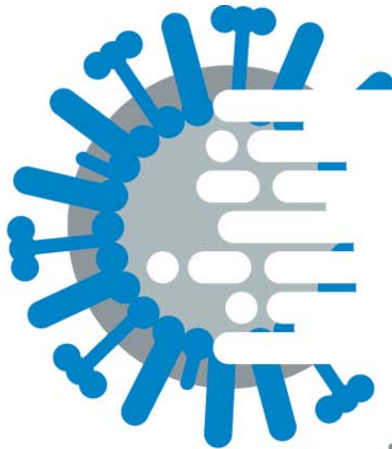

FluResearchNet.

Internal FluResearchNet Meeting 2011

September 26 – 27, 2011

Braunschweig, Germany

Funded by

Federal Ministry
of Education
and Research

TABLE OF CONTENTS

PROGRAMME..3

GENERAL INFORMATION 9

ORAL PRESENTATIONS..13

POSTER PRESENTATIONS 53

PARTICIPANTS..61

FluReseachNet PUBLICATIONS 67

Programme Internal FluResearchNet Meeting 2011

FluResearchNet Meeting Braunschweig, 26.-27. September 2011

Monday, 26.09.2011

10.00	**Registration**
11.00 - 11.15	**Welcome notes** Ludwig, S. I Münster Schughart, K. I Braunschweig
Session 1: Viral adaptation and host susceptibility	
11.15	**Key note: Molecular determinants of influenza virus host adaptation and pathogenicity** Gabriel, G. I Hamburg
11.45	**Adaptation of avian influenza viruses to growth in differentiated porcine airway epithelial cells** Punyadarsaniya, D. I Hannover
12.05	**Synergistic adaptive mutations in the HA and PA lead to increased virulence of pandemic 2009 H1N1 influenza A virus in mice.** Seyer, R. I Münster
12.25	**Analysis of the host genetic susceptibility to influenza A infections in mouse mutants** Hatesuer, B. I Braunschweig
12.45	**Identification of host genetic factors influencing host susceptibility to Influenza A infections.** Nedelko, T. I Braunschweig
13.05	**Long-term evolution of swine influenza A virus in Germany** Lange, J. I Jena
13.25 - 14.30	**Lunch**
Session 2: Pathogenesis	
14.30	**Key note: Attenuation and Virulence of Influenza Viruses** Stech, J. I Greifswald - Insel Riems
15.00	**Characterization of the neuraminidase of the H1N1/09 pandemic influenza virus** Matrosovich, M. I Marburg
15.20	**Recombinant soluble influenza A hemagglutinins bind to cell surface sialic acids of cultured cells and tissue sections** Sauer, A.-K. I Hannover
15.40	**Type II pneumocytes are the major target cells for seasonal and highly pathogenic influenza A viruses in the human lung** Wolff, T. I Berlin
16.00	**Primary Human Blood-derived Macrophages Restrict Release of Infectious Viral Particles.** Friesenhagen, J. I Hannover
16.20	**Subtype-specific pathogenicity of European swine influenza viruses in mice** Schmidtke, M. I Jena

16.40 - 17.15	**Coffee Break**
Session 3: Antiviral Strategies	
17.15	**Standard oseltamivir treatment has no therapeutic effect on H1N1 influenza virus A/Jena/5258/09 infection in mice** Seidel, N. I Jena
17.35	**Antiviral activity of the MEK-inhibitor U0126 against pandemic H1N1v and highly pathogenic avian influenza virus in vitro and in vivo** Planz, O. I Tübingen
17.55	**Biphasic action of p38 MAPK signaling in the influenza A virus induced primary and secondary host gene response** Börgeling, Y. I Münster
18.15	**Host proteases and influenza infection: characterization and inhibition** Bahgat, M. M. I Braunschweig
19.30	**Get-together**

Tuesday, 27.09.2011

Session 4: Pathogenesis II	
09.00	**Key note: HPAIV H5N1 escaping neutralisation: more than HA variation** Beer, M. I Greifswald - Insel Riems
09.30	**A single point mutation (Y89F) within the non- structural protein 1 of influenza A viruses dramatically limits lung epithelial cell tropism and virulence in mice.** Hrincius, E. I Münster
09.50	**Reassortment of wild-type H7N1 HPAIV with a H5N1 NS-segment leads to increased virulence in intravenously infected embryos of chicken and turkey** Petersen, H. I Hannover
10.10	**Restricted replication of avian influenza polymerases in mammals is rescued by adaptive mutations in NEP** Brunotte, L. I Freiburg
10.30	**Highly Pathogenic Influenza Viruses Cause an Inhibition of the Immune Response in Human Monocytes via Activation of the Rar-related Orphan Receptor alpha.** Friesenhagen, J. I Hannover
10.50	**Whole genome expression analysis as a valuable tool to host responses during the course of an influenza A virus infection** Wilk, E. I Braunschweig
11.10-11.30	**Coffee Break**
Session 5: Interferon System	
11.30	**Macrophage-expressed IFN-β mediates apoptotic alveolar epithelial injury in severe influenza virus pneumonia** Högner, K. I Gießen
11.50	**Interferon-lambda protects chicken embryos from viral infection** Rubbenstroth, D. I Freiburg
12.10	**Identification and preliminary characterization of IFN*a*-producing cells in virus-infected chickens** Bender, S. I Freiburg
12.30	**Rig-I activates the Phosphatidylinositol-3-kinase (PI3K) to promote efficient type I interferon production in response to influenza virus vRNA** Ehrhardt, C. I Münster
General network activities	
12.50	**Activities of the National Research Platform for Zoonoses** Ludwig, S. I Münster
13.00	**Discussion and closing remarks** Ludwig, S. I Münster
13.30	**Lunch**

Poster Presentation

P1 **Analysis of the antiviral ISG15 system and its role in the replication of human and animal influenza A viruses**
J. Knepper*, V. K. Weinheimer, T. Wolff

P2 **Antiviral activity of the proteasome inhibitor VL-01 against Influenza A viruses**
E. Haasbach*, E. K. Pauli, R. Spranger, D. Mitzner, U. Schubert, R. Kircheis, O. Planz

P3 **Pandemic H1N1 influenza virus carrying a Q136K mutation in the neuraminidase gene is resistant to zanamivir but exhibits reduced fitness in vivo**
D. Rubbenstroth*, M. Kaminski, and P. Stäheli*

P4 **Lung Epithelium Progenitors Show Increased Proliferation, Decreased Apoptosis and are infected during Influenza Pneumonia**
O.R. Gottschald*, J. Quantius, W. Seeger, J. Lohmeyer, S. Herold

P5 **FGF10/FGFR2b-induced alveolar epithelial repair is impaired in severe influenza virus pneumonia**
J. Quantius*, O.R. Gottschald, J. Lohmeyer, S. Herold

GENERAL INFORMATION

Date

September 26 - 27, 2011

Venue

Helmholtz Centre for Infection Research
Inhoffenstr. 7
D-38124 Braunschweig

Get Together

Restaurant i-Vent
(im i-Punkt)
Jenastieg 3-5
D-38124 Braunschweig
The restaurant is located in the 17th floor.

Organization

FluResearchNet
c/o Institute of Molecular Virology (IMV)
Center of Molecular Biology of Inflammation (ZMBE)
Westfälische Wilhelms-Universität Münster
Von-Esmarch-Str. 56
D-48149 Münster
Phone: +49 (0)2 51/83 53013
EMail: flu.2011@uni-muenster.de

Helmholtz Centre for Infection Research
Department of Infection Genetics
Inhoffenstr. 7
D-38124 Braunschweig
Phone: +49 531 6181-1102

DAS HELMHOLTZ-ZENTRUM FÜR INFEK-
TIONSFORSCHUNG IM ÜBERBLICK

Herzlich Willkommen im Helmholtz-Zentrum für Infektionsforschung! 15

ORAL PRESENTATIONS

Viral adaption and host susceptibility

Monday, September 26, 2011

Key note: Molecular determinants of influenza virus host adaptation and pathogenicity
Gabriel, G. l Hamburg

Adaptation of avian influenza viruses to growth in differentiated porcine airway epithelial cells
Punyadarsaniya, D. l Hannover

Synergistic adaptive mutations in the HA and PA lead to increased virulence of pandemic 2009 H1N1 influenza A virus in mice.
Seyer, R. l Münster

Analysis of the host genetic susceptibility to influenza A infections in mouse mutants
Hatesuer, B. l Braunschweig

Identification of host genetic factors influencing host susceptibility to Influenza A infections
Nedelko, T. l Braunschweig

Long-term evolution of swine influenza A virus in Germany
Lange, J. l Jena

Adaptation of avian influenza viruses to growth in differentiated porcine airway epithelial cells

D. Punyadarsaniya[1*], I. Hennig-Pauka[2], C. Winter[1,3], C. Schwegmann-Wessels[1], G. Herrler[1]

[1]Institute of Virology, University of Veterinary Medicine, Hannover, Germany;
[2]Clinic for swine, small ruminants and forensic medicine, University of Veterinary Medicine, Hannover, Germany
[3]Clinic for Poultry, University of Veterinary Medicine, Hannover, Germany

Swine are an important host for the epidemiology and interspecies transmission of influenza A viruses. The differentiated epithelial cells of respiratory tract are the primary target cells for influenza viruses infection. To analyze the infection of porcine airway epithelial cells by influenza viruses, we established precision-cut lung slices as a culture system for differentiated respiratory epithelial cells. A comparison of the infection by a porcine (H3N2 subtype) and an avian (H9N2 subtype) showed that the avian virus was inferior in the following parameters: (i) production of infectious virus, (ii) length of replication cycle, (iii) ciliostatic effect, and (iv) spectrum of infected cells (ciliated cells/mucus-producing cells). To analyze the adaptation of avian influenza viruses to the porcine respiratory epithelium, the H9N2 was passaged three times in precision-cut slices prepared from the porcine lung. Titration of the infectious virus released in the supernatant revealed that the virus recovered from the third passage was characterized by a shorter replication time resembling that of the porcine virus. On the other hand, the amount of infectious virus was not affected by the adapation process. Immunostaining was performed to identify infected cells, ciliated cells, and mucus-producing cells. While during the first passage co-staining of H9N2-virus-infected cells was detected only with ciliated cells, the second and third passage viruses behaved like the porcine H3N2 virus, i.e. they infected also mucus-procuding cells. These results show that the replication time and the spectrum of infected cells are the first parameters that are affected during adaptation of the avian H9N2 virus to growth in porcine respiratory eptihelial cells. The mutations that are correlated with these changes are currently determined.

Key words: respiratory epithelium, precision-cut lung slices, influenza viruses

Synergistic adaptive mutations in the HA and PA lead to increased virulence of pandemic 2009 H1N1 influenza A virus in mice.

