Oral 1

STRUCTURE AND ACTIVATION OF PARAMYXOVIRUS FUSION GLYCOPROTEINS

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Abstract: Fusion of the lipid envelope of paramyxoviruses with the host cell plasma membrane during virus entry is mediated by the F protein, a trimeric extracellular transmembrane-anchored glycoprotein. During F-mediated fusion, its fusion peptide inserts into the host cell membrane, followed by large-scale refolding from a largely globular pre-fusion conformation to a golf tee-shaped postfusion conformation. For most paramyxoviruses, the F conformational change is triggered by binding of the attachment glycoprotein (HN/H or G) to cellular receptors, which is thought to enable an interaction of F with the N-terminal stalk domain of the attachment protein. However, for Respiratory Syncytial virus (RSV) and Metapneumovirus (MPV), activation of the F protein does not require the attachment glycoprotein, suggesting a fundamentally different mechanism for fusion activation.

Here we will present negative stain EM and crystal structure analyses of the prefusion conformation of the Hendra virus F protein, a member of the henipavirus genus that is known to cause disease in horses and humans with high mortality rate. The overall fold of the Hendra F protein is remarkably similar to that of parainfluenzavirus 5 F, despite their low homology (~27% identify). The most notable differences in secondary structure occur on the Hendra F protein surface. By contrast, comparisons to the RSV F prefusion structure show more significant structural differences. These larger structural differences between RSV and Hendra/PIV5 F proteins may be linked to their different modes of activation. Structural features in key areas of the henipavirus F structure, such as the fusion peptide cleavage site, the heptad repeat A region, and the putative interaction site for G will be discussed, along with their implications for understanding the mechanism of F activation.

Disclosure of Interest: None declared

Oral 2

X-RAY STRUCTURE OF VESICULAR STOMATITIS VIRUS GLYCOPROTEIN BOUND TO A FRAGMENT OF ITS CELLULAR RECEPTOR.

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Abstract: Vesicular stomatitis virus (VSV) is the prototype of the genus Vesiculovirus of the Rhabdoviridae family. VSV encodes one transmembrane glycoprotein (G) that mediates both virus attachment to its receptor and fusion of the viral envelope with the endosomal membrane. Recently, it has been shown that VSV engages the low density lipoprotein receptor (LDLR) to gain entry into host cells and that the third cysteine rich module of the LDLR (CR3) is essential for VSV entry. To elucidate the complex interplay between the virus and the LDLR, we combined several techniques to understand molecular basis of the interaction between G and the LDLR. *In vitro,* we found that both VSV viral particle and G ectodomain can bind to purified CR3 only when G is in its pre-fusion conformation (*i.e.* at high pH). We showed that purified CR3 specifically inhibits viral infection of BHK cells by VSV in a concentration-dependent manner. We also solved the X-ray structure of the complex between G and CR3 at 3.7 Å resolution. The asymmetric unit contained one protomer of G in its native conformation associated with one CR3 domain. The attachment site of CR3 on VSV G is located on both G's central domain and pleckstrin homology domain and involves only non-conserved residues within vesiculovirus genus. These results provide a better understanding for VSV specific receptor recognition.

Disclosure of Interest: None declared

Oral 3

THE INTERACTION BETWEEN TIM-1 AND NPC1 IS IMPORTANT FOR THE CELLULAR ENTRY OF EBOLA VIRUS

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Abstract: Multiple host molecules are known to be involved in the cellular entry of filoviruses, including Ebola virus (EBOV); T-cell immunoglobulin and mucin domain 1 (TIM-1) and Niemann-Pick C1 (NPC1) have been identified as attachment and fusion receptors, respectively. However, the molecular mechanisms underlying the entry process have not been fully understood. We found that TIM-1 and NPC1 colocalized and interacted in the intracellular vesicles (most likely endosomes/lysosomes but not Golgi compartments) where EBOV glycoprotein (GP)-mediated membrane fusion occurred. Interestingly, a TIM-1-specific monoclonal antibody (MAb), M224/1, prevented GP-mediated membrane fusion and also interfered with the binding of TIM-1 to NPC1, suggesting that the interaction between TIM-1 and NPC1 is important for the filovirus membrane fusion. Moreover, MAb M224/1 efficiently inhibited the cellular entry of viruses from all known filovirus species. These data suggest a novel mechanism underlying filovirus membrane fusion and provide a potential cellular target for antiviral compounds that can be universally used against filovirus infections.
Disclosure of Interest: None declared

Oral 4

HAPLOID GENETIC SCREEN REVEALS A PROFOUND AND DIRECT DEPENDENCE ON CHOLESTEROL FOR HANTAVIRUS MEMBRANE FUSION

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Abstract: Hantaviruses cause hemorrhagic fever with renal syndrome (HFRS) in the Old World and a highly fatal hantavirus cardiopulmonary syndrome (HCPS) in the New World. No vaccines or antiviral therapies are currently available to prevent or treat hantavirus disease, and critical gaps in our understanding of how hantaviruses enter cells challenge the search for therapeutics. Here, we performed a comprehensive genetic screen in haploid human cells to identify host factors required for entry by Andes virus, a highly virulent New World hantavirus. We found that multiple genes involved in cholesterol sensing, regulation, and biosynthesis, including key components of the sterol response element binding protein (SREBP) pathway are critical for Andes virus entry. Genetic or pharmacologic disruption of the membrane-bound transcription factor peptidase/site-1 protease (MBTPS1/S1P), an SREBP control element, dramatically reduced infection by virulent hantaviruses of both the Old World and New World clades, but not by rhabdoviruses or alphaviruses, indicating that this pathway is broadly, but selectively required by hantaviruses. These results could be fully explained as arising from the modest depletion of cellular membrane cholesterol that accompanied S1P disruption. Mechanistic studies in cells and with protein-free liposomes revealed that high levels of cholesterol are specifically needed for hantavirus membrane fusion. Taken together, our results indicate that the profound dependence on target membrane cholesterol is a fundamental, and unusual, biophysical property of hantavirus glycoprotein-membrane interactions during entry.
[RKJ, LMK & LTJ contributed equally to this work]
Disclosure of Interest: None declared

Oral 5

ENDOSOME-LYSOSOME FUSION INDUCED BY THE PROTEIN COMPLEX HOPS IS CRITICAL FOR EBOLA VIRUS ENTRY

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Abstract: As a member of the virus family *Filoviridae*, Ebola virus (EBOV) represents an emerging zoonotic pathogen, which is associated with episodic but increasingly frequent outbreaks of a highly hemorrhagic fever in sub-Saharan Africa with human case-fatality rates of up to 90%. Despite

longstanding efforts, our understanding of the filovirus-host molecular interactions required for viral entry and infection remains limited.
For host cell entry, filoviral particles must undergo trafficking to late endosomes/lysosomes (LE/LY) where they gain access to essential entry host factors. Using a comprehensive genetic screen in human haploid cells we previously identified all six subunits of the HOPS (Homo-typic fusion and vacuole protein-sorting) complex as putative host factors for EBOV entry. HOPS, a highly conserved, macro-molecular complex, mediates tethering and hetero-typic fusion of LE and LY. CRISPR/Cas9-mediated knock-down of Vps18, the central protein subunit of HOPS, in human osteosarcoma (U2OS) cells, significantly and specifically reduced infection of recombinant VSV particles bearing EBOV GP. Trafficking and activity of other essential entry host factors, the filoviral receptor NPC1 and endosomal cysteine proteases, were not substantially affected by the loss of HOPS, implicating the HOPS complex as an independent entry factor in EBOV infection.
Mechanistic studies in Vps18kd cells revealed that binding, internalization and viral particle trafficking to NPC1-positive LE were not significantly affected by Vps18 depletion. Instead, a newly established assay for viral membrane fusion in intact cells revealed that EBOV GP-mediated merger of viral and cellular membranes is dramatically reduced in Vps18kd cells. In summary, our results indicate that HOPS activity is an essential prerequisite for the filoviral membrane fusion step, and suggest that HOPS-mediated endo-lysosomal fusion delivers viral particles to the intracellular sites where cytoplasmic escape takes place.
Disclosure of Interest: None declared

Oral 6
INSIGHTS INTO HUMAN METAPNEUMOVIRUS PARTICLE ASSEMBLY AND TRANSMISSION
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Abstract: The paramyxovirus human metapneumovirus (HMPV) is a leading cause of respiratory tract infections worldwide across all age groups, and is particularly devastating in the pediatric, elderly and immunocompromised populations. Despite its high prevalence and burden on human health, there is currently no treatment for HMPV infections. The process of forming new virus particles requires complex interactions between viral and cellular components and the requirements for particle production differ substantially among paramyxoviruses. Interestingly, HMPV infection in normal human bronchial epithelial cells (BEAS-2B) revealed the localization of viral proteins to two structures: long extensions that connect cells, and complex branched networks of cell-associated filaments. Actin polymerization and remodeling was found to play an important role in the production of HMPV particles, in the formation of cell-associated viral filamentous networks, and in HMPV-induced stimulation of intercellular extensions. In addition, mass spectrometric analysis on ultra-purified HMPV particles identified multiple proteins associated with the actin cytoskeleton. Studies with dominant negative Rho GTPases (RhoA, Rac1 and cdc42), the major coordinators of cellular actin dynamics and modeling, indicate a role for signaling in formation of these HMPV protein-containing structures. In addition, initial studies support a role for actin-based intercellular extensions in direct spread of the virus to uninfected cells. Finally, infection of a human airway epithelial model with HMPV resulted in alteration in the actin cytoskeleton mainly at the apical side, further supporting the involvement of the actin cytoskeletal dynamics in HMPV infection. Overall, our data reveal an important role for the actin cytoskeleton in HMPV replication and provide new insights into mechanisms of HMPV transmission.
Disclosure of Interest: None declared

Oral 7
INTRACELLULAR TRAFFICKING OF INFLUENZA VIRUS GENOME MEDIATED BY ENDOCYTIC RECYCLING COMPARTMENTS LOCATED AT THE CENTROSOME
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Abstract: After nuclear export of newly replicated influenza viral genomes, they are transported to the centrosome mediated by Y-box binding protein-1 (YB-1), which is a cellular protein involved in transcription and translation of cellular mRNAs. YB-1 accumulates in the centrosome with vRNP and forms a toroidal structure with a beads-on-a-string distribution around the centriole as like other centrosomal matrix proteins. Since we also reported that YB-1 is required for the centrosome maturation during mitosis in uninfected cells, it is likely that, under influenza virus infection, YB-1 is recruited to the centrosome by stimulating the microtubule assembly at interphases. Here we found that YB-1 activates the microtubule assembly at the centrosome for the formation of endocytic recycling compartments (ERC) in response to infection. The endocytic transport pathway through ERC is regulated by a small GTPase, Rab11a. We revealed that the amount of GTP-bound Rab11a increases in infected cells, suggesting that the membrane trafficking is stimulated via the GEF (guanine exchange factor) activity for Rab11a. Currently, analyses to clarify the functional significance of ERC formation for the transport of the viral genome to the plasma membrane are ongoing.
Disclosure of Interest: None declared

Oral 8

THE NECTIN-4/AFADIN PROTEIN COMPLEX AND INTERCELLULAR MEMBRANE PORES CONTRIBUTE TO RAPID MEASLES VIRUS SPREAD IN PRIMARY HUMAN AIRWAY EPITHELIA

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Abstract: The discovery that measles virus (MV) uses the adherens junction protein nectin-4 as its epithelial receptor provides a new vantage point to understand why measles is so contagious. We show here that in well-differentiated primary cultures of airway epithelial cells from human donors (HAE), MV infectious centers form rapidly and become larger than those of the other respiratory pathogens: human respiratory syncytial virus, parainfluenza virus 5, and Sendai virus. While visible syncytia do not form after MV infection of HAE, the cytoplasm of infected cells suddenly flows into adjacent cells, as visualized through wild type (ICB-323) MV-expressed cytoplasmic green fluorescent protein (GFP). During MV infectious center formation in HAE, trans-epithelial resistance is maintained. High-resolution video microscopy documents that GFP flows through openings that form on the lateral surfaces between columnar epithelial cells. Within 2 hours GFP flows from a secondarily infected cell to the next columnar cell, allowing for rapid infectious center formation. To assess the relevance for this process of the protein afadin, which connects nectin-4 to the actin cytoskeleton, we knocked down its mRNA. This resulted in more limited infectious center formation. We also generated a nectin-4 mutant without the afadin-binding site in its cytoplasmic tail. This mutant was less effective than human nectin-4 in promoting infection in primary cultures of porcine airway epithelia, which endogenous nectin-4 does not act as MV receptor *(J. Virol. 88, 14161, 2014)*. Thus, MV spread in HAE requires the nectin-4/afadin complex, and is based on cytoplasm transfer. We think that the MV membrane fusion apparatus opens intercellular pores that allow cytoplasm transfer but the apical cytoskeletal ring of HAE blocks pore expansion. Intercellular pores, promoting rapid epithelial spread of MV infections without detectable cytotoxicity, may contribute to the extremely contagious nature of measles.
Disclosure of Interest: None declared

Oral 9

NIPAH VIRUS C PROTEIN FACILITATES EFFICIENT VIRUS BUDDING BY RECRUITING ESCRT TO SITES OF MATRIX BUDDING

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Abstract: The reported evidence for the importance of the host ESCRT machinery in Nipah virus budding is contradictory. Although mutational analyses identified two putative late domains in the NiV-

M protein, NiV-M VLP budding was insensitive to Vps4 DN inhibition. The latter suggests that NiV budding is ESCRT-independent.

NiV-M VLP budding may not reflect the biological phenotype during live virus budding where other viral proteins may play a role. To determine if other NiV proteins contribute to efficient virus budding, we co-transfected the other viral proteins with NiV-M and found that only NiV-C significantly enhanced NiV-M budding. NiV-C co-immunoprecipitated with NiV-M and was also incorporated into VLPs. Remarkably, structural homology alignment identified a domain in NiV-C that resembled Vps28, an ESCRT-I component that serves as an adaptor between ESCRT-I-Vps23[TSG101] and ESCRT-II complexes. To interrogate the biological implications of this finding, we generated isogenic, inducible cell lines expressing either WT or DN Vps4. NiV-C enhancement of budding was abrogated only upon DN Vps4 induction. Similarly, infection with live NiV followed by induction of DN Vps4 but not WT Vps4 reduced infectious titers >3 logs at 24 hpi. Western blotting confirmed that reduced titers were due to reduced virion production.

For structural reasons, we demarcated NiV-C into N-, Mid- and C-terminal domains (NTD, MiD, CTD). Biochemical analysis revealed that the NiV-C-CTD interacts with TSG101, while mutagenesis of conserved residues in NiV-C-MiD abrogated NiV-C enhancement of budding. A chimeric NiV-C protein where NiV-C-MiD was replaced by the structurally homologous region in Vps28 maintained the ability to enhance budding. Altogether, our data show that NiV-C enhances NiV budding by recruiting ESCRT to sites of NiV-M budding, and that NiV-C is a functional mimic of host Vps28. Whether other henipavirus C proteins exhibit such convergent evolution in function is under investigation.
Disclosure of Interest: None declared

Oral 10
VIRAL BUDDING REGULATED BY A CRITICAL RESIDUE LOCATED AT THE PUTATIVE DIMERIC INTERFACE OF THE CANINE DISTEMPER VIRUS MATRIX PROTEIN
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Abstract: Paramyxoviruses rely on the matrix (M) protein to orchestrate viral assembly and budding at the plasma membrane. Growing evidences support the notion that M-oligomerization plays a critical role in virus particle formation. Recent structural data from different paramyxovirus M-proteins revealed a monomeric unit composed of N- and C-terminal modules (NTD and CTD, respectively) that exhibit an overall very similar configuration (β-sandwich) and connected via a flexible linker. Moreover, monomers assembled into dimers that were suggested to act as functional units necessary to build up higher oligomers and essential for viral budding. To elucidate the structure(s) and function(s) of the canine distemper virus (CDV) matrix protein, we developed biochemical, cellular and functional assays to investigate in details specific designed mutants. Pending on the determination of the CDV M-protein conformation, we generated a structural homology model based on the New castle disease virus (NDV) M crystal structure to direct our mutagenesis approach. Among others, we mutated a small polar residue (N138) locating at the core of the monomeric and dimeric interfaces. Unexpectedly, despite the presumed key central position of residue N138 at both binding interfaces, when substituted into a non-conservative amino acid (N138F), co-immunoprecipitation experiments revealed mostly unaltered M-M and M-actin interactions. Furthermore, confocal microscopy of the M-N138F mutant indicated wt-like accumulation at the at the cell periphery. Remarkably, however, M-N138F led to severe impairments in viral budding, as determined by virus-like particle (VLP) isolation assay. Overall, our data suggest either major structural variations between CDV and NDV M-proteins, long range effects of residue N138F in modulating the inherent ability of M to generate VLPs, and/or an impact of the latter residue on a plasma membrane-induced oligomeric re-organisation of M-dimeric units required for viral budding.
Disclosure of Interest: None declared

Oral 38

STRUCTURES OF CHANDIPURA VIRUS GLYCOPROTEIN: NEW INSIGHTS IN THE EVOLUTION AND WORKING OF THE VESICULOVIRUS GLYCOPROTEIN FUSION MACHINERY.

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Abstract: Chandipura virus (CHAV) is a member of the vesiculovirus genus. CHAV entry into susceptible cells is mediated by its single envelope glycoprotein G which is both involved in receptor recognition and fusion of viral and cellular membranes. We have determined the crystalline structure of the low pH post-fusion state of the ectodomain of CHAV glycoprotein (G) at 3.6 Å resolution. An overall comparison of this structure with the previously reported VSV post-fusion conformation, shows a high structural similarity as expected from the comparison of primary structures. Among the three domains of G, the pleckstrin homology domain appears to be the most divergent and the largest differences are confined to the secondary structure of the major antigenic site of rhabdoviruses. Finally, local differences indicate that CHAV has evolved alternate structural solutions in hinge regions between pleckstrin homology and fusion domains but also pH sensitive switches that are distinct from those that we have previously characterized in detail for VSV G.

