Poster 11

EXPLORING MARBURGVIRUS NUCLEOPROTEIN INTERACTION WITH MATRIX PROTEIN VP40

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Abstract: *Marburgvirus* (MARV) and *Ebolavirus* (EBOV) are enveloped single stranded negative sense RNA viruses able to cause lethal and transmissible diseases. There are no currently approved FDA vaccines or therapeutics available for either virus, though many platforms are under investigation. The ongoing EBOV outbreak in West Africa highlights the urgent need for additional study of these agents. The expression of MARV nucleoprotein (NP) leads to helical scaffolding required for nucleocapsid (NC) assembly. Expression of matrix protein VP40 leads to virus like particle (VLP) formation that is morphologically similar to virus particles. Upon co-expression, NP and VP40 interact and co-localize within the cytoplasm and at the cell membrane; additionally, the production of VLPs containing a core of NP occur. Previous EBOV studies have shown both termini of NP are essential for NP-VP40 interaction and NC incorporation into VLPs. We are evaluating MARV NP interaction with VP40 using 100 amino acid serial deletions of MARV NP (delNP) and WT VP40. We employ multiple techniques, with silver staining of VLP molecules and confocal microscopy being particularly valuable. Silver staining is sensitive and independent of antibody (Ab) epitope for NP probing, allowing us to visualize all delNP mutants equally. To visualize delNP mutants in microscopy, we employ N-terminally HA tagged constructs and untagged VP40, probing with anti-HA and anti-MARV VP40 Ab. During our studies we have found variation in expression levels and distribution of each delNP and differences in the propensity to colocalize with VP40. Currently, we have defined the N-terminus as not playing a role in NP-VP40 interactions and further analysis of the C-terminus data is ongoing. Understanding how NP and VP40 interact, will not only help us understand basic viral assembly but could potentially lead to a therapeutic target since disrupting NP-VP40 interaction could decrease viral load by decreasing viral assembly efficiency.

Disclosure of Interest: None declared

Poster 12

THE ARENAVIRUS MATRIX PROTEIN PPXY LATE DOMAIN DRIVES THE PRODUCTION OF DEFECTIVE INTERFERING PARTICLES

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Abstract: Arenaviruses are enveloped RNA viruses that cause severe human disease. These viruses are maintained primarily in rodent reservoirs where they establish persistent, asymptomatic infections that feature high levels of defective interfering (DI) virus particles. While DI particles are thought to play an important role in the establishment and maintenance of viral persistence, little is known regarding the mechanism for their production. The arenavirus matrix protein (Z) drives the process of viral budding, presumably through one or more encoded late domains. We discovered that the lymphocytic choriomeningitis virus (LCMV) matrix protein is phosphorylated at the terminal tyrosine residue (Y88) of its only late domain (PPXY). Recombinant (r)LCMV lacking a functional late domain due to mutation at Y88 (Y88F or Y88A to prevent phosphorylation or Y88E to mimic phosphorylation) were recoverable and, despite growing slower than WT virus, eventually reached the same peak titers as WT virus. The phosphomimetic Y88E rLCMV mutant had partially restored growth kinetics compared to the Y88A or Y88F viruses, suggesting that phosphorylation of this residue is important for Z's functionality and ultimately viral fitness. Strikingly, virus particles produced by the Y88 mutant viruses, though less in number, contained a much higher ratio of infectious particles to DI particles when compared to WT virus. Thus, our studies show for the first time that the PPXY late domain is critically required for the production of DI particles and that phosphorylation of this motif may play a key regulatory role in this process.

Disclosure of Interest: None declared

Poster 13

CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS ENTRY INTO HOST CELLS OCCURS THROUGH THE MULTIVESICULAR BODY AND REQUIRES ESCRT REGULATORS

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Abstract: Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne bunyavirus causing outbreaks of severe disease in humans, with a fatality rate approaching 30%. There are no widely accepted therapeutics available to prevent or treat the disease. CCHFV enters host cells through clathrin-mediated endocytosis and is subsequently transported to an acidified compartment where the fusion of virus envelope with cellular membranes takes place. To better understand the uptake pathway, we sought to identify host factors controlling CCHFV transport through the cell. We demonstrate that after passing through early endosomes in a Rab5-dependent manner, CCHFV is delivered to multivesicular bodies (MVBs). Virus particles localized to MVBs approximately 1 hour after infection and affected the distribution of the organelle within cells. Interestingly, blocking Rab7 activity had no effect on association of the virus with MVBs. Productive virus infection depended on phosphatidylinositol 3-kinase (PI3K) activity, which meditates the formation of functional MVBs. Silencing Tsg101, Vps24, Vps4B, or Alix/Aip1, components of the endosomal sorting complex required for transport (ESCRT) pathway controlling MVB biogenesis, inhibited infection of wild-type virus as well as a novel pseudotyped vesicular stomatitis virus (VSV) bearing CCHFV glycoprotein, supporting a role for the MVB pathway in CCHFV entry. We further demonstrate that blocking transport out of MVBs still allowed virus entry while preventing vesicular acidification, required for membrane fusion, trapped virions in the MVBs. These findings suggest that MVBs are necessary for infection and are the sites of virus-endosome membrane fusion. This work was supported by DOD/DTRA HDTRA1-12-1-0002 and gifts from the Douglass and Ewing Halsell Foundations.
Disclosure of Interest: None declared

Poster 15

EBOLA VIRUS INTERNALIZATION INTO CELLS BY MACROPINOCYTOSIS IS DEPENDENT ON AUTOPHAGY-MEDIATING PROTEINS

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Abstract: Ebola virus (EBOV) belongs to the family *Filoviridae* and is a causative agent of a severe, often fatal disease in humans. Our and others' studies demonstrated that macropinocytosis is the primary route of EBOV uptake into cells. However, little is known about the mechanism of macropinosome formation and therefore virus internalization. Here, we show that proteins that historically are known to initiate and coordinate autophagy, a catabolic process where cellular membranes serve as a source of vesicles whose role is to engulf cell contents for degradation, were also essential for EBOV infection. We show that these proteins regulated infection in a glycoprotein-dependent manner and, therefore, were affecting virus entry. Using 3D high-resolution microscopy of virus particle-cell interaction, we were surprised to find that virions localized to vesicles that contained autophagy-related proteins and a marker for macropinosomes close to the cell surface. Additionally, we observed that disruption of function of autophagy regulators blocked vesicle formation and uptake by macropinocytosis. We propose that autophagy-related proteins control EBOV entry into cells by coordinating the formation of or being an essential component of macropinosomes. This novel finding suggests new roles for autophagy-related proteins in macropinosome formation as well as EBOV entry. This work was supported by NIH R01AI063513; DOD/DTRA HDTRA1-12-1-0002; the Douglass and Ewing Halsell Foundations.
Disclosure of Interest: None declared

Poster 16

UNDIAGNOSED ACUTE VIRAL FEBRILE ILLNESSES IN SIERRA LEONE

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Abstract: Sierra Leone in West Africa is located in a Lassa fever hyper-endemic region that encompasses Guinea, Sierra Leone, and Liberia. Each year suspected Lassa fever infections result in approximately 500-700 samples being submitted to the Kenema Government Hospital (KGH) Lassa Diagnostic Laboratory located in eastern Sierra Leone. Of the samples tested, generally only 30-40% are positive for Lassa virus antigen and/or Lassa-specific IgM antibodies; therefore 60-70% of the patients are presenting with acute diseases of unknown origin. In this study we looked at samples that were malaria and Lassa virus negative. Using IgM capture enzyme-linked immunosorbent assays (ELISAs) we evaluated the patient samples for antibodies to arthropod-borne and hemorrhagic fever virus pathogens that could mimic Lassa fever presentation and occur in the region. We found evidence for IgM antibodies to dengue, West Nile, yellow fever, Rift Valley fever, Chikungunya, Ebola, and Marburg viruses, but not Crimean-Congo hemorrhagic fever virus.
Disclosure of Interest: None declared

Poster 17

DECIPHERING THE ASSEMBLY OF MULTI-SEGMENT GENOME COMPLEXES IN INFLUENZA A VIRUS (IAV)

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Abstract: Influenza A is a major cause of virus-related deaths worldwide and an important target for biomedical research. It has a segmented genome of eight single-stranded, negative sense RNAs packed into Ribonucleoproteins (vRNPs). This segmentation is the basis for reassortment between different strains with the potential to create highly virulent, even pandemic new strains. A packaging mechanism is supposed, ensuring the incorporation of (at least) one copy of each segment into a budding virion. During their transport from the nucleus to virion budding from the plasma membrane, the vRNPs are thought to “hitch-hike” on Rab11A-positive recycling endosomes (REs) and to establish multi-segment complexes based on RNA-RNA interactions, so called packaging signals.
This makes the various segments of viral RNA in infected cells a very promising target of study. Visualization of their location and colocalization during the time course of infection will allow us to learn more about the workings and potential hierarchies within such a packaging process. At the same time we get information about the abundance and inter-cell heterogeneity of vRNPs among large sets of infected cells. Further understanding of the vRNP packaging can ultimately open up paths to new antiviral medication.
Here, we will use RNA-FISH in a way in which we can overcome the spectral limits of multiplexing. Traditionally, the method is limited to one target per spectral channel (most microscopes offer three, four or rarely five channels) which prohibits the detection of all eight vRNP segments of IAV in the same cells. We can extend this by removing the fluorescent oligonucleotide probes after one round of imaging. After this, other vRNP targets can be visualized using the same colors as before which results in highly multiplexed overlay images. The method is also compatible with DAPI staining and immunofluorescence (IF) and might be of a more general interest.
Disclosure of Interest: None declared

Poster 18

THE STEM REGION OF TYPE II TRANSMEMBRANE SERINE PROTEASES IS DETERMINANT OF INFLUENZA VIRUS ACTIVATION AND A TARGET FOR ANTIVIRAL INHIBITION

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Abstract: The influenza A virus (FLUAV) hemagglutinin (HA) mediates viral binding and entry into target cells. HA is synthesized as an inactive precursor and proteolytic activation by host cell proteases is essential for FLUAV infectivity. Recently, TMPRSS2, a type II transmembrane serine protease

(TTSP), was found to be required for HA activation in the host. Moreover, several TTSPs, i.e. TMPRSS4 and HAT, were shown to activate FLUAV in vitro while other members of the TTSP family were found to be inactive, despite robust expression and auto-activation. Therefore, the aim of the present study was to identify molecular determinants in TTSPs, which control cleavage and activation of HA.
To identify determinants of HA activation, we created and functionally analyzed chimeras between TMPRSS2, which activates HA, and TMPRSS3, which fails to activate HA. For this purpose, the cytoplasmic, transmembrane or stem domains were exchanged between these proteins. We found that the exchange of the stem region abrogates HA cleavage by TMPRSS2 and endows TMPRSS3 with the ability to cleave HA. Comparable results were obtained when the stem regions of TMPRSS4 and TMPRSS3 were exchanged, indicating that the stem region is a key determinant of HA cleavage and activation by TTSPs. Immunostaining revealed that the active TMPRSS3 chimera colocalized with HA in infected cells while the wt protein did not, indicating that colocalization of TTSP with HA is essential for HA activation and is controlled by the stem region. Finally, dextran sulphate, a scavenger receptor (SR) A inhibitor, was found to block HA processing by TTSPs harboring a SR domain in their stem region while processing by TTSPs without SR domain was not affected. Collectively, our results indicate that the stem region of TTSPs can determine HA activation, potentially by controlling the ability of these enzymes to colocalize with HA, Moreover, our findings show that the stem region is a potential target for antiviral intervention.
Disclosure of Interest: None declared

Poster 19
MEASLES VIRUS IS ENDOCYTOSED AND INDUCES MACROPINOCYTOSIS IN SLAM-POSITIVE CELLS
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Abstract: The virus family *Paramyxoviridae* contains important human pathogens with a global health and economic burden. In particular, Measles virus (MeV) remains a leading cause of mortality in infants and children in the developing world, despite an efficacious vaccine is available and in use. Low coverage and the lack of specific treatment have contributed to prolonged circulation of this virus with sporadic outbreaks in developed countries. Notably, MeV and pseudotyped MeV particles (MeV-PP) have been successfully used in cancer treatment and gene therapy. These promising biomedical applications may be improved through increased knowledge of the mechanisms underpinning viral entry.
We have examined MeV and MeV-PP entry via the immune receptor; signalling lymphocyte activation molecule (SLAM CD150). Our results show that virus particles are endocytosed 20min post attachment to the cell surface. Viral entry is insensitive to pharmacological inhibitors of clathrin- and caveolin-mediated endocytosis, as well as inhibitors of acidic endosomal pH. In contrast, treatment with blebbstatin or amiloride significantly reduces entry and subsequent infection. These findings were confirmed by infection of cells expressing dominant negative forms of proteins involved in these endocytic pathways. By using scanning electron microscopy, we have shown that binding of MeV to SLAM-positive cells induces extensive but transient membrane blebbing and filopodia formation. MeV was found to induce fluid-phase uptake as well as a dramatic reorganization of the actin cytoskeleton as observed by fluorescence microscopy. The requirement of actin dynamics was also evaluated using several molecular inhibitors. The role of plasma membrane cholesterol and Rho GTPases during entry was also assessed. Together, these results suggest a previously unidentified entry mechanism for MeV that involves macropinocytosis of the viral particle.
Disclosure of Interest: None declared

Poster 20
IDENTIFICATION OF A NEURONAL RECEPTOR FOR WILD TYPE MEASLES VIRUS
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Abstract: Measles virus (MV) is one of the leading causes of death in children. MV enters the body via the respiratory tract and spreads to other tissues. In immunocompromised individuals MV can infect the CNS, primarily neurons, causing measles inclusion body encephalitis. More rarely, in apparently immunocompetent children the virus causes subacute sclerosing panencephalitis several years after childhood measles. There are no effective treatments for these conditions. Signalling lymphocytic activation molecule (SLAM) and Nectin-4 have been identified as MV cell entry receptors on immune cells and the basal surface of epithelial cells, respectively. However, the receptor for cell entry on neurons is unknown. Using a phage antibody display library we selected an antibody which inhibits wild type (WT) MV infection of SH-S-Y5Y (human neuroblastoma) and hNT2 (human teratoma cells differentiated using retinoic acid to fully differentiated neurons). The antibody immunoprecipated a protein which was identified by mass spectroscopy. Transfection of neuronal cells with siRNA specific for the receptor protein mRNA inhibited WT MV infection. Establishment of a Vero cell line (Vero cells are non-permissive for WT strains of MV) expressing the receptor allowed virus binding and infection of these cells. As previously observed for SLAM and Nectin-4, virus infection caused down-regulation of the neuronal protein in the receptor expressing Vero cell line. Furthermore, this was also observed in vivo as almost complete down-regulation of the receptor was observed in MV infected areas of human brain tissue. The neuronal receptor could represent a target to inhibit MV spread in the CNS and also allow MV vector-based treatment of neuroblastomas, a most common and deadly form of childhood cancer with poor outcome.
Disclosure of Interest: None declared

Poster 21

INFLUENZA VIRUS REPLICATION DECREASES SIALIC ACID ON THE CELL

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Abstract: Influenza virus adheres on cellular receptor (sialic acid) through viral envelope protein HA and then endosome is formed to include viral particle. Viral particle in endosome carries nearby nuclear by dynein. At viral particle binding event sialic acid plays very important role for influenza virus infection. In our previous work we showed that influenza virus binding on the membrane of G1 phase cell selectively. So we compared that sialic acid content on resting and dividing cells and found that sialic acid content on resting cell is higher than dividing cell. From these results we are interested in the sialic acid content of influenza virus infected cell. To elucidate this question we developed nanoprobe binding on a2,3- and a2,6-sialic acid. Using these probes we found that sialic acid content decrease gradually after virus binding and at 5hpi sialic acid content decreased half of uninfected cell. We performed several assays to elucidate the reason of a drop of sialic acid content in influenza virus infected cells.
Disclosure of Interest: None declared

Poster 22

ANALYSIS OF THE ASSEMBLY AND BUDDING OF LUJO VIRUS

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Abstract: Lujo virus (LUJV) belongs to Arenaviridae and causes viral hemorrhagic fever. Here, the mechanism of viral release driven by Z and GPC was analyzed. We showed that L-domains (YREL and PSAP) in Z are required for the efficient virus-like particle (VLP) release, but Tsg101 and Alix/AIP1, both are involved in ESCRT machinery, are not necessary for the VLP budding and following VLP production. Moreover, we found that LUJV GPC is cleaved by site 1 protease (S1P) at RKLM motif. GPC cleavage by S1P is not important for the GPC translocation to the cell surface, which is the platform of viral assembly and budding. We also found that GPC cleavage by S1P is critical for the GP incorporation into the VLP. These results suggested that S1P is an efficient target to combat highly pathogenic Lujo virus.

Disclosure of Interest: None declared

Poster 23
IGF2 IS INVOLVED IN THE REGULATION OF BORNA DISEASE VIRUS PRODUCTION
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Abstract: Borna disease virus (BDV) causes a long-term persistent infection in the cell nucleus, and production of the infectious particle into culture supernatant is strikingly suppressed. To elucidate the molecular mechanism controlling the viral particle production, we tried to identify host factor(s), which suppresses BDV production from infected cells. Screening of human shRNA library revealed that knockdown (KD) of IGF2 (insulin-like growth factor 2) in BDV-infected cells led to the promotion of virus production into supernatants by more than 30-fold, compared with control. IGF2 KD also enhanced a reporter protein, firefly luciferase (FLuc), expression by recombinant BDV-FLuc in infected cells. In the IGF2 KD cells infected with BDV-FLuc, the FLuc activity was rescued by overexpression of IGF2 with off-target sequence against shRNA as well as in control shRNA-transduced cells with BDV-FLuc. Moreover, IGF2 KD enhanced the efficiency of the pol II-based reverse genetics system of BDV. These results indicate that IGF2 has an important role on BDV particle production.
To evaluate if IGF2 pathway via its receptors (IGFR1 and IGFR2) is related to BDV production, recombinant human IGF2 was added to BDV-infected cells at various concentration (1-100 ng/ml). As the result, the addition of IGF2 into the culture medium had no effect on BDV production from BDV-infected cells and FLuc activity by BDV-FLuc. Immunoprecipitation assay showed that IGF2 interacted with GP1, envelope protein of BDV. Indirect fluorescent assay revealed that IGF2 was localized in the nucleus and cytoplasm of BDV-infected cells, while major localization of IGF2 was the cytoplasm in mock cells. Furthermore, IGF2 KD increased GP1 protein expression and polymerase activity. These data suggest the possibility that IGF2 might be involved in BDV regulation at multistep of the life cycle, including viral envelope production in the cytoplasmic domain and polymerase activity in the nucleus.
Disclosure of Interest: None declared

Poster 24
TETHERIN MEDIATED INHIBITION OF MEASLES VIRUS
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Abstract: Measles virus (MeV) is an established pathogen of humans typified by its high infectivity and rapid dissemination through naïve populations. A key feature of these viruses is their ability to spread by both viral-cell and cell-cell fusion; a process driven by the viruses' surface glycoproteins F (fusion) and H (haemagglutinin). Both processes contribute to viral dissemination and pathogenesis in the host; however, the mechanisms underpinning any equilibrium between budding and cell-cell fusion are poorly understood. Moreover cellular restriction of these processes and the potential for innate-immune antagonism of viral dissemination are not-well defined.

Using highly permissive cell lines we showed that MeV is inhibited by human tetherin (BST2), a well-characterised viral restriction factor that tethers nascent viral particles to the surface of infected cells. One key feature of our *in vitro* MeV infections was a large reduction in syncytia expansion, indicating a potentially novel target for tetherin restriction. Using newly established quantifiable fusion assays (based on an adapted BiFC approach) we subsequently showed that tetherin can directly inhibit MeV cell-cell fusion. By applying standard biochemical approaches we also identified one of the viral targets for tetherin that underpins this restriction. Furthermore, using a panel of tetherin mutants we established the domains of this innate immune protein that are required for this effect.

In related research we have expanded this project to other members of the morbillivirus genus, as well as other mammalian hosts identifying unique differences between the different mammalian tetherin proteins and their ability to mediate inter-species restriction of the morbillivirus genus.

These findings expand our understanding of MeV and morbillivirus virology and shed new light on the innate immune restriction of morbillivirus dissemination.
Disclosure of Interest: None declared

Poster 25
THE HEPTAD REPEAT C DOMAIN IN THE RESPIRATORY SYNCYTIAL VIRUS F PROTEIN PLAYS A KEY ROLE IN VIRUS MEDIATED MEMBRANE FUSION
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Abstract:
Introduction
Respiratory syncytial virus (RSV) is a major global pathogen, causing morbidity and mortality within paediatric populations through pneumonia and bronchiolitis. The virion surface glycoprotein F is an essential player in virus entry into cells, mediating virion-cell membrane fusion and then subsequent cell-cell fusion resulting in virus spread through multinucleate syncytia. During the fusion process, F transitions from a metastable pre-fusion state into a highly stable post-fusion form, stabilised by two heptad repeats (HR) – HRA and HRB. In this study, a third uncharacterised heptad repeat, HRC, was investigated.

Methods
An alanine scan was performed throughout the HRC helix from Lys-75 to Met-95 in an expression plasmid containing recombinant RSV F. Epithelial cells were transfected and F protein-mediated cell-cell fusion was assessed by two distinct fusion assays. Protein expression, processing, cell surface representation and protein confirmation were assessed through western blot and flow cytometry.

Results
Seven of the mutants completely abrogated F-mediated fusion, eight displayed reduced fusion and six mutants increased cell-cell fusion. When these phenotypes were mapped to the published pre-fusion structure, a striking pattern was observed in that mutants that enhanced fusion aligned on one helical face, while mutants that abrogated fusion lay on another.

