2018

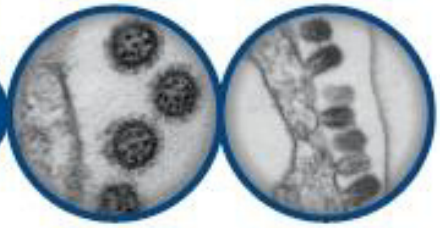
Confirmed speakers

Wendy Barclay | London, UK
John Briggs | Cambridge, UK
Takeshi Noda | Kyoto, Japan
Guus Rimmelzwaan | Hanover, Germany
Martin Schwemmle | Freiburg, Germany

6th International Influenza Meeting

Highlight Topic:

Visualizing Flu



02 – 04 September

Venue:

University of Muenster, Castle
Schlossplatz 2 | 48149 Muenster
Germany

FluResearchNet.

German Research Platform for Zoonoses

Scientific committee

Stephan Ludwig | Münster
Klaus Schughart | Braunschweig
Peter Stäheli | Freiburg

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GENERAL INFORMATION

Scientific Committee
Stephan Ludwig, Münster
Klaus Schughart, Braunschweig
Peter Stäheli, Freiburg

Offical Language
The official language of the meeting is English. Simultaneous translation will not be provided.

Poster Presentations
Posters are to be mounted between 15.00 and 21.00 p.m. on Sunday afternoon, September 2nd. Posters are to be removed between 14.30 and 16.30 p.m. on Tuesday, September 4th.

Meals
Lunches will be provided in the Foyer of the castle of Münster, as indicated in the program. Evening meals will be provided as part of the social program. You are invited by the organizers.

Social Program
The Opening reception will take place at the venue on Sunday, September 2nd and will start at 19.00 p.m.
Address: Universität Münster, Schlossplatz 2, D-48149 Münster

The Conference Dinner will take place at Schlossgarten Cafe. The restaurant is located in 2 minutes walking distance directly behind the venue – you will reach it by leaving the castle's backdoor and walking straight through the castle garden. The dinner starts at 19.00 pm.

Organization
FluResearchNet and German Research Platform for Zoonoses
c/o Institute of Molecular Virology (IMV)
Center of Molecular Biology of Inflammation (ZMBE)
Westfälische Wilhelms-Universität Münster
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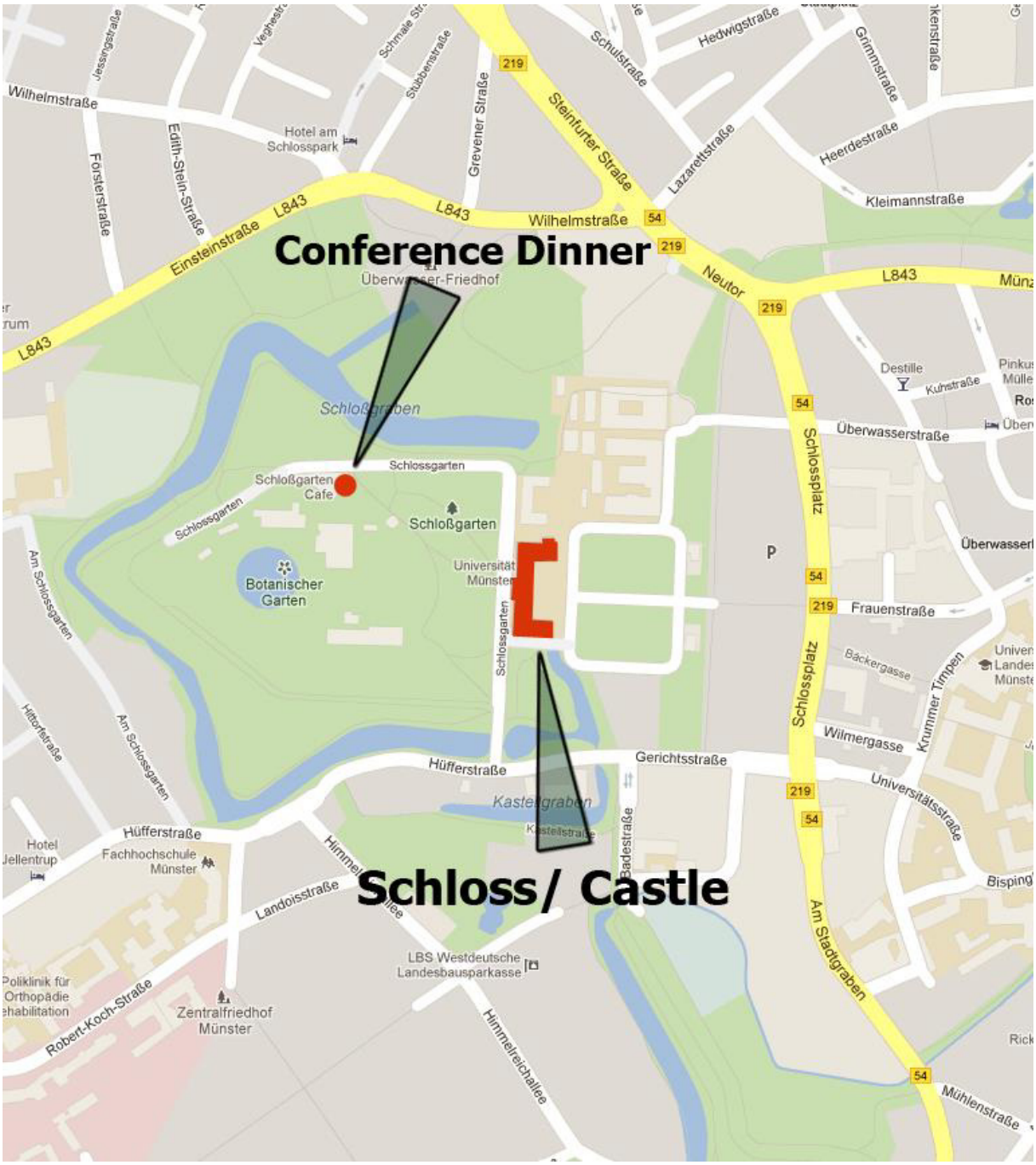
VENUE


Conference Dinner
Schloss/ Castle
Wilhelmstraße
Einsteinstraße
L843
Steinfurter Straße
Neutor
Überwasser-Friedhof
Schloßgraben
Schlossgarten
Schloßgarten Cafe
Schloßgarten
Botanischer Garten
Universität Münster
Schlossplatz
Überwasserstraße
Frauenstraße
Bäckergasse
Wilmergasse
Gerichtsstraße
Hüfferstraße
Kastellgraben
Universitätsstraße
Am Stadtgraben
Mühlenstraße
Hotel am Schlosspark
Fachhochschule Münster
LBS Westdeutsche Landesbausparkasse
Zentralfriedhof Münster
Robert-Koch-Straße
Landoisstraße
Himmelreichallee
Badestraße
Krummer Timpen
Am Schlossgarten
Hittorfstraße
Försterstraße
Edith-Stein-Straße
Grevener Straße
Schulstraße
Hedwigstraße
Grimmstraße
Heerdestraße
Lazarettstraße
Kleimannstraße
Destille
Kuhstraße
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ORAL PRESENTATIONS

Opening

Sunday, September 02 2018

Keynote lecture: Packaging of segmented genome in influenza virus

Takeshi Noda, Kyoto, Japan

Session 1

VISUALIZING FLU

Chair: Stephan Ludwig, Muenster, Germany

Monday, September 03 2018

Keynote lecture: Enveloped viruses revealed by cyro-electron tomography
John Briggs, Cambridge, United Kingdom

Visualizing influenza and parainfluenza virus infection non-invasively in living mice with non-attenuated reporter viruses
Charles Russel, Memphis, USA

The structure of the Influenza A Virus Genome
David L.V. Bauer, Oxford, United Kingdom

Molecular Anatomy of an Influenza Virion
Edward Hutchinson, Glasgow, United Kingdom

Visualizing influenza and parainfluenza virus infection non-invasively in living mice with non-attenuated reporter viruses

Charles Russel[1]

[1] St. Jude Children's Research Hospital, Memphis, USA

Infection has classically been measured by titering infectious virus recovered from euthanized animals or respiratory washes. In contrast, bioluminescence imaging measures in living animals the expression of luciferase, a marker for the extent of infected cells. It is a powerful tool for studying virus-host interactions yet luciferase insertions attenuate replication and virulence. Propagating lung samples having the highest ratios of bioluminescence-to-titer, we used directed evolution of a luciferase-expressing pandemic H1N1 (pH1N1) 2009 influenza A virus in mice to restore fitness and increase bioluminescence signal. Mouse-adapted virus had 10-fold higher bioluminescence signal compared to wild-type and had wild-type-like replication and virulence in mice. Fitness was restored by PA-D479N and PB2-E158G amino-acid mutations and PB2 non-coding mutations C1161T and C1977T, which collectively increased mRNA transcription. The adapted reporter virus will be a useful tool for noninvasive imaging of pH1N1 influenza virus infection and its clearance while analyzing virus-host interactions and developing new therapeutics and vaccines. We are currently using the virus to study in pharmacologically immunosuppressed mice prolonged infection, immune reconstitution, and clearance. Future studies will explore treatment options in the immunocompromised host, building on our previous studies using a non-attenuated Sendai reporter virus (murine parainfluenza virus 1). Collectively, our studies demonstrate the unique power of visualizing viral infection non-invasively in living animals.

The structure of the Influenza A Virus Genome

David L.V. Bauer[1], Bernadeta Dadonaite[1], Brad Gilbertson[2], Sanja Trifkovic[2], Steven Rockman[2], Alain Laederach[3], Lorena Brown[2], Ervin Fodor[1]

[1]University of Oxford, United Kingdom
[2]University of Melbourne, Australia
[3]University of North Carolina, USA

The IAV genome consists of eight single-stranded viral RNA (vRNA) segments contained in separate viral ribonucleoprotein complexes (vRNPs) that are packaged together into a single virus particle. The structure of the vRNA is believed to play a role in assembling the different vRNPs into budding virions and in directing reassortment between established human influenza viruses and influenza viruses harboured in the animal reservoir. Reassortment can lead to the emergence of novel influenza strains to which there is little pre-existing immunity in the human population. While previous studies have revealed the overall organisation of the proteins within vRNPs, characterisation of vRNA structure using conventional structural methods is hampered by limited resolution and an inability to resolve dynamic components.

We have employed multiple high-throughput sequencing approaches to generate the first global high-resolution structure of the IAV genome. We find that different IAV genome segments acquire distinct RNA conformations and form both intra- and inter-segment RNA interactions inside influenza virions. We demonstrate that this extensive network of RNA-RNA interactions is required for the assembly of the viral RNA genome and virus replication. We then used our detailed map of IAV genome structure to provide the first direct evidence for how inter-segment RNA interactions drive vRNP co-segregation during reassortment between different IAV strains. This work is a roadmap both for the development of antivirals targeting key RNA interactions and for the creation of a framework to 'risk assess' reassortment potential to better predict the emergence of new pandemic influenza strains.

Molecular Anatomy of an Influenza Virion

Edward Hutchinson[1], Naina Nair[2], Daniel Goldfarb[1], Swetha Vijayakrishnan[1], James Streetley[1], Svenja Hester[4], Pippa Harvey[1], Elizabeth Sloan[1], Terry Smith[3], David Bhella[1]

[1] Centre for Virus Research, MRC-University of Glasgow, United Kingdom
[2] School of Art Glasgow, United Kingdon
[3] University of St Andrews, United Kingdom
[4] University of Oxford, United Kingdom

Influenza virions are complex and vary in their shape and composition. As a result, no single method can describe them in detail. We therefore applied a multidisciplinary approach, generating a highly detailed model of an average influenza virion that provided a basis for assessing natural variation.

By combining cryo-electron tomography, proteomics, lipidomics and molecular modelling, we reconciled measurements of virion morphology with the identity, quantity and structures of virion components. Our pseudoatomic model revealed a crowded membrane with a distinctive lipid composition and an interior densely packed with both viral and host proteins.

We next used our model to assess the effects of natural virion variation. First, we considered the range of influenza virion morphologies. We found that elongated 'bacilliform' virions have an equivalent surface area to spherical virions, but that transition from one to the other would constrain the viral genome complex and force it to become disordered. Consistent with this, we could not observe a '7+1' arrangement of genome segments in spherical virions. Second, we examined how influenza virions vary between hosts. We showed that the incorporation of some proteins, notably tetraspanins, varies drastically between hosts but that this variation does not compromise infectivity. Third, we examined changes over time. We found that as an infection progresses influenza virions package exponentially more of the immunosuppressive protein NS1.

Thus, by comparison to a detailed model of an average virion we showed that the same influenza virus produces different virions from different hosts, and at different times during an infection.

Session 2

INNATE IMMUNITY

Chair: Martin Schwemmle, Freiburg, Germany

Monday, September 03 2018

IFN-λ enhances influenza immunity by stimulating TSLP release during intranasal immunization
Ye Liang, Freiburg, Germany

Oncolytic influenza virus infection restores immunocompetence of lung tumor-associated alveolar macrophages
Dörthe Masemann, Muenster, Germany

Phosphorylation of TRIM28 enhances the expression of IFN-β and proinflammatory cytokines during HPAIV infection of human lung epithelial cells
Tim Krischuns, Muenster, Germany

An Infection-Triggered SUMO Switch Controls Induction of an Antiviral Program by TRIM28
Nora Schmidt, Zurich, Switzerland

Interleukin-1β paves the way for protective lung-resident memory T cells: implications for a universal flu vaccine?
Dennis Lapuente, Bochum, Germany

IFN-λ enhances influenza immunity by stimulating TSLP release during intranasal immunization

Ye Liang[1], Daniel Schnepf[1], Jan Becker[1], Karolina Ebert[2], Yakup Tanriver[2], Valentina Bernasconi[3], Hans Henrik Gad[4], Rune Hartmann[4], Nils Lycke[3], Peter Stäheli[1]

[1] Institute of Virology, Medical Center University of Freiburg, Germany
[2] Institute of Medical Microbiology, Medical Center University of Freiburg, Germany
[3] Department of Microbiology and Immunology, University of Gothenburg, Sweden
[4] Department of Molecular Biology and Genetics, Aarhus University, Denmark

Interferon-λ (IFN-λ) acts on epithelial cells and mediates innate antiviral protection of mucosal surfaces. Here we report that IFN-λ can also enhance adaptive immunity following infection of the respiratory tract. Mice deficient in IFN-λ signaling showed an impaired antibody response after influenza virus infection. We further found that subunit vaccines enriched with IFN-λ induced strongly enhanced IgG1 and IgA antibody responses in wild-type mice compared with IFN-λ-free vaccines if administrated by the intranasal route. No such adjuvant effect of IFN-λ was observed if the vaccines were administrated by the subcutaneous or intraperitoneal routes. IFN-λ triggered the synthesis of thymic stromal lymphopoietin (TSLP) in epithelial cells of the upper airways which targeted migratory dendritic cells and boosted antigen-dependent germinal center reactions in draining lymph nodes and spleen. The IFN-λ/TSLP axis not only induced strongly increased responses to influenza subunit vaccines but also enhanced survival after lethal virus challenge. Thus, IFN-λ plays an important role in potentiating adaptive immune responses which initiate in the upper airways and it has great potential to increase the effectiveness of mucosal vaccines.

Oncolytic influenza virus infection restores immunocompetence of lung tumor-associated alveolar macrophages

Dörthe Masemann[1], Katharina Köther[1], Meike Kuhlencord[2], Georg Varga[3], Johannes Roth[2], Brian Dennis Lichty[4], Ulf Rüdiger Rapp[5], Viktor Wixler[1], Stephan Ludwig[1]

[1] Institute of Molecular Virology, University of Muenster, Germany
[2] Institute of Immunology, University of Muenster, Germany
[3] Department of Pediatric, Rheumatology and Immunology, University Children´s Hospital Muenster, Germany
[4] Department of Pathology and Molecular Medicine, McMaster Immunology Research Centre, McMaster University, Hamilton, Canada
[5] Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany

The majority of all lung cancers belong to the subtype of non-small-cell lung carcinomas (NSCLCs), which are known to be insensitive to chemotherapy and radiation. Non-small-cell lung cancer is the most frequent type of lung cancer and demonstrates high resistance to radiation and chemotherapy. Additionally, these tumors develop a highly immunosuppressive tumor-microenvironment as an immune evasion mechanism. Genetic analysis has revealed oncogenic activation of the Ras/Raf/MEK/ERK signaling pathway to be a hallmark of NSCLCs, which promotes influenza A virus (IAV) infection and replication in these cells. Thus, we aimed to unravel the oncolytic and immunostimulatory properties of IAV infection against NSCLCs in an immunocompetent model in vivo. Using Raf-BxB transgenic mice that spontaneously develop NSCLCs based on lung-specific c-Raf onkogene expression, we demonstrated that infection with low-pathogenic IAV leads to rapid and efficient oncolysis, eliminating 70% of the initial tumor mass. Interestingly, IAV infection of Raf-BxB mice caused a functional reversion of highly immunosuppressed tumor-associated lung macrophages into a M1-like pro-inflammatory active phenotype that additionally supported virus-induced oncolysis of cancer cells. Altogether, our data demonstrate for the first time in an immunocompetent in vivo model that oncolytic IAV infection is capable of restoring and redirecting immune cell functions within the tumor microenvironment of NSCLCs, indicating that controlled infection with attenuated oncolytic IAV might be a potential approach for therapy of NSCLCs in patients.

Phosphorylation of TRIM28 enhances the expression of IFN-β and proinflammatory cytokines during HPAIV infection of human lung epithelial cells

Tim Krischuns[1], Franziska Günl[1], Lea Henschel[1], Marco Binder[2], Joschka Willemsen[2], Sebastian Schloer[3], Ursula Rescher[3], Vanessa Gerlt[1], Gert Zimmer[4], Carolin Nordhoff[1], Stephan Ludwig[1], Linda Brunotte[1]

[1] Institute of Molecular Virology, University of Muenster, Germany
[2] Division Virus-Associated Carcinogenesis (F170), German Cancer Consortium, German Cancer Research Center (DKFZ), Germany
[3] Institute of Medical Biochemistry, University of Muenster, Germany
[4] Institute of Virology and Immunology (IVI), Switzerland

Human infection with highly pathogenic avian influenza viruses (HPAIV) is often associated with severe tissue damage due to hyperinduction of interferons and proinflammatory cytokines. The reasons for this excessive cytokine expression are still incompletely understood, which has hampered the development of efficient immunomodulatory treatment options. The host protein TRIM28 associates to the promoter regions of over 13.000 genes and is recognized as a genomic corepressor and negative immune regulator. TRIM28 corepressor activity is regulated by post translational modification, specifically phosphorylation of S437, which modulates binding of TRIM28 to the heterochromatin-binding protein HP1. Here, we identified TRIM28 as a key immune regulator leading to increased IFN-β and proinflammatory cytokine levels during infection with HPAIV. Using avian- and human- derived influenza A virus strains as well as HPAIV, we could demonstrate that strain-specific phosphorylation of S473 is induced by a signaling cascade constituted of PKR, p38 MAPK and MSK1 in response to RIG-I-independent sensing of viral RNA. Furthermore, using chemical inhibitors as well as knockout cell lines, our results suggest that phosphorylation of S473 facilitates a functional switch leading to increased levels of IFN-β, IFN-γ and other cytokines. In summary, we have identified TRIM28 as a critical factor controlling excessive expression of type I and II IFNs as well as proinflammatory cytokines during HPAIV infection. In addition, our data indicate a novel mechanism of PKR-mediated IFN-β expression, which could lay the ground for novel treatment options aiming at rebalancing dysregulated immune responses during severe HPAIV infection.

