In addition, effects of QAACs on aggregation processes as well as microaggregate properties will be studied. The overall goal is to improve our understanding of the fate of QAACs in the soil system in order to better assess the risk of resistance gene development and thus potential threats to humans and the environment.

Impact of farming practice on segetal species composition in central Hessen/ Germany

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The research about the impact of farming practice on segetal species is part of a project in which an innovative combine harvester is tested as an approach for non-chemical weed control. This study focuses on the initial conditions of segetal species' soil seed bank and current vegetation before the use of the new harvester. Data analysis is based on the identification of seedlings of the soil seed bank samples and the current vegetations' species in the field. Data basis are 42 fields in two landscape areas and two farming practices (conventional and organic) in the central part of the German state Hessen. Among several environmental factors being tested the farming practice showed a significant impact on species numbers in soil seed bank samples and current vegetation as well. Differences in species numbers were higher in soil seed bank samples than in current vegetation. Species richness was significantly higher in organic farming practice than in conventional farming. Determination methods for species (soil seed bank, current vegetation) showed significant differences in three out of four variants of the field trial. Lower species numbers in the in-field-identification might be caused by the competition for environmental resources like water and light between weed and crop in the field. Ordering arable species along a gradient of farming intensity resulted in 10 species indicating moderate intensive or extensive farming conditions. However, only two species could be stated significantly as indicators for a higher intensity level in farming. Farming practice was ascertained as the major impact factor on arable species diversity and individual numbers found in the fields and soil seed bank samples. Arable species communities showed higher species richness in organic fields than in the conventional ones.

Forest soil nanoparticles and colloids enhance delivery of phosphorus into sinks mimicking plant roots

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The strong binding and hence low mobility of phosphorus in soils limits phosphorus uptake of plants. In soils, natural nanoparticles and –colloids, the smallest fraction of the soil solid phase, bind more phosphorus than the larger soil particles due to their large specific surface area. At the same time, these particles can carry phosphorus to plant roots by diffusion, as has been shown for hydroponic systems in the literature. However, the mobility of nanoparticles and colloids in hydroponic systems is much larger than in soils, in which these particles are trapped in small pores, thin water films and at water-air interfaces. The magnitude and the kinetics of phosphorus delivery by nanoparticles and colloids into a phosphorus sink mimicking plant roots was tested using the Diffusive Gradients in Thin-Films (DGT) technique under water-unsaturated conditions. Nanoparticles and colloids were extracted from three forest soils differing in parent material and compared with the respective bulk soil and the colloid-free extraction residue. The nanoparticles and colloids extracted from a phosphorus-poor podzolic sandy soil strongly enhanced phosphorus transport into the sink compared to bulk soil and the colloid-free soil extraction residue. In contrast, the phosphorus delivery into the sink by nanoparticles and colloids was smaller than phosphorus release and transport from bulk soil for a soil developed on dolomite rock. No difference was found for a soil with intermediate phosphorus-stocks developed from gneiss. We make the case that the phosphorus delivery into the root-like sinks by nanoparticles and colloids depends on particle size and composition, speciation and soil pH.

Effects of ocean acidification on key physiological processes of the reef-building coral *Porites cylindrica*

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Oceans have more than doubled their uptake of atmospheric CO_2 over the last half-century, which is expected to increase even further. Higher levels of dissolved CO_2 trigger shifts in seawater carbonate chemistry and lower pH, causing a phenomenon known as ocean acidification (OA), which threatens coral reefs. Reef-building corals are particularly vulnerable to OA due to challenges to build their carbonate skeleton through calcification. Under normal conditions, skeletal growth is generally prioritised over tissue growth and closely coupled with coral photosynthesis (the major process of energy acquisition in reef-building corals). Under OA, calcification in reef-building corals is reduced. However, it is currently unknown whether tissue growth follows a similar decreasing general trend. Additionally, effects on photosynthesis remain unclear, with species showing different and sometimes opposing responses. Thus, despite knowledge that OA directly affects coral growth processes and photosynthesis, an understanding of whether OA alters the interaction between these metabolic processes is still wanting. In this study, we performed a microcosm experiment to simultaneously investigate the OA effects on calcification, tissue growth, and photosynthesis of the reef-building coral species *Porites cylindrica*. We hypothesized that OA would induce lower calcification rates in *P. cylindrica* and tested whether tissue growth and photosynthesis reflected calcification effects. Specifically, coral fragments were maintained in two pCO_2 conditions (~400 µatm and ~1000 µatm) for three months. Photosynthetic rates were assessed using respirometry, tissue growth using 3D scanning, and calcification using buoyant weight technique. Results from this study will further inform of the coupled OA effects on key coral physiological processes and their interplay under a climate change scenario. Such knowledge could have implications for better understanding and predicting coral community productivity on future coral reefs and changes in their structural integrity.