Conclusion
It is clear that interactions made by residues of HRC play a pivotal role in the fusion process and it is postulated that HRC is crucial in maintaining the delicate stability of the pre-fusion form. The clustering of these residues and proposed mechanism of action provide opportunities that will direct further research and insights that may prove beneficial to anti-viral drug and vaccine design.
Disclosure of Interest: None declared

Poster 26
LOW PH IS SUFFICIENT TO TRIGGER ANDES HANTAVIRUS FUSION ACTIVATION
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Abstract: The hantavirus membrane fusion process is mediated by the Gc envelope glycoprotein from within endosomes. In this work, cell-free *in vitro* systems were established to characterize hantavirus fusion activation. Acidification was sufficient to trigger the interaction of virus-like particles (VLPs) with liposomes. Low pH also induced Gc multimerization rearrangements leading to highly resistant Gc homotrimers with the characteristics of a stable post-fusion conformation. No acid-dependent oligomerization changes were detected for the trypsin-sensitive Gn companion protein.

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Disclosure of Interest: None declared

Poster 27

RNA-POLYMERASE OF INFLUENZA VIRUS: A SERIES OF TEMPERATURE-SENSITIVE MUTANTS REVEALS A ROLE OF THE PA LINKER IN NUCLEAR TARGETING OF THE PB1-PA DIMER

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Abstract: The influenza virus RNA-dependent RNA polymerase catalyses genome replication and transcription within the cell nucleus. Efficient nuclear import and assembly of the polymerase subunits, PB1, PB2, and PA are critical steps in virus cycle. We investigated the function of the PA linker (residues 197-256), located between its N-terminal endonuclease domain and its C-terminal structured domain that binds PB1, the polymerase core. A large series of PA linker mutants engineered by codon substitutions and deletions exhibited a temperature-sensitive (*ts*) phenotype (reduced viral growth at 39.5°C vs. 37°C/33°C), suggesting an alteration of folding kinetic parameters. The *ts*-phenotype was associated to a reduced efficiency of replication/transcription of a pseudo-viral reporter RNA in a minireplicon assay. Using a fluorescent-tagged PB1, we observed that *ts*- and lethal PA mutants do not efficiently recruit PB1 to reach the nucleus at 39.5°C. A protein complementation assay using PA mutants, PB1 and b-importin IPO5 tagged with fragments of the *Gaussia princeps* luciferase showed that increasing the temperature negatively modulates the PA-PB1 and the PA-PB1-IPO5 interactions or complex stability. The selection of revertant viruses allowed the identification of different types of compensatory mutations located in one or the other of the three polymerase subunits. Several substitution and deletion *ts*-mutants were shown to be attenuated and able to induce antibodies in mice. Taken together, our results identified a PA domain as being critical of PB1-PA nuclear import and as a “hot spot” to engineer *ts*-mutants that could be used to design novel attenuated genetically stable vaccines.
Disclosure of Interest: None declared

Poster 28

MEASLES VIRUS APPROPRIATES THE ADHESIVE INTERFACE OF NECTIN-4 TO ENTER THE RESPIRATORY EPITHELIUM

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Abstract: Many viruses utilize cell adhesion molecules of the immunoglobulin superfamily as receptors. In particular, viruses of different classes exploit nectins. The large DNA viruses, herpes simplex and pseudorabies viruses, use ubiquitous nectins 1 and 2. The negative-strand RNA virus measles virus (MeV) uses tissue-specific nectin-4, and the positive-strand RNA virus poliovirus uses nectin-like 5 (necl-5), also known as poliovirus receptor. These viruses contact the BC, C'C'', and FG loops on the upper tip of their receptor's most membrane-distal domain. This location corresponds to the newly defined canonical adhesive interface of nectins, but how viruses utilize this interface has remained unclear. Here we show that the same key residues in the BC and FG loops of nectin-4 govern binding to the MeV attachment protein hemagglutinin (H) and cell entry, nectin-4 homodimerization, and heterodimerization with nectin-1. On the other hand, residues in the C'C'' loop necessary for homo- and heterotypic interactions are dispensable for MeV-induced fusion and cell entry. Remarkably, the C'C'' loop governs dissociation of the nectin-4 and H ectodomains. We provide formal proof that H can

interfere with the formation of stable nectin-1/nectin-4 heterodimers. Finally, while developing an alternative model to study MeV spread, we observed that polarized primary pig airway epithelial sheets cannot be infected. We show that a single amino acid variant in the BC loop of pig nectin-4 fully accounts for restricted MeV entry. Thus, the three loops forming the adhesive interface of nectin-4 have different roles in supporting MeV H association and dissociation and MeV-induced fusion.
Disclosure of Interest: None declared

Poster 29

ROLE OF RNA/RNA INTERACTIONS IN THE PACKAGING AND REASSORTMENT OF THE INFLUENZA A VIRUS GENOME

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Abstract: Influenza A viruses (IAVs) cause annual influenza epidemics and occasional severe pandemics. Their genome is segmented into eight vRNAs, which offers evolutionary advantages but complicates genomic packaging. Accumulating evidence supports the existence of a selective packaging mechanism, but its molecular mechanism remain unclear.
Combining electron tomography with *in vitro* assays, we showed that the eight vRNAs of IAVs build up networks of interactions, which, surprisingly, are different for a human H3N2 (MO) and an avian H5N2 (EN) virus (Fournier *et al*. (2012) *Nucleic Acids Res* 40:2197-209, Gavazzi *et al.* 2013 *Nucleic Acids Res* 41:1241-54). Using silent *trans*-complementary mutations, which destroy an intermolecular interaction when introduced in a single vRNA but restore it when introduced simultaneously in the two vRNAs, we could show that the interaction between vRNAs PB1 and NS of the EN virus is required for optimal replication and vRNA packaging, and specifically, for co-packaging of the interacting vRNAs (Gavazzi *et al.* 2013 *PNAS* 110: 16604-9).
Unexpectedly vRNA/vRNA interactions are poorly conserved; a fact that should affect genetic reassortment (Gerber *et al.* 2014 *Trends Microbiol* 22:446-55). Accordingly, we observed that genetic reassortment between MO and EN viruses is strongly limited by suboptimal compatibility between the vRNA packaging signals (Essere *et al.* 2013 *PNAS* 110:E3840-8).
Disclosure of Interest: None declared

Poster 30

A HEAD-STALK CONNECTING LINKER OF DEFINED LENGTH FAVORS ACCURATE MEASLES VIRUS HEMAGGLUTININ TETRAMERIZATION AND EFFICIENT MEMBRANE FUSION TRIGGERING

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Abstract: The measles virus membrane fusion apparatus consists of a receptor binding (hemagglutinin, H) protein tetramer and a fusion (F) protein trimer. Different receptors, which contact the H-protein head through partially overlapping but substantially different binding surfaces, trigger the same output: F-trimer refolding and membrane fusion. Different models of how the fusion-activation signal is transmitted consider either dramatic rearrangements of the attachment protein heads around the tetrameric stalk or more limited conformational changes through connecting linkers down into the stalk. We previously characterized a central fusion-activation segment of the tetrameric H-stalk (residues 59-154) H-stalk Hthat connects with the base of the F-trimer and transmits the triggering signal. Here we ask whether a 17-residue linker (residues 167-183) connecting the stalk with the individual heads is critical for signal transmission. This linker was unresolved in all five H crystal or co-crystal structures and may align the heads with the stalk. To understand its role of we subjected it to different types of mutagenesis. Extensive alanine substitutions had no effect on fusion triggering, suggesting that sequence identity is not critical for fusion-triggering function. Shortening or lengthening linker length reduced or completely abrogated fusion-trigger function, while length compensation restored it. We then characterized the mechanism of function loss. Mutated H proteins were efficiently transported to the cell surface, but

certain linker alterations resulted in accumulation of high molecular weight H-oligomers with reduced fusion-trigger capacity. Thus, a defined length of the head-stalk connecting linker is critical for accurate H-tetramerization and efficient fusion triggering. The flexible 17-residue linker may allow sufficient H-head movement about the stalk to achieve a triggering-permissive arrangement while limiting improper H-oligomer aggregation.
Disclosure of Interest: None declared

Poster 31
FORWARD GENETIC SCREENING TO IDENTIFY HOST FACTORS REQUIRED FOR BUNYAVIRUS ENTRY
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Abstract: Bunyaviruses are a family of negative-sense RNA viruses that can be borne by their arthropod or rodent vectors into livestock and human hosts. Their tripartite genomes are packaged into spherical or icosahedral virions with four viral proteins: the glycoproteins Gn and Gc, nucleocapsid, and polymerase. The *Bunyaviridae* family includes the causative agents of many important human pathogens, such as Rift Valley fever virus (RVFV) and Crimean-Congo hemorrhagic fever virus (CCHFV). To date, there are a paucity of FDA-approved vaccines or therapeutics for these diseases, and the bunyavirus life cycle within the mammalian host is incompletely characterized. We have utilized a forward genetic screen to identify host factors involved in the entry of bunyaviruses into mammalian cells. In a haploid genetic screen using RVFV, we identified a suite of enzymes involved in glycosaminoglycan (GAG) biogenesis as well as several components of the cis-oligomeric Golgi (COG) complex, one of the central components of Golgi trafficking. Using a variety of biochemical and genetic approaches, we showed that both pathogenic and attenuated RVFV strains required GAGs for efficient infection on some - but not all - cell types, with the block to infection being at the level of virion attachment. Examining other members of the bunyavirus family for GAG-dependent infection suggests that the interaction with GAGs is not universal among bunyaviruses, indicating that these viruses, as well as RVFV on certain cell types, employ additional as-yet-to-be-identified virus attachment factors and/or receptors. We performed a second screen using Bunyamwera virus (BUNV), which is the prototypical model virus of study for the *Bunyaviridae* family, in which we identified members of endosomal trafficking pathways. The requirement for these factors also varied amongst the bunyaviruses we examined, highlighting the nonuniformity of entry mechanisms within this incredibly diverse family of viruses.
Disclosure of Interest: None declared

Poster 32
MOLECULAR CHARACTERIZATION OF THE INTERACTION BETWEEN THE CELLULAR MOTOR PROTEIN KIF13A AND THE LASSA VIRUS MATRIX PROTEIN Z
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Abstract: Lassa virus (LASV), a member of the *Arenaviridae* family, exits its host cell by budding from the plasma membrane, a process that is mainly driven by the viral matrix protein Z. We have previously demonstrated that the kinesin family member 13A (KIF13A), a plus-end-directed microtubule-dependent motor protein, is involved in the intracellular transport of Z protein to the site of virus budding. Plasmid-driven overexpression of KIF13A results in an increased localization of Z at the cell periphery, while functional blockage of endogenous KIF13A by overexpression of a dominant-negative mutant or KIF13A-specific siRNA causes a perinuclear accumulation and decreased production of both Z-induced

virus-like particles and infectious LASV. Although a functional role of KIF13A in Z trafficking is well established, the molecular mechanism of the Z/KIF13A interaction remains elusive. KIF13A is characterized by an N-terminal motor domain, a forkhead-associated (FHA) domain, and five predicted coiled-coil domains. Different cellular cargo molecules have been shown to directly interact with KIF13A, such as the serotonin type 1A receptor 5HT(1A)R that binds to the FHA domain. In contrast, transport of the mannose 6-phosphate receptor (M6PR) from the TGN to the plasma membrane requires an interaction between KIF13A and the adaptor protein AP-1 for bridging M6PR/KIF13A association. However, we show that AP-1 is not involved in Z-KIF13A interaction. To identify domains within KIF13A that are important for interaction with Z, we created a series of KIF13A deletion constructs for *in vitro* analysis of Z binding. Biochemical and microscopic analysis indicate that while the FHA domain is dispensable for Z/KIF13A interaction, Z binding requires both a C-terminally located coiled-coil motif and adjacent amino acids. These results provide a novel interaction domain for KIF13A-mediated cargo transport.

Disclosure of Interest: None declared

Poster 33

LENTIVIRUS-BASED RSV PSEUDOTYPES (RSVPP) AS MODEL TO STUDY RSV CELL ENTRY AND FOR SCREENING OF ENTRY INHIBITORS

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Abstract: Background:

Retroviral budding is governed by expression of group-specific antigen (Gag-proteins) and proceeds in the absence of expression of cognate viral envelope proteins. This feature has been used to create retroviral pseudotypes incorporating envelope proteins from numerous non-retroviral families. These particles closely mimic receptor interactions and early cell entry stages of these latter viruses. Respiratory Syncytial Virus (RSV) is a clinically important virus causing upper and lower respiratory tract disease. Apart from a humanised monoclonal antibody against the RSV fusion protein (Synagis) the only licensed antiviral treatment is ribavirin. Therefore, the management of RSV-infected patients is hampered by limited availability of antivirals.

Results:

We created codon optimized expression constructs for RSV F, G and SH genes of the RSV-long strain. Co-transfection of these expression constructs along with lentiviral expression constructs for gag and pol genes and lentiviral vectors encoding luciferase or GFP reporter genes into 293T cells resulted in the production of infectious RSVpp. Generation of pseudotypes was monitored by inoculation of naïve RSV-permissive host cells and determination of reporter gene expression. Incorporation of F and G into these particles was readily detected by Western blotting. RSVpp infection (like infection by wild type RSV particles) was independent of endosomal acidification but was neutralized by Synagis and by competition with heparin. Screening a small compound library of molecules with known antiviral activity against enveloped viruses like hepatitis C virus (HCV) and vesicular stomatitis virus (VSV) a plant-derived molecule with antiviral activity against RSVpp infection was identified.

Conclusion:

RSVpp mimic key early infection steps of wild type RSV particles. Therefore, this model is a powerful system to dissect molecular pathways of RSV cell entry and for identification of RSV-specific entry inhibitors.

Disclosure of Interest: None declared

Poster 34

OSELTAMIVIR EXPANDS QUASISPECIES OF INFLUENZA VIRUS THROUGH CELL-TO-CELL TRANSMISSION

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Abstract: The population of influenza virus consists of a huge variety of variants, called quasispecies, due to error-prone replication. The expansion of quasispecies provides viruses with a great opportunity for their escape from anti-viral drugs and adaptation to new environments. Oseltamivir, a neuraminidase inhibitor, is one of most effective drugs against influenza virus. Previously, we reported that progeny virions of influenza virus become infected to adjacent cells via cell-to-cell transmission pathway in the presence of oseltamivir. During cell-to-cell transmission, progeny virions are enriched on the plasma membrane between infected cells and their adjacent cells, and viruses become infected to adjacent cells at high multiplicity. Co-infection with viral variants may rescue recessive mutations with each other. Thus, it was assumed that the cell-to-cell transmission causes expansion of virus quasispecies. We have demonstrated that temperature-sensitive mutations remain in progeny viruses even at non-permissive temperature by co-infection in the presence of oseltamivir. This is possibly due to a multiplex infection through the cell-to-cell transmission by the addition of oseltamivir. Further, by the oseltamivir treatment, the number of missense mutation introduced by error-prone replication was increased in a passage-dependent manner. Taken together, we propose that oseltamivir expands influenza virus quasispecies via cell-to-cell transmission, and may facilitate the viral evolution and adaptation.
Disclosure of Interest: None declared

Poster 35

ANALYSES OF CELL ENTRY AND FUSION MECHANISMS OF SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME VIRUS

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Abstract: Severe fever with thrombocytopenia syndrome (SFTS) is an emerging fatal hemorrhagic fever caused by a newly identified Phlebovirus in the *Bunyaviridae* family, SFTS virus (SFTSV). Despite the medical importance of this disease, there are no vaccines or effective therapies against SFTS. Studies on the virus-host cell interactions are expected to contribute in development of antiviral strategies. In this study, we have developed a pseudotype vesicular stomatitis virus (VSV) bearing the unmodified Gn/Gc glycoproteins of SFTSV (SFTSVpv) and analyzed host cell entry of SFTSV. SFTSVpv generated in 293T cells exhibited high infectivities in various mammalian cell lines. A pH-dependent endocytosis of SFTSVpv was confirmed by the use of lysosomotropic agents. Infectivities of SFTSVpv were neutralized by a serial dilution of the convalescent patient sera. Entry of SFTSVpv and growth of SFTSV were increased in Raji cells expressing not only C-type lectin dendritic-cell-specific intercellular adhesion molecules 3-grabbing nonintegrin (DC-SIGN) but also DC-SIGN-related (DC-SIGNR) and liver and lymph node sinusoidal endothelial cell C-type lectin (LSECtin). 25-hydroxycholesterol (25HC), which is the soluble oxysterol metabolite, inhibited the cell entry and the membrane fusion of SFTSV. These results indicate that the SFTSVpv developed in this study can be used not only to study SFTSV glycoproteins in the entry process but also to develop neutralization test for diagnosis of SFTS. Furthermore, 25HC may have a potential for antiviral agent against SFTS.
Disclosure of Interest: None declared

Poster 36

CANINE DISTEMPER VIRUS MEMBRANE FUSION ACTIVATION: ROLE OF CRITICAL RESIDUES OF CD150/SLAM

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Abstract: The entry machineries of measles virus (MeV) and canine distemper virus (CDV) are composed of tetrameric attachment proteins (H) and fusion proteins, which co-operate with a host cell receptor to trigger membrane fusion for cell entry. Currently, two receptors involved in morbillivirus pathogenesis are known: SLAM and Nectin-4. While the recently determined atomic structure of MeV H bound to SLAM clearly highlighted the binding interface, the study additionally spotlighted two distinct

tetrameric assemblies of the complex. Interestingly, in one of these conformations (V-shape) two SLAM molecules were “sandwiched” between two discrete H-head domains, thus spotlighting two binding interfaces (“front and back”). Here we investigated the functional relevance of both binding sites in promoting membrane fusion in the context of CDV H and canine SLAM. Alanine-scanning mutagenesis identified 5 critical regulatory residues in the “front” binding site of SLAM. Among those, substituting position 123 (E123) led to the most pronounced impact on fusion-promotion, although the interaction with H remained unaltered. Consequently, this inferred a key role of this specific residue in transmitting a productive signal to the H-heads domain required for membrane fusion triggering. Conversely, while mutagenesis of 3 strategically selected residue of the “back” binding site of SLAM did not display any significant reduction in fusion-triggering, mutating these residues in the context of a partially-defective “front” SLAM mutant led to considerable additive fusion-support impairments. Importantly, the latter loss-of-function correlated with impaired physical interaction with CDV-H. Altogether, while our data suggest a functional impact of both SLAM binding sites in triggering membrane fusion machineries, they additionally support the idea that the V-shape conformation of H may represent of “post-receptor-binding” state required for a sustained activity of membrane fusion machineries.
Disclosure of Interest: None declared

Poster 37
SEQUENTIAL CONFORMATIONAL CHANGES IN THE MORBILLIVIRUS ATTACHMENT PROTEIN INITIATE THE MEMBRANE FUSION PROCESS
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Abstract: With the ultimate aim to develop *pan*-morbillivirus fusion inhibitors, we characterized in this study a potent neutralizing monoclonal antibody. The antibody recognizes the ectodomain of the membrane-bound tetrameric attachment (H) protein, which together with the fusion protein and a host cell receptor executes plasma membrane fusion for cell entry. The H-ectodomain consists of a short F-binding/activating stalk region that supports receptor-contacting head domains. Molecular characterization of the identified mAb epitope (which locates in the C-terminal stalk section called “spacer”), enabled us to unravel two sequential conformational changes occurring in CDV H-tetramers that stand at the core of the molecular mechanism translating receptor binding to F-triggering. Our findings additionally provided mechanistic evidence supporting the notion that both rearrangements are triggered upon receptor-induced “de-activation” of an auto-repressed state assumed by H before receptor binding. This locked state may be temporarily stabilized by a critical “head-to-spacer” interaction that enables H/F interaction, while preventing premature F-activation by the inherent H-stalk’s triggering bioactivity. Receptor-contact then disrupts the “head-to-spacer” interaction, which in turn “unlocks” the stalk, allowing it to rearrange and trigger F. Overall, our data deliver essential mechanistic insights supporting and extending the recently hypothesized “safety catch” model of morbillivirus membrane fusion activation. Furthermore, beyond having identified the “spacer” module as the key candidate element in the H-stalk domain coordinating the dynamics of the “safety catch” mechanism, our data further suggested that despite strong overall structural conservation between different paramyxovirus envelope glycoproteins, the presence or absence of “spacer” in attachment protein stalks emerges as a key indicator for the F-triggering strategy applied by different paramyxovirus family members.
Disclosure of Interest: None declared

Poster 47
STRUCTURE OF THE L-PROTEIN OF VESICULAR STOMATITIS VIRUS FROM ELECTRON CRYOMICROSCOPY
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Abstract: We have obtained a 3.9 Å resolution structure of the vesicular stomatitis virus (VSV) large polymerase protein by electron cryomicroscopy. As source of polymerase we expressed and purified L in complex with a portion of its phosphoprotein cofactor (P). The structure shows five L protein domains -- three (the RNA-dependent RNA polymerase, a mRNA capping domain, and a methyltransferase domain) with assigned enzymatic activity and two (a connector between the capping and methyltransferase domains and a C-terminal domain) that appear to have largely organization roles. The structure obtained likely represents an early initiation complex, and suggests a domain rearrangement occurs shortly after initiation.
Disclosure of Interest: None declared

