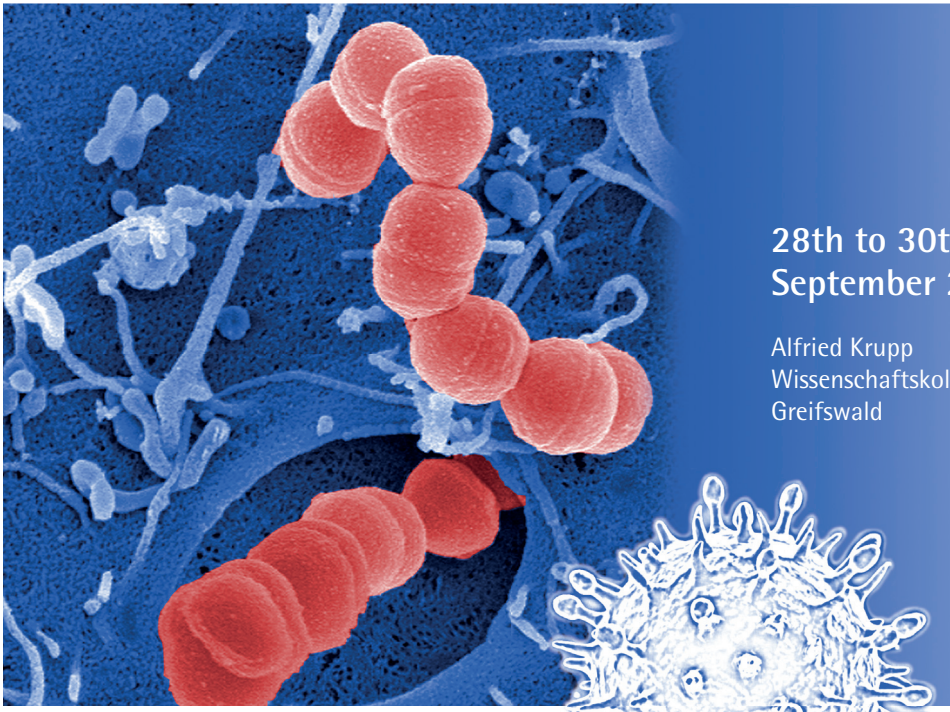

28th to 30th
September 2016

Alfried Krupp
Wissenschaftskolleg
Greifswald

1st SUMMER SCHOOL
"INFECTION BIOLOGY"

Alfried Krupp Wissenschaftskolleg
Greifswald

GRK 1870
Bacterial Respiratory Infections

ERNST MORITZ ARNDT
UNIVERSITÄT GREIFSWALD

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Seit 1456

DFG Deutsche
Forschungsgemeinschaft

FRIEDRICH-LOEFFLER-INSTITUT
FLI
Bundesforschungsinstitut für Tiergesundheit
Federal Research Institute for Animal Health

Welcome note

Dear colleagues,

It is a great pleasure to welcome you to the 1st Summer School on "Infection Biology". This conference is a joint effort of the DFG Research Training Group 1870 "Bacterial Respiratory Infection - Common and Specific Mechanisms of Pathogen Adaptation and Immune Defense", the Friedrich-Loeffler-Institut (FLI), Federal Research Institute for Animal Health, and the Alfried Krupp Wissenschaftskolleg Greifswald.
This truly interdisciplinary event addresses established experts and promising young scientists working in microbiology, virology and infection biology. The topics include state-of-the-art presentations on bacterial and viral interactions with the human host as well as recent advances in the understanding of co-infection mechanisms and pathophysiology. The program of the Summer School combines keynote lectures, oral presentations by experts in the field and young investigators, workshops and posters by the PhD students of the RTG1870 and FLI. Moreover, a panel discussion on Ethics and the Dual-use issue in biological sciences will be held by prominent researchers. Additionally, several informal get-togethers will facilitate the scientific exchange between invited speakers and participants and aim to establish new contacts or collaborations.
We are sure that the historic environment of Greifswald - a former member of the Hanseatic League - combined with the modern aspects of a university town at the Baltic Sea will be the perfect place to create a stimulating atmosphere of exciting talks, inspiring discussions, and scientific curiosity. We are pleased to welcome you in Greifswald and wish you a successful and stimulating conference.

With kind regards,
Yours sincerely,

Prof. Dr. rer. nat.
Sven Hammerschmidt

Chair of the Department Genetics of Microorganisms and Speaker of the DFG-GRK1870

Prof. Dr. rer. nat. Dr. med. vet. h. c.
Thomas C. Mettenleiter
President of FLI, Insel Riems
Chair of the Institute of Molecular Virology and Cell Biology at FLI

Prof. Dr. rer. nat.
Bärbel Friedrich

Academic Director of the Alfried Krupp Wissenschafts-kolleg

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Sven Hammerschmidt (Greifswald)
Thomas C. Mettenleiter (Greifswald – Insel Riems)

18:15 **Keynote Lecture:** Influenza: Virus-Host-Bacterium
Hans-Dieter Klenk (Marburg)

19:00 Dinner

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Venue: **Alfried Krupp Wissenschaftskolleg Greifswald**
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09:00 Culture systems for differentiated airway epithelial cells and their infection by swine influenza viruses
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09:45 Colonization and invasion strategies of Streptococcus suis in the porcine respiratory tract
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09:40 Bat influenza virus chimeras as basis for the development of a new type of vaccine backbone for livestock vaccination
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15:45 Coffee break
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16:45 Detection and characterization of pathogens by high-throughput sequencing
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18:00 Farewell
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Thomas C. Mettenleiter (Greifswald - Insel Riems)
18:30 Barbecue

Venues

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Martin-Luther-Straße 14
17489 Greifswald
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Friedrich-Loeffler-Institut
Suedufer 10
17493 Greifswald – Insel Riems
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Influenza: Virus-Host-Bacterium

Klenk HD

Institute for Virology, Philipps-University Marburg, Marburg, Germany

The high genetic variability of influenza-A-viruses comprising many hemagglutinin and neuraminidase subtypes is the prime determinant of the complex ecology and epidemiology of these agents. Whereas only some subtypes have been observed with man, swine, horse and other mammals, a large spectrum is found in birds. In fact, it is now widely accepted that waterfowl and shore birds are the natural hosts of most influenza-A-viruses and that the viruses observed in other species are derived from these avian strains. Host range as well as pathogenesis and virus transmission are the result of a complex interplay between many host factors and all viral proteins among which, however, the polymerase and the hemagglutinin appear to play particularly prominent roles. Complication of influenza infection by bacterial co-infection is a common phenomenon. Desialylation of epithelial cells by the viral neuraminidase promoting adhesion of the bacteria to exposed cryptic oligosaccharide receptors and damage of virus-infected cells allowing bacterial invasion of the respiratory epithelium are among the mechanisms by which viral infection paves the way for bacterial infection. On the other hand, bacterial proteases have been shown to activate the hemagglutinin and, thus, to enhance virus growth. Finally, evidence is increasing that commensal bacteria stimulate the immune response against the virus indicating that the microbiome of the host may also play a beneficial role in influenza infection.

Contact: Hans-Dieter Klenk
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Culture systems for differentiated airway epithelial cells and their infection by swine influenza viruses

Herrler G,[1] Valentin-Weigand P[2]

[1]Institute for Virology, University of Veterinary Medicine Hannover, Hannover, Germany
[2]Institute of Microbiology, University of Veterinary Medicine Hannover, Hannover, Germany

The airway epithelium is the primary barrier to infection by respiratory pathogens. The barrier function depends on specialized cells such as ciliated cells and mucus-producing cells. These specialized cell types are not available as immortalized cells. To analyze the infection of differentiated airway epithelial cells, primary cell cultures from the respiratory tract of the respective species have to be prepared. We introduce two culture systems that we have applied to investigate the infection by porcine pathogens: precision-cut lung slices (PCLS) and air-liquid interface (ALI) cultures. The specific features of each system will be explained.
In the second part, results of infection studies with influenza viruses will be presented. Here, we show that PCLS can be used to analyze the viral effect on the ciliary activity of the epithelial cells. The ciliostatic effect may even serve as a virulence marker of the respective virus strain. Data on bacterial mono-infection and co-infection by Streptococcus suis and swine influenza viruses will be presented in a second contribution.
Our data demonstrate that cultures of differentiated airway epithelial cells may provide results that cannot be addressed with immortalized cells.

Contact: Georg Herrler
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Colonization and invasion strategies of Streptococcus suis in the porcine respiratory tract

Valentin-Weigand P,[1] Herrler G[2]

[1]Institute of Microbiology, University of Veterinary Medicine Hannover, Hannover, Germany
[2]Institute for Virology, University of Veterinary Medicine Hannover, Hannover, Germany

Streptococcus suis colonizes the epithelium in the airways. While local infection often remains asymptomatic, severe or even fatal diseases occur when streptococci become invasive and spread to different sites in the infected host. The main niche of S. suis is reservoir for a heterogeneous community of bacteria and viruses. However, little is known about interactions of S. suis with its primary host cells and other microorganisms in its environment. We have established porcine respiratory air-liquid interface cultures (ALI) from the porcine lung to analyze interactions of S. suis with their primary target cells at conditions which are close to the in vivo situation. In particular, we focused on the role of the cytolysin suilysin. We found that suilysin contributes to adherence to airway cells, loss of ciliated cells, host cell apoptosis, and invasion. We propose that the high efficiency of S. suis-induced apoptosis and invasion is due do the high local concentration of the toxin under ALI conditions. Recently, we extended these studies by analyses of co-infections of S. suis and swine influenza virus (SIV). We could show in a porcine precision-cut lung slice model that pre-infection of respiratory epithelial cells by SIV promoted adherence and colonisation of S. suis in a two-step process. In the initial stages these effects were dependent on bacterial encapsulation, whereas at a later stage of infection SIV promoted S. suis adherence and invasion by damaging ciliated epithelial cells. Concluding, the ALI- and PCLS co-infection model established here revealed novel insights into the dynamic interactions between S. suis, SIV and primary host cells.

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Subversion of Inflammatory Responses by the Pneumococcus

Weiser JN

Department of Microbiology, New York University School of Medicine, New York, USA

Much of the mortality attributed to influenza virus is due to secondary bacterial pneumonia, particularly from Streptococcus pneumoniae. We find that prior influenza infection enhances the two critical steps in pneumococcal pathogenesis; colonization of the nasopharynx and host-to-host transmission.

Influenza accelerates bacterial replication in vivo, and sialic acid, a major component of airway glycoconjugates, is identified as the host-derived metabolite that stimulates pneumococcal proliferation. Influenza infection increases sialic acid and sialylated mucin availability and enhances desialylation of host glycoconjugates. Pneumococcal genes for sialic acid catabolism are required for influenza to promote bacterial growth. Decreasing sialic acid availability in vivo by genetic deletion of the major airway mucin Muc5ac or mucolytic treatment limits influenza-induced pneumococcal replication.

We adapted an infant mouse model to elucidate host determinants of transmission of S. pneumoniae from inoculated index mice to uninfected contact mice. In the context of co-infection with influenza A virus, the pneumococcus is transmitted among wildtype littermates. Mice deficient for TLR2 are colonized to a similar density but transmit S. pneumoniae more efficiently than wildtype animals and show a decrease in expression of interferon α and higher viral titers. The greater viral burden in tlr2-/- mice correlates with heightened inflammation, and is responsible for an increase in bacterial shedding from the mouse nose. Taken together, these results suggest that the innate immune response to influenza virus promotes bacterial shedding, allowing the bacteria to transit from host to host.

Nakamura, S, KM Davis and JN Weiser. Synergistic stimulation of type I interferons during influenza co-infection promotes Streptococcus pneumoniae colonization in mice. Journal of Clinical Investigation. 121(9):3657-65. 2011

Siegel, SJ, and JN Weiser, Influenza promotes pneumococcal growth during co-infection by providing host sialylated substrates as a nutrient source. Cell Host & Microbe. 16:55-67. 2014

Richard, AL, SJ Siegel, J Erikson, and JN Weiser. TLR2 signaling decreases transmission of Streptococcus pneumoniae by limiting bacterial shedding in an infant mouse influenza A co-infection model. PLoS Pathogens. 10:e1004339. 2014

Contact: Jeffrey N. Weiser
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Insights into influenza virus infections with and without bacteria

Wolff T

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Influenza viruses are one the most significant causes of vaccine-preventable respiratory disease triggering annual epidemics and occasional pandemics. Clinical findings associated with influenza range from asymptomatic infection to severe or even life-threatening conditions such as pneumonia or acute respiratory distress syndrome. Regular influenza can be complicated by co-infection with a wide range of bacteria including S. pneumonia, S. aureus or S. pyogenes, which is associated with more severe symptoms and increases in mortality.
Our laboratory has focused in the past on multiple host cell interactions of influenza viruses that sabotage the innate defense of the host, in particular the type I interferon (IFN) response. This antagonistic activity is mainly executed by the pleotropic viral NS1 protein that suppresses upregulation of type I IFN genes by targeting the intracellular RNA sensor RIG-I, as well as activation of the latent IFN-inducible dsRNA-dependent protein kinase R.
In my presentation I will summarize basic molecular, structural and epidemiological knowledge on influenza viruses that has accumulated since their initial isolation in the early 1930ies. This will be followed by considerations of findings as to the role of type I IFN in influenza pneumonia and biology, as well as to NS1's mode of action in blocking IFN-dependent effector functions.