An Infection-Triggered SUMO Switch Controls Induction of an Antiviral Program by TRIM28

Nora Schmidt[1], Patricia Domingues[1], Corinna Patzina[1], Filip Golebiowski[1], Michael H. Tatham[2], Ronald T. Hay[2], Benjamin G. Hale[1]

[1] Institute of Medical Virology, University of Zurich, Switzerland
[2] Centre for Gene Regulation and Expression, University of Dundee, United Kingdom

Dynamic post-translational modification of diverse proteins with SUMO (small ubiquitin-like modifier) is critical to orchestrate cellular recovery from genomic damage, proteotoxic stress and pathogen insult. Using a quantitative affinity proteomics approach, we surveyed the pan-viral host SUMOylation response and identified a spectrum of common and unique SUMO remodelling events that are mounted during influenza A and B virus infections, as well as during viral innate immune stimulation. Notable among common infection-triggered events was the complete loss of SUMO-modified TRIM28, a multifunctional host E3 ligase that acts as a SUMO-dependent transcriptional co-repressor and restriction factor for several endogenous and exogenous retroviruses, as well as some DNA viruses. Loss of SUMOylated TRIM28 during influenza virus infection did not correlate with stress-induced phospho-regulated proteasome-mediated degradation of TRIM28 or activation of canonical antiviral RNA sensors, but could be mimicked by the forced action of selected deSUMOylating enzymes, which are known redox stress-sensors. Using a CRISPR/Cas9-based knockout/reconstitution strategy, combined with system-wide transcriptomics, we found that deSUMOylated TRIM28 potentiates induction of the host antiviral interferon response during infection, and thereby acts to limit efficient influenza virus replication. Our data suggest that virus-triggered deSUMOylation of TRIM28 contributes to cellular innate immune defences by derepressing expression of host genes involved in the antiviral response.

Interleukin-1β paves the way for protective lung-resident memory T cells: implications for a universal flu vaccine?

Dennis Lapuente[1,2], Michael Storcksdieck genannt Bonsmann[1], Andre Maaske[1], Viktoria Stab[1], Vanessa Heinecke[1], Katarina Liedtke [1], Rebecca Heß [1], Astrid Westendorf [3], Wiebke Bayer[4], Christina Ehrhardt [5], Matthias Tenbusch [1,2]

[1] Department of Molecular and Medical Virology, Ruhr-University Bochum, Bochum, Germany
[2] Institute of Clinical and Molecular Virology, University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nürnberg, Germany
[3] Institute of Medical Microbiology, University Hospital Essen, University Duisburg-Essen, Essen, Germany
[4] Institute for Virology, University Hospital Essen, University Duisburg-Essen, Essen, Germany
[5] Institute of Molecular Virology, Center for Molecular Biology of Inflammation, Westfaelische Wilhelms-University Muenster, Muenster, Germany

Tissue-resident memory T-cells (TRM) are increasingly recognized as important, cross-reactive immune component in the protection against influenza A viruses (IAV). However, there is a lack of specific vaccine strategies to elicit potent TRM responses. Here, we evaluated vector-encoded IL-1β as genetic adjuvant in intranasal adenoviral vector immunizations against IAV.
First of all, we showed that IL-1β enhances antibody and T-cell responses, most pronounced in lung TRM populations. In consequence, vaccination with the adjuvant established superior protection against divergent H1N1, H3N2 and H7N7 infections. Specifically, a coordinated action of both CD4+ and CD8+ T-cells was required to mediate optimal cross-reactive immunity, but this protection was independent of circulating T-cells. From a mechanistic perspective, we demonstrated that IL-1β activates several essential checkpoints in the formation of lung TRM including (i) immediate infiltration of immune cells into the lung, i.e. TRM-priming CD103+ DCs, (ii) broad lung inflammation consisting of cytokines, chemokines and adhesion molecules and (iii) lung infiltration of TRM precursors with an increased expression of CD69 und CD103 but only in presence of local antigen. Bone marrow transfer experiments showed that IL-1β-induced TRM-development requires IL-1 receptor signaling in both stromal and hematopoietic cells but with stronger contribution of the latter ones. Importantly, analysis of respiratory parameters and lung barrier function showed no detrimental effects of the adjuvanted immunization.
Our data reveal the multifaceted effects of mucosal IL-1β on the induction of TRM and thus contribute to the basic understanding of local immunity which might pave the way for efficient TRM-inducing IAV vaccines.

Session 3

Evolution and Emerging Viruses

Chair: Stephan Pleschka, Gießen, Germany

Monday, September 03 2018

Keynote lecture: Influenza-like virsues from bats
Martin Schwemmle, Freiburg, Germany

Eurasian avian-like swine influenza viruses harbor increased pandemic potential due to MxA escape mutations in their nucleoprotein
Philipp P. Petric, Freiburg, Germany

Risk assessment of fifth-wave H7N9 influenza A viruses in mammalian models
Xiangjie Sun, Atlanta, USA

Evolution of influenza A virus nucleoprotein is influenced by the E3 ubiquitin ligase activity of the interferon-inducible Tripartite Motif (TRIM)22 protein
Elisa Vicenzi, Milan, Italy

Eurasian avian-like swine influenza viruses harbor increased pandemic potential due to MxA escape mutations in their nucleoprotein

Philipp P. Petric[1], Dominik Dornfeld[1], Ebrahim Hassan[1], Roland Zell[2], Martin Schwemmle[1]

[1] University Medical Center Freiburg, Germany
[2] Jena University Hospital, Germany

To cross the human species barrier, influenza A viruses (IAV) of avian origin have to overcome the interferon-induced host restriction factor MxA by acquiring distinct mutations in their nucleoprotein (NP). We recently demonstrated that North American classical swine IAV are able to escape MxA restriction partially. Here, we investigated whether the Eurasian avian-like swine IAV lineage currently circulating in European swine would likewise evade restriction by human MxA. We found that the NP of the isolate A/swine/Belzig/2/2001 (Belzig) exerts increased MxA escape similar in extent to human IAV NPs. Mutational analysis revealed that the MxA escape mutations in Belzig-NP differ from the known MxA resistance cluster of the North American classical swine lineage and human-derived IAV NPs. A mouse-adapted avian IAV of the H7N7 subtype encoding Belzig-NP showed significantly enhanced viral growth in both MxA-expressing cells and MxA-transgenic mice compared to control viruses lacking the MxA escape mutations. Similarly, growth of recombinant Belzig virus was only marginally affected in MxA-expressing cells and MxA-transgenic mice compared to Belzig mutant viruses lacking MxA escape mutations in NP. Phylogenetic analysis of the Eurasian avian-like swine IAV revealed that the NP amino acids required for MxA escape were acquired successively and were maintained after their introduction. Our results suggest that circulation of IAV in the swine population can result in the selection of NP variants with a high degree of MxA resistance, thereby increasing the zoonotic potential of these viruses.

Risk assessment of fifth-wave H7N9 influenza A viruses in mammalian models

Xiangjie Sun[1], Jessica Belser[1], Claudia Pappas[1], Joanna Pulit-Penaloza[1], Nicole Brock[1], Hui Zeng[1], Hannah Creager[1], Amanda Lewis [1], Wun-Jun Shieh [1], Thomas Stark[1], John Barnes [1], Terrence Tumpey [1], Taronna Maines [1]

[1] Centers for Disease Control and Prevention, Atlanta, GA, U.S.A

The fifth-wave of the H7N9 influenza epidemic in China was distinguished by a sudden increase in human infections, an extended geographic distribution, and the emergence of highly pathogenic avian influenza (HPAI) viruses. Genetically, some H7N9 viruses from the fifth-wave have acquired novel amino acid changes at positions involved in mammalian adaptation, antigenicity, and HA cleavability. In our study, several low pathogenic avian influenza (LPAI) and HPAI H7N9 human isolates from the fifth epidemic wave were assessed for their pathogenicity and transmissibility in mammalian models, as well as their ability to replicate in human airway epithelial cells. We found that a LPAI virus exhibited a similar capacity to replicate and cause disease in two animal species as viruses from previous waves. In contrast, HPAI H7N9 viruses possessed enhanced virulence, causing greater lethargy and mortality, with an extended tropism for brain tissues in both ferret and mouse models. These HPAI viruses also showed signs of adaptation to mammalian hosts by acquiring the ability to fuse at a lower pH threshold compared with other H7N9 viruses. All of the fifth-wave H7N9 viruses were able to transmit among cohoused ferrets but exhibited a limited capacity to transmit by respiratory droplets. Furthermore, deep sequencing analysis revealed that the H7N9 viruses sampled after transmission showed a reduced amount of minor variants, suggesting a potential purifying selection may take place during H7N9 transmission in ferrets. Taken together, we conclude that the fifth-wave HPAI H7N9 viruses have gained the ability to cause enhanced disease in mammalian models, and with further adaptation may acquire the ability to cause an H7N9 pandemic.

Evolution of influenza A virus nucleoprotein is influenced by the E3 ubiquitin ligase activity of the interferon-inducible Tripartite Motif (TRIM)22 protein

Elisa Vicenzi[1], Isabel Pagani [1], Andrea Di Pietro[1], Alexandra Oteiza[2], Nadir Mechti[2], Nadia Naffakh[3]

[1] IRCCS-Ospedale San Raffaele, Milan, Italy
[2] CNRS, UMR5235, DIMNP, University of Montpellier, France
[3] Pasteur Institute, Paris, France

Influenza A virus (IAV) continuously mutates under both intrinsic and immunologically-driven selections. We previously identified TRIM22 protein as a restriction factor of seasonal but not of pandemic (pdm) H1N1 viruses. As TRIM22 restriction is due to a direct interaction with the viral nucleoprotein (NP) leading to its ubiquitination and proteasome degradation, we evaluated the evolution of NP lysine (K) residues that are target of TRIM22 E3 ubiquitin ligase activity from 1918 to 2009.

By sequence alignment, we identified four arginine (R) residues in NP of 1918 pdmH1N1 at position 98, 293, 422 and 446. These four R residues were progressively replaced by K residues in seasonal H1N1 strains since 1936 and were present from 1977 to 2009 when four R residues were reintroduced in the 2009 pdmH1N1. Single R-to-K or K-to-R mutations of pdm and seasonal NP, respectively, did not result in either gain or loss of TRIM22 restriction activity as measured in a viral polymerase activity assay. However, the combination of four R-to-K or K-to-R substitutions determined either a progressive gain or loss of TRIM22-dependent restriction, respectively. Introduction of four R into seasonal NP of H1N1 reconstructed by reverse genetics resulted in a loss of TRIM22 restriction concomitantly with a loss of NP ubiquitination by this restriction factor.

Our present findings indicate that TRIM22 is a component of an innate immunity barrier against zoonotic introduction of IAV in humans and suggests that adaptive mutations in NP should be carefully monitored as part of a surveillance effort to predict the potential occurrence of future pandemic IAV infections.

Session 4

VIRAL REPLICATION

Chair: Thorsten Wolff, Berlin, Germany

Monday, September 03 2018

Phosphorylation of tyrosine 132 of influenza A virus matrix protein 1 is essential for efficient viral genome packaging and particle assembly
Angeles Mecate-Zambrano, Muenster, Germany

Kinetic analysis of the influenza A virus HA/NA balance reveals contribution of NA to virus-receptor binding and NA-dependent rolling on receptor-containing surfaces
Hongbo Guo, Utrecht, The Netherlands

Mutational analysis of the M2 ion channel proteins of avian and bat influenza A viruses
Gert Zimmer, Bern, Switzerland

Fusion peptides mediate influenza viral fusion via two sequential mechanisms
Peter Kasson, Charlottesville, USA

Phosphorylation of tyrosine 132 of influenza A virus matrix protein 1 is essential for efficient viral genome packaging and particle assembly

Angeles Mecante-Zambrano[1], Guiscard Seebohm[2], Darisuren Anhlan[1], André Schreiber[1], Lilo Greune[3], Stephanie Grothe[4], Nora Caroline Stein[4], Ludmilla Wixler[1], Alexander Schmidt[3], Klaus Langer[4], Simone König[5], Tianlai Shi[6], Stephan Ludwig[1], Yvonne Börgeling[1]

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[2] Institute for Genetics of Heart Diseases (IfGH), Department of Cardiovascular Medicine, University Hospital Muenster, Muenster, Germany
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[5] Core Unit Proteomics, University of Muenster, Muenster, Germany
[6] Immunology, Inflammation and Infectious Diseases (I3) DTA, Roche Pharma Research and Early Development, Roche Innovation Center Basel, Switzerland

Rapid development of resistance of influenza A viruses (IAV) to currently available drugs emphasizes the urgent need for novel therapeutics. The highly conserved matrix protein 1 (M1) is a master regulator of the virus life cycle and its multifunctionality is most likely regulated by posttranslational modifications. Phosphorylation of M1 tyrosine 132 (Y132) was previously suggested to be essential for virus fitness, as viruses carrying a mutation at this site could not be rescued. Based on overexpression data, it was hypothesized that this might be due to defective nuclear entry of M1. In the present study, we were able to rescue a virus mutant carrying an alanine at Y132 allowing for analysis of the role of this phosphorylation site during genuine infection.
WSN M1 Y132A showed strongly decreased viral replication compared to wild type. While we did not detect any reduced nuclear import, coarse M1 protein clusters were observed at the plasma membrane in late stages of infection. Interestingly, M1 Y132A association to membranes was not altered, but deeper characterization revealed a defect in M1 recruitment to IAV assembly sites in lipid raft domains, which resulted in a diminished structural stability of viral progeny and the presence of filamentous particles. Importantly, WSN M1 Y132A showed random defects in viral genome packaging, resulting in an increased production of non-infectious progeny.
These findings indicate that phosphorylation of M1 Y132 is crucial at late stages of IAV replication, and that efficient particle assembly including genome packaging is triggered by Y132 of the M1 protein.

Kinetic analysis of the influenza A virus HA/NA balance reveals contribution of NA to virus-receptor binding and NA-dependent rolling on receptor-containing surfaces

Hongbo Guo1, Huib Rabouw1, Wenjuan Du1, Meiling Dai1, Floor van der Vegt1, Ryan McBride2, James C. Paulson2, Raoul J. de Groot1, Frank J. van Kuppeveld1, Erik de Vries1, Cornelis A. de Haan1.

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Influenza A virus (IAV)-sialic acid (SIA) receptor interactions determine viral fitness and host tropism. The dynamics of binding, determined by a receptor-binding hemagglutinin (HA), a receptor-destroying neuraminidase (NA) and a complex in vivo receptor repertoire, are crucial but poorly understood. Biolayer interferometric analysis revealed virtually irreversible IAV binding to surfaces coated with synthetic sialosides or engineered sialoglycoproteins in the absence of NA activity, making equilibrium-binding models not applicable. Both HA and NA contributed to the initial binding rate. Extreme avidity resulting from multiple low-affinity HA-SIA interactions gave rise to a dynamic binding mode, in which NA activity was driving rolling of virus particles over the receptor surface until receptor density was sufficiently decreased to allow virus dissociation. Addition of competing receptors or antibodies also induced virus dissociation. Quantitative BLI analysis enabled functional examination of the HA/NA balance which governs this dynamic and motile interaction that is expected to be crucial for penetration of the mucus layer and subsequent infection of cells.

Mutational analysis of the M2 ion channel proteins of avian and bat influenza A viruses

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The matrix protein 2 (M2) is a multifunctional protein, which plays a crucial role in influenza A virus entry and egress. In this study, we investigated the role of M2 protein in the context of a glycoprotein (G)-deficient vesicular stomatitis virus (VSVΔG) encoding the three envelope proteins hemagglutinin (HA), neuraminidase (NA) and M2 of A/chicken/Rostock/8/34 (H7N1), a highly pathogenic avian influenza A virus. We found that M2 ion channel activity was essentially required to obtain an infectious chimeric virus, most likely because this preserves the native conformation of HA in the acidic milieu of the secretory pathway. VSVΔG(HA,NA,M2) virus was sensitive to amantadine, a well-known inhibitor of the ion channel protein. The mutation S31N in the pore-forming transmembrane domain rendered the virus resistant to amantadine, but also affected virus fitness. Analysis of a series of C-terminally truncated M2 proteins revealed that certain regions of the cytoplasmic domain are important for ion channel activity, while mutation of key amino acid residues in the amphipathic helix (F47A, F48A), in the cholesterol binding motif (Y52A, Y57A), and the acylation site (C50S) did not significantly affect M2 activity. The highly diverse M2 proteins of the recently discovered bat influenza viruses H17N10 and H18N11 were unable to rescue infectious VSVΔG(HA-NA-M2), suggesting that they do not have ion channel activity. However, a single amino acid change (N31S) was sufficient to recover infectious virus. In conclusion, VSVΔG(HA,NA,M2) proved to be a powerful vector system to functionally characterize the ion channel proteins of various influenza A viruses.

Fusion peptides mediate influenza viral fusion via two sequential mechanisms

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Cell entry by influenza virus is mediated by the hemagglutinin protein. Longstanding mutagenesis experiments indicate that, while refolding of hemagglutinin into a coiled-coil structure is required for fusion, intramembrane activity of the fusion peptides is also required. However, the mechanism for this and precisely how fusion peptides act within membranes to drive viral fusion has been elusive. Using a combination of single-virus fusion kinetics from fluorescence microscopy and molecular dynamics simulations, we are able to explain the activity of hemagglutinin fusion peptides via two sequential mechanisms, one primarily affecting fusion stalk formation and one affecting fusion pore formation. Additional data allows us to assign the deficiencies of commonly described fusion-peptide mutants to one or the other mechanism. This study for the first time yields atomic-resolution models of fusion pore formation by influenza and a predictive model for how fusion peptide mutants act.

Session 5

VACCINES & ANTIVIRALS

Chair: Oliver Planz, Tübingen, Germany

Tuesday, September 04 2016

Keynote lecture: Cytotoxic T lymphocytes to influenza virus: Cat and mouse
Guus Rimmelzwaan, Hanover, Germany

Bispecific Fc gamma receptor engaging molecules directed against the conserved viral M2 ectodomain protect against influenza A virus infections
Dorien De Vlieger, Ghent, Belgium

The stalk domain of influenza pH1 HA tolerates substitutions that may confer decreased susceptibility to broadly neutralizing antibodies
Alfred Ho, London, United Kingdom

Viral activation of the Raf/MEK/ERK kinase cascade promotes nuclear export of viral ribonucleoproteins (RNPs) by regulating matrix protein binding to the RNPs
André Schreiber, Muenster, Germany

Prodrugs of the Phosphoribosylated Forms of Hydroxypyrazinecarboxamide Pseudobase T-705 and its De-Fluoro-Analogue T-1105 as Potent Influenza Virus Inhibitors
Evelien Vanderlinden, Leuven, Belgium

Bispecific Fc gamma receptor engaging molecules directed against the conserved viral M2 ectodomain protect against influenza A virus infections

Dorien De Vlieger[1], Katja Hoffmann[2], Lien Van Hoecke[1], Inge Van Molle[3], Remaut Han[3], Hartmut Hengel[2], Bert Schepens[1], Xavier Saelens[1]

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Influenza virus infections cause 3 to 5 million cases of severe illness and 250 000 to 500 000 deaths each year. The best way to prevent disease is vaccination. However due to the short immune memory and antigenic drift in the viral hemagglutinin and neuraminidase protein, vaccines need to be administered yearly. Therefore, and as a measure against pandemic influenza outbreaks, antivirals are indispensable in the battle against influenza virus infections. Here, we describe the development of a new antiviral strategy based on the use of bispecific single domain antibodies (VHHs) termed BiFEs (Bispecific Fcg Receptor Engaging molecules), that can simultaneously bind an influenza A virus infected cell and an immune effector cell. The BiFEs were constructed by linking a VHH directed against the conserved ectodomain of the influenza M2 protein (M2e) to a second VHH directed against the mouse Fc gamma receptor I (FcgRI), mouse FcgRIV or human FcgRIIIa protein. BiFEs were recombinantly produced in Pichia pastoris. Using a newly developed cell-based activation assay, we demonstrated the specific and highly selective activation of individual FcgRs in the presence of the BiFEs and influenza A virus-infected cells. In addition, the BiFEs promoted phagocytosis of influenza-infected cells by macrophages. Importantly, BiFEs directed against M2e and mouse FcgRI or –RIV protected BALB/c mice against challenge with influenza X47 (H3N2) virus. These results, together with the ease of production in yeast and the high stability, demonstrate the potential of the BiFEs as a new antiviral treatment option for influenza virus infections.

The stalk domain of influenza pH1 HA tolerates substitutions that may confer decreased susceptibility to broadly neutralizing antibodies

Alfred Ho[1], Pramila Rijal[2], Alain Townsend[2], Leo Poon[3], Mike Skinner[1], Wendy Barclay[1]

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[3] The University of Hong Kong, Hong Kong

The highly-conserved influenza hemagglutinin stalk domain is a favoured target for broadly neutralising antibodies (bnAbs), which are heralded as a new class of therapeutic biologics that would inhibit many different strains and subtypes of influenza A viruses. Based on structural and bioinformatics analyses of multiple bnAb-HA co-crystal structures, we rationally designed influenza virus libraries altered in pdm09 H1 HA stalk epitope residues. Our work revealed that this region can accommodate a greater sequence diversity than previously thought, challenging prevailing dogma that the stalk domain is intransient.