High microplastic turnover rates in the freshwater mussel Dreissena sp.

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In recent years, microplastic pollution has received increasing attention and environmental contamination with microplastic particles (i.e., plastic particles <5 mm) has been reported globally. In aquatic systems, a major concern is the ingestion by filter-feeding organisms. In addition to adverse health effects for the filter-feeders themselves, they can act as a starting point for the trophic transfer of microplastics and associated toxins. An important, high capacity filter feeder in European and American freshwater systems is the mussel Dreissena sp., for which first studies have described physiological effects of microplastic exposure. However, the influence of different concentrations and exposure times on the turnover rates of microplastic particles is unknown. In this study we used a fully crossed design to examine the effect of varying concentrations of microplastic fragments as well as different ingestion and egestion periods on the uptake of plastic particles. The different concentrations tested had only a small influence on the number of particles found in the soft tissue of the mussels, with even low concentrations resulting in high numbers of microplastics in some specimens. In addition, longer ingestion periods led to increasing numbers of microplastics, demonstrating that microplastics seem to be accumulating in the tissue. Interestingly, the results also suggest that microplastic particles are egested quickly after exposure, indicative of a high turnover rate. Such high turnover rates of Dreissena sp. might facilitate the transfer of microplastics from the water column to the benthos, thus changing the flow of microplastic particles in the environment. In combination with the demonstrated accumulation potential, these mechanisms can lead to the build-up of both microplastic particles and associated toxins, resulting in negative effects, possibly also for a variety of organisms at higher trophic levels.

Reef-building corals may not develop mechanisms to prevent microplastic uptake

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Microplastic (MP) pollution poses an increasing threat to marine species and ecosystems. Animals that trap suspended particles, like corals, can be particularly affected by physical contact or ingestion. Recently, it has been shown that actively feeding corals may prefer MP over natural food. However, little is known about the MP feeding behaviour of reef-building corals, which are additionally supplied with nutrients by symbiotic algae. These corals mainly rely on energy derived through photosynthetically active symbionts (so-called zooxanthellae) and may therefore feed more selectively. Moreover, it is unknown whether corals might develop mechanisms to prevent MP uptake after long-term exposure. The aim of this study was therefore to assess the potential for long-term acclimatisation to MP exposure in zooxanthellate, reef-building corals, by determining MP feeding rates. Four coral species (Acropora muricata, Porites lutea, Pocillopora verrucosa, and Heliopora coerulea) were exposed to MP over a period of 15 months, and MP feeding rates were compared with those of natural food (brine shrimp eggs). The results showed that the long-term exposure to MP did not change the feeding behaviour of the corals. This suggests that reef-building corals may discriminate between natural food and MP, probably triggered by chemical stimuli emanating from the particles. However, even after long-term exposure, they do not develop mechanisms to fully avoid the capture and ingestion of MP. They could therefore suffer sustained energy losses due to MP exposure, which might explain health impairment in corals associated with MP pollution.

Application of artificial inteligence algorithms for the prediction of maximum event water fractons in stream water on multivariate events in a developed catchment in Germany

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Quantifying maximum event water fractions of stream water during precipitation events based on isotopic hydrograph separation gives insight into runoff generation mechanisms and hydrological response behavior of a catchment. The proper estimation of maximum event water fractions based on a set of independent multivariate variables would allow the quantification of event and pre-event water contributions during peak streamflow even at times when no direct measurements of isotopes are available. Here we estimate event water fractions in stream water over 40 precipitation events. A mobile field laboratory was set up to measure high-resolution (20 min) stable isotopes of water by laser spectrometry. Artificial Neural Networks (ANN) and Support Vector Machine (SVM) models were developed to model the same information. We consider precipitation and antecedent wetness hydrometrics such as precipitation depth, precipitation intensity and soil moisture of different depths as independent variables measured in the same high-temporal resolution. An important issue is the reduction of the deviation between observations and simulations in both the training and testing set of the models. In order to minimize this difference, various combinations of hyperparameters such as number of neurons in hidden layers, learning rate and various kernel functions are studied. A k-fold cross validation analysis is performed to find the best configuration. Further constraints in the iteration procedure are considered to avoid overfitting. The study was carried out in the Schwingbach Environmental Observatory (SEO), Germany. Results indicate a good performance of the optimized ANN and SVM models. However, the SVM model clearly outperformed the ANN model in terms of performance and its stability under different train and test splits. The optimized SVM was able to better capture the dynamic of the maximum event water fractions and the predicted values were closer to the corresponding observed values with fewer variations over the cross validation repetitions compared to those of the optimized ANN model.