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Biosafety – Risk assessment on research of viral-bacterial co-infections

Mertsching J

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A risk assessment procedure is the process of evaluating the biological risks arising from a biohazard considering the adequacy of any existing controls, and deciding whether or not the risk is acceptable. The goal of a risk assessment is to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person's exposure to an agent, the likelihood that such exposure will cause a laboratory-acquired infection (LAI), and the probable consequences of such an infection. The information gathered by risk assessment will provide a guide for the selection of appropriate biosafety levels and microbiological practices, safety equipment, and facility safeguards that can prevent LAIs.

After an introduction on risk assessment and containment principles the presentation will discuss risk assessment aspects on research of viral-bacterial co-infections. As an example, in case of human infections with influenza virus it is known that most infections are caused by mildly pathogenic viruses which rarely cause life-threatening disease in healthy people, but some individuals with a weakened immune system can experience severe complications. Increased virulence has been attributed to a variety of factors, including enhanced susceptibility to co-infection with bacteria. Therefore one major focus of current infection research is to understand the mechanisms that facilitate dual infections of virus and bacteria.

The presentation will answer the question whether we have to consider a synergistic risk when conducting viral-bacterial co-infections in an experimental setting.

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Animal Biosafety

Teifke J

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The number of institutions that keep a variety of animal species for research purposes on infectious, especially zoonotic diseases is growing worldwide. The same is true for public awareness and concerns about occupational health, environmental protection, and last but not less important, animal welfare and for the body of regulations on these issues. All these fields are covered by the term "Animal Biosafety". When working with infected animals, especially livestock species, specific care must be taken to prevent the spread of disease. Thus, in-vivo research requires each investigator and all laboratory personnel to take additional safety precautions in the primary containment of large animal rooms to minimize the incidence of laboratory acquired infections. To ensure a safe environment for those who work with infected animals it is crucial that the occupational and environmental health and safety program of an institution is drawn up in close cooperation with the Institutional Animal Care and Use Committee (IACUC) and an Institutional Biorisk Committee (IBC) which coordinates and surveys all issues of biosafety and biosecurity. This places significant responsibility on employers to ensure both the appropriate level of animal care and protection of the employees but also of agriculture involved in animal research.
This presentation provides information on the use of experimentally infected animals housed in the high containment research facilities at the Friedrich-Loeffler-Institut (FLI) with a focus on settings where these animals harbor and propagate zoonotic pathogens or infectious agents of high veterinary significance for their use in translational research.

1. Fontes B. Institutional responsibilities in contamination control in research animals and occupational health and safety for animal handlers. ILAR J. 2008;49(3):326-37.
2. Abad, FX; Solanes, D; Domingo, M. Animal Biosafety Level 3 facility - enhancements when dealing with large animals. In: Working in Biosafety Level 3 and 4 laboratories : a practical introduction / ed. by Manfred Weidmann, Nigel Silman, Patrick Butaye and Mandy Elschner. - Weinheim : Wiley Blackwell, 2014: 31-39.
3. Frasier D, Talka J. Facility design considerations for select agent animal research. ILAR J. 2005;46(1):23-33.

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Dual Use Research of Concern: Biosafety, Biosecurity, Responsibility

Mettenleiter TC

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Dual Use Research of Concern (DURC) is defined as life science research that, based on current understanding, can be reasonably anticipated to provide knowledge, information, products or technology that could be directly misapplied to pose a significant threat with broad potential consequences to public health and safety, agricultural crops and other plants, animals, the environment, materiel, or national security.
In addition DURC implicates a decision between 'freedom of research' vs. 'responsibility of science'.
Scientists have a professional responsibility for their research and its implications. Therefore they have to take into account dual use research issues and concerns. Awareness of the implications of their work and the various ways in which information and products could be misused has to be raised at the institutional level but also in education of young scientists at the university. Risk mitigation measures are key to minimize potential breaches in biosecurity and/or misuse of results from respective research. Research institutions are responsible in regard to the research topics of their staff. Furthermore they have to implement the structural requirements to sensitize their researchers and support them in the ethical assessment of DURC.
A joined committee of DFG and Leopoldina has developed guidelines and assists research institutions by their implementation. Ethical committees in the different institutions will further assist and minimize possible constraints while assuring the highest level of misuse prevention.

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The hidden lipoproteome of Staphylococcus aureus

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Lipoproteins are attached to the outer leaflet of the cell membrane by a diacylglycerol moiety that is covalently linked to a conserved, N-terminal cysteine residue in the lipoprotein sequence. Positioned at the interface between the bacterium and its environment, lipoproteins fulfill many important functions in nutrient uptake, signaling, cell division, surface stress response, redox balance, and host-pathogen interactions. S. aureus devotes about two percent of its total coding capacity to lipoproteins. However, despite their importance, little is known about the majority of S. aureus lipoproteins.
In the present study, we deleted 20 S. aureus genes encoding poorly characterized lipoproteins or lipoproteins of unknown function and screened the respective mutants for phenotypes in order to gain initial evidence for the possible biological function of the targeted lipoproteins.
Growth experiments in different media and under different stress conditions identified lipoprotein mutants sensitive to heat stress, cell wall active antibiotics, and with altered hemolysis activity. Further inspection of selected mutants using light microscopy showed that the two heat sensitive strains had a strong tendency to aggregate in large cell clusters. Closer inspection using electron microscopy revealed a severe defect in cell division as the basis for cell cluster formation.
Experiments are under way to identify the molecular basis for the cell division defect and to identify the link between the lipoprotein and the cell wall division machinery.

Contact: Anica Beyer
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Insights into the role of the transcription termination factor Rho of Staphylococcus aureus

Nagel A,[1] Nicolas P,[2] Debarbouille M,[3] Michalik S,[1] Hertlein T,[4] Depke M,[1] Guerin C,[2] Hiron A,[3] van der Kooi-Pol MM,[5] Palma Medina LM,[1] Murr A,[1] Pané-Farré J,[6] Hecker M,[6] Ohlsen K,[4] Msadek T,[3] van Dijl JM,[5] Völker U,[1] Mäder U[1]

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[4]*Institute for Molecular Infection Biology, University of Würzburg, Germany*
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In a comparative OMICs-study we analyzed S. aureus HG001, a derivative of the strain NCTC 8325, under multiple experimental conditions. Data of the tiling array transcriptome analysis [1] revealed a relatively low abundance of antisense RNAs in the S. aureus wild type, where they overlap only 6% of the coding genes. As known from previous studies, the transcription termination factor Rho plays a major role in suppressing antisense transcription in E. coli [2] and B. subtilis [3], and indeed there is a remarkable overall increase in antisense transcription in the absence of Rho in S. aureus. In contrast to results reported for E. coli, elevated antisense transcription significantly affected sense transcript levels. Proteome analysis of cytoplasmic and secreted fractions showed significant differences in the abundance of several proteins, namely increased amounts of SaeSR-dependent virulence factors like extracellular adherence protein (Eap) and fibronectin-binding proteins (FnbA and FnbB) in the rho mutant. Elevated expression of the regulon controlled by the SaeSR two-component system perfectly confirmed the mRNA data of the tiling array study. Our data suggest that under conditions of Rho deficiency the SaeSR regulatory system of S. aureus is activated by a so far unknown mechanism leading to the observed induction of the SaeSR regulon. Currently, we use different infection models to further explore the impact of the higher levels of SaeSR-dependent virulence factors.

[1] Mäder et al.; PLOS Genet. 2016; 12(4):e1005962.
[2] Peters et al.; Genes Dev. 2012; 26(23):2621-33.
[3] Nicolas et al.; Science 2012; 335(6072):1103-6.

Contact: Anna Nagel
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Bat influenza virus chimeras as basis for the development of a new type of vaccine backbone for livestock vaccination

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Influenza-A-viruses (IAVs) are important pathogens for a broad range of animals like birds, horses, dogs and pigs. Beside seasonal infections in humans leading to annual epidemics, IAV-spillover infections from animals to humans can result in severe infections with a huge economically impact. Moreover, livestock and companion animals are acting as mixing vessels for different IAVs, bearing the risk that reassortants are able to cross the species barrier. The most recent 2009 influenza pandemic in humans is e.g. based on an avian-swine-human quadruple reassortant virus. Effective and safe IAV live vaccines for animals do not exist with very few exceptions for pigs outside of Europe. This is mainly due to the risk of possible reassortment events between vaccine strains and wild type IAV-strains, and partly because of the pathogenicity associated with live vaccine strains especially in very young animals. However, we recently succeeded to generate chimeric viruses containing six out of the eight bat influenza A-like H17N10 virus genes, with the remaining two genes encoding the haemagglutinin and neuraminidase proteins of a prototypic IAV. These viruses grow in mammalian and avian cells, but exhibited limited replication in chicken. Most importantly, these bat chimeras fail to reassort with other IAV. Therefore, bat influenza A-like viruses are to our opinion a very promising vector backbone for the development of a new generation of live IAV vaccines. Determining and characterizing different chimeric combinations with improved properties for live vaccines using in vitro and in vivo settings are our objects of research.

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Proteomic Analysis of Lymphocytes after Infection and Transformation with the oncogenic Gallid Herpes Virus 2

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[2]Institute of Virology - Freie Universität Berlin, Berlin, Germany

Annually, huge economic losses in poultry industry are encountered due to infections with Marek's-disease-virus (MDV), an alpha-herpesvirus. In chickens MDV causes a variety of symptoms, including neurological disorders and immunosuppression. Notably, MDV induces transformation of lymphocytes, leading to malignant T-cell lymphomas in visceral organs with mortalities of up to 100 %. However, the transformation process is not fully understood. Previous in-vitro studies concerning MDV pathogenesis have been mainly conducted on primary fibrocytes or kidney cells, due to the short lifespan of primary lymphocytes in cell culture. Hence, molecular mechanisms of transformation have so far not been analyzed in natural target cells in-vitro. Our aim was to determine changes in host proteomes after infection and transformation with MDV. A recently established cultivation system prolongs the lifespan of primary lymphocytes through addition of cytokines to the growth medium. This allows us to infect T-cells and B-cells in-vitro and to conduct quantitative proteomic analysis. First LC-MALDI TOF MS/MS analysis of laser-dissected tumor revealed typical proteins associated with cancer and infectious diseases. In addition, many viral proteins and changes in host proteome were detected in infected primary B-cells. Now, additional deep statistical analysis is needed to further evaluate changes in host cell proteomes.

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Exploration of Viral Life Cycles by Fluorescent Virus Imaging

Finke S

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Spatiotemporal resolution of dynamic infection processes is required to understand mechanisms of virus replication and pathogenesis. With the development of methods to genetically label virus infected cells or even individual virus particles for fluorescence microscopy, important advances have been made in direct observation of virus infection processes in "live virus imaging" approaches.
For neurotropic rabies virus (RABV), strategies of virus particle labeling and use of such fluorescent virions to investigate dynamic processes in primary neuron cultures will be demonstrated by imaging of receptor binding, co-internalisation of receptors and virus ligand as well as quantitative analysis of subsequent intra-axonal transport processes.
More downstream in the viral life cycle, live RABV imaging also has been used to visualize and quantify dynamic transport processes related to virus assembly and release. These live imaging approaches have allowed us for the first time to directly analyze anterograde transport of newly formed virus in axons and the development of novel, more sophisticated concepts of intra-neuronal spread of RABV beyond the established doctrine, which so far relied on the assumption of exclusive retrograde axonal RABV transport in infected neurons.

Contact: Stefan Finke
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Molecular pathogen-host interaction in human lung tissue

Hocke AC,[1] Zscheppang K,[1] Berg J,[1] Faykhova D,[1] Nerlich A,[1] Mieth M,[1] Opitz B,[1] Becher A,[1] Szymanski K,[1] Rückert, JC,[2] Neudecker J,[2] Tönnies M,[3] Bauer TT,[3] Eggeling S,[5] Schimek M,[5] Wolff T,[4] Suttorp N,[1] Hippenstiel S[1]

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Pneumonia is a leading cause of death worldwide and mortality rates are constant since decades. Streptococcus pneumoniae (S. pneumoniae) is the most common bacterial respiratory pathogen causing community-acquired pneumonia and preceding influenza infections regularly pave the way for even more severe bacterial infections. The pathogens directly induce the host immune system, which involves cellular damage and death. However, the underlying mechanisms differ between pathogens, species, and get complex in viral-bacterial co-infections.

Therefore, we established a human lung tissue model by which viral and bacterial infections alone as well as in combination are analysed by e.g. molecular, biochemical, or microscopic procedures. Next to lab strains, the model serves to investigate clinically isolated pathogens, which allows for comparison of different virulance factors.

