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Venue:

Helmholtz Zentrum für Infektionsforschung
Inhoffenstr. 7
38124 Braunschweig
Building X, Forum X0.13

Cell Biology of emerging viral infections

Thursday, June 22nd

12:00 - 13:00 Welcome Lunch
13:00 - 13:15 Opening

13:15 - 14:00 **Keynote Lecture 1**
Increasing preparedness by networking: The network "Rodent-borne Pathogens"
Rainer G. Ulrich, Friedrich-Loeffler-Institut, Greifswald Riems, Germany

14:00 - 14:20 Molecular Basis for the Host Adaptation of Zoonotic Orthohantaviruses
Laura Menke

14:20 - 14:40 Exploring Hantavirus Replication by Fluorescence In situ Hybridization
Roland Schwarzer

14:40 - 15:30 Coffee break and poster viewing

15:30 - 16:15 **Keynote Lecture 2**
Title: tba
Nicole Tischler, Fundación Ciencia & Vida, Universidad San Sebastián, Santiago, Chile

16:15 - 16:35 Molecular determinants of Hepatitis C virus core protein assembly
Titas Mandal

16:35 - 16:55 Respiratory Viruses interacting with cells: The importance of electrostatics
Andreas Herrmann

16:55 - 17:30 Coffee break and poster viewing

17:30 - 18:15 **Keynote Lecture 3**
Novel antiviral strategies against SARS-CoV-2
Ulfert Rand, Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany

18:15 - 18:35 Isothermal amplification based nucleic acid detection of SARS-CoV-2 via lateral flow assay and a semi-quantitative readout technique
Saloni Agarwal

18:35 - 18:55 Unveiling the Impact of Latent Cytomegalovirus Infection in Healthy Individuals
Liang Zhou

19:00 Dinner at Restaurant Konak, Leipziger Str. 232, 38124 Braunschweig

Friday, June 23rd

08:30 - 09:15 **Keynote Lecture 4**
Structure and function of the hantavirus L protein
Maria Rosenthal, Bernhard-Nocht-Institut für Tropenmedizin, Hamburg, Germany

09:15 - 09:35 In situ imaging of Rift Valley fever virus using electron cryo-tomography
Jan Hellert

09:35 - 09:55 Cholesterol depletion in cell line models of HIV persistence promotes latency reversal
Eva Müller

09:55 - 10:40 **Keynote lecture 5**
Molecular mechanisms regulating the interactions between retroviral HIV-1 Gag precursors and genomic RNA during the retroviral assembly
Serena Bernacchi, CNRS, Institut de Biologie Moléculaire et Cellulaire, Strasbourg Cedex ,France

10:40 - 11:15 Coffee Break

11:15 - 11:35 CD73: A Key Player in HIV Persistence
Hannah S. Schwarzer-Sperber

11:35 - 12:20 **Keynote Lecture 6**
Defective viral genomes – friends or foe?
Benjamin Nilsson-Payant, Twincore, Hannover, Germany

12:20 Closing

Talk Abstracts

- *Laura Menke, HZI Braunschweig*

Molecular Basis for the Host Adaptation of Zoonotic Orthohantaviruses

Human-pathogenic orthohantaviruses are rodent-borne pathogens that do not cause obvious pathogenic effects within their natural hosts. Upon transmission to humans, European orthohantaviruses can cause hemorrhagic fever with renal syndrome of high severity. Despite the disease burden, our knowledge about the infection mechanism and the influence of adaptive amino acid exchanges within the envelope proteins remains incomplete.
Focusing on virus entry, our goal is to investigate orthohantavirus infection in human and rodent-derived cell models. We use a combination of both single-cell microscopy as well as proteomics to identify receptor candidates and to clarify the signaling pathways that are triggered by viral attachment and entry within the different cell lines. In parallel, we have started to comparatively characterize the different host cell lines with respect to their morphology and physiology, most importantly their endocytic pathways, by using selected orthohantavirus strain infections. The overall infectivity of viral strains will be quantified by different microscopy techniques. To increase the validity of our observations, we will complement our imaging results with quantification by flow cytometry. To correlate cell-type dependent effects with viral adaptation, a further focus of our work is to investigate the spatial and temporal evolution of the orthohantavirus glycoprotein complex (GPC), a main determinant of viral tropism. This study will build the basis to further elaborate the functional consequences of adaptative amino acid exchanges with the help of GPC-pseudotyped Vesicular Stomatitis Viruses (pVSV).