Differential effects of a recurrent flooding on the flooding tolerance of eight hardwood floodplain forest saplings

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Alluvial floodplain forests became rare in many parts of Europe, due to anthropogenic changes such as dikes and transformation to settlements and agricultural areas. The restoration of floodplain forests is an important goal in alluvial restoration, but a difficult task due to the complex environmental conditions of floodplains. The zonation of woody species in floodplains is mainly determined by hydrological conditions, not only within one year but also during the previous year. Therefore, tolerance to flooding can be regarded as a key factor for the successful establishment of woody species. We examined whether a past flooding event of three different durations showed an improvement on the flooding tolerance of eight woody floodplain forest saplings after a recurrent flooding of nine weeks, under controlled common garden conditions. The individuals of the experiment already experienced either no flooding event or a partial flooding event of three different durations (three, six or nine weeks) the previous year. In a next step, these fourteen-month-old saplings were again either exposed to no flooding or a partial flooding of nine weeks. We assessed survival, foliar injury and growth in terms of plant height, number of leaves and stem diameter. We also included a long-term recovery period. In general, the individuals showed no improvement in the flooding tolerance to a recurrent flooding event irrespective of its previous flooding duration. Contrary to the expectations, there was rather a negative effect on the flooding tolerance especially of the less flooding tolerant species. Differences in species response to flooding can be explained mainly by their ability to react to the resulting stress with morphological, physiological and metabolic adaptations. Furthermore, we could show that the inclusion of a certain recovery period is very important in order to assess flooding tolerance and therefore avoid misjudgments.

Reconstructing agriculture and industrial activity with lake sediments

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The suitability of lake sediment cores to reconstruct past inputs and regional pollution and usage patterns of pesticides has been shown on multiple occasions. This can come in handy especially if the data situation is either unreliable or scarce. So as in the former German Democratic Republic (GDR). While the officials of the GDR released statistics concerning industrial and agricultural activity, these data don't cover the whole period of the state's existence. Using ten sediment cores of ten lakes in east Germany, these statistics were counter-checked. We analyzed them for heavy metals using ICP-OES and for organochlorine pesticides (OCP) like DDT and HCH (lindane) using a miniaturized liquid extraction technique in conjunction with head space solid phase micro extraction (HS-SPME) and GC/MS. Trace element concentrations follow an over-regional pattern and are indicative of activity far outside the GDR's territory. The OCP concentrations, however, are indicative of local developments. In the lakes' profiles, several regional features but also national policies or reactions thereto are visible.

Section 10 - Clinical Sciences

Schedule of Section 10
TuWednesday, 30th September 2020

Part 1	**Chairperson: Veronika Lehner**
13:15 - 13:25	**Wenjie Sheng**
	Evaluating the immunogenic cell death responses by chemotherapy in ovarian cancer cell lines
13:25 - 13:35	**Chaoyu Zhang**
	Development of a universal two-step pre-targeting approach for precise detection and treatment of ovarian cancer
13:35 - 13:45	**Michaela Melzer**
	Influence of extracellular matrix on TGF-β-induced tenogenesis in mesenchymal stromal cells
13:45 - 13:55	**Alina Hagen**
	Scalable production of equine platelet lysate for multipotent mesenchymal stromal cell culture
Part 2	**Chairperson: Rebecca Hasseli**
14:30 - 14:40	**Carla Doll**
	Identification of chronic diseases in equine tendons by magnetic resonance imaging
14:40 - 14:50	**Jiawen Yong**
	Adiponectin Interacts In-Vitro with Cementoblasts Influencing Cell Migration, Proliferation and Cementogenesis partially through the MAPK signaling pathway.

14:50 - 15:00	**Reem Jamous**
	Ex Vivo Bone Organ Culture Mimicking Defect Healing In Vivo
15:00 - 15:10	**Veronika Lehner**
	When structure matters and matter structures: Nano-3D-printed biphasic polymer scaffolds in a 3D cell culture model
Part 3	**Chairperson: Reem Jamous**
15:45 - 15:55	**Rebecca Hasseli**
	It's burning - Neuropathy in Inflammatory Rheumatic Diseases
15:55 - 16:05	**Jessica Zilli**
	Quantitative histologic evaluation of the CA hippocampal fields' pyramidal cell layer in cats
16:05 - 16:15	**Anca-Laura Amati**
	C-reactive protein-mediated inhibition of ATP-induced inflammasome activation
16:15 - 16:25	**Ruth Charlotte Dartsch**
	Notch1 signalling in alveolar regeneration and fibrotic repair
16:25 - 16:35	**Michael John Cekay**
	Impact of pulmonary arterial hypertension on overall survival in lung cancer patients
Keynote Section 10	**Chairperson: Veronika Lehner**
17:00 - 17:30	**Dr. Herbert Schiller, Helmholtz-Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt** Single cell analysis as a basis for a better understanding of lung injury and repair