We found a unique cellular tropism of different influenza subtypes for type II pneumocytes and showed that these cells also contribute to the inflammatory response against S. pneumoniae. Whereas influenza cause a non-inflammatory apoptosis of these cells during its replication, pneumococci cause a pneumolysin dependent lytic cell death showing signs of apoptosis and necrosis. Moreover, pneumococcal pneumolysin induce IL-1beta in alveolar macrophages leading to protective epithelial GM-CSF release. In contrast, influenza triggers a strong interferon response interfering with pneumococcal activation of the IL-1/GM-CSF-axis, whereas cell death induction remained unaffected.

The use of living human lung tissue excellently serves for the investigation of basic molecular mechanisms determining the development of infectious diseases. Thereby, it reveals differences to mice with regard to the IL-1/GM-CSF-axis and is useful to test pharmacological intervention.

1. Influenza A viruses target type II pneumocytes in the human lung.
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In submission

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Detection and characterization of pathogens by high-throughput sequencing

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The advent of the various high-throughput sequencing technologies has enabled a plethora of investigations. For infectious disease research and diagnostics especially the detection of novel and the in-depth characterization of known pathogens are of importance.
High-throughput sequencing based metagenomics has gained impact in human and veterinary science and pathogen detection. The power of high-throughput sequencing-driven metagenomic approaches to identify infectious agents is defined by the extreme amount of sequencing information that can now be obtained in a single experiment with novel sequencing instruments at relatively low cost. Importantly, the obtained sequence information can be useful for downstream analyses like (RT-)qPCR screening of additional samples for instance for confirmation, quantification, and epidemiological purposes.
High-throughput sequencing also enables the precise determination of complete genome sequences of viruses, bacteria, and parasites. Like the increase in the importance for pathogen detection, the determination of complete genome sequences by HTS is becoming the state-of-the-art for the characterization of pathogens. This not only makes the precise determination of strain relations possible but also the in depth characterization of pathogens for instance with respect to the presence of specific virulence factors, host range determinants, or antibiotic resistance genes. Moreover, the enormous sequencing depth that can be achieved also enables the investigation of viral populations and the monitoring of viral evolution at the full-genome level. In addition, transcriptome analysis without prior knowledge of the genome for the design of DNA microarrays is now feasible, offering insight into pathogen physiology or pathogen-host interaction.

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Proteomics and Immunoproteomics - lessons learned from S. aureus host-pathogen interactions

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Staphylococcus aureus is a pathogen that plays a major role in community-acquired infections. Treatment of these infections becomes more complicated due to the spread of antibiotic resistance. With high sensitive methods like nLC-MS/MS a new level of data quality becomes available for analysis of so far unresolved questions in host-pathogen. To find new meaningful diagnostic targets for immunoproteomics, extended in vitro and in vivo analyses are necessary to characterize S. aureus specific targets in respect to their suitability. In total more than 1800 proteins were quantified during infection and about 200 used for further immunoproteomics studies from different case control cohorts.
The results and the pros and cons of both methods (proteomics and immunoproteomics) will be discussed.

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Platelet activation and aggregation provoked by Staphylococcus aureus secreted proteins

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Staphylococcus aureus is an opportunistic human pathogen provoking a wide range of severe community-acquired and nosocomial infections amongst others infective endocarditis (IE) and disseminated intravascular coagulopathy (DIC). Both clinical manifestations constitute an uncontrolled activation of both platelets and coagulation cascade resulting in thrombocytopenia. S. aureus - platelet interactions occur either directly or indirectly via recruitment of serum components. Bacterial factors inducing platelet activation or aggregation are mostly secreted proteins possessing ECM binding activity.
Fifty-six recombinant secreted or surface-localized staphylococcal proteins were screened for their capacity to activate platelets, measured by the activation markers P-selectin and αIIbβ3 conformation, using whole blood, platelet-rich-plasma (PRP), and washed platelets in buffer from a constant set of donors. Micropattern protein array (MiPA) chips were functionalized with His6-tagged staphylococcal proteins to assess the interactions on a single cell level.
This study confirmed the potential of the SERAM (secretable expanded repertoire adhesive molecules) protein Eap to induce platelet activation and aggregation. In addition, this study further identified the chemotaxis inhibitory protein CHIPS, the formyl peptide receptor-like 1 inhibitory protein FLIPr, all involved in immune evasion, as well as the major autolysin Atl as potent platelet activators. Furthermore, the domains of Atl and the extracellular adherence protein (Eap), responsible for platelet activation could be narrowed down.

Taken together, this study identified two members of the SERAM family (Eap and Atl) and two additionally secreted proteins of S. aureus as platelet activators and aggregators. These results emphasize the importance and diversity of S. aureus-platelet interactions.

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Modelling the spread of African swine fever

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African swine fever (ASF), caused by the ASF virus (ASFV) of the family of Asfarviridae, is one of the most important animal diseases affecting both domestic pigs and wild boar. It has arrived in the eastern countries of the European Union Lithuania and Poland in early 2014 and has been circulating in the Baltic states and Poland since then. Most cases of ASF occur in the wild boar population with only few cases in domestic pig holdings. As there is neither treatment nor preventive vaccination available at the moment, economic consequences for an introduction of ASF in Germany might be severe. Therefore in case of an introduction of ASF in Germany immediate and effective control measures are needed.
To improve understanding of a potential spread of African swine fever in Germany the aim of this project is to use simulation models to simulate said spread – in wild boar as well as in the domestic pig population. These spatio – temporal models are using stochastic methods to reflect the biological diversity of the disease as much as possible.
Using the outcome of those simulations, we want to estimate the epidemiological and economic consequences for Germany in case of an ASF introduction.

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Expression of viral immunogens by pestivirus replicon vectors

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Emerging infectious diseases are occurring at an increased frequency worldwide. Most standard vaccination technologies are limited in their ability to respond rapidly to emerging viruses. The Innovative Medicine Initiative started therefore the "Zoonoses Anticipation and Preparedness Initiative (ZAPI)". The main objective is to develop a universal platform for the rapid identification and expression of viral immunogens / domains by using non-mammalian systems.

Pestivirus replicons, autonomously replicating defective RNA genomes that are not able to generate infectious particles, will be used as vector for the expression of Schmallenberg virus (SBV), Rift Valley fever virus and Middle-East Respiratory Syndrome Coronavirus immunogens, immunogenic domains and peptides.

Here, we describe the construction and characterization of the atypical pestivirus Bungowannah virus (BV) with deletions within the structural proteins, and the nonstructural proteins Npro, p7 and NS2, respectively, creating up to 5 kb of space for insertion of heterologous sequences. For proof of principle, eGFP was inserted into the deleted regions or was expressed from a second cistron which was inserted into the 3' noncoding region of the replicon genomes. Nanoluciferase expressing constructs were generated to compare the replication efficiency of the BV replicons. The most efficient replicons were selected for the expression of an immunogenic domain of the SBV Glycoprotein Gc. Furthermore, we could show, that BV replicons are able to replicate in a broad range of ruminant cell lines and in cell lines of mouse, bat, African green monkey and human origin.

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Staphylococcus aureus lipase 1 – a novel staphylococcal immunomodulatory factor?

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Staphylococcus aureus is a common commensal but can also cause severe infections. This ambiguity is usually explained by the multifaceted interactions between the bacterium and the immune system. S. aureus produces various virulence factors that interfere with different parts of the immune system. Still, the human immune system is usually able to keep the bacterium at bay, and various studies show that a partially protective immunological memory can be established. For instance, B cells produce antibodies against most extracellular staphylococcal proteins. However, there is one protein standing out: The Staphylococcus aureus lipase 1 (Lip), a secreted lipolytic enzyme which is highly abundant and very conserved, elicits only extremely low levels of specific IgG and IgM antibodies in humans. The goal of this project is to unravel the reasons for the observed antibody gap.

First results indicate that Lip has a strong mitogenic effect on T lymphocytes. Stimulation with active Lip induced very strong proliferation of T cells, suggesting polyclonal T cell activation. This effect was abolished when using a nonfunctional S408A mutant. In this case, the proliferation indices as well as Lip-specific precursor frequencies were comparable to those of typical S. aureus recall antigens.

The T cell activating effect therefore appears to be dependent on the enzymatic function, while typical staphylococcal superantigens rely on their structure.

The next steps towards a better understanding of Lip's function will involve a closer look at the prerequisites for proliferation induction as well as the functional characterization of the proliferating T cells.

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Simple, quick and cost-efficient: A universal RT-PCR for amplifying and sequencing the genome of foot-and-mouth disease virus.

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Foot- and mouth disease (FMD) comes along with debilitations and restrictions for livestock industries all over the world. Because of its high contagiosity prevention strategies are of great importance. The foot-and-mouth disease virus (FMDV) is included in the family of Picornaviridae, genus Aphtovirus, possessing a small single-stranded RNA genome of 8.4 kilobases (kb). Generation of whole genome sequences is of increasing importance due to the growth of international nucleotide databases. It provides epidemiological tracing of virus transmission and is supportive in case of virus disease control strategies. This study describes the development and examination of a quick, universal and cost-efficient RT-PCR assay to generate the sequence of the whole polyprotein of the FMDV genome. The novel RT-PCR method was evaluated using twelve different virus strains from cell culture, covering all seven serotypes of FMDV. Additionally, different sample materials were tested to mimic diagnostic or rather field samples. All primer pairs showed a robust amplification with high sensitivity and with no evidence of a better performance for Eurasian serotypes than for SAT serotypes.
In summary, the described assay is suitable for the generation of FMDV sequences covering the whole polyprotein to be used in phylogenetic analysis, quick serotyping and antigenic characterisation.

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Cell adhesion molecules (integrins) modulate Flavivirus infection in mouse cell lines

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To infect the host cells, viruses must interact with a broad range of molecules which includes their cellular receptors and molecules that support virus replication. Flaviviruses like Dengue, West Nile, Usutu, Yellow fever and Zika virus have caused recent outbreaks around the world. Up to now, few molecules were characterized as Flavivirus receptor(s) or able to modulate flavivirus infection. Integrins are a family of transmembrane proteins expressed in many cell types with important functions like cell migration, attachment, mitoses and apoptosis. Previous studies postulated that West Nile virus uses integrin to enter into the cells and further studies from our group showed that integrins modulate West Nile virus infection. In order to elucidate if integrins are involved in Flavivirus infection we infected integrin deficient mouse fibroblasts and CHO cells, a flavivirus resistant cell line, with different Flaviviruses. In these sets of experiments we evaluated: (i) virus binding; (ii) replication kinetics; (iii) internalization and (iv) replication. Our results show that integrins are not the main flavivirus receptor and its expression in CHO cells did not confer Flavivirus permissiveness. Also, integrins are not involved in Flavivirus binding neither internalization into the cell. Replication was slightly impaired in cells deficient for β1 and β3 integrin subunits and strongly impaired in αVβ3 integrin deficient cells. In summary, integrins are not involved in the first steps of Flavivirus infection (binding and internalization) but somehow integrins modulate Flavivirus replication. The exact mechanism how integrins modulate flavivirus replication is unknown and subject of investigation.

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Comparative pathogenicity study of European bat lyssavirus 1 isolates in the mouse model

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European bat lyssavirus 1 (EBLV-1) is the most common of the four lyssavirus species present in the bat population in Europe and known to have caused rabies in a variety of mammals including humans. The genome of EBLV-1 isolates displays a certain heterogeneity, visible in their division into two genetic distinct sublineages as well as in insertions and deletions which were found in recent years. The impact of these genetic differences on the pathogenicity of EBLV-1 has never been assessed even though it was observed for other lysaviruses, i.e. Lagos bat virus. Therefore we compared the pathogenicity of eight EBLV-1 isolates, representing both sublineages as well as known insertions and deletions, in the mouse model using different routes of inoculation. Besides survival, we also investigated the amount of viral antigen present in different brain areas of mice which succumbed to rabies. Results show that differences in the pathogenicity of the isolates exist when inoculated intranasally or intramuscularly with a low dose. Furthermore variations in the incubation periods could be observed following intramuscular inoculation. Clinical symptoms as well as the viral loads in different parts of the brain depend on the inoculation route. We therefore conclude that results obtained with one EBLV-1 isolate cannot be generalized for the whole species and studies using only single isolates as representatives need careful interpretation.

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Establishment of serological assays for the detection of henipavirus specific antibodies in pigs

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Hendra and Nipah virus (HeV; NiV) are enveloped, single-stranded negative-sense RNA viruses in the Henipavirus genus, Paramyxoviridae family. Henipavirus infections were first reported in the 1990's causing severe and often fatal outbreaks in domestic animals and humans in the Australasian region. Recently, there has been first serological evidence for the presence of henipa-like viruses in African fruit bats, pigs and humans.

Truncated forms of henipavirus attachment (G) proteins were expressed in a novel expression system based on the stable transfection of the eukaryotic parasite Leishmania tarentolae. Both G proteins were applied in Enzyme-linked Immunosorbent Assay (ELISA) for the detection of henipavirus specific antibodies in serum samples of pigs experimentally infected with HeV or NiV.

HeV and NiV G proteins were efficiently purified via Strep-tag affinity chromatography with total protein yields of about 1 mg per litre of cell culture. For NiV challenged pigs, seroconversion was detectable 14 days after infection in the NiV G based ELISA whereas the HeV G ELISA only detected cross-reactive henipavirus specific antibodies at day 20 post infection. Sera of HeV infected pigs collected 28 days after infection cross-reacted with the NiV G but, as expected, displayed higher reactivity in the HeV G based assay.