- *Roland Schwarzer, Institute for Translational HIV Research, University Hospital Essen*

- *Titas Mandal, University of Potsdam*

Molecular determinants of Hepatitis C virus core protein assembly

Lack of fundamental knowledge of the molecular mechanisms involved in the Hepatitis C Virus (HCV) life cycle within the infected host cells hinders the development of specifically aimed therapeutic approaches. Several studies suggest that the interaction between HCV core protein (C) and lipid interfaces is crucial to produce HCV particles in infected cells, but this process was not characterized in detail yet and the lipids which might be involved in the assembly of the HCV remain unknown. To fill this knowledge gap, we are quantitatively characterizing the interaction between C and host cells lipid structures in an in vitro biophysical model system that closely mimics lipid droplet (LD) and endoplasmic reticulum (ER) lipid environments. Using modern fluorescence-based microscopy methods (including super-resolution microscopy and fluorescence fluctuation analysis) methods, we monitor protein-lipid and protein-protein interactions driving C oligomerization, under well-defined and controlled conditions. We have so far confirmed the intracellular localization of C and its partition between the lipid monolayer and bilayer in hepatic cells depending on the maturation state. This, in turn, has served as a model for our in vitro localization studies. We have studied C binding dynamics on model LDs as a function of lipid headgroup, protein concentration, and time. The investigation is being further supplemented and extended with studies performed directly in living cell models. By combining the information derived from the model and cellular systems, we will be able to clarify the molecular mechanisms driving HCV capsid formation.

- *Andreas Herrmann, Freie Universität Berlin*

Respiratory Viruses interacting with cells: The importance of electrostatics

The COVID-19 pandemic has rekindled interest in the molecular mechanisms involved in the early steps of infection of cells by viruses. Compared to SARS-CoV-1 which only caused a relatively small albeit deadly outbreak, SARS-CoV-2 has led to fulminant spread and a full-scale pandemic characterized by efficient virus transmission worldwide within a very short time. Moreover, the mutations the virus acquired over the many months of virus transmission, particularly those seen in the Omicron variant, have turned out to result in an even more transmissible virus. Here, we focus on the early events of virus infection of cells. We review evidence that the first decisive step in this process is the electrostatic interaction of the spike protein with heparan sulfate chains present on the surface of target cells: Patches of cationic amino acids located on the surface of the spike protein can interact intimately with the negatively charged heparan sulfate chains, which results in the binding of the virion to the cell surface. We show that these events can be expressed as a semi-quantitative model by calculating the surface potential of different spike proteins using the Adaptive Poison-Boltzmann-Solver. The positive surface potential caused by the cationic patches increased markedly from the original Wuhan strain of SARS-CoV-2 to the Omicron variant. The surface potential thus enhanced leads to a much stronger binding of the Omicron variant as compared to the original wild-type virus. Finally, we briefly digress to other viruses and show the usefulness of these electrostatic processes and calculations for cell-virus interactions more generally.

- *Saloni Agarwal, University of Potsdam*

Isothermal amplification based nucleic acid detection of SARS-CoV-2 via lateral flow assay and a semi-quantitative readout technique