C-reactive protein-mediated inhibition of ATP-induced inflammasome activation

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Interleukin-1β (IL-1β) is a potent pro-inflammatory cytokine of innate immunity. While IL-1β is involved in host defense against infections, it also plays a central role in the pathogenesis of life-threatening systemic inflammation triggered in about a third of the patients undergoing major open surgery and accounting for a 13-fold increase in postoperative mortality. Hence, controlling the systemic inflammation induced by surgical trauma is of outstanding biomedical relevance. IL-1β release by mononuclear phagocytes can be induced by Toll-like receptor agonists, followed by stimulation with extracellular ATP, an important danger signal in surgically induced sterile inflammation. Infections activate numerous additional ATP-independent pathways of IL-1β release. Increased systemic IL-1β stimulates the hepatic expression of C-reactive protein (CRP), a pentameric acute phase protein and commonly used clinical marker for inflammation. The pro-inflammatory functions of CRP are mediated by its association with molecules containing a phosphocholine (PC) head-group exposed on the surface of damaged cells. Anti-inflammatory properties of CRP were also described but poorly understood. We demonstrated that native CRP inhibits the ATP-mediated release of IL-1β from human monocytic cells at concentrations typical for mild inflammation in human patients. The activity of CRP depends on the presence of soluble endogenous ligands. CRP/ligand complexes activate metabotropic functions at nicotinic acetylcholine receptors (nAChRs), suppress activation of the ATP receptor P2X7 and, hence, inflammasome assembly and IL-1β release. Our data suggest that CRP/ligand complexes protect against ATP-mediated inflammation, while sparing ATP-independent pathways that are typically induced by pathogens. We therefore plan to develop CRP-based therapeutic strategies preventing ATP-induced inflammation. Our further goals are to investigate the anti-inflammatory properties of patient CRP and to identify its endogenous ligands. We will use this knowledge to develop modified CRP that is devoid of pro-inflammatory activity but prevents ATP-induced IL-1β release, therefore dampening ATP-mediated sterile inflammation without increasing the risk of sepsis.

Impact of pulmonary arterial hypertension on overall survival in lung cancer patients

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Lung cancer (LC) is one of the major causes of morbidity and mortality worldwide. Our group recently discovered that pulmonary hypertension (PH) is present in a significant proportion of lung cancer patients. To investigate the incidence and impact of PH in lung cancer, we retrospectively analyzed lung cancer patients in our center from the years 2017 to 2019. Measurements of pulmonary artery (PA) and ascending aorta (A) diameter (size) from the images acquired with high-resolution computed tomography revealed that 246 of 555 (44.32%) lung cancer patients had a mean PA size of ≥28mm, assumed to indicate PH. In addition, we calculated the PA/A ratio, a parameter suggested as robust indicator of PH. Notably, 124 of 555 patients (22.34%) had a PA/A ratio ≥1. In 106 patients who had available echocardiographic data, PH was further confirmed by increased systolic pulmonary artery pressure (sPAP) (37.90 ±14.12mmHg) in patients that had a PA/A ratio ≥1, as compared to those with PA/A ratio <1 (28.81 ±8.28mmHg). To investigate the impact of PH on the survival of lung cancer patients, we evaluated the progression free survival (PFS) and overall survival (OS) in these patient cohorts. Importantly, the median PFS was significantly shorter in patients with lung cancer and PH when a cutoff PA size of ≥28mm was used (median PFS PA <28mm = 265 days; median PFS PA ≥28mm = 191 days), and the difference was even more prominent, when a cutoff PA/A ratio of ≥1 was employed (median PFS PA/A ratio <1 = 268 days; median PFS PA/A ratio ≥1 = 143 days). In corroboration, median OS was significantly reduced for patients with PA size ≥28 mm (413 days vs 562 days) and PA/A ratio ≥1 (209 days vs 562 days) compared to PA size <28mm and PA/A ratio >1, respectively.