R. Seyer[1*], E.R. Hrincius[1], D. Ritzel[2], M. Abt[2, +], H. Marjuki[3], J. Kühn[4], T. Wolff[2], S. Ludwig[1], C. Ehrhardt[1]

[1] Institute of Molecular Virology (IMV), ZMBE, Von Esmarch-Str. 56, D-48149 Muenster, Germany; [2] Robert Koch Institute, FG17, Nordufer 20, D-13353 Berlin, Germany; +, present address: Institute for Medical Microbiology and Hygiene; University Medical Centre Mannheim; Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany; [3] Division of Virology, Department of Infectious Diseases, St, Jude Children`s Research Hospital, Memphis, TN 38105, USA; [4] Institute of Medical Microbiology, Clinical Virology, Von Stauffenberg Strasse 36, D-48151 Muenster, Germany

Influenza impressively reflects the paradigm of a viral disease in which continued evolution of the virus is of paramount importance for annual epidemics and occasional pandemics in humans. The pandemic outbreak of the H1N1variant (H1N1v) virus in 2009 caused relatively mild symptoms in the majority of patients. However, the high mutation rate of influenza viruses may facilitate to the emergence of future H1N1v strains that exhibit higher pathogenicity and cause more severe outbreaks of respiratory disease. Because of the continuous threat of novel influenza outbreaks it appears important to gather further knowledge about viral pathogenicity determinants. Here, we explored the adaptive potential of the H1N1v isolate A/Hamburg/04/09 (HH/04) by sequential passaging in mice lungs. Three passages in mice lungs were sufficient to dramatically enhance pathogenicity of HH/04, as evidenced by increased mortality in mice. Sequence analysis identified four nonsynonymous mutations in the third passage virus. Using reverse genetics, three synergistically acting mutations were defined as pathogenicity determinants, comprising two mutations in the hemagglutinin (D222G and K163E), whereby the HA(D222G) mutation was shown to determine receptor binding specificity, and the PA(F35L) mutation increasing polymerase activity. In conclusion, synergistically action of all three mutations results in a mice lethal pandemic H1N1v virus. Our results highlight the potential of H1N1v to rapidly adapt to a new mammalian host within a few passages, clearly indicating the potential for the emergence of highly pathogenic H1N1v variants.

Key words: pandemic H1N1variant (H1N1v), mice-adaptation, pathogenicity determinants, polymerase activity

Analysis of the host genetic susceptibility to influenza A infections in mouse mutants

B. Hatesuer[1*], H. Wu[1], M. Tantawy[1], K. Schughart[1]

[1]Helmholtz Center for Infection research

Infections with the influenza A virus are responsible for the most common diseases of the respiratory tract of humans and cause serious illness and even death of affected people. The course and severity of disease is critically influenced by the virulence of the pathogen, but also by the susceptibility of the host. Here, many unknown host genetic factors play an important role and determine survival or death of the infected organism. In previous studies, we found that DBA/2J mice are highly susceptible to H1N1 and other influenza A subtypes. On the other hand, infected C57BL/6J mice exhibited much less severe symptoms and survived the infection. Genome-wide gene expression analysis showed the activation and down-regulation of many genes in the lung after infection in both strains. Based on these observations and the information in the literature we started to assess the role of individual genes for the host defense against influenza infections in mouse mutant lines.

So far, *Rag2* (recombination activating gene 2), *Irf7* (interferon regulatory factor 7) and *Ifi27l2a* (interferon, alpha-inducible protein 27 like 2A) knockout mice showed a more susceptible phenotype after influenza infection compared to C57BL/6 wild type mice. On the other hand, a mutation in a protease gene resulted in a highly resistant phenotype.

Identification of host genetic factors influencing host susceptibility to Influenza A infections

Tatiana Nedelko[1], Heike Kollmus[1], Barkha Srivastava[1], and Klaus Schughart[1, 2].

[1]Department of Infection Genetics, Helmholtz Centre for Infection Research, Inhoffenstr. 7, 38124 Braunschweig, Germany.
[2]University of Veterinary Medicine Hannover, Bünteweg 2, 30559 Hannover, Germany.

Genetic factors that are associated with increased susceptibility of the host to influenza virus infection or disease are largely unknown. Here, we studied the host response to influenza A virus (PR8 H1N1) infection in two inbred mouse strains – C57BL/6J and DBA/2J, and a large set of lines from the BXD recombinant inbred population. We characterized in detail the host response in the susceptible strain (DBA/2J) and resistant mouse strain (C57BL/6J) in terms of viral load, cytokine/chemokine profiles, lung pathology and genome-wide gene expression. In both mouse strains, chemokines, cytokines and interferon-response genes were up-regulated, indicating that the main innate immune defense pathways were activated. Infection of more than 50 recombinant inbred strains (BXD) allowed to identifying Quantitative Trait Loci (QTLs) that contribute susceptibility or resistance to virus infection. By analyzing survival, time to death and bodyweight loss as traits, we identified QTL peaks on chromosomes 5, 16, 17 and 19. We are currently mining these intervals for candidate genes, which may control these traits.

Long-term evolution of swine influenza A virus in Germany

J. Lange[1*], A. Philipps[1*], A. Krumbholz[1*], C. Steglich[1*], S. Bergmann[1*], S. Motzke[1*], M. Groth[2], S. Taudien[2], M. Platzer[2], R. Dürrwald[3], R. Zell[1]

[1]Dept. of Virology and Antiviral Therapy, Jena University Hospital; [2]Genome Analysis Group, Leipniz Institute for Age Research, Fritz Lipmann Institute, Jena; [3]IDT Biologika GmbH, Dessau-Rosslau

More than 200 porcine influenza A viruses from a collection of some 450 strains have been sequenced with conventional and next generation sequencing (NGS) techniques. The majority of the strains were obtained in the ongoing longitudinal swine influenza surveillance of the IDT Biologika that started in 2003. At present some 60–80 strains are collected each year and sequenced. Two NGS sequencers were assessed, the FLX genome sequencer of Roche and the GAII of Illumina. Using the FLX GS and 454 pyrosequencing chemistry, a test run yielded some 450 influenza virus-specific high quality reads (mean 330 nt). Nine contigs representing 66–99 per cent of the influenza virus segments (mean 88.6%) were de-novo assembled, and coverages ranged between 21-38fold (mean 26.2fold). The Illumina technique yielded up to 30 million reads per run with a length of 76 nt and 13–26 million reads (on average 87%) that could be assigned to an indexed virus isolate. After optimisation, the number of virus-specific reads increased from 5.8 % to 26.9 %. Analysis of 18 virus genomes per run is routinely achieved in a multiplex approach with an efficiency greater 99% of the influenza virus genome and coverages ranging from 22.7-14,650fold per nucleotide position.
Phylogenetic analysis of these isolates revealed evolution of swine influenza virus since its emergence in 1979. There is evidence of reassortments and occasional introduction of human seasonal and pandemic strains. Molecular as well as serologic data indicate antigenic drift and suggest the need to replace vaccines every 8-10 years.

Key words: next generation sequencing, Illumina, phylogenetic analysis, swine influenza surveillance

Pathogenesis

Monday, September 26, 2011

Key note: Attenuation and virulence of Influenza viruses

Stech, J. l Greifswald - Insel Riems

Characterization of the neuraminidase of the H1N1/09 pandemic influenza virus

Matrosovich, M. l Marburg

Recombinant soluble influenza A hemagglutinins bind to cell surface sialic acids of cultured cells and tissue sections

Sauer, A.-K. l Hannover

Type II pneumocytes are the major target cells for seasonal and highly pathogenic influenza A viruses in the human lung

Wolff, T. l Berlin

Primary human blood-derived macrophages restrict release of infectious viral particles.

Friesenhagen, J. l Hannover

Subtype-specific pathogenicity of European swine influenza viruses in mice

Schmidtke, M. l Jena

Characterization of the neuraminidase of the H1N1/09 pandemic influenza virus

J. Uhlendorff, T. Gerlach, L. Kühling, V. Laukemper, T. Matrosovich, V. Czudai-Matwich, F. Schwalm, H.-D. Klenk, M. Matrosovich*

Institute of Virology, Philipps University, Marburg

The 2009 pandemic H1N1 influenza virus (H1N1pdm) is a reassortant virus containing the gene of the hemagglutinin (HA) of a North American swine virus and the gene of the neuraminidase (NA) of a Eurasian avian-like swine H1N1 virus. Here we compared properties of the NA of H1N1pdm with those of NAs of H1N1 avian, swine and seasonal human viruses.
The H1N1pdm NA displayed an avian-virus-like sialidase activity with respect to 2-3-linked sialic acids and swine-virus-like sialidase activity with respect to 2-6-linked sialic acids. The NAs of avian viruses and early Eurasian swine viruses possessed strong hemadsorption activity. By contrast, no hemadsorption activity was detected in the NA of H1N1pdm and other swine viruses analyzed. To assess potential biological significance of observed distinctive catalytic and hemadsorption activities of the NA of H1N1pdm, we generated recombinant viruses that shared seven genes of H1N1pdm A/Hamburg/5/09 and contained the NA gene from representative avian, swine and seasonal human virus strains. The viruses harboring avian, Eurasian swine and seasonal human NAs eluted more slowly from red blood cells, were more sensitive to neutralization by human airway mucins and replicated less efficiently in differentiated cultures of human airway epithelial cells as compared with the viruses containing the NA of H1N1pdm and the NA of North American triple reassortant swine virus A/Ohio/2/07.
Our data indicate that functional properties of the NA of H1N1pdm are closer to those of H1N1 swine viruses than to those of H1N1 avian and seasonal human viruses.

Key words: neuraminidase, sialidase activity, hemadsorption, mucins

Recombinant soluble influenza A hemagglutinins bind to cell surface sialic acids of cultured cells and tissue sections

A.-K. Sauer*, M. Bohm, C. Schwegmann-Weßels, G. Herrler
Institute for Virology, University of Veterinary Medicine Hannover

The interaction of Influenza viruses with host cells is mediated by the hemagglutinin (HA). This viral surface glycoprotein recognizes sialic acid residues as a receptor determinant. Most avian influenza viruses preferentially bind to α2,3-linked sialic acids whereas human viruses prefer α2,6-linked sialic acids. To distinguish between these two linkages, the lectins MAA and SNA were used in many studies. Due to the huge diversity of oligosaccharides and the different HA subtypes, the two plant lectins are not sufficient to characterize the binding sites of influenza HA.

We therefore use soluble recombinant HAs of different subtypes in comparison with the classic lectin staining to investigate receptor distribution on different cell lines and sections of avian trachea and porcine lung shown by immunofluorescence.

Binding of the H9 and the H7 subtypes differs between cell lines and species of the tissue section. Although most cell lines show an equal intensity of the SNA and MAAII stain, the soluble H9 shows a stronger binding compared to the soluble H7. On avian trachea sections on the other hand the twofold amount of H9 is needed to achieve a quality of binding comparable to that of the H7 binding. On porcine lung sections, showing mainly SNA staining on the apical surface, H7 does not bind at all whereas H9 shows clear binding but not as widely distributed as the SNA stain.

These experiments show that a plant lectin staining alone cannot sufficiently depict the distribution of the cellular interaction partners of influenza A viruses. Soluble hemagglutinins should be a valuable tool to visualize potential influenza binding sites on cells and tissues.

Key words: soluble hemagglutinins, sialic acids, binding

Type II pneumocytes are the major target cells for seasonal and highly pathogenic Influenza A viruses in the human lung

V. K. Weinheimer[1], A. Becher[2], J. Knepper[1], T. Bauer[3], M. Tönnies[3], A. Hocke[2], S. Hippenstiel[2], T. Wolff [1 *]

[1]Div. of Influenza/Respiratory Viruses, Robert Koch Institut, Berlin, Germany; [2]Medizinische Klinik m. S. Infektiologie Charité, Berlin, Germany; [3]HELIOS Klinikum Emil von Behring, Berlin, Germany

The emergence of the pandemic H1N1-2009 influenza A virus and the continuous threat by highly pathogenic avian H5N1 strains highlight the significance of zoonotic virus transmissions. However, we still do not understand the basis for the strong pathology induced by H5N1 viruses in human lung. To explain the distinct pathological changes the hypothesis has been put forward that H5N1 virus infections inhibit repair processes and trigger dysregulated immune responses by targeting alveolar type II pneumocytes. However, it is uncertain whether this is a valid possibility as the cellular tropism of human and animal influenza viruses in human lung has not been systematically studied.