Covid-19 pandemic led to much collateral during the last 3 years and on-going. RT-PCR has been the gold-standard for diagnostics of the virus in oro-nasopharyngeal swab samples. Alongside RT-PCR, antigen-detection based self-test kits emerged rapidly for quick tests and surveillance, globally. Alternatively, much effort was focused on developing rapid, efficient, and inexpensive alternative POCT diagnostic techniques in contrast to expensive and time-consuming RT-PCR and not-so sensitive Ag-self-test-based diagnostics. Isothermal amplification techniques like LAMP and RPA proved to be highly efficient for DNA/RNA amplification with similar efficacy to PCR. Paper based LFA platform when applied for reading-out LAMP amplified labelled products, served as an inexpensive and efficient POCT diagnostic setup for result interpretation by naked eye. The LAMP-LFA synergism can be digitally interpreted and eventuated in giving the range of concentration of viral load in the sample tested.
LAMP-LFA setup was established using cDNA and RNA of SARS-CoV-2 N-gene by introducing Biotin-dUTPs and FITC-LF primer during the LAMP amplification. The LAMP amplification of cDNA was optimised for 10 min with 96% accuracy and 15 min for RNA with 82% accuracy. To improve the efficacy of the setup, FITC was incorporated during LAMP amplification tagged to LF primer and biotin was later introduced via a specific DNA probe for the LAMP amplified region of the N-gene. This alternative strategy was tested with the heat-inactivated throat swab samples of SARS-CoV-2 positive and negative patients, and RT-LAMP-LFA was observed to be 93% accurate. The results of LFA were readout via a smartphone based IVD-device, which recorded relative intensities of the test and control bands on the LFA. The data collected showed a linear correlation (R2= 0.8) between the viral load and intensity of the test band.
Overall, we demonstrate a rapid, efficient, inexpensive, POCT platform with smartphone based digital readout biosensor. The demonstrated POCT LAMP-LFA biosensor is a versatile diagnostic platform for nucleic-acid based diagnosis. The synergistic technique could be eventually adapted to a variety of

pathogens like bacterial, fungal, viral. The digitalisation of LFA results could potentially lead to a ‘‘POCT-at home test’’ setup with the accuracy of a lab-based test.

- *Liang Zhou, HZI CIIM Hannover*

Unveiling the Impact of Latent Cytomegalovirus Infection in Healthy Individuals

Latent cytomegalovirus (CMV) infection is a prevalent persistent viral infection that can have long-lasting effects on the immune system. While primary CMV infection is typically asymptomatic and controlled by the host immune response, the virus can remain latent in certain cells for years without causing symptoms. This cohort consisted of 317 healthy individuals of Western European ancestry, including 75 CMV serology positive individuals.

Our goal was to comprehensively investigate the impact of latent CMV infection by employing advanced omics techniques. These techniques included immune cell subset profiling, DNA methylation analysis, inflammatory protein measurements, metabolite detection, and single-cell RNA sequencing(with data obtained from 38 individuals, including 9 CMV-positive individuals).

At the functional level, our findings revealed minimal differences between CMV-positive and CMV-negative individuals in terms of metabolite and protein profiles. However, at the molecular level, we observed profound changes in epigenetic and transcriptional patterns, particularly within the CD8+ T cell subset. These findings were further substantiated through cell count analysis, which demonstrated significant increases in CD8+ T cell numbers during latent CMV infection. Furthermore, CD8+ effector memory cells were identified as potentially playing a crucial role in latent CMV infection.

In summary, this study provides novel insights into the impact of latent CMV infection at multiple levels, including functional, molecular, and cellular aspects. By unraveling these mechanisms, we hope to enhance our understanding of the course and outcome of latent CMV infection, ultimately contributing to improved management and clinical outcomes.

- *Jan Hellert, Leibniz Institut für Virologie, Hamburg*

In situ imaging of Rift Valley fever virus using electron cryo-tomography

I am presenting electron cryo-tomograms of cells infected with Rift Valley fever virus strain MP-12. Among others, these data show the membrane architecture of intracellular viral assembly sites, structural details of budding virions, and the arrangement of nuclear filaments formed by the virus' major virulence factor NSs.

- *Eva Müller, Institute for Translational HIV Research, University Hospital Essen*

Cholesterol depletion in cell line models of HIV persistence promotes latency reversal

HIV persists in latent cells despite effective anti-retroviral therapy (ART). "Shock-and-kill" aims to reactivate latent HIV and eliminate infected cells. However, clinical trials of latency reversal agents (LRAs), successfully tested in cell culture, often failed to sufficiently reverse latency or had severe side effects. Better LRAs and a deeper understanding of HIV latency are needed to achieve remission. It is known that increased cholesterol efflux mediated by ABCA1 impedes the disease progression. Furthermore, statin treatment reduces virologic failure in ART patients suggesting a role of lipid metabolism in viral persistence. Therefore, we strive to investigate how the lipid metabolism