Poster 48
PHOSPHORYLATION OF MARBURG VIRUS NP INFLUENCES REPLICATION AND ASSEMBLY
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Abstract: Marburg virus (MARV) causes severe hemorrhagic fevers among humans and nonhuman primates. The nucleoprotein NP, together with the viral proteins VP30, VP35, VP24 and the polymerase L, form the nucleocapsid that encapsidates the viral RNA. Previous studies have shown that NP is phosphorylated at C-terminal serine and threonine residues. While in infected cells both phosphorylated and unphosphorylated NP can be detected, only the phosphorylated form is incorporated into released viral particles, suggesting a role of the phosphorylation in virogenesis. Seven phosphorylation regions have been identified in NP, some of which have already been studied more in detail.
To study function of phosphorylation of S619 (region VII), two mutants were constructed replacing S619 by aspartate (NP S619D) or alanine (NP S619A) to mimic either a constantly phosphorylated or non-phosphorylated state of the residue. Substitution of NP by NP S619A led to increased rates of transcription and/or replication while the phenotype of NP S619D did not significantly differ from wtNP in an infectious virus-like particle assay. Recombinant viruses carrying the same mutations were successfully rescued and characterized. recMARV NP S619D was more efficiently infecting cells and outgrew recMARV NP S619A in a growth competition assay. Non-phosphorylated S619 therefore seems to be crucial for efficient transcription and/or replication of the viral genome while phosphorylation of S619 leads to enhanced release of viral particles. Taken together, the data suggest that dynamic phosphorylation of S619 regulates the switch from RNA synthesis to assembly of progeny virions.
Disclosure of Interest: None declared

Poster 49
THE MOLECULAR MECHANISM OF INFLUENZA VIRUS MEMBRANE SCISSION
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Abstract: Influenza A virus is an enveloped, negative sense RNA virus which causes annual epidemics and major pandemics. Assembly and budding of new viral particles is a complex and multistep process, of which, many aspects remain unclear, despite many years of research. Influenza virus M2 protein is a homotetrameric transmembrane protein, containing three domains: ecto domain, transmembrane domain and cytoplasmic tail (CT). In the final stage of budding it has been shown that M2 mediates membrane scission through an amphipathic helix (AH), which is formed by the first 17 amino acids of the protein's CT, however the exact mechanism by which membrane scission occurs is not known. Using a collection of biochemical and biophysical techniques we have investigated the structure of the M2 AH and assessed its function in viral assembly and budding. Using Nuclear Magnetic Resonance we have determined the solution and membrane-bound structure of the M2 AH. We see that the M2 AH

is helical in solution but is stabilized upon lipid binding. Further biophysical characterization reveals that the M2 AH requires a defined lipid environment for efficient membrane interaction. Using lipid nanotubes of specific lipid compositions we see that membrane insertion of the M2 AH causes nanotube constriction and scission. These results define the biophysical mechanism of membrane scission that is necessary for the completion of influenza virus budding.
Disclosure of Interest: None declared

Poster 50

ULTRA-STRUCTURE AND PH DEPENDENT CONFORMATIONAL CHANGES OF THE LASSA VIRUS GLYCOPROTEIN

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Abstract: Lassa virus (LASV), from the family *Arenaviridae*, is an enveloped negative-strand RNA virus, which utilizes rodents as a natural host reservoir and is endemic throughout many parts of West Africa. Due to the high associated mortality rates (approximately 15%) and absence of any known therapeutics to treat human infection, LASV has been classified as a category level 4 biohazard pathogen. The glycoprotein (GP) of LASV, which consists of the stable signal peptide (SSP), GP1, and GP2 glycoprotein subunits, is responsible for initiating attachment to and subsequent fusion with the host cell. As understanding of the molecular architecture of LASV GP is key for understanding the mechanism of viral entry, we subjected the GP spike to electron microscopy analysis. Using techniques in cryo-electron tomography and subtomogram averaging, we solved the GP structure from chemically fixed LASV to 12 Å resolution, the highest resolution viral glycoprotein spike structure solved to date using this method. The structure reveals that the LASV GP ectodomain forms a globular trimeric structure, which extends into the viral interior. Following virus internalization into the host cell through GP attachment to the α-dystroglycan receptor, fusion of viral and host cell membranes is thought to be mediated at an unusually low pH of 3-4.5 via the host cell lysosome-resident receptor, LAMP1. To investigate the conformational changes of the GP during this process, we also solved structures of the GP at pH 5 and pH 3. At pH 5, only minor conformational differences were observable and 9% of the GP ectodomain mass was lost, which may correspond to detachment of the SSP. At pH 3, we observed major conformational changes including loss of the GP1 from the GP spike, a result which we confirm biochemically. By comparing the GP structures at neutral and low pH, we attribute the densities of GP to heterodimeric GP1 and GP2, and present the GP structure at its intermediate and post-fusion state.
Disclosure of Interest: None declared

Poster 51

BIOINFORMATICS TOOLS TO PREDICT PROTEIN-PROTEIN INTERACTIONS IN INFLUENZA VIRUS

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Abstract: Influenza virus belongs to Orthomyxoviridae family and is lipid enveloped with negative sense single stranded RNA segmented genome. The envelope includes several viral specific proteins that orchestrate virus entry into the cell via membrane fusion reaction as well as progeny virions assembly and budding at the plasma membrane. Yet, there are no 3-D data available for transmembrane and/ or cytoplasmic domains of surface antigens hemagglutinin (HA), neuraminidase (NA) and an ion channel M2. Only two thirds of membrane-associated matrix protein M1 (NM-domain) is crystallized and resolved. We now aimed to predict amino acid residues participating in envelope proteins interactions under the viral membrane using bioinformatics tools. It was hypothesized that due to spatial limitations the residues located at the protein-protein interface should evolve in accordance. To find correlated mutations, we (1) loaded all available amino acid sequences of HA, NA, M2, M1 from the Influenza

Research Database (http://www.fludb.org/); (2) excluded repeated/ defected sequences; (3) aligned the unique ones; (4) applied the EvFold program (http://evfold.org) searching non-transitive correlations/ co-evolving amino acid positions for the protein pairs of interest. Among the amino acid position pairs found by the program we manually picked up those having the Direct Information score above 0.1. The position pair frequencies were calculated. Finally, we selected one, two and three potentially interacting amino acid position pairs for the HA-M2, HA-M1 and M1-M2 protein pairs, respectively that are topographically/ physicochemical consistent as well as highly evolutionary sustainable. Site-directed disturbance of those interactions might assist in future in developing live attenuated virus vaccines of new generation. The experimental verification of our predictions using PCR mutagenesis and reverse genetics approaches are now in progress.
Disclosure of Interest: None declared

Poster 53
INVESTIGATING THE ROLE OF THE DIMERISATION OF VESICULAR STOMATITIS VIRUS PHOSPHOPROTEIN
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Abstract: Vesicular stomatitis virus (VSV) belongs to the family *Rhabdoviridae* of the nonsegmented negative-sense RNA viruses (NNV). The genome contains five transcription units that code for five structural proteins. Three proteins are involved in RNA synthesis: the RNA polymerase (L), the nucleoprotein (N) and the phosphoprotein (P). N assembles into a polymeric form that encapsidates the viral genome, forming an long helical N-RNA complex that serves as a template for viral transcription and replication. P is essential for viral replication. In particular, by binding to both L and polymeric N, P positions the polymerase on the N-RNA complex and keeps it attached when the polymerase moves along the template. P of NNV is an oligomeric protein. In paramyxoviruses P forms tetramer, whereas biophysical studies from our lab showed that VSV P forms dimer in solution. The protein has a modular organisation with two intrinsically disordered regions and two folded domains, and is thus highly flexible. In this work, we address the question of the role of the oligomerization of P. It has been proposed that P moves by cartwheeling along the N-RNA template, carrying the L subunit, a mechanism that requires an oligomeric P. However, rabies virus P deleted of its dimerization domain remains functional in an *in cellula* transcription assay, suggesting that dimerization is dispensable for RNA synthesis. To explore further the role of the dimerization, we are investigating the effect of the deletion of VSV P dimerization domain. To this purpose, we constructed a P variant deleted of its central region, PΔ107-177. We used SAXS and SEC-MALLS-RI to investigate its structural properties, yeast-two- hybrid experiments to investigate its ability to interact with itself and with N in the cellular context and a minigenome assay to evaluate the effect of this deletion on VSV transcription and replication. Finally, we generated a recombinant virus that harbors a truncated PΔ107-177.
Disclosure of Interest: None declared

Poster 54
DECIPHERING THE FORMATION OF SOLUBLE AND FUNCTIONAL POLYMERASE COMPLEXES OF MONONEGAVIRALES
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Abstract: *Mononegavirales* that include several human and animal pathogens have a conserved gene order and use elaborate molecular machinery made of at least four molecular partners for their transcription and replication. These partners have coevolved with acquisition of a permanent and entire encapsidation of the genome by the nucleoprotein (N) and the use this N-RNA complex as a template for the viral polymerase made at least of a phosphoprotein (P) and large enzymatic protein (L). Not only P is required for L function, but L expression is stabilized by P, the underlying molecular mechanism of

which is unknown. In fact, the formation of a soluble, stable and functional polymerase complex from measles (MeV), Nipah (NiV) and vesicular stomatitis (VSV) viruses requires three proteins, L, P and the cellular chaperon heat shock protein 90 (HSP90). Contrary to other viruses such as Influenza virus (*Orthomyxoviridae* family), Bamboo mosaic virus (*Tymovirales* order) or hepatitis B virus (*Hepadnaviridae* family), HSP90 acts as a true transient chaperon since its activity is dispensable for the stability and functions of the polymerases of the three mononegavirales. Evidence supporting this finding includes: (i) ubiquitination of L and its degradation by the proteasome, (ii) colocalization and co-immunoprecipitation of HSP90 with the viral polymerase in infected cells, (iii) analysis by western blot of soluble and insoluble L proteins, (iv) analysis of L expression fading overtime, (v) a new L folding assay performed "in cellula", (vi) functional analysis of the polymerase in the context of the viral cycle in infected cells. Moreover, MeV and NiV L proteins subtly differ with VSV L protein which shows a fairly good expression level in the absence of P provided that HSP90 is active. Finally, we will report the mapping of P regions important in supporting formation of mature polymerase with at least three regions that coordinately act, each of them assuming a distinct function.
Disclosure of Interest: None declared

Poster 55
STRUCTURAL AND FUNCTIONAL ANALYSES OF THE MUMPS VIRUS PHOSPHOPROTEIN DOMAINS
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Abstract: Mumps virus (MuV) is a human pathogen in the *Paramyxoviridae* family that causes an acute infection with symptoms ranging from parotitis to mild meningitis and severe encephalitis. The nucleoprotein (NP), phosphoprotein (P), and large protein (L) make up the NP-P-L complex, which is essential for viral RNA synthesis. P proteins of paramyxoviruses consist of N-terminal (P_N), central/oligomerization (P_O), and C-terminal (P_C) domains. Like many other negative-stranded RNA viruses (NSVs), MuV P_C has a nucleocapsid binding domain (NBD). Recently, an additional NBD was found in MuV P_N and functions in uncoiling the nucleocapsid. The unique antiparallel orientation of MuV P tetramer formation (with two N-terminal and two C-terminal P_O at each end) allows for both P_N and P_C to be located on each end of the tetramer, however functionality of P_O has not been examined for viral RNA synthesis. MuV P mutants were generated to elucidate the structural and functional roles of the P domains in viral transcription and replication. Oligomerization of P_N and P_C was required for full activity in a minigenome system. Furthermore, disulfide bond engineering was utilized to examine the orientation in P dimer formation. In addition, the L-binding domain of MuV P was investigated using confocal microscopy and biochemical analysis.
Disclosure of Interest: None declared

Poster 56
IDENTIFICATION OF RESIDUES IN THE C-TERMINUS OF LASSA VIRUS L PROTEIN BEING IMPORTANT FOR TRANSCRIPTION BUT NOT REPLICATION
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Abstract: The human pathogenic Lassa virus belongs to the family of *Arenaviridae*. The viral proteins are encoded by a single-stranded bi-segmented RNA genome in ambisense orientation. During virus infection the ~250-kDa L protein mediates transcription of the genes and replication of the viral genome segments. This protein has different domains, one of which is known to have an RNA-dependent RNA-polymerase function (center), another one is carrying an endonuclease activity (N-terminus). The remaining parts of the L protein have not been functionally described yet.
Transcription of the viral genes is dependent on a mechanism called cap-snatching, whereas the replication is thought to be primer-independent. Using a combination of the Lassa virus replicon system and identification of RNA products by Northern blotting we were able to identify specific residues in the C-terminus of the L protein, which are important for transcription but dispensable for genome replication.

Thus, the C terminus of Lassa virus L protein is involved in the mRNA synthesis process, potentially by mediating cap binding.
Disclosure of Interest: None declared

Poster 57
INFLUENCE OF CHANGES IN DIFFERENT DOMAINS OF AN AFRICAN BAT HENIPAVIRUS FUSION PROTEIN ON SURFACE EXPRESSION AND BIOLOGICAL ACTIVITY
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Abstract: So far, zoonotic henipavirus outbreaks in humans only occurred in Australia (Hendra virus) and Southeast Asia (Nipah virus, NiV). However, henipavirus-like RNA was also isolated from fruit bats in Africa. Though live virus could not be isolated yet, the full-length genome of prototype African bat henipavirus GH-M74a was successfully sequenced from bat samples. To evaluate the potential of GH-M74a to replicate in henipavirus-susceptible cells, we functionally characterized its surface glycoproteins as major determinants for virus entry and cell-to-cell spread. In addition to some minor functional defects in the G protein, the GH-M74a fusion protein was found to display a significantly reduced surface expression and decreased cleavage rates, resulting in a very limited biological activity compared to functional NiV F protein. In this study, we aimed to define if these properties can be attributed to specific protein domains in the GH-M74a F protein. We therefore analyzed several mutants with deletions in the unusually long pre-SP region. We also generated chimeric GH-M74a F proteins in which different domains (F2, F1 subunits, transmembrane domain, cytoplasmic tail) were replaced by the corresponding regions of functional NiV F protein. All mutants were analyzed for total protein expression, F cleavage rates, intracellular distribution, surface expression and fusion activities. Interestingly, none of the minor or major mutations resulted in an increased GH-M74a functionality. This suggests that the reduced expression and limited fusion activity of GH-M74a F protein are caused by intrinsic structural properties in more than one protein domain. As African henipaviruses are only distantly related to their Asian and Australian counterparts, likely multiple changes in structurally important parts are needed for the evolution of a fusion protein with biological activities similar to that of their pathogenic relatives.
Disclosure of Interest: None declared

Poster 58
FUNCTIONAL AND STRUCTURAL STUDIES OF THE INFLUENZA C VIRUS RNA-DEPENDENT RNA POLYMERASE
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Abstract: Influenza viruses encode an RNA-dependent RNA polymerase responsible for transcription and replication of the viral genome. Transcription involves the generation of a primer, which is produced by recognition of host pre-mRNAs and cleavage 10-13 nucleotides downstream of the cap. Recently, atomic resolution structural data for influenza A and B polymerases has become available, but details of the molecular mechanisms involved in transcription and replication remain obscure. Influenza C virus causes occasional mild respiratory disease in humans. Similar to influenza A and B viruses, its polymerase is composed of three subunits: PB1, PB2 and P3 the last two which carry out cap binding and endonuclease activities, respectively. Despite displaying low sequence identity with influenza A, alanine mutations of the equivalent active site residues in PB2 and P3 abolish transcription, which suggests cap snatching is conserved within influenza viruses. In order to further our understanding of this complex, we investigate the structural biology of Influenza C polymerase, which can be stably expressed and purified. The introduction of direct electron detectors has significantly improved the ability to obtain near-atomic resolution information of protein complexes by electron microscopy. This tool could help us shed light on influenza polymerases, which remain an attractive therapeutic target.
Disclosure of Interest: None declared

Poster 59
REGULATION OF INFLUENZA VIRUS RNP COMPLEX FORMATION
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Abstract: The RNA genome of influenza A virus consists of eight single stranded negative sense vRNA segments. The vRNA segments are bound by viral RNA polymerase at the 5' and 3' ends and the remaining viral RNA is associated with nucleoprotein (NP) to form a viral ribonucleoprotein (vRNP) complex. These vRNP complexes carry out both viral transcription and replication. NP, in the RNP complex, forms oligomers through an interaction between the tail-loop and binding-groove of neighbouring monomers and binds to the viral RNA in a non-sequence specific manner. We propose that NP oligomerisation and RNA binding must be regulated in order for efficient RNP complex formation to occur, however, little is known about the regulation of NP in the viral life cycle. A phosphorylation site within the oligomerisation binding-groove of NP has been identified in both influenza A and influenza B viruses and we hypothesised that phosphorylation at these sites may regulate oligomerisation. Our results show that constitutive phosphorylation within the binding-groove inhibits NP oligomerisation leading to inhibition of ribonucleoprotein activity and viral growth. Inhibiting phosphorylation at this site results in unregulated NP oligomerisation, which also reduces RNP complex activity and viral growth. We therefore propose that during the viral lifecycle NP oligomerisation is regulated by reversible phosphorylation at a site within the oligomerisation binding-groove.
Disclosure of Interest: None declared

Poster 60
ANTIGENIC PROPERTIES OF HANTAVIRUS NUCLEOCAPSID (N) PROTEIN AND ITS APPLICATION TO ANTIGEN FOR IMMUNOCHROMATOGRAPHIC (ICG) TEST.
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Abstract: Hantaviruses are classified to the *Hantavirus* genus of the *Bunyaviridae* family. At least 24 virus species that represent sero- and genotypes have been registered within the genus *Hantavirus*. Of which, four virus species, Hantaan, Seoul, and Dobrava/Belgrade viruses are causative agents of hemorrhagic fever with renal syndrome (HFRS). Puumala virus is a causative agent of nephropathia epidemica (NE). While, Sin Nombre, Andes and related viruses are causative agents of hantavirus pulmonary syndrome (HPS). Thottapalayam virus (TPMV) is a shrew borne hantavirus and antigenically and genetically most distant from other hantaviruses, but pathogenicity to human is unknown. Since hantavirus N protein is the most abundant in the virion and immunodominant, N protein has been applied as a diagnostic antigen. Epitope mapping study with panel of monoclonal antibodies indicated that linear epitopes are locating at the N–terminal about 100 amino acids. Truncated recombinant Ns of HFRS, NE and HPS causing hantaviruses and TPMV that lacked the N–terminal region reduced the reactivity to immune sera against homologous virus. These results indicated that the region is immunodominant antigen, and the basic structure of N-terminal part of N protein may be common among viruses within the genus *Hantavirus*. The N–terminal regions of HFRS and HPS causing viruses have been expressed by *E. coli* and applied as diagnostic antigens for immunochromatographic (ICG) test. The ICG test was evaluated with human and rodent sera obtained in endemic countries. Sensitivity to detect hantavirus-specific IgG antibodies was same or even higher than to ELISA. The intensity of ICG test test line is weaker in the heterologous combination. Thus, the recombinant N protein based ICG test is considered as an useful test for differential diagnosis among HFRS, NE and HPS virus infection.
Disclosure of Interest: None declared

Poster 61
INVOLVEMENT OF GRAF1/ARHGAP26 AND RHO FAMILY GTPASES IN THE GROWTH OF HUMAN PARAINFLUENZA VIRUS TYPE 2

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Abstract: We previously reported that LPA, an activator of Rho family GTPases (RhoA, Cdc42, and Rac1), promoted syncytium formation by human parainfluenza virus type 2 (hPIV-2) (Tsurudome *et al.*, 2008, *J. Virol.*). However, it remains unknown whether these GTPases affect the hPIV-2 growth. Here, we show that the hPIV-2 growth was promoted by LPA, while NSC23766, a Rac1-specific inhibitor, did not affect the hPIV-2 growth, indicating the possible involvement of RhoA and/or Cdc42.
Rho family GTPases are regulated by guanine nucleotide exchange factors and GTPase-activating proteins (GAPs). We hypothesized that Graf1/ARHGAP26, one of GAPs, might mediate the Rho-hPIV-2 interaction, since it regulates RhoA and Cdc42 but not Rac1. We have shown that hPIV-2 growth was inhibited in HEK293 cells stably expressing Graf1 whereas its knockdown restored this growth, suggesting negative regulation of hPIV-2 growth by Graf1. To elucidate the hPIV-2-Graf1 interaction, we investigated their subcellular localization. hPIV-2 infection changed Graf1 localization from an homogenous distribution within the cytoplasm to granule formation. Graf1 co-localized with hPIV-2 P, NP, and partially with V, all components of the viral RNP. Immunoprecipitation studies are in progress to examine the binding of Graf1 with each hPIV-2 protein.
Disclosure of Interest: None declared

Poster 62
STRUCTURE OF SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME VIRUS NUCLEOPROTEIN IN COMPLEX WITH SURAMIN REVEALS THERAPEUTIC POTENTIALS
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Abstract: Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease caused by SFTS virus (SFTSV). Lack of vaccines and inadequate therapeutic treatments have made the spread of the virus a global concern. Viral nucleocapsid protein (N) is essential for its transcription and replication. Here, we present the crystal and solution structures of N from SFTSV and its homologs from Buenaventura (BUE) and Granada (GRA) viruses. The structures reveal that phleboviral N proteins fold into a compact core domain and an extended N-terminal arm that mediates oligomerization. Structures of the tetrameric, pentameric and hexameric assemblies of N proteins explain structural basis for their oligomerization observed in solution. A triple mutant of SFTSV-N, R64D/K67D/K74D, lost its ability to bind RNA and consequently failed to activate transcription and replication. Screening of small molecules to abrogate RNA-binding ability of SFTSV-N identified Suramin as a potent inhibitor with a K_d of 0.44 μM. Molecular details of the binding of Suramin with SFTSV-N were obtained via a crystal structure refined to 2.3 Å resolution. Suramin binds in a positively charged cavity, a likely viral RNA binding pocket, running along the inner edge of the ring-like assembly of SFTSV-N. Suramin could inhibit the replication of SFTSV in Vero cells. Interestingly, Suramin binds only phleboviral Ns. Our structural, mutagenesis and inhibition studies indicate that phleboviral Ns adopt a highly conserved architecture and use a similar strategy for tethering RNA. Therefore, a common Suramin-based therapeutic approach targeting SFTSV-N and its homologs could be developed for containing phleboviral outbreaks.
Keywords: Crystal structure; Severe fever with thrombocytopenia syndrome virus (SFTSV); Nucleoprotein (N); Small-molecule inhibitors; Suramin
Disclosure of Interest: None declared