We recovered 27 virus mutants, some of which are observed in natural isolates at low frequency. Both in MDCK cells and also in a stringent primary human airway epithelial (HAE) cell culture system, the amino acid mutations in stalk residues often did not incur any fitness cost. Our in-silico residue mutational scanning predicted at least ten mutants from our rescued mutant pool that may reduce or abolish bnAb binding. To investigate this further, we tested binding and neutralization of the mutants by a panel of bnAbs. Although there were no large-effect (>>10-fold) escape mutations, we found small-effect mutations that modestly decreased the neutralization of mutant virus by specific bnAbs. In summary, mutations which may confer escape from bnAbs are tolerated in the highly-conserved HA stalk, highlighting vulnerabilities in universal flu vaccines and viral therapeutics which need to be addressed.

Viral activation of the Raf/MEK/ERK kinase cascade promotes nuclear export of viral ribonucleoproteins (RNPs) by regulating matrix protein binding to the RNPs

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[1] Institute of Molecular Virology, University of Muenster, Germany
[2] Institute of Cell Dynamics and Imaging, University of Muenster, Germany

It was already shown that viral infection leads to the activation of a variety of signaling processes in the infected cells. Some of these activities are necessary for an efficient viral replication. The dependence of the Influenza A virus (IAV) on cellular signaling pathways leads to the opportunity of a novel antiviral strategy by targeting host factors that are essential for viral replication. It was already shown that viral infection induces the Raf/MEK/ERK kinase cascade for an efficient nuclear export of newly synthesized viral ribonucleoproteins (vRNP) and that this mechanism can be blocked with specific MEK-inhibitors. Such antiviral strategies are reducing the possibility of inducing viral resistance and enable a larger timeframe for a further antiviral treatment. However, the detailed mechanism how cellular kinases contribute to the nuclear export of the viral genome is still enigmatic. Here we shed first light on the mode of action by investigating the role of the Raf/MEK/ERK/RSK/MSK signaling pathway for the interaction of vRNPs with the viral M1 protein at the chromatin by using specific inhibitors against MEK (CI-1040), RSK (BI-D1870) and MSK (SB747651A) (Haasbach et al., (2017) Antiviral. Res. 2017 142:178-184).

Prodrugs of the Phosphoribosylated Forms of Hydroxypyrazinecarboxamide Pseudobase T-705 and its De-Fluoro-Analogue T-1105 as Potent Influenza Virus Inhibitors

Evelien Vanderlinden[1], Johanna Huchting[2], Matthias Winkler [2], Hiba Nasser [2], Lieve Naesens[1], Chris Meier[2]

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[2] Hamburg University, Germany

The nucleobase analogue T-705 (6-fluoro-3-hydroxy-2-pyrazinecarboxamide; favipiravir) is a unique antiviral drug possessing broad anti-RNA virus activity and a high barrier for resistance. When we compared the influenza virus inhibition by T-705 and its non-fluorinated analogue T-1105, the latter proved to be four-fold more potent in Madin-Darby canine kidney (MDCK) cells. In an enzymatic RNA elongation assay with influenza virus-derived viral ribonucleoproteins, T-1105 ribosyl 5'-triphosphate (RTP) was even six-fold superior to T-705 RTP (IC50 values: 0.48 µM vs. 2.7 µM) in inhibiting GTP incorporation into viral RNA.
We previously reported (Naesens et al., Mol Pharmacol 84, 615-629, 2013) that human hypoxanthine guanine phosphoribosyltransferase (HGPRT) is crucial to convert T-705 and T-1105 into their ribosyl-5'-monophosphates which are then further phosphorylated to the active RTP metabolites. Since both pyrazine derivatives are poor HGPRT substrates, we applied our DiPPro and TriPPPro prodrug approaches to increase the intracellular RTP levels.
We demonstrated efficient T-1105-RDP- and -RTP-release from the DiPPro- and TriPPPro-compounds by esterase activation. Using crude enzyme extracts, we saw rapid phosphorylation of T-1105-RDP into T-1105-RTP. In sharp contrast, phosphorylation of T-1105-RMP was not seen, indicating a yet unrecognized bottleneck in T-1105's metabolic activation. Accordingly, DiPPro- and TriPPPro-compounds displayed improved cell culture activity against influenza A and B virus, which they retained in HGPRT-deficient MDCK cells, indicating that they release a phosphoribosylated metabolite inside the cells. DiPPro-T-1105-RDP showed four-fold higher potency in suppressing one-cycle viral RNA synthesis versus T-1105. Hence, our T-1105-RDP- and -RTP-prodrugs improve antiviral potency and achieve efficient metabolic bypass.

Session 6

PATHOGENESIS

Chair: Peter Stäheli, Freiburg, Germany

Tuesday, September 04 2018

The 2nd sialic acid-binding site of influenza A virus neuraminidase contributes to the hemagglutinin-neuraminidase-receptor balance
Wenjuan Du, Utrecht, The Netherlands

Evaluation of the zoonotic potential of H18N11 virus variant tested in the ferret model
Marco Gorka, Greifswald – Isle of Riems, Germany

TMPRSS2 is the major HA-activating protease for IAV, but not for IBV in the human respiratory tract
Hannah Limburg, Marburg, Germany

The balance between intrinsic cellular innate, host innate and host adaptive immune responses matters for the outcome of influenza virus respiratory infection
Viktor Wixler, Muenster, Germany

The 2nd sialic acid-binding site of influenza A virus neuraminidase contributes to the hemagglutinin-neuraminidase-receptor balance

Wenjuan Du[1], Vera Nijman[1], Hongbo Guo[1], Jennifer Doedt[2], Zen Li[3], Geert-Jan Boons[3], Frank J.M. van Kuppeveld [1],Erik de Vries[1], Mikhail Matrosovich[2], Cornelis A.M. de Haan[1]

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It is generally accepted that the catalytic activity of influenza A virus (IAV) neuraminidase (NA) needs to match the receptor-binding activity of the corresponding hemagglutinin (HA) and the sialic acid (SIA)-receptor repertoire of the host. What this HA-NA-receptor balance entails at the molecular level is, however, not known. NA of avian, but not human viruses contain a 2nd SIA-binding site (2SBS), adjacent to the catalytic site, which contributes to sialidase activity against multivalent substrates. It is not known to what extent the 2SBS contributes to the HA-NA-receptor balance of virus particles. Here, we analysed the NA of 1957 H2N2 pandemic virus with and without a functional 2SBS (referred to as human and avian-like N2, respectively). Recombinant avian-like N2 was much more active than human N2, but only when multivalent substrates containing α2,3-linked SIAs were used, in agreement with the increased binding of this N2 to these receptors. When introduced in human H3N2 viruses, avian-like N2 resulted in altered plaque morphology and decreased replication when compared to human N2. The importance of the 2SBS for receptor binding of and cleavage by virus particles was analysed by kinetic bio-layer interferometry assays. A functional 2SBS contributed to virion-receptor binding and NA-dependent self-elution in a HA- and receptor-dependent manner. In conclusion, the 2SBS is an important determinant of the HA-NA-receptor balance. The rapid loss of a functional 2SBS in pandemic viruses probably served to balance the altered receptor-binding properties of the corresponding HA and host receptor-repertoire.

Evaluation of the zoonotic potential of H18N11 virus variant tested in the ferret model

Marco Gorka[1], Kevin Ciminski[2], Martin Schwemmle[2], Martin Beer[1], Donata Hoffmann[1]

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Influenza A viruses (IAV) are important zoonotic pathogens that cause epidemic outbreaks in birds, swine and other mammals. In 2012 and 2013 two 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. 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. Whether or not these viruses are able to infect further mammalian species including the model species for human influenza pathogenesis: the ferret is currently unknown. By reverse genetic techniques a H18N11 virus was generated. Passaging in vitro (canine cell culture) selected an H18N11 variant virus (rP11) with two mutations within the HA (K170R and N250S) and a stop codon in NA (G107X) protein. Ferrets were experimentally inoculated to check for the zoonotic potential of the variant virus. Viral genome was detected in the upper respiratory tract, lung and brain but transmission to ferrets in direct contact was excluded. All inoculated ferrets euthanized 7 days post infection or later seroconverted. Therefore, we assume, the variant virus is poorly adapted to ferrets and does have a low zoonotic potential, since low level of replication was obviously demonstrated.

TMPRSS2 is the major HA-activating protease for IAV, but not for IBV in the human respiratory tract

Hannah Limburg[1], Dorothea Bestle, Harshavardhan Janga, Leon Schulte, Hong Moulton, David A. Stein, Eva Böttcher-Friebertshäuser

[1] Institute of Virology, Philipps-University Marburg, Germany

Cleavage of influenza A virus (IAV) and influenza B virus (IBV) hemagglutinin (HA) activation by host proteases is essential for virus infectivity.
The HA of most influenza viruses including seasonal H1N1, H3N2 and IBV as well as the zoonotic-H7N9 virus is cleaved at a single arginine residue by trypsin-like proteases. We identified TMPRSS2 as a protease present in the human airways that activates HA with a monobasic cleavage site *in vitro*. Further studies by us and others have demonstrated that TMPRSS2 is essential for infectivity and pathogenesis of H7N9 and H1N1 in mice. In contrast, H3N2 and IBV activation and spread is independent of TMPRSS2 expression and due to so far unknown protease(s). These studies demonstrated that IAV and IBV with monobasic HA cleavage sites differ in their protease specificity in mice.
Here, we investigated the role of TMPRSS2 in activation and replication of different IAV subtypes H1-H16 and IBV in primary human bronchial (HBE) cells and alveolar type II cells (ATII) cells by knockdown of TMPRSS2 expression using the peptide-conjugated phosphorodiamidate morpholino oligomer (PPMO) T-ex5. T-ex5 treatment causes mis-splicing of TMPRSS2 mRNA and expression of a truncated inactive protease.
We found that knockdown of TMPRSS2 expression strongly suppressed proteolytic activation and spread of all tested IAV subtypes in HBE cells and ATII cells. In contrast, activation and replication of IBV was not affected by T-ex5 in the cells.
Our data suggest that TMPRSS2 is the major HA-activating protease for IAV, but not for IBV in the human respiratory tract.

The balance between intrinsic cellular innate, host innate and host adaptive immune responses matters for the outcome of influenza virus respiratory infection

Viktor Wixler[1], Dörthe Masemann[1], Rafael Leite Dantas[1], Siarhei Sitnik[1], Carolin Nordhoff[1], Ludwig Stephan[1]

[1] Institute of Molecular Virology, Westfaelische Wilhelms University (WWU), Muenster, Germany

After lung infection, influenza A viruses (IAVs) have to overcome at least three highly efficient immune defense barriers: the intrinsic cellular innate response, the host innate immune response and the host adaptive immune response. To circumvent these immune defense barriers and propagate successfully, influenza A virus proteins enter into a symbiosis with cellular proteins, altering host intrinsic pathways and gene transcription. The multifunctional adaptor protein FHL2 is a cellular protein with fine-tuning adjustment properties. It acts as a regulator of signaling cascades but also as a cofactor of transcription and controls several anti-inflammatory immune responses. Regarding IAV infection, FHL2 plays a dual role. By infecting FHL2 wildtype and knockout cells and mice, we showed that FHL2 restricted viral replication at early phases of infection but supported it at later phases. On the one side, FHL2 supported the IRF-3-dependent transcription of the Ifnb1 gene, accelerating thereby the intrinsic cellular innate response. On the other side, it restricted the migration of antigen-presenting CD11c+CD103+CD11b- dendritic cells from infected lungs into adjacent lymph nodes, decreasing thereby the recruitment of specific anti-viral CD3+CD8+IFNγ+ T lymphocytes into sites of inflammation. This effect of FHL2 was abrogated when RAG1KO mice lacking mature T lymphocytes were used.
Thus, a 1.5-fold stronger boost of the third defense barrier to influenza A virus infection in FHL2KO over wildtype mice was able to compete out the beneficial effect the influenza viruses originally had in FHL2KO mice due to the 10-fold weaker first barrier of cell intrinsic innate immune response.

Session 7

VIRUS HOST CELL INTERACTION

Chair: Klaus Schughart, Braunschweig, Germany

Tuesday, September 04 2018

Keynote lecture: The interplay between influenza polymerase and the host
Wendy Barclay, London, United Kingdom

Species comparisons identify avian ANP32A splice variants that differentially impact influenza A virus polymerase host restriction
Benjamin G. Hale, Zurich, Switzerland

LC3 punctae in IAV-infected cells do not represent double membrane autophagosomes but endosomes
Katherine Fletcher, Cambridge, United Kingdom

Decay accelerating factor as a virulence determinant in influenza A virus infection
Maria Joao Amorim, Lisbon, Portugal

Species comparisons identify avian ANP32A splice variants that differentially impact influenza A virus polymerase host restriction

Benjamin G. Hale[1], Patricia Domingues[1], Davide Eletto[1], Osvaldo Zagordi[1]

[1]University of Zurich, Switzerland

The viral RNA polymerase complex (vPol), comprising PB1, PB2 and PA, is essential for influenza A virus (IAV) replication. Cellular co-factors are necessary for vPol function, and host differences in these proteins act as barriers that limit IAV emergence into new species. ANP32A is a key host determinant of vPol efficiency that likely drives selection of mammalian-adaptive virulence motifs, such as PB2-627K: mammalian ANP32As lack a 33 amino-acid insert typically found in avian ANP32As, meaning they cannot support avian-motif (PB2-627E) IAV replication without viral adaptation. Here, we provide new insights into these selection mechanisms by functionally characterizing unique species' features of ANP32As from across the amniote clade of vertebrates. Surprisingly, our analyses suggest that inserts are a common component of both avian and crocodilian ANP32As, but that species-specific insert sequences restrict avian-signature vPol to using only the avian co-factor efficiently. We also uncovered that avian species express multiple ANP32A splice variants that differ only in insert sequence composition. Using deep sequencing, we found that chicken ANP32A harboring a 33 amino-acid insert is the predominant isoform normally expressed, but that minor insert variants with 29 or 0 amino-acids (mammalian-like, unable to enhance avian vPol activity) also exist. Strikingly, the 29 amino-acid insert lacks a hydrophobic SUMO-interaction motif (SIM)-like sequence that promotes vPol binding and is required for chicken ANP32A to fully support avian-signature IAV replication. We hypothesize that altered regulation of ANP32A splicing across species, tissues, or stress conditions could impact within-host IAV restriction and potentiation of pre-adaptation to non-avian co-factors.

LC3 punctae in IAV-infected cells do not represent double membrane autophagosomes but endosomes

Katherine Fletcher [1], Rachel Ulferts [2], Liam Lee[2], Mike Hollinshead[2], Suzanne Turner[2], Oliver Florey[1], Rupert Beale[2]

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Influenza A virus (IAV) infection causes accumulation of the autophagy protein LC3 at intracellular membranes and the plasma membrane. M2 directly interacts with LC3 and enhances its accumulation. However, the proton channel activity of the viral M2 protein is critical for LC3 lipidation. Thus it resembles LC3 lipidation in response to compounds that raise the pH of vesicles. Many pathogens encode ion channels, and some of these have been shown to affect LC3 lipidation. We propose that this phenomenon represents a novel cellular pathway detecting a 'danger' signal of abnormal pH – i.e. 'erroneous neutrality' - of intracellular vesicles.

It has been proposed that M2 prevents fusion of autophagosomes to lysosomes during IAV infection. We provide evidence that IAV-induced LC3-positive intracellular vesicles are not double-membrane autophagosomes, but endosomal single-membrane vesicles. The formation of these endosomes appears to be induced by the virus and – due to the deacidifying action of the viral M2 protein – these vesicles are targeted by a novel LC3-lipidation pathway.

We have recently shown that recruitment of the lipidation complex ATG5-ATG12/ATG16L1 in IAV-induced LC3-lipidation critically depends on the C-terminal WD40 domain of ATG16L1. This domain is dispensable for macroautophagy, but also required for lipidation complex recruitment in LC3-assisted phagocytosis and iononophore-induced LC3 lipidation. Additionally, essential macroautophagy factors such as the ULK-1 complex and phosphoinositol-3-phosphate, are dispensable for LC3-lipidation during IAV infection.

In summary, IAV-induced LC3-lipidation is clearly different to canonical autophagy in that it targets single membrane vesicles and relies on a distinct ATG16L1 recruitment pathway. To identify genes involved in this novel cellular pathway, we performed a whole genome CRISPR knock out screen. This screen confirmed that this pathway uses the canonical lipidation machinery but none of the upstream factors of canonical autophagy. Work on novel genes involved in this pathway will be presented.

Decay accelerating factor as a virulence determinant in influenza A virus infection

Maria Joao Amorim[1], Zoé Vaz da Silva, Nuno Santos[1]

[1] Instituto Gulbenkian de Ciência, Lisbon, Portugal

The complement is no longer considered a mere killer of infected cells and pathogens, but is viewed a key player in immunity. It bridges innate and adaptive responses, and orchestrates the intensity of immunological and inflammatory processes by communicating with immune cells. Interactions are beginning to be fully appreciated, and their identification is crucial, as excess complement activation is associated with severe outcomes in many infections. The complement must be selective enough to avoid mounting a potent attack against the host. The self-targeting deleterious effects of complement are avoided via a series of so called regulators of complement activation (RCA) whose function is perfect for a viral targeting. Amongst the RCAs complement decay-accelerating factor (DAF or CD55) and CD59 block the complement cascade at central and terminal points, respectively and localise ubiquitously at the apical surface of polarised cells. The lack of DAF and CD59 is associated with over-stimulation of complement resulting in increased inflammatory cytokines and worse outcomes in several models of infection and autoimmunity. We found that, conversely to observed in these models, in IAV infection the lack of DAF, but not of CD59 (used as control), mitigate the outcome of disease. Our results suggest a completely novel mechanism that bypasses the well-established immune evasion strategy of protecting virions from complement-mediate attack through incorporation of RCAs in their envelopes. In fact, our data shows that DAF deficient mice display less inflammatory signs in the lungs, and resolve the inflammation faster without affecting viral clearance. Mechanistically, we have evidence that DAF is recruiting monocytes by a process we are dissecting. Our results contribute to better define virulence factors in IAV infection and understand how components of the complement communicate with other arms of host immunity.

POSTER PRESENTATIONS

P1

Influenza: Similarities and Differences between Germany and Japan

Aziza Miriam Belkheir[1], Alexander Michael Englisch[1], Marco Gallus[1], Hirotaka Gambo[2], Hilke Maren Könemann[1], Monatsu Ota[2], Wataru Moriyama[2], Yusuke Shimizu[2], Ayumi Shinozaki[2], Wolfgang R. Ade[3], Jan Carl Becker[4], Stephan Ludwig[5], Michiaki Masuda[6], Jens Julian Storp[1], Hannes Sykora[1], Chisato Takahashi[2], Hikari Ueno[2], Takeshi Ukai[2], and Yuko Watanabe[2]

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[6]Department of Microbiology, Dokkyo Medical University, Mibu, Japan

Background and Objectives: Influenza is a common public health problem for both Germany and Japan affecting large populations every year. Since Japan was selected as a partner country for the 6th International Influenza Meeting held in Muenster, Germany, student delegates from both countries decided to conduct a joint research to compare Germany and Japan with regard to the social impact of influenza and how the influenza problems are handled.
Methods: Data were collected from web-based documents of the Robert Koch Institute of Germany and the Ministry of Health, Labor and Welfare (MHLW) and the National Institute of Infectious Diseases (NIID) of Japan. Influenza-related academic papers with a high evidence level were also retrieved.
Results and Discussion: We compared the influenza vaccination rates, the numbers of inpatients diagnosed as influenza, the numbers of influenza-associated excess deaths and the regular treatment protocols of Germany and Japan. While there are some similarities in the epidemiology of and clinical approaches to influenza in both countries, there are also aspects unique to each country possibly based upon differences in the cultural background and the health insurance systems. Since the 2009 pandemic, more attention seems to have been paid to influenza in Germany and Japan. Further comparison and analysis of the influenza prevention and control strategies in these countries may provide useful insights into the development of better countermeasures against local, as well as global, influenza outbreaks.