Finally, the henipavirus G proteins expressed in our study were successfully used to test henipavirus challenged pig sera in ELISA. Serological testing of field sera from African pigs against both Leishmania-derived henipavirus G proteins will present a valuable approach to indirectly determine the presence of henipa-like virus specific antibodies.

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Survey for the zoonotic bacteria Leptospira and Rickettsia in wild small mammal populations in Germany

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Amongst wildlife species, rodents are considered to be an important reservoir for various zoonotic bacteria. Here, we describe a Leptospira and Rickettsia survey of 4,019 investigated rodents and shrews in Germany, performed between 2010 and 2014 in rural settings in Mecklenburg-Western Pomerania, North Rhine-Westphalia, Thuringia and Baden-Wuerttemberg.
Kidney tissue samples were analyzed by a PCR targeting the lipl32 gene. Using this initial screening PCR, 524 of 3,950 (13.3%) small mammals were tested positive for Leptospira. Subsequently, a partial secY gene-specific PCR and sequencing were used to identify the Leptospira species. The presences of Leptospira kirschneri, Leptospira interrogans or Leptospira borgpetersenii were determined. MLST-based typing resulted in the identification of six sequence types. Spotted Fever Group Rickettsia-specific DNA was detected in 314 of 3,939 (8.0%) ear pinna samples, using a real-time PCR targeting the citrate synthase (gltA) gene. Typing of the rickettsial positive species was performed by a conventional PCR, targeting the partial outer membrane protein B (ompB) gene. The ompB-based typing resulted in the identification of three Rickettsia species, Rickettsia helvetica, Rickettsia felis and Rickettsia raoultii. In conclusion, leptospiral and rickettsial DNA could be found in each rodent and small mammal species with a broad geographical distribution, but without distinct host specificity. Co-infections of Leptospira and Rickettsia were found at low frequency of 0.8%. These findings underline the need for further investigations to assess the public health relevance of the different potential animal reservoirs and pathogen lineages.

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Spatial clustering of rabies virus genomes using affinity propagation clustering

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Rabies is one of the oldest known zoonosis, caused by rabies virus an important species of the genus Lyssavirus. So far, the spread of rabies virus is analyzed on regional levels, since a global phylogenetic clustering and classification system is not yet available. A phylogenetic tree calculated by Maximum Likelihood (ML) method of rabies full genome sequences suggests a space-dependent clustering. Our analyses revealed two limitations of this approach: (i) The analysis of large datasets results in highly complex dendrograms. (ii) The clustering of phylogenetic trees by visual inspection could lead to different results due to a lack of criteria for cluster definition. To overcome these limitations, we analyzed about 400 full genome RABV samples from nearly 50 different countries an interpreted the results with a new clustering approach, affinity propagation clustering.
Affinity propagation clustering is a mathematical method that uses the phylogenetic distance matrix to allocate sequences to generic clusters. In addition, the optimal number of clusters is calculated. We applied this method to the distance matrices derived from the full genome sequences, to validate evidence for spatially-dependent clustering of rabies virus sequences. The resulting cluster structure strongly corresponds to the dendrogram structure of the ML tree and indicating an objective classification model for rabies viruses.

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Metabolic and regulatory adaption of Staphylococcus aureus to different carbon sources

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Different human body sites are characterized by specific carbon sources available to S. aureus. For example, during invasive disease glucose is the major carbon source in the blood stream, whereas pyruvate may correlate with a colonizing lifestyle since it is available in significant amounts in nasal secretion. Lactate for instance is found in lungs of cystic fibrosis patients were it is secreted by Pseudomonas aeruginosa during co-colonization with S. aureus. In addition, lactate may also function as a signal of the hosts' state of immune defense since lactate is produced in significant amounts by proliferating T-cells at the site of infection. Finally, for many intracellular pathogens glycerol has been demonstrated as a major carbon source during host cell invasion and thus may have an equally important role for S. aureus during intracellular survival which is an important step in the development of chronic S. aureus infections.
For a better understanding of S. aureus adaptation processes to different host-environment relevant carbon sources we are using a combined metabolomic and proteomic approach. S. aureus was grown in a synthetic medium with glucose, pyruvate, lactate or glycerol as sole carbon source. To also investigate the impact of carbon catabolite repression in this process, we included a catabolite control protein A mutant in our analyses and exposed S. aureus to combinations of glucose with either pyruvate, lactate or glycerol. Quantitative data will be presented describing the protein repertoire and extracellular metabolic profile of S. aureus when grown with different carbon sources.

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EVAg – European Virus Archive goes global: a unique biological resource in the field of virology

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During the past century and especially the last decade with upcoming new technologies, a large number of pathogenic viruses with emerging potential have been discovered and isolated. These viruses are routinely used for basic research, epidemiological studies, disease control, development of diagnostics as well as for therapeutic purposes like the generation of novel vaccines and antivirals. Therefore, besides appropriate skills and capacities, the availability of suitable high quality reference material is crucial. In 2009, a FP7 project called "European Virus Archive" (EVA) was conceived whereby the overall objective was to constitute a European network of high calibre centres with the appropriate expertise to identify, collect, amplify, characterise, standardise, authenticate, and distribute viruses. EVA has been operating satisfactorily and about 2000 products have been distributed worldwide. In order to continue and expand its activities the H2020 project "European Virus Archive goes Global" (EVAg; http://www.european-virus-archive.com/) has been founded by the European Commission, and consist of an international group of 25 laboratories. The ultimate goal of this non-profit organisation is to become the largest virus collection worldwide and provide eligible access to valuable high quality guaranteed viruses of all risk groups as well as virus-derived materials like viral genomes and proteins, suitable cell lines, antigen-specific sera or monoclonal antibodies, and to unique services including access to high biosafety level laboratories and animal facilities. Accordingly, EVAg is mainly involved in the development and distribution of diagnostic reference material e.g. during the emergence of viruses such as MERS-CoV or Zika virus.

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Pneumococcal fitness and virulence gene regulation by the TCS08

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Background: Streptococcus pneumoniae (pneumococci) is equipped with virulence factors that are coordinated by various regulatory systems. Among these systems, the Two-Component regulatory Systems (TCS) play a major role in pneumococcal fitness and virulence. Hence, the impact of TCS08 and its effect on the regulation of virulence and metabolism in S. pneumoniae was evaluated in this study. Methods: To assess the impact of the TCS08 in pneumococci, isogenic mutants (Δrr08, Δhk08 and Δtcs08) were generated. MBP-tagged recombinant HK08 and RR08 were purified by affinity chromatography. Functional assays (phostag-acrylamide, electromobility shift assay (EMSA), Transcriptomics) and a pneumonia model were performed. Results: A substantially increased expression of the PavB protein was detected only for the hk08-mutant. The phosphotransfer assays with TCS08 proteins suggests autophosphorylation of RR08 and a phosphatase activity of HK08. The EMSA with purified RR08 and a pavB promoter DNA-fragment illustrated binding of non- and phosphorylated RR08. The microarray analysis showed regulation of different genes involved in virulence and metabolism. Finally, in vivo infections in the acute pneumonia mouse model displayed a lower virulence capacity for the Δhk08 and Δtcs08 mutants but not Δrr08. Conclusion: These data show that TCS08 regulates genes involved in pneumococcal pathogenicity and metabolism. Additionally this study suggests that non-phosphorylated RR08 functions as a repressor while the phosphorylated RR08 acts as an activator for the expression of PavB. Finally, Δhk08 and Δtcs08 mutants had a lower virulence and were unable to progress post-intranasal infection from the lungs to the blood in mice.

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Entry and reassortment potential of the newly discovered bat influenza A-like viruses

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Influenza A viruses (IAV) are important zoonotic pathogens that cause epidemic outbreaks in poultry, wild birds, swine and other mammals. In 2012 and 2013 two bat influenza A-like virus genomes were found in little yellow-shouldered fruit bats (Sturnira lilium) in Guatemala and flat-faced fruit bats (Artibeus planirostris) in Peru, provisionally designated as H17N10 and H18N11. Infectious virus particles, however, were never isolated and attempts to generate infectious viruses by reverse genetic approaches failed so far. Furthermore conventional IAV hemagglutinins (HAs) bind canonical sialic acid-containing receptors. In contrast, biochemical and structural studies indicated that influenza A-like H17 does not. In fact, H17 and H18 HAs are unable to bind and hemagglutinate red blood cells, and are therefore atypical HAs. Besides, the neuraminidase (NA) works as a counterpart to HA in the life cycle of conventional IAV. It is an enzyme that cleaves sialic acids from their sugar backbone to facilitate the release of newly synthesized viral particles from infected cells. Bat influenza A-like NAs however lack any detectable neuraminidase activity. These two differences lead to the suggestion, that bat influenza A-like viruses use other entry mechanisms, compared to conventional IAV. With a first series of experiments we aim to identify the functional entry receptor. We also hypothesize that NA-deleted classical IAVs are perfect surrogate viruses to study either bat NA or bat HA or both bat proteins. To achieve this, bat HA and the NA will be integrated in NA-deleted H5 and H7 viruses by a reverse genetics approach.

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A natural pair of low and high pathogenicity avian influenza virus H7N7 in Germany

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Avian influenza viruses threaten poultry production worldwide. Highly pathogenic avian influenza viruses (HPAIV) cause devastating losses in gallinaceous poultry and, due to zoonotic propensities, also pose a threat to public health. The last outbreak of HPAIV H7N7 in Germany (one holding in Emsland) dates back to summer 2015. This virus likely arose by spontaneous mutation from a low pathogenic (LP) H7N7 precursor virus detected in a neighbouring chicken holding. It remains to be elucidated how, when and why such mutations occur. The availability of this native LP/HP pair of viruses enables further work into the mechanisms of HPAIV generation in a project that started in April 2016:

- The presence of HP mutations in LPAIV H5 and H7 quasispecies from field samples will be examined by RT-qPCR and NGS.
- Competing co-infections of LP and HP AIV H7N7/Emsland will be carried out in chickens and ducks to determine the minimal percentage of HP viruses in a mixture required to induce disease.
- Generation of HP AIV H7N7/Emsland from its LP precursor by serial passages of LP virus in eggs and cell cultures of different avian species and in vivo will be attempted in order to gain insights into mutation mechanisms.

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Arenavirus Life Cycle Modelling Systems: Development and Application

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Arenaviruses are responsible for a number of viral hemorrhagic fever (VHF) diseases endemic to Africa and South America. These viruses are transmitted from rodents to humans via aerosols, particularly those contaminated with rodent excreta, and in some cases possibly also ingestion of contaminated food. VHF-causing arenaviruses are classified as biosafety level (BSL) 4 agents, which poses significant challenges for both research and the development of antivirals. Our goal was to develop a variety of systems that can be safely used under BSL1/2 conditions for both basic and applied research applications.
To this end minigenomes (genome analogues that replace the viral proteins with reporter proteins such as GFP or Luciferase) were constructed for Junín virus. These constructs were then expressed together with the viral polymerase complex proteins (NP and L) in mammalian cells to facilitate viral transcription/replication, resulting in reporter protein synthesis, which serves as a read-out for these processes. Further, to expand this system, the viral proteins responsible for budding and entry (GP and Z) were co-expressed along with the minigenome components, leading to the generation of transcription and replication-competent virus-like particles (trVLPs), which can then be used to infect target cells.
Using these assays we have conducted analyses looking at aspects of basic virus biology (e.g. transcription/replication efficiency as a pathogenic determinant), performed applied screens for potential antiviral compounds and host factors important for the viral lifecycle, and most recently adapted these assays for the detection of neutralizing antibodies.

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Interaction of Matrix Proteins with Nuclear ANP32B Is Conserved Among Henipa- and Avulaviruses

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Recently, the nuclear cell factor acidic leucine-rich nuclear phosphoprotein 32 family member B (ANP32B) has been identified as a nuclear interactor of Hendra and Nipah virus (HeV, NiV; genus Henipavirus) matrix proteins. Although the detailed mechanism still has to be resolved, the specificity of this interaction and re-localisation of the matrix proteins (M) strongly suggested that ANP32B plays an important role in host manipulation by henipaviruses.
In order to see, whether interaction with ANP32B represents a conserved feature among paramxyxoviruses, we here investigated whether M proteins of Newcastle Disease Virus (NDV, genus Avulavirus, subfamiliy Paramyxovirinae),Bovine Respiratory Syncytial Virus (BRSV, genus Pneumovirus, subfamiliy Pneumovirinae) and Measles virus (MV, genus Morbillivirus, subfamily Paramyxovirinae) also interact with ANP32B. Notably, ANP32B was successfully co-purified with NDV M and, similar to henipavirus M proteins, NDV M accumulated in the nucleus in an ANP32B dependent manner. In contrast, no co-purification of BRSV M and MV M with ANP32B was observed, indicating that both does not interact with ANP32B. Our data strongly support the hypothesis, that ANP32B is part of a conserved host cell manipulatory mechanism of Henipa- and Avulaviruses. Ongoing mutational analysis are performed to identify conserved binding motifs and to further unravel the mechanism behind.