Poster 63
NEW INSIGHTS INTO THE RESPIRATORY SYNCYTIAL VIRUS N0-P COMPLEX
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Abstract: As for all the members of *Mononegavirales,* the genomic RNA of Respiratory Syncytial Virus (RSV) is tightly bound to the viral nucleoprotein (N) and maintained as a helical N-RNA ribonucleoprotein (RNP) complex called the nucleocapsid (NC). This NC is used as a template for transcription and replication by the RNA-dependent RNA polymerase complex (RdRp) consisting of the L (Large protein) polymerase and its cofactor P (Phosphoprotein). Within this complex, two types of interactions between P and N proteins are required for polymerase activity. First, specific recognition of the viral RNA-N matrix by the L polymerase - a prerequisite for viral transcription and replication - is mediated by the P protein, which interacts with both L and N proteins. By analogy with orthologues from viruses of the same family, P is also expected to play the role of chaperon to maintain the neo-synthesized N in a non-polymerized, monomeric RNA-free form, named N^0. Recently, we engineered a recombinant mutated RSV N protein that behaves as wild-type N^0, i.e. monomeric, unbound to RNA but still capable to bind P. We used this N mutant designated N^{mono} as a substitute for N^0 and found that the N-terminal residues 1-30 of P are sufficient to interact with N^{mono}. Ala-scan of residues (1-30) of P allowed the identification of hydrophobic residues as critical for this interaction. The periodicity of these residues suggests that this domain of P folds into an alpha helix upon binding to N. We thus searched for a complementary surface on N by combining biochemical (GST pull-down assay) and cellular (minigenome assay) approaches. We also generated N- and C-terminal truncated forms of N in order to proceed to crystallographic trials of the N^0-P complex. Our results suggest that RSV uses a singular mechanism to maintain a pool of neosynthesized N^0 competent for specific encapsidation of its genomic RNA.
Disclosure of Interest: None declared

Poster 64
CRYO-ELECTRON MICROSCOPY STRUCTURES OF LA CROSSE ORTHOBUNYAVIRUS POLYMERASE IN PRESENCE AND ABSENCE OF VIRAL RNA
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Abstract: *Bunyaviridae* is the largest family of segmented negative strand viruses which also include *Orthomyxoviridae* and *Arenaviridae*. Central to their viral cycle is the RNA-dependent RNA polymerase which replicates and transcribes the genome segments within circular ribonucleoprotein particles (RNPs). Here we describe a cryo-electron microscopy reconstruction of the full length La Crosse polymerase in complex with viral RNA, together with a reconstruction of its apo truncated form (delta-Cterminal construct). Combined with the X-ray structure determined in our group, we provide a partial pseudo-atomic model of the La Crosse polymerase. Flexible regions of the polymerase, which are likely to undergo significant movements during replication/transcription, correspond to the endonuclease, the RNA-binding domain and the C-terminal region. This is reminiscent to what is seen in influenza polymerase suggesting that, in addition to their conserved architecture, these polymerases could have common mechanisms of actions.
Disclosure of Interest: None declared

Poster 65
PB1-F2 INFLUENZA A VIRUS PROTEIN BETA-AGGREGATED SECONDARY STRUCTURE: SYNCHROTRON INFRARED AND FLUORESCENT MICROSCOPY IN INFECTED-CELLS
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Abstract: PB1-F2, a small accessory protein of Influenza A Virus (IAV), is considered as a factor of virulence. PB1-F2 has no structure in aqueous solution but can switch from α-helical conformation to β-sheet secondary structure depending on the hydrophobicity of the environment. Moreover, PB1-F2 has a strong propensity to oligomerize. PB1-F2 amyloid fibers and soluble β-oligomers were detected in IAV-infected cells. However, the putative role of the PB1-F2 β-aggregates in the virus cycle remains unclear and new techniques were needed to detect them properly in the viral context. In the present

study, we investigated the formation of PB1-F2 β-aggregates in IAV-infected cells at the single cell level using synchrotron radiation. Fourier-transform infrared (IR) and deep UV (DUV) microscopy are non-invasive techniques for monitoring biochemical changes *in situ* in cells and tissues. Human epithelial pulmonary cells (A549) and monocytic cells (U937) were infected with a wild-type IAV and its PB1-F2 knock-out mutant and harvested at different time post-infection. IR spectra were recorded in each condition and processed to evaluate the change in the component band of the spectra corresponding to the amide I (secondary structure) and the CH region (membranes). The data obtained were analyzed by component principal analysis and confirmed the presence of an IR specific β-aggregates signature only in IAV-infected cells expressing PB1-F2 in a cell- and time-dependent fashion. Taking advantage of the high frequency of tryptophan (Trp) residues in the sequence of PB1-F2, the increase of the auto-fluorescent signal of Trp recorded by DUV microscopy was correlated with the accumulation of the β-aggregates in IAV-infected cells. Furthermore, PB1-F2 compromises the integrity of the cellular membranes in a cell-type dependent manner. These data should provide further insight into the PB1-F2 structure-function relationship and help to decipher its role in the pathogenicity of the virus.
Disclosure of Interest: None declared

Poster 66

FINE MAPPING AND CHARACTERIZATION OF THE BINDING DOMAIN OF THE HRSV PHOSPHOPROTEIN WITH THE M2-1 PROTEIN

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Abstract: The RSV genome is transcribed into 10 mRNAs by the RNA-dependant RNA polymerase complex (RdRp). M2-1 protein is a transcription antiterminator which increases the processivity of the RdRp during transcription. M2-1 is recruited to RNA transcription sites by the phosphoprotein P. Since protein-protein interactions are a target for antiviral compounds, our objective is to obtain the crystallographic structure of the M2-1—P complex. The atomic structure of full-length tetrameric M2-1 is now available. However, since P is a naturally disordered protein, it is not possible to use full-length P for that purpose. The aim of this work was to finely characterise the M2-1 binding domain of P and to use this domain for co-crystallization trials. The M2-1-binding domain of P was previously mapped to residues 100-120 by internal deletions by Mason et al. By using NMR, we identified P residues ~ 90-100 as a region interacting with M2-1. Using recombinant proteins and deletions, the M2-1 binding site was finely mapped to amino acid residues 93-110. The role of amino acid residues in M2-1—P interaction was investigated by site-directed mutagenesis and pull-down assays, and the impact of these mutations on viral transcription was evaluated *in cellula* using an RSV minigenome. The results highlighted the critical role of some residues located in this region. The role of P oligomerization for M2-1—P interaction was also investigated.
Disclosure of Interest: None declared

Poster 67

NMR STUDY OF THE STRUCTURE AND INTERACTIONS OF THE HUMAN RESPIRATORY SYNCYTIAL VIRUS PHOSPHOPROTEIN

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Abstract: NMR allows to study the structure, dynamics and interaction properties of globular proteins, but also intrinsically disordered proteins and regions, which are not accessible to other structural techniques. Here we are focusing on the phosphoprotein (P), which is the main polymerase co-factor and necessary for both viral transcription and replication. As compared to other P proteins of *Mononegavirales*, hRSV P is rather short and does not comprise domains with stable tertiary fold outside the central trypsin-resistant tetramerization domain. Sequence analysis of P predicts the presence of a helical oligomerization domain and large disordered N-and C-terminal extensions. This domain arrangement is confirmed by NMR. Moreover we used backbone chemical shift analysis and ^{15}N relaxation experiments to show that transient helices are formed in the N- and C-termini of P. At the C-terminus, nearly completely formed helices seem to prolong the oligomerization domain. At the N-

terminus transiently formed helices coincide with the binding sites for the RSV nucleoprotein in the N0:P complex and for the transcription co-factor M2-1, as shown by NMR interaction experiments. Finally to investigate possible long-range contacts between the flexible ends of P we have made paramagnetic relaxation enhancement measurements.
Keywords: respiratory syncytial virus, polymerase activity, phosphoprotein, M2-1.
Disclosure of Interest: None declared

Poster 78

CHARACTERIZATION OF THE ENDONUCLEASE DOMAIN OF HANTAAN VIRUS L POLYMERASE

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Abstract: Arenaviruses, Bunyaviruses and Orthomyxoviruses have evolved mechanism to obtain the caps from host mRNAs in order to prime their own replication, a process called “cap-snatching”. The capping protects the viral mRNA from degradation and prevents its recognition by some receptors of the host innate immune system. La Crosse orthobunyavirus (LACV) L polymerase has an endonuclease activity essential for cap-snatching that constitute a prominsing drug target. Here we modeled the active site of the putative endonuclease domain of Hantaan virus (HTNV) and Andes virus (ANDV) L polymerases using the determined structure of LACV. Based on our model, we propose that HTNV L residues H36, E54, D97, E110 and T112 correspond to the key residues of LACV active site, H34, D52, D79, D92 and K94. Using HTNV L constructs comprising the N-terminal domain, we could show that a single mutation in the putative catalytic site of HTNV L, such as H36, E54 or D97, efficiently rescues the expression of the construct, validating our model of the catalytic site. Moreover, we have found that the co-expression of the putative HTNV L endonuclease domain suppresses mRNA and protein expression, as illustrated by the decreased activity of the Nanoluciferase reporter, either co-expressed from an independent plasmid, or fused to HTNV L. This finding is in line with a recently described similar effect of the N-terminus of ANDV (Heinemann P., et al., 2013, J.Virol 87:6975). Using these tools, we could quantify the relative ratio of expression between wild type and endonuclease dead L. Furthermore, these results provide a basis for the development of a cell-based screening assay to identify new inhibitors of L’s endonuclease activity and hence hantavirus transcription.
Disclosure of Interest: None declared

Poster 79

PARAINFLUENZA VIRUS TYPE 2 AND 5 “CHIMERIC” MINIREPLICONS REVEAL REGULATORY SIGNALS FOR VIRAL GENE EXPRESSION IN THE LEADER SEQUENCE

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Abstract: Polymerase activity of nonsegmented negative-strand RNA viruses is controlled by genome-encoded *cis*-acting elements, such as start-stop signals for replication/transcription that regulate viral gene expression. We previously found that the polymerase complex of human parainfluenza virus type 2 (hPIV2) can utilize the minigenome of parainfluenza virus type 5 (PIV5), and that this activity is lower than that using a normal hPIV2 minigenome. This is a suitable system for seeking new regulatory signals for parainfluenzaviral RNA expression, because the lower activity of hPIV2 polymerase is determined by the PIV5 RNA sequences. We constructed a number of chimeric hPIV2/PIV5 luciferase encoding minigenomes that are basically hPIV2 minigenomes whose leader, 5’ and 3’ UTR of mRNA and trailer sequence are partially replaced with those of PIV5. By analyzing luciferase activity using hPIV2 NP, P and L in BSR T7/5 cells, we found that the PIV5 leader sequence has a negative effect on the hPIV2 polymerase. Further mutagenesis identified that the domains responsible for this effect are positions at 25-30 and 37-42 of PIV5 leader sequence. In particular, replacement of nucleotides 26 and 27 of the hPIV2 leader sequence with those of PIV5 resulted in the most significant decrease of hPIV2 polymerase activity. These results reveal that a regulatory signal for polymerase activity is encoded in the internal region of the leader sequence of the parainfluenza viral genome RNA.

Disclosure of Interest: None declared

Poster 80

NEW INSIGHTS INTO THE NONCONSERVED NONCODING REGION OF THE SUBTYPE-DETERMINANT HEMAGGLUTININ AND NEURAMINIDASE SEGMENTS OF INFLUENZA A VIRUSES

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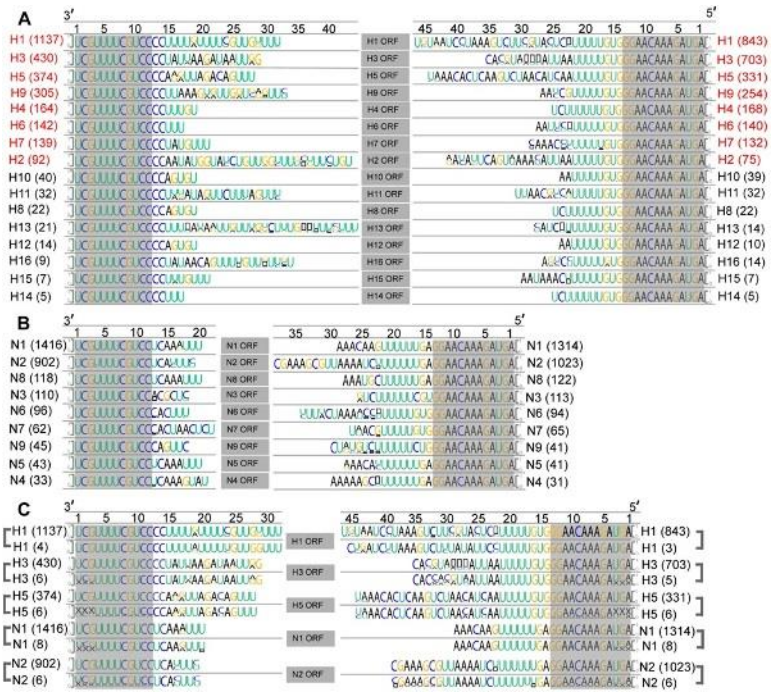
Abstract: The non-coding regions (NCRs) of the eight RNA segments of influenza A virus consist of the highly conserved promoter region and the non-conserved segment-specific NCRs at both the 3′ and 5′ ends. The roles of the segment-specific NCRs of the eight segments have been extensively studied. However, the diversities in the same region of the two subtype-determinant HA and NA segments received little attention. In this report, we bioinformatically analyzed all available NCRs of HA and NA vRNAs of influenza A viruses from NCBI Influenza Virus Resource Database and found that nucleotides in the segment-specific NCRs of HA and NA vRNAs are subtype-specific and varied significantly in sequence and length at both the 3′ and 5′ ends among different subtypes. In addition, we also found that all HA ssNCRs contain common 2-3 HA-specific nucleotides at both the 3' and 5′ promoter region. We then systematically studied the biological significance of the HA subtype-specific NCRs (HA ssNCRs) of those common HA subtypes (H1-H7 and H9) in the context of WSN (H1N1) reverse genetics system. We found that the HA ssNCRs play a critical role in HA vRNA virion incorporation. Upon the HA vRNA incorporation, the 3′ end HA ssNCR plays a more critical role than the 5′ end HA ssNCR and no stringent compatibility between the two ends is required. Furthermore, our data imply that, in addition to particular nucleotide(s), the length of the HA ssNCR is involved in regulating HA vRNA incorporation efficiency. These results provide new insights into the HA segment virion incorporation that is critical for the emergence of epidemic and pandemic influenza A virus strains.

Picture:



Disclosure of Interest: None declared

Poster 81

VIRUS-LIKE PARTICLE SYSTEM IDENTIFIES THE ENDONUCLEASE DOMAIN OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS

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Abstract: Bunyavirus transcription requires priming by 5'capped oligonucleotides, which are cleaved from host cell mRNAs by cap-snatching. As for arenavirus and orthomyxovirus, the endonuclease involved is located in the viral polymerase N-terminus. For the bunyaviruses LaCrosse (LACV, *Orthobunyavirus*) and Rift Valley fever (RVFV, *Phlebovirus*), functional analysis confirmed the essential role of the N-terminal residues D79 and D111, respectively (Klemm *et al.*, JVI, 2013). However, for Crimena-Congo hemorrhagic fever virus (CCHFV, *Nairovirus*), sequence alignments predicted the endonuclease domain to be more central, near position 700 (Morin *et al.*, PLoS-Pathogens, 2010).
We tested this hypothesis using our recently developed virus-like particle (VLP) system (Devignot *et al.*, JVI, in press). Cells were transfected with plasmids encoding CCHFV polymerase L (wild-type or mutated in conserved amino acids), nucleoprotein N, glycoproteins and a virus reporter-minigenome. Minigenome transcription by the plasmid-encoded L is reflected by reporter activity in cell lysates. Minigenome replication is assessed after transfer of supernatants onto new cells: If the VLP supernatants contained replicated minigenome, they could be transcomplemented with wild-type L and N, leading to reporter activity.
Among the L mutants tested, most striking results were obtained with the mutation at position D693, corresponding to LACV D79 and RVFV D111. While transcription activity was entirely abolished for D693A, replication and the ability to produce VLPs were preserved.
Those results show that D693 is a central amino acid of CCHFV endonuclease: The active center is indeed around position 700, as predicted. This emphasizes the unique structural organisation of nairovirus polymerases within the bunyavirus family.
Disclosure of Interest: None declared

Poster 82

SINGLE-CELL ANALYSIS AND STOCHASTIC MODELING REVEAL LARGE CELL-TO-CELL VARIABILITY IN INFLUENZA A VIRUS INFECTION

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Abstract: Viral infections can be initiated by a small number of infectious particles or even a single virion. In these cases, successful replication of the virus relies on reactions that comprise very few molecules, e.g., a single copy of the viral genome and a handful of proteins. However, such reactions are subject to stochastic fluctuations inherent to all biochemical reactions (intrinsic noise), which can cause large cell-to-cell heterogeneity of the infected cell population. Moreover, individual host cells may differ in their basic properties such as protein content or cell cycle stage introducing additional variations (extrinsic noise).
To analyze how this variability affects influenza A virus (IAV) infections we performed a single-cell analysis of adherent Madin-Darby canine kidney (MDCK) cells infected with influenza virus A/Puerto Rico/8/34 (H1N1). Experimental results reveal a large heterogeneity regarding the cell-specific virus yield ranging from 1 to 970 plaque forming units. The intracellular viral genome levels (vRNA(-)) vary by three orders of magnitude. Moreover, the segmentation of IAV genomes seems to increase the susceptibility of their replication to noise since the level of different genome segments can vary substantially within a cell.
To interpret the results systematically, we derived a stochastic mathematical model of the intracellular viral life cycle. In combination, our experimental data and simulations show that the variability in vRNA levels is caused by both, extrinsic noise and stochastic fluctuations intrinsic to viral RNA synthesis. In addition, simulations demonstrate that an abortion of virus entry and the random degradation of vRNAs can result in a high number of non-productive cells after single-hit infection. Hence, these results shed

light on the variability of IAV infection at the single-cell level, which may play an important role during the early stages of an infection, where the virus establishes itself in a new host.
Disclosure of Interest: None declared

Poster 84
MEASLES VIRUS DEFECTIVE-INTERFERING RNAS: ORIGIN AND MULTIPLICATION
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Abstract: Defective-interfering viral genomes (DI-RNAs) can form during infection by negative strand RNA viruses and outgrow full-length viral genomes, modulating the severity and duration of infection. We observed that C protein-deficient measles virus (MV-C^{KO}) generates high levels of copyback DI-RNAs (Pfaller et al., 2014, J. Virol. 88, 456-468). These DI-RNAs accumulate at cytoplasmic viral replication sites and form RNA structures with double-stranded character (dsRNA), thereby leading to autophosphorylation of protein kinase R (PKR) and induction of cellular stress and innate immune responses. Using reverse genetics we documented the *de novo* generation of copyback DI-RNAs from a series of independent rescue events for vaccine (vac2) and wild-type (IC323) MV as early as passage 1 after virus rescue. MV-C^{KO} viruses exhibited a higher frequency and variation of generated DI-RNAs than parental viruses, suggesting that C is a processivity factor of the viral polymerase. We obtained nucleotide sequences of 65 individual DI-RNAs and from this information predicted their structural features, breakpoints, re-initiation sites and length. Several DI-RNA showed clusters of A-to-G or U-to-C transitions. Neighboring nucleotides flanking mutation sites were characteristic of those favored by adenosine deaminase acting on RNA 1 (ADAR1), which catalyzes the C6 hydrolytic deamination of adenosine to produce inosine in dsRNA, referred to as A-to-I-editing. DI-RNAs were incorporated into virus particles, as demonstrated by northern blot analysis of purified virions. The C^{KO}-phenotype is dominant: in interferon-incompetent lymphatic cells DI-RNAs derived from vac2-C^{KO} suppressed replication of vac2, as shown by co-infections with viruses expressing different fluorescent reporter proteins and quantification of their expression levels. In contrast, co-infection with a C protein-expressing virus did not rescue the suppressive phenotype of DI-RNAs.
Disclosure of Interest: None declared