P2

HIGHLY PATHOGENIC H5N1 INFLUENZA A VIRUS SPREADS EFFICIENTLY IN HUMAN PRIMARY MONOCYTE-DERIVED MACROPHAGES AND DENDRITIC CELLS

Veera Westenius[1], Sanna M. Mäkeläa[1], Ilkka Julkunenb[2], Pamela Österlund[1]

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As a zoonotic reservoir, avian-origin influenza A viruses pose a constant threat to humans. Two current virus strains with pandemic potential, H5N1 and H7N9, are both highly pathogenic in humans with mortality of 60% and 40%, respectively. To characterize the highly pathogenic influenza virus infection in human monocyte-derived macrophages and dendritic cells (DCs) weused human isolates of highly pathogenic H5N1/2004 and H5N1/1997 and low pathogenic H7N9/2013 avian influenza viruses in comparison with a seasonal H3N2/1989 virus. We noticed that the H5N1 viruses have an overwhelming ability to replicate and spread in primary human immune cell cultures, and even the addition of trypsin to induce the efficient cleavage of HA did not equalize the infectivity of H7N9 or H3N2 viruses to the level seen with H5N1 viruses. H5N1 virus stocks contained more often propagation-competent viruses than the H7N9 or H3N2 viruses, and human DCs and macrophages maintain 1 000 and 10 000 –fold increase in the production of infectious H5N1 virus, respectively. Both analyzed highly pathogenic H5N1 viruses showed multicycle infection in human DCs and macrophages whereas the H3N2 and H7N9 viruses were incapable of spreading in immune cells. Interestingly, the vast replication of the H5N1 virus greatly enhanced the cytokine gene expression ("cytokine storm"), which may in part explain the high pathogenicity of H5N1 virus infection in humans.

P14

Prolonged evolution of virus-specific memory T cell immunity post severe avian influenza A (H7N9) virus infection

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Since 2013, influenza A/H7N9 has emerged as the commonest avian influenza subtype causing human infection, and is associated with a high fatality risk. However, the characteristics of immune memory in patients who have recovered from H7N9 infection are not well understood. We assembled a cohort of forty-five H7N9 survivors and followed for up to 15 months after infection. Humoral and cellular immune responses were analyzed in sequential samples obtained at 1.5-4 months, 6-8 months and 12-15 months post-infection. H7N9-specific antibody concentrations declined over time while frequencies of virus-specific IFN-γ, IL-2 or TNF-α secreting T cells were higher at 12-15 months post infection than at earlier time points. Elevated levels of antigen-specific CD8+ T cells expressing lung-homing marker CD49a were observed at 6-8 months after H7N9 infection compared to samples obtained at 1.5-4 months. Our findings indicate the prolonged reconstruction and evolution of virus-specific T cell immunity, and provide implications for T-cell directed immunization strategies.

P15

Filamentous morphology of recombinant A/WSN/33 (H1N1) virions detected with novel electron microscopy protocols

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Pleiomorphic morphology (spherical, ellipsoidal, filamentous or irregular) of virions is one of the characteristics of Influenza virus. Filamentous phenotype is thought to be favorable for virus transmission in nature. As was shown for several virus strains, it is supported by specific amino acid changes in the matrix M1 protein and might be influenced by some host cellular components. The method of the sample preparation for the electron microscopy analysis might also change the virions shape. Finally, it is not known whether the recombinant virus produced from eight plasmids via the wide-spread reverse genetics technique demonstrate quite the same morphology as the native virus or the laboratory strain. Now we compare the morphology of recombinant A/WSN/33 (H1N1) virus grown in various hosts with that of the laboratory strain. We analyzed both wild type recombinant virus and two M1 mutants. To avoid the virus morphology distortion, we used a number of sample preparation protocols including (1) gentle concentration of virions by low speed filtration of the virus-containing allantoic fluid/ cell culture medium through Amicon 100K filters or (2) fixation of virus particles within the allantoic fluid/ cell culture medium with glutaric aldehyde before ultracentrifugation. We found that (1) the M1 of control recombinant virus contains two amino acid substitutions, Ser126Cys and Ile219Val, compared to the Flu database and laboratory strain. This control recombinant virus gathered in the mixed HEK 293T/ MDCK cell culture and further passaged twice in embryonated chicken eggs demonstrated 11% filamentous phenotype (the remainder particles are spherical/ellipsoidal); (2) the control recombinant virus collected from MDCK cells instead of chicken embryos is 98% spherical; (3) the reverse substitutions Cys126Ser/Val219Ile in M1 got via site-directed mutagenesis raised the portion of filamentous particles till 22% if the virus was collected from chicken embryos; (4) an amino acid substitution Ala209Thr in M1 decreased the portion of filamentous particles accumulated in chicken embryos till 3%. In conclusion, (1) the pleiomorphic morphology of recombinant influenza virus is affected by both the M1 protein sequence and combination of some host components; (2) the recombinant A/WSN/33 (H1N1) virus may demonstrate (partially) filamentous phenotype in contrast to the well-known "spherical" (over 98%) phenotype of A/WSN/33 (H1N1) laboratory strain; (3) the amino acid substitutions Ser126Cys/ Ile219Val observed in the M segment of recombinant virus are not the reason of the recombinant virus abnormal phenotype compared to the laboratory strain.

P17

Staphylococcus aureus and influenza virus: interaction of pathogens requires interplay of cells via alveolus-on-a-chip model

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Background and objectives
Postinfluenza models of Staphylococcus aureus pneumonia demonstrate the severe outcome of a coinfection associated with substantial morbidity and mortality for patients. To date, investigations concerning microbial infections of the lung are usually carried out in animal models. However, lung anatomy and physiology as well as composition of the immune system differ significantly between rodents and men.
To investigate cell processes between epithelial, endothelial and immune cells after influenza virus/ S. aureus coinfection, we aimed to establish a human alveolus-model recreating a reactive tissue-tissue interface between the vascular endothelium and the airway-facing epithelium.
Methods/Results
For this reason, MOTiF biochips were seeded with human endothelial cells on the vascular site and with epithelial cells and macrophages on the airway site (Figure 1). This organoid was cultured for up to 14 days with a robust and stable air-liquid interphase under dynamic flow conditions. Barrier integrity was proven by transepithelial electrical resistance (TEER) measurements and permeability assays. Expression and localization of cell-type specific markers and functional proteins was proven by immunofluorescence. Viral and bacterial infection occurs trough airway site with further designation of acute phase of invasion and early immune response thereafter up to 8 hours, depending of multiplicity of infection (MOI).
Dynamic conditions for maintaining ALI allow a stable barrier with high transepithelial resistance and an intact vascularity. We will provide evidence for an increase of barrier integrity after introduction of macrophages proven by TEER measurement and permeability tests. Our data indicate an stable surfactant production of alveolar epithelial cells type II. Subsequent infection has been successfully established and pathogenicity factors can be investigated.
Conclusions
We established a functional, biochip-based human in vitro alveolus model that is suitable for investigation of complex co-infections. Separated airway and vascular chambers allow an infection with a pathogen from the airway site. Thereby inducing an immune response, it is possible to observe migration of immune cells from the vascular site into the infected sites to study species-specific mechanism of pathogens.
Figure 1: Schematic composition of the alveolus model. The cavity in the chip is divided by a porous membrane into an upper and a lower chamber. The membrane serves as a scaffold for cells. Vascular endothelial cells are cultured beneath the membrane in the lower chamber. Alveolar epithelial cells and macrophages are co-cultured on top of the membrane. Nutrient and oxygen supply is achieved by perfusion of the vascular chamber. After reaching confluency cells are co-cultured at an air-liquid-interphase for up to 14 days.

P18

Host responses to influenza virus infection in the peripheral blood are similar in mice and men

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Influenza virus (IV) infections represent a very serious public health problem. At present, no established biomarkers exist to support diagnosis for respiratory viral infections. Mouse human cross-species comparisons are often compromised by the fact that animal studies concentrate on the infected lungs whereas in humans almost all studies use peripheral blood from patients. In addition, human studies do not consider genetic background as variable although human populations are genetically very diverse. Therefore, we performed a cross-species gene expression study of the peripheral blood from human patients and from the Collaborative Cross (CC) mouse population after IV infection. The recently established CC is a mouse genetic reference population derived from eight genetically different founder strains including classical lab strains as well as mouse models for human diseases like diabetes and obesity. In addition, three wild-derived strains strongly enhance the genetic diversity. Inbreeding for more than 20 generations has led to the CC strains whereby their genetic diversity is similar to that of the human population. We demonstrate that changes of gene expression after influenza infection in individual genes are highly similar in mice and humans. The top-regulated genes in humans are also differentially regulated in mice. We conclude that the mouse is a highly valuable *in vivo* model system to validate and to discover candidate genes, which can be used as biomarkers in humans. Furthermore, mouse studies allow confirmation of findings in humans in a well-controlled experimental system to understand the function of human candidate genes.

P19

Detection and characterisation of influenza virus RNA polymerase dimers using Bimolecular Fluorescence Complementation (BiFC)

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Influenza virus encodes a heterotrimeric RNA-dependent RNA polymerase (RdRP), composed of subunits PB1, PB2 and PA. The RdRP carries out both transcription and replication of the viral RNA genome segments in the context of ribonucleoproteins (RNPs). Replication of negative-sense viral RNA is a two-step process, progressing via a positive-sense complementary RNA intermediate. The mechanism of viral genome replication is mostly unknown, though there are multiple reports indicating RdRP-RdRP interactions may be central for the process. Purified RdRPs from human and avian influenza A viruses both form dimers of heterotrimers in solution. Using a combination of X-ray crystallography and SAXS analysis our group has identified the interface involved in RdRP dimerization, which is primarily located on the PA C-terminal domain. We establish a bimolecular fluorescence complementation (BiFC) assay to monitor intermolecular interactions between RdRPs in cells expressing viral RNPs. Using this system we confirm the existence of RdRP dimers in the context of actively replicating RNPs. Mutating amino acid residues at the identified dimer interface causes loss dimerization and inhibition of RNA replication in minigenome assays. These data suggest that dimerisation of RdRP via the PA C-terminal domain is important for replication of the viral RNA genome.

P26

Antigenic properties of the recent human influenza A (H3N2)viruses isolated in MDCK and MDCK-Siat1 cells

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Keywords: influenza, A(H3N2), etiology, virus isolation, antigenic analysis
METHODS: Virus isolation in MDCK and MDCK-Siat1 cells, identification, antigenic analysis in HI(hemagglutination inhibition) and MN (microneutralization) assays with the panel of rat and ferret polyclonal- post-infection antisera,
RESULTS: During the epidemic season 2016-2017 influenza A(H3N2) viruses were dominant among the influenza A and B viruses that were derected in Saint-Petersburg. rt-PCR diagnostics of influenza indicate that A(H3N2) viruses comprised 55%, A(H1N1)pdm09 - 0,8%, influenza B Victoria viruses – 36%, B Yamagata lineage– 0,2%, A unsubtyped - 8%. 150 samples were chosen for virus isolation in two cell cultures in parallel. Overall, 107 strains were isolated in MDCK cells and 116 strains in MDCK-Siat1 cells.
In recent years influenza A(H3N2) viruses isolated either in MDCK or MDCK-Siat cells are difficult to characterize in HI assay due to their week or absent ability to agglutinate human RBCs. All isolated strains were titrated with human RBCs in the presence of 20nM Oseltamivir carboxylate. 11,1% of the viruses isolated in MDCK-Siat1 cells and 30.6% MDCK- variant viruses did not show the drop in titre in the presence of oseltamivir added to circumvent NA-mediated binding to the RBCs. However, 29,6% MDCK-Siat1 viruses and 18,1% MDCK viruses had 2-fold drop titre; 4-fold drop titre was registered for 18,5% MDCK-Siat1 viruses and 14,3% MDCK strains.
Strains isolated in parallel were subjected to the comparative antigenic analysis in HI-assay with 20nM oseltamivir carboxylate or MN-assay. HI-assay was carried out for the strains, which had sufficient titre in the presence of 20 nM Oseltamivir carboxylate. Viruses isolated from the same samples in MDCK-Siat1 or MDCK cells have shown similar results in HI assay. All analyzed strains were recognized by the antisera raised against A/Hong Kong/4801/14 (MDCK isolate) and A/St. Petersburg/80/2014 at the titres within 1 to 4-fold of homologous titre and were not recognized by the antisera raised against strains of 3C.3a group - A/ Switzerland/9715293/13 (CE) and A/Stockholm/06/14 (CE).
Conclusion: Antigenic properties of recent human influenza A(H3N2) viruses do not differ between the strains isolated in MCK-Siat1 or MDCK cells.

P28

Influenza winter 2017/2018 winter season in Umbria (Italy): influenza virus circulation and vaccine immunogenicity

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The 2017/2018 winter season was characterized by a high and persistent influenza virus activity. A co-circulation of A/H3N2, A/H1N1 and B Influenza viruses was observed and their genetic and antigenic characterization induced WHO to recommend the inclusion of new strains of influenza A/H1N1 and B viruses in the 2018/2019 influenza vaccine.
We studied the 2017/2018 influenza virus circulation in Umbria, a little Italian region, examining 131 throat swabs. Eighty-five (65%) were positive: 38 A/H1N1, 4 A/H3N2 and 42 B influenza viruses. Lineage determination (genetic and antigenic tests) of some of the circulating B influenza virus evidenced that they were mainly B/Yamagata not included in 2017/2018 trivalent vaccine (TIV). Vaccine immunogenicity was examined in 38 elderly people (mean age 85 years, range 65-98) living in a nursing home after 2017/2018 TIV administration. Although high pre-vaccination haemagglutination inhibiting (HI) titers were found before vaccination against all the 3 vaccine antigens, the vaccine was able to induce in most instances significant antibody titer increases evaluated as protective titers (HI ≥40) and geometric mean titers (GMT) satisfying at least 2 of the 3 the European Medicine Agency criteria. Moreover we examined the possibility of TIV induced B cross-lineage protection, comparing HI titers against the vaccine B antigen (B/Victoria/lineage) and against different B/Yamagata/lineage strains (the new B antigen for 2018/2019 vaccine (B/Phuket/3073/2013) and 4 Yamagata-like strains isolated in Umbria). A moderate ability of influenza vaccine with a B/Victoria component to enhance antibodies against B/Yamagata-like viruses was observed, however the responses were lower and less satisfactory. Similar results were found examining neutralization antibody titers.
Our results evidenced the ability of 2017/2018 TIV of inducing satisfactory response in elderly institutionalized people. Moreover we found a moderate ability of 207/2018 influenza vaccine containing B/Victoria component to enhance antibodies against circulating B/Yamagata-lineage viruses. These data support the 2017/2018 interim vaccine efficacy against influenza B/Yamagata observed in a Spanish and in a European multi-country study (Euro Surveill.2018;23(9):pii=18-00086), underlying the opportunity of increasing the very limited use of influenza quadrivalent vaccines, containing both B/Victoria and B/Yamagata-lineage strains

P29

Reassortment of internal protein-encoding gene segments of Eurasian H9N2-type avian influenza A virus (IAV) with co-circulating highly pathogenic H5N1- and H5N8-type IAV strains: Impact on viral replication and pathogenicity in mammalian systems in vitro and in vivo

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Humans are susceptible to infection with influenza A, B and C viruses. Influenza A viruses (IAV) represent worldwide circulating pathogens that cause annual epidemics and occasionally worldwide pandemics, infecting millions of people. In parallel, several high pathogenic avian influenza viruses (HPAIV) and low pathogenic avian influenza viruses (LPAIV) have (occasionally) crossed the species barrier from birds to mammals/humans upon genetic reassortment (frequently with LPAIV/H9N2 strains) and/or adaptive mutations. This was observed for HPAIV/H5N1, HPAIV/H5N6, LPAIV/H6N1, LPAIV- and HPAIV/H7N9, LPAIV/H9N2, and LPAIV/H10N8, which have successfully infected humans since 1997 causing sporadic infections and/or fatalities. Increasing evidences show establishment of stable lineages of HPAIV/H5N1, HPAIV/H5N8 and LPAIV/H9N2 viruses in chickens worldwide - especially Egypt (EGY) and Germany (GER). This raises concerns that reassortment among these three highlighted strains could generate novel viruses with the ability to cross the species barrier to mammals. For early risk estimation we investigated the impact of genetic exchange between intensively circulating LPAIV/H9N2(EGY/GER) and HPAIV/H5N1(EGY) or HPAIV/H5N8(GER). Based on our results we discuss the influence of specific reassortments on the zoonotic potential of Egyptian HPAIV/H5N1 and German HPAIV/H5N8 in mammals in vitro and in vivo.

P30

The HA cleavage is necessary for the M2 channel activation in influenza A virus

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Non-infectious influenza A virus possessing uncleaved HA0 is activated by trypsin via the HA0→HA1/2 cleavage. The cleaved HA1/2 provides virus entry into target cell where the intravirion acidification through the M2 channel plays important role. We have suggested that HA0 plugs the M2 channel and its cleavage into HA1+HA2 by trypsin is responsible for a functional priming of M2 channel [Zhirnov et al. Virology, 492:187-196; (2016)]. After this publication, Dr. Petr Chlanda has proposed an alternative and interesting idea that trypsin can cleave and activate both the HA0 and the M2 in parallel [P.Chlanda, Virology, 509: 131-132 (2017)]. Moreover, the M2 molecule is known to have two Arg residues in the N-terminal exomembrane domain, as potential trypsin targets. To test this idea experimentally, we treated noninfectious HA0 virus with increasing concentrations of trypsin and monitored changes in virus infectivity and in electrophoretic patterns of M2, HA0, and HA1/2 by western-blot analysis. Virus activation was revealed to arise to maximum levels already at low and mild trypsin concentrations (0,5-10 μg/ml) in parallel with the HA0→HA1/2 cleavage. In contrast to the HA0 cleavability, the M2 was found to be noncleavable and resistant to trypsin even at concentrations as high as 25 μg/ml and more. This clear link between the HA0→HA1/2 cleavage and virus activation at the intact uncleaved M2 protein compromises the Chlanda's idea of the M2 independent proteolytic activation. By the other side, these data support our concept that (i) uncleaved HA0 plays down-regulatory role for the M2 ion channel in virus particle and (ii) the HA0 proteolytic transition to HA1/2 is a prerequisite for the M2 channel functional priming prior to its activation by low pH that is critically important for virus uncoating process.

P31

A novel negative-strand polarity protein NSP of influenza A virus in infected mice

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Influenza A virus belongs to enveloped viruses with a negative polarity RNA genome consisting of 8 RNA segments, which transcribe a template synthesis of positive-sense mRNAs translating 14 unique viral proteins with splicing and translational shift mechanisms in some mRNAs. Through this classical negative polarity pathway, the 8'th NS segment encodes the anti-interferon NS1 protein (mw 27 kDa) and the nuclear export NEP protein (14 kDa). Surprisingly, an alternative open reading frame (ORF) for the synthesis of the third viral protein (NSP – "negative-strand polarity protein"; m.w. 17-25 kDa in different virus strains), was identified in the NS segment/ This ORF suggests an additional positive polarity genome strategy for influenza A virus. Earlier it was shown that full-length virion polarity RNA segment NS of influenza A virus initiated synthesis of the NSP protein in vitro translation system derived from rabbit reticulocyte, indicating a messenger function of the virion RNA [Zhirnov et al. 2017; Dokl Biochem Biophys.,473(1):122-127]. Whether the NSP protein can be synthesized in the whole body under infection with influenza A virus is not yet known. Here, in order to test this idea, the formation of lymphocytes specific to this protein was studied in mice after sequential infections with influenza A viruses H1N1 and H3N2. The formation of T-lymphocyte specific clones recognizing a peptide domain in the central region of the NSP protein (amino acid positions 81-119) was found to develop in mice infected with influenza A viruses. These observations additionally confirm that expression of the NSP gene and synthesis of the corresponding protein occur in the animal organism under infection with influenza A virus. The obtained data further support the concept that RNA genome of influenza A virus has a bipolar (ambisense) strategy.