We have also solved a second crystal structure (3Å resolution) which contains two distinct conformations, corresponding to intermediate states of G during the low-pH induced conformational change, arranged in a flat tetrameric assembly exposing fusion loops. Together with electron microscopy and tomography, this work reveals the chronological order of the structural changes in the protein. Furthermore, using native mass spectrometry, we have demonstrated that after dissociation of the pre-fusion trimer into monomers, vesiculovirus fusion glycoprotein can re-associate into dimeric assemblies. Together with mutagenesis studies of the crystalline dimeric interface, those data indicate that the tetramer is biologically relevant and is most probably involved in the initial stages of the fusion process.

Disclosure of Interest: None declared

Oral 39

NEW STRUCTURE-BASED STABILIZATION OF RESPIRATORY SYNCYTIAL VIRUS FUSION PROTEIN SHEDS LIGHT ON THE FUSION MECHANISM

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Abstract: Respiratory syncytial virus (RSV) causes acute lower respiratory tract infections and is the leading cause of infant hospitalizations. Recently, a promising vaccine antigen based on the RSV fusion protein (RSV F) stabilized in the native prefusion conformation has been described. Here we report an alternative, structural and mechanism-based approach to arrest RSV F in the prefusion conformation, based on the prevention of hinge movements in the first refolding region and the elimination of proteolytic exposure of the fusion peptide. A limited number of unique mutations were identified that stabilized the prefusion conformation of RSV F and dramatically increased expression levels. The role of each mutation is explained by crystallographic studies.

This highly stable prefusion RSV F elicited neutralizing antibodies in mice and induced full protection against viral challenge in cotton rats in contrast to RSV F in the postfusion conformation. Additionally, we elucidated the function of an enigmatic polypeptide fragment of F which is unique for RSV (p27) that is not present in other Paramyxoviruses. Crystallographic and biochemical analysis of the prefusion variants provide insight into the maturation and triggering mechanism of this highly complex molecular fusion machinery.

Disclosure of Interest: None declared

Oral 40

MOLECULAR RECOGNITION OF HUMAN EPHRINB2 CELL SURFACE RECEPTOR BY AN EMERGENT AFRICAN HENIPAVIRUS

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Abstract: The discovery of African henipaviruses (HNVs) related to pathogenic Hendra (HeV) and Nipah (NiV) viruses from Southeast Asia and Australia presents an open-ended health risk. Cell receptor usage by emerging African HNVs at the stage of host cell entry is a key parameter when considering the potential for spillover and infection of human populations. The attachment glycoprotein from a Ghanaian bat isolate (GhV-G) exhibits <30% sequence identity with Asiatic NiV-G/HeV-G. Here, through functional and structural analysis of GhV-G, we show how this African HNV targets the same human cell-surface receptor (ephrinB2) as the Asiatic HNVs. We first characterized this virus-receptor interaction crystallographically. Compared to extant HNV-G-ephrinB2 structures, there was significant structural variation in the six-bladed β-propeller scaffold of the GhV-G receptor-binding domain but not the Greek key fold of the bound ephrinB2. Analysis revealed a surprisingly conserved mode of ephrinB2 interaction that reflects an ongoing evolutionary constraint amongst geographically distal and phylogenetically divergent HNVs to maintain the functionality of ephrinB2 recognition during virus-host entry. Interestingly, unlike NiV-G/HeV-G, we could not detect binding of GhV-G to ephrinB3. Comparative structure-function analysis further revealed several distinguishing features of HNV-G function: a secondary ephrinB2 interaction site that contributes to more efficient ephrinB2-mediated entry in NiV-G relative to GhV-G, and cognate residues at the very C-terminus of GhV-G (absent in Asiatic HNV-Gs) that are vital for efficient receptor-induced fusion but not receptor binding *per se*. These data provide molecular-level details for evaluating the likelihood of African HNVs to spill-over into human populations.

Disclosure of Interest: None declared

Oral 41

STRUCTURAL ANALYSIS OF INFLUENZA VIRUS MEMBRANE FUSION BY ELECTRON CRYOTOMOGRAPHY

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Abstract: Entry of Influenza virus into its host cell occurs via fusion of the viral membrane with its encapsulating endosomal membrane. Fusion is triggered by the low pH induced conformational change of haemagglutinin (HA) resulting in the extrusion of fusion peptides which insert into the endosomal membrane. Once the membranes have fused the viral RNP package can be released into the cell cytosol.
We have performed a low pH time course on a mixture of Influenza viral particles and liposomes and studied the ensuing fusion events by cryo Electron Tomography.
We observe snapshots of pre-fusion attachment, fusion, and post-fusion structures in three-dimensions. We correlate changes in the viral glycoprotein, viral membrane and virion interior leading to a structural picture of their role in membrane fusion.

Disclosure of Interest: None declared

Oral 42

THE USE OF SHOTGUN GLYCOMICS TO IDENTIFY ENDOGENOUS RECEPTORS FOR INFLUENZA VIRUSES IN NATURAL TISSUES

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Abstract: The hemagglutinin glycoprotein (HA) of influenza A virus is responsible for mediating virus attachment to host cells via glycan receptors containing terminal sialic acid. It is known that the type of terminal sialic acid linkage can confer species tropism, as avian and human strains preferentially bind ∝2,3-linked sialic and ∝2,6-linked sialic acids, respectively. To better define other characteristics of endogenous glycans that serve as viral receptors, we have been exploring glycan recognition in tissues that are naturally infected by influenza such as the pig lung. For these studies we utilized the novel technology of "shotgun glycomics" to identify natural glycan receptors. The total released N- and O-glycans from pig lung glycoproteins and glycolipid-derived glycans, were fluorescently-tagged, separated by multidimensional HPLC, and individual glycans were covalently printed on glass slides to generate pig lung shotgun glycan microarrays. We examined the binding of a panel of viruses representative of a range of HA subtypes and swine, avian, and human hosts. All viruses tested interacted with one or more sialylated N-glycans, but not O-glycans or glycolipid-derived glycans, and each virus demonstrated novel and unexpected differences in endogenous N-glycan recognition as compared to the structures bound on synthetic glycan microarrays. The results illustrate the repertoire of specific, endogenous N-glycans of pig lung glycoproteins for virus recognition, and offer a new direction for studying endogenous glycan functions in viral pathogenesis.
Disclosure of Interest: None declared

Oral 43

THE F SPECIFICITY OF THE PARAINFLUENZA VIRUS HN IS NOT DEFINED SOLELY BY THE PRIMARY STRUCTURE OF THE HN STALK DOMAIN

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Abstract: Virus-specific interaction between the attachment protein (HN) and the fusion protein (F) is considered prerequisite for the induction of membrane fusion by parainfluenza viruses. Our previous chimeric analyses of the HN and F of parainfluenza 5 (PIV5), human parainfluenza virus 2 (HPIV2), and simian virus 41 (SV41) suggested that this HN-F interaction was mediated by particular amino acids of the HN stalk domain and those in the F head domain. Unexpectedly, however, we recently found that an SV41 F-specific chimeric HN (designated SCA), composed of HPIV2 HN head domain and SV41 HN stalk domain, could not induce cell-cell fusion of BHK cells when coexpressed with an SV41 HN-specific chimeric F (designated No. 36), whose 21 amino acids in the head domain were derived from SV41 F and the rest derived from PIV5 F. Interestingly, substitution of 13 amino acids at or around the dimer interface of HPIV2 HN-derived head domain of SCA with the SV41 HN counterparts resulted in a chimeric HN, which was able to induce fusion with No. 36 but not with SV41 F. More interestingly, substitution of two amino acids out of the 13 amino acids of SCA resulted in another chimeric HN, which could induce fusion either with No. 36 or with SV41 F, similarly to SV41 HN. These observations suggest that substitutions of particular amino acids at or around the HN dimer interface exert allosteric effect on the conformation and/or flexibility of the HN stalk domain, which results in drastic conversion of the F specificity.
Disclosure of Interest: None declared

Oral 44

NEAR-ATOMIC STRUCTURE OF THE HELICAL MEASLES VIRUS NUCLEOCAPSID

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Abstract: Measles is virus causing a highly contagious human disease. We determined the structure of the helical Measles Virus nucleocapsid formed by the folded domain of the Measles Virus nucleoprotein encapsidating an RNA. This 4.3 Å resolution cryo-electron microscopy reconstruction is

the first near-atomic resolution three-dimensional structure of a helical nucleocapsid of a negative-strand RNA virus. The resulting pseudoatomic model of the Measles Virus nucleocapsid offers important insights into the mechanism of nucleoprotein-RNA assembly. It explains why each nucleoprotein of Measles Virus binds six nucleotides whereas the Respiratory Syncytial Virus nucleoprotein binds seven. Our structure provides a rational basis for further analysis of Measles Virus replication and transcription, and reveals potential targets for drug design.
Disclosure of Interest: None declared

Oral 46

IMPACT OF AMINO ACID SUBSTITUTIONS IN THE P-BINDING DOMAIN OF THE MEV NUCLEOPROTEIN ON VIRAL RNA SYNTHESIS

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Abstract: Measles virus possesses a non-segmented, single stranded RNA genome of negative polarity that is encapsidated by the nucleoprotein (N) within a helical nucleocapsid (NC) that acts as the template for transcription and replication. Viral RNA synthesis is ensured by the RNA-dependent RNA polymerase made of the large protein (L) and the phosphoprotein (P), with P serving as an essential tethering factor between L and the nucleocapsid. The N protein consists of a structured N-terminal moiety (N_{CORE}, aa 1-400) that wraps the viral genome, and a C-terminal domain (N_{TAIL}, aa 401-525) that is intrinsically disordered. While N_{CORE} contains all the regions necessary for self-assembly and RNA-binding, N_{TAIL} mediates the polymerase anchoring onto the NC via binding of its MoRE (aa 489-506) to the C-terminal X domain (XD, aa 459-507) of the P protein. The extent to which the N_{TAIL}-XD interaction strength may influence the dynamic of the viral RNA synthesis by the polymerase is poorly known. We thus undertook a mutational study, and combined biochemical analyses, molecular dynamics (MD) simulations and measurements of RNA synthesis by mutated viruses bearing substitutions within the N_{TAIL} MoRE. Using a protein complementation assay (PCA) based on split-GFP reassembly, we found that the MoRE is poorly evolvable in its ability to bind XD, with most amino acid substitutions leading to dramatic drops in the interaction strength. Six individual amino acid substitutions within the MoRE were further analyzed and found to exhibit a distinct impact on the N_{TAIL}/XD binding strength as measured by split-GFP PCA in E. Coli, split-luciferase in human cells and ITC using purified recombinant proteins, with a very good correlation among the three methods. When introduced into recombinant viruses, they differently affect the rate of viral RNA accumulation. The mechanisms by which two key residues stabilize the N_{TAIL}/XD complex were further analyzed by molecular dynamic simulations.
Disclosure of Interest: None declared

Oral 68

STRUCTURE AND FUNCTION OF THE RNA SYNTHESIS MACHINERY OF VESICULAR STOMATITIS VIRUS

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Abstract: The RNA synthesis machinery of vesicular stomatitis virus (VSV) comprises a genomic RNA completely encased within a nucleocapsid protein sheath and associated with the viral polymerase. The viral components of the polymerase are the catalytic subunit – a 240 kDa large (L) protein – and a phosphoprotein cofactor that binds L and the N-RNA template. The L protein contains the RNA dependent RNA polymerase (RdRp) and an unconventional suite of capping enzymes that produce the fully capped and methylated mRNA. The capping reactions are carried out by a

GDP:polyribonucleotidyltransferase (PRNTase) which transfers the nascent monophosphate RNA onto a GDP acceptor to form a GpppApA cap structure with subsequent methylation by a dual specificity ribose 2'-O and guanine-N7 mRNA cap methyltransferase (MTase) to yield GpppAmpA and then 7mGpppAmpA. In addition to the unconventional mechanism of mRNA cap formation, the reactions also differ in their need for a sequence specific signal in the RNA chain, and require a nascent mRNA chain length of 31-nucleotides. The critical active site residues for the enzymes have been mapped within L and are universally conserved among the nonsegmented negative strand (NNS) RNA viruses. We have determined by electron cryomicroscopy the structure of VSV L. The density map, at a resolution of 3.8 Å, has led to an atomic model for nearly all of the 2109-residue polypeptide chain, which comprises three enzymatic domains RdRp, PRNTase, and MTase and two structural domains. The RdRp resembles the corresponding enzymatic regions of dsRNA virus polymerases and influenza virus polymerase. A loop from the capping domain projects into the catalytic site of the RdRp, where it appears to have the role of a priming loop and to couple product elongation to large-scale conformational changes in L.
Disclosure of Interest: None declared

Oral 69
DIVERGENT SEGMENTED NEGATIVE STRAND VIRAL POLYMERASES HAVE THE SAME ARCHITECTURE AND MODE OF REGULATION BY VIRAL RNA
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Abstract: Segmented negative-strand RNA virus polymerases transcribe and replicate the viral RNA (vRNA) within a circular ribonucleoprotein particle (RNP). We present cryo-EM and X-ray structures of respectively apo- and vRNA bound La Crosse orthobunyavirus (LACV) polymerase that give atomic resolution insight into how such RNPs perform RNA synthesis. Apart from interesting idiosyncratic differences, the overall architecture of the monomeric LACV polymerase is strikingly similar to that of the heterotrimeric influenza polymerase, despite high sequence divergence. The LACV 3′ and 5′ vRNA extremities are sequence specifically bound in separate sites on the polymerase. The 5′ end binds as a stem-loop and allosterically orders functionally important polymerase active site loops. Identification of distinct template and product exit tunnels allows proposal of a detailed model for template-directed RNA synthesis within a circularised RNP that could apply to all related viruses. The results will aid development of replication inhibitors of these diverse, serious human pathogenic viruses
Disclosure of Interest: None declared

Oral 70
X-RAY STRUCTURE AND ACTIVITIES OF AN ESSENTIAL MONONEGAVIRALES L-PROTEIN DOMAIN
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Abstract: Human metapneumovirus (hMPV) is a paramyxovirus of *Pneumovirinae* subfamily closely related to Respiratory Syncytial virus (RSV). Like RSV, hMPV is highly contagious and causes respiratory-tract disease.
This virus encodes the large protein (L) whose the primary sequence of L contains six conserved regions (CRs I-VI). L harbours enzymatic activities for nucleotide polymerization (CR-III) and for each step of mRNA cap formation (CR-V and CR-VI).
Bioinformatic analyses have predicted CR-VI as a methyltransferase (MTase), activity involved in the cap synthesis. Nevertheless, the boundaries of CR-VI are not well defined, nor if CR-VI acts on its own or in conjunction with other domains and if it carries both N7 and 2'O MTase activities. In addition, the lack of L protein high-resolution structure prevents mechanistic understanding of all its activities. We

have study hMPV CR-VI+ domain, the CR-VI domain associated with the "+" unconserved region. We success to solve its crystal structure and investigated its MTase activity.
The crystal structure of CR-VI+ was solved to 2.2 Å and shares some characteristics with the SARS-Coronavirus and *Flavivirus* 2'O MTases. *In vitro*, CR-VI+ binds and methylates RNAs containing the conserved start sequence of hMPV transcripts. CR-VI+ methylates first the 2'O and then the N7 position, this uncommon methylation order also occurring in VSV. By directed mutagenesis we determined that all catalytic tetrad residues K-D-K-E are essential for the 2'O methylation whereas only the aspartate D_{181} is important for the N7 methylation. This last observation is also recovered in *Flavivirus*. CR-VI+ also exhibits nucleotide triphosphatase activity, converting GTP into GDP, which is requiring during the first step of the cap synthesis.
To conclude, CR-VI+ harbours 3 of the 4 enzymatic activities needed to form a complete cap structure $^{m}GpppN_{m}$ and represents an attractive target for the structure-based design of antiviral compounds.
Disclosure of Interest: None declared

Oral 71
STRUCTURE OF THE INFLUENZA C VIRUS RNA-DEPENDENT RNA POLYMERASE
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Abstract: The RNA-dependent RNA polymerase of influenza viruses (FluPol) is a large heterotrimeric machine that performs both viral transcription and genome replication. For transcription initiation, FluPol uses capped primers, derived from cellular RNAs through a snatching mechanism involving cap-binding and endonuclease domains within the polymerase. In contrast, replication is initiated *de novo* from both genomic (vRNA) and anti-genomic (cRNA) templates. These distinct activities likely correspond to different FluPol conformations, which are regulated by the varying association of FluPol with other viral or host proteins and the conserved 5′ and 3′ extremities (promoters) of vRNA or cRNA. Thus, even though structures of influenza A and B polymerases in complex with vRNA promoters have recently been reported, these still offer only limited snapshots into the mechanisms of FluPol.

