Cellular Polarity and Viral Infection

Research Aims: The laboratory is interested in how intestinal epithelial cells (IECs) that line the surface of the gut can tolerate the presence of the commensal microflora and at the same time recognize and fight enteric pathogens.

Background: Intestinal epithelial cells (IECs) constitute the primary barrier that separates us from the outside environment. These cells are faced with a major challenge: they have to tolerate the presence of the commensal microbiota while maintaining full responsiveness against enteric pathogens. How IECs achieve such balance and tailored innate immune response has only become to be appreciated. Understanding these mechanisms is mandatory to design new strategies of intervention against enteric pathogens and enteric diseases which often result from unappropriate response against the gut microflora. The mechanisms by which IECs can tolerate the presence of the commensal bacteria present in the gut have started to be revealed. Cellular polarity through segregation of pattern recognition receptors (PRRs) have been proposed to be one mechanism leading to the tolerance of the commensal microbiota in mouse models. PRRs recognizing bacteria have been reported to be localized at the basolateral side of IECs which prevent the recognition of bacteria located at the apical side of IECs (lumen side of the gut). Exactly how IECs coordinate this polarization of functions remains unclear. Similarly, whether similar mechanisms are established in human have not been addressed and whether polarization extent to all class of PRRs is not known. Recently, the presence of large amount of viruses in the gut has begun to be appreciated. Whether IECs have developed similar mechanisms to tolerate the presence of these enteric viruses remain to be characterized. Importantly, the potential role of these viruses in maintaining gut homeostasis has not been addressed.

Research Highlights:
Viral strategies to infect intestinal epithelial cells: Reovirus is a widely used model virus to study antiviral innate immune response and pathogenesis. During the natural course of infection, virion particles are converted in the lumen of the gut to intermediate subviral particles (ISVPs) by proteolytic digestion. The reasons for this conversion remained unclear. However, we recently found that ISVPs have developed specific strategies to not trigger innate immune response in IECs. On the contrary, infection of IECs by virions induces a strong immune response.
response that leads to cellular death. Interestingly, we found that ISVP infected cells secrete a pro-survival factor that protects IECs against virion induced cellular death. We propose that conversion of reovirus to ISVPs in the lumen of the gut is a viral strategy to initiate primary infection by subverting IECs innate immune system and by counteracting cellular-death pathways.

**Polarized antiviral response IECs, strategies to tolerate the intestinal commensal flora:**
IECs are structurally polarized with an apical side facing the lumen of the gut and a basolateral side facing the lamina propria side. While the luminal side is constantly exposed to the commensal flora, the basolateral side is sterile. One way for IECs to tolerate the presence of the ever-present microbial flora is to segregate their innate immune function to the basolateral side and be “blind” from their apical side. We found that the extent of immune response is a function of the site of infection in human IECs. Infection of IECs from the basolateral side triggered a stronger response of IECs compared to infection of the apical side. Using several human IECs culture model and human gut organoids, we are currently identifying the molecular mechanisms that lead to this polarized antiviral response and are testing whether it could be a strategy to avoid excessive response against the commensal flora while maintaining full responsiveness against viruses that have passed the epithelium barrier.

**Functional differences in type I versus type III interferon mediated immunity in IECs:**
While type I interferon (IFN) mediated immunity is ubiquitous to all cells, type III IFN mediated immunity is confined to epithelial cells due to the restricted expression of its receptor. It is currently believe that both IFNs have redundant functions; however, the epithelium specificity of type III IFN strongly suggests that both IFNs must have functional differences at epithelial surfaces. In the lab we have defined, for the first time, functional differences between type I and type III IFN signaling in epithelial cells and we propose that type III IFN signaling is specifically tailored to efficiently combat infection without inducing an excessive pro-inflammatory response.

**Group Composition:**
- Dr. Megan Stanifer, Post-doc
- Dorothee Albrecht, Technician
- Pranav Shah, PhD student
- Delia Bucher, PhD student
- Kalliopi Pervolaraki, PhD student
- Marta Fratini, PhD student
- Markus Mukenhijn, PhD student
- Christian Kischnick, PhD student

**Last Year Publications:**
- Boulant S. et al., 2015, *Viruses*, review Dynamics of virus-receptor interactions in virus binding, signaling, and endocytosis
- Odendall C. et al., 2014, *Nat Immunol* Diverse intracellular pathogens activate type III interferon expression from peroxisomes
- Grinevich V. et al., 2015, *book chapter* “Somatic transgenesis (Viral vectors)”