P33

TRIM 25 nuclear translocation is hampered by localisation to influenza A virus non-structural protein 1 cytosolic aggregates

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Recently, research efforts focus on the role of viral RNA species in the context of host antiviral signalling inhibition. Imaging studies give invaluable insights into complex RNA-protein and protein-protein interactions visualized directly at their side of action.
Among the IAV proteins the non-structural protein 1 (NS1) of influenza A virus (IAV) is described to be involved in manifold processes aiming the blockage of host defence. The interaction with double-stranded RNA and /or RNA helicase, retinoic acid inducible gene I (RIG-I), impedes the activation of transcription factors initializing antiviral signalling cascades. This block is enhanced by interacting with tripartite motif containing protein 25 (TRIM25), an ubiquitin ligase which is a mediator of innate virus recognition through the interferon regulatory factor 3 (IRF3) pathway. We found TRIM25 accumulation on NS1 cytosolic aggregates which results in diminished levels of nuclear TRIM25, which is required for the block of viral RNA elongation.
Applying confocal and super-resolution fluorescence microscopy and immunoprecipitation our findings revealed strain specific characteristics of NS1 cytosolic aggregation and confirmed localisation of TRIM25 to these structures at late stages of viral replication when comparing WT IAV and recombinant IAV lacking NS1 expression.
With the help of a sophisticated imaging approach we were able to detect IAV vRNA and TRIM25 at nanoscopic resolution combined with the diffraction limited detection of NS1 for the first time. Contrary to the postulated interaction between TRIM25 and IAV vRNA, our results evince IAV vRNA not being involved into the TRIM25-NS1 cytosolic complex in Panama/2007/1999 infected A549 cells at 16 hours post infection.

P36

Inhibition of RIPK3-dependent cell death as immune antagonism

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The risk of emerging pandemic influenza A viruses (IAV) that approach the devastating 1918 strain motivates finding strain-specific host-pathogen mechanisms. During infection, dendritic cells (DC) mature into antigen-presenting cells that activate T cells, linking innate to adaptive immunity. DC infection with seasonal IAVs, but not with the 1918 and 2009 pandemic strains, induces global RNA degradation. Here we show that DC infection with seasonal IAV causes immunogenic RIPK3-mediated cell death. Pandemic IAV suppressed this immunogenic DC cell death. Only DC infected with seasonal IAV, but not with pandemic IAV, enhanced maturation of uninfected DC and T cell proliferation. In vivo, circulating T cell levels were reduced after a pandemic, but not seasonal, IAV infection. Using recombinant viruses, we identified the HA genomic segment as the mediator of cell death inhibition. These results identify a novel process of pandemic virus subversion of the immune response.

P37

Manipulation of cellular lipid balance as a potent antiviral target for enveloped viruses

Jonas Goretzko, Sebastian Schloer,

The release of viral genome into the host cytoplasm and its translocation to the nucleus is strongly dependent on the fusion of Influenza A virus (IAV) envelope with host endosomal membranes. Recent publications have already delineated the importance of cellular cholesterol levels on the infectivity of IAV, as interferons (IFN) are also impairing cholesterol levels and its cellular distribution in endosomal/lysosomal (LE/L) compartments. Here, we analyzed the antiviral virility of itraconazole (Itra) and posaconazole (Posa) in the context of IAV and vesicular stomatitis virus (VSV) infection. Both compounds are well-known antifungal agents affecting cholesterol levels in host membranes. By increasing the cholesterol levels in LE/L Itra and Posa impair virus entry. Treatment either with Itra or Posa inhibits a very early step in IAV life cycle, namely prior to the transport of vRNPs to the nucleus. Because the IFN system is a crucial factor in limiting virus infection and also affecting LE/L cholesterol distribution, we further checked the antiviral potency of both compounds in three different mammalian cell lines including Vero cells, lacking an IFN-system. Strikingly, viral replication was also reduced in Vero cells treated with Itra or Posa suggesting that the antiviral effect is not only caused by modulating the IFN system, but could be improved in the presence of an IFN system as gene expression analysis of A549 cells revealed. We further elucidated the antiviral potency of Itra against IAV infection in mice. Intra-gastral application of Itra significantly reduces mortality and viral burden in the respiratory tract of IAV-infected mice. Our results established a protective function of Itra and Posa against IAV by impairing the LE/L cholesterol balance and by modulating the IFN system.

P38

Mutations in conserved NA residues of H5N1 naturally isolated from humans modulated sialidase activity and virulence in mice but not in chickens

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HPAIV H5N1 of clade 2.2.1 is endemic in Egypt since 2006 and 2 distinct clades have evolved: clade 2.2.1.1 in commercial poultry and clades 2.2.1.2 and 2.2.1.2a in humans and poultry. Compared to the neuraminidase (NA) of the parental 2.2.1 viruses, avian viruses in clade 2.2.1.1 possessed one mutation (I168T) and human-like viruses in clades 2.2.1.2 and 2.2.1.2a had 4 mutations (A46D, L204M, S319F and S430G) and 16 mutations, respectively. Here, recombinant 2.2.1.2a viruses carrying different NA resembling those in clade 2.2.1, 2.2.1.1 or 2.2.1.2 or single mutations were generated. In vitro, no or minimal impact on replication in cell cultures, plaque size, cleavability, receptor binding activity (RBA) and oseltamivir resistance was observed. Viruses with human-like NA had significantly lower NA activity than viruses with avian-like NA. Reduced NA activity of 2.2.1.2a was due to L204M. Insertion of L204M in H1N1, H5N1 and H7N1 viruses also reduced the NA activity. Over 97% (n=8053) of NA sequences in the GenBank possessed L204. All inoculated chickens died within 3 dpi. In mice, virus with L204M exhibited lower virulence and did not kill all animals, whereas S319F and S430G increased the virulence without remarkable difference in the cellular immune response. Together, H5N1 viruses in humans acquire NA mutations to maximize fitness in mammals without impact on replication in poultry.

P39

High virulence of a natural H4N2 avian influenza virus with a polybasic cleavage motif after mutation in the hemagglutinin or reassortment with highly pathogenic H5N1 virus

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According to the definition of the OIE, highly pathogenic (HP) avian influenza viruses (AIVs) are any AIV with an intravenous pathogenicity index (IVPI) ≥1.2, or H5 and H7 viruses with a polybasic cleavage site (CS) in the hemagglutinin (HA). However, according to this definition non-H5/H7 viruses with a polybasic CS, which were rarely reported from nature, are classified as low pathogenic (LP). In 2012, an H4N2 virus with a polybasic CS (322PEKRRTR/G329) and IVPI of 0 was isolated from quails in California. Here, we investigated the virulence of this virus after (1) insertion of point mutations in the CS, (2) reassortment with HPAIVs and (3) passaging in embryonated chicken eggs. All gene segments of H4N2 virus were cloned and threonine at position 327 in the CS was changed either to arginine (R327) or lysine (K327). Beside the wild-type (wt) virus, a mutant possessing the CS of a HP H5N2-strain and viruses carrying R327 or K327 in the CS with or without gene segments from HPAIVs A/swan/Germany/R65/2006(H5N1) or A/chicken/Germany/AR1385/2015(H7N7) were generated. Chickens were inoculated via intravenous (IV) or oculonasal (ON) routes. HPAIV H5N1 carrying either the wildtype H4 HA or variants at position 327, and the wt virus after 20 egg passages exhibited IVPI > 2.0. The IVPI values of the other viruses ranged from 0 to 0.6 indicating low virulence. Together, the H4N2 virus with a polybasic CS may shift to high virulence, resembling H5/H7 viruses after few genetic changes in the HA or serial passages.

P42

A dose-response approach using mixed dose-groups shows immune-potentiating capacity and improved efficacy in ferrets of the cationic liposomal CAF09 adjuvant in an H7N9 influenza whole inactivated virus vaccine challenge study

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Background
Often contribution of an adjuvant to vaccine efficacy is shown in studies using one or up to three different doses. The suboptimal vaccine dose is determined in a prior dose finding study or based on literature. In either case, reproducibility is a problem, which affects the window to show improvement by the adjuvant. Another limitation is that these studies provide information within a limited dose range. Statistical analysis is restricted to comparing the groups, while interpolation would predict for a wider dose range. However, more doses are required for such an approach. Using multiple doses and fewer (1-2) animals per dose requires a different housing strategy. Historically, groups in challenge studies are housed separately, since non-protected placebo-animals may re-infect protected vaccinated animals. Here we tested the cationic adjuvant CAF09 in combination with a whole inactivated virus (WIV) vaccine against influenza H7N9 in a dose-response study.
Methods
20 ferrets were allocated to 4 cages of 5 ferrets. Vaccines were administered twice, three weeks apart with 5 different doses ranging from 0.94 – 15 µg HA with 2Log steps. Two cages received the H7N9 WIV only and the other two cages the CAF09 adjuvanted variant. Two weeks after last vaccination ferrets were intra-tracheally challenged with H7N9 influenza and ferrets were sacrificed after 5 days.
Results
Ferrets vaccinated with the vaccine alone show a clear dose-response on functional antibody titers clinical parameters, virus replication and pathology of the lung. The adjuvanted vaccine showed a dose-response on antibody titers after first vaccination, but all doses reached a plateau after booster vaccination. The adjuvanted-vaccine also provided near to complete protection at the lowest doses and full protection at the highest doses.
Conclusions
Thus, the dose-response approach using mixed dose-groups shows a clear dose-dependent effect of the vaccine alone and an immune potentiating effect and a strong contribution to the efficacy of the CAF09 adjuvant. However, the study design can be further improved by including a few lower doses and a placebo, such that also for the adjuvanted vaccine suboptimal effects are obtained. Moreover, re-infection of ferrets by using mixed dose-groups does not seem to play a role, since clear dose-response effects are visible for virus replication.
This study is a proof of concept of the dose-response approach, a strategy that provides results over a wider range of doses using a similar or lesser number of animals as single or multiple dose comparison studies, respectively. This allows for better investigation of adjuvant contribution and further on better clinical study design.

P43

Activating proteases of a novel emerging H7N9 influenza A virus

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Activation of the hemagglutinin (HA) of influenza A viruses by host proteases is a prerequisite for infectivity. The HA of low pathogenic avian influenza A (LPAI) H7N9 viruses is cleaved at a single arginine by TMPRSS2. In 2016, the HA of the H7N9 virus mutated into highly pathogenic avian influenza A (HPAI) variants with multiple basic amino acids at the cleavage site. These novel viruses have caused severe human infections and poultry farm outbreaks in China.
We investigated the changes in protease specificity of H7N9 HA with a monobasic compared to multibasic cleavage sites with regard to the cleavability by different proteases and structural aspects. Therefore, 42 FRET-substrates derived from the HA cleavage sites of H1-H18 with a focus on various H7 subtypes were synthesized and tested with recombinant enzymes in a fluorescence assay. Based on this screening, the contribution of several serine proteases of the trypsin-like and subtilisin family in the activation of the HPAI H7N9 HA was studied in cell culture. The novel multibasic HA of HPAI H7N9 viruses is more susceptible to be cleaved by a broader spectrum of proteases compared to the LPAI variant. However, siRNA-mediated knockdown of furin in HeLa cells leads to a clearly reduced HA activation compared to a control. Furthermore, a comparative model shows an extended HA cleavage loop for the multibasic compared to the monobasic variant which is required for furin cleavage.
Taken together, these data suggest that furin is the major processing protease of the newly emerged HPAI H7N9 virus.

P44

Monitoring of influenza: Whole-Genome Sequencing to provide insights into disease severity

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Every winter, seasonal influenza causes substantial morbidity and mortality and has a significant impact on the economy. Moreover, a new influenza virus subtype can arise and cause a pandemic, with devastating effects on public health, healthcare systems, economy and sometimes also agricultural systems. Therefore, there exists a need for rapid and accurate characterization of the highly dynamic genomes of the influenza viruses for the prevention and mitigation of influenza. High-throughput molecular approaches offer new possibilities for influenza monitoring and global pandemic preparedness. By determining the whole genome of influenza virus, higher resolution evolutionary patterns can be revealed, knowledge of reassortment events and emerging mutations across all genes are provided and information on intra-host diversity of the virus is obtained. This information can lead to a better understanding of genetic changes in all segments during various seasons, possible antiviral resistance, tropism markers, antigenic characteristics, virulence and reassortment events.
We aim to develop a generic whole genome sequencing workflow for influenza A and B viruses to improve the characterization of circulating influenza strains in humans for routine surveillance. Therefore, two published multiplex RT-PCR protocols were improved to generate amplicons of the 8 influenza segments of swab samples, including three universal primers to amplify influenza A segments and a cocktail of 8 primers to amplify B segments. The generated amplicons were used as templates for Illumina MiSeq sequencing. Three viral loads based on the RT-qPCR results of the swab samples, namely high ($16 < Ct < 20$), moderate ($20 < Ct < 30$) and low ($30 < Ct < 35$) Ct of the main human influenza A subtypes, A(H1N1) and A(H3N2), and B were used to assess if all types of influenza can be amplified with the optimized multiplex RT-PCR and to estimate the limit of detection to detect all genome fragments using the whole workflow.
This optimized workflow will be used to sequence a representative subset of 172 samples of the subtype H3N2 from the 2016-2017 influenza season in Belgium corresponding to different degrees of disease severity. This subset will be analysed for specific mutations in the consensus sequence that might be associated with severe influenza cases. These protocols will also be used for the routine surveillance of circulating strains in Belgium. In the future, the quasispecies composition of clinical influenza virus isolates will also be analysed to try to identify a possible relationship between sequence polymorphisms and the reported disease severity.

P45

Co-infection of influenza A virus and Streptococcus pneumoniae in laboratory mouse

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Influenza viruses are the cause of highly contagious infectious diseases. Their genetic variability allows them to grow every year in human population, emerging to local epidemics, sometimes pandemics. Frequently associated bacterial co-infections are responsible for higher morbidity and mortality rates during the spread of influenza. Bacteria, commonly colonizing the upper respiratory tract mucosa, can spread to the lower parts of the respiratory tract and into the lungs. These bacterial co-infections cause middle ear inflammation, sinusitis, bronchitis, even severe necrotizing pneumonia resulting in death of the host. The severity of infection and the probability of developing pneumonia is multifactorial and includes several host and pathogenic properties, including the viral and bacterial strain, inoculum size, host immune system and time between exposure to influenza virus and bacteria.
In our work, we monitored the development of secondary bacterial infection with *Streptococcus pneumoniae* in laboratory mice primary infected with influenza virus A/NT/60/68 (H3N2). *Streptococcus pneumoniae* are one of the most common agents of secondary bacterial infections. Seven days after infection with the influenza virus, the mice were secondarily infected with different doses of bacteria. At each time interval we monitored the development of secondary bacterial infection by the *in vivo* bioimaging method. We also detected the presence of virus and bacteria *ex vivo* in various organs of mice.

Work was supported by grants: VEGA 2/0106/17, VEGA 2/0146/15, VEGA 2/055/18.

***Keywords:** Influenza A; Streptococcus pneumoniae; co-infection**

P46

Trypsin-like serine proteases in the lower respiratory tract of mice and their putative role in proteolytic activation of influenza A and B viruses

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Cleavage of the hemagglutinin (HA) precursor protein into its subdomains by host cell proteases is essential for virus infectivity, spread and pathogenicity. Seasonal influenza A and B viruses possess a monobasic HA cleavage site with a single arginine and are activated by trypsin-like proteases. TMPRSS2, a type II transmembrane serine protease (TTSP), activates HA with monobasic cleavage site *in vitro* and turned out to be an essential host cell factor for pathogenicity of H7N9 and H1N1 in mice *in vivo*. Cleavage activation of H3N2 instead, is less dependent on TMPRSS2 and due to additional so far unknown trypsin-like protease(s). Activation of influenza B virus has been demonstrated to be independent on TMPRSS2 in mice, as well.
These results revealed that influenza viruses can vary in their sensitivity to different proteases and therefore exhibit diversified protease specificity. Here, we analyze the protease repertoire in airway tissues of the lower respiratory tract of mice (trachea, bronchi and lung) by RNA sequencing in order to screen for further HA activating proteases. Comparison of these data with expression profiles in immortalized mouse lung epithelial cells (MLE-15), a negative cell culture system for HA cleavage, enables evaluation of protease candidates. Based on transcriptomic data, selected protease candidates present in murine airways and but absent in MLE-15 cells were analyzed for HA activation in cell culture experiments. Identification of further HA activating proteases could lead to novel therapeutic targets for influenza treatment.

P48

Homology-based modeling of the C-terminal inner region of pandemic H1N1 Influenza virus hemagglutinin

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Influenza virus hemagglutinin cytoplasmic (intraviral) domain plays crucial role in membrane fusion promotion during virus entry and participates in progeny virions assembly. The structure of this C-terminal region located inside the virion under the viral membrane has not been determined by crystallographic methods yet. The ab-initio PepFOLD 3.5 method of 3D-modeling predicts that the NH2-FWMCSNGSLQCRICI-COOH fragment of hemagglutinin from pandemic H1N1 influenza virus (residues 552 – 566) forms a beta hairpin. In nature, three fatty acid residues are linked to side chains of three cysteine residues within this fragment, and the only relatively hydrophilic region is obviously a loop having sequence SNGSLQ. The aim of this study is to model 3D structure of the hemagglutinin inner region with the help of homology-based methods. Using the MOTIF search server (https://www.genome.jp/tools/motif/MOTIF2.html) that utilizes an algorithm developed for Kyoto Encyclopedia of Genes and Genomes (KEGG) we found a protein (human m-calpain form II; PDB ID: 1kfu) possessing the same sequence motif SNGSLQ. The motif is situated between two beta strands and has a beta-turn formed by amino acid residues NG. Using the calpain-2 3D-structure as a template we further built a 3D model for the hemagglutinin inner region FWMCSNGSLQCRICI via SWISS-MODEL server (https://swissmodel.expasy.org/). According to our modeling predictions, the region forms a beta-hairpin with a beta-turn around the glycine. The loop SNGSLQ is stabilized by five hydrogen bonds between following amino acid residues: S1 and G3; S1 and S4; S4 and Q6 (including three hydrogen bonds found by Protein Interactions Calculator (http://pic.mbu.iisc.ernet.in/) between hydroxyl and amide groups from side chains of the respective residues). The obtained model could be used in future for in silico experiments to search for short peptide blockers of virus pathogenesis. The work was supported by Russian Foundation for Basic Research grant 18-54-00019 (to L.V.Kordyukova) and Belarusian Republican Foundation for Fundamental Research grant B18R-113 (to V.V.Khrustalev).

P49

Kinetics of the T cell response against influenza A virus is influenced by the site of infection

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Influenza A virus (IAV) infects millions of people each year, resulting in respiratory disease with symptoms ranging from a mild common cold to a severe fatal viral pneumonia. Vaccines may protect against multiple IAV subtypes by targeting conserved intracellular epitopes of IAV. By using ferrets, we can assess how the T cell response against IAV is influenced by the site of induction, which IAV proteins are more likely to evoke an immune response and which proteins are involved in cross-protection.
In a recent study, we infected ferrets (n=28) intranasal (i.n.) or intratracheal (i.t.) with H2N2 or PBS and analyzed samples from pre-infection and 14 days post infection. We found that i.n. infection with H2N2 invoked a stronger virus-specific T cell response in the blood. However, more CD8+ T cells could be detected in the bronchoalveolar lavage of i.t. infected ferrets. T cells showed strong responses against peptides of the conserved H2N2 proteins PA, PB1 and PB2, which corresponds with our observation that T cells of H2N2 infected animals cross-react to H1N1.
These results imply that the site of vaccination influences the T cell response, which can contribute to the development of more efficient IAV vaccines against seasonal and pandemic IAV.

P50

IAV-NS1 inhibits global transcription of the host cell and is associated with nuclear Chromatin/DNA

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To study the function of NS1 independent of an IAV infection, we expressed a NS1 estrogen receptor (ERT) fusion-protein that is inducible by tamoxifen. Incubation of NS1ERT expressing cells with tamoxifen at least partially complements the attenuated replication of IAVs lacking NS1 suggesting that a functionally active NS1 is induced. Addition of tamoxifen to cells expressing NS1ERT from diverse IAV subtypes (apart from IAV Puerto Rico/8/34) induces a cytopathic phenotype, the activation of JNK and apoptosis. A NS1 deletion mutant demonstrates that apoptosis induction is solely a function of the C-terminal effector domain of NS1. The transfection of reporter constructs indicates that NS1 inhibits the expression host genes. The analysis of nascent RNA by click-it chemistry shows that upon 4-hydroxy-tamoxifen (OHT) addition to NSERT expressing cells global transcription is attenuated, most efficiently by the C-terminal domain of NS1 alone suggesting that transcriptional inhibition lastly leads to apoptosis in tamoxifen induced, NS1ERT expressing cells. We further show that NS1 is closely associated with the chromatin fraction of the host nucleus. Nuclear localization of NS1 is independent of its interaction with CPSF4. Overexpression of CPSF4 suggests that it counteracts the inhibition of expression by NS1. We speculate that NS1 association to host chromatin enables NS1 to interact with CPSF4 which results in global transcriptional inhibition of host genes.