Notch1 signalling in alveolar regeneration and fibrotic repair

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Idiopathic pulmonary fibrosis (IPF) is the most abundant idiopathic interstitial pneumonia with a fatal median survival worse than many solid cancers. A major and early hallmark of disease pathogenesis is a chronic repetitive type II alveolar epithelial cell (AECII) injury resulting in an aberrant wound healing process and progressive scarring of the lung parenchyma. Two approved drugs slow the progression of lung function decline via targeting mostly the aberrant fibrotic repair but not type II alveolar epithelial cells, as the primary progenitor cells of the alveolar space. Notch signalling, a highly conserved developmental pathway has been shown to be of fundamental importance in lung development. Furthermore, Notch signalling is persistently activated after major lung injury and promotes aberrant alveolar regeneration and alveolar cyst formation reminiscent of microscopic honeycombing in IPF. We previously identified Notch signalling in AECII in the Bleomycin model of lung fibrosis as well as human IPF and hypothesize a critical role for Notch1 in the IPF AECII progenitor cell subpopulation. Furthermore, assuming that not all AECII possess progenitor cell characteristics we aim to identify the Notch-responsive epithelial subpopulation and its role in alveolar regeneration.
To further elucidate the potential role of Notch1 signalling in human healthy Donor- and IPF alveolar epithelial regeneration and differentiation, we established a human alveolosphere forming assay from Donor- and IPF-AECII isolated from lung transplants or lung explants, respectively. Our first preliminary results show a highly reduced alveolosphere forming capacity of the overall mature HTII-280+ FACS sorted IPF-AECII population compared to healthy Donor-AECII. Furthermore, we will elucidate the contribution of Notch1 signalling for AECII proliferation and differentiation by global as well as specific Notch1-receptor blockade to elucidate Notch1 as a potential therapeutic target to facilitate AECII regeneration in IPF.

Identification of chronic diseases in equine tendons by magnetic resonance imaging

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In equine medicine, tendon diseases are prevalent reasons for an early retirement. Especially the forelimb superficial digital flexor tendon is affected. During regeneration, a framework of collagen type III is initially formed, which is subsequently replaced by collagen type I. This occurs through the interplay of matrix metalloproteinases and their inhibitors, the tissue-inhibitors-of-metalloproteinases. As tendon tissue is hypocellular and low-vascularized, regeneration in the sense of restitutio ad integrum is impossible, and stiffer connective tissue is formed. As a result of its lacking elasticity, reinjury occurs frequently. Especially chronically damaged tendons have a poor prognosis. An injection of multipotent mesenchymal stromal cells (MSC) into the tendon lesion has emerged as a treatment with promising results. Their effect is based on anti-inflammatory and possible anti-fibrotic mechanisms, which could be advantageous to avoid the fibrosis in tendon disease. However, anti-fibrotic MSC mechanisms are not well understood yet.
The overall aim of the project is to analyze the extracellular matrix remodelling by MSC in an in vitro model of naturally occurring chronic tendon disease. To establish this cell culture model, dezellularized scaffolds made of equine tendons with chronic disease are required. Horse limbs are collected from a local abattoir and screened for chronic tendon disease by MRI. In a first step, to standardize the recognition of tendon disease, limbs were examined by MRI and the tendons were subsequently assessed histologically by a trichrome staining which differentiates healthy collagen from scar tissue. The histological sections were also examined using scores that included inflammatory cells, fibrocytes and the collagen fiber arrangement in a hematoxylin eosin staining. MRI results are compared with the results of the histological assessment. In the next step, tendon disease will be graded based on MRI only and tendons will be harvested for scaffold production, to be used in the cell culture model.

Scalable production of equine platelet lysate for multipotent mesenchymal stromal cell culture

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The clinical application of multipotent mesenchymal stromal cells (MSC) has gained tremendous attention in veterinary medicine, especially in equine medicine, yet many open questions remain. One critical issue is the in vitro cultivation of the MSC before their clinical use, as culture conditions could impact on cell quality and efficacy of therapies. Currently, using fetal bovine serum (FBS) as a supplement to the basal medium is the gold standard for *in vitro* cultivation of equine MSC. However, the use of FBS is afflicted with several problems. First, the extraction of FBS is controversially discussed due to ethical reasons. Further disadvantages are the rising costs, because of the consistent increase in MSC application, and the different composition of the batches. Finally, in addition to the risk of an immune reaction to the bovine antigens in xenogenously used FBS, there is also the hazard of transmission of bovine pathogens. For this reason, several alternatives are being tested including horse serum, serum-free supplements, and platelet lysate. The latter led to promising results but is far from being regularly used in equine MSC culture. Interestingly, human MSC for clinical application are already cultured with human platelet lysate as supplement. However, in contrast to the level of knowledge and clinical implementation in human cell therapies, there are still significant deficits in veterinary medicine, especially in terms of standardization and scalability of equine platelet lysate production. The aim of this study was to establish a scalable protocol for equine platelet lysate (ePL) production and to test the obtained ePL for its suitability for equine MSC culture. Whole blood was collected in 500 ml blood bags from the jugular vein of 20 healthy horses and was used to prepare platelet lysate by a buffy coat method. The technique appeared standardizable, platelets were retained and leukocytes widely removed.