Poster 85
A SINGLE RNA SEQUENCE SWITCHING THE GENESIS OF INFLUENZA RNA POOLS - FISHING FOR SVRNA AND MORE

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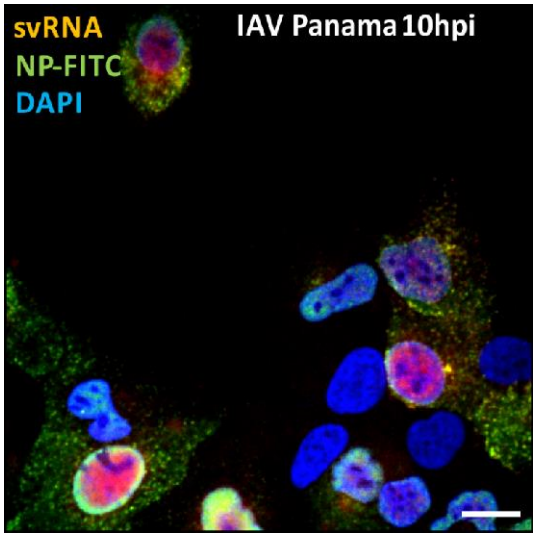
Abstract: The genome of the Influenza A virus (IAV, *Orthomyxoviridae*) is divided into eight single ribonuclear protein segments (vRNPs) consisting of negative sense RNA (vRNA) covered with the IAV nucleoprotein (NP). IAV infects cells via endocytosis, whereas upon fusion of the viral envelope with the endosomal membrane the vRNPs are released into the cytoplasm and are transported into the nucleus.
In the early state of infection, mRNA is directly transcribed from vRNPs for translation of viral proteins. Simultaneously, vRNAs are used by the viral polymerases to produce complementary RNAs (cRNAs) in a stochastic manner. Such cRNAs are transcribed at the 3' end to short viral RNAs (svRNAs), which lead to increased cRNA-production by allosteric modification of the polymerases to mediate enhancement of vRNA-replication. Covered with NP, the newly synthesized vRNAs are transported to

the cell membrane for budding of new viruses. To form an infectious virus, it is presumed that all eight different vRNPs have to assemble into a single viral envelope. Interestingly, it is not known when and in which order the different vRNPs concur and assemble.
In this work, we study important steps of the genesis of the Influenza RNA genome - the switch from protein synthesis to vRNP replication and the vRNP assembly. Fluorescence *in situ* hybridization (FISH) with forced-intercalation probes (FIT-probes) targeting the svRNAs was performed to visualize the reported switch in IAV-infected cells. To resolve the vRNP-assembly at single cell level, a modified version of FISH is introduced, allowing the detection of more RNA targets than the number of separate detection channels available. In a first FISH-cycle, up to 5 sets of FISH-probes targeting each different vRNP species are detected in separate fluorescence channels simultaneously. Afterwards, the signal is removed and the same cells are re-labeled with probes targeting the remaining vRNPs and with additional cell markers.
Picture:



Disclosure of Interest: None declared

Poster 86
UNCONVENTIONAL MRNA CAPPING ENZYME, GDP POLYRIBONUCLEOTIDYLTRANSFERASE, OF VESICULAR STOMATITIS VIRUS
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Abstract: The multifunctional L protein (2109 amino acids) of vesicular stomatitis virus (VSV) catalyzes unconventional mRNA capping by the GTPase and GDP polyribonucleotidyltransferase (PRNTase) activities. The latter activity transfers pRNA from pppRNA to GDP through the covalent enzyme-pRNA intermediate to generate a capped RNA (GpppRNA), whereas eukaryotic mRNA capping enzyme, RNA guanylyltransferase (GTase), transfers GMP from GTP to ppRNA through the covalent enzyme-GMP intermediate to form GpppRNA. In addition, the guanosine recognition mechanism of PRNTase appears to be different from that of GTase. The covalent pRNA attachment site in the L protein has been mapped to a histidine residue at position 1227 (H1227), which is part of the histidine-arginine (HR) motif in the conserved sequence block V of the L protein. We performed site-directed mutagenesis of conserved and semi-conserved amino acid residues surrounding the HR motif to examine their participation in the PRNTase reaction. We found that some amino acid residues (*e.g.*, G1100, T1157, W1188, H1227, R1228, F1269, Q1270) in collinear sequence motifs, GxxxP, [Y/W][I/L/V]GSxT, W, HR, and [F/Y]Q, are specifically required for the PRNTase activity in the step of L-pRNA intermediate formation, but not for the GTPase activity. Furthermore, we analyzed the effects of these mutations on transcription using an *in vitro* reconstituted transcription system. Interestingly, these cap-defective mutants synthesized the

leader RNA, but exhibited aberrant stop-start transcription using cryptic termination and initiation signals within the *N* gene, resulting in sequential synthesis of ATP or GTP-initiated N mRNA fragments including a 3′-polyadenylated RNA. These results suggest that the collinear sequence motifs constitute the active site of the PRNTase domain and co-transcriptional pre-mRNA capping is required for accurate selection of termination and initiation sites to synthesize full-length mRNAs.
Disclosure of Interest: None declared

Poster 87
INFLUENZA NUCLEOPROTEIN RESIDUES REQUIRED FOR VIRAL GENE EXPRESSION EXPOSE ANTIVIRAL TARGET
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Abstract: Influenza nucleoprotein (NP) is a structural component of the vRNP that also interacts with both viral and host factors to regulate viral RNA expression. NP is conserved among influenza A isolates, making NP interactions compelling antiviral targets. Here we report characterization of an NP mutant with glycine substitutions at five accessible and conserved residues within the body domain of NP, termed NPbd3, the third of five body domains mutants constructed and examined, but the only mutant non-functional for viral gene expression.

We confirmed expression and localization of wild type (WT) NP and NPbd3 by both cellular fractionation followed by western blot, and NP-GFP fusions observed through fluorescence microscopy. Electrophoretic mobility shift assay (EMSA) with purified NP protein confirmed NPbd3 bound nucleic acids as WT-NP. Blue native (BN) gel electrophoresis of protein extracts followed by western blot demonstrated the ability of NPbd3 to form oligomers. Although NPbd3 was expressed in the cell, localized to the nucleus, found in oligomers, and bound nucleic acid *in vitro*, NPbd3 was defective for viral RNA expression in reconstituted vRNPs and cRNPs as assessed by reverse transcription and quantitative polymerase chain reaction (RT-qPCR).

To investigate this region of the NP body domain further, single amino acid glycine substitution mutants were cloned. Analysis of NP single mutants revealed that all were nearly as functional as WT-NP, suggesting these accessible amino acids in the NP body domain play a redundant role. A double glycine substitution mutant revealed the two amino acids that are responsible for the non-functional phenotype. We hypothesize these two residues are important for NP interaction with the viral RNA dependent RNA polymerase complex, specifically PB2. Our studies demonstrate disruption of this accessible domain should block viral RNA expression and support studies of antivirals targeting this highly conserved NP body domain.
Disclosure of Interest: None declared

Poster 88
FUNCTIONAL INTERROGATION OF PARAMYXOVIRINAE GENOMES WITH EFFICIENT REVERSE GENETICS
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Abstract: Paramyxoviruses (PVs) have not been amenable to whole genome interrogation via transposon mutagenesis due to inefficient reverse genetics. To perform saturation transposon mutagenesis in PVs, which requires ~10^5-10^6 independent rescue events, we first increased the rescue efficiency for *Paramyxovirinae*. Addition of ribozymes to generate bona fide 3’ and 5’ genomic termini

coupled with the use of an optimized T7 polymerase gene increased rescue efficiency ~1,000 fold to ~1 in 10^2-10^3 transfected cells.

We used our improved T7opt-HhRbz reverse genetics system to interrogate the genetic and functional plasticity of PVs by generating saturating genome-wide insertional mutagenesis libraries for the *Paramyxovirinae*: Measles (*morbillivirus*), Sendai (*respirovirus*), Mumps (*rubulavirus*), Nipah (*henipavirus*), and Newcastle disease virus (*avulavirus*). A neutrally-placed extra stop codon (+3 nucelotides) was added in the various template genomes such that only PV genomes containing a single 15 nucleotide transposon would fulfill the "rule of six" and be efficiently rescued. For each PV, $>10^5$ individual insertional mutants were recovered, pooled, rescued en masse, and passaged twice.

We used Illumina sequencing (10^8 reads) to map the positions of the transposons in the resulting mutant viral libraries. For MeV, insertions in the N/P, P/M, and M/F intergenic regions were the most over-represented. Insertions in the N, P, and M genes were also easily recovered (P/V/C>N~M), but insertions in the F and H genes were severely underrepresented. The relative inelasticity of MeV env glycoproteins may be due to constraints on the interaction of H with multiple protein-based receptors, and may also explain its relative lack of antigenic drift. Ongoing comparative analysis of conserved and unique regions where insertions are tolerated between G/H- and HN-bearing PVs will shed light on the constraints on antigenic variance among PVs that used protein or glycan-based receptors, respectively.
Disclosure of Interest: None declared

Poster 89

RAPID LONG RANGE SLIDING OF RNA DEPENDENT RNA POLYMERASES ON VIRAL GENOME TEMPLATES VISUALIZED BY PHOTOACTIVATABLE LOCALIZATION MICROSCOPY

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Abstract: RNA dependent RNA polymerases (RdRPs) are packaged within negative strand non segmented RNA viruses to transcribe and replicate their genome template. RdRPs are tightly associated with purified genome templates and only initiate transcription at the 3' end of the template. RdRPs mechanism of redistribution along the genome template has not been clear. Using VSV RdRPs fused to photoactivatable Dendra proteins and virion purified genome templates we report tracking of single RdRPs along genome templates. Our observations show rapid sliding of single RdRPs with diffusion coefficients as high as 10^6 nm^2/Sec and distances as long as 800 nanometers. These observations unmask a fundamental mechanism of redistribution of polymerases on the genome templates.
Disclosure of Interest: None declared

Poster 90

THE ROLES OF PHOSPHORYLATION OF THE NUCLEOCAPSID PROTEIN OF MUMPS VIRUS IN REGULATING VIRAL RNA TRANSCRIPTION AND REPLICATION

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Abstract: While phosphorylation of the nucleoprotein has been shown to play important roles in transcription and replication of other negative strand RNA viruses, the role of phosphorylation of mumps virus (MuV) nucleocapsid protein (NP) has not previously been studied. MuV is a paramyxovirus with a negative sense, non-segmented RNA genome. The viral RNA genome is encapsidated by the nucleocapsid protein (NP), which forms the ribonucleoprotein (RNP). The RNP serves as a template for both transcription and replication of the viral RNA. In this study, we investigated the role of NP phosphorylation on MuV RNA synthesis. We first confirmed the phosphorylation of NP in MuV infected cells through the use of radioactive labeling. After confirming phosphorylation, we determined putative phosphorylation sites using liquid chromatography-mass spectrometry (LC-MS) and *in silico*

modeling. Nine serine or threonine residues were individually mutated to alanine. Mutation of the serine residue at position 439 to alanine (S439A) was found to reduce phosphorylation by over 90% in transfected cells. Using the MuV minigenome system developed in our lab, we were able to show that NP-S439A increased activity, while four other mutants produced lower activity and four had similar activity when compared to wild-type NP. MuV containing the NP-S439A mutation was rescued and phosphorylation of NP was shown to be reduced by 90%. The virus containing the NP-S439A mutation was able to grow to a higher titer than wild-type virus, but there was some lag in virus produced shortly after infection. The virus was shown to have enhanced viral RNA synthesis and viral protein production at early time points after infection, indicating that phosphorylation at the major phosphorylation site of NP plays an important role in down-regulating viral RNA synthesis. **Disclosure of Interest**: None declared

Poster 91

THE ABERRANT GENE-END TRANSCRIPTION SIGNAL OF THE M GENE OF HPIV3 DOWN-REGULATES F PROTEIN EXPRESSION AND THE F-SPECIFIC ANTIBODY RESPONSE IN VIVO

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Abstract: Human parainfluenza virus type 3 (HPIV3), a paramyxovirus, is a major viral cause of severe lower respiratory tract disease in infants and children. HPIV3 gene transcription initiation and termination is controlled by conserved gene-start (GS) and gene-end (GE) sequences, respectively, resulting in monocistronic mRNAs. The GE transcription signal of the HPIV3 matrix (M) protein gene is identical to those of the nucleoprotein and phosphoprotein genes except that it contains an apparent 8-nucleotide insert. This was associated with an increased synthesis of a readthrough transcript of the M gene and the downstream fusion (F) protein gene. However, its effects on F protein expression and on *in vitro and in vivo* infection and immunogenicity were not known. We hypothesized that this insert may down-regulate the expression of F protein by interfering with termination/reinitiation at the M-F gene junction, thus promoting the production of M-F readthrough mRNA at the expense of monocistronic F mRNA. To test this hypothesis, two similar recombinant HPIV3 viruses were generated from which this insert in the M-GE signal was removed. The M-GE mutants exhibited a reduction in M-F readthrough mRNA and an increase in monocistronic F mRNA. This resulted in a substantial increase in F protein synthesis in the infected cells as well as enhanced incorporation of F protein into virions. The efficiency of mutant virus replication was similar to that of wild-type (wt) HPIV3 both *in vitro* and *in vivo*. However, the F protein-specific serum antibody response in hamsters was increased for the mutants as compared to wt HPIV3. Thus, in wt HPIV3 the aberrant M-GE signal operates a previously undescribed mechanism that reduces the expression of a major neutralization and protective antigen, resulting in reduced immunogenicity. Repairing the M-GE signal should provide a means to increase the antibody response to a live attenuated HPIV3 vaccine without affecting viral replication and attenuation.
Disclosure of Interest: None declared

Poster 93

CHARACTERIZATION OF AVIAN METAPNEUMOVIRUS ATTACHMENT GLYCOPROTEIN IN A CELL LINE DEFICIENT IN O- GLYCOSYLATION

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Abstract: Expression and glycosylation of avian metapneumovirus (AMPV) G protein were examined in eukaryotic cell lines by transient-expression method using a constructed recombinant vertebrate transfer vector carrying the G gene. The transiently expressed G gene products migrated in SDS-PAGE, and showed premature form bands at molecular weights of 43, 45 and 58-90 kilodaltons (kDa) and a fully processed mature form around 110kDa, which represented the unglycosylated precursor, N-glycosylated intermediate, and O glycosylated mature forms, respectively. The presence of O-linked sugars in mature G protein was confirmed by using an O-glycosylation deficient Chinese hamster ovary

(CHO ldlD) cell line, in which the G protein could not be processed to the mature form unless the exogenous Gal and GalNAc were supplemented, and the lectin binding assay with peanut agglutinin, which bound only to O-linked sugars. In addition, tunicamycin treatment and adding galactose (Gal) and N-acetylgalactosamine (GalNAc) to asparagine-linked (N-linked) and serine- or threonine-linked (O-linked) oligosaccharides on glycoproteins provided additional evidence to support the N and O-linked glycosylation of G protein. Furthermore, indirect immunofluorescence staining assay demonstrated that the expressed G protein at cell surface displayed an intracellular transport of G protein.
Disclosure of Interest: None declared

Poster 94
VISUALISING THE SITES OF INFLUENZA VIRUS RNA SYNTHESIS.
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Abstract: Influenza A viruses contain eight negative sense, single stranded RNA gene segments which are packaged into the virus in the form of viral ribonucleoprotein complexes (vRNPs). Each vRNP contains a single viral RNA (vRNA) encapsidated by nucleoprotein with the 3´ and 5´ conserved termini bound to the viral RNA-dependent RNA polymerase complex. Unlike most RNA viruses, whose replication cycle occurs solely in the cell cytoplasm, the synthesis of influenza RNA occurs in the nucleus. Both transcription and replication is undertaken by the influenza polymerase complex but through two distinctly different mechanisms. Viral transcription (vRNA→mRNA) is primed by short capped RNA structures acquired by the viral polymerase through endonucleolytic cleavage of cellular pre-mRNAs and is terminated by the addition of a poly(A) tail. Replication, on the other hand, is a primer independent process, which occurs through a full length complementary RNA (cRNA) intermediate (vRNA→cRNA→vRNA). While many biochemical techniques have been employed to characterise viral RNA synthesis, no direct evidence of the nuclear sites of viral RNA synthesis is currently available. We have developed a technique to label nascent viral RNA using modified alkyne-containing nucleotides (ethynyl-UTP; E-UTP). Nascent transcripts are labelled by a “click” reaction between the E-UTP incorporated into nascent viral RNA and azides linked to fluorescent dyes. We have verified that both *in vitro* and *in vivo* the influenza polymerase can incorporate the E-UTP into viral RNA. We are using this technique to visualise the discrete sites of viral RNA synthesis and to identify the sub-nuclear compartments where viral RNA synthesis occurs. This technique will also allow us to use dual-colour dSTORM super-resolution microscopy to investigate the cellular components present at the sites of viral RNA synthesis.
Disclosure of Interest: None declared

Poster 95
PARTICIPATION OF INTERNAL SEQUENCES WITHIN THE 5’ AND 3’ NON CODING REGIONS IN ARENAVIRUS RNA TRANSCRIPTION AND REPLICATION
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Abstract: Tacaribe virus (TCRV) is the prototype of the New World group of the *Arenaviridae* family. The TCRV genome comprises two segments named S (small) and L (large), each encoding two proteins in an ambisense orientation separated by an intergenic region. The RNA segments contain untranslated regions (UTRs) at their 5’ and 3’ ends whose length ranges from 30 to 77 nucleotides (nt). The terminal 19-nt sequence at the 3’ end, which is highly conserved in each segment and across arenaviruses, shows sequence complementarity to the corresponding 5’ terminal sequence allowing the formation of a panhandle structure. While these terminal sequences are known to contain signals required to promote viral polymerase binding and activity, the role of internal regions within the 3’ and 5’ UTR remains poorly understood. Using a TCRV S-like minigenome assay, we found that internal deletions

within the genomic 5' UTR were associated with a strong inhibition in the synthesis of minigenome and anti-minigenome RNAs without affecting the relative proportion of mRNA. In contrast, internal deletions within the genomic 3' UTR had a less pronounced effect. Substitution of the entire internal sequence at the 5' UTR by the corresponding region from other arenaviruses did not affect the RNA minigenome/mRNA levels. In addition, when a conserved stem-loop structure predicted at the genomic 5′ UTR was deleted, the levels of minigenome expression were dramatically reduced. Similar results were observed upon deletion of an 8-nt sequence located nearby the predicted stem-loop. Substitution of this short sequence with an unrelated sequence partially restored minigenome levels, while mutagenesis of nucleotides predicted to stabilize the stem-loop structure had no effect. Overall, our results suggest that the internal sequence within the genomic 5' UTR is involved in viral RNA replication and as a consequence in mRNA transcription.
Disclosure of Interest: None declared

Poster 96
HENDRA L IS NOT FUNCTIONALLY COMPATIBLE IN CHIMERIC RNPS
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Abstract: Spillover infections of Hendra virus (HeV) and Nipah virus (NiV) from the natural host, bats, to terrestrial mammals and humans are associated with high mortality rates and severe morbidity. HeV surveillance of bat urine in 2009 lead to discovery of a novel *Henipavirus*, Cedar virus (CedPV). CedPV infection has not been associated with disease outbreaks in terrestrial mammals nor humans. Ferrets are a realistic model of HeV and NiV human disease due to similarities in kinetics of viral growth in the respiratory tract and spread to the brain. Indeed, CedPV is likely to be apathogenic as virus growth in the lungs of experimentally infected ferrets was limited and moreover induced neutralizing antibodies.
Comparison of genes and proteins across Henipavirus members may help elucidate the mediators for subclinical disease attributable to CedPV infection. The HeV and NiV phosphoprotein (P) genes, as with other members of the *Paramyxovirinae*, contain an RNA editing site that facilitates co-expression of V and W proteins from a single ORF. In contrast, CedPV P does not possess an editing site and hence is unable to produce these proteins, which have been shown to antagonise the innate immune response. In combination with nucleoprotein (N), the large protein (L) and the viral RNA, P also constitutes the ribonucleoprotein complex (RNP) by acting as a chaperone to prevent dimerization of unassembled N, N_0, and by recruiting L to the RNP interface.
With the use of minigenome luciferase reporter assays, we measured the transcriptional activity of chimeric RNPs containing combinations of CedPV and HeV N, P and L proteins. Our data reveals that interchange of N and P proteins with CedPV L enables minigenome transcription, while chimeric RNPs with HeV L are not functional. This finding suggests only particular HeV chimeras may be rescued via reverse genetics. Further studies are required to characterize the RNP-L interaction for its evaluation as a target for replication inhibitors.
Disclosure of Interest: None declared

Poster 97
QUANTIFICATION AND KINETICS OF INDIVIDUAL RNA SEGMENTS IN HANTAVIRUS INFECTED CELLS.
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Abstract: Worldwide Hantaviruses of the *Bunyaviridae* family causes 50-100,000 human infections annually. Most of the cases are hemorrhagic fever with renal syndrome in Eurasia, although a few hundred cases of Hantavirus (cardio) pulmonary syndrome are reported from the Americas.
Bunyavirus particles are enveloped with a spherical shape that includes segmented negative stranded RNA genomes. The genome is divided into three individual RNA segments, denoted S, M, and L (small, medium, and large).