P51

The critical role of hemagglutinin (HA) in transmission of duck-origin H5Nx low-pathogenic avian influenza viruses in chickens

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In wild waterfowl avian influenza viruses (AIV) primarily replicate in the gastrointestinal tract and are shed into the environment at large quantities facilitating transmission to domestic poultry. Continuous circulation of H5 and H7 AIVs in domestic poultry may favour the evolution of highly pathogenic AIVs (HPAIV). To understand the adaptation process of AIV in poultry, we experimentally infected chickens via the intratracheal route with a number of low-pathogenic AIV (LPAIV) H5Nx and H7Nx isolates from wild waterfowl. While most H7Nx LPAIV were transmitted to contact animals without prior adaptation, none of the H5Nx LPAIV was passed to the sentinels. Interestingly, H5N1 and H5N8 HPAIV isolates were not transmitted to sentinel chickens when the HA proteolytic cleavage site was changed from a polybasic to a monobasic motif. Genetic reassortment of a non-transmittable H5N1 virus with either a transmittable H7N7 virus or a chicken-adapted H5N2 virus revealed that the hemagglutinin (HA) is critical for efficient virus shedding and transmission. Mutations in HA changing the pH threshold of fusion, the proteolytic cleavage site or potential glycosylation sites did not enhance virus transmission. However, recombinant H5N1 harboring the globular HA head domain from a chicken-adapted H5N2 was efficiently transmitted. Mutational analysis demonstrated that adaptations in the receptor-binding pocket (130-loop, 190-helix, and 220-loop) of HA are critical for H5 virus transmission in chickens. These findings suggest that duck-origin H5 viruses exhibit different receptor-binding activities than chicken-adapted viruses. Whether these adaptive mutations have an impact on receptor specificity or receptor affinity is currently under investigation.

P56

Single domain antibodies to probe the antiviral function of interferon-induced MX1 proteins

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For centuries, our immune system has been trapped in an evolutionary arms race with viruses. This relationship has shaped our immune defenses and led to the evolution of several specialized anti-viral proteins. An example of these are the Mx proteins: large GTPases that are part of the antiviral response induced by type I and III interferons, and thus belong to the first line of defense against viral infections. Despite the importance of these proteins, little is known about their anti-influenza activity. Therefore, we aim to elucidate the interactions between human and mouse Mx proteins and influenza vRNPs by using single domain antibodies (VHHs) as tools.

We have identified 20 VHHs directed against human MxA and 20 against mouse Mx1. These VHHs have been purified from the culture medium of Pichia pastoris transformants and are currently being characterized in vitro for their capacity to interfere with the GTPase activity of MxA and Mx1. Co-transfection of several of the MxA-specific VHHs with MxA results in an increase of the percentage of infected cells after infection with Influenza A SC35M (H7N7). Other MxA VHHs seem to decrease the percentage of infected cells and yet another set of VHHs appear to have no effect.

In the near future, we also aim to visualize the interaction between Mx proteins and IAV by high resolution microscopy techniques, using directly labeled VHHs as imaging tools. Overall, our findings may lead to novel insights in the mechanism of IAV restriction of human and murine Mx proteins.

P57

HDACs INHIBITOR TRICHOSTATIN A ENHANCES DISEASE SEVERITY AND LUNG PATHOLOGY IN INFLUENZA A/H3N2-INFECTED MICE

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Histone deacetylases (HDACs) are epigenetic factors modulating gene expression related to numerous biological processes including inflammation. All mechanisms can turn on/off the transcription of genes creating a network of reinforcing or counteracting signals in the cell. Thus, the application of molecules - inhibitors/activators that alter their activity might influence in an unexpected manner pathological condition such as the influenza infection.
Herein, as a pilot study, we selected Trichostatin A (TSA) as a standard substance known to inhibit HDACs to test primarily our hypothesis that it might affect pathology in influenza-infected mice. TSA was administered subcutaneously at a dose of 10 mg/kg/day to ICR mice in a 5 day-lasting course. We followed-up the survival, as well as lung viral titers, macroscopic pulmonary changes and histology on the 5th day p.i. A group treated with the optimal dose of 10 mg/kg/day reference compound oseltamivir phosphate served to compare the antiviral responses in vivo.
We found that TSA failed to exert protective effect in experimental influenza A H3N2-infected mice. The survival of TSA-treated and infected animals was lower than in the placebo control. Indeed, TSA administration increased lung score indicative for severe exudate formation 2 times more as compared to the reference antiviral. Lung viral titer in TSA-treated mice with flu exceeded that in the untreated infected mice by 0.67 Lg. Correspondingly we observed alterations in the lung histology showing increased inflammation, fibrosis and respiratory distress syndrome. In the control group the dose of 10 mg/kg oseltamivir phosphate lead to 80% mice survival, decreased lung pathology and virus titer.

P58

Establishment of in vitro assays for the potency of the influenza vaccines based on the macrophage activations

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Potency of inactivated influenza vaccines have been measured as a content of hemagglutinin (HA) antigen possessing the biological activities by single radial immunodiffusion (SRID) assay based on antigen–antibody interactions. Although this assay is a golden standard for measuring vaccine potency, it takes long time to prepare appropriate strain-specific reagents. Therefore, we developed a new potency assay for inactivated whole virion vaccines, requiring no specific antibody, based on the activation of NF-kB/AP-1 or ISG in vitro culture of human macrophages. Inactivated influenza whole virion vaccines induced macrophage activation in a dose-dependent manner. This activity was also well correlated with the HA antigen content by the standard SRID assay and serum antibody titer and protection from challenge virus infection in vaccinated mice when the vaccine was subject to heat inactivation. These observations demonstrate the newly developed assay is useful for measuring the potency and evaluating the stability of inactivated whole virion vaccines.

P60

Identification of key kinases required for influenza A virus entry as novel drug targets

Annika Hunziker, Emilio Yángüez, Silke Stertz

Although annual epidemics of seasonal influenza affect around 10% of the global population, current treatment options are limited and development of new antivirals is urgently needed. Here, we reveal key kinases required for influenza A virus (IAV) entry as potential novel drug targets. Using SILAC-based quantitative phosphoproteomics we quantified over 3000 IAV-induced phosphorylation changes that occur within minutes of infection. We developed a protocol to identify cellular kinases responsible for these changes and show that inhibition of selected kinases, such as the G protein-coupled receptor kinase 2 (GRK2), leads to decreased IAV replication. Focusing on GRK2 as potential drug target, we found that specific GRK2 inhibitors significantly reduced replication of seasonal and pandemic IAVs in primary human airway cultures and in mice. Ongoing studies using quantitative phosphoproteomics aim to compare the phosphorylation signature induced by different IAV strains, including seasonal human and avian viruses in order to identify additional drug targets with the potential to inhibit a broad spectrum of influenza viruses.

P61

The role of F(ab)2 and Fc fragments of HA2-specific antibodies in the recovery from influenza infection

Karolína Tomčíkova, Mária Vozárová, Margaréta Fogelová, František Kostolanský, Eva Varečková

Antibodies (Ab) are crucial molecules in the protection of organism against influenza infection. Nowadays, elicitation of cross-protective antibodies targeting the HA stalk domain becomes a promising approach to develop a universal vaccine. Besides virus neutralizing (VN) Abs, the broadly reactive non-neutralizing antibodies contribute to elimination of virus and recovery from influenza infection. In comparison with strain specific VN Abs, anti-HA2 antibodies involve their Fc-dependent effector function to mediate the antiviral protection. Many studies focus on the role of epitope specificity of anti-stem Abs and on the mechanisms stimulated by their Fc fragment effector function.
In our work, we employed fragmentation of monoclonal antibody specific to HA2 gp of influenza A virus by ficin protease. Ficin showed to be the most effective proteolytic enzyme for digestion of mouse IgG1 molecules to obtain bivalent fragments F(ab)2, retaining their antigen binding activity, and Fc fragments responsible for their effector function. We prepared such fragments from two HA2 specific MAbs, the one of which had fusion-inhibition activity. These MAbs were used for examination of the contribution to protection mediated by paratope, i.e. F(ab)2 fragment and their Fc fragments to the clearance of virus from mice infected with lethal dose of IAV.

This work was supported by VEGA grants: 2/0146/15, 2/0106/17, 2/0055/18

P62

Development and optimization of the assay for screening the compounds inhibiting endonuclease activity and disrupting cap-binding of influenza A polymerase

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[1]IOCB AS CR

Influenza virus A circulates in birds and mammals and causes severe infectious disease with potential fatal outcomes. Virus circulates worldwide and triggers annual epidemics that affect from 3 to 5 million people each year (WHO, 2014). There are two classes of anti-influenza drugs available: neuraminidase and M2 channel inhibitors. Increasing of resistance against these two types of inhibitors along with potential emergence of new viral strains emphasize an unmet need for new inhibitors.
Therefore, we aim to develop a high-throughput assay for screening of compounds targeting Influenza RNA polymerase, particularly, its cap binding and endonuclease domains. The screening methods are planned to be based on AlphaScreen technology and recently published DIANA assay (Navrátil et al., 2016).
In our laboratory, we have expressed and purified recombinant cap binding domain of PB2 subunit with C-terminal His-tag and endonuclease domain of PA subunit with N-terminal GST fusion, both from pandemic isolate A/California/07/2009 H1N1. For AlphaScreen assay we designed a biotinylated probe based on published nanomolar endonuclease inhibitor. Binding properties of several probes with different types of linker connecting inhibitor and biotin molecules were tested by surface plasmon resonance. To find an optimal screening condition, we tested several conditions with different probe/protein ratio in presence of Mn2+, Mg2+ and reducing agent. We also designed a PB2-cap binding inhibitor probe for DIANA assay which is currently in preparation.

P63

How to care for your influenza filaments

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Clinical isolates of influenza virus exhibit a range of morphologies, from spheres with diameters of 120 nm to filaments with lengths sometimes exceeding 30,000 nm. Despite decades of laboratory studies, the functional properties of filaments are still unclear.
Early studies of filaments suggested that they could be damaged by common laboratory manipulations, potentially skewing the results of research into their properties. Assessing the impact of this damage requires analysing large numbers of filaments, but this has previously only been achievable with laborious manual counting of electron micrographs.
To improve on manual particle counting, we applied a confocal microscopy based approach that allowed us to rapidly count and measure the lengths of large numbers of filaments. We used this platform to determine whether common laboratory manipulations, including physical stressors like pipetting and chemical stressors like pH changes, could affect the number of filaments or the distributions of their lengths.
We found that most common laboratory manipulations do not noticeably affect filament populations and so are suitable for studying filamentous influenza virus. However, we found that freezing causes structural damage to filaments, an effect most apparent when freezing dilute samples. This demonstrates that confocal methods can be used to assess the basic biophysical properties of filaments. As virus samples are routinely frozen before use, we suggest caution be exercised when interpreting past studies of the properties of filamentous influenza, and use unfrozen virus preparations in future.

P64

Phylogenetic analysis of influenza viruses of the seasons 2016-2018 in Ukraine

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Background
The phylogenetic analyses of influenza viruses allow to performing the comparison between viruses and monitoring the evolution in a frame of territory. Influenza viruses have high level of mutations caused by errors of polymerase.
The aim of our work was to analyze variability of influenza viruses type B which were isolated during 2016-2018 years and to construct of phylogenetic trees.
Materials
Nasal-throat swabs taken from influenza-affected patients from different regions of Ukraine, collected during 2016-2018 years, were used in the study. Samples were analyzed using real-time polymerase chain reaction (RT-PCR). Influenza viruses were isolated in MDCK and MDCK-SIAT cell culture. The sequences of influenza viruses from other countries were received from web-site GISAID using BLAST analysis. Sequences were aligned using ClustalW algorithm. Phylogenetic analysis was performed using MEGA 7 software.
Results
All recently circulating viruses from Ukraine analyzed carried HA genes that fell into genetic group 1A, the B/Brisbane/60/2008 genetic group, the vast majority falling into a genetic group defined by two HA1 amino acid substitutions. Despite the appeared new clusters with deletions in 162-164 HA1 in different countries, we not detected it in Ukrainian isolates (B/Kyiv/367/2017, B/Kharkiv/372/2017, B/Kyiv/375/2017 and B/Kyiv/46/2018).
Conclusions
The phylogenetic analyzes of Ukrainian influenza viruses of 2016-2018 seasons of B/Victoria linage was done. A large number of unique amino acid substitutions were observed by sequencing in influenza viruses sequences HA and NA gene, which had not affect the antigenic or other functional regions.

P65

Tubulin-dependent apical transport of cellular NKA induced by influenza A virus infection

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Influenza A virus (IAV) infection may cause life-threatening conditions such as "Acute Lung Injury" (ALI) and "Acute Respiratory Distress Syndrome" (ARDS) characterized by lung edema formation, impaired gas exchange leading to death. Clearance of alveolar fluid is strongly dependent on active transport of sodium across alveolar epithelial cells. One of the main ion channels that establish an osmolytic gradient is Na+/K+-ATPase (NKA) located in the basolateral membrane. IAV-infection of primary alveolar epithelial cells (i) decreases NKA amount on the basolateral membrane of neighbouring, non-infected cells (Peteranderl C. et al., 2016) and (ii) induces NKA translocation to the apical site of infected cells. Our aim was to illuminate molecular mechanism underlying an apical presentation of NKA in IAV-infected cells.
By the use of Western blot (WB) analysis of biotynilated apical cell membrane proteins and by NKA quantification through "on cell western blot" analysis (OCWB) of cells infected with different IAV we could (i) quantitatively demonstrate NKA appearance in the apical membrane of Calu3 cells during late stages of IAV infection, which is (ii) induced by all tested IAVs. Through MEK, MLCK-, actin/microtubulin polymerization-, ROCK-, HDAC6- or kinesin-inhibition combined with OCWB / immunofluorescence analysis we could show that NKA misdistribution was blocked by interfering with the tubulin-associated transport, and its regulation. By analysis of changes in the FITC-dextran concentrations of apical and basal Calu3 culture media p.i. we could demonstrate that (iii) prevention of NKA translocation improved a vectorial water transport. This indicates that IAV-induced NKA misdistribution adds to edema formation

P67

Predominant of influenza A (H3N2) clade 3C.2a may have contributed to the low vaccine effectiveness against influenza A (H3N2) in the 2014 influenza season in Thailand

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Background: The vaccine effectiveness (VE) against influenza A (H3N2) viruses [A(H3N2)] estimated from a VE study in Thailand was 73% (95% confidence interval [CI], -14% to 94%) and 6% (95% CI, -103% to 56%) in 2013 and 2014 seasons, respectively, when the given A (H3N2) vaccine strain belong to clade 3C.1.
Methods: A total of 15 A (H3N2) viruses from the referred VE study was used for complete genome sequencing. The viruses were from vaccinated children (n=5) and unvaccinated children (n=10).
Results: Phylogenetic analysis determined that the 2013-2014 viruses belonged to clade 3C.2 (5 strains from 2013) and clade 3C.2a (10 strains from 2014), while the vaccine strains from both seasons belonged to clade 3C.1. We found that vaccinated children from 2013 and 2014 seasons were infected with clade 3C.2 and 3C.2a, respectively. Two unique mutations were observed in the clade 3C.2a viruses from 2014, which caused a potential loss of a glycosylation site on epitope A but resulted in a potential gain of a glycosylation site on epitope B on the hemagglutinin (HA) protein. This mutation was absent in the 2013 3C.2 viruses.
Conclusions: We identified infection with A(H3N2) that were a different clade compared with the vaccine strain, from vaccinated children participating in a VE study during 2013 to 2014 seasons. This suggests that infection with viruses that were a mismatch to the vaccine strain may have resulted in breakthrough infections in vaccinated children. The predominance of clade 3C.2a in 2014 may have contributed to the low VE in the 2014 season.

P68

Co-circulation of two Yamagata-lineage clades of influenza B viruses during the 2013 and 2014 influenza seasons in Thailand and implication for vaccine effectiveness

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Background: The low vaccine effectiveness (VE) against influenza B-Yamagata lineage viruses in Thailand during the 2013 (25%, 95% confidence interval [CI], -120% to 75%) and 2014 (23%, 95% CI, -236% to 82%) seasons was estimated in a VE study. The WHO recommended influenza B strains in the 2013 and 2014 Southern Hemisphere trivalent vaccine belong to Yamagata clade 3 (2013) and Yamagata clade 2 (2014), respectively.
Methods: We randomly selected 15 influenza B samples collected from vaccinated and unvaccinated children in the referred VE study. Genetic analysis was conducted to assess how closely related the 15 viruses were to the given vaccine strains.
Results: Phylogenetic analysis of the influenza B viruses sequenced showed that in 2013 and 2014, 12 tested, 9/12 (75%, 95% CI 47-91%) and 3 tested, 1 (33% CI 6-79%) did not match vaccine clade, respectively. For both years, we observed that all of the influenza B virus infections in vaccinated persons were from viruses belonging to different phylogenetic clades than the vaccine strain. Compared to vaccine strains, 4 amino acid mutations were observed at antigenic sites on the hemagglutinin 1 (HA1) protein of the viruses circulated in both seasons.
Conclusions: Two genetically different clades of influenza B-Yamagata lineage viruses were detected from a sub-set of infected children. Vaccinated individuals were infected with viruses belonging to different clades from those included in the vaccine. This implies that vaccine containing only one clade may not protect against the other clade and co-circulating of the two genetically different clades may contribute to low VE.

P69

Phylogenetic analysis of influenza viruses in Bhutan during 2008-2017

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Background: Bhutan does not currently have an influenza vaccination program. Human influenza surveillance has conducted in Bhutan since 2008. Collected samples were tested by real-time RT-PCR (rRT-PCR) to detect and identify influenza viruses. This study aimed to examine the similarity between circulating influenza viruses in Bhutan and the annual vaccine strains through phylogenetic analyses.
Methods: A total of 277 influenza rRT-PCR positive specimens collected during 2008 to 2017 included 22 influenza A(H1N1), 86 A(H3N2), 79 A(H1N1)pdm09, and 90 B viruses. Sanger sequencing was performed to obtain hemagglutinin gene (HA) sequences from selected specimens and/or virus isolates. A phylogenetic tree was constructed using HA sequences and sequences from GenBank and Global Initiative on Sharing All Influenza Data (GISAID) databases
Result: Phylogenetic analysis revealed the influenza A(H1N1) viruses belonged to clades 2B (2008-2009); A(H1N1)pdm09 viruses belonged to clades 1 (2009), 3 (2010), 4 (2009-2010), 5 (2011), 7 (2012), 6C (2012), 6B (2013-2016), and 6B.1 (2016-2017); A(H3N2) viruses belonged to clades 1 (2009), 3C.2 (2012-2014), 3C.3 (2013), 3C.2a (2014-2016), 3C.2a1 (2016-2017) and 7 (2009); B Victoria-lineage viruses belonged to clades 1A (2009-2010, 2012, 2016-2017) and 1B (2009-2011); B Yamagata-lineage viruses belonged to clades 3 (2010-2011, 2013-2014, 2016) and 2 (2012).
Conclusion: This study summarizes the epidemiology of circulating influenza viruses over a 10 year period (2008-2017) in Bhutan. Bhutan experiences the same circulating influenza strains as its neighboring countries. The data indicates influenza viruses detected in Bhutan fall in the same clades as the vaccine strains and introduction of an influenza vaccine program would benefit the country.

P70

Detection of Antiviral Resistance of Influenza Viruses in Bhutan, Nepal, the Philippines, and Thailand during 2013 to 2015

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Background: Influenza virus surveillance in Bhutan, Nepal, the Philippines and Thailand has conducted since 2008. Collected samples were tested by real-time RT-PCR (rRT-PCR) to detect influenza viruses. Antiviral resistance of randomly selected samples was also examined. This study aimed to detect antiviral resistance of samples collected from 2013 to 2015.
Methods: A total of 241 randomly selected influenza rRT-PCR positive samples included 38 influenza A(H1N1)pdm09, 139 A(H3N2), and 64 B viruses collected from 2013 to 2015 were tested by pyrosequencing and neuraminidase inhibition assay (NAI) to detect antiviral resistance. Single nucleotide polymorphisms (SNPs) was quantified in a subset of samples. Quantitative contributions of individual SNPs to changes in IC50 were fitted with additive genetic models to adjust for SNP occurrences.
Results: Only one mutation corresponding to a known antiviral resistance marker was detected from all 241 samples tested. The D197N mutation with an NAI IC50 of 14.2 nM was detected from an influenza B positive sample collected in Bhutan during 2014. SNP analysis on the sample showed two positions with mutant fractions over 20%: D197N (67%) and G407S (28%). The additive model suggests adjusted effects of those positions as 3.02 (95%CI:-0.62-6.66) and 26.05 (95%CI: 14.54-37.57), respectively. The predicted IC50 using SNPs data underestimated the observed resistance by 4.83 nM.
Conclusions: The vast majority of tested samples did not contain antiviral resistance markers. Only one sample contained a phenotypic resistance marker was detected. However, mutations known to contribute to resistance were prevalent in the minority population. Some were able to heighten IC50 at significant levels.