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Responses of bovine lung epithelial cells to Mycoplasma mycoides strains, which have different target hosts

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Contagious bovine pleuropneumonia (CBPP) caused by Mycoplasma mycoides subsp. mycoides (Mmm) is an important cattle disease in Africa. The present control measures rely on attenuated live vaccines with limited efficiency and sporadic severe side effects at the site of inoculation. The knowledge of host pathogen interactions and the protective immune mechanisms will foster the development of an improved vaccine against CBPP.
We investigated the very first host response by analyzing the transcriptome of embryonic bovine lung cells (EBL), which were co-cultivated with the highly virulent African strain Afadé (Mmm) and that of EBL cells co- cultivated with the caprine strain Mycoplasma mycoides subsp. capri (Mmc), that is non-pathogenic for cattle but the closest relative of Mmm. Co-cultured with Mmm EBL showed a reduction of cell viability. Particularly noteworthy is the upregulation of transcripts encoding interferon induced proteins, like ISG15, IFIT1 and MX1. Even Mmc led to a remarkable upregulation of genes, confirming a strong impact of this pathogen to bovine cells. However this response did not affect the viability of the eukaryotic cells. Surprisingly, the caprine mycoplasma adhered better to bovine cells than the bovine pathogen. Nevertheless, in contrast to EBL in co-cultivation with the caprine strain, EBL co-cultivated with Mmm were not able to create a protective response against the pathogen leading to cell death. Although there is a common response at both strains, the inappropriate response to Mmm led to a deleterious reaction that was not observed with Mmc, characterizing the pathogenic mechanisms of Mmm.

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Identification of a pneumococcal enzyme essential for anchoring of lipoteichoic acid to the bacterial cell surface

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Typical structures of Gram-positive cell walls are wall teichoic acids (WTA) and lipoteichoic acids (LTA) which are highly diverse and often species- and strain-specific. The role of WTA in cell morphology and division, regulation of autolysis and adhesion to host cells is e.g. well characterized for Staphylococcus aureus and Bacillus subtilis, while the role of LTA remains elusive. Teichoic acids (TA) of Streptococcus pneumoniae are unique in several aspects. First, pneumococcal WTA (pnWTA) and LTA (pnLTA) are identical in their complex repeating unit structures, suggesting a common biosynthesis pathway. Second, pnTA are highly decorated with phosphorylcholine (P-Cho), which bind non-covalently choline-binding proteins (CBPs) to the surface and are essential for bacterial growth. Based on the in silico analysis of the S. pneumoniae R6 genome, putative enzymes involved in the biosynthetic pathway of teichoic acids have been proposed. Inactivation of spd_1672, a gene in strain D39 encoding an enzyme with homology to O-antigen ligases from different Gram-negative bacteria, demonstrated the involvement of this enzyme in pnLTA anchoring to the surface of pneumococci. Chemical analysis of the cell wall of mutant D39ΔcpsΔspd1672 indicated a total loss of pnLTA compared to the isogenic wild-type. The phenotypic characterization of the mutant revealed an impact on growth and an increased autolytic activity. Alterations in cell morphology of pneumococcal wild-type and mutants were illustrated by SEM. Furthermore, quantification of selected TA associated CBPs and capsule was investigated by flow cytometry. In summary, we present here the identification of an enzyme involved in anchoring of pnLTA.

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Network “Rat-borne pathogens”: Searching for pathogen co-infections

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The Norway rat Rattus norvegicus is an important reservoir of zoonotic pathogens, such as orthopox virus (OPV) and Leptospira, but also for agents of no or unknown zoonotic potential. In addition, in Norway rats human pathogens have been detected, but rats are most likely not involved in their transmission, but may serve as a sentinel.
In a recent survey, Norway rats originating from five European countries were investigated for Leptospira spp., Rickettsia spp., OPV, and rat polyomavirus (ratPyV). Leptospira DNA was detected in 60 of 420 rats and Rickettsia DNA was found in three of 369 rats. PCR-based typing resulted in the identification of L. interrogans and Rickettsia helvetica, respectively. RatPyV DNA was detected in 103 of 421 rats. OPV DNA was detected in none of the rats, but OPV-specific antibodies in three of 388 rats. The frequency of single Leptospira and ratPyV infections and co-infections was, independent of sex, greater for adults compared to juveniles/subadults and greater at rural sites compared to urban areas.
Study results indicate a broad geographical distribution of Leptospira DNA in rats within Europe underlining the need to further investigate potential mechanisms leading to increased prevalence in rural habitats. In contrast, rickettsia and OPV infections rarely occurred in wild rat populations. The potential influence of ratPyV on the susceptibility to infections with other pathogens should be investigated in future studies. Recently, pilot studies were initiated within the network for Staphylococcus aureus, Streptobacillus moniliformis and further pathogens.

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Investigations of the phosphoproteome of Streptococcus pneumoniae

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Streptococcus pneumoniae is a human pathogen that can cause several severe and invasive infections. Due to the worldwide rise of resistance to antibiotics in pneumococci it is of great relevance to analyze not only the pneumococcal genome but also the proteome. Data about protein abundance in S. pneumoniae may provide an extensive source of information for the selection of new targets for vaccine and antibiotic development.
Protein phosphorylation is a reversible post-translational modification enabling the translation of extracellular signals into cellular responses and adaptation to a steadily changing environment. Therefore, it is of particular interest to capture precise qualitative and quantitative information about the phosphoproteome of S. pneumoniae.
S. pneumoniae has only one gene encoding a PP2C-type phosphatase, PhpP, located upstream from the stkP gene, which encodes the only membrane-associated serine-threonine kinase, StkP. PhpP and StkP are in the focus of our study. They appear to constitute a functional signaling couple in vivo and likely belong to the same complex.
For the identification and quantification of relative differential changes in the phosphoproteome of the unencapsulated wildtype (WT) and the deletion mutants ΔstkP and ΔphpP we use the metabolic labeling strategy Stable isotope labeling using amino acids in cell culture (SILAC) followed by the enrichment of phosphorylated peptides with TiO2 and mass spectrometric analysis.
First results show, that the cell devision proteins FtsZ and DivIVA are phosphorylated in the WT. Furthermore we found phosphorylation sites in some ABC-transporters and cation transporters that might play a role in various biological processes.

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Development and optimization of a GFP-expressing tetracistronic trVLP system for ebolaviruses

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Ebolaviruses are zoonotic pathogens causing severe hemorrhagic fevers with high case fatality rates. While tremendous progress developing ebolavirus countermeasures has recently been made, there are still no approved therapeutics available. This is at least partially due to safety constraints, limiting work with infectious ebolaviruses to biosafety level (BSL) 4 laboratories, which constitutes a significant limitation for both research and drug development. However, life-cycle-modelling systems, which model aspects of the ebolavirus life cycle at lower BSLs, mitigate this problem. The newest of these systems, i.e. the tetracistronic transcription and replication-competent virus-like particle (trVLP) system, which we recently developed, safely models virtually every aspect of the ebolavirus life cycle (except primary transcription) over multiple infectious cycles under BSL1 conditions by using infectious reporter-expressing trVLPs as surrogate for ebolaviruses. We have now created a new variant of this system, which uses green fluorescent protein instead of luciferase as reporter, resulting in several advantages: 1) Information regarding the absolute number of infected cells is acquired, whereas luciferase only provides a measure for the relative infection within an experimental sample, 2) the system is compatible with the high-content-imaging approaches most often used for antiviral screening, and 3) measurement is possible in living cells (facilitating multiple measurements of infection over time) and doesn't require costly reagents. We report details of the development of this system as well as its optimization, resulting in a significant improvement in the rate of infection, which is particularly relevant for high-content-imaging, but also advantageous for other experimental approaches.

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Investigations to Streptococcus pneumoniae under iron limitation

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Streptococcus pneumoniae is one of the major human pathogens, which can cause several severe diseases ranging from mild forms like sinusitis to life-threatening infections like septicemia. They can lead to high mortality and morbidity rates, especially among children under the age of five, elderly and immunosuppressed patients. Therefore it is important to understand how the pneumococcal virulence functions, not only on genome but also on proteome level.
The acquisition of iron is essential for pathogenic bacteria. Iron is involved in several key metabolic pathways both as a cofactor and redox-active catalyst. Because of the high insolubility of iron under physiological conditions, the iron concentration in its human host is restricted. Hence, the aim of this study is to analyze the changes of the proteome of S. pneumoniae under iron limitation compared to control conditions.
In order to quantify changes in protein abundances we used SILAC as a metabolic labeling technique. Furthermore, we used 2,2'-bipyridine to induce iron limitation. After cultivation, the sample were fractionated in cellular and extracellular proteins and pooled with a heavy labeled internal SILAC standard prepared under the conditions examined. Followed by LC-MS/MS analysis, the obtained data were subjected to a classical database search and statistically analyzed. In this study more than 800 of 1914 predicted pneumococcal proteins were identified. 500 proteins could be quantified, including about 130 significantly regulated proteins. A part of those proteins belong to pathogenesis- and iron-associated proteins, like the pneumococcal surface protein C, PspC, and the iron-compound ABC transporters SPD_0915 and SPD_1652.

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The Newcastle disease virus W protein – an mRNA editing product of the phosphoprotein gene

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Like all members of the Paramyxovirinae Newcastle disease virus (NDV) undergoes a co-transcriptional mRNA editing to enlarge its coding capacity. The P gene codes for a 395 amino acids (aa) phosphoprotein. By co-transcriptional insertion of one or two non-templated G nucleotides at the RNA editing site two further mRNAs are transcribed, coding for V and W proteins. The 293 aa V protein is a known interferon antagonist. However, the deduced W protein of 176 aa has so far not been demonstrated.
Here, the existence of W protein was investigated for NDV Clone 30, a lentogenic vaccine virus strain. Mass spectrometry investigations of purified virions gave the first indication for the presence of the W protein. For further confirmation, W specific synthetic peptides were selected for immunization of rabbits to generate W protein specific serum. The obtained serum was then used for indirect immunofluorescence, Western blot analyses and localization studies by electron and confocal microscopy, confirming the existence of the W protein in cell lysates as well as in purified virions.
In summary, we show for the first time the existence of the NDV W protein.

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Detection of novel viral pathogens by adaptive diagnostics to prevent epidemics

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The basis for research in infection biology is the detection and characterization of the responsible pathogen, e.g. viruses. Since there are no diagnostic tools for novel viruses they often remain undetected. Infectious diseases which cause no or only mild symptoms in their host, but lead to severe symptoms after a change of species, are particularly dangerous. Next-generation sequencing (NGS) combined with a metagenomic analysis provides an opportunity to detect and characterize new viruses. However, it provides no information about the serological host response and the development of serological assays is often complex and time-consuming. Since there is a gap between the detection of new viruses and necessary serological diagnostics, the BMBF-funded interdisciplinary project DetektiVir aims to develop a generic workflow that is applicable to all kinds of novel virus diseases. The workflow starts with a sample containing an unidentified virus, in which via NGS and metagenomic analysis viral sequences can be identified and classified. These identified viral sequences are the basis for the subsequent identification of suitable antigenic regions - by using peptide array analysis - finally resulting in a functional serological assay. Furthermore, the proposed workflow lays the foundation for future research on a detected novel virus in the field of infection biology. The first part of the workflow is now already optimized and will be presented with a few examples highlighting the strengths and weaknesses of the concept.

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Detecting KHV variants for vaccine development

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Koi herpesvirus infection (KHV-I) is a notifiable disease according to OIE (World Organisation for Animal Health) and EU. It causes high mortalities in aquaculture of Cyprinus carpio L. worldwide. While there are genetic variants among the causative agent KHV, it remains unknown how these different variants of KHV have evolved and how they spread among carp populations. Therefore, a phylogenetic method based on variable number of tandem repeats (VNTR) has been adapted to get a deeper insight into the global phylogeny of KHV. During the last two decades a considerable number of aquaculture facilities had to be closed due to KHV. By reason of this high economic impact of KHV-I, there is an urgent need of a safe vaccine in Germany. Using classical and molecular methods we are aiming at a safe and robust vaccine, which does not spread from animal to animal and which is easy to administer.

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B-cell response of mice against RVFV strain MP12

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Rift Valley Fever Virus is a zoonotic virus transmitted by mosquitos. Main hosts are ruminants but mice are found highly susceptible too. MP12 is an attenuated strain of RVFV used for vaccination of cattle and other domestic ruminants. Mice immunized with MP12 should show high antibody titers with neutralizing activity in VNT.
In former studies at the FLI RVFV strain MP12 was used in mice to generate monoclonal antibodies. Therefore, mice received several boosts after immunization and the spleens were used for the generation of monoclonals. In contrast to these studies the time line of the B-cell response based on seroconversion following a one-shot immunization of mice is in focus in this study.
After immunization serum was examined in regular time lag for antibodies against the glycoproteins Gn and Gc as well as the nucleoprotein NP. After seroconversion was confirmed a serum neutralization assay with MP12 was performed to determine if the antibodies are protective against reinfection. To further characterize the immune response to envelope proteins an ELISA based pespcan method was used to determine binding patterns to the glycoproteins Gn and Gc.
With this study the exact time point for the appearance of protective virus neutralizing antibodies can be determined. It may serve as a model for testing of other RVFV vaccines in mice.