We have now solved an apo structure of FluPol from an influenza C virus, revealing a dramatically different conformation that FluPol can adopt. Though all of the major domains resemble their counterparts in the promoter-bound structures, the arrangement of peripheral domains is remarkably different. Particularly striking is the C-terminal two thirds of PB2. Each domain here is rotated independently with respect to the promoter-bound structures, resulting in a complete re-arrangement of this part of the molecule. The apo structure is in a closed, pre-activation state, as the cap-binding site is occluded by part of PB2. Thus, as well as highlighting the flexibility of FluPol, our structure suggests a possible mechanism for its regulation by promoter RNA.
Disclosure of Interest: None declared

Oral 72
UNIQUE INTERACTIONS OF THE MUMPS VIRUS PHOSPHOPROTEIN WITH ITS NUCLEOCAPSID
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Abstract: The nucleocapsid (NC) of mumps virus (MuV) forms a stable coil. How the viral-RNA-dependent-RNA polymerase (vRdRp) gains access to the sequestered genomic RNA for viral RNA synthesis is not fully understood. The role of the MuV phosphoprotein (MuV P) in this process was systematically characterized. The C-terminal region of the nucleocapsid protein (N) contains a molecular recognition element (MoRe) that is accessible through the interstitial space between successive helical turns of NC. Comparisons between the native NC and a structure of a truncated NC (residues 1-379) suggest that the C-terminal region of the MuV N protein is located between successive

helical turns. We have found that MuV P_{CTD} (residues 286-391) bind the MoRe and other residues of N. A cryoEM structure of MuV NC- P_{CTD} complex defined its binding site in NC. Its location is similar to the P binding site of respiratory syncytial virus (a paramyxovirus) NC. As a cofactor of vRdRp, this interaction between P_{CTD} and N proteins leads to the specific recognition of NC by vRdRp. Unexpectedly, the MuV P_{NTD} (residues 1-194) was also found to bind NC in contrary to other paramyxoviruses such as measles virus and Sendai virus. More interestingly, P_{NTD} binding uncoils the helical NC of MuV. Co-expression of P_{NTD} increases gene expression in the mini-genome system. The novel mode of MuV P antiparallel association places both P_{NTD} and P_{CTD} at each end of the P tetramer. We hypothesize that P_{NTD} and P_{CTD} act in concert to induce changes in NC for viral RNA synthesis by vRdRp. P_{CTD} primarily allows vRdRp to specifically recognize NC. On the other hand, P_{NTD} primarily uncoils MuV NC to expose the "entrance" to the sequestered genomic RNA. The two functions position vRdRp perfectly at the site in NC for viral RNA synthesis.
Disclosure of Interest: None declared

Oral 73
FUNCTIONAL AND STRUCTURAL ANALYSIS OF THE INTERACTION BETWEEN INFLUENZA VIRUS POLYMERASE AND THE SPLICEOSOMAL RED-SMU1 COMPLEX
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Abstract: Influenza A virus mRNAs are synthesized by the viral RNA-dependent RNA polymerase in the nucleus of infected cells, in close association with the cellular RNA polymerase II and the mRNA processing machinery. Two proteins essential for viral multiplication, the exportin NS2/NEP and the ion channel protein M2, are produced by splicing of the NS1 and M1 mRNAs, respectively. We identified two human spliceosomal factors, RED and SMU1, that control the expression of NS2/NEP and are required for efficient viral multiplication. We found several lines of evidence that in infected cells, the hetero-trimeric viral polymerase recruits a complex formed by RED and SMU1, through interaction with its PB2 and PB1 subunits. The splicing of the NS1 viral mRNA is specifically affected in cells depleted of RED or SMU1, leading to a decreased production of the spliced mRNA species NS2, and to a reduced NS2/NS1 protein ratio. In agreement with the exportin function of NS2, these defects impair the transport of newly synthesized viral ribonucleoproteins from the nucleus to the cytoplasm, and strongly reduce the production of infectious influenza virions.
Mapping of the protein-protein interaction domains indicated that the N-terminal domain of RED (NtRED) interacts both with a large C-terminal domain of the viral polymerase subunit PB2, and with the N-terminal domain of SMU1. The NtRED-SMU1 complex was expressed in insect cells and purified. Mass spectrometry and SAXS analysis revealed a 1:2 stoechiometry. Structural data are currently being refined.
Overall, our results provide an integrated functional, biochemical and structural analysis of the interaction between influenza A virus polymerase and the spliceosomal RED-SMU1 complex, which acts as a key regulator of viral gene expression. This opens the way to the development of new antiviral drugs that inhibit viral growth by targeting the assembly of the tripartite polymerase-RED-SMU1 complex.
Disclosure of Interest: None declared

Oral 74
INFLUENZA VIRUS EXPLOITS HOST KINASES TO REGULATE NP OLIGOMERIZATION AND ASSEMBLY OF THE VIRAL REPLICATION MACHINERY
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Abstract: Influenza virus assembles large ribonucleoprotein (RNP) complexes that direct replication and transcription of the viral genome. These RNPs contain the polymerase, genomic RNA and multiple copies of nucleoprotein (NP). During RNP assembly, monomeric NP oligomerizes along the length of the genomic RNA to protect the viral genome and scaffold polymerase activities. As influenza virus NP spontaneously oligomerizes and non-specifically binds nucleic acids, a portion of NP must be actively maintained as a monomer and selectively transitioned to an oligomer during RNP assembly. This regulated RNP assembly is essential for virus replication, but how it is controlled is poorly understood. We elucidated a phospho-regulatory mechanism whereby NP oligomerization is specifically controlled by host kinases. We identified new evolutionarily conserved phosphorylation sites on influenza A NP and demonstrated that phosphorylation of NP decreased oligomerization and RNP formation. Two phosphorylation sites, S165 and S407, are located on opposite sides of the NP:NP interface. In both influenza A and B virus, mutating or mimicking phosphorylation at these sites blocked homotypic interactions and drove NP towards a monomeric form. Moreover, we have identified NP-specific host kinases that hyper-phosphorylate residues S165 and S407 and block NP:NP interactions. Highlighting the central role of this regulatory process during infection, mutating NP phospho-sites or expressing NP-specific kinases impaired RNP formation, polymerase activity and virus replication. Thus, we have shown that dynamic phosphorylation of NP is a critical regulator of RNP assembly and modulates progression through the viral life cycle. The evolutionary conservation of these phospho-sites suggests that our results are broadly applicable across viral strains and genera and reveal a global regulatory strategy for *Orthomyxoviridae.*
Disclosure of Interest: None declared

Oral 75

A PREDICTED DYNEIN LIGHT CHAIN 1 BINDING MOTIF IN RABIES VIRUS L PROTEIN IS INVOLVED IN MICROTUBULE REORGANIZATION AND PRIMARY TRANSCRIPTION

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Abstract: To investigate the intracellular distribution of rabies virus (RABV; *rhabdoviridae*) polymerase L, we expressed mCherry tagged L (mCherry-L) in the absence of other RABV proteins. Analysis of mCherry-L localization by confocal laser scan microscopy revealed that mCherry-L accumulated at microtubules (MT). Moreover, microtubule reorganization and acetylation correlated with mCherry-L accumulation at MTs, indicating that RABV polymerase L binds and leads to MT modification. In silico analysis revealed a putative dynein light chain 1 (DLC1) binding motif in L that could explain MT association through DLC1. Because DLC1 binding by polymerase cofactor P is known, those data further indicated that both components of the RABV polymerase complex are able to bind DLC1. Recombinant viruses with mutations in the respective P and L motifs showed that both motifs are required for regulation of primary transcription, indicating that DLC1 acts as an enhancer of transcription by binding to both components of the viral polymerase complex. Interestingly, DLC1 levels in infected cells were also regulated by both motifs. Finally, disruption of the L motif resulted in a cell type specific loss of MT localization, showing that DLC1 is involved in RABV L mediated reorganization of the cytoskeleton. Overall, we conclude that DLC1 acts as a transcription factor that stimulates primary RABV transcription by binding to both, RABV P and L protein. We further conclude that RABV L is able to influence MT organization and posttranslational modification. In infected neurons, MT manipulation by L may contribute to efficient intracellular transport of virus components.
Disclosure of Interest: None declared

Oral 76

RNA-BINDING OF EBOLA VIRUS VP30 IS ESSENTIAL FOR ACTIVATING VIRAL TRANSCRIPTION

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Abstract: The template for Ebola virus (EBOV) transcription and replication is the helical viral nucleocapsid composed of the viral genome, which is complexed by the nucleoprotein, NP, VP35 and VP24. While viral replication is exerted by the polymerase L and its cofactor VP35, viral transcription further requires the presence of the EBOV transcription factor VP30. So far, it is largely unclear how VP30 commits the polymerase to its transcriptional mode.
Here, we studied how RNA binding by VP30 relates to its function in transcriptional activation employing VP30 mutants with mutations in previously identified functional motifs. For analyzing VP30:RNA binding, we developed a VP30-tailored gel mobility shift assay which enabled us to quantify binding and to resolve different complex forms. As RNA substrate, we chose the 3'-terminal 154 nt of the EBOV genome comprising the 3'-leader. This RNA fragment encodes signals essential for replication and transcription, including the transcription start site and part of the 5'-UTR of the first viral gene (NP). Binding of VP30 to the genomic EBOV RNA was severely impaired by VP30 mutations that (i) mimic its fully phosphorylated state, (ii) destroy the protein's capability to form homohexamers, and (iii) disrupt the integrity of its Zinc-finger domain. The same features were previoulsy identified as being important for transcriptional activation. Furthermore, we demonstrated that the previously described VP30:VP35 interaction is RNA-dependent, but not formation of VP30 homohexamers and VP35 homotrimers. We propose an extended model according to which VP30 forms a ternary complex with the (-) RNA template and VP35, which enables the polymerase complex of VP35 and L to resist termination and dissociation when transcribing termination-active sequences in the 5'-UTRs of viral mRNAs.
Disclosure of Interest: None declared

Oral 77

IDENTIFICATION OF A NOVEL VIRAL PROTEIN EXPRESSED FROM THE PB2 SEGMENT OF INFLUENZA A VIRUS

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Abstract: Over the past two decades, several novel viral proteins have been identified that modulate viral infections *in vitro* and/or *in vivo*. However, additional as yet unidentified viral proteins may be expressed by influenza virus and may play a role in viral replication and/or pathogenicity. The PB2 segment is one of the longest influenza A virus segments and is known to encode only one viral protein, PB2. Here, we used RT-PCR targeting viral mRNAs transcribed from the PB2 segment to look for novel viral proteins encoded by spliced mRNAs.
Total RNA was extracted from 293 cells infected with A/WSN/33 (WSN). RT-PCR was performed with an oligo(dT) primer and primers specific for the PB2 mRNA. Two species of PCR product were produced: the mRNA encoding the PB2 protein and a novel spliced mRNA derived from the PB2 segment. The nucleotide sequences around the splicing donor and acceptor sites of the novel mRNA were highly conserved among human pre-2009 H1N1 viruses but not among human H1N1pdm and H3N2 viruses. A novel viral protein, termed PB2-S1, was translated from this novel mRNA in virus-infected cells. PB2-S1 localized to mitochondria and inhibited the RIG-I-dependent interferon signaling pathway. PB2-S1 also interfered with viral polymerase activity depending on its PB1 binding capability. PB2-S1-deficient viruses, however, showed similar growth kinetics in MDCK cells and virulence in mice to those of wild-type virus. The biological significance of PB2-S1 to seasonal H1N1 influenza A virus replication and pathogenicity warrants further investigation.
Disclosure of Interest: None declared

Oral 103

IDENTIFICATION OF CONSERVED PARAMYXOVIRUS T CELL EPITOPES: DOES MEASLES VACCINATION PROTECT AGAINST ZOONOTIC HENIPAVIRUS INFECTIONS?

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Abstract: Measles virus (MV) is a member of the family *Paramyxoviridae*, genus *Morbillivirus*. Morbillivirus infections induce cross-protection, largely based on cross-reactive T-cell responses. We have previously shown that MV vaccination of non-human primates provides partial protection from infection with canine distemper virus (CDV). In the context of the possible global eradication of measles, characterization of morbillivirus cross-reactive immune responses is important for assessment of the potential of animal morbilliviruses to become zoonotic threats as MV vaccination coverage decreases. Moreover, it is not clear if morbillivirus cross-reactive T-cells could provide protection against other paramyxoviruses. A panel of MV-specific human T-cell clones was tested for cross-reactivity with animal morbilliviruses. Two MV fusion glycoprotein (F)-specific T-cell clones were identified that recognized both MV- and CDV-infected autologous cells. Overlapping peptides were used to determine the minimal epitopes recognized by these clones. Two novel T-cell epitopes were identified in the MV F glycoprotein, recognized by an HLA-DQB1*0603-restricted $CD4^+$ and an HLA-B*1501-restricted $CD8^+$ T cell clone. These epitopes are located in the fusion peptide, which is not only highly conserved in the genus *Morbillivirus*, but also in other paramyxoviruses including the genus *Henipavirus*. Henipaviruses are zoonotic bat paramyxoviruses classified as BSL-4 agents that cause severe neurological disease in humans. These data suggest that, based on HLA haplotype and history of MV infection or vaccination, humans may have cross-protective T-cells that could provide partial protection against zoonotic henipavirus infections.
Disclosure of Interest: None declared

Oral 104

LYMPHOCYTIC CHORIOMENINGITIS VIRUS DIFFERENTIALLY AFFECTS VIRUS-INDUCED TYPE I IFN RESPONSE AND MITOCHONDRIAL APOPTOSIS MEDIATED BY RIG-I/MAVS

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Abstract: Arenaviruses are important emerging human pathogens maintained by non-cytolytic persistent infection in their rodent reservoir hosts. Despite high levels of viral replication, persistently infected carrier hosts show only mildly elevated levels of type I interferon (IFN-I). Accordingly, the arenavirus nucleoprotein (NP) has been identified as a potent IFN-I antagonist capable of blocking activation of interferon regulatory factor (IRF)-3 via the retinoic acid inducible gene (RIG)-I/ mitochondrial antiviral signaling (MAVS) pathway. Another important mechanism of host innate anti-viral defense is represented by virus-induced mitochondrial apoptosis via RIG-I/MAVS and IRF3. In the present study, we investigated the ability of the prototypic Old World arenavirus lymphocytic choriomeningitis virus (LCMV) to interfere with RIG-I/MAVS-dependent apoptosis. We found that LCMV does not induce apoptosis at any time during infection. While LCMV efficiently blocked induction of IFN-I via RIG-I/MAVS in response to super-infection with cytopathic RNA viruses, virus-induced mitochondrial apoptosis remained fully active in LCMV infected cells. Notably, in LCMV infected cells, RIG-I was dispensable for virus-induced apoptosis via MAVS. Our study reveals that LCMV infection efficiently suppresses induction of IFN-I, but does not interfere with the cell's ability to undergo virus-induced mitochondrial apoptosis as a strategy of innate anti-viral defense. The RIG-I-independence of mitochondrial apoptosis in LCMV infected cells provides first evidence that arenaviruses can reshape apoptotic signaling according to their needs.
Disclosure of Interest: None declared

Oral 105

UNDERSTANDING THE ANTIVIRAL ACTION OF HUMAN MXA, STRUCTURAL AND FUNCTIONAL ANALYSIS OF TWO CRUCIAL MODULES.

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Abstract: Human MxA, the product of the *MX1* gene, is an interferon-induced large GTPase and functions as an early barrier against RNA viruses, including influenza- and bunyaviruses. The domain architecture of MxA comprises an N-terminal GTPase (G) domain, a bundle signaling element and a C-terminal stalk responsible for oligomerization and recognition of the viral ribonucleoproteins. The contribution of each domain for the antiviral function is poorly understood. By mutational analysis we show that the G domains of adjacent MxA molecules dimerize, and formation of such G domain dimers controls GTP hydrolysis. GTPase activity is required for MxA release from interferon-induced intracellular pools and subsequent antiviral action. Furthermore, we identified a flexible loop L4 for viral target recognition that protrudes from the compact structure of the stalk domain. A minimal motif forms a recognition interface in L4 that mediates viral nucleocapsid binding and determines the antiviral specificity of MxA. We hypothesize that loop L4 binds viral NP, and oligomerization of MxA via the stalk allows multiple contacts, stabilizing MxA-nucleocapsid interactions. By analyzing naturally occurring amino acid exchanges in the functional domains of MxA, we identified variations that are neutral, abolish, or even dominant-negatively impact the antiviral function of the wild-type. The rare occurrence of such polymorphic variations in the *MX1* gene underlines the central role of MxA for the interferon-induced antiviral defence in humans.
Disclosure of Interest: None declared

Oral 106

TARGETING IMPORTIN-A7 AS A THERAPEUTIC APPROACH AGAINST PANDEMIC INFLUENZA VIRUSES.