The aim of this study was to investigate viral RNA replication and transcription *in vitro* and *in vivo* to improve the understanding of the infectious process of Hantaviruses. In order to do that we have examined the kinetics, levels and ratios of the different RNA segments at different time-points during the first week of infection. In essence, we have fused primer binding sequences of all three segments into one continuous synthetic DNA sequence under the control of flanking T7/Sp6 promoter regions for *in vitro* transcription. The amount of expressed and extracted RNA of infected cells was determined for a total of ten days and compared with predetermined amounts of *in vitro* transcribed S, M, and L RNA as standards for quantification by RT-PCR. From these experiments the amounts of S, M, and L at different time-points of infection in Puumala virus infected cell cultures was determined.
Our results indicate increasing amounts of viral RNA for all three RNA segments during the first week of infection. Interestingly, we observed large differences and variation in copy numbers of the individual segment during the time of observation.
Disclosure of Interest: None declared

Poster 98
POLYCISTRONIC EXPRESSION OF THE INFLUENZA A VIRUS RNA-DEPENDENT RNA POLYMERASE BY USING A 2A SELF-PROCESSING SEQUENCE
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Abstract: The RNA-dependent RNA polymerase (RdRp) of influenza A virus consists of three subunits of PB2, PB1, and PA, and catalyses both viral RNA genome replication and mRNA transcription. Cotransfection of three monocistronic expression vectors for these subunits with nucleoprotein and viral RNA expression vectors reconstitutes a functional viral ribonucleoprotein complex. However, the activity per mass of RdRp is extremely low since the expression level and the ratio of the three subunits are uncontrollable in a single transfected cell. We constructed polycistronic expression vectors for the subunits in which the three coding sequences (CDS) of the subunits were joined with the *Thosea asigna* virus 2A self-processing sequence in various CDS arrangements. PB1-PB2-PA and PB1-PA-PB2 open reading frame constructs (12A/1A2 ORF), in which PB1 CDS was placed at the most upstream, showed higher reporter activity in a luciferase-based mini-replicon assay than that observed by cotransfection of three monocistronic expression vectors. In these cases, PB1 protein was comparable with that expressed from the monocistronic PB1 vector, but the levels of PB2 and PA were very low or below detection limit. This decrease was most likely due to proteasomal degradation caused by the 2A-derived sequences attached to the amino and/or carboxyl ends of PB2 and PA. When the hygromycin B phosphotransferase CDS was placed at the most downstream of 1A2 ORF, a stable RdRp-expressing MDCK cell line was easily established in the presence of hygromycin B, indicating that acquisition of hygromycin B resistance assures the expression of upstream RdRp. We will discuss the advantages and limitations of the polycistronic expression of influenza A virus RdRp.
Disclosure of Interest: None declared

Poster 99
IDENTIFICATION OF AMINO ACIDS CRITICAL FOR POLYMERASE ACTIVITY IN THE N-TERMINAL REGION OF THE PB2 SUBUNIT OF INFLUENZA VIRUS RNA POLYMERASE
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Abstract: The influenza virus RNA-dependent RNA polymerase consists of PB1, PB2 and PA subunits. Although PB2 is known to be required for transcription via binding to 5' cap of host mRNA, its precise role in RNA synthesis is still unclear. Here, we investigated the function of the N-terminal region of PB2 in the process of transcription and replication of the viral genome. We targeted 8 clusters of basic amino acid in the N-terminal region of PB2, because negatively charged viral RNA tends to interact with basic residues. Targeted residues were mutated to alanine and 293T cells were transfected with plasmids expressing NA vRNA, NP, PB1, PA and mutant PB2, and the polymerase activity was measured by primer extension. Mutations in R124-H127 region, K140-R144 region and K331-R332 region of PB2

caused a sever decrease both in transcription and replication, whereas mutations in R268-K269 region selectively inhibited transcription. Mutations did not affect the expression of PB2 and the correct assembly of the polymerase trimeric complex, but severely impaired the accumulation of ribonucleoprotein (RNP) complexes, suggested a functional loss of the polymerase. Cross-linking studies in vitro showed that mutations in R124-H127 and K140-R144 regions significantly decreased binding to the vRNA and cRNA promoters. Furthermore, a reduced binding to the promoter led to the defect in the initiation of replication activity of the polymerase. We conclude that the N-terminal region of PB2 is involved in promoter binding and plays an important role in transcription and replication.
Disclosure of Interest: None declared

Poster 100

THE USE OF A HYBRIDIZATION-BASED PATHOGEN ENRICHMENT STRATEGY TO ENHANCE THE DETECTION AND IDENTIFICATION OF HENIPAVIRUSES.

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Abstract: The henipaviruses are negative-sense, single-stranded RNA viruses in the family *Paramyxoviridae*, containing three established species: Hendra virus (HeV), Nipah virus (NiV) and Cedar virus. HeV and NiV are zoonotic pathogens capable of causing severe illness and death in domestic animals and humans. During an outbreak, accurate virus characterization aids in diagnosis, risk factor investigation and potentially control measure implementation. Next Generation Sequencing (NGS) functions independently of the need for *a priori* knowledge about the target sequence, as opposed to conventional PCR, however, the abundance of viral RNA relative to host transcripts is typically orders of magnitude in favour of the latter. Targeted enrichment of viral genomes can therefore greatly enhance this discrepancy in the virus to host RNA ratio. We have designed a hybridization-based enrichment assay containing a set of henipvirus specific 120nt, biotinylated oligodeoxynucleotides designed to capture nucleic acid from an Illumina NGS library prepared from HeV RNA. A comparison of unenriched and enriched NGS libraries yielded 0.02% and 83.15% HeV-specific sequence reads, respectively; a 4000x enrichment of viral genetic material. In addition, we show here that due to the extensive length of the capture probes, the binding affinity for related viruses is robust, and we were also able to capture and sequence a previously uncharacterized henipavirus. As the hybridization assay is solution based, the henipavirus probe set is easily refined as more sequences become available. This assay will aid in the detection and characterization of existing and novel henipaviruses.
Disclosure of Interest: None declared

Poster 101

THE N-TERMINAL FRAGMENTS OF THE PB2 AND PA SUBUNITS SEVERELY INHIBIT RIBONUCLEOPROTEIN (RNP) ACTIVITY OF INFLUENZA A VIRUS

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Abstract: Influenza A virus has a RNA-dependent RNA polymerase (RdRp) that is composed of three subunits, PB1, PB2, and PA, which assembles with nucleoproteins (NP) and a viral RNA (vRNA) to form a ribonucleoprotein complex (RNP). Recently, we demonstrated that the combination of influenza RNP components is important for both its assembly and activity. Therefore, we questioned whether the inhibition of the RNP activity via an incompatible combination of the RNP complex could become one of methodologies for an anti-influenza drug. Thereupon, we confirmed whether the peptides/fragments mimicking one of RNP components can interfere with their formations. We found that some fragments derived from PB2 and PA subunits efficiently inhibited RNP activity. Moreover, we determined the domains and important amino acids on the fragmented PB2 and PA subunits, respectively, that were required for strong inhibitory effects of RNP activities. On the other hand, during the process of inhibitory study by PA subunit we found interesting suppression of protein expression of the RNP components. Especially, we found two residues (D108 and K134) on the fragment that were critical determinants for

the suppression. Furthermore, we identified the combination of three amino acids (P28, M86 and E100) on the PA fragment that was crucial for the strong suppression, and the minimum essential region (residues from 1 to 188) of the PA subunit that allowed its suppression. Our results suggest that the fragments derived from PB2 and PA subunits become inhibitors that targets influenza RNP activity. Moreover, our findings indicate that the fragments in this report may become candidates for an effective inhibitor of influenza RNP activity.
Disclosure of Interest: None declared

Poster 102
HOST CELL TYPE AFFECTS FORMATION OF DEFECTIVE INTERFERING PARTICLES IN WILD-TYPE AND VACCINE STRAINS OF MEASLES VIRUS
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Abstract: Copy-back defective interfering (DI) particles are formed during the replication of paramyxoviruses, including measles virus (MeV). DIs interfere with the replication of wild-type virus and have been shown to affect components of innate immunity. The formation of copy-back DIs by both vaccine and wild-type strains of MeV was examined in Vero/hSLAM, Vero, and MRC-5 (human fibroblast) cells. A DI specific RT-PCR assay detected a small copy back DI with an amplified region of approximately 200 base pairs following passage of wild-type and vaccine strains. Sequence analysis showed that the identified DI genome contained both positive and negative sense MeV genome sequences, as expected for a copy-back DI. Additionally, a similar polymerase jump point was identified in both the wild-type and vaccine strains resulting in a total size of 437 and 480 base pairs for wild-type and vaccine samples, respectively. RT-PCR analysis of additional wild-type strains also amplified a 200 base pair region within a DI, suggesting that these DIs may also have a similar jump point. The effect of the DIs in the preparations were evaluated by RT-qPCR analysis of N gene expression and demonstrated that viral replication was decreased with increasing DI presence. Further evaluation demonstrated that a copy-back DI genome was not detected in RNA isolated directly from a commercial vaccine preparation. Passage of this preparation at low MOI demonstrated DI formation in both Vero and MRC-5 cell lysates; however, not in unconcentrated MRC-5 supernatant fluid samples. Interestingly, following passage of a DI containing virus preparation in WI-38 cells, DI genomes were no longer detectable. These results suggest that host cell type and method of virus collection may play an important role in DI formation, and that MeV may have sequences within the genome that are more likely to contribute to polymerase dissociation.
Disclosure of Interest: None declared

Poster 113
SWINE IFITM PROTEINS PROTECT AGAINST INFLUENZA A VIRUS INFECTION IN VITRO
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Abstract: Due to their zoonotic potential, swine influenza viruses pose a major threat to human health and cause huge economical losses, clearly demonstrated by the swine-origin influenza pandemic in 2009.
Interferon-inducible transmembrane proteins (IFITMs) have been described as potent antiviral factors active against a whole variety of viruses, including influenza A virus (IAV). However, information on expression and antiviral potential of IFITMs present in pigs is scarce. In our study we show that IFITMs are present in several swine cell lines and use qPCR to demonstrate their inducibility upon interferon (IFN) treatment or infection. We cloned porcine homologs of IFITM1, IFITM2, IFITM3 and IFITM5 and stably introduced them into newborn pig trachea epithelial (NpTr) and newborn swine kidney (NSK) cells. Infection of those cells with IAV strains of human, avian or swine origin resulted in significantly

decreased viral titers compared to control cells. Interestingly, no differential IFITM-sensitivity for IAV strains of different origin could be observed. We further confirmed the antiviral restriction capacity of swine IFITMs via a mini-genome reporter assay in NpTr and HEK293T cells. When performing localization studies with different swine IFITMs we observed a correlation between potency of antiviral restriction and subcellular localization. While swIFITMs 2 and 3 that showed the highest antiviral potential localized predominantly to late endosomes, homologs of IFITM1 that only moderately decreased IAV infection were mainly found at the plasma membrane.
In summary we show that IFITMs are present and induced upon infection or IFN-treatment in swine cells and potently restrict IAV of different origin.
Disclosure of Interest: None declared

Poster 114

MASS-SPECTROMETRY BASED PROFILING OF PKR-INTERACTION PARTNERS IN THE COURSE OF INFLUENZA A VIRUS INFECTIONS

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Abstract: The RNA-dependent protein kinase (PKR) is an interferon induced, double-stranded RNA-activated protein kinase that plays a significant role in innate antiviral immunity. The activation of PKR involves dimerization, autophosphorylation and phosphorylation of numerous target factors. Downstream effects of PKR activation include inhibition of translation, initiation of apoptosis and the induction of transcription factors that lead to production of type I interferon.
Due to its key role in antiviral immunity many viruses have evolved mechanisms to avoid PKR initiated effects. We and others have previously described the influenza virus non-structural protein 1 (NS1) as an antagonist of PKR. We are interested in understanding the precise mechanism of PKR activation in the context of viral infection and the role of cellular and viral factors in regulating PKR activation. For this purpose we used a SILAC approach followed by LC-MS/MS analysis to identify immunoprecipitable interaction partners of PKR before and during influenza A wildtype or ΔNS1 virus infection.
In four experimental replicates we were able to detect 50 cellular PKR binding proteins. Bioinformatic analyses imply that the detected proteins are involved in cellular pathways as RNA processing, stress response and signaling. We confirmed the interaction between PKR and a subset of candidates in co-immunoprecipitation experiments and also observed intracellular co-localisation of PKR and interaction partners. Moreover, we showed that some of the identified proteins upon overexpression induce PKR phosphorylation, suggesting that these proteins are novel regulators of PKR.
It is expected that bioinformatic and functional analyses of novel PKR interaction partners will further our understanding of cellular antiviral mechanisms and their modulation by influenza A virus.
Disclosure of Interest: None declared

Poster 115

THE SMALL HYDROPHOBIC PROTEIN OF MUMPS VIRUS INFLUENCES NF-KB AND MAPK ERK SIGNALLING PATHWAYS

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Abstract: NF-kB and ERK signalling pathway play a critical role in cellular processes as cell proliferation and mitosis. Selective manipulation of these kinases provides a viral strategy to evade host defense mechanisms and to ensure efficient replication and survival of the virus.
Using a reporter gene assay the small hydrophobic (SH) protein of mumps virus (MuV) suppressed the activation of NF-κB after stimulation with TNFα, IL-1β, Poly(I:C) and Pam3CSK4, each stimulus targeting distinct cellular receptors. Activation of NF-κB by overexpression of pathway proteins in the presence of SH protein demonstrated that SH blocks the NF-κB signaling pathway downstream of MyD88, but upstream of TRAF2, TRAF6 and IKKβ. This data suggest that the SH protein interferes with an adaptor protein that serves as an interface between MyD88 and TRAF2, TRAF6 and IKKβ. Verification of the data using two recombinant mumps viruses (rMuV, rMuVΔSH) is currently underway.

Infection with wt-MuV and vaccine strain Jerry Lynn led to expression and dual phosphorylation of ERK 5 h and 10 hpi, whereas total ERK levels remained constant. In contrast to Flu and RSV, a biphasic activation of ERK was not detected. These results present for the first time that MuV targets the ERK signaling pathway. How the virus benefits from ERK activation is under current investigation.
How and to what purpose mumps virus modulates two intracellular signalling pathways will be discussed.
Disclosure of Interest: None declared

Poster 116

RIG-I RECOGNITION OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS 5′-MONOPHOSPHORYLATED GENOMIC RNA

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Abstract: In the cytoplasm, the retinoic acid-inducible gene I (RIG-I) senses the RNA genomes of several RNA viruses. RIG-I binds to viral RNA, eliciting an antiviral response via the cellular adaptor MAVS. Critical in RIG-I activation is the presence of double-stranded RNA (dsRNA) and a 5′-triphosphate (5′ppp) or -diphosphate moiety (5′pp). The RNA genome ends of Crimean-Congo hemorrhagic fever virus (CCHFV) exhibit complementary pairing to form dsRNA, but the 5′ end is monophosphorylated (5′p), similar to uncapped cytoplasmic host RNA. We found that, during CCHFV infection, RIG-I mediated a type-I interferon (IFN) response via MAVS. Interfering with RIG-I signaling reduced IFN production and IFN stimulated gene expression, and increased viral replication. Immunostimulatory RNA was isolated from CCHFV infected cells and cells actively replicating CCHFV minigenomes, and from virion preparations. RIG-I interaction with the CCHFV 5′p RNA was confirmed by co-immunoprecipitation and *in vitro* RIG-I binding to synthetic RNA oligonucleotides mimicking the monophosphorylated panhandle. Converting CCHFV 5′p to 5′ppp increased binding of RIG-I to the synthetic panhandle. Treating viral RNA with a panel of phosphatases and dsRNA-specific RNAse revealed that the complementary ends of CCHFV 5′p genome are sufficient for inducing a type-I IFN response. Altogether, these data provide evidence that 5′-terminal processing of viral RNA likely contributes to CCHFV's evading RIG-I detection, but that cells still rely heavily on RIG-I for sensing the CCHFV 5′ genome and mounting a productive antiviral response to this often lethal human infection.
Disclosure of Interest: None declared

Poster 117

RESPIRATORY SYNCYTIAL VIRUS SERUM NEUTRALIZING ANTIBODIES IN INFANTS TARGET THE PREFUSION FORM OF THE F PROTEIN MORE STRONGLY THAN THE G PROTEIN

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Abstract: Respiratory syncytial virus (RSV) is the most frequent cause for hospitalization in infants. Neutralizing antibodies (nAb) play a major role in the defense against RSV infection. The RSV G and F glycoproteins are responsible for virion attachment to target cells and fusion of the virion and cell membranes to initiate infection, as such they are the only targets for nAb. The F protein exists in two configurations. Prefusion F protein is the active form present on the surface of the virion and is responsible for causing membrane fusion to initiate infection. The F protein is in its postfusion form at the end of this process, and cannot return to the prefusion state. No studies have yet quantified the levels of serum antibodies to the prefusion F glycoprotein or to the attachment (G) glycoprotein, or the

relative roles of these serum antibodies in neutralizing the virus. We expressed and purified recombinant soluble versions of each of these proteins to characterize the antibodies in serum from acutely infected children ≤ 2 years of age, diagnosed with their first episode of RSV bronchiolitis. We established ELISA assays to quantify antibodies against the prefusion F, postfusion F and the G proteins. We found that serum from acutely infected infants contain at least 3-fold more IgG antibodies against the prefusion form of the F protein compared to the postfusion F and the G proteins. To test the neutralizing function of these antibodies we pre-absorbed sera with each of these soluble proteins before adding virus and testing its infectivity *in vitro*. Pre-adsorption with the prefusion F protein removed more of the serum neutralizing activity (50-55%). Unexpectedly, G-specific antibodies removed more neutralizing activity (35-40%) than postfusion F-specific antibodies (0-20%). Overall, our data indicate that the prefusion form of the F protein is the most important neutralizing RSV antigen, but that G-specific antibodies also play a significant role in RSV neutralization.
Disclosure of Interest: None declared

Poster 118
MISMATCHES IN RNA DUPLEXES PREVENT RIG-I ACTIVATION
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Abstract: RIG-I is a cytoplasmic sensor that recognizes dsRNA and triggers an antiviral response upon viral infection. Genomes of some negative strand viruses (such as Orthomyxoviruses, Arenaviruses and Bunyaviruses) can be found as panhandle structures. These panhandles are the viral promoter for transcription and replication. Additionally they have been described to be the structures sensed by RIG-I upon influenza infection. In their double-strand stem, these panhandles exhibit conserved mismatches localized in a key region for RIG-I binding on dsRNA. We therefore investigated if these mismatches may have a role in preventing RIG-I activation.
In this study, we compared the stimulatory effect of perfect and mismatched dsRNA at the different steps of RIG-I activation: RNA binding, ATPase activity, RIG-I oligomerization and IFNβ activation. We found that neither RNA binding, nor ATPase activity, nor RIG-I oligomerization were affected by the presence of mismatches. In marked contrast, IFNβ activation was strongly impaired when dsRNAs with mismatches were used. The mechanisms underlying this inability to activate RIG-I are under investigation.
In conclusion, these mismatches as found in panhandles of many segmented negative strand viruses may represent a general viral strategy to escape RIG-I sensing.
Disclosure of Interest: None declared

Poster 119
THE NUCLEOPROTEIN OF NEWLY EMERGED H7N9 INFLUENZA A VIRUS HARBORS A UNIQUE MOTIF CONFERRING RESISTANCE TO ANTIVIRAL HUMAN MXA.
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Abstract: The natural host of influenza A viruses (IAVs) are aquatic birds. Occasionally, these viruses cross the species barrier, as in early 2013 when an avian H7N9 virus infected humans in China. Since then, multiple transmissions of H7N9 viruses to humans have occurred, leaving experts puzzled about molecular causes for such efficient crossing of the species barrier compared to other avian influenza viruses. Interferon-induced Mx proteins are known restriction factors preventing influenza virus (IAV) replication. Unfortunately, some viruses (e.g., human IAV, e.g. A/PR/8/34) have developed some resistance, which is associated with specific amino acids in their nucleoproteins, the target of Mx function. We identified a surface-exposed cluster of amino acids in NP, comprising isoleucine-100, proline-283, and tyrosine-313, that is essential for reduced Mx sensitivity. Accordingly, two substitutions

in the NP of PR/8 [PR/8(mut)] to the Mx-sensitive amino acids (P283L and Y313F) led to attenuation in Mx1-positive mice. Serial lung passages of PR/8(mut) in Mx1 mice resulted in a single exchange of tyrosine to asparagine at position 52 in NP (in close proximity to the amino acid cluster at positions 100, 283, and 313), which partially compensates loss of Mx resistance in PR/8(mut). Intriguingly, the NP of the newly emerged avian-origin H7N9 virus also contains an asparagine at position 52 and shows reduced Mx sensitivity. N52Y substitution in NP results in increased sensitivity of the H7N9 virus to human Mx, indicating that this residue is a determinant of Mx resistance in mammals. Our data strengthen the hypothesis that the human Mx protein represents a potent barrier against zoonotic transmission of avian influenza viruses. However, the H7N9 viruses overcome this restriction by harboring an NP that is less sensitive to Mx-mediated host defense. This might contribute to zoonotic transmission of H7N9 and to the severe to fatal outcome of H7N9 infections in humans.
Disclosure of Interest: None declared

Poster 120

NSS PROTEIN OF SFTS VIRUS SUPPRESSES INTERFERON PRODUCTION THROUGH DIFFERENT WAYS FROM RIFT VALLEY FEVER VIRUS

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Abstract: SFTS virus (SFTSV) is a newly identified Phlebovirus that causes severe fever with thrombocytopenia syndrome in humans. A better understanding of the factors that govern SFTSV virulence and pathogenicity is urgently required for antiviral therapies and safe vaccines. Recognition of RNA viral pathogens by the retinoic acid-inducible gene I (RIG-I)-like receptor (RLR) family results in the activation of type I interferon (IFN) response. To avoid this response, viruses have evolved strategies that target different essential steps in the activation of host innate immunity. Here we demonstrated that SFTSV nonstructural protein NSs functioned as the interferon (IFN) antagonist mainly by suppressing TBK1/IKKε−IRF3 signaling pathway. SFTSV NSs interacted with and relocalized TANK-binding kinase1 (TBK1) into NSs-induced cytoplasmic structures, and this interaction could effectively inhibit downstream phosphorylation and dimerization of IRF3, resulting in the suppression of antiviral signaling and IFN induction. Functional sites of SFTSV NSs binding with TBK1 were then studied and results showed that NSs had lost their IFN-inhibiting activity after deleting the 25 amino acids in N-terminal. Furthermore, the mechanism of Rift Valley fever virus (RVFV) NSs blocking IFN-β response was also investigated. RVFV NSs protein could neither interact with nor co-localized with TBK1 in cytoplasm, but suppressed the expression levels and then phosphorylation and dimerization of IRF3 in the subsequent steps, finally inhibited the production of IFN-β. Altogether, our data demonstrated the probable mechanism used by SFTSV to inhibit IFN responses which was different from RVFV and point toward a novel mechanism for RVFV suppressing IFN responses.
Disclosure of Interest: None declared