It's burning - Neuropathy in Inflammatory Rheumatic Diseases

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Inflammatory rheumatic diseases (IRDs) e.g. rheumatoid arthritis (RA), spondyloarthritis and systemic sclerosis (SSc) are heterogeneous disorders, in which damage to joints, muscles and various internal organs can be observed. Moreover, peripheral neurons can be affected, including the axons and myelin sheaths, which can result in pain, burning, tingling, numbness and even paresis. Thus, more detailed knowledge about the underlying molecular processes of and specific treatment options for neuropathy (NP) in IRD are urgently needed, especially as IRD patients may develop different subtypes of NP. The aim of this project was to assess the prevalence and the individual types of NP in RA, spondyloarthritis and SSc patients, to elucidate the clinical, neurophysiological and neuropathologic features of NP, and to establish data and strategies for further clinical and basis research in bench-to-bedside translation. We investigated the relationship between clinical, serologic, electrophysiologic, sonographic and histologic findings. Baseline questionnaires and neurological and physical examination were used to screen the presence of neuropathic pain and autonomic dysfunction. Laboratory tests were performed (e.g. vitamin deficiency) to exclude other causes for NP. Electrophysiological tests were performed to differentiate demyelinating from axonal NPs. Additionally, skin biopsies are performed to detect an involvement of small fibres. Nerve ultrasonography is used to evaluate selected peripheral nerves to depict alteration of nerve sizes that could reflect inflammation and edema in NP associated with IRD. Ultrasonography of muscles were done to detect muscle atrophy due to axonal NP. Dermal fibroblasts were

isolated to analyse expression of selected inflammatory markers and adhesion molecules such as integrins. We plan to include around 100 patients in this study. Already 16 patients have been included. NPs occurs in many IRD patients, but more detailed knowledge about the pathophysiology and different types of NPs are lacking. In this study we will first investigate the prevalence and different types of NPs in RA, spondyloarthritis and SSc patients. Thirteen patients are included so far and, in these patients, different types of NPs were detected, which resulted to a change of patients' therapeutic regime.

Ex Vivo Bone Organ Culture Mimicking Defect Healing In Vivo

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Bone healing is a complicated process that goes through subsequent overlapping phases. Bone research is most commonly conducted through pre-clinical animal models and in vitro studies. However, in vitro systems fail to preserve bone integrity as seen in living bone. In vivo methods cause significant pain and distress in the animals. This study aimed to establish a whole bone organ culture to allow investigation of bone healing in a controlled manner that closely mimics the in vivo processes.
Long bones were collected from by-product breeded animals under sterile environment and then placed in an ex vivo culture system. Drill hole in the metaphyseal region was created in all the samples. Additionally, a drill hole defect filled with anti-microbial paste was used to investigate the changes. Ex vivo culture was maintained in an incubator at 37 °C and 5% CO_2. Culture medium and cell proliferation around the bones were monitored every day. Bones were harvested at dayo and day7. After harvesting, bones were embedded using freezing method. Histology and chemical analysis using Time of flight- Secondary Ion Mass Spectrometry (Tof-SIMS) was carried out.
The daily monitoring of ex vivo cell culture revealed the presence of numerous inflammatory cells during the early time points. Mesenchymal stem cells were seen in the culture medium around the whole bone organ. Whole bone organ was alive until the end of day7. Histology showed presence of erythrocytes in the bone marrow around the drill hole defect. Tof-SIMS showed the diffusion of anti-microbial paste in the bone marrow.
The preliminary results showed the survival of whole bone organ in the ex vivo condition until day7. Further trials are going on to keep bone alive after day7 though the supply of required nutrients. Moreover the project is also developing to find the alternatives through 3D bioprinting.

When structure matters and matter structures: Nano-3D-printed biphasic polymer scaffolds in a 3D cell culture model

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Osteochondral lesions (OCLs) can occur due to a trauma, or can be caused by chronic degenerative processes, such as osteoarthritis, which is the most common joint disease [1], affecting more than 242 million people worldwide [2]. According to the Robert Koch Institute, 17.9% of adults in Germany over 18 are afflicted. For those aged 65 and over, the prevalence is consider ably higher, at 48.1% in women and 31.2% in men [1]. Within the framework of the "Poly-Implant-Druck" project, a collaboration of five research institutes, a novel therapeutic approach for OCL was defined. In particular, biphasic but yet monolithic nano-3D-printed polymer scaffolds were designed to mimic the ideal geometric and mechanic properties of bone and cartilage tissue respectively. Additionally, bioactive fillings create favourable conditions for the ingrowth of local stem cells and, thereby, for the integration of the implant into the healthy surrounding tissue. Preceding in vitro experiments, within the scope of another doctoral thesis, indicated a good cytocompatibility of the osteo-phase in the scaffolds with human mesenchymal stem cells. However, since cartilaginous tissue exhibits unique properties and demands, a specialised

and innovative 3D-cell culture system was designed for this study. Micro pellets obtained by this process were cultivated under particular conditions, meeting the hypoxic needs of cartilage, by means of applying the chemical component Dimethyloxaloylglycine. Results of these experiments, using cutting-edge techniques, are of key importance for the animal study, embedded within this project, and first findings will be available by the time of the 2020 annual GGL conference taking place. In conclusion, the "Poly-Implant-Druck" project offers an innovative and promising therapeutic approach for treating one of the major health issues in our growing and aging society.