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Abstract: Viral drug resistance is believed to occur less likely if compounds are directed against cellular rather than viral proteins. In this study, we analyzed the feasibility of a crucial viral replication factor, namely importin-α7, as a potential cellular drug target to combat pandemic influenza. Surprisingly, only five viral lung-to-lung passages were required to achieve 100% lethality in importin-α7$^{-/-}$ mice that are otherwise resistant. Viral escape from importin-α7 requirement was mediated by five mutations in the viral ribonucleoprotein complex and the surface glycoproteins. Moreover, the importin-α7$^{-/-}$ mouse-adapted strain became even more virulent for wildtype mice compared to the parental strain. These studies show that targeting host proteins may still result in viral escape by alternative pathways eventually giving rise to even more virulent virus strains. Thus, therapeutic intervention strategies should consider a multi-target approach to reduce viral drug resistance.
Disclosure of Interest: None declared

Oral 107

MECHANISM OF CELL-INTRINSIC INNATE IMMUNE ANTAGONISM BY EBOLA VIRUS VP24

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Abstract: Ebolaviruses and marburgviruses are non-segmented negative sense ssRNA viruses that are members of the family *Filoviridae.* They are highly pathogenic due, in part, to their ability to potently inhibit innate immune responses. Zaire ebolavirus VP24 (eVP24) binds to karyopherin alpha (KPNA) nuclear transporters, preventing nuclear transport of PY-STAT1 and downstream activation of interferon-stimulated genes (ISGs). The related Marburg virus VP24 (mVP24) does not block interferon (IFN) responses. The exact mechanism of eVP24 inhibition was unknown and it was unclear why the inhibitory function is not shared by mVP24. The crystal structure of eVP24 in complex with the C-

terminus of KPNA5 was solved. Using this structure as a guide, we performed functional and binding studies with wild-type and mutant eVP24s to demonstrate that eVP24 recognizes a unique non-classical nuclear localization signal-binding site on the C-terminus of KPNA5. This site is also used by PY-STAT1 for nuclear transport. Our results show that eVP24 can compete with PY-STAT1 for interaction with KPNA5 at this site, inhibiting IFN signaling and the subsequent antiviral response. Structural differences between eVP24 and mVP24 explain why mVP24 cannot block IFN responses. Further analysis of the KPNA interaction with the VP24s from other ebolavirus species identified reduced binding affinity and reduced IFN inhibitory activity of the less pathogenic Bundibugyo virus VP24 (bVP24) to KPNA5 as compared to eVP24. Cumulatively, these data suggest that VP24 acts as a virulence factor and provides a guide for development of therapeutic approaches designed to counteract the VP24 immune evasion function.
Disclosure of Interest: None declared

Oral 108
HOST DEFENCE AND VIRAL COUNTER DEFENCE BY THE PLANT-INFECTING BUNYAVIRUS TSWV
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Abstract: RNA interference (RNAi or RNA silencing) plays a major role in host development and gene regulation but also acts as a antiviral defense mechanism in plants and insects. Recently this has been proven in mammals as well, however there is one major difference between the antiviral RNAi defense against viruses in plants and those in insects and mammals, which is the requirement of an RNAi amplification cycle in plants mediated by host RNA-dependent RNA polymerases (RDRs). Without this amplification, plants are not able to mount a strong antiviral RNAi response. Plant and insect viruses have evolved ways to counteract antiviral RNAi e.g. by encoding so-called RNA silencing suppressor (RSS) proteins that interfere with this pathway.
Using a transient transgene silencing assay, based on the RNAi amplification by RDRs, the Tomato spotted wilt bunyavirus (TSWV) NSs protein has been identified as RSS protein. Biochemical analysis demonstrated that this protein *in vitro* binds small and long dsRNA molecules and *in vivo* is able to suppress dsRNA and miRNA-induced gene silencing. Recently we also identified the NSs RSS protein as the effector of an intracellular innate immunity sensor (the putative NOD-like receptor (NLR) *Tsw)* from pepper plants. Its (in)direct recognition leads to a *Tsw*-mediated triggering of a downstream hypersensitive, programmed cell death response. Isolates of TSWV have been identified that escape from being recognized by *Tsw*, and their NSs proteins have additionally been observed to be compromised in their RSS activity. Further analysis revealed that some of these were only compromised in their ability to suppress local silencing, but still suppressed systemic silencing. An alanine mutant screen indicated a functional overlap in RSS and effector-functionality while mutation of a WG/GW motif and concomitant loss of RSS (and effector) activity indicated a putative interaction with AGO1, the core component of the RNA-induced silencing complex (RISC).
Disclosure of Interest: None declared

Oral 109
ATTENUATION OF LYSSAVIRUSES: BIOTYPE II AND III PHOSPHOPROTEINS CANNOT INHIBIT IRF3 ACTIVATION AND INTERFERON INDUCTION IN HUMAN CELLS
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Abstract: The phosphoprotein P of the neurotropic rabies virus (RABV) is a potent antagonist of the host interferon response in human and murine cells, preventing both PAMP receptor-mediated IFN induction and IFN-mediated JAK/STAT signalling. In addition, P is an essential factor for encapsidation and transcription of viral RNA. We have previously identified RABV P mutants defective for inhibition of IFN induction but not for the other functions of P. RABV carrying the mutations are apathogenic for mice after intracerebral injection, demonstrating that neurovirulence of RABV correlates with the capacity to

prevent activation of IRF3. We here assessed the ability of the P proteins of non-RABV lyssaviruses to interfere with IFN induction in human cells. We found that P from members of biotype I, including bat viruses like European bat lyssavirus type 1 (EBLV-1), was as effective as RABV P in preventing IFN induction in human cells. However, P from biotype II and III viruses completely failed in preventing IRF3 activation and IFN induction upon expression from plasmids or in the context of recombinant RABV carrying the heterologous P proteins. This failure in preventing virus recognition has probably implications on the degree of antiviral host defence. Current experiments involve informatics, differential pull down and mass spectrometry, in order to confirm the molecular target of RABV P instrumental in blocking IRF3 activation.
Disclosure of Interest: None declared

Oral 110
C-TERMINAL REGION OF TOSCANA VIRUS NSS PROTEIN IS CRITICAL FOR INTERFERON-B ANTAGONISM AND PROTEIN STABILITY.
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Abstract: Toscana Virus (TOSV) is a Phlebovirus belonging to the *Bunyaviridae* family responsible for central nervous system (CNS) injury in humans. TOSV non structural protein (NSs) is an IFN-β antagonist and exerts its inhibitory effect by interacting with RIG-I (Retinoic-acid Inducible Gene I), an upstream viral sensor involved in the IFN-β signalling cascade, leading to its degradation. Two C-terminal truncated NSs proteins, Δ1C-NSs (aa 1-284) and Δ2C-NSs (aa 1-287), were cloned and tested for their functional activity, showing an opposite behaviour on IFN-β signalling. While Δ1C-NSs did not show any activity leading to RIG-I degradation, promoting RIG-I-mediated IFN-β activation, Δ2C-NSs behaved like the wild-type NSs protein. Moreover, immunofluorescence of cells expressing deleted NSs evidenced a greater stability of Δ1C-NSs in comparison with the other recombinant NSs protein. In order to explain this behavior, the sequence interposed between the two constructs, corresponding to the TLQ aa string, was deleted and a new construct ΔTLQ-NSs was also tested. A significant decrease of IFN-β inhibitory effect was observed in RIG-I and ΔTLQ-NSs co-transfected cells, compared to those expressing RIG-I in combination with wt-NSs, indicating that this domain could have a role in RIG-I mediated degradation and regulation of its signalling pathway. However, both the truncated proteins were still able to interact with RIG-I, as shown by co-immunoprecipitation (Co-IP) experiments, suggesting that the domain/s involved in negative regulation of RIG-I signalling and RIG-I interaction are mapped on different regions of the TOSV NSs protein. Taken together, these results suggest that TOSV NSs C-terminal domain could be critical for NSs antagonistic properties, though the functional role of TLQ aa sequence is still to be clarified.
Disclosure of Interest: None declared

Oral 111
EVIDENCE FOR A NOVEL MECHANISM OF INFLUENZA VIRUS-INDUCED TYPE I INTERFERON EXPRESSION BY A DEFECTIVE RNA-ENCODED PROTEIN
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Abstract: Influenza A virus (IAV) defective RNAs are generated as byproducts of error-prone viral RNA replication. They are commonly derived from the larger segments of the viral genome and harbor deletions of various sizes resulting in the generation of replication incompatible viral particles. Furthermore, small subgenomic RNAs are known to be strong inducers of pattern recognition receptor RIG-I-dependent type I interferon (IFN) responses.
The present study identifies a novel IAV-induced defective RNA derived from the PB2 segment of A/Thailand/1(KAN-1)/2004 (H5N1). It encodes a 10 kDa protein ($PB2_{\Delta}$) sharing the N-terminal amino

acid sequence of the parental PB2 protein followed by frame shift after internal deletion. $PB2_{\Delta}$ induces the expression of IFNβ and IFN-stimulated genes by direct interaction with the cellular adapter protein MAVS, thereby reducing viral replication of IFN-sensitive viruses such as IAV or vesicular stomatitis virus. This induction of IFN is completely independent of the defective RNA itself that usually serves as pathogen-associated pattern and thus does not require the cytoplasmic sensor RIG-I. These data suggest that not only defective RNAs but also defective RNA-encoded proteins can act immunostimulatory and, in the case of KAN-1 infection, might contribute to the overwhelming cytokine response characteristic for highly pathogenic H5N1 viruses leading to a more severe phenotype *in vivo.* This is the first time that such a function was described for a defective RNA-encoded protein, a finding that has several important implications with regard to deciphering viral protein functions and options for immunostimulatory approaches. Furthermore, this is an example of how influenza viruses may acquire novel polypeptides with altered functions from its limited genome.
Note: These data were in part presented at different meetings before.
Disclosure of Interest: None declared

Oral 112
B-INTERFERON INDUCTION IN VSV-INFECTED CELLS: WHAT IS THE VIRAL RNA INDUCER?
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Abstract: Several lines of evidence support a role for the genomes of copy-back defective interfering virus (DI) as inducers of the cellular antiviral response in non-segmented negative strand RNA virus (NNS) infections. Chief among these is the potential for these DI genomes to form 5' triphosphate-bearing dsRNA structures that are known to be potent activating ligands for RIG-I in vitro. What triggers RIG-I activation in infected cells however has not been established with certainty. We show here that optimal IFN-b induction in A549 cells infected by vesicular stomatitis virus (VSV) does not require the presence of copy-back DI and infection with the latter by themselves does not cause induction. Infection with a recombinant VSV expressing the dsRNA-binding vaccinia virus E3L protein reduced but did not eliminate activation. On the other hand, infection with the polR VSV mutant, which overproduces viral dsRNA, or infection with recombinant viruses expressing complementary GFP transcripts, showed only marginal differences in IFN-b induction compared to wild-type virus. Interestingly, PKR was readily activated in polR virus-infected cells but not in wild type-infected cells, and this activation was completely suppressed by E3L expression. We conclude that the nature of the viral RNA(s) that leads to activation of the IFN-b response in standard VSV-infected cells does not fit current models. Possible alternatives will be discussed.
Disclosure of Interest: None declared

Oral 125
FUNCTIONS OF HUMAN PARAINFLUENZA VIRUS 3 HEMAGGLUTININ-NEURAMINIDASE RECEPTOR BINDING SITES IN FUSION ACTIVATION
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Abstract: Entry of human parainfluenza virus 3 (HPIV3) is mediated by conformational change in the fusion (F) protein when triggered by the receptor-engaged receptor binding protein, hemagglutinin-neuraminidase (HN). Attachment of HN to its sialic acid (SA) receptor activates F to undergo structural changes that lead to fusion. Two SA binding sites in the globular head of HPIV3 HN contribute to fusion with the target cell; site I is the primary SA binding/neuraminidase site and site II is the binding/F-activation site. Our hypothesis is that upon receptor engagement site II transmits an activation signal from HN's head to its stalk domain, which then induces activation of F, and that this function is distinct from simple receptor avidity and separate from the activation function of the HN stalk. We showed that the transition of HN from a 'heads-down' to 'heads-up' conformation is not coincident with activation of F, and that HN in its 'heads-up' form is present in association with F prior to receptor engagement. We show that virions interact with SA presenting membranes via HN arrays and HN/F complex arrays, and that these are unperturbed upon interaction and remain structured in the heads-up configuration

observed in free virus. To address transmission of the activation signal upon receptor engagement we dissected the contributions of each site in the HN head using chimeric proteins that bear the globular head of HPIV3 HN with specific mutations at site I or site II, paired with the stalk of measles virus hemagglutinin or Nipah virus G, or mutations at HN site II that reduce or abolish fusion while not affecting receptor engagement. The results suggest that HPIV3 HN site II bears primary responsibility for the portion of the triggering function that resides in the globular head of the receptor binding protein, and that the signal that triggers fusion is distinct from receptor recognition.
Disclosure of Interest: None declared

Oral 126
REVERSE GENETIC ANALYSIS OF BAT INFLUENZA VIRUS HARBORING THE ENTRY MACHINERY OF AN INFLUENZA A VIRUS
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Abstract: In 2012 the first complete sequences of a distantly-related influenza virus (H17N10) was discovered in bats from Central America, however due to the lack of infectious virus isolates, further characterization has been impeded. Using polymerase reconstitution and virus-like particle formation assays, we now show partial functional compatibility between bat and influenza A virus (IAV) components. Guided by these observations, we generated an infectious virus containing six out of the eight bat virus genes and having the remaining two genes encoding the HA and NA proteins of a prototypic IAV. This was only possible by including bat specific packing sequences in the HA and NA segment. The chimeric bat viruses replicated to varying degrees in a broad range of mammalian cell cultures and mice. In sharp contrast, viral growth was poor in avian cells or *in ovo*, which was rapidly overcome by the acquisition of specific adaptive mutations in gene products of the internal segments of the virus. Thus, zoonotic transmissions of bat influenza viruses to other susceptible species, including humans, cannot be excluded. Nevertheless, we can show that the bat chimeric viruses fail to reassort with classical IAV, which might further reduce this risk. Although bat NP is fully compatible in virus-like particle formation assays of prototypic IAV, we surprisingly failed to rescue a H7N7 virus (A/SC35M) with a NP H1710 segment harboring the packaging signals of SC35M (NP-SC35M/bat). To study whether the coding sequences of bat NP might cause this incompatibility, we created NP-SC35M/bat segments coding for a series of SC35M/bat NP chimeras for functional analyses. These studies revealed that this incompatibility is due to genome-packaging deficiencies caused by bat NP-specific amino acids rather than the encoding nucleotide sequence. We therefore speculate that genome-packaging sequences co-evolved with distinct features in NP differently between prototypic IAV and bat influenza viruses.
Disclosure of Interest: None declared

Oral 127
EBOLA VIRUS ENTRY INTO MACROPHAGES
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Abstract: The Ebola virus (EBOV) epidemic in Western Africa underlines that ebolaviruses constitute a severe public health threat, against which new antiviral strategies must be developed. These endeavors require a thorough understanding of virus-host cell interactions. Our research is focused on viral entry into target cells, a process that is driven by the viral glycoprotein (GP) and that depends on viral usurpation of host cell factors. However, the factors required for viral entry into macrophages, the major viral targets, are incompletely defined and were investigated in the present study.
Employing vectors bearing EBOV-GP, we could show that entry into macrophages is not promoted by TIM-1, Axl and mannose-specific lectins, which can augment entry into certain cell lines. In contrast, NPC1, integrin αV and scavenger receptor A were required for efficient GP-driven entry and EBOV invasion of macrophages. Entry factor usage and cell tropism of vectors bearing GPs of EBOV, Mayinga

strain, 1976, and Makona variant, 2014, were comparable, suggesting that both viruses should be comparably susceptible to entry inhibitors. Exposure of macrophages to interferon induced expression of IFITM proteins, which can inhibit entry of several viruses, including ebola and influenza viruses. Indeed, entry driven by all ebola-, Marburg-, and cuevavirus GPs tested was inhibited by IFITMs and evidence was obtained that IFITMs might inhibit influenza and EBOV entry by different mechanisms. Moreover, we identified an IFITM3 mutant which augmented entry driven by Lloviu virus GP, suggesting that IFITMs regulate a process which can promote or repress viral entry. Finally, we found that IFITMs and antibodies can synergistically block viral entry, suggesting that antibodies with poor neutralizing activity in vitro might still unfold antiviral activity in vivo. Collectively, we defined cellular factors, which regulate macrophage infection by EBOV and might constitute targets for intervention.
Disclosure of Interest: None declared

Oral 128
BISBENZYLISOQUINOLINES ARE POTENT ANTI-EBOLA VIRUS COMPOUNDS TARGETING HOST CELL ENTRY
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Abstract: *Ebolaviruses* are highly pathogenic viruses, causing sporadic outbreaks of rapidly progressing hemorrhagic fever in humans and non-human primates. Although Ebola virus (EBOV) is currently epidemic in West Africa killing more than 10,000 people, there is no officially approved therapy or broadly active vaccine, making it a significant public health threat. Our recent work demonstrated that endosomal calcium channels, TPCs, played a critical role in EBOV host cell entry, and drugs targeting the proteins efficiently blocked infection, with tetrandrine, a bisbenzylisoquinoline alkaloid, being the most potent. Here we are investigating structural activity relationship by testing potency of available bisbenzylisoquinolines against EBOV infection. We found that most of them inhibited infection in Huh7 cells, with IC50 ranging from 50 nM up to 500 nM. They inhibited infection of VSV-based pseudotyped virus bearing EBOV glycoproteins as well as Marburg virus glycoproteins, suggesting that they are filovirus entry inhibitors. We also found that they blocked infection of human macrophages, the primary target of EBOV *in vivo*. One of the most potent compounds, cepharanthine, significantly enhanced survival of virus-challenged mice. Cepharanthine has been clinically used in Japan for decades and we have begun testing its anti-Ebola efficacy in non-human primates. Taken together, bisbenzylisoquinolines including tetrandrine and cepharanthine constitute a group of potent EBOV inhibitors and some of them are potential antiviral drugs. This study has been supported by Disruptive Technologies, and William and Ella Owens Medical Research Foundation.
Disclosure of Interest: None declared

Oral 129
HOST CELL FACTORS INVOLVED IN MARBURG VIRUS NUCLEOCAPSID TRANSPORT
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Abstract: Endosomal sorting complex required for transport (ESCRT) machinery supports efficient budding of Marburg virus (MARV) and many other viruses. Interaction between the ESCRT machinery and viral proteins is predominantly mediated by late domains. The PSAP late domain motif of the MARV nucleoprotein NP recruits the ESCRT-I protein tumor susceptibility gene 101 (Tsg101). A recombinant MARV encoding NP with a mutated PSAP late domain ($rMARV_{PSAPmut}$) was generated and showed attenuation by up to one log compared with recombinant wild-type MARV ($rMARV_{wt}$) and formed smaller plaques. Nucleocapsids in $rMARV_{PSAPmut}$-infected cells were more densely packed inside viral inclusions and more abundant in the cytoplasm than in $rMARV_{wt}$-infected cells. A similar phenotype was detected when MARV-infected cells were depleted of Tsg101. Live-cell imaging analyses revealed that Tsg101 accumulated in inclusions of $rMARV_{wt}$-infected cells and was co-transported together with nucleocapsids. In contrast, $rMARV_{PSAPmut}$ nucleocapsids did not display co-localization with Tsg101, had significantly shorter transport trajectories, and migration close to the plasma membrane was

severely impaired, resulting in reduced recruitment into filopodia, the major budding sites of MARV. We further show that the Tsg101 interacting protein IQGAP1, an actin cytoskeleton regulator, was recruited into inclusions and to individual nucleocapsids together with Tsg101. Moreover, IQGAP1 was detected in a contrail-like structure at the rear end of migrating nucleocapsids. Down regulation of IQGAP1 impaired release of MARV. These results indicate that the PSAP motif in NP, which enables binding to Tsg101, is important for the efficient transport of nucleocapsids to the sites of budding.
Disclosure of Interest: None declared

Oral 130

HOW DO PARAMYXOVIRUSES ESTABLISH PROLONGED/PERSISTENT INFECTIONS? LESSONS FROM STUDIES ON PARAINFLUENZA VIRUS TYPE 5.