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Mapping domains of the Schmallenberg virus nonstructural protein NSm

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Schmallenberg virus (SBV) is an insect-transmitted orthobunyavirus causing severe fetal malformation, abortions and stillbirth in ruminants. While the nonstructural protein NSs is a major virulence factor, the main functions of NSm are still unknown.
To investigate the role of the integral membrane-protein NSm, containing three hydrophobic transmembrane (I, III and V) and two nonhydrophobic regions (II and IV), we generated a number of recombinants on the basis of our reverse genetics system and tested these constructs in virus rescue experiments, growth kinetics and interferon-assays.
Rescue experiments were successful with mutants containing a complete deletion of NSm domain IV, or a partial C-terminal deletion within domain II or when both deletions were combined. Mutants with partial N-terminal deletions of domain II and complete deletion of domain III, respectively, could not be rescued. All replicating single-mutants as well as NSm and NSs double-mutants displayed only mild differences in their growth properties in BHK-21 cells. Interferon-induction was only observed with double-mutants with a NSs deletion.
In conclusion, our data show that the N-terminal parts of the NSm domains II and III are essential for the generation of infectious virus, whereas domain IV and the C-terminal part of domain II are not required.

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Development of a theophylline-based riboswitches for gene regulation in two obligate intracellular pathogens Coxiella burnetii and Chlamydia abortus

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Bacterial gene expression is frequently controlled at the RNA level by regulatory elements called riboswitches. They consist of an aptamer sequence, which recognizes a specific ligand and an effector domain which controls gene expression at the transcriptional or translational level via an RNA conformational switch. Natural riboswitches bind metabolites like coenzyme B12 to regulate the respective biosynthetic pathway. Additionally, artificial riboswitches employing synthetic ligands like theophylline active in many organisms. They are attractive tools because their one component nature makes them self-sufficient, easy to implement and respond rapidly. Therefore, I want to introduce riboswitch regulation to the obligate intracellular pathogens Coxiella burnetii and Chlamydia abortus using a set of published theophylline-responsive riboswitches, shown to work in different organisms. The constructs use RFP as reporter gene, different plasmid backgrounds and promoter sequences in Escherichia coli as initial model. First, I tested the strong Tac promotor, active in all three organisms. Later, it will be replaced by organism specific promoters like the com1 promoter from C. burnetii or the ompA promoter from C. abortus. Different RSF1010-based vectors serve as shuttle plasmids for both organisms. First experiments show that the riboswitches function in E. coli and that they differ in their regulatory properties.

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Development of Novel Double-Attenuated Live Vaccines in Swine against H1N1 Influenza Strains

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Pigs are commercially relevant livestock and frequently infected with influenza viruses. The disease is of great economic relevance and furthermore bears high zoonotic risks. To reduce disease burden and minimize virus reservoirs, effective vaccination is still a key issue. However, conventional inactivated vaccines often confer insufficient protection and are strongly biased towards a predominant humoral immune response. For that reason, this project aims to improve this situation in pig husbandry by developing a live attenuated influenza virus vaccine. By reverse genetics, we generated attenuated mutants of the wild-type strain A/Bayern/74/2009 (H1N1v). We cloned all eight gene segments and introduced a non-physiological, strictly elastase-dependent cleavage site into the hemagglutinin. For this purpose, we replaced the amino acids at positions four to one (P4-P1) upstream of the actual proteolytic cleavage site by four alanine residues. In addition, we have truncated the C terminus of the non-structural protein 1 to generate a double-attenuated mutant. After we had studied the in-vitro properties, we performed the first in-vivo experiment to investigate the attenuation features of the single-attenuated virus mutant Bayern74/09/HA-Ela as well as the double-attenuated virus mutant Bayern74/09/HA-Ela/NS1-99.

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Toxoplasma gondii: Characterization of virulence factors from genetically atypical cross products

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The obligate intracellular protozoan parasite Toxoplasma gondii has a clonal population structure in Europe and North America. Among these clonal types only T. gondii strains of type I are highly virulent for immunocompetent lab mice, e.g. BALB/c. In Germany, the less virulent T. gondii type II strains prevail. The variance in mouse virulence can be explained by strain dependent polymorphisms of parasitic virulence factors that can interact differentially with the host immune system. T. gondii oocysts representing type II/III recombinant genotype were isolated from a fecal sample obtained from a naturally infected cat in Germany. From this isolate five individual parasite clones were generated as tachyzoites, each showing different combinations of type II and III allele patterns. The clones also revealed differences in virulence for BALB/c mice. The known prominent parasite virulence factors ROP18 and ROP5 that are able to inhibit the activity of mouse immunity related GTPases (IRG), were analyzed to clarify the distinct virulence behavior in BALB/c mice. The ROP18 expression differs markedly whereas the ROP5 allele is the same in the 5 clones. Furthermore, the mRNA expression profile of the cytokines IL-12, IL-10, and TNFα, which activity can be influenced by the T. gondii virulence factors GRA15 and ROP16, was determined in IFNγ activated macrophages. The results show that the parasites differ in their ability to stimulate the cytokine expression.

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Generation of recombinant Newcastle disease virus expressing Peste des petits ruminants virus surface glycoproteins

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Peste des petits ruminants (PPR) is a highly contagious disease in small ruminants as sheep and goats caused by the Peste des petits ruminants virus (PPRV) belonging to the genus morbillivirus in the family paramyxoviridae.
The surface glycoproteins hemagglutinin (H) and fusion protein (F) are considered to be the most important viral proteins to induce a protective immune response in the host since neutralizing antibodies are being generated towards H and F during infection.
In order to differentiate between infected and vaccinated animals, recombinant marker vaccines (DIVA vaccines) are useful. Therefore, we generated two recombinant Newcastle disease viruses (NDV) expressing either the PPRV H or F protein by reverse genetics. The expression of the PPRV glycoproteins was confirmed by western blot analysis of infected cells.
The two recombinant viruses replicate well in embryonated SPF chicken eggs, their replication in different sheep and goat cell lines as well as their thermostability was investigated.
In summary, two recombinant NDV are available which can be studied in an animal experiment for their fitness to protect from PPR, a disease that becomes more important because of the eradication of rinderpest and the abolition of vaccination against it which conveyed a cross protection against PPRV.

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Cell Culture Adaptation of Non-fixed Rabies Virus Strains from Fox and Dog

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Fixed Rabies virus (RABV) laboratory strains, like SAD, are generally known to infect worse via peripheral routes of inoculation compared to non-fixed isolates. Vice versa, non-fixed field isolates grow bad on standard cell cultures and need some passages to adapt. Starting with recombinant virus clones from non-adapted dog and fox field viruses (rRABV DogA, rRABV Fox 148), we performed serial passages on murine neuroblastoma cells (NA), canine epithelial cells (MDCK) and hamster kidney cells (BsrT7/5) and monitored infectious virus titers in cell culture supernatants. Since titers are increased by more than 2 logs while passaged on BsrT7/5 cells, deep-sequencing after the 10th passage was performed to show cell culture adaptation. Whereas most viral genes remained conserved, mutations accumulated in the glycoprotein. In fox and dog virus identical amino acid exchanges were identified. In addition to an amino acid exchange at position 266 present in all passaged viruses, a BsrT7/5 cell specific mutation at position 444 was selected, indicating that cell type or species specific constraints exist that restrict cell culture replication of field RABV. Confocal fluorescence microscopy further indicated that the identified mutations caused less extensive ER retention of G and more efficient virus particle release at the plasma membrane.

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Rupture Forces among Human Blood Platelets at different Degrees of Activation

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Little is known about mechanics underlying the interaction among platelets during activation and aggregation. Although the strength of a blood thrombus has likely major biological importance, no previous study has measured directly the adhesion forces of single platelet-platelet interaction at different activation states. Here, we filled this void first, by minimizing surface mediated platelet-activation and second, by generating a strong adhesion force between a single platelet and an AFM cantilever, preventing early platelet detachment. We applied our setup to measure rupture forces between two platelets using different platelet activation states, and blockade of platelet receptors. The rupture force was found to increase proportionally to the degree of platelet activation, but reduced with blockade of specific platelet receptors. Quantification of single platelet-platelet interaction provides major perspectives for testing and improving biocompatibility of new materials; quantifying the effect of drugs on platelet function; and assessing the mechanical characteristics of acquired/inherited platelet defects.

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Allergic reactions to Staphylococcal serine proteases in airway diseases

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Recently there is increasing evidence that besides commensal and invasive behavior S. aureus may also drive allergic reactions. In patients suffering from allergic disorders, e.g. atopic dermatitis and nasal polyposis, S. aureus colonization appears much more frequent. Additionally, allergic airway diseases are frequently associated with high titer IgE binding to staphylococcal superantigens. An implication of S. aureus proteins in the pathophysiology of allergic disorders has thus been suggested. However, the driving allergens of S. aureus remained elusive.
We could identify the serine protease-like proteins (Spl) A-F as major IgG4-binding proteins of S. aureus. Spl-specific IgG1 and IgG4 was quantified in sera of healthy donors (n=46) by ELISA. Spl-specific IgE was measured in serum of asthma patients (n=50) and healthy controls (n=40). Peripheral blood T cells of healthy donors (n=9) were stimulated with Spls and cytokine production was elucidated by cytometric bead array. The ability of Spls to induce allergic responses in vivo was tested in a murine asthma model.
Compared to Hla, the Spl-specific antibody response was significantly shifted toward IgG4. Spl-specific IgE was significantly elevated in serum of asthma patients. Spl-stimulation of T cells elicited a Th2/Treg-dominated cytokine profile (IL-4, IL-5, IL-10, IL-13), whereas Th1/Th17 cytokines (IFN-γ, IL-6, IL-17, TNF-α) were of low concentrations or absent. In mice, inhalation of SplD without adjuvant induced allergic lung inflammation characterized by Th2 cytokines and eosinophil infiltration.
The results indicate a role of S. aureus Spls as triggering allergens in allergic airway diseases, opening prospects for diagnosis and causal therapy of asthma.

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Immunome analysis reveals species-specific humoral immune response in sepsis patients

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Sepsis is the third leading cause of death in hospitalized patients in Germany. Blood culture is positive in less than half of the cases of clinical sepsis. Yet the identification of the causative agent is essential for a targeted antimicrobial therapy.
In this study we examined whether the ability of sepsis patients to generate a highly specific humoral immune response towards the invading pathogen might support conventional sepsis diagnosis.
In a prospective clinical trial ICU patients with suspected sepsis were recruited. Plasma samples were collected before sepsis, at diagnosis and during the infection. Plasma antibody binding to extracellular proteins of frequent sepsis pathogens was quantified using a Simple Western Assay. Dynamics in antibody binding were assessed in 74 patients. Healthy adults served as control. 2D-immunoblots were used to identify immunogenic proteins of different bacteria.
In healthy subjects, anti-bacterial IgG levels were stable over at least two months. In contrast, sepsis patients frequently exhibited dynamic antibody profiles during sepsis. An increase of specific IgG could be detected in 40% of patients with a microbiological diagnosis (21/53) and also in one third of sepsis patients without a suspected pathogen (7/21. In cases with an intraabdominal infection focus, an antibody response to more than one bacterial species was often observed.
During sepsis the adaptive immune system responds specifically to the invading pathogen. The identification of immunogenic proteins of frequent sepsis pathogens may help to develop a new serological test for pathogen detection and risk stratification in sepsis patients.

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Analysis of vaccine-induced rabies cases using deep sequencing

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Rabies is a long known zoonotic disease caused by an ssRNA(-) virus of the genus Lyssavirus. During large-scale vaccination programs of foxes, millions of oral rabies vaccine virus baits were distributed. Notably, most of them contained one of the live-attenuated SAD-derived (Street Alabama Dufferin) strains B19 or P5/88, both in vitro derivatives of SAD-Bern(orig). Post vaccination surveillance detected at least ten possible vaccine-associated fatal rabies cases in four different species: five in red foxes (Vulpes vulpes) and for each one in stone marten (Martes foina), European badger (Meles meles), and cattle (Bos taurus), respectively. It was shown that genetic relationships of SAD vaccines based on consensus sequence information could lead to biased results and the important role of low frequency variants was highlighted. In order to determine the genetic relationship of the selected vaccine-associated viruses with the vaccine strains used for the vaccination campaign in the respective region, we conducted deep sequencing. For data analysis beyond consensus distances we used the frequency of each nucleotide at each position of the viral genome to calculate pairwise Manhattan distances from a nucleotide frequency alignment of the sequenced strains. Results were visualized using two-dimensional non-metric distance scaling. Isolates from vaccine-induced rabies cases clustered tightly together, but surprisingly none of the SAD-B19-related cases grouped around their parental vaccine batches. Instead these isolates showed similarities to other in vitro derivatives of SAD-Bern(orig) indicating an in vivo selection process favoring only a portion of the variants/haplotypes within SAD-B19 vaccines.