Poster 121

GENERATION OF RECOMBINANT SCHMALLENBERG VIRUS NUCLEOCAPSID PROTEIN AND ITS APPLICATION IN SEROLOGICAL TESTS

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Abstract: In 2011, an unidentified disease in cattle was first reported in Germany in a farm near the town of Schmallenberg (Hoffmann et al., 2012). Metagenomic analysis identified a novel *Orthobunyavirus*, which subsequently was isolated from blood specimens of infected animals. This new virus was called Schmallenberg virus (SBV) after the place of origin of the collected samples. Clinical symptoms of diseased cows include fever, reduced milk yield and diarrhoea. Also, SBV infection has been implicated in many cases of severely malformed bovine and ovine offspring.
To develop improved reagents for SBV serology, a high-level yeast expression system was employed to produce recombinant SBV nucleocapsid (N) protein. Recombinant SBV N protein was investigated as an antigen in SBV-specific IgG enzyme immunoassay and used for generation of monoclonal

antibodies (MAbs). Yeast-expressed SBV N protein was reactive with anti-SBV IgG-positive cow serum specimens collected from different farms of Lithuania. After immunization of mice with recombinant SBV N protein, four MAbs were generated. The MAbs raised against recombinant SBV N protein reacted with native viral nucleocapsids in SBV-infected BHK cells by immunofluorescence assay. The reactivity of recombinant N protein with SBV-positive cow serum specimens and the ability of the MAbs to recognize virus-infected cells confirm the antigenic similarity between yeast-expressed SBV N protein and native viral nucleocapsids. Our study demonstrates that yeast expression system is suitable for high-level production of recombinant SBV N protein and provides the first evidence on the presence of SBV-specific antibodies in cow serum specimens collected in Lithuania.
Disclosure of Interest: None declared

Poster 122
LLOV VP24 AND VP35 PROTEINS FUNCTION AS INNATE IMMUNE ANTAGONISTS IN BAT AND HUMAN CELLS
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Abstract: Cuevavirus, a new genus within the *Filoviridae* family, consists of a single species *Lloviu cuevavirus*. The Lloviu virus (LLOV) was discovered in dead *Miniopterus schreibersii* insectivorous bats in Spain. Live LLOV was not isolated from the bat carcasses. However, virus sequence detected in several tissue samples permitted amplification of the complete genome, minus an estimated 700 nucleotides at the extreme 5' end, by PCR. Open reading frames (ORFs) encoding the seven common filoviral proteins (GP, NP, VP24, VP30, VP35, VP40, and L) were present in the LLOV genome. Although similar to Ebola viruses (EBOV) and Marburg viruses (MARV), whether LLOV can infect humans or cause human disease is not known. Therefore, we sought to determine how LLOV proteins function in human versus bat cells. Specifically, whether innate immune evasion functions carried out by the EBOV VP35 and VP24 proteins and MARV VP35 and VP40 proteins are employed by LLOV proteins, as these functions are thought to contribute to viral pathogenesis. Using interferon (IFN)-β and IFN-stimulated gene 54 (ISG54) promoter gene assays, we demonstrate that the LLOV VP35 and LLOV VP24 proteins inhibit IFN production and signaling, respectively, in both human and bat cells. Subsequent analysis revealed LLOV VP35 to be multi-functional, inhibiting virus-induced phosphorylation of IFN regulatory factor 3 (IRF3), in human and bat cells, and dsRNA-dependent protein kinase R (PKR) in human cells. Additionally, we show that LLOV VP24 inhibits IFN signaling by preventing the nuclear accumulation of STAT1 in human and bat cells, as determined by an immunofluoresence assay, and that LLOV VP24 can compete with tyrosine phosphorylated STAT1 for binding to karyopherin alpha 5 (KPNA5). Taken together, the data suggests that LLOV VP35 and VP24 function in a similar manner to EBOV VP35 and VP24 proteins as innate immune antagonists.
Disclosure of Interest: None declared

Poster 123
DISTINCT REGIONS OF THE NIV C PROTEIN ARE INVOLVED IN SUPPRESSING VIRAL REPLICATION AND THE HOST IFN-B RESPONSE
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Abstract: Nipah virus (NiV) is a highly pathogenic paramyxovirus, and continues to cause annual outbreaks of fatal encephalitis in humans. The C protein of NiV has been shown to inhibit host responses by way of reducing early virus replication. Protein alignments of paramyxovirus C proteins indicate several conserved residues shared across henipaviruses and morbilliviruses. We generated recombinant NiVs containing several mutations of these conserved residues, and show that at least two distinct regions of the NiV C protein contribute to suppressing host IFN-β responses in primary cells.
Disclosure of Interest: None declared

Poster 124

IDENTIFICATION OF CELLULAR FACTORS INFLUENCING INFLUENZA A VIRUS REPLICATION IN PRIMARY HUMAN RESPIRATORY CELLS

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Abstract: Influenza A virus (IAV) is a high priority NIAID pathogen that can cause seasonal epidemic infections every year, and pandemic infections, which occur every 10 to 50 years. Seasonal influenza results in up to 35,000 (US) and 500,000 (worldwide) deaths annually. Large-scale siRNA screens of IAV infection have identified many host proteins that participate in virus replication, such as MXA, IFITM3 and GBF1. However, the majority of these screens were performed using a single strain of IAV, H1N1 to infect cancer cell lines in a single cycle of viral replication. In our study, we hypothesize that strains of IAV with different levels of virulence interact with the host in a strain-specific fashion. Therefore, we aim to identify cellular factors that influence viral replication and are common to or exclusive of three different IAV strains (H1N1, H2N3 and H5N1) displaying increasing degrees of virulence. For that, we have selected a set of ~ 1000 interferon stimulated genes and optimized the siRNA screen conditions using H5N1 to infect primary human respiratory cells. The screen was optimized to detect virus spreading, and therefore, proteins that act on early and later steps of replication were identified. As a result, we validated thirty proteins affecting viral replication in early stages and ten additional factors affecting the budding and spreading of the virus. Our future steps include the further mapping of the genes to the viral life cycle.

Disclosure of Interest: None declared

Poster 145

VIRO-INDUCED-COMPARTIMENTALIZATION : THE CASE OF NEGRI BODIES FORMED IN RABIES VIRUS INFECTED CELLS

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Abstract: Replication and assembly of many non-related viruses occur in specialized intracellular compartments known as viral factories. These neo-organelles concentrate viral proteins and nucleic acids to facilitate viral replication, and might prevent the activation of cell-intrinsic defenses. Rabies virus (RABV) which belongs to the *Rhabdoviridae f*amily, induces the formation of spherical cytoplasmic inclusions, termed Negri bodies (NBs). NBs, are made of N and P proteins, and contain also the polymerase L and the chaperon HSP70. At early time post-infection, there is a limited number of NBs per cell (1 or 2) which grow with time (1-3 µm diameter), then smaller inclusions are detected at later time. We have previously shown that NBs contain all viral RNAs species and are sites of viral transcription and replication (Lahaye et al. 2009). By electron-microscopy, NBs appear to be associated with membranes most probably derived from the endoplasmic reticulum and viral particles have been detected around NBs suggesting that NBs could also constitute viral assembly sites. These results all together provide evidence that NBs are viral factories. To analyse the morphogenesis and trafficking of NBs, we have performed live cell imaging by using recombinant fluorescent viruses. We have highlighted some events of fragmentation that could be the source of smaller structures and some fusions of CNs that may explain the increase in size of the inclusions during infection. Whereas NBs are poorly dynamic, very small dynamic structures (less than 200nm) are ejected from NBs. They could correspond to newly synthesized nucleocapsids. Their movement is independent of actin microfilaments but depends of the microtubule network. We propose that the nucleocapsids are derived from early NBs and are rapidly transported along microtubules throughout the cell. They can either reach the plasma membrane and form new viral particles or be used as templates for the formation of late viral factories.

Disclosure of Interest: None declared

Poster 146

DISSECTING CANINE DISTEMPER VIRUS PATHOLOGY AND PATHOGENESIS AT THE LIGHT OF NATURALLY-OCCURRING HEMAGGLUTININ FUNCTIONAL CHANGES

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Abstract: Starting in mid-2009 a major canine distemper (CD) epidemic was observed in Swiss wildlife. Although Switzerland was not new to CD outbreaks, the current epidemic was standing out because of the high morbidity and mortality, the number of species affected and the extension of the affected areas of the country. A thorough pathological and virological investigation highlighted unusual features of the disease with prominent polioencephalitis, differently from the classic leucoencephalitis and a novel molecular signature of the Swiss strains consistent with higher cell surface expression and SLAM binding efficiencies than well-characterized demyelinating CD virus (CDV) strains such as A75/17. Furthermore a family pet dog with unambiguous vaccination history developed the disease with similar features to those observed in wild carnivores including the red fox (*Vulpes vulpes*), the Eurasian badger (*Meles meles*) other mustelids (*Martes* sp) and for the first time the Eurasian lynx (*Lynx lynx*). Interesting, following the major outbreaks we observed CDV infection in unusual hosts including rodents and a domestic cat. In order to better investigate the possible molecular basis of such an apparent plastic virus we dissected the naturally occurring amino acid (aa) changes of closely related, but distinct CDV strains in the attachment protein (H). The results showed that most of the naturally occurring changes have a minimal functional impact on the CDV H, however few of them may have profound functional consequences. Strikingly, these severely functional impacting aa changes occur together with other of compensatory nature. The final outcome is the emergence of distinct Hs with overlapping functional parameters. This compensatory system might partially explain the ability of CDV to escape the immune system pressure of the host and to adapt to new hosts, while maintaining an overall constant fitness.

Disclosure of Interest: None declared

Poster 147

UBR BOX N-RECOGNIN-4 (UBR4) IS A HUMAN HOST FACTOR REQUIRED BY MAMMALIAN INFLUENZA A VIRUSES DURING LATE STAGES OF THE VIRAL LIFE CYCLE.

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Abstract: As intracellular pathogen, influenza A virus (IAV) is dependent on a large set of cellular factors to complete its life cycle. Recently, several studies have been performed to identify host proteins required by IAV. Surprisingly, the overlap of host factors between the different screens was lower than anticipated. Likely, this is the result of variations in experimental procedure and selection methods of the individual studies. We revisited the raw data of four different published siRNA screens and identified by computational analysis an additional set of cellular proteins that are required by IAV. Amongst others, UBR box N-recognin-4 (UBR4), a member of the N-recognin family, was a promising candidate which we chose for follow-up studies. UBR4 was identified as an interaction partner of the viral ion channel protein M2. Furthermore, siRNA-mediated knockdown of UBR4 protein levels reduced growth of the IAV strain A/WSN/33 in a lung epithelial cell line. Notably, depletion of UBR4 in mouse lungs using peptide-phosphorodiamidate morpholino oligonucleotides (PPMOs) impaired IAV growth *in vivo* resulting in significantly reduced virus titers in lung homogenates compared to control. Further

experiments suggested that UBR4 is required during a late stage of the viral life cycle as nucleoprotein (NP) expression or the growth of a single cycle reporter virus remained unaffected by UBR4 knockdown. Besides A/WSN/33, an H1N1 virus, the H3N2 virus strains A/Udorn/72 and A/HongKong/68 were shown to be sensitive to UBR4 depletion. Interestingly, avian influenza strains appear to be less dependent on UBR4 expression. These data indicate that the requirement for UBR4 could be a prerequisite for adaptation of influenza viruses to the mammalian host.
Disclosure of Interest: None declared

Poster 148

RANDOM MUTAGENESIS OF INFLUENZA A PB2 RESIDUES 701 AND 702 EXHIBIT HETEROGENEOUS TEMPERATURE SENSITIVITY AND VIRAL GROWTH IN DIFFERENT HOST SYSTEMS

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Abstract: Influenza A viruses are transmissible through different host species. Emergences of novel influenza A infections in humans are of serious public health concerns.. Polymerase basic protein 2 (PB2) is believed to play a crucial role to cross species barrier. Several comprehensive studies about mutations in PB2 (E627K, D701N, K702R, etc.) have been shown to enhance the growth rate of avian influenza viruses (H7N9 and H5N1) in mammalian system. However, characterization of mutations other than asparagine at position 701 and arginine at position 702 are yet to be investigated. A system of random mutagenesis targeting PB2 residues 701 and 702 were established to generate the recombinant viruses in human embryonic kidney 293T cells, then passaged in Madin-Darby canine kidney (MDCK) cells or embryonated chicken eggs to study the viability of rescued viruses in different host systems. Total 31 isolated viruses (including wild type, A/Puerto Rico/8/1934) were then performed a screening by viral polymerase activity assay in human (293T) or avian (chicken fibroblast DF-1) cells. Heterogeneous temperature sensitivity was observed in both mammalian and avian systems for various mutants. The polymerase activity of mutant 701D702G in DF-1 cells was substantially higher than that of 293T cells. Furthermore, the growth kinetics of mutants in MDCK cells and DF-1 cells were affected by altering residues 701 and 702, implicating that the mutations can determine the virulence in different hosts. Structural predictions by Geno3D showed positive correlation between surface charge distributions and polymerase activity in 293T cells (Pearson r, p<0.0001). In this study, a panel of PB2-701/702 mutants was developed for the investigations of viral replication kinetics, host specificity and the plasticity of PB2.
Disclosure of Interest: None declared

Poster 149

CHARACTERIZATION OF THE HEMAGGLUTININ OF BAT-DERIVED INFLUENZA VIRUSES

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Abstract: Recently, influenza virus-like RNA genomes were detected from bats in Central and South America. Nucleotide sequences of hemagglutinin (HA) and neuraminidase (NA) gene segments of the viruses were phylogenically distinct from those of previously known influenza A virus subtypes (i.e., H1-H16 and N1-N9), and thus these newly found bat-derived influenza viruses were proposed as H17N10 and H18N11 subtypes. While influenza A viruses are one of the important zoonotic pathogens causing high morbidity disease in humans, pigs, houses, poultry, and so on, biological properties of these novel bat-derived influenza viruses are largely unknown since infectious viruses have not been isolated yet.

To gain insight into the mechanisms underlying entry of the virus into cells, we generated vesicular stomatitis viruses (VSVs) pseudotyped with the bat influenza virus glycoproteins, HA and NA, and screened several mammalian cell lines including bat-derived cells. We found that VSVs pseudotyped with H17 and H18 HAs efficiently infected particular bat cell lines but not MDCK (canine), Vero E6 (monkey), and HEK293 (human) cells, all of which are commonly used for influenza A virus propagation. Chemical modification of the susceptible bat cells with several enzymes and inhibitors revealed that cellular entry of bat-derived influenza virus HAs required the endosomal acidification for membrane fusion and that they did not utilized sialic acids which are known to serve as receptors of the other known influenza viruses. Our data suggests the limited potential of bat-derived influenza viruses to infect other mammalians including humans.
Disclosure of Interest: None declared

Poster 150
HOST SIGNALING PATHWAYS INVOLVED IN ANTIBODY-DEPENDENT ENHANCEMENT OF EBOLA VIRUS INFECTION
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Abstract: Antibody-dependent enhancement (ADE), a phenomenon in which viral infectivity is increased by virus-specific antibodies, is observed *in vitro* for a large number of viruses. ADE of Ebola virus infection has been shown to depend on the cross-linking of virus-antibody or virus-antibody-complement complexes to cell surface Fc or complement receptors, leading to enhanced infection of susceptible cells. These receptors are known to activate various signaling pathways that lead to the reorganization of the actin cytoskeleton and membrane remodeling. In this study, we examined whether the Fc receptor-dependent ADE entry of Ebola virus requires host cell signaling. ADE-dependent infectivity of vesicular stomatitis virus pseudotyped with Ebola virus glycoprotein (VSV-EBOV GP) was first compared using Jurkat cells transduced with cDNA encoding full-length Fc gamma receptor IIa (FcγRIIa) or mutant FcγRIIa lacking its cytoplasmic tail (FcγRIIaΔCT). We found that infectivity of VSV-EBOV GP was significantly enhanced in cells expressing full-length FcγRIIa in the presence of an ADE antibody, whereas the enhancement of infectivity was limited in FcγRIIaΔCT-expressing cells. To investigate possible signaling pathways downstream of FcγRIIa in the ADE entry of EBOV, ADE-dependent and -independent infectivities of VSV-EBOV GP were compared using K562 cells naturally expressing FcγRIIa in the presence of several inhibitors of FcγRIIa-mediated signaling pathways. We found that the ADE-dependent infection was exclusively reduced by R788 and PP2, inhibitors of Syk or Src family PTKs. These results suggest that the FcγRIIa-mediated signaling pathways are important for ADE of Ebola virus infection.
Disclosure of Interest: None declared

Poster 151
COLOR-FLU: MULTI-SPECTRAL FLUORESCENT REPORTER INFLUENZA VIRUSES AS POWERFUL TOOLS FOR IN VIVO IMAGING ANALYSIS
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Abstract: Influenza A virus is one of the main inhaled pathogens that causes acute inflammation in the respiratory tract. In particular, highly pathogenic avian H5N1 viruses cause severe infections in humans, often with fatal outcomes. Even though many studies have addressed the pathogenicity of influenza viruses, the mechanism of influenza pathogenesis remains unclear. In this study, we generate influenza viruses expressing multi-spectral fluorescent proteins (Color-flu viruses) to study influenza virus infection in *in vivo* models. We show that Color-flu viruses stably and highly express the fluorescent

proteins in infected animals and are useful for various types of microscopic and flow cytometric analyses.
The lung macrophage is thought to play a pivotal role in inducing innate immune responses to influenza virus infection yet it also triggers lethal damage to lung tissue. To analyze the kinetics of lung macrophages, we developed time-lapse imaging analysis of mouse lungs infected with Color-flu viruses, and investigated the dynamic interaction between macrophages and virus-infected cells. We also analyzed the gene expression profiles of sorted macrophages from the lungs of Color-flu virus-infected mice by using a DNA microarray. The microarray analysis revealed high-level expression of type-I interferons by Color-flu-positive macrophages.
Our data show that Color-flu viruses are powerful tools to analyze influenza virus infections *in vivo* to better understand influenza pathogenesis and the host immune system.
Disclosure of Interest: None declared

Poster 152
IDENTIFICATION OF HOST FACTORS INVOLVED IN INFLUENZA VIRUS REPLICATION
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Abstract: Viruses rely on host cellular functions for their replication cycle. Genome-wide screens are increasingly used to identify host factors and pathways that are involved in virus replication. Although several such screens have been conducted for influenza viruses, very few validation or mechanistic follow-up studies have been performed. We therefore conducted a systematic study to elucidate physical and functional host-viral interactions in influenza virus-infected cells. We identified 1,292 host proteins that co-precipitated with influenza virus proteins, 323 of which markedly affected virus replication as assessed in an RNAi-based assay. We performed systematic mechanistic studies for 91 selected host factors to identify the step(s) in the viral life cycle affected by each host factor. Such information would be useful to gain further insights into the mechanisms of influenza virus replication. Moreover, the testing of compounds that inhibit the functions of the host targets identified in our study revealed several drugs that significantly reduced influenza virus replication *in vitro*. Thus, virus-host interactome screens are powerful strategies to identify target host factors and guide antiviral drug development.
Disclosure of Interest: None declared

Poster 153
OPENING THE CAGE ON INFLUENZA REPLICATION IN HUMAN MACROPHAGES
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Abstract: Macrophages are white blood cells, part of the innate immune system, recruited in response to an infection or damage within the body. The role of these cells is to survey for any possible threats, mediate the host response and engulf invading pathogens. Seasonal influenza infection, one such pathogen, is a major contributor to global morbidity and mortality from SARI (Severe acute respiratory infection). The potential for the seasonal virus to transform, resulting in highly pathogenic strains from a small number of mutation events is a serious, on going concern. The 2009 pandemic, for example, which caused relatively mild disease led to an estimated 285,000 deaths worldwide with the burden of mortality falling heavily on previously healthy individuals.

Influenza requires host transcription machinery to replicate its genome and produce functional mRNA. Capped Analysis of Gene Expression (CAGE) was used to analyse mRNA from influenza-infected

macrophages from multiple donors at 4 time-points over the course of infection. This uniquely sensitive technique allowed for observation of flu mRNA production, giving insights into the progression of influenza replication in these immune cells.

Most interestingly, this technique allowed us to observe the so-called 'cap snatched leader sequences' - host mRNA sequences, stolen by flu to facilitate mRNA production in the host cell - unveiling some interesting results, including an apparent bias toward particular RNA sequences.