**External Funding:**
- DFG SFB1129: Integrative analysis of pathogen replication and spread.
- European FP7, Marie Curie integration grant

**Teaching Activities:**
- Molecular Virology Master Student Practical
- HBIGS course, practical
- Molecular Virology Post-transcriptional regulation tutorial
- DKFZ progress in cancer research
- DKFZ journal club: Infection and Cancer
- Frontiers in Biosciences Master Student lecture
- International Graduate School: Pathogen-Host Interactions at Cellular Barriers, Muenster, Germany
Research aims:

**Aim 1. Deciphering oxytocin circuits modulating pain perception**

In our study, we detected a subset of ~30 parvocellular OT neurons which simultaneously terminate on magnocellular OT neurons and neurons of deep layers of the spinal cord. Evoked OT release from these parvocellular OT neurons suppresses nociception and promotes analgesia in an animal model of inflammatory pain. Our findings show that small groups of parvocellular neurons interact with magnocellular neurons to build in the spinal cord - organized OT circuits controlling diverse body functions (Eliava et al., Neuron, 2016).

**Aim 2. Identification of oxytocin neuron ensembles confronting fear**

We developed a technique, “virus-mediated Genetic Activity-Induced Tagging (vGAIT) of cells”, which is based on the activity-dependent expression of the immediate early gene c-fos in virally infected OT neurons. Using vGAIT we labeled OT neurons, which had been activated by fear expression (OTFear neurons) and found that only a small fraction of OTFear neurons (10-12%) was labeled. Blue-light stimulation of axons of OTFear neurons within the CeA rapidly profoundly reversed the freezing behavior, while pharmacogenetic inhibition of OTFear neuronal somas by Clozapin-N-oxide significantly inhibited fear extinction. Our results provide evidence for functional specialization within the central OT system and open perspectives for dissection of OT ensembles modulating distinct forms of socio-emotional behavior, such as threat and empathy. (The manuscript is in submission.)

**Aim 3. Dissection of central mechanisms of stress-induced inhibition of reproductive physiology**

Pharmacological studies since the early 1980’s have demonstrated that the main neuropeptide of the stress response, corticotropin-releasing hormone (CRH), inhibits the activity of the hypothalamic-pituitary-gonadal (HPG) axis in mammals via its predominant receptor type 1 (CRHR1). We aimed to analyze this phenomenon by genetic means. Thus, we deleted CRHR1 in GnRH neurons exposed to restraint stress or an intraperitoneal injection of the bacterial endotoxin lipopolysaccharide. To our surprise, we found a preserved stress-induced decrease of luteinizing hormone (LH) levels. Similarly, the application of CRH in acute brain slices did not change membrane potentials or action potential firing of GnRH neurons. Moreover, the stress-induced suppression of LH levels could not be prevented when CRH-R1 was genetically removed in GABA neurons or all neurons, indicating that CRH-R1 activation has no indirect effect of GnRH mediated LH release after stress induction. However, the infusion of CRH itself prevented the decline of LH levels in basal condition in

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Schaller Research Group on Neuropeptides

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mice lacking CRHR1 in all neurons. Our results challenge the prevailing concept of a CRH/CRHR1-mediated inhibition of HPG axis activity under acute stress and suggest the possibility of distinguishing between CRHR1-signaling in basal vs. acute stress conditions. (The manuscript is in preparation.)

**Group Composition:**
- Dr. Marina Eliava, Postdoctoral Fellow
- Dr. Androniki Raftogianni, Postdoctoral Fellow
- Dr. Xinying Liu, Postdoctoral Fellow
- Dr. Andrey Rozov, Postdoctoral Fellow
- Miriam Silva de Gouveia (Kernert), PhD student
- Ferdinand Althammer, PhD student
- Diego Benusiglio, PhD student
- Ana Almeida, PhD student
- Yan Tang, Guest scientist
- Jonas Schimmer, Bachelor student
- Judith Müller, Technical Assistant
- Elke Lederer, Technical Assistant

**Last Year’s Publications (Original articles):**

**Reviews:**

**Book chapters:**

**External Funding:**
- DFG, German Research Foundation grant GR 3619/4-1, 2012-2015.

**Awards and Patents:**
- Human Frontier Research Grant

**Teaching Activities:**
- Habilitation, July 2015
- Lecture for PhD students at the Interdisciplinary Center of Neuroscience (IZN), University of Heidelberg, 2015.
- Neuroscience summer lecture series for Master students, University of Heidelberg, June 2015.
- Practical courses for Bachelor students, University of Heidelberg, June 2015.
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2012 - present: Group Leader
Schaller Research Group at the University of Heidelberg and the DKFZ

2005 - 2010: Senior Scientist, National Institutes of Infectious Diseases, Japan

2001 – 2005: PhD at Tokyo University, Japan

Norovirus Study Group

Research Aims: The purpose of my research group is to better understand norovirus capsid flexibility with respect to receptor binding interactions and virus evolution with the ultimate aim of developing norovirus antivirals.