contributes to HIV latency and reversal. We utilized J-Lat cells, a cell line model of HIV latency. J-Lats are HIV-infected T cells, harboring a GFP reporter gene under control of the HIV promoter (A72 cells), or in addition the key HIV transcriptional regulator tat (A2 cells). In these cells, viral transcription is low at baseline but can be reactivated by LRAs (e.g. PMA/I). We depleted cholesterol pharmacologically, while stimulating cells with PMA/I. Furthermore, we knocked-down cholesterol related genes (ABCA1, NPC1, NPC2) using CRISPRi, which were tested and confirmed by qPCR. We demonstrated that manipulation of the lipid metabolism fosters viral reactivation in combination with PMA/I in J-Lat cells, independent of tat. Different drugs, affecting cholesterol metabolism at different stages show similar phenotypes, indicating that the enhanced viral transcription is caused by cholesterol depletion rather than drug off-target effects. In addition, we identified suitable sgRNAs for specific knockdown of ABCA1, NPC1 and NPC2. These sgRNAs are now used to create knock-downs in J-Lat cells. Using our newly established CRISPRi cell line system, we will further decipher the contribution of specific proteins of the lipid and cholesterol metabolism on HIV transcription.

- *Hannah S. Schwarzer-Sperber, Institute for Translational HIV Research, University Hospital Essen*

CD73: A Key Player in HIV Persistence

Poster Abstracts (alphabetical order)

- *Abraham Ayanwale, Medizinische Hochschule Hannover*

The Arenaviridae are a family of negative-sense single-stranded RNA viruses within the larger Bunyavirales order. Although rodents are the natural reservoir species of the Arenaviridae family, some viruses are known to be able to cross the species barrier and infect humans. In humans Arenaviruses can have a wide spectrum of disease manifestations, ranging from (chronic) asymptomatic infections to severe hemorrhagic fever with high case-fatality rates. To date, there are no approved vaccines or antivirals against any Arenavirus with the exception of the live-attenuated Candid#1 vaccine against Junin virus. Due to the severe threat to global health that Arenaviruses pose, it is therefore critical to gain a better understanding of the primary immunological mechanisms that underlie viral disease and that could explain why some viruses do not cause any apparent symptoms whilst others cause highly pathogenic disease.
Here, we compared the host response to the highly pathogenic Lassa virus (LASV) and the mostly apathogenic Tacaribe virus (TCRV) in human lung epithelial A549 cells. We observed a robust type I/III interferon (IFN) response in cells infected with TCRV early in infections, while cells infected with LASV hardly exhibited any differential gene expression. The induction of the IFN response was further validated using a wide range of other apathogenic Arenaviruses. In order to determine the pathogen recognition receptors involved in the detection of Arenaviruses, we infected A549 cells containing knockouts for RIG-I or MDA5 and saw a clear loss of IFN activity in cells lacking RIG-I. Interestingly, however, when cells were transfected with viral RNA extracted from purified TCRV stocks, the genomic RNA of TCRV did not appear to induce a significant induction of IFN signalling compared to known inducers of IFN such as poly(I:C) or RNA extracted from influenza A virus stocks. This suggests that genomic viral RNA might be modified to prevent IFN induction, but that in the context of active infection RIG-I still is able to detect viral PAMP.
Understanding the different processes that occur during Arenaviruses infection and replication, leading to immune detection or immune evasion of different viruses, is essential for our attempts to develop effective and specific novel antiviral strategies combating highly pathogenic diseases caused by Arenaviruses.

- *Daniel Bourquain, Robert Koch-Institute*

In vitro characterization of high- and low-pathogenic genotypes of Dobrava-Belgrade Orthohantavirus reveals differences in replication kinetics and innate immune stimulation

In Europe, the autochthonous Dobrava-Belgrade virus (DOBV) is the most virulent hemorrhagic fever with renal syndrome (HFRS)-causing pathogen with a lethality of up to 12%. The DOBV species has evolved into genetically closely related genotypes which differ remarkably in their virulence to humans. However, the underlying molecular mechanisms of these differences in pathogenicity are still unknown. In vivo, hantaviruses replicate mainly in endothelial cells and the pathogenesis of HFRS is defined by the disruption of the endothelial barrier leading to hemorrhages and kidney damage. Therefore, we characterized the replication and immunomodulation of low-pathogenic DOBV genotype Kurkino (DOBV-Aa) and highly pathogenic genotypes DOBV Sochi (DOBV-Ap) and Dobrava (DOBV-Af) in vitro in primary human pulmonary microvascular endothelial cells (HPMEC) and in podocytes. Interestingly, clear differences separating low- and high-pathogenic genotypes in terms of replication kinetics and immune stimulation could be observed in both cell types. Low-pathogenic DOBV-Aa infection resulted in an early induction of a strong antiviral interferon (IFN) response, which led to termination of viral replication. In contrast, highly pathogenic DOBV-Af and -Ap induced a weaker and delayed IFN-response or exhibited a higher tolerance towards IFN allowing productive