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Abstract: Although paramyxoviruses primarily cause acute infections, under certain circumstances paramyxoviruses can establish prolonged or persistent infections *in vivo*. However little is understood at the molecular level as to how persistent papramyxovirus infections are established and maintained. In a prototypical response to an acute infection it would be expected that the adaptive immune response, in particular the CTL response, would eliminate all infected cells within a few weeks. In prolonged/persistent infections this does not happen. One contributing possibility in the establishment of prolonged/persistent infections may be that in at least some cells virus replication and protein synthesis is dampened down to such a degree that the adaptive immune response cannot recognize and kill such cells. Using parainfluenza virus type 5 (PIV5) of tissue culture cells as our model, we have begun to investigate the role of viral factors and host cell defense mechanisms (the interferon response) that can influence the establishment and maintenance of persistent infections. These studies have revealed that; i) the IFN response can aid in the establishment of persistence but is not required for maintenance, ii) the overall level of virus transcription in persistently infected cells is significantly less than in acutely infected cells, iii) the viral transcriptional gradient varies under different conditions, iv) there is cellular heterogeneity in the level of virus protein synthesis within a persistently infected cell populations, and v) virus activity in clones of cells fluxes between active and repressed states. These studies challenge our understanding of the control of paramyxovirus transcription and replication, and raise the questions as to whether at least some paramyxoviruses have evolved specific mechanisms to establish prolonged/persistent infections *in vivo* in order to maintain a reservoir of infected individuals within a population.
Disclosure of Interest: None declared

Oral 131

IDENTIFICATION OF EBOLA VIRUS AND SUDAN VIRUS DEFECTIVE VIRAL GENOMES IN SERUM OF EXPERIMENTALLY INFECTED NONHUMAN PRIMATES

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Abstract: Defective viral genomes (DVGs) are sub genomic molecules that have incomplete coding capacity and propagation is dependent on helper viruses. DVGs arising both *in vitro* and *in vivo* have been described for a variety of viruses, during persistent and acute infections. DVGs have been shown to influence pathogenesis, possibly through a robust induction of interferon or suppression of innate immune response. Ebola virus (EBOV) DVGs have been observed *in vitro* following serial passage at high MOI, but currently there is no evidence of DVGs during *in vivo* EBOV infections. Recently, ultra deep sequencing has been utilized as a tool to identify the presence of DVGs. Briefly, following sequencing and bioinformatic analysis, the observed read depth at each locus is related to the abundance of that particular locus in the original sample. We sequenced EBOV and Sudan virus (SUDV) from serum harvested from experimentally infected nonhuman primates (NHPs) that succumbed to disease. Surprisingly, there was a dramatic increase in read depth associated with the

5' end of the viral genomes that is characteristic of some forms of DVGs. Further analyses revealed that this 5' enrichment pattern was not detected in the viral inoculum. Interestingly, the DVGs appeared only after five days post infection. Further analyses are underway to characterize and quantify the DVGs, and investigate their role in virus infection.
Disclosure of Interest: None declared

Oral 132
DIFFERENTIAL INHIBITION OF MACROPHAGES AND DENDRITIC CELLS BY PATHOGENIC AND NONPATHOGENIC ARENAVIRUSES

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Abstract: Resting macrophages and dendritic cells (DCs) recognize danger signals upon viral infections and undergo a maturation process to become immune effector and accessory cells. The mechanisms by which macrophages and DCs are activated and/or inhibited by viral infections have not been well understood for many viruses. Arenaviruses are negative-strand RNA viruses that can cause hemorrhagic fever diseases in humans with limited treatment options and infect macrophages and DCs as early target cells. We report here the first mechanistic characterization of how arenaviruses affect the DC/macrophage maturation. Pathogenic but not nonpathogenic arenaviruses can inhibit the maturation and functions of human monocyte-derived macrophages, due to the pathogenic Z protein N-terminal domain (NTD) that we have recently shown to selectively inhibit the intracellular RIG-i-like receptor (RLR) pathway (Xing et al., J Virol 2015). In contrast, both pathogenic and nonpathogenic arenaviruses inhibit the maturation of human monocyte-derived DCs via the conserved NP RNase activity that we have previously shown to degrade immunostimulatory RNAs and block the production and release of type I IFNs into the cell culture milieu (Qi et al., Nature 2010; Jiang et al., J Biol Chem 2013). Exogenous addition of IFNs can overcome DC inhibition by arenaviruses but cannot reverse macrophage inhibition by pathogenic Z NTD. Using recombinant Pichinde virus (rPICV) carrying NP RNase mutant and/or a pathogenic Z NTD, we show that arenaviruses mainly employ NP RNase activity to inhibit DCs and rely on pathogenic Z to inhibit macrophages. The cell type-dependent immune suppression activities by pathogenic Z and NP RNase are further demonstrated by lentiviral vector-mediated expression of individual proteins in human primary macrophages and DCs. Our results provide important insights into arenavirus-induced immune suppression and pathogenesis and reveal novel knowledge of virus-macrophage/DC interactions.
Disclosure of Interest: None declared

Oral 133
ACUTE LUNG INJURY RESULTS FROM INNATE SENSING OF VIRUSES BY AN ER STRESS PATHWAY

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Abstract: Incursions of new pathogenic viruses into humans from animal reservoirs are occurring with alarming frequency. The molecular underpinnings of immune recognition, host responses, and pathogenesis in this setting are poorly understood. We studied pandemic influenza viruses to determine the mechanism by which increasing glycosylation during evolution of surface proteins facilitates diminished pathogenicity in adapted viruses. ER stress during infection with poorly glycosylated pandemic strains activated the unfolded protein response (UPR), leading to inflammation, acute lung injury, and mortality. Within this cascade of events, the activation of ER stress kinases and subsequent induction of inflammatory pathways such as MAP kinase signaling was causative for innate immune

responses. Seasonal strains or viruses engineered to mimic adapted viruses displaying excess glycans on the hemagglutinin (HA) did not cause ER stress, allowing preservation of the lungs and survival. We propose that ER stress resulting from recognition of non-adapted influenza viruses with poor glycosylation on the HA head is utilized to discriminate “non-self” at the level of protein-processing and to activate immune responses, with unintended consequences on pathogenesis. Interestingly, these differences were not due to alterations in viral replication or accumulation of viral material, indicating a distinct mechanism from traditional virus UPR activation pathways which cause stress through overloading of the ER with unfolded proteins. Understanding the mechanism of ER stress mediated induction of innate immunity should improve strategies for treating acute lung injury from zoonotic viral infections.
Disclosure of Interest: None declared

Oral 134

CELLULAR RNA EDITING ENZYME ADAR1 INTERACTS WITH INFLUENZA A VIRUS PROTEINS DURING INFECTION AND ENHANCES VIRUS REPLICATION THROUGH INHIBITION OF IRF3

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Abstract: Cellular RNA editing enzyme adenosine deaminase acting on RNA 1 (ADAR1) is an interferon-inducible protein reported to act as a pro-viral factor for several viruses, including HIV-1, measles and influenza A virus. ADAR1 is an RNA binding protein which catalyses the conversion of adenosine to inosine in double stranded RNA. Evidence of non-specific editing of influenza and measles virus RNA has been found, although the significance of this is unknown. Influenza NS1 interacts with ADAR1 and infection causes re-localisation of ADAR1 from the nucleoplasm to the nucleolus. Here, we show that NS1 is both necessary and sufficient for this re-localisation. A mutant A/PR/8/34 NS1 protein which cannot bind to RNA or supress the activation of interferon regulatory factor 3 (IRF3) also failed to induce ADAR1 relocalisation. siRNA knock-down of ADAR1 decreased A/PR/8/34 replication, but also induced IRF3 phosphorylation even in the absence of virus infection. This suggested the hypothesis that ADAR1 is pro-viral through down-regulation of IRF3 activation, with the corollary that this mechanism would not apply to strains of virus that control innate responses downstream of IRF3 phosphorylation. Supporting this, replication of A/Udorn/72 (a virus that unlike A/PR/8/34, does not control IRF phosphorylation) was insensitive to ADAR1 knock-down. We conclude that ADAR1 supresses the phosphorylation and activation of IRF3, thereby enhancing the replication of influenza strains sensitive to the IRF3 signalling pathway.
Disclosure of Interest: None declared

Oral 135

MECHANISMS OF PARAINFLUENZA VIRUS DISSEMINATION, PATHOGENESIS, AND TRANSMISSION IN A MOUSE MODEL

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Abstract: Respiratory viral infection is a dynamic process. Known host-pathogen interactions include early host innate responses, dissemination within the respiratory tract, host- and virus-based pathogenic responses, transmission to a new host, adaptive immune clearance, and reinfection if immunity is incomplete, wanes, or becomes suppressed. To study host-pathogen interactions in individual animals, we developed the first non-invasive bioluminescence imaging system for a negative-strand RNA virus using Sendai virus in a mouse model. Bioluminescence imaging revealed that contact transmission involves the transit of infectious particles from the donor upper respiratory tract (URT) to the recipient URT. In contrast, infections in recipient mice after airborne transmission are anisotropic, initiating in the nasopharynx or trachea and then remaining localized or disseminating. Local immune responses and protection from reinfection are determined in part by the magnitude of primary infection within a given respiratory tissue. A distinction between URT and LRT infection extends to transmission

and pathogenesis. Transmission localizes to the URT while pathogenesis is determined by the magnitude of infection in the lungs and the resultant host response. We developed an immunocompromised host model by depleting lymphocytes with cyclophosphamide as is done in leukemia therapy. Both immunocompetent and lymphocyte-depleted mice supported high levels of infection in the URT, while infection in immunocompromised animals spreads deeper and to a greater extent in the lungs. Restoration of neutrophils by G-CSF treatment starting 2 d.p.i. delays spread of infection into the lungs without accelerating clearance. T-cell transplant therapy is currently being studied. Overall, bioluminescence imaging in living animals illuminates a dichotomy between the URT and LRT and also reveals a novel determinant of infection, pathogenesis, and immunity: the initial site and dose of inoculation.
Disclosure of Interest: None declared

Oral 136
INTERFERON-LAMBDA CONTROLS THE SPREAD OF INFLUENZA VIRUSES FROM THE UPPER RESPIRATORY TRACT TO THE LUNGS AND RESTRICTS VIRUS TRANSMISSION IN MICE
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Abstract: Previous infection studies have invariably assigned a minor role to interferon-λ (IFN-λ) in the control of influenza viruses in the lungs of mice. However, we came to a completely different conclusion when performing experiments in which we administered the virus selectively to the upper respiratory tract. Under such experimental conditions, influenza virus replication was largely restricted to the nasal tissue in wild-type mice. In contrast, in mice lacking functional receptors for IFN-λ, influenza virus was frequently observed to spread to the lungs. Interestingly, the virus spread more frequently to the lungs in mice lacking functional receptors for IFN-λ than in mice lacking functional receptors for IFN-α/β, indicating that IFN-λ is more critical for virus restriction at the mucosal barrier.
We further observed that the Udorn (H3N2) strain of influenza A virus is readily transmitted upon contact among mice lacking functional receptors for both IFN-α/β and IFN-λ. Contact transmission of this virus was also efficient when mice lacking functional receptors for IFN-λ were employed. In contrast, wild-type mice and mice lacking functional receptors for IFN-α/β transmitted the virus only with greatly reduced efficacy. These findings demonstrate that the crucial role of IFN-λ in influenza virus restriction is only getting apparent under experimental conditions which imitate the physiological route of virus infection.
Disclosure of Interest: None declared

Oral 137
AN EPITHELIAL INTEGRIN REGULATES THE AMPLITUDE OF PROTECTIVE LUNG INTERFERON RESPONSES TO INFLUENZA AND SENDAI VIRUS INFECTIONS
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Abstract: Integrins facilitate intercellular movement and communication. Unlike the promiscuous activities of many integrins, ß6 integrin is restricted to damaged and developing epithelia and partners exclusively with integrin aV to modulate acute lung injury (ALI). Given that ALI is a complication of respiratory infection, we used mice lacking ß6 integrin (ß6 KO) to probe the role of the epithelial layer in controlling the lung microenvironment during viral infection. Surprisingly, we found ß6 KO mice were resistant to lethal infections with influenza and Sendai viruses, and displayed resistance to Streptococcus pneumoniae infection alone and after prior influenza infection, the key cause of death from influenza. Even obese mice, which are extremely susceptible to developing severe and often lethal influenza infection, showed enhanced protection when ß6 was inhibited. Resistance in the absence of epithelial ß6 integrin was caused by intrinsic priming of the lung microenvironment by type I interferons through a mechanism involving transforming growth factor-ß regulation and cross-talk with alveolar macrophages. We propose that during infection, the upregulation of ß6 on epithelia leads to activation of lung localized TGF-ß1 activity, which then suppresses the production of interferons by alveolar

macrophages providing an advantage to the pathogen. Our studies provide a novel model to understand how the epithelia may regulate the lung microenvironment during infection. Further, targeting ß6 integrin and/or TGF-ß1 activity may provide a means to improve outcomes in lung microbial infections.
Disclosure of Interest: None declared

Oral 138
THE INFLUENZA A VIRUS PROTEIN NS1 INHIBITS NLRP3 INFLAMMASOME-MEDIATED INTERLEUKIN-1BETA SECRETION

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Abstract: Recognition of viral infections through the innate immune system plays a key role in limiting early viral replication and triggering the adaptive immune responses. We previously demonstrated that sensing of cellular distress induced by the influenza A virus M2 protein triggered the formation of the nucleotide-binding oligomerization domain (NOD)-like receptor family, pyrin domain-containing 3 (NLRP3) inflammasome by recruiting apoptosis-associated speck-like protein containing a CARD (ASC) and pro-caspase-1. This event leads to the activation of caspase-1 that cleaves immature forms of the proinflammatory cytokines, IL-1β and IL-18 for secretion. The nonstructural protein 1 (NS1) of influenza virus is known to inhibit innate antiviral responses by antagonizing the type I interferon response. However, the function of NS1 in regulating the NLRP3 inflammasome remains to be defined. Here, we demonstrate that the NS1 protein of influenza virus interacts with NLRP3 and its adaptor protein ASC. As a result, NS1 inhibited the formation of ASC specks required for full activation of the inflammasome and suppressed inflammasome-mediated IL-1β secretion. Our results reveal a mechanism by which the NS1 protein of influenza virus directly suppresses NLRP3 inflammasome-mediated IL-1β secretion.
Disclosure of Interest: None declared

Oral 139
MAPPING OF A REGION IMPORTANT FOR SHUT-OFF ACTIVITY IN THE PA-X PROTEIN OF INFLUENZA A VIRUS

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Abstract: The influenza A virus genome comprises eight segmented RNAs from which at least 17 viral proteins are expressed in virus-infected cells. The PA segment encodes the viral polymerase protein PA and the novel viral protein PA-X. PA-X is expressed as the result of a ribosomal frameshift. Thus, PA-X consists of the N-terminal endonuclease domain of PA (191 amino acid residues) and a C-terminal PA-X-specific domain (61 amino acid residues). PA-X decreases host and viral mRNA accumulation via its shut-off activity, which dependents on its N-terminal endonuclease activity. Although PA has the N-terminal endonuclease domain, it has lower shut-off activity than PA-X, suggesting that other regions of PA-X are also important for the shut-off activity.
Unlike PA-X, the N-terminal 252 amino acid residues of PA (PA_252) have little shut-off activity, indicating that both the N-terminal endonuclease activity and the C-terminal PA-X-specific region are important for the shut-off activity of PA-X. To determine the amino acid residues in the PA-X-specific region that are important for its shut-off activity, we constructed PA-X mutants and evaluated their shut-off activities in a luciferase assay. We found that the N-terminal 15 PA-X-specific amino acid residues, particularly 6 basic amino acid residues, are important for the shut-off activity. These 6 basic amino acids enabled a PA_252 to suppress protein expression at a level comparable to that of wild-type PA-X. These residues were also highly conserved among all influenza A viruses. Our findings support the concept that these basic amino acids are required for the shut-off activity of PA-X.

Disclosure of Interest: None declared

Oral 140

THE FOCAL ADHESION KINASE IS INVOLVED IN RABIES VIRUS INFECTION THROUGH ITS INTERACTION WITH THE VIRAL PHOSPHOPROTEIN P

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Abstract: Rabies is a fatal human disease caused by viruses of the genus Lyssavirus belonging to the family Rhabdoviridae. The single-stranded negative-sense RNA of the rabies virus encodes five proteins : nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G), and a polymerase (L). To maintain efficient virus replication, some of them are multifunctional, such as the phosphoprotein P. This viral protein plays an essential role in viral transcription and replication and has been identified as an interferon antagonist. As a successful viral infection relies on the recruitment of cellular factors, we have searched for P partners using a yeast two-hybrid screen. This approach allowed us to identify the focal adhesion kinase (FAK) as a cellular partner of P. The binding involved the 106-to-131 domain, corresponding to the dimerization domain of P and the C-terminal domain of FAK containing the proline-rich domains PRR2 and PRR3. The P-FAK interaction was confirmed in infected cells by coimmunoprecipitation and colocalization of FAK with P in Negri bodies (NB), which are the inclusion bodies formed by viral infection and considered as the viral factories. By alanine scanning, we identified a single mutation in the P protein that abolishes this interaction. The mutant virus containing a substitution of Ala for Arg in position 109 in P (P.R109A), which did not interact with FAK, is affected at a posttranscriptional step involving protein synthesis and viral RNA replication. Furthermore, FAK depletion inhibited viral protein expression in infected cells. Altogether our data provide the first evidence of an interaction of RABV with FAK that positively regulates infection. Interestingly, the positive role of FAK in viral RNA synthesis is correlated with its interaction with P and association with the NBs, namely the sites of viral transcription and replication.