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Influence of extracellular matrix on TGF-β-induced tenogenesis in mesenchymal stromal cells

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Tendon injuries are frequently occurring diseases in horses and humans. Mostly, they are caused by overload, microdamage and inflammation. It was shown that the Achilles tendon of humans is comparable to the superficial flexor tendon of horses. Therefore, it is partly possible to transfer research results between species. In studies in horses, it was shown that treatment with mesenchymal stromal cells (MSC) has a positive effect on tendon healing. However, the cellular mechanism of action, responsible for repair and regeneration of tendon issue, is not completely known. Part of it may be achieved by tenogenic differentiation, which probably results in the replacement of tenocytes and the production and modulation of the extracellular matrix (ECM).

The transforming growth factor (TGF)-β, which is signalling through intracellular smad molecules, is a potent paracrine mediator of tenogenic induction. It is known that the tenogenic effect of TGF-β can be altered by the presence of ECM. Yet, there is no detailed information about the signaling pathways. A potential way is via rho/Rock cascade activated by integrin beta receptor. This receptor can be bound by collagen I, which builds up to 95% of tendon matrix. In addition, the rho/ROCK pathway, which acts on the cytoskeleton, was repeatedly reported as an intracellular mediator in tenogenic differentiation. Its importance has been demonstrated under ECM influence and mechanical stimulation. Inhibition of rho/ROCK signal transduction by Y-27632 prevented tenogenic differentiation triggered by mechanical stimulation or the ECM. These results suggest that rho/ROCK inhibition could also affect TGF-β-induced tenogenic differentiation. The aim of this study is to investigate this possible inhibitory effect of rho/ROCK on TGF-β-induced tenogenic differentiation in both human and equine MSC.

Evaluating the immunogenic cell death responses by chemotherapy in ovarian cancer cell lines

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Ovarian cancer is the most lethal disease among gynecological tumor and the majority patients present at advanced stages. The gold standard treatment for ovarian cancer is an intensive surgical cytoreduction, followed by a cytostatic treatment. Some of the cytostatic agents has the ability to induce immunogenic cell death (ICD), which initiates host immunity against targeted cancer cells. Here, the treated cancer cells release death signals, which have the ability to promote maturation of immature DCs, which engulf cancer-specific antigens that are released from the ruptured tumor cell.

ICD elicited by chemotherapeutics results in long-lasting protective antitumor immunity and has demonstrated as potential source of cancer vaccine immunogen and reducing drug resistant. To investigate the effect of cytostatic agents in inducing ICD in ovarian cancer, different cytostatic agents will be used to treat different ovarian cell lines and the presence of ICD markers in treated human ovarian cells will be investigated.

Here, the cell surface expression levels of calreticulin and heat shock proteins (Hsp) 70, and Hsp90 will be analyzed by flow cytometry using calreticulin, Hsp70 and Hsp90 specific monoclonal antibodies. The high mobility group box 1 (HMGB1) level in the culture supernatants of treated ovarian cancer cells will be evaluated also in vitro using HMGB1 ELISA kit. Furthermore, the extracellular ATP concentrations in the culture supernatants will be measured by a lucifer-

in-based ATP Assay. Furthermore, the maturation of dendritic cells will be investigated in vitro by analyzing the expression of dendritic maturation surface markers CD80, CD86 and HLA-DR.

Adiponectin Interacts In-Vitro with Cementoblasts Influencing Cell Migration, Proliferation and Cementogenesis partially through the MAPK signaling pathway.