viral replication. Using cell-based assays of IRF- and NFκB-activation, we could show that the potent IFN response towards DOBV-Aa was highly dependent on immune recognition via the cellular RLR-MAVS-signaling pathway. Contrastive to the IFN response, a strong induction of the NFκB-driven proinflammatory immune response was detected in DOBV-Af and Ap-infected cells, which was not induced by DOBV-Aa. The observed differences in the stimulation of IFN- and proinflammatory responses may affect DOBV pathogenesis. The weak antiviral IFN-response may allow a more efficient replication of the highly-pathogenic DOBV genotypes while strong inflammation may promote immune pathogenesis and tissue damage.

- *Lukas Broich, HZI Braunschweig*

A novel approach to study virus-receptor interaction in super-resolution microscopy

Influenza A viruses (IAV) bind cells using the viral protein hemagglutinin recognizing sialylated plasma membrane glycans, IAVs primary attachment factors (AF). Since AFs cannot fulfill a signaling function, the virus needs to activate downstream factors in order to trigger endocytic uptake. The epidermal growth factor receptor (EGFR) was shown to be activated and transmit IAV entry signals (Eierhoff, PLoS Path, 2010) but how IAV engages and activates EGFR remained unclear.
We have previously used quantitative super-resolution microscopy to study the lateral organization of IAV attachment factors as well as its functional receptor at the scale of the virus-cell interface (<100 nm) (Sieben, PLoS Path, 2020). A question that remains is how viruses engage with cellular receptors in live cells and how receptor binding is translated into a local signaling event. However, this interaction is inherently difficult to image due to the transient nature of virus-cell binding before entry. We are thus developing a new assay to image virus-cell interaction for a prolonged period by immobilizing intact viruses on glass coverslips. This new tool allows us to study receptor diffusion using single-molecule imaging as well as the recruitment of cellular adaptor proteins to the bound virus particles in live cells. To provide a more general platform, we further establish fluorescent nanoparticles conjugated with viral spike proteins as virus surrogates.

- *Lea Gabele, HZI Braunschweig*

Mechanistic single-cell investigation of neuroinflammation induced by influenza A virus infection

Lea Gabele[1,2], Shirin Hosseini[1,2], Kristin Michaelsen-Preusse[2], Nele Rieke[3], Christian Sieben[3], Martin Korte[1,2]

[1]Department of Cellular Neurobiology, Zoological Institute, TU-Braunschweig, Germany
[2]Helmholtz Centre for Infection Research, Neuroinflammation and Neurodegeneration Group, Braunschweig, Germany
[3]Helmholtz Centre for Infection Research, Nanoscale Infection Biology Group, Braunschweig, Germany

Influenza A virus (IAV) infection can lead to cerebral manifestations ranging from mild cognitive impairment to encephalopathy, potentially increasing the risk for neurodegenerative diseases. Some IAV strains, such as avian H7N7 subtype, are neurotropic and capable of invading and infecting resident cells in the brain. IAV-induced production of proinflammatory cytokines leads to activation of microglia, the brain-resident immune cells, thereby potentially triggering neuronal damage and dysfunction. Microglial characteristics such as phagocytosis rate, activity and morphology are sex-dependent. Therefore, a better understanding of IAV neurotropism and the mechanisms leading to deleterious neurological manifestations in a sex-specific manner is of great importance. Here, we performed in vitro experiments using primary hippocampal cultures from male and female mice. To analyze the role of microglia and to be closer to the hippocampal physiology, we added microglia of