P71

MX1 requirements for influenza A virus restriction

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Type 1 interferons (IFNs) are produced by infected cells upon detection of pathogenic agents and are the first line of defence against viral infections. IFNs induce the expression of hundreds of IFN-stimulated genes (ISGs), both in infected and neighbouring cells. The products of these ISGs in turn induce in cells a potent antiviral state, capable of limiting viral replication. The dynamin-like, high-molecular weight GTPases MX1 and MX2 play a significant role in the IFN-induced inhibition of viral replication. Human MX1(or MxA) is a restriction factor of broad antiviral activity, able to inhibit influenza A virus (FLUAV) and a great diversity of RNA and DNA viruses at different stages of their life cycles. Human MX2 (or MxB) is notably able to inhibit HIV-1 and herpes viruses. Although the antiviral activity of human MX1 has been studied extensively, the molecular mechanism of action remains largely unsolved. MX1 and MX2 are 63% identical at the amino acid level, share a similar domain organization and their crystal structures are almost practically superimposable. Taking advantage of chimeras between MX1 and MX2 in which their different domains have been swapped, as well as point mutants, we have notably identified a new motif required for influenza A restriction by MX1. Importantly, some MX1/MX2 chimeric proteins are highly active against influenza A viruses but not in the context of minireplicon assays. Additional ongoing efforts to better characterize MX1's requirement for influenza A restriction and mechanism of action will be presented.

P72

Aniline-based inhibitors of influenza H1N1 virus acting on hemagglutinin-mediated fusion

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The influenza virus hemagglutinin (HA) is responsible for fusion between the viral and endosomal membranes during influenza virus entry. This fusion process can be blocked by compounds interfering with the acid-induced conformational change of HA.
After identifying two series of easily accessible anilines as inhibitors of influenza A/H1N1 virus, extensive chemical synthesis and analysis of the structure-activity relationship were performed. In Madin-Darby canine kidney cells infected with A/H1N1 viruses, the lead compound, **9d**, displayed a 50% effective concentration of 1.5 to 5.5 μM and an antiviral selectivity index of 30. Inhibition of polykaryon formation in HA-expressing cells indicated that **9d** and its analogue **14a** interfere with low pH-induced membrane fusion mediated by the H1 and H5 (group 1) HA subtypes. Virus resistance as well as NMR experiments with the lead molecule **9d** demonstrated that it interferes with HA-mediated f1usion by binding to the HA stem and preventing its refolding at low pH. Molecular dynamics simulations suggest that ligand **9d** is able to fill the "TBHQ pocket" (1) in the HAs of A/PR/8/34 and A/Virginia/ATCC3/2009. This implies that the "TBHQ pocket" represents a common and particularly relevant site for small-molecule HA fusion inhibitors, although distinct chemotypes are required to address the different polarity of this cavity in group-1 versus group-2 HA subtypes.
1) Russell et al., Proc. Natl. Acad. Sci. U. S. A. **2008**, 105, 17736-17741.

P76

Overcoming the resistance to influenza A virus infections in mice carrying a deletion in the host protease TMPRSS2 by exchange of amino acids in the hemagglutinin

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Background:
Annual epidemics and occasional pandemics by influenza A virus (IAV) pose a severe threat to human health. Host cell factors that are required for viral spread but not for cellular survival represent ideal targets for anti-viral therapies. The cleavage activation of the influenza virus hemagglutinin (HA) by host cell proteases is essential for viral infectivity. Thus, host proteases represent very suitable target for the development of anti-viral drugs.

Methods:
We infected a mouse strain carrying a knock-out (KO) mutation in the host protease TMPRSS2 with IAVs carrying mutations in the HA and determined pathology and virus replication in infected lungs.

Results:
We showed that deletion of the HA-activating protease gene, *Tmprss2*, in knock-out (KO) mice inhibits spread of mono-basic H1N1 influenza viruses. Lung pathology was strongly reduced and mutant mice were protected from weight loss, death and revealed reduced viral load. After infection with influenza A virus expressing a H3, body weight loss and survival was as severe in *Tmprss2* KO mutants an in wild type mice. Modifications in the HA loop and interacting amino acids influenced viral replication and pathology in *Tmprss2* KO mice. Body weight loss and survival of *Tmprss2* KO mice was dependent upon cleavability of the mutated HA protein by the host protease.

Conclusions:
Our results validate the host protease TMPRSS2 as a potential drug target for anti-viral therapy.

P77

Understanding influenza filament formation through multi-modal imaging strategies

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Wild type influenza A viruses (IAVs) typically form a pleomorphic population of viral particles, ranging from filaments that can be several microns in length to 0.1 micron diameter spheres. However, spherical particles predominate in laboratory-adapted strains, filamentous particles are present in a high proportion of clinical isolates. Despite the wealth of knowledge about the virus life cycle derived from laboratory-adapted spherical strains, the basics of elongated and filamentous virus particle formation and their functions remain poorly understood. A particular challenge is linking ultrastructural details of membrane and protein trafficking to the formation of elongated and fully filamentous structures that can be microns in length.

In order to unravel the mechanism of filament formation we used a multi-modal imaging approach, combining high-resolution cryo-EM tomography, traditional scanning and transmission electron microscopy, and super-resolution light microscopy. To this end, we have optimized a system by which we can analyze filament budding using a filamentous IAV, cells thin enough at the viral budding site for electron beam penetration, and multiple viral protein antibodies for immunofluorescence. This has allowed us to obtain quantitative data on the viral budding site, as well as to generate high resolution images of filament formation. Using these methods, we are characterizing membrane trafficking during viral budding in detail, as well as identifying global patterns in viral and host protein localization. This will allow us to generate an accurate picture of the co-option of cellular processes into the influenza budding site, thereby providing fundamental information about the formation of the biologically normal and clinically-relevant influenza virions.

P78

Interferon-beta stimulation by aberrant influenza virus RNAs is sequence dependent

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The influenza A virus (IAV) genome consists of eight segments of negative strand viral RNA (vRNA). These segments are replicated in the nucleus of the host cell by the viral RNA dependent RNA polymerase (RdRp) in the context of nucleoprotein (NP)-coated viral ribonucleoprotein (vRNP) complexes. In addition to making full-length copies of the vRNA segments, the RdRp generates a range of aberrant RNA products resulting from internal deletions, such as defective interfering RNAs and <125 nt-long mini viral RNAs (mvRNA). The cytoplasmic RNA sensor retinoic acid-inducible gene-I (RIG-I) can detect mvRNA molecules and trigger innate immune responses, such as interferon (IFN)-β expression. Such responses are important in the outcome of viral disease. To understand which type of mvRNAs contribute most to innate immune signalling, we here performed vRNP reconstitutions of different mvRNA-like templates with polymerases of different IAV strains. We measured mvRNA binding to RIG-I and the induction of IFN-β promoter activity, and found that the IAV mvRNA sequence strongly influences IFN-β promoter activity in a template sequence-dependent manner, independent of RIG-I binding. This effect is conserved for the RdRps of the 1918 Spanish Flu, an H5N1 avian IAV, or the lab-adapted WSN IAV. Our results suggest that different mvRNAs contribute differently to RIG-I activation and IAV induced immune responses. Subsequently, sequence differences between flu strains could be indicative of their immunogenicity, representing an interesting avenue for further investigation.

P81

Characterization of the entire landscape of peptides presented by MHC during influenza virus infection

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Influenza viruses (IV) infection is a public health concern worldwide. Currently, all available vaccines as well as antiviral drugs that target the virus itself are prone to resistance. Lesson learned from previous pandemic outbreaks is that people with a preexisting cellular immune response are either protected or developed less severe disease against the infection. This observation leads to propose new vaccine approaches by activation of cellular immune response against influenza virus. Nevertheless, it is unknown whether antivriral drug treatment will lead to alteration in the cellular immune response against influenza viruses. In this regard, the proposed study investigated whether antiviral drugs will alter the quality of the cellular immune response against the virus. A ligandome analysis was performed to characterize the entire landscape of peptides presented by MHC molecules. This analysis investigates not only the alterations of presented viral peptides, which may influence the immune response, but also the alterations in cellular proteins expression, e.g. as a consequence of treatment of antiviral compounds. This knowledge is necessary for drugs directed against intracellular targets as well as devising new approach against IV infection. However, further investigations are required.

P82

Increased pathogenicity in mice of a mouse-adapted influenza H7N9 virus was associated with delayed host innate immune responses

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An avian influenza virus A/Anhui/1/2013 (Anhui; A/H7N9) was adapted in mice (Anhui-M), which showed higher pathogenicity than did the original strain (Anhui-E). Fifty % of lethal doses of each virus were 2.5 x 10^4 pfu/50 µL (Anhui-E) and 50 pfu/50 µL (Anhui-M), respectively. When mice were inoculated nasally with 4 µl (40xLD_{50}) of each virus, which volume allows the inoculated virus spread restricted to the upper respiratory tract (URT), Anhui-E induced little weight loss in the mice while the animals infected with Anhui-M exhibited marked weight loss. Next, the expression of type I IFN-associated genes and IFN-α production after 10xLD_{50}/50µL of each virus infection in the mice lungs were compared between the two viruses. On day 1 after infection, Anhui-M induced these responses at a lower level than did Anhui-E, but no significant differences of them were shown in 3 days after infection. Furthermore, when mice received poly(I:C) pre-treatment to prepare for type I IFN induction in the respiratory tract, the mice recovered earlier from the fatal lung infections than in non-treated mice. These results suggested that the increased lung pathogenicity of Anhui-M was associated with the delayed host innate immune responses to a small dose of the virus. Six virus clones from Anhui-M had two amino acid substitutions in the PA protein (T97I and L268F) or in the HA protein (A143T and A196E). Further study for the relationship between the pathogenicity and the virus mutations of Anhui-M is in progress in regard to the suppression of host innate immunity.

P83

Tyrosine kinase 2 is not required for interferon-λ mediated signaling and protection against lethal Influenza A virus infection in mice

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Binding of interferons (IFN) to their respective surface receptors activates JAK kinases such as JAK1, JAK2 and TYK2. Upon activation these kinases mediate the phosphorylation of STAT molecules, which eventually promotes the expression of a large number of IFN-stimulated genes (ISGs) encoding antiviral resistance factors. TYK2 is associated with both the IFNAR1 and the IL-10Rβ receptor chain, each forming a subunit of either the type I or type III IFN receptor complex.
Here we aimed to clarify the role of TYK2 in the signaling pathways of type I (IFN-α/β) and type III IFNs (IFN-λ) using primary airway epithelial cells and primary mini-gut organoids derived from mice harboring defective or functional alleles for Tyk2.
Both primary culture systems readily responded to IFN-λ by upregulating ISG expression irrespective of whether they carried functional or defective Tyk2 alleles. In contrast, IFN-α mediated ISG induction was severely diminished in cells with no functional TYK2. When mice were treated with IFN-α or IFN-λ before intranasal infection with a lethal dose of influenza A virus, IFN-λ efficiently protected from disease irrespective of whether the mice carried functional or defective Tyk2 alleles. In contrast, IFN-α was only protective in animals carrying a functional Tyk2 gene, while Tyk2-deficient animals succumbed to the infection.
We conclude that Tyk2 deficiency severely limits IFN-α signaling but does not affect IFN-λ mediated ISG induction in respiratory and intestinal epithelial cells or protection against a lethal infection of the respiratory tract.

P85

Postnatal priming of interferon and metabolic response programs determines the host's responsivity to influenza infections later in life

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The morbidity and mortality of newborn infants from influenza infections is low compared to older children and adults. It is largely unclear what reprogramming of immunity causes the higher susceptibility to severe influenza infections with increasing age.
We challenged human respiratory and innate immune cells from healthy newborns and adults with the H1N1/California/04/2009 virus. Next to detailed virus-addressing studies we performed global transcriptomic analyses using a variety of state-of-the-art computational methods. While no differences could be observed regarding viral infectibility and progeny, the inflammatory response of neonatal primary airway epithelial cells, macrophages and monocytes upon influenza A virus (IAV) infection was generally dampened compared to adults. A system biology approach revealed that the differential anti-IAV response of human monocytes was primarily linked to differential programming in newborns and adults at baseline. Genes related to antigen presentation and myeloid differentiation were in fact less inducible but shaped the age-dependent anti-IAV response by opposing and strong basal expression differences. In contrast, IFN response genes and energy supply-related metabolic pathways were comparably strong induced but in neonates parallelly shifted to significantly lower expression levels than in adults suggesting training with increasing age.
We conclude that the course and outcome of influenza infections is less driven by the viral load but determined by the host's inflammatory response. The priming of IFN response and metabolic gene modules during environmental adaptation might represent the critical determinant of how the host's responsivity to influenza infections evolves later in life.

P88

Suspected spillover of A/H1N1 (2009) from humans to swine in Ghana: Current status of molecular surveillance

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The emergence of the A/H1N1 (2009) human pandemic influenza virus that had its origin in pigs caused the most influenza pandemic. This has refocused the world's attention on the possible emergence of zoonotic influenza viruses from pigs. While the human population is in the post-pandemic era with A/H1N1 (2009) now being an endemic seasonal influenza virus, the virus, then referred to as H1N1pdm, has spilled back in pigs in Europe, Asia, and America. Very little information is available from sub-Saharan Africa. We aimed to assess the possible circulation of H1N1pdm in pigs in Ghana. A total of 1200 nasal swabs was collected from 75 pig herds apparent healthy under intensive care in the Ashanti region in two seasons from 2016 -2017. RNA was extracted from all samples and RT-qPCR targeting a conserved region of the influenza A virus (IAV) matrix gene performed. Positive samples were tested for five HA and three NA subtypes, respectively, including H1N1pdm by subtype-specific RT-qPCR. IAV was detected in 2% (12/600) and 0.8% (5/600) of rain and dry season samples, respectively. The overall incidence of IAV was 1.4% (17/1200). All M gene-positive samples also tested positive for H1N1pdm.
Our results confirm the circulation of influenza A H1N1pdm in pigs in Ghana. Virus isolation and full genome sequencing have been initiated to clarify the origin of these viruses as possible spillover infections from humans. Regular swine surveillance is required to provide timely information on possible endemic H1N1pdm infections in swine, and the putative generation of reassortant viruses with pandemic potentials that may emerge from pigs.

P90

Influenza A virus M2 protein interacts with cellular Na,K-ATPase: Does it have a pathophysiological role in influenza pneumonia?

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Influenza A Virus (IAV) infections of the lower respiratory tract can induce viral pneumonia resulting in acute lung injury (ALI/ARDS) with fatal outcome. Characteristics of an IV-induced pneumonia are an alveolar epithelial cell (AEC) damage and accumulation of protein-rich edema fluid in the alveolar compartment impairing gas exchange. Depending on a sodium gradient established by the basolateral Na,K-ATPase (NKA) and the apical epithelial sodium channel (ENaC) edema fluid is removed from the alveolar space under normal conditions. However after IV-infection a decreased alveolar fluid clearance was observed.
In primary AEC it was shown that an IAV-infection leads to a mistargeting of the NKAα1-subunit to the apical cell membrane, but to a reduced NKA expression in the non-infected neighbouring cells. Co immunoprecipitation (co-IP) studies identified the viral M2 protein as a binding partner of NKAα1. To study the pathophysiological implications of this virus-host interaction we characterized the NKA binding site in the viral M2 protein. In a mutational approach we were able to identify three amino acids in the cytoplasmic tail abutting the transmembrane domain as critical for NKAα1 binding. Recombinant seasonal IAV with impaired NKAα1 binding was slightly attenuated for replication in vitro and ex vivo. It is currently investigated for mistargeting of the NKAα1 to the apical side of cell and the impact on edema clearance in a polarized Calu 3 cell model.
In addition to established roles in intracellular genome release and virus budding our data suggest a further function of the IV M2 protein in relocalizing the cellular NKAα1, which is likely to contribute to pathophysiological effects in IV infection. Investigating the impact of the M2/NKA interaction on the impaired edema clearance could help to better understand this outcome of an IV infection in the future.

P91

Endosomal cholesterol accumulation is a host cell-protective mechanism inhibiting endosomal escape of influenza A virus

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With annual epidemics occurring in all parts of the world and the risk of global outbreaks, IAV infections remain a major threat to public health. Infected host cells detect viral components and mount an interferon (IFN)-mediated response to restrict virus propagation and spread of infection. The contribution of endosomal cholesterol levels, especially in the context of the IFN-induced antiviral response, has remained controversial so far. Here we report that the elevation of endosomal cholesterol accumulation is part of the IFN response and plays a pivotal role in the early antiviral defense. We demonstrate that inducing endosomal cholesterol accumulation is antiviral in non-IFN primed cells, restricting incoming IAV particles, impairing mixing of IAV/endosomal membrane lipids, and inhibiting IAV endosomal escape. Our results establish a protective function of LE/L cholesterol accumulation and suggest endosomal cholesterol balance as a possible antiviral target.

P92

Interferon response is essential in containing human pathogenic Bourbon virus, an influenza-like, tick-borne Orthomyxovirus

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A hitherto unknown viral pathogen was isolated in 2014 from a patient in eastern Kansas who died with high viremia shortly after disease symptoms developed (Kosoy et al., 2015). Additional cases with mild but also fatal outcomes have since occurred in the US. The novel virus, designated Bourbon virus (BRBV), is an influenza-like virus belonging to the genus of tick-borne Thogotoviruses in the *Orthomyxoviridae*. Recent tick surveillance studies of the CDC confirmed the prevalence of BRBV in the affected US regions. Here, we analyzed the pathology of Bourbon virus infection in mice and found an unexpected high sensitivity of the virus to the host interferon (IFN) system. Infected standard laboratory mice did not show disease symptoms or viral replication. However, in mice carrying defects in the type I and type II IFN system the virus grew to high titers and caused severe pathology. In cell culture experiments, Bourbon virus was blocked by antiviral agents like ribavirin and T705-favipiravir. Our data show that type I and II IFNs play a critical synergistic role in inhibiting BRBV suggesting that fatal outcomes in humans may be caused by defects in the patients' innate immune defence. Furthermore, our findings indicate that patients would benefit from an already approved antiviral treatment.

P95

Generation of a human and mouse ex vivo lung culture model for translational studies on respiratory viruses

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Ex vivo lung culture models provide an excellent tool to investigate basic and translational research questions. Here, we have established a human lung culture model using human lung tissue from patients undergoing lung surgery and an analogous mouse *ex vivo* lung culture model to study important aspects of viral replication, pathogenesis and the cellular antiviral immune response towards influenza A virus infection. Our experimental data demonstrate that patient-derived lung tissue supports viral replication. RT-PCR analysis revealed up regulation of IFN-ß as well as antiviral ISGs upon infection. In addition, we can show that human lung tissue is susceptible to the actions of the cell culture approved inhibitor of viral replication bafilomycin. Transferring this model to mouse lung tissue will reduce animal experiments and allow us to perform translational studies in genetically modified mouse lung tissue in the future.