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Current clinical evidences suggest that circulating Adipokines such as Adiponectin can influence the ratio of orthodontic tooth movement. Here, we aimed to investigate the effect that Adiponectin exert on cementoblasts (OCCM-30) and especially on the intracellular signaling molecules of Mitogen-activated protein kinases (MAPK). The expression of the Adiponectin Receptor 1 (AdipoR1) and 2 (AdipoR2) were verified by Real-Time PCR (RT-PCR), Western Blot (WB) and Immunofluorescence (IF). OCCM-30 cells (M. Somerman, NIH, Maryland) were stimulated with Adiponectin (Prospec) and mineralization was measured by Alizarin Red S (Sigma-Aldrich) staining and by colorimetric analysis using a spectrophotometer (xMarkTM, Bio-Rad) after Cetylpyridinium Chloride (Sigma-Aldrich) addition. The expression of Alkaline Phosphatase (AP), as well as Bone Sialoprotein (BSP), Osteocalcin (OCN) and Osteoprotegerin (OPG) were evaluated by RT-PCR. Cell migration and proliferation were measured by a standard wound healing assay and MTS Elisa Kit (Promega). Activation of MAPKs were evaluated by WB. Moreover, the MAPK inhibitors of P38 (SB203580, InvivoGen), ERK 1/2 (PD98059, Calbiochem) and JNK (SP600125, InvivoGen) were used. We demonstrated that OCCM-30 cells express AdipoR1 and AdipoR2. Alizarin Red S staining revealed that Adiponectin increases mineralized nodule formation and quantitative AP activity in a dose-dependent manner. Adiponectin up-regulates the mRNA levels of AP, BSP, OCN as well as OPG. Adiponectin also increases the migration and proliferation of OCCM-30 cells. Moreover, Adiponectin induces a transient activation of JNK, P38, ERK1/2 and promotes the phosphorylation of STAT1 and STAT3. The activation of Adiponectin-mediated migration and proliferation was attenuated after pharmacological inhibition of P38, ERK1/2 and JNK in different degrees. Adiponectin promotes in-vitro OCCM-30 cell migration, proliferation as well as cementogenesis. One of the underlying mechanisms is the activation of MAPK signaling pathway. Adiponectin may play a vital role on cementoblasts, suggesting a potential novel role in the orthodontic treatment.

Development of a universal two-step pre-targeting approach for precise detection and treatment of ovarian cancer

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Ovarian cancer is the eighth most common cause of cancer death in women worldwide. This cancer is mostly diagnosed at advanced stage and shows a high mortality due to limited screening strategies. Antibody-drug conjugates (ADCs) are a novel anti-tumor therapy, which combines specificity of monoclonal antibody and chemotherapy. Unfortunately, the generation of current ADCs are relying on using random conjugation methods. These methods generate heterogeneous products, resulting in inconsistent pharmacokinetic, efficacy and safety profiles. Furthermore, ADCs are associated with prolonged circulation, slow clearance and Meinhold-Heerlein poor tissue penetration.

To overcome these limitations, we are trying to establish a two-step pre-targeting approach, taking the advantages of enzymatic site-specific conjugation strategy based on the SNAP-tag technology and specific binding ability of coiled coils. Here, a single chain antibody (scFv) will be fused genetically with coiled coil (ZipPLUS), while toxic agent Monomethyl auristatin E (MMAE) will be conjugated to another coiled coil (ZipMINUS) through SNAP-tag technology, which is able to bind ZipPLUS specifically.

The targeting activity of the pre-targeting complex will be confirmed in vitro using flow cytometry and fluorescence microscopy by conjugated ZipMINUS with fluorescence dye instead of MMAE.

To determine the therapeutic properties of the pre-targeting complex, the scFv-ZipPLUS will be applied to ovarian cancer cells followed by applying ZipMINUS-SNAP-MMAE. The cell viability and induction of programed cell apoptosis cells will be determine using XTT cell viability assay and caspase assay.

Quantitative histologic evaluation of the CA hippocampal fields' pyramidal cell layer in cats

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Cats are known to be affected by hippocampal sclerosis (HS), potentially causing antiepileptic drug (AED) resistance. This can occur as a consequence of longstanding seizure activity secondary to trauma, inflammatory or idiopathic diseases or even be primary. In humans, temporal lobe resection is a standardized therapy in patients with refractory temporal lobe epilepsy and HS is a frequent finding.
Diagnosis of HS relies mostly on reduced pyramidal cell density at hippocampal specimen, which may differ throughout the hippocampal axis, also in healthy cats. For this reason, normal reference values on cellular density and cytoarchitecture for the different parts of the feline hippocampus are needed to establish standardized examination and diagnosis of feline HS. In Veterinary Medicine such data are still missing, therefore the purpose of this study is to evaluate feline hippocampal pyramidal layer cellular density and cytoarchitecture at different levels.
Twenty cats, presented in the Small Animal Clinic-JLU in Giessen, were enrolled. The cats were euthanized for reasons unrelated to the study. Cadavers were eligible if they were free from intracranial clinical signs ante-mortem. From formalin fixed brains three transverse sections (dorsal, middle and ventral part) of each left hippocampus were obtained. From each specimen histological (Haematoxylin-eosin and Nissl staining) and immunofluorescence studies with antibodies against NeuN to detect neurons combined with GFAP for glial cells were performed and cells evaluated. Statistical comparison of the cell populations in the three transverse sections, distinguishing CA1, CA2, CA3, CA4 areas and dentate gyrus, will be performed. Human standard recommendations for the diagnosis of HS were observed during the assessment. Studies are ongoing and final results are not available.
As surgical treatment for epileptic cats with AED resistance may become as in humans a therapy option in the future, this study will help in the standardized examination of hippocampal specimen for HS.

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