the respective sex to the cultures. In this project, we aim to characterize the neuropathological mechanisms of IAV infection in detail. Our preliminary results indicate that the phagocytosis rate of microglia increase 6 hours post infection (hpi), especially in male cultures. Moreover, three distinct microglial morphology types with sex-specific densities, including ramified, bushy, and amoeboid forms, were observed in female and male cultures following IAV infection. In cultures prepared from male mice, a lower infection rate compared to cultures derived from females were observable. To further characterize the neurovirulence of H7N7 IAV, we are currently investigating synaptic stripping by analyzing neuronal spine density as well as the frequency of dendritic spine types following H7N7 IAV infection. The results of this study may pave the way for a better understanding of the mechanisms involved in the immediate and long-term neurological consequences of IAV infection in order to develop appropriate prevention strategies.

- *Lea Hamberger, Institute of Virology, University Medical Center Freiburg*

Bat influenza A virus H18N11 infects human monocyte-derived macrophages and alters macrophage function

In recent years, bats received increasing attention as hosts for several emerging zoonotic viruses, including Marburg, Ebola, SARS and MERS coronaviruses. Paradoxically, however, bats have long been neglected as a potential reservoir for influenza A viruses (IAVs). After the discovery of the previously unknown IAV subtype H18N11 in rectal swabs from fruit bats in Peru in 2010, this subtype was subsequently also identified in bats from several other South American countries. Unlike classical IAV strains, H18N11 does not use sialic acid-linked receptors for cellular entry but instead uses major histocompatibility class II (MHCII) molecules found primarily on antigen-presenting cells.
We have previously shown that human MHCII molecules function as a receptor for H18-mediated cell entry. Therefore, we hypothesized that human leukocytes could be susceptible to H18N11 infection.
Here, we show that human monocyte-derived macrophages are highly susceptible to H18N11 and infection results in productive viral replication. In addition, following infection macrophages loose CD11b and CD206 expression, indicating a switch to a more pro-inflammatory macrophage-phenotype. Isolated human lymphocytes were significantly less susceptible to H18N11 infection than monocyte-derived macrophages and infection of B and T cells did not result in the release of progeny virus, demonstrating that infection of human lymphocytes is not productive.
Overall, these results demonstrate that H18N11 has the ability to infect and replicate in human leukocytes, with macrophages being more susceptible to infection than lymphoid cells.

- *Fawad Khan, HZI Braunschweig*

Human Cytomegalovirus (HCMV) is a betaherpesvirus that latently infects the majority of the human population and can cause severe complications in immunocompromised people. While antivirals targeting HCMV are available, drug resistance frequently develops. CD8 T-cells specific against HCMV antigens (pp65, IE1) presented by HLA class-I molecules are well studied and may provide efficient virus control in Immunotherapeutic settings. The ability of TCR Tg CD8 T cells to neutralize HCMV and control its spread, was monitored in a real-time automated fluorescent microscopy. The phosphoprotein pp65 is a late gene encoding for a tegumental protein, but also for a highly Immunodominant epitope. We noticed that pp65-specific CD8 T cells were able to control HCMV infection as early as 6 hpi. The early recognition indicated that pp65 is expressed before the late phase of the virus cycle. Monitoring of pp65 gene expression dynamics by reporter fluorescent genes expressed by its promoter revealed that pp65 was detectable as early as 6 hpi, and that a second and much larger bout of expression occurs by 48 hpi. Phosphonoacetic acid blocked this second bout, but not the first one. Hence, our data suggest that pp65 acts as an early virus gene for immunological purposes.

- *Annett Petrich, University of Potsdam*

Investigating virus-host interactions at a single cell level using quantitative fluorescence microscopy

Annett Petrich & Salvatore Chiantia

- *Nele Rieke, HZI Braunschweig*

The Influenza A Virus tropism and replication kinetics in primary hippocampal cells

Despite their primary target being the respiratory tract of infected mammalian hosts, some IAV strains were able to reach and replicate in the CNS of infected mice in vivo . Both, this neurotropic strain as well as some non-neurotropic strains were able to induce cognitive long- and short-term impairments after infection (Hosseini et al., 2018).

Aims:
I) To unravel the IAV infection and replication kinetics and to better understand IAV neurotropism.
II) To compare and understand the inflammatory profile upon IAV infection in lung and CNS.