Disclosure of Interest: None declared

Oral 141

NUCLEAR FUNCTION OF THE RESPIRATORY SYNCYTIAL VIRUS MATRIX PROTEIN

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Abstract: Respiratory syncytial virus (RSV) matrix protein (M) is a major virion structural protein and plays a key role in virus assembly. We have shown previously that RSV M is localized in the nucleus of infected cells early in infection. Using various *in vitro* and transfected cell systems, we have elucidated the nuclear localization signal (NLS) and import pathway of M as well as the CRM-1 dependent nuclear export signal (NES) (1, 2). We now report on our ongoing work on the nuclear function of M and the role of phosphorylation in regulating its nuclear localization; both essential for optimal virus replication and assembly.

The bipartite NLS of M consists of two basic regions separated by 14 residues, KK^{156} and RNK^{172}. Together, these motifs are essential for nuclear import and function to inhibit cellular transcription via complex interactions including binding to DNA and probably facilitate nuclear retention of M. RSV carrying a mutation in these residues has deficient replication kinetics. The previously described RNA binding regions (3) are not involved in inhibition of host transcription as elucidated by in-cell transcription assays in transfected cells expressing mutant M. Significantly, a mutant RSV deficient in DNA binding, has delayed replication kinetics, does not inhibit transcription at early times in cell culture and is severely restricted *in vivo*. The nucleocytoplasmic transport of M is regulated by CK2 phosphorylation

at S^{95} and T^{205}; mutation of both residues to non-phophorylatable alanine results in loss of regulated nuclear transport and reduced titres in cell culture.
Our data show that the regulated nuclear localization of M protein is essential for initial inhibition of cellular transcription in RSV infection which appears to be critical for optimal replication of the virus.

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Disclosure of Interest: None declared

Oral 142

INFLUENZA A VIRUS PROTEIN PB1-F2 TRANSLOCATES INTO MITOCHONDRIA VIA TOM40 CHANNELS AND IMPAIRS INNATE IMMUNITY.

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Abstract: Mitochondria contribute to cellular innate immunity against RNA viruses. Mitochondrial-mediated innate immunity is regulated by signalling molecules that are recruited to the mitochondrial membrane, and depends on the mitochondrial inner membrane potential (Δψm). Here we examine the physiological relevance of Δψm and the mitochondrial-associating influenza A viral protein PB1-F2 in innate immunity. When expressed in host cells, PB1-F2 completely translocates into the mitochondrial inner membrane space via Tom40 channels, and its accumulation accelerates mitochondrial fragmentation due to reduced Δψm. By contrast, PB1-F2 variants lacking a C-terminal polypeptide, which is frequently found in low pathogenic subtypes, do not affect mitochondrial function. PB1-F2-mediated attenuation of Δψm suppresses the RIG-I signalling pathway and activation of the NLRP3 inflammasome. PB1-F2 translocation into mitochondria strongly correlates with impaired cellular innate immunity, making this translocation event a potential therapeutic target.

Disclosure of Interest: None declared

Oral 143

A NOVEL HOST FACTOR PROMOTES THE NUCLEAR EXPORT OF INFLUENZA VIRAL RIBONUCLEOPROTEIN COMPLEXES

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Abstract: Influenza virus RNA segments (vRNAs) associate with viral nucleoprotein (NP) and three polymerase subunits (PB2, PB1, PA) to form viral ribonucleoprotein (vRNP) complexes. After vRNP replication in the nucleus, the vRNP interacts with the viral M1 and NS2 proteins and is exported to the cytoplasm as a vRNP-M1-NS2 complex. However, the export mechanisms of vRNP are not fully understood. Here, we found that a novel PB2-interacting protein, KIAA0664, is involved in vRNP nuclear export. KIAA0664 interacted with PB2 and was translocated to the nucleus in infected cells and in PB2-expressing cells, even though KIAA0664 is normally located only in the cytoplasm. RNAi-mediated depletion of KIAA0664 led to a significant reduction in influenza virus progeny. Interestingly, KIAA0664 suppression inhibited the nuclear export of vRNP, M1, and NS2, causing their nuclear accumulation; however, viral protein and viral RNA expression levels were not affected. These results suggest that KIAA0664 is involved in vRNP nuclear export. Experiments are underway to further analyze the localization of KIAA0664 and its effects on vRNP nuclear export.

Disclosure of Interest: None declared

Oral 144

UNRAVEL MOLECULAR MECHANISMS OF INFLUENZA NS1 PROTEIN AND HOST CELL PKR INTERACTION BY USING LIVE CELL MULTICOLOR FLIM-FRET AND ANISOTROPY

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Abstract: Influenza A virus is the causative agent of the annual Flu epidemic and global pandemics. Of crucial interest for combating infection is the interaction between the main virulence factor NS1 (non structural protein 1) and the host innate immune system, more specifically to the immune effector protein PKR (protein kinase R). For replication, influenza viruses have to block PKR otherwise PKR will bind viral RNA leading e.g. to global stop of translation. Both proteins PKR and NS1 consist of a double stranded RNA (PKR) or a more general RNA binding domain (NS1), it is also known that both proteins can directly interact with each other. Since decades inhibition of PKR by NS1 is well known, but the molecular mechanism of inhibition still has to be unraveled. To address the latter, we applied live cell microscopy techniques like multicolor FLIM-FRET (fluorescence lifetime imaging with Förster resonance energy transfer) and anisotropy using a combination of three fluorophores to analyse interaction between PKR, NS1 and dsRNA simultaneously.
Our results show first that dsRNA mimicking viral RNA is an essential mediator for interaction between viral NS1 protein and host cell PKR. Second dimerization of NS1 is essential for interaction with PKR and with dsRNA. Third we found that transfecting NS1 leads to an increase of non-interacting PKR in presence of dsRNA, in combination with a change in distance between PKR and dsRNA. Our study not only identified for the first time organisational principles of the PKR-NS1-dsRNA complex in a cell-based FLIM-FRET system but also let us propose the novel hypothesis that both proteins are competing for RNA binding sites, so that NS1 is able to mask viral RNA allowing virus replication. In upcoming experiments we will perform microscale thermophoresis measurements to detect changes in the binding constant between PKR and dsRNA in presence of various NS1 constructs.
Disclosure of Interest: None declared

Oral 198

PROLONGED MULTIPHASIC B AND T CELL RESPONSES DURING MEASLES VIRUS INFECTION AND RNA CLEARANCE

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Abstract: Prolonged Multiphasic B and T cell Responses during Measles Virus Infection and RNA Clearance

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Measles is an acute viral disease associated both with immune suppression and development of life-long immunity. Clearance of measles virus (MeV) involves rapid elimination of infectious virus during the rash and slow elimination of viral RNA from blood and lymphoid tissue after the rash. To better characterize clearance of viral RNA and immune responses during recovery, we analyzed the cellular sites of RNA persistence and the appearance and function of MeV-specific T cells and antibody secreting cells in rhesus macaques over 6 months after infection with wild type MeV. MeV RNA was present in peripheral blood mononuclear cells and lymph node cells. IFN-g and IL-17-producing cells appeared in circulation in 3 waves approximately 2-3, 8 and 18-24 weeks after infection. The largest numbers of IFN-g-producing cells were present early while the largest numbers of IL-17-producing cells appeared late. Intracellular cytokine staining showed that IL-17-producing cells were both $CD4^+$ (Th17) and $CD8^+$ (Tc17) cells that expressed transcription factor RORgt and showed specificity for both the hemagglutinin and nucleocapsid proteins. Plasmablasts producing MeV-specific antibody appeared in

blood 2 weeks after infection, peaked at 8 weeks and were still present at 6 months. Therefore, the evolution of the immune response during MeV clearance is prolonged with a potentially important and previously unrecognized role for IL-17-producing cells.
Disclosure of Interest: None declared

Oral 199
IMMUNE BIOMARKERS OF EBOLA VIRUS DISEASE IN HUMANS
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Abstract: The current Ebola virus disease (EVD) outbreak in west Africa has highlighted the need for identification of biomarkers of EVD outcome in humans. Clinical monitoring of EVD patients in treatment centers, as well as evaluation of the therapeutic effect of experimental drugs and vaccines, are just two examples of interventions requiring reliable markers of disease progression. Here we present data obtained in Guinea since the beginning of the outbreak, and in the context of the diagnostic activities of the European Mobile laboratory (www.emlab.eu). Analysis of immune parameters revealed significant upregulation of cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), in CD8 T cells of acute EVD patients. CTLA-4 upregulation was statistically correlated with poor outcome and high viremia, and was reversed during recovery of an EVD case imported into Europe for medical treatment. To determine the relevance of these findings for the pathophysiology of EVD in Africa, we built an immunology laboratory at Donka Hospital in Conakry, Guinea, and investigated the kinetics of the T cell immune response in EVD patients receiving medical care at the Ebola treatment center (ETC) in Coyah. Using state of the art multiparametric flow cytometry, we evaluated the kinetics of the immune response against EVD in patients from the time of admission to the ETC until death or discharge.
Our results indicate that fatal EVD is associated with strong immunosuppression, characterized by lack of CD8 T cell degranulation and proliferation, and a significant upregulation of CTLA-4. Conversely, recovery from EVD is associated with downregulation of CTLA-4 in CD8 T cells, which is correlated with vigorous CD8 T cell proliferation, cytotoxic activity and virus clearance from blood. Our findings highlight the importance of CD8 T cell immunity for EVD survival, and point out at CTLA-4 as a novel immune biomarker of EVD outcome.
Disclosure of Interest: None declared

Oral 200
DISSECTING THE DYNAMICS OF INFLUENZA VIRUS TRANSMISSION.
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Abstract: The efficiency of Influenza transmission through the air may be determined by: 1. Replication in the donor host and emission of virus into the environment. 2. Survival of infectious particles in the environment. 3. Ability to establish of a new infection in the recipient host. The dynamics of these processes can inform public health policy: for example in stage 1, at what time during their infection is a host most contagious?
We previously showed in the ferret model, a pH1N1 2009 virus was transmitted more efficiently to animals exposed to donors early in infection (day 1-2) than later (day 5-6) (Roberts et al. 2013). Due to

the large numbers of animals required, we did not map the contagious period more accurately. We have now developed a methodology for measuring the infectious virus shed into the air from infected hosts. We measured airborne virus from infected ferrets at frequent intervals during 7 days. This allows us to understand the dynamics of shedding, the role of clinical signs and how genetic variations affect contagiousness, adding a new dimension to the ability to compare transmissibility of viruses in the ferret model.
The ability of an influenza virus to transmit between ferrets correlates with low ferret infectious dose, 10 infectious particles or less (Roberts et al. 2012). Mutation of the sialic receptor specificity increased the FID50 to above 100 infectious units, abrogating transmissibility. The dose at transmission that initiates infection in the recipient host is low, so a tight evolutionary bottleneck might exist at transmission. To investigate, we infected ferrets with a mixture of tagged influenza viruses. For respiratory droplet transmission the bottleneck is small and a mean number of 3 virions are transmitted. The dose during contact transmission is more variable and sometimes higher, allowing mixtures of viruses to be transmitted, so by direct contact, viruses carrying drug resistance were transmitted even with a small fitness cost.
Disclosure of Interest: None declared

Oral 201
SHED GP OF EBOLA VIRUS RENDERS MONOCYTES PERMISSIVE TO INFECTION
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Abstract: Ebola virus (EBOV), a member of the Filoviridae family, causes lethal hemorrhagic fever in man and monkeys, displaying up to 90% mortality rates. During EBOV infection a significant amount of surface glycoprotein GP is shed from infected cells in a soluble form (shed GP) that is readily detected in the blood of human patients and experimentally infected animals.
We have previously shown that shed GP can lead to activation of non-infected macrophages and DCs, however its role in the potential activation of monocytes, the most abundant Antigen Presenting Cell (APC) type in the blood, has still not been determined. Here we show for the first time that, EBOV shed GP is not only capable of binding to monocytes but also of inducing monocyte differentiation into macrophage-like cells and leading to release of proinflammatory cytokines. Importantly, monocytes treated with shed GP were also shown to downregulate anti-viral factors including IFTIM and to upregulate crucial factors for viral entry, such as Cathepsine B and the EBOV intracellular receptor NPC1. Furthermore, using infectious EBOV, and contrary to data usually seen with untreated monocytes, we now highlight the ability of shed GP to render monocytes permissive to Ebola virus infection.
Overall our data show that Ebola virus shed GP is able to modulate the phenotype of monocytes and importantly to extend the range of EBOV cellular targets through rendering monocytes permissive to infection, thus contributing to the EBOV high pathogenicity.
Disclosure of Interest: None declared

Oral 202
HOST AND VIRAL DETERMINANTS OF PROTEOLYTIC ACTIVATION OF INFLUENZA VIRUSES
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Abstract: Cleavage of the hemagglutinin (HA) protein of influenza virus is essential for virus infectivity, and it is mediated by a host cell protease(s) *in vivo*. Using TMPRSS2 knockout (KO) mice we and other two groups recently demonstrated that TMPRSS2 expressed in the respiratory tract is the main protease for the HA cleavage of H1N1 and H7N9 influenza A virus (IAV) strains in mice (Sakai et al. J Virol 2014; Hatesuer et al. PLoS Pathog 2013; Tarnow et al. J Virol 2014). Our group and Hatesuer et

al. showed that TMPRSS2 is also essential for proteolytic activation of H3N2 IAVs, while Tarnow et al. showed that it is not critical for activation of H3N2 IAV.
We demonstrated that after passages in TMPRSS2 KO mice, H3N2 IAV, which originally failed to be activated well in TMPRSS2 KO mice, became highly pathogenic for the mice by loss of an oligosaccharide at the HA stalk region. These data demonstrated that oligosaccharide modifications at the HA stalk region modulate protease specificity of IAV.
We also analyzed influenza B virus (IBV) strains. A mouse-adapted IBV replicated efficiently in TMPRSS2 KO mice as well as in wild-type mice. Non mouse-adapted human isolates of IBV also spread, albeit less efficiently than the mouse-adapted IBV, similarly in TMPRSS2 KO and wild-type mice. These data demonstrated that, unlike IAV, IBV intrinsically employs a host protease different from TMPRSS2 for HA activation *in vivo*. The clear difference in protease specificity may partly contribute to the host range differences between IAV and IBV.
Disclosure of Interest: None declared

Oral 225
ENGINEERED ONCOLYTIC NEWCASTLE DISEASE VIRUS SHOWS GREAT PROMISES FOR VIROTHERAPY
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Abstract: The clinical development of mesogenic Newcastle disease virus (NDV) as oncolytic virus has been hampered by its select agent status due to its pathogenicity in chickens. We have genetically engineered the mesogenic 73T strain to reduce its chicken virulence while maintaining its oncolytic potency. This was achieved by modification of the fusion protein cleavage site and extension of the HN-L intergenic sequence. Sequence insertion at the HN-L junction down-regulates overall viral gene expression and reduces viral titers in embryonated chicken eggs and avian cells. Interestingly, although the HN-L insertion results in the reduced L polymerase protein, all the other viral genes are upregulated in mammalian cells and viral replication in mammalian cells is not affected. In addition to attenuating the virulence in birds we have developed a system whereby we can insert transgenes to enhance the therapeutic potential of NDV. As a proof of concept we have inserted granulocyte/macrophage colony-stimulating factor (GM-CSF) to enhance the immune modulatory properties of NDV through enhanced antigen presentation. The addition of a therapeutic transgen has no effect on viral growth or oncolytic potential.
We show that NDV$^{GM\text{-}CSF}$ selectively replicates in and kills a wide variety of human tumor cell lines. NDV$^{GM\text{-}CSF}$ administered systemically selectively replicates in tumors as shown by luciferase reporter virus imaging and by viral titration, which leads to significant tumor growth inhibition in a HT1080 fibrosarcoma xenograft model. NDV treatment causes profound changes in the immune suppressive microenvironment resulting in long-lasting anti-tumor immune responses in syngeneic tumor mouse models. The therapeutic efficacy of recombinant NDV$^{GM\text{-}CSF}$ in syngeneic mouse models is greatly enhanced when combined with immune checkpoint inhibitors. Our data demonstrated that the NDV is a promising oncolytic virus for further clinical development.
Disclosure of Interest: None declared

Oral 226
PAN-RESISTANCE MECHANISM OF RESPIRATORY SYNCYTIAL VIRUS AGAINST DIVERSE SMALL-MOLECULE ENTRY INHIBITORS
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Abstract: Respiratory syncytial virus (RSV) is responsible for the majority of infant hospitalizations due to viral disease. Despite its clinical importance, no vaccine against RSV disease or effective antiviral therapy is available. Several classes of RSV entry inhibitors are considered for human use and currently in preclinical or clinical development. We have identified a small-molecule RSV entry inhibitor targeting

the viral fusion (F) protein. Biochemical, structural, and functional characterization of the inhibitor spotlighted two F microdomains involved in viral escape from inhibition. Mutations in these domains caused unique pan-resistance against the compound and the panel of chemically unrelated entry inhibitors considered for human therapy. Resistance hot-spots are located in physical proximity only in the prefusion form of RSV F. We demonstrate that escape mutations lower the barrier for prefusion F triggering, mediating resistance through an accelerated RSV entry kinetics. One resistant RSV recombinant remained fully pathogenic in a mouse model of RSV infection, challenging the clinical potential of currently developed RSV entry inhibitors. To circumvent F pan-resistance in drug discovery campaigns, we have developed replication-competent influenza virus and RSV reporter strains expressing nano and firefly luciferase, respectively. The RSV reporter carries the signature resistance mutation. Co-infection of cells with these strains allows the simultaneous identification of ortho- and paramyxovirus-specific hits and broad-spectrum antivirals. Miniaturized to 384-well plate format and fully validated, we have applied this protocol to a 60,000-entry library. After counterscreens, a panel of confirmed influenza virus-specific, RSV-specific, and broad-spectrum hits with sub-micromolar antiviral potency emerged. Mechanistic characterization and lead development are ongoing, but pilot analyses confirmed that none of the hit classes is sensitive to RSV F pan-resistance.
Disclosure of Interest: None declared

Oral 227
INHIBITION OF THE ANDES HANTAVIRUS FUSION PROCESS
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Abstract: Rodent-borne hantaviruses can cause hantavirus pulmonary syndrome or hemorrhagic fever with renal syndrome in humans. To enter cells, hantaviruses fuse their envelope membrane with host cell membranes of the endocytic pathway. Previously, we showed that the Gc glycoprotein is the viral fusion protein and that it shares characteristics with class II fusion proteins. The ectodomain of class II fusion proteins is composed of three domains rich in beta sheets. A stem region connects these three domains with a transmembrane anchor to the viral membrane. These fusion proteins can be inhibited by the addition of exogenous domain III (DIII) and the stem region which are thought to interact against the core trimer during the transition from the pre-fusion to the post-fusion structure of the fusion protein. Here, we predicted and synthesized DIII with and without the predicted stem region of Gc from Andes hantavirus (ANDV) based on our previous model structure. Gc DIII was soluble, presented disulfide bridges and beta sheet secondary structure, confirming the *in silico* model. Coincubation of ANDV with the exogenous Gc fragments lead a 60% decrease of viral infection and complete inhibition was reached by the exogenous Gc fragments when the virus entered the cell by fusion with the plasma membrane. As a whole, the results show that Gc fragments blocked viral cell entry at a late stage of the membrane fusion process, after the trimerization of Gc and before the hemifusion of membranes.
Funding: FONDECYT 1140050 and CONICYT basal funding PFB-16
Disclosure of Interest: None declared

Oral 228
INHIBITORS OF CELLULAR KINASES WITH BROAD-SPECTRUM ANTIVIRAL ACTIVITY FOR HEMORRHAGIC FEVER VIRUSES
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Abstract: Host cell kinases, in particular those in the PI3K/Akt pathway, have been implicated in the replication of several biosafety level 4 (BSL-4) viruses. To identify a host protein that might be a target for a broad-spectrum antiviral therapy, and to shed light on conserved virus-host interactions, we tested

163 cellular kinase inhibitors for their ability to impede the replication of multiple BSL-4 viruses. Two compounds with broad-spectrum activity were identified.