P96

Phosphorylation of serine 205 of influenza A virus NS1 protein as determinant of adaptation and functional evolution

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Influenza A virus (IAV) infections are still a major burden of mankind. High evolution rates as well as the ability to infect a wide range of hosts leads to seasonal epidemics and occasional pandemics. IAV non-structural protein 1 (NS1) is a multi-functional protein that plays diverse roles during virus replication and has been linked to host adaptation. Currently circulating H1N1 viruses in humans show six amino acid changes compared to the swine origin pandemic (pH1N1) virus, which entered the human population in 2009 and eradicated previously circulating H1N1 viruses. In this regard, NS1 of pre-pandemic H1N1 showed high prevalence of serine (S) at position 205 while pH1N1 exhibits an asparagine (N) at this position. Over the course of adaptation in humans, pH1N1 re-acquired S205 suggesting an important role in host adaptation. Interestingly, we found NS1 S205 to be phosphorylated during pre-pandemic H1N1 virus infection. To analyze the importance of S205 phosphorylation in viral replication and host adaptation, we substituted S205 of a pre-pH1N1 to non-phosphorylatable glycine, aspartic acid to mimic constitutive phosphorylation as well as pH1N1 asparagine. Phosphorylation mutants replicated less efficiently compared to wild type. Furthermore, these mutants showed decreased amounts of viral proteins, which can be attributed to a diminished expression of viral mRNAs. So far, we hypothesize that tight temporal regulation of S205 phosphorylation is needed for efficient viral replication and might be re-gained as determinant of functional evolution during adaptation of pH1N1 to the human host, which will be focus of future studies.

P99

Replication of bat chimeric influenza viruses is efficiently inhibited by human MxA

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The interferon-induced and antivirally active human GTPase MxA represents a major interspecies-barrier for influenza A viruses (IAVs) of avian origin that has to be overcome in order to establish a new lineage in the human population. As a consequence, all human-adapted IAVs, including the pandemic 1918 and the pH1N1 virus as well as all their descendants, encode adaptive mutations in their nucleoproteins allowing MxA escape. In contrast, avian IAVs lack such amino acids. Intriguingly, bat Mx1 from Carollia perspicillata and Sturnira lilium (both hosts of bat IAV) was previously shown to be antivirally active against avian IAV with comparable efficiency as the human counterpart MxA. To evaluate whether bat IAV are thus resistant to human MxA, we infected cells stably expressing either functionally active MxA or the antivirally inactive MxA mutant MxAT103A with bat chimeric viruses, designated SC35M-H17N10 and PR8-H18N11. These viruses encode the internal proteins of either H17N10 or H18N11 plus surface glycoproteins of classical H7N7 or H1N1, respectively. Interestingly, viral growth of both chimeric viruses was completely abrogated in MxA expressing cells but not in the MxAT103A expressing control cell line. Consistently, while PR8-H18N11 was highly pathogenic in mice lacking a functional Mx protein, MxA transgenic mice were resistant to infection. These surprising findings suggests that the adaption process of bat IAV to bat Mx proteins did not result in MxA resistance, which is why bat IAV are like avian IAV highly sensitive to human MxA.

P100

Whole-genome characterization of influenza A viruses circulated in Russian Federation in 2015-2018 epidemic seasons

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BACKGROUND: Implementation of NGS technology in the framework of influenza surveillance in Russia significantly improved identification of possible factors that could influence the course of epidemics and the impact of internal genes evolution on pathogenicity and transmission of influenza viruses. Whole-genome sequencing of influenza A viruses selected by the epidemic periods and geography spread (30 regions) was performed.
METHODS: rRT-PCR, whole-genome amplification, Illumina sequencing, phylogenetic analysis.
RESULTS: All analyzed influenza A(H1N1)pdm09 viruses belonged to phylogenetic group 6B.1 and demonstrated the slow antigenic drift. A set of mutations was revealed in internal genes of Russian A(H1N1)pdm09 viruses leading to amino acid substitutions: D2E and E125D (transport of host mRNA) in NS1 protein; M83I (nuclear transport signal site) in NEP protein; M105T in NP protein; Q208K in M1 protein; N204S in PA-X protein (possible virus-host interactions). The incidence rate of these substitutions increased from about 10% in 2014-2015 to 85% in 2015-2016 and up to 100% in 2017-2018.
A(H3N2) sequenced viruses consistently clustered in two groups corresponding to 3C.2a and 3C.2a1 on all trees, with several intra-group reassortant exceptions (HA and PA genes).
Some A(H3N2) viruses had 11 and 25 aa truncated PB1-F2 protein, belonged to subgroup 3C2a.1, possessed I58V substitution in HA1 and clustered together on phylogenetic trees for all genome segments. PB1-F2 truncation is seldom observed in A(H3N2) viruses (the incidence rate less than 1% during the last decade). The functional significance of this change may be related to the virulent properties of the virus or its transmission ability.

P102

Phenotypic and genotypic analysis of influenza viruses susceptibility to antivirals during the 2017-2018 epidemic season in Russian Federation

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Being constantly evolving influenza viruses can develop the resistance to the most common used antivirals, such as neuraminidase inhibitors. In this study we show the results of phenotypic and genotypic analysis of influenza A and B viruses isolated in the 2017-2018 season in Russia susceptibility to the neuraminidase inhibitors oseltamivir and zanamivir and M2 proton channel inhibitor rimantadine.
The total 404 influenza viruses were studied to determine 50% inhibitory concentration (IC50) using fluorescent assay (MUNANA), 176 belonged to A(H1N1)pdm09 subtype, 84 –A(H3N2), 142 were influenza B/Yamagata lineage and 2 were B/Victoria lineage.
Only one strain of A(H1N1)pdm09 subtype had shown highly reduced inhibition (HRI) to oseltamivir, confirmed by presence of H275Y aminoacid substitution in the neuraminidase gene. Also, one of influenza B strains (Yamagata lineage) had shown reduced inhibition (RI) to oseltamivir. The overall frequency of influenza viruses resistant to neuraminidase inhibitors was 0,5%, similar to what has been observed in recent global studies.
The total 260 influenza A viruses tested demonstrated resistance to rimantadine.

P103

Infection-induced heterosubtypic immunity against influenza virus correlates better with humoral than cellular immunity in TIV-vaccinated mice and benefits from innate immune activation.

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Conventional influenza vaccines aim at the induction of virus-neutralizing antibodies. However, influenza vaccine efficiencies are often low. We investigated to what extent infection-permissive immunity provided by a seasonal trivalent inactivated influenza virus vaccine (TIV) could modulate disease and virus-induced host responses after infection with H1N1 virus that matches the vaccine. More than one TIV vaccination is needed to induce high serum HI titers efficiently in mice. However, single TIV administration already protected from vast morbidity after H1N1 infection, even in the presence of lung virus titers. Contrary to negative control mice, complete loss of alveolar macrophages, as well as pulmonary infiltration of Ly6c+ monocytes and release of pro-inflammatory cytokines and chemokines was prevented in TIV-vaccinated animals. We also show that induction of germinal center B cells and tissue-resident CD8+ T cells in the lung after H1N1 infection correlates with protection during reinfection with a lethal dose of a H3N2 virus but is negatively impacted by TIV vaccination. On the other hand, sera from TIV vaccinated animals that received H1N1 infection outperform sera from animals that either received H1N1 infection or a TIV vaccine, but not both, in an in vivo microneutralisation assay with H3N2 virus. Cross-protective sera were not able to inhibit red blood cell hemagglutination by H3N2 virus. These results suggest that, contrary to H1N1 virus-exposed non vaccinated animals, TIV vaccinated animals that were exposed to H1N1 virus rely more on cross-reactive serum antibodies than on cellular immunity for protection during lethal H3N2 reinfection. Finally, we show that innate immune activation through intranasal administration of Sendai virus defective interfering RNA, an antagonist of the innate RNA sensor RIG-I, can synergize with H1N1 virus- and/or TIV vaccine-induced pre-existing immunity to enhance protection during lethal H3N2 infection.

P104

vRNAsite: Prediction and evaluation of viral RNA-RNA interaction sites between influenza A wild type and mutant vRNAs

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Seasonal influenza A virus (IAV) epidemics are difficult to counter with vaccination. This is due to the unique packaging of the eight viral RNA (vRNA) segments and the ability to create reassortants between different strains. One theory about packaging involves vRNA-vRNA interactions (RRI) between different segments [1,2], however to our knowledge there has been no attempt to computationally analyze the involved mechanisms. Here, we present vRNAsite which predicts possible RRIs between all IAV vRNAs.

vRNAsite takes the original and mutated segments as input and a sliding window between any two vRNAs calculates minimum free energy (MFE) dependent scores for each single nucleotide pairing via RNAcofold [3]. Resulting scores are used to evaluate possible interaction sites as well as differences between WT and mutant RRIs. Our first results show multiple sites with promising scores hinting at possible RRIs. We validated one of these sites with an *in vitro* experiment involving the avian H5N2 virus [2]. Here, only four compensatory point mutations between segment 2 (PB1) and 8 (M) led to a significantly attenuated viral reproduction when one of the two mutants was present. An introduction of both mutant vRNAs restored the viral reproduction to WT levels.

With this work we demonstrate that a computational approach for the packaging of IAV is essential. We will further develop vRNAsite with the goal of predicting complete packaging networks for IAV. This might yield the ability to predict the pathogenic potential and reproductive capacity of reassortant IAVs, which would lead to an improvement in IAV vaccination development.

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P105

Intradermal DNA vaccination harboring a combination of conserved HA-peptides eliminates/reduces viral shedding and overcomes maternally derived antibodies

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Swine influenza viruses (SIVs), though not causing abundant mortality, need to be controlled since swine can act as mixing vessels favoring genome reassortment of diverse influenza viruses. To prevent possible emergences of new influenza virus variants, good vaccination strategies remain crucial. We focused on the use of potential conserved immunogenic hemagglutin peptide (HA-peptide) acting as multivalent vaccines and have reported their use in vaccine formulations. In the present work we combined different conserved HA-epitopes expressed along with flagellin, an adjuvant that interferes with innate immune response, reverse-translated to a pCDNA3.1(+) plasmid and were used as vaccines against SIV infection. Vaccination studies were performed in conventional farm pigs sero-positive to SIV (maternal derived antibodies, MDA) as well as in pigs selected for absence of MDA (sero-negative) to SIV. Vaccine efficacy was evaluated based on virus detection, immune response elicited and protection conferred to pH1N1 or SwH3N2 virus challenge. Our results demonstrated complete elimination and/or significant reduction in viral shedding in both experimental conditions within the first week after challenge suggesting vaccine efficacy against both the SIV subtypes (H1 and H3). It also demonstrates that maternally derived antibodies (MDA) were not an obstacle for the vaccine approach employed. An elevated boost in antibodies both against H1 and H3 in sera and BALF were detected in vaccinated animals along with strongly increased mucosal IgAs. Additionally, vaccinated animals mounted strong neutralizing antibodies in BALF. We consider that the vaccine formulation described here could potentially be used as a multivalent vaccine against influenza viruses.

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Trivalent Live Attenuated Influenza Vaccine (LAIV3) Induces multifaceted antibody response against H1N1 in children

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Influenza is a major respiratory pathogen and vaccination is the main method of prophylaxis. In 2012, the trivalent live attenuated influenza vaccine (LAIV3) was licensed in Europe for use in children. Vaccine-induced antibodies directed against the main viral surface glycoproteins, haemagglutinin (HA) and neuraminidase (NA), play an important role in limiting virus infection. Antibodies to the HA inhibit virus attachment to the host cell receptors and can be measured by the Haemagglutinin inhibition (HI) assay. Neutralizing antibodies (microneutralizing (MN) antibody, virus neutralizing antibody (VN)) as well as non-neutralizing antibodies (ADCC antibody) are also associated with reduce influenza viral infection. The objective of this study was to dissect the overall antibody responses induced after LAIV3 immunization to the influenza A viruses in children and adults.

Plasma was collected at regular time intervals from 20 children and 20 adults pre- and post-LAIV3 vaccination (up to a year) and analysed using a variety of serological assays. Previously we published that LAIV3 elicited H3-head and low levels of H1 stalk specific antibody responses in children, supporting the prophylactic use of LAIV in children [1]. In our recent data, we further showed no increase in MN antibody response post LAIV vaccination against H1N1. H1N1 stalk specific neutralizing and NI antibodies were boosted in children after LAIV. A trend of an increase in ADCC antibodies was observed after vaccination although not significant. Overall no increase in traditional serological HI and MN antibodies was observed in adults or children, however H1 NI and stalk specific antibodies increased in the children. The results indicate that these methods are more sensitive to qualitative changes in serum antibodies. This warrants that further exploration of using the HIN1 method for evaluating vaccine efficacy after LAIV vaccination.

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P107

Induction of mannitol metabolism in influenza A virus infected cells

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Influenza A virus (IAV) infection leads to pathogenetically important metabolic changes in host cells, but a comprehensive functional analysis of substrate utilization in IAV-infected cells has not been performed. We adapted the Phenotype MicroArrayTM for mammalian cells system to study changes in host cell respiration during IAV infection, based on simultaneous analysis of metabolism on 367 energy substrates in real time. Basal cell respiration increased upon infection of A549 cells with two different H1N1 isolates. In infected cells, respiration was lower on polysaccharides but higher on polyols as substrates, most significantly when mannitol was offered as carbon source. Remarkably, adding mannitol to cells growing in medium with or without glucose significantly increased replication of both strains. Metabolic flux analyses with C13-labeled mannitol revealed that infected cells, but not uninfected cells, acquired the ability to take up mannitol and use it as a carbon source for glycolysis, Krebs cycle intermediates, and amino acid synthesis. Remarkably, when cells were grown in the presence of both glucose and mannitol, nearly all lactate was derived from mannitol once glucose was depleted. Microarray analysis revealed that mannitol led to a significant reduction of the expression of mRNAs encoding the key enzymes of the polyol pathway, i.e. sorbitol dehydrogenase and aldo-keto reductase. These results provide the, to our knowledge, first evidence that higher eukaryotic cells can use extracellular mannitol as a carbon source and suggest that this pathway (1) is highly inducible by IAV and (2) may depend on enzymes distinct from the classical polyol pathway.

P108

Aconitate decarboxylase and itaconic acid reduce inflammation and host susceptibility in influenza A virus infection

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Aconitate decarboxylase 1 (ACOD1) catalyzes the conversion of cis-aconitate to itaconic acid, and its activity is highly regulated in sterile inflammation and host responses to some bacterial pathogens. Very little is known about its role in anti-viral defenses. We have therefore used targeted deletion of ACOD1 in mice and THP-1 cells, as well as exogenous addition of itaconic acid and dimethyl-itaconic acid, to study the role of the ACOD1-itaconic acid axis in host defenses against influenza A virus (IAV). ACOD1 mRNA was highly expressed in wild-type mouse lungs 48 h post infection with IAV (H1N1)PR/8/34. Weight loss, mortality, and histopathologic changes in lung were higher in ACOD1-/- than in +/+ mice. Infected ACOD1-/- THP-1 cells exhibited higher IAV hemagglutinin expression and higher inflammation, particularly in type I IFN-regulated pathways, than wild-type cells. This effect was reversed upon exogenous addition of itaconic acid or dimethyl-itaconic acid, and it correlated with suppression of pathogenetically important pathways, but increased release of the anti-inflammatory polypeptide IL-1ra. In infected A549 cells, exogenous addition of both compounds markedly reduced IFN responses and TLR signaling pathways without affecting viral replication. Computational network analysis identified the NFkB inhibitors IkBa and IkBz as master regulators of the immunomodulatory effects. These results provide first evidence that the ACOD1-itaconic acid axis constitutes a crucial link in protective host responses to IAV infection, likely due to limiting inflammation and associated end-organ damage. Furthermore, they suggest that itaconic and dimethyl-itaconic acid merit further evaluation as adjunct, immunomodulatory treatments in influenza infection in humans.

P112

PD0184264, an active metabolite of the MEK-inhibitor CI-1040 shows superior pharmacokinetics and antiviral activity against influenza virus

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Drugs directed against influenza virus directly show the tendency to induce resistance. We have previously shown that influenza virus (IV) highjack cellular factors for its own purpose and that the nuclear RNP export is strongly dependent on the virus-induced activation of the Raf/MEK/ERK signal pathway. Thus, this pathway is most favorable for antiviral intervention, because it is required by the virus to cross intracellular barriers, such as the nuclear membrane. We have shown that the MEK inhibitor CI-1040 demonstrated antiviral activity against IV in cell culture and in the mouse model. CI-1040 is a MEK Inhibitor that was originally developed by Pfizer for anti-tumor therapy. Here, we show that ATR-002 (PD0184264), the active metabolite of CI-1040, is more efficient in inhibiting IV propagation *in vivo* due to superior pharmacokinetics. We compare the kinase inhibitory potential of CI-1040 and ATR-002 in a cell free and cell dependent assay. Furthermore, antiviral activity of both compounds is demonstrated *in vitro* and after infection of mice with H1N1pdm09.
Treatment of mice with 25mg/Kg ATR-002 resulted in almost complete virus clearance in the lung. This effect could only be observed when 150mg/kg CI-1040 was used. Pharmacokinetic studies revealed that the ATR-002 appears to be clearly better absorbed in the antiviral mouse model compared to the mother compound. We now use ATR-002 as a candidate for further development in the direction to clinical investigations.

P113

The flavonoid Tiliroside demonstrates antiviral potential against influenzavirus by interfering with the viral entry into the host cell

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Viral infections, such as influenza, are a major cause of respiratory disease worldwide with high morbidity/mortality and enormous costs. Because influenza vaccination success is not always present and less than 25% of the population is effectively protected by influenza vaccine, antiviral therapy is an essential tool for controlling influenza viral infection. At present, specific and licensed antiviral drugs are broadly available against influenza virus from only one class of drugs (neuraminidase inhibitors). Their general effectiveness is discussed controversially. Their prophylactic use is limited due to possible side effects and resistance. Therefore, there is a clear and unmet need for antiviral drugs to treat or prevent influenza virus infections.
We were able to show that an extract of the gray-haired rockrose plant is antiviral against influenza and rhinoviruses. The mechanism of action is based on the extract preventing the virus from entering the cell. Furthermore, we succeeded in characterizing the herbal ingredient responsible for the antiviral effect. It is the flavonoid tiliroside. We were able to demonstrate the antiviral activity of tiliroside against influenzavirus alone and in combination with MEK-inhibitors. Time of addition experiments were performed to scrutinize the mode of action. Furthermore, EC_{50} values show the antiviral potential of tiliroside.
Based on our results so far, we see great potential to advance the preclinical development of tiliroside. The next steps will be pharmacokinetic and antiviral activity investigations in mice.

P114

ERK-phosphorylation, a valuable Biomarker to invest the effectiveness of MEK-inhibitors as Antivirals against Influenza

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The interest is increasing rapidly in the use of biomarkers and surrogate markers as primary measures of the effectiveness of investigational drugs in definitive drug trials. We develop MEK-inhibitors as antivirals against influenza. Here, the status of ERK-phosphorylation represents a perfect surrogate marker of the drug effectiveness.
There are various tools available to detect ERK-phosphorylation. The most sensitive one is the analysis of the phosphorylation sites of ERK using mass spectrometry. This method is very time and cost intensive. Another approach is to analyze the phosphorylation either with Western Blot Analysis (WBA) or with ELISA. The latter is a quantitative method but not very sensitive. WBA is more sensitive but only a qualitative method.
WES (Simple Western™), a relatively new analysis approach uses capillary electrophoresis to identify and quantitate a protein of interest and in addition also the phosphorylation status of a protein. We have used this method to characterize, the phosphorylation status of ERK.
We present a method, where lymphocytes can collected from a blood samples that will be used for pharmacokinetic (PK) analysis. Simply after centrifugation of the blood sample, the cell free plasma will be collected for PK analysis and normally cells would be thrown away. From these cells we collect the lymphocytes that will be analyzed for ERK phosphorylation. This allows the direct correlation of drug plasma concentration with the status of ERK-phosphorylation and consequently MEK activity.

P115

SENSITIVITY OF 2015 KAZAKHSTAN INFLUENZA VIRUSES TO CHEMOTHERAPY DRUGS

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One of the most important characteristics of influenza viruses is resistance to specific medicines. Practice shows that it is impossible to select an etiotropic antiviral drug effective against the whole variety of circulating viruses.
The purpose of this work was to study the resistance of the Kazakhstan strains of influenza virus to commercial chemotherapy drugs with different mechanisms of action. Studies were conducted on new isolates of the influenza A/H1N1 viruses isolated in 2015. Sensitivity to influenza drugs was assessed by the level of inhibition of reproduction of 100 EID50 (50% embryo infectious dose) of the virus by different drug concentrations in chick embryos.
It was established that the 2015 Kazakhstan strains of the influenza A/H1N1 viruses are sensitive to tamiflu and resistant to arbidol and ingavirin. With respect to remantadine, both sensitive and resistant variants have been detected among the viruses studied which indicates the heterogeneity of the influenza virus strains circulating in Kazakhstan. The results obtained indicate the need to monitor the epidemiological surveillance and study drug resistance in viruses - infectious agents.

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