Methods:
A primary Co-culture consisting of neurons, astrocytes and microglia derived from mouse E17.5 hippocampi is infected with neurotropic rSC35M (H7N7) IAV for different time points. The infection rate is determined in this complex cell culture system with a self-written Python and ImageJ tool that is based on ImageJ StarDist Plugin.

Results:
- Cell-type dependent IAV infection- and replication kinetics
- Early microglial death upon IAV infection

- *Ruixia Wang, Freie Universität Berlin*

Characterization and functional analysis of a c-type lysozyme gene from obscure puffer Takifugu obscurus

Lysozyme (Lyz) is an alkaline enzyme that can hydrolyze mucopolysaccharides in bacteria and is highly conserved across vertebrates and invertebrates. In this study, a c-type lysozyme gene (named ToLyz) from the obscure puffer Takifugu obscurus was cloned and characterized. Sequence analysis showed that ToLyz had a high similarity with LyzC of Takifugu rubripes. Phylogenetic analysis indicated that ToLyz clustered with Lyzs from other teleost fishes. Quantitative real-time PCR (RT-qPCR) analysis revealed that ToLyz mRNA was mainly expressed in the liver. The transcript level of ToLyz gene was significantly upregulated after Staphylococcus aureus and Vibrio harveyi challenge. The optimal pH and temperature of recombinant ToLyz protein (rToLyz) lytic activity was detected to be 7.5 and 35 ℃, respectively. rToLyz showed obvious antibacterial and bacterial binding activity to a Gram-positive bacterium (S. aureus) and three kinds of Gram-negative bacteria (Aeromonas hydrophila, Edwardsiella tarda, and V. harveyi) at different time points. In order to understand the effect of lyz on bacterial cell wall, the morphological changes of V. harveyi cells treated with rToLyz were observed under the scanning electron microscope, which further confirmed the antibacterial and bacteriolytic activity of rToLyz. Taken together, our current study indicated that ToLyz was involved in the immune response to bacterial infection in obscure puffers.

- *Lisa Welker, Institut de Biologie Moléculaire et Cellulaire (IBMC) & Institut de génétique et de biologie moléculaire et cellulaire (IGBMC)*

Characterization of the molecular mechanisms involved in HIV-1 viral particles assembly

Lisa Welker [1,2], Julien Batisse [1], Charlotte Bussienne [1,2], Jean-Christophe Paillart [2], Roland Marquet [2], Salvatore Chiantia [3], Marc Ruff [1] & Serena Bernacchi [2]

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In cytoplasm, HIV-1 Pr55Gag (Gag) precursor selects the viral genomic RNA (gRNA) by specifically interacting with its 5'UTR region. Then, via the myristoylated matrix domain of Gag, the Gag-gRNA complex is localized to the assembly sites at the plasma membrane (PM). During assembly, 2500 copies of Gag oligomerize onto the entire gRNA, suggesting that the interactions between Gag and the gRNA 5'UTR must become non-specific at this stage. We aim to characterize the molecular factors involved in this change of Gag-gRNA binding properties that promote viral assembly. These factors could be either the anchoring of Gag to the PM, and/or its capability to multimerize at the assembly sites.
We first produced, in mammalian cells, myristoylated Gag proteins (Myr-Gag) wild type (WT) and three mutants at different levels in the capsid multimerization domain. The proteins were then stably associated with supported lipid bilayers (SLBs) mimicking the lipid composition of the PM inner leaflet. Following fluorescent labeling of the molecules, we characterized the in vitro binding parameters (Kd) between a panel of viral RNAs (vRNAs) and SLB-bound Myr-Gag proteins using fluorescence microscopy. In contrast to what we previously demonstrated by in solution experiments simulating the conditions for gRNA cytoplasmic selection, the resulting Kd values revealed that Myr-Gag WT bind with similar affinity all the tested vRNAs. These preliminary results therefore suggest that the interaction with the membrane induces changes in Gag-gRNA binding properties.
Interestingly, these experiments on SLBs, also highlighted that two out the three Myr-Gag multimerization mutants are strongly impaired in their capability to bind the vRNA, revealing the importance of different symmetry contacts for Gag-vRNA interactions at the membrane.
In the next step, we will assess the influence of Gag multimerization on the gRNA cytoplasmic selection step. Overall, this exhaustive in vitro analysis will provide the first quantitative assessment of the influence of Gag-PM anchoring, and its capacity to multimerize, on Gag-gRNA binding properties.