OSU-03012 (also known as AR-12), a reported inhibitor of 3-phosphoinositide-dependent protein kinase 1, inhibited the growth of Lassa (LASV), Ebola (EBOV), Marburg (MARV) and Nipah (NiV) viruses. For each, the 50% effective concentration (EC_{50}) was approximately 0.5 µM, whereas the 50% cytotoxic concentration (CC_{50}) ranged from 3-8 µM, depending on the cell type, indicating a specific antiviral effect. BIBX 1382, an inhibitor of epidermal growth factor receptor tyrosine kinase, blocked the replication of LASV, EBOV and MARV (but not NiV) with EC_{50} values around 2 µM, and CC_{50} values from 15-60 µM. Both OSU-03012 and BIBX 1382, at concentrations well below their respective CC_{50}'s, reduced LASV and EBOV viral titers by 2-3 log $TCID_{50}$/ml. Both were active in multiple cell lines. Neither compound inhibited the assembly and release of LASV Z protein virus-like particles, neither did they block replication of an EBOV mini-genome. BIBX 1382, but not OSU-03012, did however, inhibit LASV- and EBOV-glycoprotein-dependent entry. These compounds implicate targets for potential broad-spectrum therapeutic intervention and may be used as tools to understand conserved virus-host interactions.
Disclosure of Interest: None declared

Oral 229
AN IN VITRO RNA SYNTHESIS ASSAY FOR MEASLES VIRUS DEMONSTRATES THAT NON-NUCLEOSIDE ANALOGS BLOCK POLYMERASE INITIATION
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Abstract: The minimal RNA synthesis machinery of nonsegmented negative-strand (NNS) RNA viruses comprises a genomic RNA encased by a nucleocapsid protein (N), associated with the RNA dependent RNA polymerase (RdRp). The RdRp activity resides within the large protein (L) that engages the N-RNA template *via* a phosphoprotein (P) cofactor. We expressed and purified the L-P complex of the Khartoum Sudan (KS) strain of measles virus (MV^{KS}) and demonstrate that the polymerase transcribes synthetic RNA templates *in vitro*. Earlier work identified two non-nucleoside inhibitors, 1-Methyl-N-[4-(1-piperidinylsulfonyl)phenyl]-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide and (S)-1-methyl-N-(4-((2-(2-morpholinoethyl)piperidin-1-yl)sulfonyl)phenyl)-3-(trifluoromethyl)-1H-pyrazole-5-carboxamide, that interfere with viral replication in cell culture. We now directly show that those inhibitors block initiation and elongation of RNA synthesis by the MV^{KS} polymerase *in vitro* with an IC_{50} of approximately 100 nM. Previous work had also identified several mutations in the L gene that were associated with resistance by selection for growth of related morbilliviruses in the presence of those inhibitors. Here we demonstrate that two of those substitutions T776A and T751I alter the IC_{50} of the inhibitor 10 and 1000 fold, respectively, and that the combination of mutations display complete resistance. We confirmed those observations by generating a recombinant MV^{KS} with an altered L protein containing both mutations which was also completely resistant. These mutations flank the active site GDNQ motif of the RdRp consistent with an allosteric mechanism of inhibition similar to the non-nucleoside inhibitors of HIV-1 reverse transcriptase. This work provides a direct demonstration that the previously identified non-nucleoside inhibitors block initiation of RNA synthesis *in vitro*, and precisely defines the contribution of resistance mutations to viral growth in cell culture and polymerase activity *in vitro*.
Disclosure of Interest: None declared

Oral 230
DELETION OF THE RESPIRATORY SYNCYTIAL VIRUS M2-2 ORF YIELDS A HIGHLY ATTENUATED LIVE VACCINE CANDIDATE WITH INCREASED IMMUNOGENICITY IN YOUNG CHILDREN
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Abstract: RSV infects and causes disease early in life, and reinfections in the first years of life are common. Vaccines are needed to protect from pediatric RSV disease. In previous work we showed that deletion of the respiratory syncytial virus (RSV) M2-2 coding sequence resulted in down-regulation of viral RNA replication, and up-regulation of RNA transcription and antigen synthesis, identifying M2-2 as an RNA regulatory factor. M2-2 deletion yielded an attenuated virus with the potential for increased immunogenicity due to high-level expression of the antigenic determinants G and F. We evaluated RSV with M2-2 deletion as live-attenuated intranasal vaccine in a clinical phase I study, which sequentially enrolled adults, seropositive children 12-59 months of age, and RSV seronegative children 6-24 months of age. The vaccine candidate was well tolerated in all cohorts. In seronegative infants and children, vaccine virus shedding was significantly reduced compared to a previous vaccine candidate without M2-2 deletion, while the serum antibody response was significantly higher. These results may be due to up-regulated gene transcription and antigen synthesis in absence of M2-2, resulting in increased immunogenicity per infectious viral unit. 19 of 20 vaccinees developed RSV neutralizing serum antibody. Surveillance over the following RSV season showed that several vaccinees had substantial antibody increases in absence of reported RSV illness, suggesting that the vaccine efficiently primed for anamnestic responses to secondary RSV infection, yet protected against RSV disease. In summary, RSV with M2-2 deletion was highly restricted in replication, and yields a safe candidate vaccine with increased immunogenicity. The M2-2 deletion seemed to uncouple replication from immunogenicity, which may help to overcome a major conundrum in the development of safe and immunogenic pediatric RSV vaccines. Further clinical evaluation of vaccine candidates with M2-2 deletion is in progress.
Disclosure of Interest: None declared

Oral 231

THE STRUCTURALLY RELATED FUSION PROTEINS OF HUMAN RESPIRATORY SYNCYTIAL VIRUS AND METAPNEUMOVIRUS ARE ANTIGENICALLY AND IMMUNOGENICALLY DISSIMILAR

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Abstract: The fusion (F) proteins of human respiratory syncytial virus (hRSV) and the highly related human metapneumovirus (hMPV) enable fusion of the viral and cell membranes at the initial stages of their respective infectious cycles and are the main target of neutralizing antibodies.

Both hRSV_F and hMPV_F experience remarkable structural changes during transit from the metastable prefusion conformation to a highly stable postfusion form. Significant structural information has recently been gained about the refolding process of hRSV_F by solving the structures of soluble prefusion and postfusion forms. Although much less is known about hMPV_F, partial information indicates that it shares structural characteristics with hRSV_F.

Despite the noted structural resemblance and partial amino acid identity, both hRSV_F and hMPV_F have limited antigenic identity reflected in few cross-neutralizing monoclonal antibodies (MAbs). Furthermore, only very limited cross-reactivity is seen in polyclonal responses against various forms of prefusion or postfusion F proteins. More importantly, while depletion of human sera with postfusion hRSV_F removes only a small fraction of neutralizing antibodies, the same procedure depletes most of the neutralizing antibodies directed against hMPV_F, indicating substantially different conformational requirements. Finally, different structural forms of hRSV_F have been shown to induce significantly different antibody responses in mice. All these data provide new insights into hRSV_F and hMPV_F based on structural studies that should contribute to the development of efficient vaccines against these important human pathogens.
Disclosure of Interest: None declared

Oral 232

HUMAN MONOCLONAL ANTIBODY DEVELOPMENT FROM FILOVIRUS SURVIVORS IN UGANDA

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Abstract: In the last two decades multiple outbreaks of Sudan virus (SUDV), Bundibugyo virus (BDBV) and Marburgvirus (MARV) have occurred in different regions of Uganda. With the assistance of our collaborators at the Uganda Virus Research Institute, we located individuals confirmed to have been infected and survived filovirus infection (PCR and virus-specific antibody positive results). After securing both country (Uganda Science and Technology Council) and international (Helsinki agreement) approval, we secured blood samples from survivors as well as negative control individuals from the specific outbreak regions in multiple collections spanning the last three years. With these samples we aim to develop human monoclonal antibody-based therapies for treatment of filovirus infection. We performed hybridoma fusions using purified peripheral blood mononuclear cells (PBMC) from the survivors to generate cell lines to produce human monoclonal antibodies. Additionally, we have extracted RNA from patient PBMC for further use and genetic analysis of the humoral immune response. To date we have screened >40,000 fusions, generated >400 human virus-specific IgG producing hybridomas with ~50 that neutralize live SUDV or MARV *in vitro*. We have initiated production of large quantities of purified monoclonals of interest for assessment in the filovirus mouse models. These ongoing efforts have the potential to generate biological therapeutics for the treatment of filovirus infections and will contribute to our overall understanding of protective human humoral immune responses generated during filovirus infection.

Disclosure of Interest: None declared

Oral 233

A RECOMBINANT NOVIRHABDOVIRUS EXPRESSING PORTIONS OF DENGUE VIRUS (DENV) E GLYCOPROTEINS AS VACCINE AGAINST DENV IN A MICE MODEL

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Abstract: Dengue Virus (DENV) is a mosquito-transmitted Flavivirus and a major threat for tropical regions. Primo-infection by one of the four circulating serotype of this virus is usually associated with mild-disease flu-like symptoms and recovery from infection provides a lifelong immunity against the specific serotype. However, subsequent infections by other serotypes may lead to severe dengue haemorrhagic fever. The main challenge for development of an effective vaccine against DENV is to induce a balanced immune response between serotype with the induction of neutralizing-antibody against all four serotypes. Development of a vaccine against DENV is a priority for endemic regions. Effectiveness as well as manufacturing assessment will be determinant towards this goal.

The Viral Haemorrhagic Septicemia Virus (VHSV) is a fish Novirhabdovirus which replicates at low temperature and is naturally inactivated over 20°C which abrogate the need for an inactivation process and insure safety in mammals. A reverse genetic system for VHSV has been developed in our laboratory and in a previous study, we demonstrated the potential of a recombinant VHSV (rVHSV) as a vaccine against another Flavivirus, West Nile Virus, in mice (Nzonza A *et al.,* 2014).

Using insertion cassette developed and patented by our laboratory, allowing the expression of any antigen at the rVHSV viral surface, we managed to produce rVHSV that express complete or fragment of the DENV envelope E protein of each serotype. We demonstrated the expression of the DENV epitopes on the viral particle by western blot assays and electron microscopy observations through immunogold labeling. Immunogenicity of the recombinant viruses and antibody specificity for each serotype was evaluated in mice.

The use of fish Novirhabdoviruses as recombinant vaccine platforms have the potential to induce an effective, safe and adaptable immune response against DENV at an affordable cost.

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Disclosure of Interest: None declared

Oral 234

BRAIN ORGANOTYPIC CULTURE MODEL AS A USEFUL TOOL FOR DRUGS DISCOVERY AND SCREENING AGAINST NEUROTROPIC VIRUSES

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Abstract: Measles virus (MeV) induces an acute lung disease in humans that spontaneously resolves within a few weeks. In rare cases, it can infect the central nervous system (CNS) and persist there, leading to the severe or lethal neuro-degenerative diseases, measles inclusion body encephalitis (MIBE) and subacute sclerosing panencephalitis (SSPE), several months or years respectively after the primary infection. Despite numerous studies of the cellular and molecular biology of MeV CNS infection, the neuropathogenesis of these entities remains poorly understood and no treatments are presently available. We initially analyzed anti-MeV approaches in SLAM transgenic mice, which develop a lethal acute neurological syndrome after intranasal infection with wild type MeV strains. However, ethical concerns limit the use of animals for screening large numbers of drugs. Thus, in this study we developed, characterized, and standardized a brain organotypic culture (OBC) that is useful for screening of new antivirals. We report that all major CNS cell types, including neurons, astrocytes, oligodendrocytes and microglia, as well as the three-dimensional brain sub-structures themselves are preserved in the slices at least for 7 days in culture. In this model MeV specifically targeted neurons (among cells expressing the entry SLAM receptor) and propagated mostly via infection of neurons throughout the brain slices. The OBC was then used to test various drugs for their antiviral activity. The HSP90 inhibitor 17-DMAG significantly reduced both viral replication and viral spread *ex vivo*. Peptides derived from the HRC domain of the MeV fusion protein efficiently blocked virus entry and dissemination in the OBC, with the most active peptide also protecting suckling SLAM transgenic mice from MeV infection *in vivo*, showing the correlation between the *ex vivo* and *in vivo* approaches. These results underline the potential utility of the OBC model for screening new antivirals.
Disclosure of Interest: None declared

Oral 235

NONSPREADING RIFT VALLEY FEVER VIRUS INFECTION OF HUMAN DENDRITIC CELLS RESULTS IN DOWNREGULATION OF CD83 AND FULL MATURATION OF BYSTANDER CELLS

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Abstract: Rift Valley fever virus (RVFV) replicon particles, also referred to as nonspreading RVFV (NSR), are highly immunogenic, inducing strong humoral and Th1-polarised cellular immune responses. The present work was aimed to gain insight into the underlying mechanisms of NSR-mediated immunity. Recent work demonstrated that wild-type RVFV efficiently targets and infects dendritic cells (DCs), via the C-type lectin DC-SIGN. We found that infection of DCs with NSR results in apoptosis of infected DCs and full maturation of bystander DCs, as evidenced by upregulation of CD83. CD83 is the most prominent marker for DC maturation and upregulation of this molecule is essential for priming of naïve T cells. Whereas NSR-infected DCs displayed upregulation of CD40, CD80, CD86, MHC-I and MHC-II, CD83 was downregulated after initial upregulation following infection. Downregulation of CD83 was not associated with reduced mRNA levels or impaired CD83 mRNA transport from the nucleus and could not be prevented by inhibition of the proteasome or endocytic degradation pathways, suggesting that CD83 downregulation occurs at the translational level. Our results suggest that bystander DCs play an important role in NSR-mediated immunity via cross-presentation.
Disclosure of Interest: None declared

Oral 236

IMPROVING THE THERAPEUTIC TREATMENT OF ACUTE RESPIRATORY VIRUS INFECTION VIA INTRAVENOUS LIPOSOMAL DELIVERY OF SIRNA TO THE LUNG.

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Abstract: RNA interference is one of the fastest developing fields in biological science and has been demonstrated as a promising therapy for a wide range of viral infections. This highly specific suppression can be achieved by the introduction of short-interfering RNA (siRNA) into mammalian systems, resulting in the effective reduction of viral load. Unfortunately, this has been hindered by the lack of a good delivery system, which is especially the case in the treatment of respiratory diseases as increased fluid production and tissue remodelling during infection complicate the direct intranasal route of administration.

We have developed a nanoparticle *in vivo* delivery system that should be highly efficient at delivering siRNA intravenously to both lung epithelial and endothelial cells, the target of most respiratory viral infections. Firstly, labelled nanoparticles were examined for their bio-distribution and ability to deliver to the lung in mice after tail vein injection. Following this, nanoparticles containing siRNA targeting the phosphoprotein (P) gene of respiratory syncytial virus (RSV) were administered intravenously to the mice and examined for therapeutic efficacy during virus infection.

Here we show that we were able to efficiently deliver siRNA to the cells of the murine lung by intravenous liposomal administration and that siRNA against RSV resulted in targeted knockdown with over an 80% reduction in RSV P gene expression observed in the lung. This correlated with a reduction in RSV protein levels as seen by immunohistochemistry.

Taken together, our approach overcomes the significant barriers seen with intranasal delivery of siRNA and classic antivirals during infection and is a significant advance in our ability to delivery siRNA overall. This work demonstrates an attractive alternate therapeutic delivery strategy for the treatment of acute respiratory viral diseases.