Here, we employed human lung organ cultures in which the complexity and spatial arrangement of cell types is preserved to study the replication and tropism of human, avian and porcine influenza A viruses. Highly-pathogenic H5N1 and human-adapted viruses propagated efficiently, whereas classical swine and low pathogenic avian viruses replicated poorly. Nevertheless, all viruses were detected almost exclusively in type II pneumocytes with some additional staining in alveolar macrophages. Surprisingly, low and high pathogenic avian viruses provoked a pronounced cyto- and chemokine response while human and pandemic H1N1 viruses were poor cytokine inducers. These findings show that differences in the pathogenic potential of influenza A viruses in the human lung cannot be attributed to a distinct cell tropism. Rather, high or low viral pathogenicity appears to be associated with a strain-specific capacity to productively replicate in type II pneumocytes and to cope with the induced cytokine response.

Key words: influenza A virus, cellular tropism, human lung, H5N1

Primary human blood-derived macrophages restrict release of infectious viral particles.

J. Friesenhagen[1,3*], D. Viemann[3], Yvonne Börgeling[2], Eike Hrincius[2], S. Ludwig[2], J.Roth[1].

[1]Institute of Immunology, University of Muenster, Germany.
[2]Institute of Virology, University of Muenster, Germany.
[3]Department of Pediatric Pulmonology, Allergology and Neonatology, Medical School Hannover, Germany.

Characteristics of systemic infections with highly pathogenic avian influenza viruses (HPAIV) such as H5N1 are cytokine burst and sepsis. Supposing that macrophages, classic amplifiers of inflammatory responses, are involved in these events, we investigated the role of human blood-derived macrophages in influenza virus infection and their contribution to cytokine release and viral replication. Macrophages were infected with low pathogenic H1N1 or high pathogenic H7N7 and H5N1 subtypes. Viral propagation of all influenza strains but especially of HPAIV was impaired in macrophages. Moreover, macrophages did not perform an effective innate immune response during HPAIV infections compared to H1N1 virus shown by transcriptome analyses. Induction of inflammatory cytokines as well as anti-virally acting type I interferons was significantly attenuated in H5N1- and H7N7-infections contradicting a primary role of this cell type for the cytokine burst. Surprisingly, in human macrophages we found a specific suppression of Influenza A virus M2 protein expression. According to the complete lack of M2 expression the replication cycle was most completely aborted in H5N1-infected cells and resulted in an abrogation of inflammasome activation. Our data point to a strategy of HPAIV to avoid initial inflammatory responses of macrophages. Bypassing this first line defense might facilitate virus spreading and infection of other cell types promoting the cytokine storm during the systemic stage of disease.

Key words: macrophages, H5N1 infection, inflammasome, M2 protein

Subtype-specific pathogenicity of European swine influenza viruses in mice

M. Schmidtke[1*], K. Bauer[1], K. Pfarr[1], D. Topf[1], H. Braun[1], M. Richter[1], R. Dürrwald[2], P. Wutzler[1]

[1]Institute of Virology and Antiviral Therapy, Jena University Hospital, Jena, Germany;
[2]IDT Biologika GmbH, Am Pharmapark, Dessau-Roßlau, Germany;

H1N1, H1N2, and H3N2 swine influenza viruses are endemic in Europe and present in 80-90% of pig herds in Germany. Even though their high economic impact on animal husbandry and effect on human health as well, the information on phenotypic properties of circulating virus strains is poor until now. Here, the pathogenicity of isolates of all three subtypes was compared in BALB/c mice.
Mice were intranasally infected with 10^4, 10^5, and 10^6 $TCID_{50}$ of individual virus isolates. Lethality, pathology score, weight loss kinetics (till day 21 p.i.), virus titers in lung and trachea as well as histopathology of lung (d4 and d21 p.i.) were used as parameters to compare the pathogenicity of individual isolates. To evaluate viral properties that could allow effective spread of swine influenza A viruses in mice, HA and NA gene sequences were determined. Interestingly, there was no significant difference in virus titers. But, behaviour, weight loss, and lung histopathology differed markedly between the three subtypes. The strongest disease resembling severe seasonal influenza with pneumonia was observed after infection with H1N1 isolates. H1N2 isolates were less pathogenic but also induced loss of body weight, illness, and a strong inflammatory response in mouse lungs after using the highest infection dose. In mice infected with H3N2 viruses no or mild illness occurred. Receptor use and HA glycosylation correlated with the observed strong subtype-specific differences in pathogenicity of swine influenza A viruses in mice. To explain the observed strong differences in inflammatory response in mouse lungs further studies are needed.

Key words: pathogenicity, swine influenza, mice

Antiviral Strategies

Monday, September 26, 2011

Standard oseltamivir treatment has no therapeutic effect on H1N1 influenza virus A/Jena/5258/09 infection in mice

Seidel, N. l Jena

Antiviral activity of the MEK-inhibitor U0126 against pandemic H1N1v and highly pathogenic avian influenza virus in vitro and in vivo

Planz, O. l Tübingen

Biphasic action of p38 MAPK signaling in the influenza A virus induced primary and secondary host gene response

Börgeling, Y. l Münster

Host proteases and influenza infection: characterization and inhibition

Bahgat, M. M. l Braunschweig

Standard oseltamivir treatment has no therapeutic effect on H1N1 influenza virus A/Jena/5258/09 infection in mice

N. Seidel[1*], H. Braun[1], M. Richter[1], A. Krumbholz[1], P. Wutzler[1], M. Schmidtke[1]

[1]Institute of Virology and Antiviral Therapy, Jena University Hospital, Jena, Germany;

In 2009 a new pandemic H1N1 influenza A virus (H1N1v) emerged containing the NA and M gene segment of Eurasian swine influenza A viruses. Like the gene donors, H1N1v isolates are adamantane-resistant. So, neuraminidase inhibitors (NAI) e.g. oseltamivir and zanamivir represent the only option for treatment and new drugs are urgently needed. Here, oseltamivir was utilized as control drug to establish and validate a mouse model with H1N1v for antiviral studies.

Within a single round of infection with influenza virus A/Jena/5258/09, a lethal virus in mice was generated (ma-Jena/5258). After producing a virus stock in MDCK cells, 10^6 $TCID_{50}$ of ma-Jena/5258 were applied to infect BALB/c mice. Oseltamivir was administered orally at 10 mg/kg/d from day 0-4, twice daily. Survival, body weight and general condition were monitored for 21 days p.i. To determine lung parameters like weight, virus load, and histopathological score some animals were dissected on day 4 and 21 p.i. Ma-Jena/5258 induced in oseltamivir-treated as well as placebo-treated animals a severe disease with strong body weight loss, impairment of general conditions, and lung histopathology leading to 10-20% mortality. The lack of oseltamivir efficacy *in vivo* was surprising because ma-Jena/5258 was proved to be NAI susceptible *in vitro.* Moreover, sequence analysis of hemagglutinin and neuraminidase gene segments did not reveal known mutations leading to reduced drug susceptibility or even resistance.

Obviously, higher oseltamivir concentrations are necessary to medicate H1N1v infections in mice.

Key words: oseltamivir, pandemic influenza, mouse model

Antiviral activity of the MEK-inhibitor U0126 against pandemic H1N1v and highly pathogenic avian influenza virus in vitro and in vivo

K. Droebner[1], S. Pleschka[2], S. Ludwig[3] and O. Planz[1,4*]

[1]Friedrich-Loeffler-Institut, Institute of Immunology, Paul-Ehrlich-Str. 28, D-72076 Tübingen, Germany [2]Institute for Medical Virology, Justus-Liebig-Universität Giessen, Germany [3]Institute of Molecular Virology (IMV), University of Muenster, Germany [4]Eberhard Karls University, Department of Immunology, Tübingen, Germany

The emergence of the 2009 H1N1 pandemic swine influenza A virus is a good example of how this viral infection can impact health systems around the world in very short time. The continuous zoonotic circulation and reassortment potential of influenza A viruses (IAV) in nature represents an enormous public health threat to humans. Beside vaccination antivirals are needed to efficiently control the spreading disease. In the present work we investigated whether the MEK inhibitor U0126, targeting the intracellular Raf/MEK/ERK signalling pathway, is able to suppress propagation of the 2009 pandemic IV H1N1v as well as highly pathogenic avian influenza viruses (HPAIV) in cell culture and also *in vivo* in the mouse lung. U0126 showed antiviral activity in cell culture against all tested IAV strains including oseltamivir resistant variants. Furthermore, we were able to demonstrate that treatment of mice with U0126 via the aerosol route led to (i) inhibition of MEK activation in the lung (ii) reduction of progeny IAV titers compared to untreated controls (iii) protection of IAV infected mice against a 100x lethal viral challenge. Moreover, no adverse effects of U0126 were found in cell culture or in the mouse. Thus, we conclude that U0126, by inhibiting the cellular target MEK, has an antiviral potential not only *in vitro* in cell culture, but also *in vivo* in the mouse model.

Key words: Influenza virus, U0126, MEK-inhibitor, Antiviral

Biphasic action of p38 MAPK signaling in the influenza A virus induced primary and secondary host gene response

Börgeling, Y.[1*]; Schmolke, M.[1,2]; Viemann, D.[3,4]; Roth, J.[3]; Ludwig S.[1]

[1]Institute of Molecular Virology (IMV), ZMBE, Von Esmarch-Str. 56, D-48149 Muenster, Germany; [2]present address: Mount Sinai School of Medicine, Department of Microbiology, New York, USA; [3]Institute of Immunology, Westfälische Wilhelms-Universität Münster, Germany; [4]present address: Department of Pediatric Pulmonology, Allergology and Neonatology, MH Hannover, Germany

Highly pathogenic avian influenza viruses (HPAIV) induce severe inflammation in poultry and men. There is still an ongoing threat that these viruses may acquire the capability to freely spread as novel pandemic virus strains that may cause major morbidity and mortality. One characteristic of HPAIV infections is the induction of a cytokine burst that strongly contributes to viral pathogenicity. It has been suggested, that this cytokine overexpression is an intrinsic feature of infected cells and involves hyperinduction of p38 mitogen activated protein kinase (MAPK). Here we investigate the role of p38 signaling in the antiviral response against HPAIV in endothelial cells, a primary source for cytokines during systemic infections.
Global gene expression profiling of HPAIV infected endothelial and epithelial cells in the presence of the p38-specific inhibitor SB202190 revealed, that inhibition of p38 leads to reduced expression of interferons (IFN) and other cytokines after A/Thailand/1(KAN-1)/2004 (H5N1) and A/FPV/Bratislava/79 (H7N7) infection in both cell types. Furthermore, the expression of interferon stimulated genes (ISGs) after treatment with IFN or conditioned media from HPAIV infected cells was decreased when the target cells were preincubated with SB202190 or dominant negative MKK6 was expressed. Finally, promoter analysis confirmed a direct impact of p38 MAPK on the IFN-enhanceosome and ISG-promoter activity. These observations show, that p38 acts on two levels of the antiviral IFN response: Initially the kinase regulates IFN induction and at a later stage p38 controls IFN signaling and thereby expression of IFN-stimulated genes.