Background:
Human noroviruses are the dominant cause of outbreaks of gastroenteritis around the world and infect all age groups. There are no antivirals or vaccines against noroviruses, mainly because these viruses cannot be grown in cell culture. The prevention and treatment of human norovirus are of major public health concerns.

Research Projects (published* and ongoing):
1. Structural Basis for Norovirus Inhibition by Human Milk Oligosaccharides*. We showed that components of breast milk (termed 2'FL and 3FL) could bind to the norovirus particles and block the binding to HBGAs. We determined the atomic resolution structure of the binding interactions of 2'FL and 3FL with the protruding domain. Our data showed that 2’FL and 3FL structurally mimicked the HBGA, and bound at the identical site on the protruding domain. We also wrote a review article explaining these kinds of interactions. We are now planning to follow up this study with additional norovirus types and their corresponding interactions.

2. Structural evolution of the emerging 2014/15 GII.17 noroviruses*. Recently, the GII.17 noroviruses have increased in prevalence worldwide. We solved the protruding domain structures of an earlier non-prevalent GII.17 and two recent prevalent GII.17 viruses. We suspect that changes in the HBGA pocket may have led to an increase in their prevalence. We are now following up this study with the development of antivirals that can target these new strains.

3. Structural Constraints on Human Norovirus Binding to Histo-Blood Group Antigens*. We analyzed several noroviruses that bind poorly HBGA. We showed that a single amino acid was likely responsible for the lack of HBGA interactions. However, a nearby loop also likely influenced the interactions. These findings complicate the development of antivirals that are directed to the HBGA pocket - as it is clear that some noroviruses may not require HBGAs for an infection.

4. Production of Human Norovirus Protruding Domains in E. coli for X-ray Crystallography*. We prepared a method paper explaining our in-house technique for the preparation and purification of norovirus protruding domains.

5. Structural analysis of a chimeric sapovirus capsid*. We solved the cryo-EM structure of a sapovirus particle and identified the different domains. The chimeric particle was designed from two different sapovirus strains and we showed that the particle had improved cross-
reactivities - indicating a broad-range vaccine could be developed using this kind of design.

6. Structural analysis of bovine norovirus protruding domain*. We solved the protruding domain structure of a bovine norovirus and compared the other noroviruses. We found that the bovine protruding domain was similar to human norovirus structure and weakly cross-reacted against human norovirus antisera. These results suggest that bovine and human noroviruses may have shared epitopes. However, the bovine norovirus HBGA binding pocket was different and indicated that these viruses may not infect humans - luckily.

7. Development of norovirus antivirals. We are preparing several antiviral candidate molecules for the preclinical trials, i.e., Nanobodies, citrate, and modified antibodies. We setup a murine noroviruses cell culture system to test the effects and provide a proof of concept. We have also prepared a new library of Nanobodies and plan to test these in the cell culture.

**Group Composition:**

- Anna Koromyslova, Postdoc
- Sylvie Doerflinger, PhD student
- German Monogarov, PhD student
- Vasili Morozov, Postdoc
- Anna Koromyslova (Jun 2013 - May 2016), Bishal Singh (Mar 2013 - Feb 2016) and Mila Leuthold (Apr 2013 - Mar 2016), (all graduated PhD)
- Lisa Hefele (Oct 2015 - May 2016), (Master Thesis)
- Bianca Moncanu (08.06.2015 - 10.09.2015) and Clara-Marie Gürth (03.08.2015 - 03.10.2015) (Master Rotation)
- Michelle Hass, Celina Geiß, Imme Roggenbach, Stefan Bassler, and Juliane Graf (04.04-2016 - 04.07.2016) (Bachelor Thesis)

**Publications (all CHS funding):**


**Patent (now under international review process)**


**Presentations:**

Interfaculty Institute of Biochemistry, Tübingen, Norovirus Structural studies, January 18th, 2016.

**External Funding:**

DFG Research unit (VIROCARB) 3 years

**Organizational functions:**

1. Frontiers in Biosciences, Molecular Virology, Teaching, Pymol concepts, Feb 2016

Proteostasis in Neurodegenerative Disease

Research Aims: The goal of our work is the mechanistic understanding of events triggering protein misfolding and the subsequent impact of aggregated protein species on cellular homeostasis, with a major focus on Aβ and Tau-mediated neurodegeneration.