Our results hint at a possible novel mechanism for replication of influenza in mammalian immune cells.
Disclosure of Interest: None declared

Poster 154
BST-2/TETHERIN IS A DETERMINANT OF ORTHOBUNYAVIRUS HOST RANGE
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Abstract: Several recently emerging viruses belong to the *Bunyaviridae*, the largest family of RNA viruses, including causative agents of significant diseases of humans, livestock and crops. Within this family, the genus *Orthobunyavirus* includes more than 170 named viruses of medical and veterinary importance. Different orthobunyaviruses infect different host species but there is a very limited understanding on what determines the host species range of these pathogens. In this study we discovered that BST-2/tetherin, an interferon stimulated gene constitutively expressed in some cell types, restricts orthobunyavirus replication in a host specific manner and thus contributes to determining virus host range. In particular, the human orthologue of BST-2/tetherin restricts orthobunyaviruses with ruminant tropism but not human viruses while the converse is observed with the ruminant BST-2/tetherin orthologues. In addition, we show that BST-2/tetherin blocks replication of orthobunyaviruses by reducing envelope incorporation in viral particles egressing from infected cells and this mechanism of action is species-specific. This work provides new insight into the variables that determine orthobunyavirus host range and will help us to dissect the adaptive changes that these viruses require to cross the species barrier and emerge into new species.
Disclosure of Interest: None declared

Poster 155
EXPLOITING THE REVERSE GENETIC SYSTEM FOR SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME VIRUS TO STUDY NSS FUNCTION
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Abstract: Severe fever with thrombocytopenia syndrome virus (SFTSV) is an emerging tick-borne pathogen that was first reported in China in 2009. Phylogenetic analysis of the viral genome showed that SFTS virus represents a new lineage within the *Phlebovirus* genus, distinct from the existing sandfly fever and Uukuniemi virus groups, in the family *Bunyaviridae*. SFTS disease is characterized by gastrointestinal symptoms, chills, joint pain, myalgia, thrombocytopenia, leukocytopenia, and some hemorrhagic manifestations with a case fatality rate of about 2 to 15%. Recently we reported the development of reverse genetics systems to study STFSV replication and pathogenesis. We developed and optimized functional T7 polymerase-based M- and S-segment minigenome assays, which revealed errors in the published terminal sequences of the S segment of the Hubei 29 strain of SFTSV. We then generated recombinant viruses from cloned cDNAs prepared to the antigenomic RNAs both of the minimally passaged virus (HB29) and of a cell culture-adapted strain designated HB29pp. The growth

properties, pattern of viral protein synthesis, and subcellular localization of viral N and NSs proteins of wild-type HB29pp (wtHB29pp) and recombinant HB29pp viruses were indistinguishable. We also show that the viruses fail to shut off host cell polypeptide production. Transient transfection and wild-type infections report that SFTSV NSs antagonizes the IFN response in unique manner among phleboviruses. Utilizing the reverse genetics system, we have attempted to generate NSs-deletant viruses and assess the effect on host innate immunity and viral replication. The robust reverse genetics system described is a valuable tool for the design of therapeutics and the development of killed and attenuated vaccines against this important emerging pathogen.
Disclosure of Interest: None declared

Poster 156

COMPARATIVE RESPIRATORY SYNCYTIAL VIRUS INFECTION OF HUMAN UPPER AND LOWER RESPIRATORY TRACT AIRWAY EPITHELIUM

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Abstract: Respiratory syncytial virus (RSV) is a major pathogen that primarily infects airway epithelium. Most infants suffer mild upper respiratory tract (URT) symptoms, while approximately one third progress to lower respiratory tract (LRT) involvement. However, little is known about relative RSV cytopathogenesis in infant URT versus LRT.
To address this, we generated well-differentiated nasal (WD-PNEC) and bronchial (WD-PNEC) epithelial cell cultures from the same individuals and infected them with a recent RSV clinical isolate. RSV tropism, infectivity, cytopathology, growth kinetics, cell sloughing, apoptosis, and cytokine/chemokine responses were determined.
RSV infection in both cultures was restricted to apical ciliated cells and occasional non-ciliated cells, but not goblet cells, without causing gross cytopathology. Infection resulted in apical release of progeny virus, increased apical cell sloughing, apoptosis and occasional syncytia. RSV growth kinetics and peak titers were higher in WD-PBECs, coincident with higher ciliated cell contents, cell sloughing and slightly compromised tight junctions. However, pro-inflammatory chemokine and lambda interferon responses were similar for both cultures.
RSV induced remarkably similar cytopathogenesis and pro-inflammatory responses in WD-PNECs and WD-PBECs that reproduce many hallmarks of RSV pathogenesis in infants. WD-PNECs may provide an authentic surrogate model with which to study RSV cytopathogenesis in infant airway epithelium.
Disclosure of Interest: None declared

Poster 157

INFECTION OF EPITHELIAL CELLS IS REQUIRED FOR EFFICIENT MORBILLIVIRUS TRANSMISSION

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Abstract: The importance of viral receptors in morbillivirus transmission from infected to naïve hosts is not fully understood. To gain insights in the roles of the immune cell receptor signaling lymphocyte activation molecule (SLAM) and the epithelial cell receptor nectin4, we performed canine distemper virus (CDV) transmission studies with wild type or receptor blind viruses by co-housing infected and naïve ferrets. Wild type virus was transmitted efficiently to naïve contacts when animals were co-housed as early as 3 days after initial infection. Transmission was most effective starting 6-7 days after inoculation, immediately prior the onset of clinical signs. Contact animals succumbed to disease approximately one week later than infected animals, indicating that infection via natural transmission followed the same kinetics as seen after intranasal inoculation. To investigate the importance of receptor usage for transmission, animals were infected with the respective receptor blind viruses and

co-housed with naïve animals after 3 days. Nectin4-blind virus was only transmitted between one infected/contact pair, while none of the other contact animals became infected. The single transmission event occurred between days 17 and 21 after infection, and no signs of disease were observed in the infected or the contact animal, indicating that the virus did not acquire compensating mutations. In the SLAM-blind group, only one of the infected animals developed a low-level, transient viremia on day 7 post-infection, and none of the naïve contacts was infected. We have thus formally demonstrated that efficient transmission to naïve contacts coincides with the onset of clinical signs in the infected individual, and is dependent on nectin4-mediated infection of epithelial tissues.
Disclosure of Interest: None declared

Poster 158

ANTIBODY-INDUCED INTERNALIZATION OF RSV F PROTEIN EXPRESSED ON THE SURFACE OF INFECTED CELLS AND CELLS EXPRESSING A RECOMBINANT PROTEIN

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Abstract: Similar to several other viruses, RSV expresses viral proteins on the surface of infected cells. Previous studies have shown that upon binding of polyclonal RSV-specific antibodies to RSV antigens expressed on the surface of infected HEp-2 cells, internalization may occur of these RSV antigen-antibody complexes.
For further elucidation of the viral protein(s) involved in these processes and confirmation of the mechanism of internalization, RSV F transfected cells were used. Transfected cells were incubated with polyclonal RSV-specific antibodies at 37°C during different times, followed by fixation, permeabilization, staining with secondary labeled antibodies and analysis of the cells by confocal fluorescence microscopy. Addition of polyclonal RSV-specific antibodies resulted in a clear uptake of viral protein-antibody complexes in a time-dependent manner. The process was rapid and stagnated after 60 minutes. By using RSV-infected cells and F-specific monoclonal antibodies, the involvement of the RSV F protein in this internalization process was further confirmed in the context of RSV infection. Also a clear reduction in surface expression was observed. Pre- and post-fusion specific antibodies were used to determine whether this process is epitope-dependent. Also Palivizumab has the potential to induce internalization of RSV F proteins. Experiments with specific endocytic inhibitors and co-transfection with dominant negative proteins showed that antibody-induced RSV F internalization is clathrin-dependent. Immunofluorescence experiments with infected HEp-2 cells indicate recycling of RSV F proteins from endosomal compartments to the surface after antibody-induced internalization.
It remains to be determined if this process affects the progress of infection and clearance of RSV F specific antibodies. Furthermore, experiments are ongoing to analyze whether internalization of RSV F-antibody complexes interferes with antibody-dependent complement-mediated lysis.
Disclosure of Interest: None declared

Poster 159

ANTIBODIES DIRECTED TOWARDS NEURAMINIDASE CONTROL DISEASE IN A MOUSE-MODEL OF INFLUENZA

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Abstract: Influenza A virus (IAV) carries two major surface glycoproteins; the hemagglutinin (HA) and the neuraminidase (NA), both important for successful virus entry and release from cells. The immune response towards NA, in comparison to HA, is far less studied. We aimed to isolate monoclonal antibodies (mAb) with antiviral activity against the N1 subtype of IAV to gain a greater understanding of the Ab response to IAV NA. Mouse hybridomas were generated expressing mAb directed towards N1 NAs. One set of mAbs, including 7D3, could all bind to recombinant NA derived from H1N1 viruses; A/USSR/1977, A/Singapore/1986, A/New Caledonia/1999, A/Brisbane/2007 and A/Belgium/2009 (Bel/09), but were unable to inhibit NA activity. In contrast, mAb CD4 was able to (i) bind NA, (ii) inhibit the NA activity, and (iii) reduce plaque size formation of both Bel/09 and NIBRG-14 (a 6:2 reverse

genetic H5N1 virus expressing the HA and NA of A/Vietnam/1194/2004). mAb 7D3 and CD4 were selected for testing their ability to protect against disease in a mouse model of influenza virus infection. Administration of mAb 7D3 did not alter morbidity and mortality of mice infected with lethal doses of any N1 IAV tested. Mice treated with mAb CD4 however, were protected from a lethal infection with Bel/09 and NIBRG-14. Interestingly, mAb CD4 significantly reduced lung-viral loads in mice infected with Bel/09 but not NIBRG-14. These results suggest that anti-NA Abs that have NA inhibitory activity can control IAV infection.
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Disclosure of Interest: None declared

Poster 160

QUANTITATIVE PROTEOMIC ANALYSIS OF PROTEIN SIGNATURES RELATED TO PERMISSIVE VS. NON-PERMISSIVE INFLUENZA A VIRUS INFECTIONS IN HUMAN HOST CELLS

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Abstract: Human and most avian influenza A virus (IAV) strains differ largely in their replication efficiency in and activation of human cells despite successful cell entry. We hypothesize that the distinct outcome of an IAV infection with a given virus strain is determined by the differential interplay between specific host and viral factors, which remain to be defined in their entity.
By using mass spectrometry-based quantitative proteomics we aim to characterize the sets of cellular and viral factors whose abundance is specifically up- or down-regulated in permissive vs. non-permissive IAV infection, respectively. Our analysis involves a “Spike-in SILAC” approach in human A549 cells that are highly permissive for seasonal H3N2 IAV, but restrict replication of avian H3N2 IAV. This approach allows the quantitative comparison of strain-specific changes in the host cell proteome.
We quantified about 5500 proteins and identified distinct sets of cellular factors influenced by infections with the human or the avian strain, respectively. Most proteins were regulated in a similar way for both virus strains, but also candidates with distinct changes in permissive and non-permissive infection were found. Rapid apoptotic cell death was ruled out as restriction factor for avian IAV replication in human cells. Ongoing studies involve the validation of differentially regulated proteins and bioinformatic as well as functional analyses to elucidate their roles in IAV infection and their potential contributions to IAV adaptation to human cells. In conclusion, we will present initial results of a comprehensive analysis of complex host pathogen interaction networks by using systems biology tools. We expect to identify key parameters related to efficient IAV replication and host specificity and thereby to further our understanding of IAV biology.
Disclosure of Interest: None declared

Poster 162

MUTATION OF SERPINE1 IN MICE INCREASED SUSCEPTIBILITY TO INFLUENZA A VIRUS INFECTION DUE TO INCREASED VASCULAR LEAKAGE AND VIRUS DISSEMINATION

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Abstract: Influenza A viruses (IAV) cause a contagious respiratory disease in human and animals. Besides intensive studies on the virus and its virulence factors, the biological mechanisms of the host-pathogen interactions are still only partially understood. Recent studies showed that IAV alter the plasminogen conversion pathway after infection, which induces hyperfibrinolysis and results in lung injury. *Serpine1* (PAI-1) is a member of the serine protease inhibitor (serpine) family that inhibits plasminogen activators, which reduce the fibrosis in lung. Deficiency of SERPINE1 can lead to bleeding due to unstable hemostatic plug formation. Here, we studied virus dissemination and host susceptibility to IAV infection in *Serpine1*$^{-/-}$ mutant mice. Our results showed that *Serpine1*$^{-/-}$ mutant mice were highly susceptible to A/Puerto Rico/8/34 (PR8, H1N1) infections compared to wild type mice. Knock-out

animals exhibited increased body weight loss, enhanced lethality and higher viral loads on day 5 post infection (p.i.). We also infected mice with A/WSN/33 (WSN), which carrying a neuraminidase that binds and converts plasminogen into plasmin and enhances viral hemagglutinin cleavage. The knock-out mice lost more body weight and showed higher mortality compared to wild type mice although virus titers were similar in WSN infection. Furthermore, red blood cells leaked into the alveolar space in *Serpine1*$^{-/-}$ mutant mice after infection with both PR8 and WSN which was associated with increased vascular permeability. Finally, we detected viral RNA in kidneys of infected *Serpine1*$^{-/-}$ mice indicating viral dissemination from the lung into the blood stream. In summary, our results suggest that due to the loss of PAI-1, PR8 can replicate to higher titers and cause a more severe outcome. WSN by itself can convert plasminogen into plasmin which results in an even more severe phenotype than observed by PR8. Thus, the *Serpine1* gene may plays an important role in lung injury and viral dissemination.
Disclosure of Interest: None declared

Poster 163

STREPTOCOCCUS SUIS AFFECTS THE REPLICATION OF SWINE INFLUENZA VIRUS IN PORCINE TRACHEAL CELLS

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Abstract: Swine influenza viruses (SIV) are important pathogens affecting pigs of all ages. Secondary infection by *Streptococcus suis* may enhance the severity of disease in piglets infected by SIV resulting in substantial economic losses. To date, the molecular basis of the interplay between SIV and *S. suis* still remains unclear. In order to understand the interaction between SIV and *S. suis*, we established an *in vitro* co-infection model based on newborn pig trachea cells (NPTr). Two SIV variants A/sw/Bad Griesbach/IDT5604/2006 H1N1 and A/sw/Herford/IDT5932/2007 H3N2 were used to compare subtype differences. Our previous studies showed that the H3N2 strain had a higher replication rate and induced a stronger ciliostatic effect in pig precision-cut lung slices compared to H1N1 strain. Wild type *S. suis* serotype 2 strain 10 (wt) and an noncapsulated mutant strain (Δcps) were used as secondary infectious agents in this study. NPTr cells were first inoculated with SIV, followed by bacterial inoculation. The course of infection was monitored by immunofluorescence microscopy and by determining the virus titers at different time points. Our results show that the viral hemagglutinin expressed on the surface of virus-infected cells interacted with α2,6-linked sialic acids of the capsular polysaccharide and thus enhanced the binding of *S. suis* and facilitated bacterial infection. On the other hand, the release of H1N1 and H3N2 SIV were delayed when NPTr cells were co-infected with *S. suis*. Depending on the affinity to sialic acid, gradual differences in the interaction between SIV and *S. suis* were detected. Moreover, most of the wt bacteria adhered to SIV infected cells. These findings indicated that *S. suis* and SIV affect each other in the infectious behavior in swine respiratory epithelial cell. Furthermore, this interaction is mediated by hemagglutinin of influenza viruses that recognizes α2,6-linked sialic acid on the capsular polysaccharide of *S. suis.*
Disclosure of Interest: None declared

Poster 164

THE EFFECT OF STREPTOCOCCUS SUIS CO-INFECTION ON THE INFECTION OF WELL-DIFFERENTIATED PORCINE RESPIRATORY EPITHELIAL CELLS BY SWINE INFLUENZA VIRUSES

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Abstract: Respiratory diseases in swine are responsible for high economic losses in pig industry worldwide. A major factor responsible for severe virus infections may be viral-bacterial co-infections. Influenza A viruses are a major cause of acute respiratory disease in pigs which may play an important role in the interspecies transmission of influenza viruses. *Streptococcus suis* (*S. suis*) is an emerging

zoonotic agent. It is one of the most important bacterial pathogens affecting the porcine airways causing invasive diseases.
The co-infection studies are expected to provide experimental data how *S. suis* affects infection by influenza viruses with high or low virulence and reveal to what extent the bacterial infection enhances the severity of infection by SIV.
The results show that (i)Primary infection by SIV facilitates adherence and colonization of encapsulated *S. suis*. (ii)Airway epithelium damage induced by infection of SIV promotes colonization and invasion of both encapsulated and non-encapsulated *S. suis*. (iii)Encapsulated *S. suis* affects infection by SIV and reduces the amount of infectious virus released into the supernatant.
We observed that adherence and invasion of *S. suis* on PCLS was efficiently promoted by SIV pre-infection. The PCLS model shows much promise for investigating microbial and host factors to determine the complex mechanisms and dynamics involved in bacterial-viral co-infections.
Disclosure of Interest: None declared

Poster 165
ADVERSE OUTCOMES OF SEASONAL INFLUENZA A VIRUS INFECTION IN PREGNANT MOUSE MODEL
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Abstract: Clinical data have demonstrated increased morbidity and mortality of pregnant women and their infants when infected with seasonal or pandemic influenza. During the 2009 H1N1 pandemic, pregnant women accounted for 5% of mortalities related to infection while only making up about 1% of the general population. The increased susceptibility of pregnant women to flu has been attributed to immunologic changes to “tolerate” the developing fetus. These systemic changes are often characterized by a suppression of cell-mediated immunity and a Th1-Th2 shift in humoral immune responses. However, the underlying mechanisms of the adverse effects of infection are not clearly understood. Using BALB/c mice we investigated the consequences of sublethal infection with H1N1 influenza virus during mid to late gestation. Influenza virus infection resulted in reduced weight gain over the course of pregnancy and a shorter gestation time compared to non-infected pregnant mice. Lung viral loads in pregnant mice were 8-fold higher compared to non-pregnant controls at 4 days post-infection indicating less effective host immune responses. Infection significantly reduced the number of healthy offspring and increased the likelihood of small for gestational age or stillborn pups. In order to better understand the combined interactions of pregnancy and infection on the innate immune response, the responses were analyzed in sera, lungs, placentas, and fetuses. Substantial modifications in compartmentalized levels of cytokines and chemokines were observed between pregnant and non-pregnant infected mice. These changes may reflect heterogeneity of organ-specific DCs programmed for regulating immunity at the maternal-fetal interface. The results indicate that the mouse model can provide insights into the mechanisms of enhanced influenza pathogenesis during pregnancy.
Disclosure of Interest: None declared

Poster 166
DEFECTIVE VIRAL GENOMES AS CRITICAL DANGER SIGNALS DURING RESPIRATORY SYNCYTIAL VIRUS INFECTION
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Abstract: Many clinically relevant viruses, including influenza (IAV) and the respiratory syncytial virus (RSV), block the host antiviral response allowing the virus to replicate to high titers before intervention of the immune system. The mechanism that mediates the transition from stealth virus replication to active antiviral immunity is unknown.
Defective viral genomes (DVGs) that are generated during virus replication at high titers are strong immunostimulators. Many viruses produce DVGs, but until recently they were considered an

epiphenomenon of *in vitro* virus replication. Immunostimulatory (i)DVGs stimulate signaling by RIG-I and MDA5 even in the presence of virus-encoded antagonist of innate immunity. In pioneer work, we showed that natural accumulation of iDVGs in the lung during infection with Sendai virus (SeV) or with IAV was necessary for a potent antiviral response. Importantly, production of the primary antiviral cytokine IFN beta was limited to the lung cellular fraction containing iDVGs. We have identified a specific RNA structure of a SeV defective genome that is essential for the strong stimulatory activity of this molecule. Notably, *in vitro* synthetized oligonucleotides containing this motif show potent immunostimulatory ability *in vivo* demonstrating the potential of these molecules to be used as novel immunostimulants.
In respiratory secretions from children infected with RSV detection of DVGs associated with enhanced expression of transcripts for type I IFNs and other antiviral genes in humans. Infection of human tissue demonstrated that iDVGs can trigger antiviral immunity in most humans and the host factors determine the rate of accumulation of iDVGs during infection. In mice, RSV iDVGs accelerated the onset of antiviral immunity, reduced virus load, and prevented weight loss and lung pathology. Overall, our data suggest that iDVGs have a critical role in stimulating antiviral immunity and determining clinical outcome during RSV infection.
Disclosure of Interest: None declared

Poster 167

ENTRY-DEPENDENT INNATE SIGNALING OF INFLUENZA H1N1 VIRUSES IN HUMAN MACROPHAGES

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Abstract: Host responses to influenza A virus infection are mediated by immune and non-immune cell populations. Resident and infiltrating monocyte-derived macrophages (MDM) play a central role in the early innate immune responses during infection. We hypothesized that the closely-related clinical isolates of the 2009 pandemic H1N1 (H1N1pdm) associated with distinct clinical outcomes, fatal (A/KY/180/10) and nonfatal (A/KY/136/09), induce different early host responses in human macrophages. To address our hypothesis, the kinetics of infection and innate immune responses were assessed in “resting” MDM at 8, 24 and 36 hours post-infection (hpi). While MDM infected with different isolates showed similar increased virus titers over time, their pro- and anti-inflammatory gene expression profiles differed markedly between the lethal (KY180) and nonlethal (KY136) isolates. At 8 hpi, KY136-infected MDM showed significantly greater levels of IFNγ, CCL5, CXCL11, CXCL10, TNF, IDO, IRF3, and IRF7 as well as pattern-recognition receptors RIGI, TLR3, TLR7, compared to KY180-infected cells. IL10, TGFβ and PPARγ were not increased in response to viral isolates tested. By 24 hpi, all viruses had similar profiles, although the magnitude of the response was lower in cells infected by KY180. To determine the mechanism driving the delayed the pro-inflammatory response by KY180, viruses were examined for potential differences in levels of intracellular viral RNAs, pH sensitivity, apoptosis, and finally, the pathway of viral entry. The only difference was mapped to the route of entry. KY180 had greater sensitivity to macropinocytosis inhibitors and electron microscopy revealed a greater number of KY180 viruses have a filamentous shape. Moreover, pro-inflammatory signaling depended on the route of entry and amino acid determinants within the HA1.
Disclosure of Interest: None declared