Disclosure of Interest: None declared

Oral 237

A NOVEL RNA VIRUS VECTOR SYSTEM FOR RNAI THERAPIES BASED ON BORNA DISEASE VIRUS

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Abstract: RNA interference (RNAi) mediated by small RNAs emerged as a promising technique for gene therapy. However, stable and long-term expression of small RNA molecules, such as microRNA (miRNA), is a major concern for application of RNAi techniques *in vivo*. Borna disease virus (BDV) belonging to the *Bornaviridae* family within the non-segmented, negative strand RNA viruses establishes a long-lasting persistent infection without obvious cytopathic effects. Furthermore, unique among animal RNA viruses, BDV transcribes and replicates in the cell nucleus. These features reveal that BDV could be an ideal candidate for generating the novel RNA virus vector system that can persistently express functional small RNAs *in vivo*. In this study, we introduce the establishment of a novel BDV vector carrying a cassette sequence for expressing the miRNA precursor in an intercistronic noncoding region between the viral phosphoprotein (P) and matrix (M) genes. The recombinant BDV (rBDV) having the miR-155 precursor sequence, rBDV-miR-155, stably expressed miR-155 for a long period of time in cultured cells and efficiently silenced the reporter gene containing miR-155 target sequence in the 3'-untranslated region. Multiple insertion of the miR-155 precursor sequence into the cassette region in the vector was shown to enhance the silencing effect of the vector. We also demonstrate that the miR-155 cassette sequence is replaceable to any miRNA sequences of interest and that such BDV vectors can efficiently suppress the expressions of both exogenously introduced target genes and endogenous genes. Our results reveal that BDV could be a novel RNA virus vector for safe delivery and long-term stable expression of RNAi drugs *in vivo*.

Disclosure of Interest: None declared

Oral 238

POST EXPOSURE PROTECTION AGAINST ZAIRE AND SUDAN EBOLAVIRUS IN LETHAL MOUSE MODELS BY HUMAN IMMUNOGLOBULINS PRODUCED IN TRANSCHROMOSOMAL CATTLE

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Abstract: Recent studies have demonstrated that polyclonal IgG obtained from immunized/filovirus-infected non-human primates (NHP) provides complete protection against challenge with filoviruses in NHPs when given up to 48 hours post exposure. The goal of this research was to produce fully human polyclonal antibodies (pAbs) that could offer prophylactic and therapeutic protection against ebolaviruses. We show that DNA vaccination of transchromosomal bovines (TcBs) with codon-optimized DNA vaccines expressing the glycoprotein (GP) genes of *Zaire ebolavirus* (EBOV), and *Sudan ebolavirus* (SUDV) produces fully human pAbs with robust neutralizing activity. Two TcBs were vaccinated via intramuscular electroporation (IM-EP) a total of four times. After two vaccinations GP-specific antibody responses (titers > 1000) against both viruses were detected by ELISA, and virus-specific neutralizing antibody responses (pseudovirion neutralization assay PsVNA80 titers >1000) were detected after three vaccinations. Human immunoglobulins (IgG) were purified from plasma collected 8 days following the third vaccination. Eight of ten BALB/c mice receiving a dose of 100mg/kg one day after lethal challenge with mouse adapted EBOV survived. Similarly, eight of nine interferon receptor 1 knockout (-a/-b) mice receiving a dose of 100mg/kg one day after lethal challenge with SUDV survived. These data are the first to demonstrate that human IgG produced by TcBs can protect against filovirus infection post-exposure and provides proof of concept of the utility of this approach.

Disclosure of Interest: None declared

Oral 278

NUCLEOTIDE SEQUENCE CONSERVATION IN PARAMYXOVIRUSES; THE CONCEPT OF CODON CONSTELLATION.

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Abstract: The stability and conservation of the sequences of RNA viruses in the field and the high error rates measured in vitro are paradoxical. The field stability indicates that there are very strong selective constraints on sequence diversity. The nature of these constraints is discussed. Apart from constraints on variation in cis-acting RNA and the amino acid sequences of viral proteins, there are other ones relating to the presence of specific dinucleotides such CpG and UpA as well as the importance of RNA secondary structures and RNA degradation rates. Recent other constraints identified in other RNA viruses such as effects of secondary RNA structure on protein folding or modification of cellular tRNA complements are also discussed. Using the family Paramyxoviridae I show that the codon usage pattern (CUP) is (i) specific for each virus species and (ii) that it is markedly different from the host - it does not vary even in vaccine viruses that have been derived by passage in a number of inappropriate host cells. The CUP might thus be an additional constraint on variation and I propose the concept of codon constellation to indicate the informational content of the sequences of RNA molecules relating not only to stability and structure but also to the efficiency of translation of a viral mRNA resulting from the CUP and the numbers and position of rare codons.

Disclosure of Interest: None declared

Oral 284

DIVIDE AND CONQUER: CREATION OF A FOUR-SEGMENTED RIFT VALLEY FEVER VACCINE VIRUS

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Abstract: Like all bunyaviruses the Rift Valley fever virus comprises a small (S), medium (M), and large (L) RNA segment of negative polarity. This segmentation confers evolutionary advantages by enabling genome reassortment events with related viruses but complicates genome packaging. To produce infectious virus, at least one of each genome segment should be packaged into a single virion. We hypothesised that increasing the complexity of genome packaging could be an effective strategy to develop live-attenuated bunyavirus vaccines. Using reverse-genetics we succeeded in the construction of four-segmented RVFV (RVFV-4s) variants. These viruses are created by splitting the M-genome segment, which normally encodes a polyprotein precursor that is co-translationally cleaved into the structural glycoproteins Gn and Gc, into two M-type segments encoding either Gn or Gc. RVFV-4s grows efficiently in tissue culture but is unable to disseminate and cause disease in mice, even in de presence of the main virulence factor NSs. A vaccination experiment in lambs subsequently showed that RVFV-4s is able to induce protective immunity after a single shot. Using state-of-the-art single molecule fluorescence *in situ* hybridization techniques we obtained clues about the molecular mechanisms responsible for the observed attenuation of RVFV-4s. Altogether, splitting of a bunyavirus glycoprotein precursor gene is considered an effective strategy to construct bunyavirus vaccines.
Disclosure of Interest: None declared

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A RAPID CELL-FREE STRATEGY TO DETERMINE THE LIKELY IMPACT OF VIRAL EVOLUTION ON ANTIGEN DETECTION SYSTEMS

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Abstract: *Marburgvirus* (MARV) is a negative-strand RNA virus capable of causing unpredictable outbreaks of transmissible and often lethal disease in resource poor equatorial Africa. MARV ecology, evolution, spillover and diagnosis all have implications for safeguarding human health in this region. Four unique llama single domain antibodies (sdAb) specific for viral nucleoprotein (NP) had previously been isolated by antibody selections on live virus preparations and mapped to the C-terminal 95 amino acids. Structural analysis of two sdAb indicated they recognize two alpha-helices that form a “V” platform that are conserved in all MARV isolates since 1967. However, our best sdAb has failed to yield structural data and a third alpha helix underneath the V is prone to evolution. In order to determine whether these variations would impact sdAb-NP interaction, we produced wild-type and variants of the NP C-terminus using conventional *in vivo* methods of protein expression and monitored binding to sdAb by ELISA. Using a neutravidin coat to orient NP in the capture assay, we determined that all four sdAb were able to detect NP mutants at levels equivalent to wild-type. To accelerate the screening process for variants and to bypass the need for cloning, sequencing, and large-scale virus antigen protein production and purification, we are transitioning the scheme to an *in vitro* cell-free protein expression system. The implications of this strategy would be the ability to rapidly predict the likely impact of viral evolution on antigen detection methods by synthetic gene and *in vitro* antigen assembly. The route would be particularly useful where Genbank sequences are rapidly made available, yet where access to diverse viral isolates in the field may be slow and/or the viruses difficult to cultivate. That our sdAb appear unaffected by evolution of NP bodes well for their use as diagnostic and surveillance tools since they recognize MARV strains of the past, present and potentially future.
Disclosure of Interest: None declared

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EFFICIENT CLONING OF COMPLETE NNSV FULL-LENGTH GENOMES BY REC-E / REC-T MEDIATED RECOMBINATION TO CREATE GENETICALLY MODIFIED FIELD ISOLATES.

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Abstract: Reverse genetics for NSVs let to an outstanding boost in virus research as targeted mutagenesis of virus genomes allowed direct proof of concepts in virus replication and pathogenesis. Although reverse genetics technology had been further developed over time, still a bottleneck in many systems is the limited number of cloned cDNA genomes. In particular, in the case of non-segmented NSVs used cDNA clones are often generated from cell culture adapted or attenuated vaccine strains, leading to a limited usability in pathogenesis research. In case of rabies virus (RABV) most reverse genetics work has been performed with recombinant viruses derived from attenuated vaccine strains or mouse adapted virulent virus.
Here we developed a system in which cDNA copies of complete virus genomes from field RABV strains (from dog, fox and raccoon) were directly cloned into reverse genetics vectors by RedE/T mediated recombination. The use of Rac phage derived recombination enzymes RedE and RedT allowed rapid and highly efficient cloning with up 80-90% correctly recombined virus cDNAs. Because the RedE/T recombination approach works independently of genome size and needs no restriction endonucleases, no *a priori* sequence information about the clone is required except for the 50 terminal nucleotides. Hence, the system is highly flexible, can be easily adapted to other NSVs and allows not only efficient cDNA cloning but also rapid recovery of different recombinant viruses strains. Exemplary, we show the rescue of different field RABV strains that now enable us to perform pathogenesis research with genetically modified field virus strains in their respective hosts. This may be an important step in the identification of pathogenesis factors, host adaptive mutations and attenuation strategies beyond conventional mouse models.
Disclosure of Interest: None declared

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IDENTIFICATION OF A NOVEL SWINE H1N2 INFLUENZA A VIRUS IN CHILE

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Abstract: Influenza A Virus (IAV) circulates endemically in swine and human populations, and other species in nature, representing a constant concern to public health and animal production systems worldwide. Although Chile has strong ecological barriers and high stringency regarding livestock crossing across its borders, since 2009 IAV has been consistently detected in pigs. Surveillance of IAVs has therefore been of increasing concern for the swine industry in Chile, currently the 6th largest exporter of this meat in the world. We characterized the diversity of swine IAV (SwAIV) and its prevalence in local swine farms. Animals were found to be susceptible to infection at 50 to 110 days of age. Serological studies showed that there is 91-59% prevalence to IAV, with ~35.2% positivity to H1N1-2009 pandemic (pH1N1) virus. Genotyping identified the circulation of 4 IAV subtypes in Chile, with at least two strains circulating per production site, which included SwH3N2, pH1N1-like, SwH1N2, and a reassortant H1N2 containing a classical swine Hemagglutinin (cSwH1) and N1 derived from the pH1N1 strain. Full-genome sequence analysis of 25 representative viruses revealed at least 2 human-to-swine introductions of the pH1N1 strain within the last 4 years. Importantly we identified a novel antigenic H1N2 cluster that is unique to Chile and that differs from the H1 clusters seen in North America. This is the first comprehensive study of the diversity and origin of swine IAV in Chile, demonstrating the value of IAV surveillance in South America, a poorly studied region of the world.
Disclosure of Interest: None declared

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THE EFFECT OF RIFT VALLEY FEVER VIRUS P78 PROTEIN ON VIRUS REPLICATION, SPREAD AND DISSEMINATION IN THE MOSQUITO VECTOR

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Abstract: Rift Valley fever is an important zoonotic disease in Africa causing a huge economic and public health burden by affecting humans and livestock. Rift Valley fever virus (RVFV) is transmitted to its host via the bite of an infected mosquito causing a variety of symptoms ranging from influenza like to lethal haemorrhagic fever and encephalitis. In the mosquito vector RVFV causes asymptomatic lifelong persistent infections. Persistence and transovarial transmission of the virus in the mosquito vector are important factors for maintaining RVFV in nature, especially during inter-epidemic episodes. Additionally, RVFV has been found to possess a wide vector range in naturally infected mosquitoes as well as in laboratory infections, indicating a great risk of introducing the virus into virgin territories.
As a member of the *Bunyaviridae* family RVFV has a three-segmented genome. The medium (M) segment encodes a polyprotein that is cleaved into the two major glycoproteins Gn and Gc, which form spikes on the viral envelope and two additional proteins, NSm and P78. The 78 kDa glycoprotein P78 is a stable fusion product between the viral glycoprotein Gn and the cytosolic NSm protein. P78 has been found to be a minor component of virions released from mosquito cells and to contribute to virus dissemination in the mosquito vector. Using reverse genetics to generate P78 knockout viruses, we analysed the differential impact of P78 expression on virus replication in mammalian and mosquito cells. While P78 has no impact on virus growth in mammalian cells it impacts virus titres in mosquito cells during acute as well as persistent infection. Furthermore, we characterized the subcellular localisation, determined the effect of P78 on virus particle composition and demonstrated that P78 facilitates cell-to-cell spread. These results, taken together with the fact that a P78 deletant virus remains fully virulent in a mouse model, underline that P78 is a mosquito specific virulence factor.
Disclosure of Interest: None declared

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IN VITRO EVOLUTION OF PERSISTENT RABIES VIRUS.

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Abstract: Although RABV does not exhibit a strong CPE in cell culture or in vivo, longer infection is harmful to the cell. For RABV vectors used as tools especially in neuronal tracing approaches less cytotoxic viruses would be desirable allowing for larger experimental time windows.
In order to study mechanisms involved in RABV cytotoxicity we infected HEK-293T cells, which are usually killed upon RABV infection in less than a week, with the RABV SAD eGFP. The majority of the cells died, however a few single GFP-positive cells survived. Being initially strongly attenuated in their growth kinetics, the cells recovered and remained GFP-positive. After passaging the persistently infected cells for several weeks infectious RABV was isolated from the supernatant and, most importantly persistently infected new cells.
By deep sequencing of isolated persistent viruses we found several point mutations compared to the parental SAD eGFP virus. Recombinant viruses with only single point mutations were generated and interestingly some showed a phenotype comparable to the isolated virus mutant mixture.
While some point mutations lead to a significant attenuation of the respective recombinant viruses, others are still able to grow to high titers. Notably, persistent viruses produce less IFN-inducing RNAs.
We are currently working on the identification of the mechanisms behind the persistent phenotype. Therefore we are comparing PAMP RNAs of wildtype and persistent RABVs in our model system and aim at transferring our findings to mESC-derived neuronal cultures as well as in vivo experiments.
Disclosure of Interest: None declared

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IMPACT OF EBOLA VIRUS GENOMIC DRIFT ON THE EFFICACY OF SEQUENCE-BASED CANDIDATE THERAPEUTICS

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Abstract: Ebola virus (EBOV) was, until recently, a rarely encountered human pathogen that caused disease among small populations with extraordinarily high lethality. At the end of 2013, EBOV initiated an unprecedented disease outbreak in West Africa causing thousands of deaths. Recent studies revealed the genomic changes this particular EBOV variant undergoes over time during human-to-human transmission. We will highlight the changes that might influence the binding efficacy of the sequence-based therapeutics, such as siRNAs, PMOs, and antibodies, suggesting that their effectiveness should be re-evaluated against the currently circulating strain. Moreover, the impact of EBOV genomic drift on the efficacy of sequence-based therapeutics was also observed during passive immunotherapy NHP trials. We characterized a mechanism of escape in two animals that succumbed to EBOV challenge despite treatment with MB-003, a plant-derived monoclonal antibody cocktail used effectively as a treatment of Ebola viral infection in NHPs. We observed two clusters of mutations that resulted in non-synonymous mutations in the target sites of the mAbs. These changes were linked to a reduction in antibody binding and later confirmed to render the virus not neutralizable by the mAb cocktail. Four of the 5 identified SNPs were shared among the two animals, suggesting that genetic drift could be a potential cause for treatment failure in NHPs or humans. Individual components of an antibody cocktail should be selected so each targets a different, unlinked viral protein domain to minimize the possibility of virus escape. Similar combinatorial approaches of therapeutics that differ in their antiviral mechanism might be needed to ensure the success of sequence-based therapeutics.
Disclosure of Interest: None declared

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MODELING THE EVOLUTIONARY TRAJECTORIES OF AN EXPANDING MORBILLIVIRUS GENUS

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Abstract: Multiple barriers restrict cross-species transmission of viruses, but receptor-mediated entry is a critical first step in any adaptation process. A defining feature of morbilliviruses is their recognition of two cellular receptors (CD150 and PVRL4) by distinct, but overlapping binding sites on the hemagglutinin (H) glycoprotein. Binding to CD150 occurs at the cell membrane of highly motile immune cells whereas interaction with PVRL4 is spatially restricted at the basolateral side of less motile epithelial cells. The availability of atomic structures of the measles virus H glycoprotein permitted computer-aided design of docking models for closely related morbilliviruses from domestic and wild animals. The models were used to predict a) if novel viruses identified in the Americas are indeed “true” morbilliviruses and b) which amino acids in the H glycoprotein can be altered to change tropism. Predictions were tested using stable cells lines expressing CD150 from a range of animal species and pseudotyped viruses expressing the modified H glycoproteins. These cell-to-cell and virus-to-cell studies delineated critical amino acids involved in receptor binding. Mini-genome replication/transcription assays showed heterotypic utilization of the nucleocapsid, phospho- and large proteins is feasible. Phylogenetic analysis of full-length genome sequences also substantiated their designation as “true” morbilliviruses confirming that the genus is significantly larger than currently thought. Viruses which are shed for protracted periods of time were identified in animals. This may have important epidemiological consequences and shows the need to reassess our view of long-term paramyxovirus persistence which focuses on the central nervous system. Such forward and reverse genetics approaches, alongside molecular modeling assists in understanding morbillivirus evolution and sheds light on the cross-species and zoonotic potential of this important group of human and animal pathogens.
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