- *Hannah Wullenkord, HZI Braunschweig*

A chemical toolbox to study virus-receptor interactions at the single-molecule level

Virus-host cell interaction is a fast and dynamic process. Early events such as virus-cell binding, signal transduction and endocytosis take place on a sec-mins time scale, but are very hard to study experimentally using apical infection with viruses suspended into the cell culture medium. Consequently, our current knowledge of the first minutes of virus infections is severely limited due to the transient nature and small scale of the process.
We aim at establishing a chemical toolbox to overcome the limitations of these current methods to study virus-cell interactions. To enable high-resolution imaging of both viruses and host cellular proteins, viruses should be immobilized onto planar glass surfaces for a basolateral infection.
Here we present the data on the successful attachment of model particles, including virus-like beads, as well as clinically relevant strains of Influenza A virus onto thin glass slides. Importantly, host lung

epithelial A549 cells displayed fast adherence and robust growth on the viral-coated glass slides, indicating the compatibility of this system with established cell culture techniques
Additionally, we want to establish a model where the surface-bound viruses can be detached during imaging to directly visualize the early events of virus infections. The dynamic control of virus adhesion will be achieved by a variety of stimuli including light or chemical triggers. This technique can be applied for subsequent investigations on viral entry, viral-induced cellular responses, and potential therapeutic targets. The ability to observe and analyze these processes in real time will enable dynamic analysis and open new avenues for innovative research in virology and infectious diseases.

- *Enyu Xie, HZI Braunschweig*

Dissecting the Role of Cellular Actin Regulation in Respiratory Syncytial Virus Infection

The cell cortex is a thin network of actin filaments beneath the plasma membrane that ensures mechanical stability and morphogenesis of the cell. Additionally, it serves as a physical barrier viruses have to overcome during infection. Assembly of cortical actin filaments is driven by F-actin nucleators, such as formins and the Arp2/3 complex. The Arp2/3 complex is a central regulator of cortical actin assembly and plays a crucial role in endocytosis. Despite a wealth of evidence supporting the requirement of dynamic actin reorganization for virus infection and replication, little is known about how viruses control actin remodeling during their replication.

Human respiratory syncytial virus (hRSV) is a severe health risk to people and causes seasonal epidemics worldwide. hRSV is an enveloped single-negative-strand RNA virus, belonging to the Pneumoviridae family of the Mononegavirales order. After binding to cell surface receptors, hRSV activates various signaling pathways to enter host cells via macropinocytosis or direct membrane fusion. At later stages of infection, hRSV might hijack actin networks to export the viral ribonucleoprotein complex from the cytoplasm to assembly sites. Aside from this, the formation of viral filaments and virion egress are supported by actin redistribution. Moreover, it has been shown that hRSV uses a filopodia-driven cell-to-cell spreading mechanism, which relies on modulating actin dynamics in an Arp2/3 complex-dependent manner. However, the molecular factors involved in those processes remain to be discovered.

Here we use CRISPR/Cas9-mediated disruption of the essential Arp2/3 complex subunit Arp2 in A549 lung epithelial cells, to dissect the impact of Arp2/3 complex removal on hRSV infection and replication. We found that Arp2 knockout strongly affects hRSV replication, at both early and late stages of infection. Interestingly, additional phenotypic characterizations of Arp2 loss of function also revealed reduced rates of host cell proliferation. In addition, and quite surprisingly, Arp2 knockout enhanced macropinocytosis in A549 cells. This phenotype was accompanied by altered cortical actin structures, established using structured illumination microscopy and scanning electron microscopy. Future studies will address the potential mechanistic connections between these phenotypes.

How to find us:

From Braunschweig main station:

Bus line 421 or Tram line 1 from central station to stop "Stöckheimer Markt". After getting off at Stöckheimer Markt, either walk to Inhoffenstraße or continue by bus in the direction of Wolfenbüttel Linden, get off at Senefelderstraße, and walk the remaining short distance to Inhoffenstraße. Walking from Stöckheimer Markt, turn left into "Mascheroder Weg" and after 500m turn right into "Inhoffenstraße".