Key words: MAPK p38, Influenza A Virus, HPAIV, cytokine storm

Host proteases and influenza infection: characterization and inhibition

Mahmoud M. Bahgat[1*] and Klaus Schughart[1]

[1]Department of Infection Genetics and University of Veterinary Medicine, Helmholtz Center for Infection Research, Inhoffenstrasse 7, 38124 Braunschweig, Germany
Email: Mahmoud-Bahgat.Riad@helmholtz-hzi.de

Proteases represent key players in establishment of influenza infection due to their role in cleavage of viral hemagglutinin. Although previous studies demonstrated the implication of transmembrane proteases in such processes, other proteases might also be involved. Here, we quantified the activities of trypsin-like proteases in homogenates of lungs from mice with various susceptibilities to influenza infection using specific peptide substrates. Results showed up-regulation in the measured activities post infection in comparison to non-infected mice. When the molecular weights of these activities were visualized by zymography, results indicated up-regulation of several proteolytically active peptides of a broad range of molecular weights suggesting involvement of more than one enzyme. The involvement of several proteases in the influenza infection was also confirmed quantitatively at the transcriptional level. The nature of such a protease family was further confirmed using specific inhibitors. Individual and cocktailed standard serine protease inhibitors blocked A/Puerto Rico/8/34 (H1N1; PR8) and A/Seal/Massachusetts/1/80 (H7N7; SC35M) propagation in cell culture and in mice. Exploring such proteases as possible alternative drug targets for development of new anti-influenza therapeutics especially in the light of the emergence of resistance to the known anti-influenza drugs will be discussed.

Key words: Influenza A viruses, trypsin like serine proteases, serine protease inhibitors

Pathogenesis II

Tuesday, September 27, 2011

Key note: HPAIV H5N1 escaping neutralisation: more than HA variation
Beer, M. l Greifswald - Insel Riems

A single point mutation (Y89F) within the non- structural protein 1 of influenza A viruses dramatically limits lung epithelial cell tropism and virulence in mice.
Hrincius, E. l Münster

Reassortment of wild-type H7N1 HPAIV with a H5N1 NS-segment leads to increased virulence in intravenously infected embryos of chicken and turkey
Petersen, H. l Hannover

Restricted replication of avian influenza polymerases in mammals is rescued by adaptive mutations in NEP
Brunotte, L. l Freiburg

Highly pathogenic Influenza viruses cause an inhibition of the immune response in human monocytes via activation of the Rar-related orphan receptor alpha.
Friesenhagen, J. l Hannover

Whole genome expression analysis as a valuable tool to host responses during the course of an influenza A virus infection
Wilk, E. l Braunschweig

A single point mutation (Y89F) within the non- structural protein 1 of influenza A viruses dramatically limits lung epithelial cell tropism and virulence in mice.

Eike R. Hrincius [1*], Lisa Gensler[1], Ann-Katrin Henneke[1], Darisuren Anhlan[1], Jon McCullers[2], Stephan Ludwig[1] and Christina Ehrhardt[1]

[1]Institute of Molecular Virology (IMV), ZMBE, Von Esmarch-Str. 56, D-48149 Muenster, Germany
[2]St. Jude Children's Research Hospital, Department of Infectious Diseases, 262 Danny Thomas Place, Memphis, TN 38105-3678

The non-structural protein 1 (A/NS1) of influenza A viruses (IAV) harbors several *src homology domain* (SH)-binding motifs (bm) (one SH2bm and two SH3bm), which mediate interaction with cellular proteins. In contrast to the sequence variability of the second SH3bm, the tyrosine 89 within the SH2bm is highly conserved among different IAV strains. This prompted us to evaluate the necessity of this SH2bm for IAV virulence. In an *in vivo* mouse model, we observed a dramatically reduced body weight-loss and reduced mortality upon infection with the A/NS1 Y89F mutant in comparison to wild-type virus. Infectious titers in the lung and bronchoalveolar-lavage fluid (BALF) were also reduced in comparison to wild-type virus. Concomitantly, we observed decreased cytokine, chemokine and immune cell levels in the lung and BALF as well as less severe pathological changes, reflecting reduced levels of virus-titers. Interestingly the replication of the A/NS1 mutant in mouse lung was overall reduced and strongly restricted to alveoli and if any marginally to bronchioli. In contrast, wild-type virus infection led to virus antigen positive areas in tracheal, bronchus, bronchiole and alveolar epithelium. Finally, wild-type virus infection resulted in a dramatic destruction of the bronchiole epithelium in clear contrast to infection with the A/NS1 mutant. Here, a slightly hypertrophic but entirely intact bronchiole epithelium was observed.
Taken together, we could show that disruption of the highly conserved SH2bm within the A/NS1 results in decreased virus distribution in the mouse lung and dramatically reduces virulence illustrating the necessity of the SH2bm for IAV induced pathogenicity.

Key words: influenza A virus, NS1 protein, SH binding motif, virulence, immune response

Reassortment of wild-type H7N1 HPAIV with a H5N1 NS-segment leads to increased virulence in intravenously infected embryos of chicken and turkey

H. Petersen[1*], Z. Wang[2], S. Pleschka[2], S. Rautenschlein[1]

[1]Clinic for Poultry, University of Veterinary Medicine Hannover, Germany; [2]Institute for Medical Virology, Justus-Liebig-University Giessen, Germany

H5N1-type highly pathogenic avian influenza viruses (HPAIV) have spread from Asia to Europe and have caused high mortality among various bird species. Also H7-type HPAIV are known to cause great fatalities in poultry populations with the last incidence in Germany in 2003. Since both subtypes are co-circulating among wild birds and poultry, reassortment between H7- and H5-type HPAIV is a possible threat not only for poultry but also for susceptible mammalian species including humans. Reassortment and transmission in poultry species could change their pathogenic and pandemic potential.
In this study we compared the HPAIV wild-type (wt) A/FPV/Rostock/34 (H7N1) and the reassortant FPV NS VN, carrying the NS segment of HPAIV A/Vietnam/1203/2004 (H5N1) in embryonated eggs of chicken and turkey for their potential to replicate in these two poultry species and induce lesions. After intravenous inoculation, embryo mortality, pathological lesions, virus replication and IFN-induction were quantified in different embryonic organs and blood samples at different time points post infection. HPAIV infection of chicken and turkey embryos led to 100% mortality in the first 24 hours post infection. More severe lesion development was observed for FPV NS VN compared to FPV wt in infected embryos of both species. Virus quantification by qRT-PCR revealed comparable organ tropisms between chicken and turkey but with faster propagation of FPV NS VN in turkey embryos.
Our results indicate that reassortment between avian H7-type HPAIV and H5-type HPAIV can indeed result in new viruses that show altered growth characteristics, host tropism and increased virulence in different poultry species.

Key words: Reassortment, HPAIV, turkey, chicken, host tropism

Restricted replication of avian influenza polymerases in mammals is rescued by adaptive mutations in NEP

B. Mänz[1,2]; L. Brunotte[1*]; M. Schwemmle[1]

[1]Department of Virology, Institute for Medical Microbiology and Hygiene, University of Freiburg, Germany
[2]Institute for Virology, University of Marburg

Zoonotic transmission of avian influenza viruses into the human population is dependent on adaptive mutations in the viral polymerase in order to achieve high-level replication in the new host. We provide evidence that the requirement for adaptation is caused by a restricted vRNA synthesis in human cells of avian polymerases that lack the human signature lysine 627 in the PB2 protein. Characterization of the pathogenicity factors of the highly pathogenic human-derived H5N1 isolate A/Thailand/1(KAN-1)/2004 (KAN-1) revealed that the observed replication defect is only partially compensated by adaptive mutations in the KAN-1 polymerase and that the decisive role to stimulate replication could be attributed to the nuclear export protein (NEP). We can show that KAN-1 and also other human avian influenza isolates, lacking lysine 627, acquired adaptive mutations in NEP, which strongly enhanced the restricted polymerase activity of avian influenza viruses in human cells, whereas NEP of avian isolates did not. Our data suggest that avian influenza viruses, when crossing the species barrier, acquire adaptive mutations in NEP to escape restricted replication in the mammalian host.

Highly pathogenic Influenza viruses cause an inhibition of the immune response in human monocytes via activation of the Rar-related orphan receptor alpha.

J. Friesenhagen[1,3*], D. Viemann[3], Yvonne Börgeling[2], Mirco Schmolke[2], S. Ludwig[2], J.Roth[1].

[1]Institute of Immunology, University of Muenster, Germany.
[2]Institute of Molecular Virology (IMV), ZMBE, Von Esmarch-Str. 56, D-48149 Muenster, Germany
[3]Department of Pediatric Pulmonology, Allergology and Neonatology, Medical School Hannover, Germany.

Characteristic symptoms of infection with highly pathogenic avian influenza viruses (HPAIV) are cytokine storm and systemic spreading of disease. Considering monocytes as possible initiators of a cytokine storm, we analyzed signaling pathways induced in influenza infected human monocytes. Primary human monocytes were infected with low pathogenic influenza virus strain PR8 and with two HAPIVs, FPV and KAN-1. The cell response was analyzed by qRT-PCR, FACS staining, Western Blot and Affymetrix gene array for three independent blood donors. Surprisingly, microarray analysis revealed that HPAIV cause a reduction of immune responses in human monocytes. We identified the Rar-related orphan receptor alpha (RORα) as a transcription factor activated by influenza virus infection. RORα knockout mice were used for further studies and for establishment of a stable transfected ER-Hoxb8 RORα knockout cell line. RORα negatively interferes with NF-κB signaling as we have shown by Western Blot in human as well as in mouse monocytes. Additionally, we could show that immune responses during influenza infection of monocytes directly depend on activation of RORα. ER-Hoxb8 RORα knockout cells showed a much stronger response to influenza virus infection than WT cells. We could also identify a novel role of RORα for interferon signaling. Physiological relevance of our findings was confirmed by *in vivo* experiments with heterozygous RORα knockout mice which showed higher susceptibility to influenza infection than WT mice. In conclusion, we found a novel mechanism used by HPAIVs to bypass an important part of the innate immune system in order to facilitate systemic spreading of disease.

Key words: H5N1, monocytes, immune response, RORα

Whole genome expression analysis as a valuable tool to host responses during the course of an Influenza A virus infection

C. Pommerenke[1], E. Wilk1[1*], B. Srivastava [1], A. Schulze[1], K. Schughart[1]

[1]Department of Infection Genetics, Helmholtz Centre for Infection Research, Braunschweig and University of Veterinary Medicine, Hannover

Murine models enable us to study systematically the infection with Influenza A and its consequences for the host. We performed a comprehensive systematic description of all aspects of the host response within one experimental setting. For these studies, we infected C57BL/6J mice with the H1N1 PR8 virus and analyzed the transcriptome of whole lungs from days 1 to 60 after infection using Agilent´s mouse 4x44k microarrays. Our analyzes revealed that various pathophysiological host responses, like the activation of the innate immune system, the switch from the innate to the adaptive immune response and the establishment of immunity is well reflected in the kinetics of gene expression patterns and were confirmed by flow cytometry and histology. These results will not only provide a valuable basis for a systems biology modeling of the normal course of an infection but also to allow to unravel important alterations of the host response in mutant mice or deleterious host responses to highly virulent virus subtypes.