Background:
Most age-associated neurodegenerative diseases, in addition to cancer and metabolic diseases, present with protein aggregates as a pathological feature, suggesting that they may share common proteotoxic mechanisms, involving cellular impairment due to the misfolding and accumulation of proteins. Therefore, it is necessary to understand the mechanisms of aggregation-associated cell toxicity to enable the design of therapeutic intervention strategies.

Like many other neurodegenerative diseases, overwhelming evidence suggests that Alzheimer’s Disease (AD) is a disorder of protein misfolding and aggregation. AD is characterized by a loss of brain volume, especially in the cortical and hippocampal regions and by the presence of two distinct types of aggregates: intracellular neurofibrillary tangles (NFT), composed of the microtubule-associated protein tau, and extracellular senile plaques, mainly composed of the Amyloid-beta (Aβ) peptide.

Research Highlights:
Characterization of Aβ seeding:
Using a series of AD-associated Aβ peptide variants as well as engineered peptide variants, we were able to generate transgenic Drosophila models that capture the intrinsic aggregation characteristics of different Aβ conformers and generate distinct Aβ strains. Most recently, we were able to establish a fully genetically-encoded Aβ seeding model, resulting in the development of neurotoxicity for a selection of seeding-competent Aβ variants, when secreted from neurons in the Drosophila brain. Our data mimic the human disease progression, showing the ability of Aβ to form distinct strains in vivo, both showing clear characteristics of amyloid structure, but having distinct impacts on neuronal integrity. Our data strongly support the amyloid seeding hypothesis and provide the AD community with a powerful Drosophila AD model as a suitable in vivo model system to explore the specific characteristics of different Aβ modifying factors.
Mechanism of tau propagation:
Following the spreading hypothesis, we recently analyzed whether tau species can be secreted from cells, potentially pointing towards the possibility of a transcellular tau propagation mechanism. For this purpose, we analyzed the cell culture medium using differential centrifugation, immunoprecipitation and western blotting. Our findings indicate the accumulation of tau species in the extracellular space, particularly for phosphorylated tau variants. Importantly, we could show that this tau material is mainly full-length and accumulates as soluble protein, while very little insoluble or vesicle-associated tau was observed. We are very intrigued about these data, as different tau variants have been proposed as biomarkers in the CSF of presymptomatic AD patients. To date, it is unclear whether these specific phosphorylated tau variants are liberated upon neuronal death or by other mechanisms. Importantly, subsequent analyses of our model showed that the extracellular tau does not result from cell death or unspecific cell lysis in our system, but that the secretion is a time- and concentration-dependent process. To compare this secretion mechanism with the conventional ER/Golgi secretion pathway, we generated a tau E14 variant harboring a specific secretion peptide (SP-E14). As expected, this tau construct is efficiently secreted from cells and accumulates as soluble protein in the medium. With this control, we then tested several chemical compounds known to inhibit the conventional secretion mechanism. As described in the literature for other secreted proteins, these chemical inhibitors efficiently block the signal peptide-driven secretion of SP-E14, but we were not able to block the secretion of tau E14 expressed in the cytoplasm. Together, our data illustrate the secretion of soluble phosphorylated tau species from neuronal cells. We are currently in the process of determining the physicochemical properties of secreted tau and cellular components driving this process, and to validate these findings in different mammalian cell culture systems.

Publications:

Group Composition:
- Dr. Tobias Rasse, Research Associate
- Michelle Eisel, Technical Assistant
- Nina Dräger, PhD Student
- Taxiahris Katsinelos, PhD Student
- Ramona Sowade, PhD Student
- Zeenna Stapper, PhD Student
- Tairi Aljand, PhD Student
- Eliana Nachman, PhD Student
- Shabab Hannan, PhD Student

External Funding:

Teaching Activities:
- MSc Neuroscience Lecture
- Biosciences Bachelor Student Practical
Molecular Mechanisms of Tumor Invasion

Research Aims: Elucidating the molecular mechanisms of brain tumor cell invasion.

Background:
High-grade gliomas are among the most deadly of all human cancers reflected by a median patient survival of less than 12 months (Barbus and Tews et al., 2011). Invasive growth and early infiltration of the surrounding healthy brain is a hallmark of glioma (Giese, 2003). This invasive nature mainly accounts for their resistance to current treatment modalities. We are studying three different paradigms, which mediate brain tumor invasion and resistance:

- Alteration of the extracellular matrix;
- Expression of pro-invasive endogenous driver proteins; and
- Cross talk of tumor with stroma cells, which then support tumor growth and invasion.