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Molecular Requirements for Nuclear Egress of Herpesviruses

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Herpesviruses use a vesicle-mediated process for translocation of newly formed capsids from the nucleus to the cytoplasm where final virus maturation proceeds. For this, capsids bud at the inner nuclear membrane, acquiring a primary envelope which is subsequently lost after fusion with the outer nuclear membrane (ONM) thereby releasing the capsids into the cytoplasm. This vesicle-mediated translocation engaging the nuclear membranes was long considered as unique for herpesviruses but recent data suggest a similar, yet not well characterized regular cellular pathway.

For herpesviruses vesicle budding and scission is solely orchestrated by the nuclear egress complex consisting of the two viral proteins designated as pUL31 and pUL34 in the alphaherpesviruses. This was shown not only in eukaryotic cells but also in model membrane systems indicating that no other viral or cellular protein is required for the formation of primary enveloped virions located in the perinuclear space. However, it is still completely unknown which viral and/or cellular proteins mediate the release of the capsids to the cytosol orchestrating the fusion of the primary virion envelope with the ONM

To test which proteins might be involved, we will isolate primary enveloped virions from the perinuclear space. This is not an easy task but we already established cell lines stably expressing a tagged pUL34 to use this in affinity chromatography. The enriched primary enveloped virions will be then analyzed by mass spectrometry to identify the putative fusion machinery in the nuclear envelope which might also mediate nuclear translocation of cellular components.

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Validation of Gene Candidates Obtained by Comparative Prediction

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Many hundreds of strains have recently been sequenced for many bacterial species. This provides an opportunity to improve gene finding by exploiting this variation as well as it poses the challenge of consistent annotation of these strains.
An extension of the gene finder Augustus allows the simultaneous prediction of genes in several aligned bacteria genomes. These comparative version decides for gene candidates by a score that is based on the one from single-genome Augustus, that is bases on a conditional random field, but also incorporates evidence for selection. It rewards a consistent annotation of aligned regions using a phylogenetic tree of the genomes. A dual-decomposition algorithm is used to approximize the maximal score [König 2015].
We applied comparative Augustus to all strains with a complete assembly on NCBI of each of the 5 bacterial species Staphylococcus aureus, Streptococcus pneumoniae, Burkholderia pseudomallei, Bacillus subtilis and Escherichia coli. To this end the genomes were aligned with Cactus [Paten 2011] for every species. As input for Cactus we passed nearly star-shaped trees. We found that the genes that are unqiue to the Augustus gene set have a higher percentage of homologs in the species specific NCBI protein databases than the genes that are unique to the RefSeq annotations. Further, we used other information like occurrence of a recognisable ribosome binding site motif, gene length and quantitative RNA data (if available) as indicatior for correctly predicted genes.

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Rat hepatitis E virus in Europe: Looking for viral and bacterial co-infections

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A rat-associated hepevirus, rat hepatitis E virus (ratHEV), was initially detected in Norway rats (Rattus norvegicus) from Hamburg, Germany, and subsequently detected in rats from three other cities in Germany and in rats from Vietnam, the US, Indonesia, China, Denmark and France.
Here, we report on a survey of additional Norway rats from Germany, Denmark, France, Switzerland, Austria and Hungary. RatHEV-specific real-time and conventional RT-PCR investigations revealed the presence of ratHEV at the majority of investigated sites in all six countries. In contrast, application of a human pathogenic HEV genotype 1-4-specific real-time RT-PCR did not detect any infections with these genotypes. No difference in age or sex related ratHEV infection status was detected. RatHEV prevalences in towns and rural sites did not differ significantly from high density urban areas, but prevalence in zoological gardens was significantly lower compared to urban areas. Coinfections with rat polyomavirus (ratPyV) and Leptospira spp. were detected in 15 of 46 and four of 47 ratHEV-infected rats, respectively. In two rats triple infections with ratHEV, ratPyV and Leptospira, all originating from one zoological garden, were detected.
In conclusion, our investigation shows a broad geographical distribution of ratHEV in Norway rats from Europe and its presence in all four habitat types investigated. Future investigations will have to evaluate potential phylogeographical reasons for ratHEV divergence, incursion of ratHEV with invading individuals and to identify putative influences of co-infections by other pathogens to the frequency and outcome of ratHEV infections.

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Virulence determinants of recent avian influenza viruses of subtype H7N7 in chickens

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Avian influenza viruses (AIV), a member of the family Orthomyxoviridae, have a multipartite single-strand RNA genome with eight gene segments coding for at least 10 viral proteins. AIV are classified into 16 hemagglutinin (HA1-HA16) and 9 neuraminidase (NA1-NA9) distinct subtypes. Some H5 and H7 viruses exhibit high virulence in poultry. These highly pathogenic (HP) strains evolve from low pathogenic (LP) progenitors by reassortment and/or point mutations. Changing the monobasic HA cleavage site (CS) in the LP viruses to a polybasic motif is essential to activate HP virus in multiple organs by furin-like proteases causing up to 100 % mortality. In 2015, both LP and HP H7N7 AIVs were isolated from poultry in Germany. Genetic relatedness of both viruses and virulence markers of the HP phenotype in chickens will be investigated using reverse genetics and animal experiments. Therefore, the eight segments of LP and HP viruses will be amplified, cloned in pHWSccdB and used for transfection of HEK293T cells to generate recombinant viruses. In addition to the insertion of a polybasic CS in the LP virus, gene segments of the HP virus will be swapped to determine the minimum constellation that is required for exhibiting high virulence in chickens.

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Metabolic impact of pneumolysins of Streptococcus pneumoniae on human epithelial cells

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The interplay between the metabolism of human cells and pathogenic bacteria is a crucial pivot-point in infection related processes. Many pathogenic bacteria secret virulence factors into the medium to gain advances over other organisms and to interfere with the host cell response. To gain deeper insight into physiological adaptations during the contact of Streptococcus pneumoniae and eukaryotic cells, an in vitro model will be investigated in which the impact of pneumolysins of S. pneumoniae on the metabolism of human epithelial cells will be monitored. Not much is known about the metabolic impact of such virulence factors and a structured metabolome and cell biological study is of particular interest to gain more knowledge about the cellular mechanisms involved in these infections related processes. We will analyze the influence of different pneumolysins on the host cells metabolism. By using bioanalytical techniques like NMR spectroscopy we will investigate the composition of metabolites in extracellular and intracellular samples. In order to connect the metabolic impact to the infection in the host cell methods of multivariate statistical analysis such as PCA and O-PLS will be used. SPME-GC/MS analytics will be employed to analyze lower concentrated compounds and selected volatile metabolites. HPLC methods for the analysis of cellular stress response related metabolites like glutathione are developed in our laboratory and will be used for the investigation of host cell responses. In a future approach we will also include selected primary cell models to analyze the influence of virulence factors like pneumolysis on the epithelial cell metabolism.

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Development of recombinant live virus vaccines against koi herpesvirus (KHV)

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KHV (Cyprinid herpesvirus 3) is a member of the family Alloherpesviridae, which belongs to the order Herpesvirales, and causes a world-wide occurring fatal disease of common carp and koi. To obtain safe live vaccines, we started to generate gene deletion mutants of KHV. Previous studies have shown that the viral thymidine kinase (TK, ORF55) and dUTPase (DUT, ORF123) are dispensable in cell culture, but relevant for virulence in carp. However, attenuation of single mutants based on a KHV field isolate was incomplete, and their in vitro replication was inefficient. Therefore, the TK and DUT genes were now deleted singly and in combination from a cell culture-adapted virus strain (KHV-Taiwan). These mutants grew to high titers, and animal experiments will be done to evaluate their protective efficacy.
To obtain "DIVA" vaccines which permit serological differentiation between immunized and infected fish, we have deleted the genes of two antibody-inducing, but obviously nonessential envelope glycoproteins of KHV (ORF148/149). Another envelope protein of KHV (pORF99) is apparently essential, and trans-complementing cell lines have to be prepared for propagation. The resulting deletion mutant might then be used as a "DISC" vaccine, which is able to replicate in carp, but does not produce infectious virus progeny.

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Interaction of the Foot-and-Mouth Disease Virus with the Innate Immune System

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The Foot-and-Mouth Disease is a highly contagious disease of cloven-hoofed animals. It is characterized by lameness and vesicular lesions on tongue, snout and between cloves. The etiologic agent, the Foot-and-Mouth Disease Virus (FMDV), belongs within the Picornaviridae family to the genus Aphthovirus. Its genome is composed of a single-stranded positive-sense RNA and codes for one polyprotein. The first translated viral protein is the Leaderprotease (LPro) which autocatalytically cleaves itself from the nascent polyprotein. Another protease encoded by the viral genome, the 3C protease (3CPro), cleaves the nascent polyprotein in precursor and mature viral proteins. In addition, both proteases have indicated impact on the transcription and translation machinery and the innate immune response.

To understand the interaction of FMDV with the innate immune system we analyzed the influence of diverse viral proteins on differentially activated IFN-I pathways. Therefore a HEK-293T cell culture system was used and Luciferase reporter assays were performed.

We characterized the LPro mediated inhibition of translation (eIF4G cleavage) and additional specific effects on IFN-I signaling cascades. In our analyses both viral proteases show inhibitory effects on different pathways of IFN-I signaling. Thus, LPro and 3CPro of FMDV interfere with the innate immune system.

To further characterize the interaction of viral proteins with cellular mechanisms more detailed studies are planned to identify the cellular targets of the viral proteins and to clarify the detailed mechanisms of interference with cellular protein translation and the innate immune system.

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Newcastle Disease Virus mediates tumor rejection by activation of NK cells but the adaptive immune response prevents relapse in a murine model of pancreatic cancer

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Background: Pancreatic cancer is projected to be the 2nd cause of cancer-related deaths worldwide by 2030 and has the worst prognosis of all solid malignancies. In 1957 it was first reported that Newcastle Disease Virus (NDV) has oncolytic properties on tumor cells.
Methods: A single dose of NDV was administered intravenously seven days after orthotopic tumor inoculation of DT6606PDA or Panc02 cells in mice. Tumor growth was measured and immune response was analyzed using flow cytometry. Staining of frozen tumor sections revealed infiltration of leukocytes. Western Blot analysis determined expression of PLC proteins on tumor cells.
Results: A single treatment with NDV inhibited DT6606PDA tumor growth in mice and prevented tumor recurrence for a period of three months. Tumor infiltration and activation of NK cells, cytotoxic and helper T-cells was enhanced. NDV induced melting of Panc02 tumors till d7 pi but recurred henceforth displaying unrestricted tumor growth and active inhibition of tumor-specific immune response. Re-isolated Panc02 cells showed enhanced expression of FoxP3 and TGF-β but neither expression of MHC I nor Rae-1δ.
Conclusion: NDV is able to reject tumors displaying high immunogenicity and moderate proliferation (DT6606PDA) by mounting an anti-tumor immune response but if non-immunogenic variants accomplish outgrowth (Panc02) anti-tumor response is prevented and relapse occurs. This study underpins the importance of an adaptive immune response which can be initiated by NDV to mediate long-term tumor surveillance apart from direct oncolysis.

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Survival of S. pneumoniae in sputum of ICU patients.

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Introduction:
Streptococcus pneumoniae and Staphylococcus aureus are known causative agents of ventilator-associated pneumonia (VAP), which leads to increased morbidity and mortality of intensive care unit (ICU) patients. To date, it is not known to which stresses these bacteria are exposed in airways of ventilated patients. The present studies were therefore aimed at establishing an ex vivo assay that mimics the in vivo situation through incubation of bacteria in sputum samples collected from different ICU patients.
Material and methods:
Wild-type S. pneumoniae TIGR4 and different virulence-attenuated mutants were incubated in sputum samples from mechanically ventilated ICU patients from the University Medical Center Groningen. Next, bacterial survival in the sputum samples was tested by plating. S. pneumoniae was also used in a sputum spotting assay, where sputum was spotted on a blood agar plate and the inhibition zones were compared to those observed for Streptococcus anginosus and S. aureus.
Results and Conclusions:
The tested S. pneumoniae strains showed different rates of survival in sputa from different patients. Intriguingly, the spotting assay revealed major differences in the bactericidal activity of sputa from different patients. Our ongoing research is aimed at assessing both patient parameters that affect bacterial survival in sputum as well as the bacterial gene repertoire needed for survival.

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Macrophage cell death during infection with Burkholderia pseudomallei

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Macrophage cell death is an intrinsic immune defense mechanism in response to microbial infection. It can remove the replicative niche of intracellular pathogens and induces immune attack. However, uncontrolled cell death likely contributes to tissue damage, which may enhance bacterial dissemination. Apoptosis, necroptosis and pyroptosis constitute the three major cell death modes and are tightly regulated to ensure adequate immune reactions to invading pathogens.
We recently provided evidence that infection of primary murine macrophages with the cytosolic pathogen Burkholderia pseudomallei and causative agent of melioidosis leads to early induction of caspase-1/11-dependent pyroptosis, a highly inflammatory and lytic cell death. We identified the B. pseudomallei type three secretion system (T3SS) rod protein as a stimulator of caspase-1. In the absence of caspase-1 and -11 delayed activation of alternate cell death pathways such as inflammatory silent apoptosis might play a role during infection.
This project will focus on additional caspase-1/11-independent cell death pathways in macrophages. We want to clarify the contribution of apoptotic caspases to resistance against B. pseudomallei. This study aims to identify and characterize bacterial factors leading to cell death induction. Finally, we will analyze the crosstalk between different forms of macrophage death.