Key words: whole genome expression analysis, kinetic study, murine influenza infection model

Interferon System

Tuesday, September 27, 2011

Macrophage-expressed IFN-β mediates apoptotic alveolar epithelial injury in severe influenza virus pneumonia

Högner, K. l Gießen

Interferon-lambda protects chicken embryos from viral infection

Rubbenstroth, D. l Freiburg

Identification and preliminary characterization of IFNα-producing cells in virus-infected chickens

Bender, S. l Freiburg

Rig-I activates the Phosphatidylinositol-3-kinase (PI3K) to promote efficient type I interferon production in response to Influenza virus vRNA

Ehrhardt, C. l Münster

Macrophage-expressed IFN-β mediates apoptotic alveolar epithelial injury in severe influenza virus pneumonia

Katrin Högner[1], Stefan Pleschka[2], Thorsten Wolff[3], Ulrich Kalinke[4], Dieter Walmrath[1], Johannes Bodner[5], Stefan Gattenlöhner[6], Werner Seeger[1], Juergen Lohmeyer[1] and Susanne Herold[1]

[1]University of Giessen Lung Centre (UGLC), Department of Internal Medicine, Division of Pulmonary and Critical Care Medicine and Infectious Diseases, Justus-Liebig-University, Giessen, Germany
[2]Institute of Medical Virology, Justus-Liebig-University, Giessen, Germany
[3]Robert Koch-Institute, FG17, Berlin, Germany
[4.]Institute for Experimental Infection Research, Twincore Centre for Experimental and Clinical Infection Research, Hannover, Germany
[5]Clinic of Thoracic Surgery, Department of Operative Medicine, Justus-Liebig-University, Giessen, Germany
[6]Department of Pathology, Justus-Liebig-University, Giessen, Germany

Influenza virus (IV) pneumonia is characterized by a rapid progression to lung failure and high mortality. Although lung macrophages (AM) have been linked to a role in host defence against viral infections it has been shown that macrophage express TRAIL (Tumor necrosis factor related apoptosis inducing ligand), a proapoptotic protein, that contributes to lung damage upon IV infection in mice. Additionally, the antiviral acting protein IFN-β, which is secreted by alveolar epithelial cells (AEC), as well as macrophages under inflammatory conditions, was proven to be a potent TRAIL inducer. Furthermore the IFN-β dependent upregulated protein Protein Kinase R (PKR), recognizing dsRNA, has been described to induce apoptosis. The mechanisms that regulate TRAIL expression and the distinct role of IFN-β and PKR in this context remain elusive. Within this project we focus on the intracellular signalling cascades (PKR and downstream events), the role of IFN-β in TRAIL upregulation and induction of apoptosis in AEC upon IV infection.
PR/8 infection of wild-type (wt) murine AM *in vitro* resulted in a dose-dependent upregulation of IFN-β and TRAIL mRNA and enhanced protein levels. The level of upregulation was dependent on the used Influenza virus strain, corresponding to their described pathogenicity. TRAIL and IFN-β were released from PR/8 infected wt AM, but not from PKR-deficient AM, showing impaired NF-κB activity. In addition, blockade of IFN-β and NF-κB in PR/8 infected wt AM resulted in reduced TRAIL expression. Furthermore

cocultivation of PR/8 infected PKR, IFNAR (type I interferon receptor) or TRAIL deficient AM with uninfected epithelial cells reduced their apoptosis compared to epithelial cells combined with infected wt AM. Moreover, TRAIL mRNA was found highly upregulated in wt and in PKR-deficient AM upon IFN-β stimulation. Additionally PR/8 and X31 induce TRAIL and IFN-β in human AM and TRAIL was detected in AM isolated from BAL of ventilated H1N1ν patients. Chimeric mice transplanted with $PKR^{-/-}$, $IFNAR^{-/-}$ or $TRAIL^{-/-}$ bone marrow cells showed reduced TRAIL and IFN-β levels in BAL upon PR/8 infection as well as reduced epithelial cell apoptosis in comparison with wt transplanted mice. The lung leakage was reduced in $IFNAR^{-/-}$ and $TRAIL^{-/-}$ bone marrow chimerics. Furthermore the body weight of TRAIL-/- chimerics was increased. In addition late treatment with IFN-β increased the TRAIL concentration in BAL, the apoptosis of epithelial cells as well as the lung leakage of PR/8 infected wt mice. In contrast pretreatment with IFN-β led to reduced virus titers and higher barrier function upon PR/8 infection *in vivo*.

These data demonstrate that PKR and NF-κB dependent IFN-ß release from PR/8-infected AM is an important auto/paracrine inducer of macrophage TRAIL expression in murine and human AM. Reduced IFN-β levels resulting in reduced TRAIL levels improve the clinical outcome *in vivo* whereas IFN-β application during inflammation showed the opposite effect suggesting that IFN-β present at late timepoints during inflammation mediates epithelial injury in severe influenza virus pneumonia.

Interferon-lambda protects chicken embryos from viral infection

A. Reuter[1,4], D.Rubbenstroth[1*], C. Winter[2], S. Kothlow[3], P. Stäheli[1]

[1]Department of Virology, University of Freiburg, Freiburg, Germany; [2]Institute for Virology, University of Veterinary Medicine Hannover, Hannover, Germany; [3]Department of Veterinary Sciences, University of Munich, Munich, Germany; [4]International Max Planck Research School for Molecular and Cellular Biology, Freiburg

Interferon-lambda (IFNλ) contributes to virus resistance of mammals by inducing interferon-stimulated genes in epithelial cells of mucosal surfaces. In contrast, little is known about the antiviral activity of IFNλ in avian species.
In this study we used a retroviral vector to constitutively express chicken IFN-λ (chIFNλ) in cultured cells and whole chicken embryos. In chIFNλ-mosaic-transgenic chicken embryos prominent expression of Mx, an ISG frequently used as marker of IFN responsiveness, was detected in tissues, such as intestine, lung, trachea and kidney that contain large numbers of epithelial cells. Retroviral vector-mediated expression of chIFNλ strongly reduced viral yields after *in ovo* infection of chicken embryos with various influenza A virus strains, Newcastle disease virus or infectious bronchitis virus. Enhanced virus resistance was also observed in primary kidney cells and tracheal organ cultures from chIFNλ-mosaic-transgenic chicken embryos, but not in chIFNλ-expressing chicken fibroblasts. These results suggest that, similar to its mammalian counterpart, chIFNλ confers antiviral protection mainly to epithelial cells in chickens. Interestingly, chicks mosaic-transgenic for chIFNλ hatched less efficiently than control animals and died within the first week after hatch for yet unclear reasons.

Key words: chicken, innate immunity, interferon-lambda, antiviral activity

Identification and preliminary characterization of IFNα-producing cells in virus-infected chickens

S. Bender[1*], B. Meyer[1], D. Rubbenstroth[1], S. Härtle[2], B. Kaspers[2], P. Staeheli[1]

[1]Department of Virology, University of Freiburg, Freiburg, Germany; [2]Department of Veterinary Sciences, University of Munich, Munich, Germany

Little is known about the innate antiviral immune response of domestic chickens. How chickens are able to produce high levels of type I interferon (IFN) in response to infection with viruses that carry inhibitors of IFN production, such as the non-structural protein 1 (NS1) of influenza A virus, is a question of particular interest. Therefore, the aim of this study was to identify and characterize cells in tissues of chickens which produce large amounts of IFNα in response to viral infection.

Ex vivo stimulation of primary chicken splenocytes with Newcastle disease virus or a toll-like-receptor 7 (TLR-7) agonist resulted in high levels of type I IFN in the culture supernatant. Using a monoclonal anti-chicken-IFNα antibody and flowcytometric intracellular cytokine staining we were able to detect a small population of IFN-producing cells in stimulated cultures. Further characterization showed that the IFN-producing cells were positive for CD45 and MHC-II.

Small numbers of chIFNα-producing cells were also detected in the spleen of chickens infected with the highly pathogenic avian influenza virus (HPAIV) strain A/Cygnus cygnus/Germany/R65/2006 (H5N1) or treated with a TLR-7 agonist. Immunohistochemical analysis revealed that the chIFNα-producing cells in HPAIV-infected spleens were negative for viral antigen but usually located in close proximity to infected cells.

Our data indicate that a small population of immune cells is responsible for the production of high amounts of chIFNα in virus-infected chickens and may represent specialized producers of type I IFNs.

Key words: chickens, innate immunity, type I interferon, flowcytometry, splenocytes

Rig-I activates the Phosphatidylinositol-3-kinase (PI3K) to promote efficient type I interferon production in response to Influenza virus vRNA

C. Ehrhardt*, E. R. Hrincius, R. Dierkes D. Anhlan, V. Wixler, St. Ludwig

Institute of Molecular Virology (IMV), ZMBE, Von Esmarch-Str. 56, D-48149 Muenster, Germany

The phosphatidylinositol 3-kinase (PI3K) is activated upon influenza A virus infection in a biphasic manner. An early and transient induction of PI3K signalling is induced by viral attachment to cells and promotes virus entry. In later phases of the infection cycle the kinase is activated by direct interaction with the viral NS1 protein, leading to prevention of premature apoptosis. Besides these virus-supporting functions, it was also suggested that PI3K signalling is essential for complete activation of interferon regulatory factor 3 (IRF-3) in response to synthetic dsRNA and IAV infections. However, a direct role of PI3K signalling in the immune response to influenza virus infections was not described yet. Here we show that accumulation of vRNA in cells infected with either influenza A or B viruses results in PI3K activation. Furthermore, expression of the RNA receptors Rig-I and MDA5 was increased upon stimulation with virion extracted vRNA or IAV infection. Using siRNA approaches, Rig-I was identified as the pathogen receptor necessary for influenza virus vRNA sensing and subsequent PI3K activation in a TRIM25 and MAVS signalling dependent fashion. Rig-I induced PI3K signalling was further shown to be essential for complete IRF-3 activation and consequently induction of the type I interferon response. These data identify PI3K as a factor that is activated as part of the Rig-I mediated anti-pathogen response to enhance expression of type I interferons.

Key words: influenza A virus; PI3K; vRNA; Rig-I

POSTER PRESENTATIONS

Analysis of the antiviral ISG15 system and its role in the replication of human and animal influenza A viruses

J. Knepper*, V. K. Weinheimer, T. Wolff

Div. of Influenza/Respiratory Viruses, Robert Koch Institute, Berlin, Germany

Influenza A viruses (IAV) infect a wide range of avian and mammalian species. However, there is still only incomplete knowledge about the virus- and host-encoded factors enabling animal IAV strains to adapt to the human host. Previous data showed variations in the upregulation of type I IFN and IFN-induced genes like ISG15 after infections with human, avian and porcine IAV. The underlying mechanisms responsible for these different immune responses are subject of our investigation. The ISG15 system shows strong antiviral effects, thus several viruses evolved ISG15 evasion mechanisms. ISG15 is a ubiquitin-like polypeptide that is covalently attached to both host and viral proteins mediated mainly by the ligases Ube1L, UbcH8 and Herc5. We hereby hypothesize that the ISG15 system plays an important role in controlling IAV replication and possibly its virulence and species specificity.
Our initial investigations showed that ISG15 is strongly induced in A549 cell cultures upon IAV infection. Interestingly, this induction occurred predominantly in uninfected cells of the culture, whereas little ISG15 was detected in the initially infected cells. Transfection-based experiments demonstrated that the sole expression of the viral polymerase with viral RNA was sufficient to induce ISG15, which was inhibited by co-expressed viral NS1 protein. These findings indicate an important role for the viral NS1 protein in counteracting the antiviral ISG15 system.
Future studies will characterize the role of ISG15 in the replication of WT and mutant IAV by gain- and loss-of-function approaches. Key findings will be validated in a previously established *ex vivo* lung infection model.