Research Highlights:
Correlated magnetic resonance imaging and ultramicroscopy (MR-UM) as a tool kit to assess the dynamics of glioma angiogenesis and resistance

A fundamental hallmark of glioma progression is angiogenesis. Tumor blood vessels differ in structure from their normal counterparts for reasons that need to be investigated in more detail. Compounds that block the formation of blood vessels have been developed for treating gliomas. However, although many of these compounds show promising effects in preclinical trials, clinical trials on humans have been less successful. Having the ability to image the blood vessels in high detail during preclinical trials would help to reveal how treatments that inhibit blood vessel formation work and how tumors might develop resistance to these drugs, i.e. by pronounced tumor invasion. However, studying tumor blood vessels remains a challenge due to technical restrictions: techniques that are able to capture how the vessels change over time are unable to show individual cells in much detail, and vice versa. Magnetic resonance imaging (MRI) is a versatile tool that can monitor how the blood vessel system of a tumor changes over time in living animals. On the other hand, ultramicroscopy is able to determine the structure of single cells of a particular type. We have combined these techniques and developed an imaging platform that allows the formation of tumor blood vessels to be precisely mapped in the setting of a preclinical study. It also enables detailed investigations into how the structure of the blood vessels is altered by anti-angiogenic treatments. Using this approach on mice with gliomas, we demonstrated that drugs that inhibit the formation of the blood vessels that supply tumors also cause the blood vessels to take on a more normal structure. Furthermore, treating the mice with a single inhibitory drug was unable to stop tumor
growth, mirroring the situation in humans (Breckwoldt, Bode et al., eLIFE, 2015).

Outlook: Currently, new inhibitors are being developed, offering the possibility of combined treatments that may be more effective than using a single drug on its own. Our imaging platform will allow the therapeutic effects obtained by these new treatments to be analyzed in detail during further preclinical studies.

Glioma invasion along white matter tracts - role of the Unfolded Protein Response (UPR)
The invasion of the surrounding healthy brain tissues by glioma tumors does not happen randomly. It has been found to be associated with distinct anatomical structures such as the basement membranes of blood vessels. With regards to migration and invasion, the inhibitory myelin pathways also serve as essential structures for glioma invasion. The currently used standard therapies, such as radio and alkylating chemotherapy, target dividing cells. However, the invading cells do not divide, since the responsible components of the cytoskeleton direct mobility and cell division mechanisms, but not both at the same time (“go or grow” hypothesis). These cells are therefore therapy-resistant, which creates a major problem for treatment. New therapeutic agents can help stop the invasion and make the cells more susceptible to established therapeutic methods. In this connection, the UPR shows great potential as a goal for therapeutic interventions. We investigate the UPR in glioma in the SUPR-G consortium (www.supr-g.org).

Ultramicroscopy as a novel tool to unravel the tropism of AAV gene therapy vectors in the brain
Recombinant adeno-associated viral (AAV) vectors have rapidly advanced to the vanguard of gene therapy in the last years. rAAV vectors are broadly used for gene transfer and numerous naturally occurring serotypes have been used to target cells in different tissues and organs including the brain and spinal cord. Hence, a strong need for fast and dynamic methods exists which efficiently unravel viral tropism in non-dissected intact whole organs, especially the brain. Ultramicroscopy allowed for a rapid analysis of marker fluorescence expression in neurons with intact anatomical projections deep inside the uncut adult rodent brain in a defined anatomical structure. Our data allow a better understanding of AAV-based hippocampal transduction opening new avenues for modeling and treatment of neurodegenerative disorders in the mouse brain.

Group Composition:
- Dr. Julia Bode, Postdoctoral Fellow
- Peter Wirthschaft, PhD student
- Anika Simon, PhD student
- Rakesh Sharma, PhD student
- Himanshu Soni, PhD student
- Fabio Dietrich, Technician
- Rebecca Van Laack, Master student
- Fabian Braun, Bachelor student
- Tatjana Alexander (HiWi)

Last Year Publications (2015):

External Funding:
- BMBF e:Med: Systems biology of the Unfolded Protein Response in Glioma (SUPR-G; 01ZX1401A; Coordinator Björn Tews); 1.500 000 €; subproject funding for Tews: 319 161 €.

Teaching Activities:
- DKFZ Major Cancer Biology
- University of Heidelberg: Methoden der Biochemie und Molekularbiologie (seminar)