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Proteome profiling of Burkholderia pseudomallei quorum sensing mutants

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Burkholderia pseudomallei, the causative agent of melioidosis, is a Gram-negative soil bacterium found in tropical areas. B. pseudomallei employs several acyl-homoserine lactone (AHL)-mediated quorum sensing (QS) systems, which activate specific sets of genes depending on cell density [1,2]. The genome of B. pseudomallei encodes genes for three QS systems with one luxI and one luxR homologue, respectively, and additionally three orphan luxR homologues [3]. The luxI homologues encode AHL-synthases producing specific AHLs binding to their respective luxR-coded transcriptional regulator, which in turn regulates the expression of virulence-related genes [4]. The aim of this study was to dissect the impact of the different QS systems on gene expression and pathogenicity of B. pseudomallei.
As a starting point single, double and triple deletion mutants were constructed and sub-proteome fractions of wild-type and mutant strains were subjected to mass spectrometry analysis aiming on the identification of QS-target genes. Cytosolic proteins were identified by the DIA approach IMSE, whilst extracellular and surface-associated proteins were analyzed by GeLC MS/MS. Moreover, the AHL-spectrum of the different mutants was analyzed by mass spectrometry and the mutants were characterized with respect to different phenotypes. Our comprehensive proteome analysis revealed a significant number of QS-regulated genes, some of which have been already described as QS-regulated in other Gram-negative opportunistic pathogens, e.g. Burkholderia cenocepacia [5] and are known to be involved in pathogenicity. We are currently investigating the virulence potential of the QS-mutants in different pathogenicity models.

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Optimizing murine infection models by using mouse-adapted S. aureus strains

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In general, to study the pathogenicity of S. aureus human-adapted S. aureus strains are used in murine infection models. However, S. aureus is highly host-specific. Several virulence factors, such as PVL, CHIPS, SCIN, are human-specific and require non-physiological concentrations in the mouse model or do not work at all. We have recently reported that mice are natural hosts of S. aureus. Our data show that murine S. aureus strains belong to unique clonal clusters and seem to be well adapted to their host. The use of such mouse-adapted S. aureus strains may be a promising tool to develop more clinically relevant infection models.
To determine whether mouse-adapted S. aureus strains can be used to optimize murine infection models, S. aureus strains isolated from wild and laboratory mice were compared to the human S. aureus strain Newman. We used two different infection models, pneumonia and bacteremia, and analyzed the survival and the bacterial load in several organs.
There were significant differences in the survival rate between the strains in both infection models. Furthermore, the bacterial load within the organs differed between the strains. These results indicate that mouse-adapted S. aureus strains have a better fitness in murine infection models and can cause more severe disease than S. aureus Newman. Notably, a CC49 isolate from a wild mouse was highly virulent in laboratory mice and is in the focus of ongoing studies. With our study we hope to improve mouse infection models that are required to study S. aureus - host interaction.

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Hyperfusogenic mutations in the herpesvirus fusion protein – in search of a super-fuser

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Enveloped viruses utilize membrane fusion to infect and spread in their hosts. Entry is initiated by binding of the virus to specific cellular receptors and fusion with the cellular membrane is catalyzed by a viral fusion protein. While many viruses encode only one protein capable of performing attachment and fusion, herpesviruses require four different proteins: a receptor-binding protein and the conserved fusion-machinery composed of glycoprotein (g)B and the gH/gL complex. Although gB possesses typical features of a class III fusion protein, it is unable to execute membrane fusion on its own but depends on gH/gL. However, although gB and gH/gL are crucial for entry, the alphaherpesvirus Pseudorabies Virus (PrV) is able to perform limited cell-to-cell spread in absence of gL. Passaging experiments of gL-deleted PrV resulted in a mutant which efficiently entered cells in absence of gL. Among others, this mutant carries mutations in gB (gBB4.1) resulting in efficient fusion with only gH.
Aim of the present study was to investigate whether additional amino acid changes might convert gB into an autonomic fusion protein. For this purpose, we introduced additional mutations into PrV gBB4.1 which had been described to enhance fusogenicity of herpes-simplex and varicella-zoster viruses. To test the activity of these mutants we used a transfection-based fusionassay. Our results show that introduction of additional mutations into gBB4.1 further enhanced fusogenicity. Despite the excessive fusion-activity, this gB mutant still depends on gH, indicating that raising the fusogenic potential is not sufficient to compensate for gH function.

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Cell-surface localized pneumococcal proteins – new insights into the abundance and immunogenicity

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Question and aims. Pneumococcal next-generation vaccines would be serotype-independent and protein-based formulations. The pneumococcal cell surface is decorated with several clusters of proteins, namely choline-binding proteins (CBP), sortase anchored or (trans-)membrane associated proteins, lipoproteins and non-classical surface proteins. Highly conserved proteins from different clusters representing potential candidates for an innovative vaccine were analyzed regarding their abundance on the pneumococcal surface and immunogenicity.
Methods. The cell-surface abundance of proteins was examined by flow cytometry using protein specific polyclonal IgGs generated in mice. The relative antibody titers from these mouse sera were determined by ELISA and antibody titers were also analyzed with the Luminex® FlexMap3D® technique. In addition, the immunogenicity of the selected proteins was screened with convalescent sera from patients infected with different pneumococcal serotypes.
Results. The Luminex-based immunoproteomics approach showed that nearly all proteins are immunogenic when administered to mice. Analysis of the convalescent patient sera revealed 5 proteins from different surface protein clusters which induced high antibody titers during pneumococcal infections. These proteins most likely also showed a high abundance on the pneumococcal cell surface in flow cytometry. Interestingly nearly all antibodies also bound to the encapsulated strain D39.
Conclusion. The analyzed pneumococcal surface proteins are highly conserved and immunogenic. Besides, surface abundance of some proteins is high and partly correlates with the observed immunogenicity. Therefore, these proteins represent promising candidates for a protein-based conjugate or subunit vaccine, which are urgently needed to combat pneumococcal infections.

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Syndromic surveillance of dairy cows using daily activity and milk yield data

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Syndromic Surveillance is a near real time approach to identify a disease outbreak or other conditions affecting the behavior of a group of animals. The central target is the collection, analysis, interpretation and dissemination of automatically generated and health- related data. In growing and efficiently managed farms technology such as sensors on animals and data collection during daily work becomes an important monitoring tool for the farm operator (e.g. for heat detection).

The objective of this study is to assess the suitability of activity and milk yield data as a surveillance tool concerning health and management events. Datasets are obtained from two commercial dairy farms on a daily base.

In total 1,800 cows are already equipped with accelerometers (IceQubes; IceRobotics Ldt.) and the daily milk yield is recorded in the herd management software DairyComp305 (Valley Agricultural Software).

The integration of these data in a database is the basis to demonstrate normal trends of activity and milk yield in parity, lactation stage and holding conditions.

Specific deviation patterns are expected for the occurrence of individual disease or management events.

By defining a useful threshold as an acceptable result of sensitivity and specificity, the application of a health alert system in a running dairy farm system has to be proven.

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Identification and molecular characterization of highly immunogenic mycobacterial lipopeptides

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A number of recent publications describe mycobacterial lipopeptides as a novel class of highly immunogenic lipid antigens in mycobacteria infected humans (Seshadri et al., JI, 2013; Bastian et al., JI, 2008), bovines (Van Rhijn et al., EJI, 2009) and guinea pigs (Kaufmann et al., JI, 2016). Previous data of our group show, that these lipopetides are presented by MHCII and induces a strong CD4+ T-lymphocytes immune reaction in TB-infected or BCG vaccinated individuals. We could demonstrate that the reaction of these lymphocytes is specific for mycobacteria of the Mycobacterium Tuberculosis Complex (MTC).
The aim of the presented PhD project is to identify the molecular identity of these antigens.
To this end we will establish mono- or oligoclonal T-lymphocyte lines of PPD reactive humans and/or BCG sensitized guineapigs.
In parallel, we will fractionate whole lipid extracts of M.tuberculosis using phase separation and column chromatography techniques.
With the antigen specific T-cell lines we will test the obtained fractions and will identify those that contain highly immunogenic lipopeptides.
In a next step we will characterize the molecular identity of the antigens.
The identification of the molecular nature of the immunogenic lipopeptides is a prerequisite to further elucidate the role of the antigens in the fine-tuned host pathogen interplay. In addition, it will be assessed to what extent these antigens contribute to the Tuberculin-Skin-Reaction, i.e. whether they can be exploited as diagnostic antigens , or whether they could serve as components of a future TB-vaccine.

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Establishment of an antibiotic signature library for Staphylococcus aureus

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Worldwide spread of antibiotic resistance greatly impairs treatment of bacterial infections. Therefore, antibacterial agents with new mechanisms of action and new innovative and cost-effective techniques for the characterization and validation of new drugs are urgently needed. Gel-based and gel-free proteomics have emerged as valuable tools to study the physiology of microbes under antibiotic stress conditions. Our study aims at the creation of a comprehensive antibiotic signature library for Staphylococcus aureus. Protein signatures of S. aureus treated with well characterized antibiotics will be used to identify cellular targets and the mode-of-action of new anti-microbial compounds.

S. aureus HG001 was exposed to various concentrations of antibiotics with well-defined molecular targets to determine their minimal inhibitory concentrations (MIC). Multiples of the MICs were tested in growth experiments to identify antibiotic concentrations that reduced the bacterial growth but did not inhibit growth completely in order to map the specific antibiotic stress response. Cytosolic protein extracts of S. aureus HG001 treated with antibiotics were analyzed using a gel-free LC-IMSE approach in combination with the Hi3 method for absolute protein quantification.

Although each antibiotic showed an individual protein expression profile, signature proteins specific for a common drug target were identified (e.g. cell wall, ribosome, replication machinery). These subsets of proteins whose expression levels are characteristic for a specific antibiotic treatment have been designated as "proteomic signature".

We established a workflow for a comprehensive antibiotic stress proteome signature library of S. aureus. Using this pipeline, new compounds can be evaluated to gain insight into their mode-of-action.

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Immune mechanisms involved in anti-viral immune response of European bats against relevant Lyssaviruses

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Lyssaviruses are the causative agents of rabies, a fatal zoonotic disease of nerve system. European bats harbor two specific types of Lyssaviruses, European Bat Lyssavirus (EBLV-1 and -2). Although cases of rabies caused by EBLV 1 or 2 are described there are no reports about epidemics in bats. Due to the long co-evolution between bats and EBLVs innate immune pathways might contribute to this disease resistance in European bats.

We focus on specific physiology of bats, which might lead to their 'special' immune response against lyssaviruses. Using established cell lines from M. myotis (M. myotis) brain (MmBr), nervus olfactorius (MmNol), and nasal epithelium (MmNep) we simulate the transmission of lyssaviruses along the aerosol infection route. The anti-viral effects, induction of interferons (IFNs)/interferon stimulated genes (ISGs) and expressions of viral receptors in each cell line will be investigated.

Bats are the only mammals which actively fly. Their flight ability enables a higher body temperature. High body temperatures ranges increase the rate of multiple immune responses in bats, and might stimulates innate immune response, such as IFNs to repress lyssavirus replication. Using M myotis cell lines we investigate lyssavirus replication in different temperatures to simulate bat's daily activity and their hibernation in vitro. We build growth curve to find different sensitivities of cell lines, simulate RNA-viral infection with Toll like receptor (TLR) agonists, and incubate cell lines in aerosol infection pathway by 39°C, 37°C, 25°C, to simulate the daily activity of bats, and by 4°C to simulate the hibernation of bats.

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Identification of gene signatures associated with foot-and-mouth disease virus infection by transcriptome analysis

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Foot-and-mouth disease virus (FMDV) is the etiological agent of a dramatic disease that affects cloven-hoofed animals. The pathogenesis of this disease is nowadays still not fully understood; in particular, it is unknown why some animals become persistently infected, despite being vaccinated. Therefore, there is the need to identify at the transcriptome level both virus and host genes involved in FMDV acute and persistent infection, in order to find the target genes to develop novel vaccines. Transcriptome shotgun sequencing (RNA sequencing) has rapidly become the leading tool to study how the transcriptome evolves, in particular how the genes are differentially expressed during virus infection. In a time series experiment, BHK-21 cells have been infected with FMDV serotype O1 Manisa at a multiplicity of infection (MOI) of 1, microscopically monitored for cytopathic effect and samples have been collected at different times post infection. From each time point, the total RNA was extracted and the poly(A)mRNA was isolated; the latter was subsequently used to establish cDNA libraries. For each library, RNA sequencing was performed both with Ion Torrent PGM and with Illumina MiSeq platforms. The differentially expressed genes have been identified statistically among the chosen time points. Through the differentially expressed genes we have found genes coding for proteins involved in viral replication, apoptotic processes, protein localization and transport, regulation of cell growth and cellular responses to various stress stimuli. In order to confirm the transcriptome results, differentially expressed genes will be selected for further investigations.

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