Key words: ISG15, influenza A virus, IFN

Antiviral activity of the proteasome inhibitor VL-01 against Influenza A viruses

E. Haasbach[1,2*], E. K. Pauli[2], R. Spranger[2], D. Mitzner[2,3], U. Schubert[2,3], R. Kircheis[2], O. Planz[1,4]

[1]Friedrich-Loeffler-Institut, Institute of Immunology, Paul-Ehrlich Str. 28, 72076 Tuebingen, Germany
[2]ViroLogik GmbH, Innovation Centre for Medical Technology and Pharmaceuticals, Henkestr. 91, 91052 Erlangen, Germany
[3]Clinical and Molecular Virology, Friedrich-Alexander-University of Erlangen-Nuernberg, Schloßgarten 4, 91054 Erlangen, Germany
[4]University of Tuebingen, Interfaculty Institute for Cell Biology, Department of Immunology, Auf der Morgenstelle 15, 72076 Tuebingen, Germany

The appearance of highly pathogenic avian influenza A viruses of the H5N1 subtype being able to infect humans and the 2009 H1N1 pandemic reveals the urgent need for new and efficient countermeasures against these viruses. The long-term efficacy of current antivirals is often limited, because of the emergence of drug-resistant virus mutants. A growing understanding of the virus-host interaction raises the possibility to explore alternative targets involved in the viral replication. In the present study we show that the proteasome inhibitor VL-01 leads to reduction of influenza virus replication in human lung adenocarcinoma epithelial cells (A549) as demonstrated with three different influenza virus strains, A/Puerto Rico/8/34 (H1N1) (EC_{50} value of 1.7 µM), A/Regensburg/D6/09 (H1N1v) (EC_{50} value of 2.4 µM) and A/Mallard/Bavaria/1/2006 (H5N1) (EC_{50} value of 0.8 µM). In *in vivo* experiments we could demonstrate that VL-01-aerosol-treatment of BALB/c mice with 14.1 mg/kg results in no toxic side effects, reduced progeny virus titers in the lung (1.1 ± 0.3 log_{10} pfu) and enhanced survival of mice after infection with a 5-fold MLD_{50} of the human influenza A virus strain A/Puerto Rico/8/34 (H1N1) up to 50%. Furthermore, treatment of mice with VL-01 reduced the cytokine release of IL-α/-β, IL-6, MIP-1 β, RANTES and TNF- α induced by LPS or highly pathogen avian H5N1 influenza A virus. The present data demonstrates an antiviral effect of VL-01 *in vitro* and *in vivo* and the ability to reduce influenza virus induced cytokines and chemokines.

Key words: Influenza A virus; proteasome inhibitor; drug-resistance

Pandemic H1N1 influenza virus carrying a Q136K mutation in the neuraminidase gene is resistant to zanamivir but exhibits reduced fitness *in vivo*

D. Rubbenstroth[1*], M. Kaminski[1], and P. Stäheli[1*]

[1]Department of Virology, University of Freiburg, Freiburg, Germany

Neuraminidase inhibitors are routinely used for the treatment of severe influenza A virus infections. Oseltamivir resistance became widespread in seasonal H1N1 influenza viruses in 2007/2008, and oseltamivir-resistant pandemic H1N1 viruses (pH1N1) also seem to retain a high degree of fitness. Zanamivir resistance is observed only infrequently in influenza viruses and little is known about the ability of pH1N1 to tolerate resistance mutations.
In this study we identified a glutamine-to-lysine mutation at position 136 (Q136K) of the neuraminidase which confers zanamivir resistance. The mutation was acquired during passage in MDCK cells in the presence of increasing concentrations of zanamivir. Introduction of this mutation into the wild-type virus by reverse genetics resulted in enhanced resistance against zanamivir in a neuraminidase activity assay. The oseltamivir resistance of this mutant was not increased. Growth kinetics on MDCK cells revealed no significant differences between the mutant (rHH-Q136K) and the wild-type virus (rHH-wt). However, when growth kinetics experiments were performed on MDCK cells over-expressing α-2,6-sialic acids (MDCK-SIAT), replication of rHH-Q136K was markedly delayed as compared to rHH-wt. Guinea pigs experimentally infected with rHH-Q136K had only moderate viral titres in nasal washings and did not transmit the virus to contact-exposed animals.
Our data shows that pandemic H1N1 viruses may gain zanamivir resistance under vigorous selective pressure. However, such neuraminidase mutations seem to result in dramatic losses in viral fitness. Therefore, it is unlikely that zanamivir-resistant mutants of pH1N1 become widespread in the human population.

Key words: pandemic H1N1; zanamivir resistance, neuraminidase, transmission

Lung epithelium progenitors show increased proliferation, decreased apoptosis and are infected during Influenza pneumonia

O.R. Gottschald[1*], J. Quantius[1], W. Seeger[1], J. Lohmeyer[1], S. Herold[1]

[1]Department of Internal Medicine II, Uniklinikum Giessen, Germany

Influenza virus can induce severe damage of the alveolar epithelium leading to pulmonary oedema and respiratory failure. Therefore, during the disease an efficient alveolar repair is crucial for recovery.

In this study, we analysed a distinct alveolar epithelial cell population (EpCAM^{high} CD49f^{high} CD24^{low}) during influenza infection. These cells were previously described to have progenitor/stem cell characteristics, being resistant to damage, proliferating after injury in vivo and being multipotent in clonal assays. We found this population of local progenitors, displaying the immunophenotypic profile ProSPC^{int} CCSP^{pos} Sca1^{int} CD104^{high} and CD34^{high}, located at the level of bronchioles in the distal respiratory tract. During influenza infection, this population showed a higher proliferation rate, peaking at day 7 p. i., than other populations of lung epithelial cells. In addition, the progenitor cells showed the lowest degree of infection induced apoptosis. Different tested influenza virus strains exhibited the highest tropism for this population, as demonstrated by NP (nucleoprotein) staining. Interestingly, the NP positive progenitor cells showed lower proliferation rates than the NP negative counterparts. When comparing different influenza virus strains (A/PR/8/34 H1N1, A/X-31 H3N2, A/California/7/2009 H1N1), we found differences in the progenitor cell proliferation rate which could be correlated to the disease severity and the percentage of infected alveolar epithelial cells. In summary, during influenza infection the local lung epithelial progenitor cell fraction was found to have higher proliferation and lower apoptosis rates compared to other lung epithelial cell subsets and all influenza virus strains tested showed a preferential tropism the progenitor cell population.

Key words: lung epithelial progenitors, lung injury and repair

FGF10/FGFR2b-induced alveolar epithelial repair is impaired in severe influenza virus pneumonia

J. Quantius[1*], O.R. Gottschald[1], J. Lohmeyer[1], S. Herold[1]

[1]Department of Internal Medicine II, Uniklinikum Giessen, Germany

Influenza virus pneumonia is associated with apoptotic damage of the alveolar epithelial barrier and pulmonary edema. Efficient alveolar repair crucial for recovery is initiated by a coordinated mesenchymal-epithelial cross-talk resulting in proliferation of epithelial progenitor cells and transit-amplifying cells. Cell lineage tracing studies in lung injury models suggest that the adult lung comprises epithelial progenitor cells which are located at bronchioalveolar duct junctions and are resistant to damage, proliferate after injury in vivo and are multipotent in clonal assays. Although FGF10 plays a major role in lung development it is also known to have reparative and anti-apoptotic potential and contributes to the proliferation of precursor cells in vitro.
Analysis of CD31- CD45- lung cells according to established surface marker expression reveals different epithelial populations with proliferative potential. Intratracheal application of recombinant FGF10 stimulates the proliferation of these epithelial populations in vivo. However, treatment with recombinant FGF10 directly before or four days post influenza virus infection had no proliferative or anti-apoptotic effect towards epithelial cell populations. Analysis of endogenous FGF10 and its receptor FGFR2b show a significant downregulation after influenza virus infection which might contribute to defective alveolar repair and progression of lung injury after severe influenza virus pneumonia.

Identification of the signaling pathways involved in influenza virus-induced downregulation of the reparative FGF10-FGFR2b axis and analysis of the role of mononuclear phagocytes may result in new potential targets for intervention in severe influenza virus-induced lung injury.

Key words: Lung injury, progenitor cells, alveolar repair, FGF10

PARTICIPANTS

A

B

Bahgat, Mahmoud M.
Helmholtz-Zentrum für Infektionsforschung
Braunschweig, Germany
Mahmoud-Bahgat.Riad@helmholtz-hzi.de

Beer, Martin
Friedrich-Loeffler-Institut
Greifswald-Insel Riems, Germany
Martin.Beer@fli.bund.de

Bender, Silke
Universitätsklinikum Freiburg
Freiburg, Germany
silke.bender@uniklinik-freiburg.de

Benninger, Gerlinde
Nationale Forschungsplattform für Zoonosen
Münster, Germany
benninge@ukmuenster.de

Bergmann, Silke
Helmholtz-Zentrum für Infektionsforschung
Braunschweig, Germany
silke.bergmann@helmholtz.hzi.de

Börgeling, Yvonne
Westfälische Wilhelms-Universität Münster
Münster, Germany
borgelin@uni-muenster.de

Brunotte, Linda
Universitätsklinikum Freiburg
Freiburg, Germany
linda.brunotte@uniklinik-freiburg.de

C

Czudai-Matwich, Volker
Philipps-Universität Marburg
Marburg, Germany
czudai@staff.uni-marburg.de

D

Darsaniya, Punyadarsaniya
Tierärztliche Hochschule Hannover
Hannover, Germany
darsaniya_p@yahoo.de

Della-Beffa, Cristina
Helmholtz-Zentrum für Infektionsforschung
Braunschweig, Germany
cristina.della-beffa@helmholtz-hzi.de

Dengler, Leonie
Helmholtz-Zentrum für Infektionsforschung
Braunschweig, Germany
Leonie.Dengler@helmholtz-hzi.de

E

Ehrhardt, Christina
Westfälische Wilhelms-Universität Münster
Münster, Germany
ehrhardc@uni-muenster.de

F

Foth, Sylvana
Helmholtz-Zentrum für Infektionsforschung
Braunschweig, Germany
Sylvana.Foth@helmholtz-hzi.de

Friesenhagen, Judith
Medizinische Hochschule Hannover
Hannover, Germany
jf1510@aol.com

G

Gabriel, Gülsah
Heinrich-Pette-Institut
Hamburg, Germany
guelsah.gabriel@hpi.uni-hamburg.de

Gottschald, Oana
Justus-Liebig-Universität Gießen
Gießen, Germany
Oana.R.Gottschald@innere.med.uni-giessen.de

H

Haasbach, Emanuel
Friedrich-Loeffler-Institut
Tübingen, Germany
haasbach@gmail.com

Haller, Otto
Universitätsklinikum Freiburg
Freiburg, Germany
otto.haller@uniklinik-freiburg.de

Härtle, Sonja
Ludwig-Maximilians-Universität München
München, Germany
Sonja.Haertle@lmu.de

Hartmann, Sandra
Tierärztliche Hochschule Hannover
Hannover, Germany
sandra.hartmann@tiho-hannover.de

Hatesuer, Bastian
Helmholtz-Zentrum für Infektionsforschung
Braunschweig, Germany
bastian.hatesuer@helmholtz-hzi.de

Henneberg, Berenike
Helmholtz-Zentrum für Infektionsforschung
Braunschweig, Germany
Berenike.Henneberg@helmholtz-hzi.de

Hippenstiel, Stefan
Charité Berlin
Berlin, Germany
Stefan.Hippenstiel@charite.de

Högner, Katrin
Justus-Liebig-Universität Gießen
Gießen, Germany
Katrin.Hoegner@innere.med.uni-giessen.de

Hrincius, Eike
Westfälische Wilhelms-Universität Münster
Münster, Germany
eikehrincius@googlemail.com

I

J

K

Kaiser, Annette
Tierärztliche Hochschule Hannover
Hannover, Germany
annette.kaiser@tiho-hannover.de

Knepper, Jessica
Robert Koch-Institut
Berlin, Germany
KnepperJ@RKI.de

Kochs, Georg
Universitätsklinikum Freiburg
Freiburg, Germany
georg.kochs@uniklinik-freiburg.de

Kollmus, Heike
Helmholtz-Zentrum für Infektionsforschung
Braunschweig, Germany
Heike.Kollmus@helmholtz-hzi.de

L

Lange, Jeannette
Friedrich-Schiller-Universität Jena
Jena, Germany
Jeannette.Lange@med.uni-jena.de

Ludwig, Stephan
Westfälische Wilhelms-Universität Münster
Münster, Germany
ludwigs@uni-muenster.de

M

Matrosovich, Mikhail
Philipps-Universität Marburg
Marburg, Germany
M.Matrosovich@gmail.com

May, Mathias
Helmholtz-Zentrum für Infektionsforschung
Braunschweig, Germany
Mathias.May@helmholtz-hzi.de

Mehnert, Nora
Helmholtz-Zentrum für Infektionsforschung
Braunschweig, Germany
Nora.mehnert@helmholtz-hzi.de

N

Nedelko, Tatiana
Helmholtz-Zentrum für Infektionsforschung
Braunschweig, Germany
Tatiana.Nedelko@helmholtz-hzi.de

O

P

Petersen, Henning
Tierärztliche Hochschule Hannover
Hannover, Germany
henning.petersen@tiho-hannover.de

Planz, Oliver
Eberhard Karls Universität Tübingen
Tübingen, Germany
oliver.planz@uni-tuebingen.de

Pleschka, Stephan
Justus-Liebig-Universität Gießen
Gießen, Germany
stephan.pleschka@mikro.bio.uni-giessen.de

Pommerenke, Claudia
Helmholtz-Zentrum für Infektionsforschung
Braunschweig, Germany
Claudia.Pommerenke@helmholtz-hzi.de

Q

Quantius, Jennifer
Justus-Liebig-Universität Gießen
Gießen, Germany
Jennifer.Quantius@innere.med.uni-giessen.de

R

Rautenschlein, Silke
Tierärztliche Hochschule Hannover
Hannover, Germany
silke.rautenschlein@tiho-hannover.de

Röll, Susanne
Ludwig-Maximilians-Universität München
München, Germany
S.Roell@tiph.vetmed.uni-muenchen.de.

Rubbenstroth, Dennis
Universitätsklinikum Freiburg
Freiburg, Germany
Dennis.Rubbenstroth@uniklinik-freiburg.de

S

Sauer, Anne-Kathrin
Tierärztliche Hochschule Hannover
Hannover, Germany
Anne-Kathrin.Sauer@tiho-hannover.de

Schmidtke, Michaela
Friedrich-Schiller-Universität Jena
Jena, Germany
michaela.schmidtke@med.uni-jena.de

Schughart, Klaus
Helmholtz-Zentrum für Infektionsforschung
Braunschweig, Germany
klaus.schughart@helmholtz-hzi.de

Schwegmann-Weßels, Christel
Tierärztliche Hochschule Hannover
Hannover, Germany
christel.schwegmann@tiho-hannover.de

Schwemmle, Martin
Universitätsklinikum Freiburg
Freiburg, Germany
martin.schwemmle@uniklinik-freiburg.de

Seidel, Nora
Friedrich-Schiller-Universität Jena
Jena, Germany
Nora.Seidel@med.uni-jena.de

Seyer, Roman
Westfälische Wilhelms-Universität Münster
Münster, Germany
r.seyer@uni-muenster.de

Stäheli, Peter
Universitätsklinikum Freiburg
Freiburg, Germany
peter.staeheli@uniklinik-freiburg.de

Stech, Jürgen
Friedrich-Loeffler-Institut
Greifswald-Insel Riems, Germany
juergen.stech@fli.bund.de

T

Tantaway Mohamed
Helmholtz-Zentrum für
Infektionsforschung
Braunschweig, Germany
mohamed.tantawy@helmholtz-hzi.de

U

V

Viemann, Dorothee
Medizinische Hochschule Hannover
Hannover, Germany
viemann.dorothee@mh-hannover.de

W

Wilk, Esther
Helmholtz-Zentrum für
Infektionsforschung
Braunschweig, Germany
Esther.Wilk@helmholtz-hzi.de

Wolff, Thorsten
Robert Koch-Institut
Berlin, Germany
wolfft@rki.de

X

Y

Z

Zell, Roland
Friedrich-Schiller-Universität Jena
Jena, Germany
Roland.Zell@med.uni-jena.de

FluReseachNet

PUBLICATIONS

2010 - 2011

Abd El Rahman S, Winter C, El-Kenawy A, Neumann U, **Herrler G.** Differential Sensitivity of Well-Differentiated Avian Respiratory Epithelial Cells to Infection by Different Strains of Infectious Bronchitis Virus. (2010). *J Virol.* **84**(17):8949-52.

Abt M, de Jonge J, Laue M, **Wolff T**. Improvement of H5N1 influenza vaccine viruses: influence of internal gene segments of avian and human origin on production and hemagglutinin content. (2011). *Vaccine.* **29**(32):5153-62.

Alberts R, Srivastava B, Wu H, Viegas N, Geffers R, Klawonn F, Novoselova N, Zaverucha do Valle T, Panthier JJ, **Schughart K.** Gene expression changes in the host response between resistant and susceptible inbred mouse strains after influenza A infection. (2010). *Microbes Infect.* **12**(4):309-18.

Alberts, R. and **Schughart, K.** High throughput gene expression analysis and the identification of expression QTLs. (2010). Chapter in: Gene Discovery for Disease Models: John Wiley & Sons, Inc., Hoboken, New Jersey, U.S.A., *in press*.

Anhlan D, Grundmann N, Makalowski W, **Ludwig S,** Scholtissek C. Origin of the 1918 pandemic H1N1 influenza A virus as studied by codon usage patterns and phylogenetic analysis. (2011). *RNA* **17**(1):64-73.

Bahgat MM, Błazejewska P, **Schughart K.** Inhibition of lung serine proteases in mice: a potentially new approach to control influenza infection. (2011). *Virol J.* **8**:27.

Bauer K, Dürrwald R, Schlegel M, Pfarr K, Topf D, Wiesener N, Dahse HM, Wutzler P, **Schmidtke M**. Neuraminidase inhibitor susceptibility of swine influenza A viruses isolated in Germany between 1981 and 2008. (2011). *Med Microbiol Immunol.* Jun 19. [Epub ahead of print]

Bertram S, Glowacka I, Blazejewska P, Soilleux E, Allen P, Danisch S, Steffen I, Choi SY, Park Y, Schneider H, **Schughart K,** Pöhlmann S. TMPRSS2 and TMPRSS4 facilitate trypsin-independent spread of influenza virus in Caco-2 cells. (2010). *J Virol.* **84**(19):10016-25.

Blazejewska P, Koscinski L, Viegas N, Anhlan D, **Ludwig S, Schughart K.** Pathogenicity of different PR8 influenza A virus variants in mice is determined by both viral and host factors. (2011). *Virology.* **412**(1):36-45.

Chase GP, Rameix-Welti MA, Zvirbliene A, Zvirblis G, Götz V, **Wolff T**, Naffakh N, Schwemmle M. Influenza Virus Ribonucleoprotein Complexes Gain Preferential Access to Cellular Export Machinery through Chromatin Targeting. (2011). *PLoS Pathog.* Sep;7(9):e1002187. Epub 2011 Sep 1.

Chase G, Wunderlich K, Reuther P, **Schwemmle M**. Identification of influenza virus inhibitors which disrupt of viral polymerase protein-protein interactions. (2011). *Methods.* Aug 16. [Epub ahead of print]

Deng Q, Wang D, Xiang X, Gao X, Hardwidge PR, Kaushik R, **Wolff T,** Chakravarty S, Li F. Nuclear localization of influenza B polymerase proteins and their binary complexes. (2011). *Virus Res.* **156**(1-2):49-53.

Droebner K, **Pleschka S, Ludwig S, Planz O**. Antiviral activity of the MEK-inhibitor U0126 against pandemic H1N1v and highly pathogenic avian influenza virus in vitro and in vivo. (2011). *Antiviral Res*. Aug 11. [Epub ahead of print]

Dudek SE, Luig C, Pauli EK, Schubert U, **Ludwig S.** The clinically approved proteasome inhibitor PS-341 efficiently blocks influenza A virus and vesicular stomatitis virus propagation by establishing an antiviral state. (2010). *J Virol.* **84**(18):9439-51.

Dürrwald R, Krumbholz A, Baumgarte S, Schlegel M, Vahlenkamp TW, Selbitz HJ, Wutzler P, **Zell R.** Swine influenza A vaccines, pandemic (H1N1) 2009 virus, and cross-reactivity. (2010). *Emerg Infect Dis.* **16**(6):1029-30.

Ehrhardt C, Seyer R, Hrincius ER, Eierhoff T, **Wolff T, Ludwig S.** Interplay between influenza A virus and the innate immune signaling. (2010). *Microbes Infect.* **12**(1):81-7. Review.

Eierhoff T, Hrincius ER, Rescher U, **Ludwig S,** Ehrhardt C. The epidermal growth factor receptor (EGFR) promotes uptake of influenza A viruses (IAV) into host cells. (2010). *PLoS Pathog.* **6**(9).

Ferreira HL, Pirlot JF, Kaspers B, **Kothlow S**, van den Berg T, Lambrecht B. Development of specific enzyme-linked immunosorbent assays to evaluate the duck immune response after experimental infection with H5N1 and H7N1 low pathogenic avian influenza viruses. (2010). *Avian Dis.* **54**(1 Suppl):660-7.

Gao S, von der Malsburg A, Paeschke S, Behlke J, **Haller O,** Kochs G, Daumke O. Structural basis of oligomerization in the stalk region of dynamin-like MxA. (2010). *Nature* **27**; 465(7297):502-6.

Giannecchini S, Clausi V, Di Trani L, Falcone E, Terregino C, Toffan A, Cilloni F, **Matrosovich M,** Gambaryan AS, Bovin NV, Delogu M, Capua I, Donatelli I, Azzi A. Molecular adaptation of an H7N3 wild duck influenza virus following experimental multiple passages in quail and turkey. (2010). *Virology.* **408**(2):167-73.

Grienke U, **Schmidtke M,** Kirchmair J, Pfarr K, Wutzler P, **Dürrwald R,** Wolber G, Liedl KR, Stuppner H, Rollinger JM. Antiviral potential and molecular insight into neuraminidase inhibiting diarylheptanoids from Alpinia katsumadai. (2010). *J Med Chem.* **53**(2):778-86.

Haasbach E, Droebner K, Vogel AB, **Planz O.** Low-Dose Interferon Type I Treatment Is Effective Against H5N1 and Swine-Origin H1N1 Influenza A Viruses In Vitro and In Vivo. (2011). *J Interferon Cytokine Res. 2011* Feb 16. [Epub ahead of print].

Haasbach E, Pauli EK, Spranger R, Mitzner D, Schubert U, Kircheis R, **Planz O**. Antiviral activity of the proteasome inhibitor VL-01 against influenza A viruses. (2011). *Antiviral Res.* **91**(3):304-13.

Haller O, Gao S, von der Malsburg A, Daumke O, Kochs G. Dynamin-like MxA GTPase: Structural insights into oligomerization and implications for antiviral activity. (2010). *J Biol Chem.* **285**(37):28419-24

Herold S. Pathogenesis, symptoms and treatment of virus influenza. (2011). *Pharm Unserer Zeit.* 40(2):115-9. Review. German. No abstract available.

Hrincius ER, Wixler V, **Wolff T,** Wagner R, **Ludwig S,** Ehrhardt C. CRK adaptor protein expression is required for efficient replication of avian influenza A viruses and controls JNK-mediated apoptotic responses. (2010). *Cell Microbiol.* **12**(6):831-43.

Hrincius ER, Dierkes R, Anhlan D, Wixler V, **Ludwig S**, Ehrhardt C. Phosphatidylinositol-3-kinase (PI3K) is activated by influenza virus vRNA via the pathogen pattern receptor Rig-I to promote efficient type I interferon production. (2011). *Cell Microbiol.* Sep 8. doi: 10.1111/j.1462-5822.**2011**.01680.x. [Epub ahead of print]

Karlas A, Machuy N, Shin Y, Pleissner KP, Artarini A, Heuer D, Becker D, Khalil H, Ogilvie LA, Hess S, Mäurer AP, Müller E, **Wolff T,** Rudel T, Meyer TF. Genome-wide RNAi screen identifies human host factors crucial for influenza virus replication. (2010). *Nature.* **463**(7282):818-22.

Kirchmair J, Rollinger JM, Liedl KR, Seidel N, Krumbholz A, **Schmidtke M**. Novel neuraminidase inhibitors: identification, biological evaluation and investigations of the binding mode. (2011). *Future Med Chem.* **3**(4):437-50.

Klenk HD, Garten W, **Matrosovich M.** Molecular mechanisms of interspecies transmission and pathogenicity of influenza viruses: Lessons from the 2009 pandemic. (2011). *Bioessays.* **33**(3):180-8.

Krawitz C, Abu Mraheil M, Stein M, Imirzalioglu C, Domann E, **Pleschka S,** Hain T. Inhibitory activity of a standardized elderberry liquid extract against clinically-relevant human respiratory bacterial pathogens and influenza A and B viruses. (2011). *BMC Complement Altern Med.* **11(1)**:16.

Krumbholz A, Lange J, **Dürrwald R,** Hoyer H, Bengsch S, Wutzler P, **Zell R.** Prevalence of antibodies to swine influenza viruses in humans with occupational exposure to pigs, Thuringia, Germany, 2008-2009. (2010). *J Med Virol.* **82**(9):1617-25.

Krumbholz A, Philipps A, Oehring H, Schwarzer K, Eitner A, Wutzler P, **Zell R.** Current knowledge on PB1-F2 of influenza A viruses. (2010). *Med Microbiol Immunol.* Oct 16. Epub ahead of print].

Lang C, Palzer A, **Dürrwald R,** Selbitz HJ, Ritzmann M. [Analysis of the situation of swine influenza in the districts Upper Bavaria, Swabia, Freiburg and Tübingen based on antibody profiles of piglets from Bavarian breeding farms]. (2010). *Berl Munch Tierarztl Wochenschr.* **123**(9-10):385-91. German

Liu Y, Childs RA, Matrosovich T, Wharton S, Palma AS, Chai W, Daniels R, Gregory V, Uhlendorff J, Kiso M, Klenk HD, Hay A, Feizi T, **Matrosovich M.** Altered receptor specificity and cell tropism of D222G hemagglutinin mutants isolated from fatal cases of pandemic A(H1N1) 2009 influenza virus. (2010). *J Virol.* **84**(22):12069-74.

Ludwig S. Disruption of virus-host cell interactions and cell signaling pathways as an anti-viral approach against influenza virus infections. (2011). *Biol Chem.* **392**(10):837-47.

Luig C, Köther K, Eva Dudek S, Gaestel M, Hiscott J, Wixler V, **Ludwig S.** MAP kinase-activated protein kinases 2 and 3 are required for influenza A virus propagation and act via inhibition of PKR. (2010). *FASEB J.* **24**(10):4068-77.

Ma W, Brenner D, Wang Z, Dauber B, Ehrhardt C, Högner K, **Herold S, Ludwig S, Wolff T,** Yu K, Richt JA, **Planz O, Pleschka S.** The NS segment of an H5N1 highly pathogenic avian influenza virus (HPAIV) is sufficient to alter replication efficiency, cell tropism, and host range of an H7N1 HPAIV. (2010). *J Virol.* **84**(4):2122-33.

Mänz B, Götz V, Wunderlich K, Eisel J, Kirchmair J, Stech J, Stech O, Chase G, Frank R, **Schwemmle M.** Disruption of the viral polymerase complex assembly as a novel approach to attenuate influenza a virus. (2011). *J Biol Chem.* **286**(10):8414-24.

Mänz B, **Matrosovich M,** Bovin N, **Schwemmle M.** A polymorphism in the hemagglutinin of the human isolate of a highly pathogenic H5N1 influenza virus determines organ tropism in mice. (2010). *J Virol.* **84**(16):8316-21.

Mordstein M, Neugebauer E, Ditt V, Jessen B, Rieger T, Falcone V, Sorgeloos F, Ehl S, Mayer D, Kochs G, **Schwemmle M,** Günther S, Drosten C, Michiels T, **Staeheli P.** Lambda interferon renders epithelial cells of the respiratory and gastrointestinal tracts resistant to viral infections. (2010). *J Virol.* 2010 **84**(11):5670-7.

Muhammad S, Haasbach E, Kotchourko M, Strigli A, Krenz A, Ridder DA, Vogel AB, Marti HH, Al-Abed Y, **Planz O,** Schwaninger M. Influenza virus infection aggravates stroke outcome. (2011). *Stroke.* **42**(3):783-91.

Nordmann A, Wixler L, **Ludwig S,** Wixler V. Monoclonal antibodies against the PB1-F2 protein of H1N1 influenza A virus. (2010). *Hybridoma (Larchmt).* **29**(4):321-6.

Penski N, **Härtle S**, Rubbenstroth D, Krohmann C, Ruggli N, Schusser B, Pfann M, Reuter A, Gohrbandt S, Hundt J, Veits J, Breithaupt A, Kochs G, Stech J, Summerfield A, Vahlenkamp T, Kaspers B, **Staeheli P**. Highly pathogenic avian influenza viruses do not inhibit interferon synthesis in infected chickens but can override the interferon-induced antiviral state. (2011). *J Virol.* **85**(15):7730-41.

Pinto R, **Herold S**, Cakarova L, Hoegner K, Lohmeyer J, **Planz O**, **Pleschka S**. Inhibition of influenza virus-induced NF-kappaB and Raf-MEK-ERK activation can reduce both virus titers and cytokine expression simultaneously in vitro and in vivo. (2011). *Antiviral Res.* May 27. [Epub ahead of print]

Robb NC, Chase G, Bier K, Vreede FT, Shaw PC, Naffakh N, **Schwemmle M**, Fodor E. The influenza A virus NS1 protein interacts with the nucleoprotein of viral ribonucleoprotein complexes. (2011). *J Virol.* **85**(10):5228-31.

Schusser B, Reuter A, von der Malsburg A, Penski N, Weigend S, Kaspers B, **Staeheli P, Härtle S**. Mx is dispensable for interferon-mediated resistance of chicken cells against influenza A virus. (2011). *J Virol.* **85**(16):8307-15.

Sipo I, Knauf M, Fechner H, Poller W, **Planz O,** Kurth R, Norley S. Vaccine protection against lethal homologous and heterologous challenge using recombinant AAV vectors expressing codon-optimized genes from pandemic swine origin influenza virus (SOIV). (2011). *Vaccine.* **29**(8):1690-9.

Skarlas T, Zevgiti S, Droebner K, Panou-Pomonis E, **Planz O,** Sakarellos-Daitsiotis M. Influenza virus H5N1 hemagglutinin (HA) T-cell epitope conjugates: design, synthesis and immunogenicity. (2010). *J Pept Sci.* Nov 30. [Epub ahead of print]

Steel J, **Staeheli P,** Mubareka S, García-Sastre A, Palese P, Lowen AC. Transmission of pandemic H1N1 influenza virus and impact of prior exposure to seasonal strains or interferon treatment. (2010). *J Virol.* **84**(1):21-6.

Steidle S, Martínez-Sobrido L, Mordstein M, Lienenklaus S, García-Sastre A, **Stäheli P,** Kochs G. Glycine 184 in Nonstructural Protein NS1 Determines the Virulence of Influenza A Virus Strain PR8 without Affecting the Host Interferon Response. (2010). *J Virol.* **84**(24):12761-70.

Versteeg GA, Hale BG, van Boheemen S, **Wolff T,** Lenschow DJ, García-Sastre A. Species-specific antagonism of host ISGylation by the influenza B virus NS1 protein. (2010). *J Virol.* **84**(10):5423-30.

Viemann D, Schmolke M, Lueken A, Boergeling Y, Friesenhagen J, Wittkowski H, **Ludwig S, Roth J.** H5N1 virus activates signaling pathways in human endothelial cells resulting in a specific imbalanced inflammatory response. (2011). *J Immunol.* **186**(1):164-73.

Vogel AB, Haasbach E, Reiling SJ, Droebner K, Klingel K, **Planz O.** Highly pathogenic influenza virus infection of the thymus interferes with T lymphocyte development. (2010). *J Immunol.* **185**(8):4824-34.

Wang Z, Robb NC, Lenz E, **Wolff T**, Fodor E, **Pleschka S.** NS reassortment of an H7-type highly pathogenic avian influenza virus affects its propagation by altering the regulation of viral RNA production and antiviral host response. (2010). *J Virol.* **84**(21):11323-35.

Wu H, Haist V, Baumgärtner W, **Schughart K.** Sustained viral load and late death in Rag2-/- mice after influenza A virus infection. (2010). *Virol J.* **28**;7:172.

Wunderlich K, Juozapaitis M, Mänz B, Mayer D, Götz V, Zöhner A, **Wolff T, Schwemmle M,** Martin A. Limited compatibility of polymerase subunit interactions in influenza A and B viruses. (2010). *J Biol Chem.* **285**(22):16704-12.

Wunderlich K, Juozapaitis M, Ranadheera C, Kessler U, Martin A, Eisel J, Beutling U, Frank R, **Schwemmle M.** Identification of high-affinity PB1-derived peptides with enhanced affinity to the PA protein of influenza A virus polymerase. (2011). *Antimicrob Agents Chemother.* **55(2):**696-702.

Zielecki F, Semmler I, Kalthoff D, Voss D, Mauel S, Gruber AD, Beer M, **Wolff T.** Virulence determinants of avian H5N1 influenza A virus in mammalian and avian hosts: role of the C-terminal ESEV motif in the viral NS1 protein. (2010). *J Virol.* **84**(20):10708-18.

Zhirnov OP, Matrosovich TY, **Matrosovich MN**, Klenk HD. Aprotinin, a protease inhibitor, suppresses proteolytic activation of pandemic H1N1v influenza virus. (2011). *Antivir Chem Chemother.* **21**(4):169-74.

Zimmermann P, Mänz B, **Haller O, Schwemmle M**, Kochs G. The viral nucleoprotein determines Mx sensitivity of influenza A viruses. (2011). *J Virol.* **85**(16):8133-40.