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Abstracts of Invited Speakers

Public Health Response

IL 01
Building a critical level of surveillance readiness and early warning systems in the control of VHFS in Africa

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Background: Epidemics of Viral hemorrhagic fevers (VHFs), mainly Ebola and others (like Lassa fever in West Africa) have had devastating health, social and economic consequences on the affected communities and countries. Late detection of the epidemics and late or inadequate response including poor coordination has been identified as amplifiers. One of the key proven strategies used by WHO in stopping the recent Ebola outbreak in DRC (May 2017) was Surveillance and Coordination.

Objective: One of the main challenges to stopping the VHFs epidemic on the African continent therefore lies in the ability of infectious disease outbreak control experts to put in place a functional Surveillance/Early Warning Systems (EWS).

Strategy: Surveillance involves establishing data gathering systems and timely dissemination of these for public health action. Early detection and prompt response to VHF outbreaks is critical. This will entail being able to carry out early case identification, including processing information collected from suspicions and rumors. Ideally, a corresponding timely response should follow the sequence. A timely response is just as crucial for successful outbreak control as early detection.

Required resources and essential activities: From the community level and primary health care facilities, identified focal persons should be trained to work with Disease Surveillance and Notification Officers (DSNOs) in case detection and timely reporting. The DSNOs will then transmit the data or information gathered through the fastest means to outbreak response teams at the local, Zonal headquarters and Federal levels. This will require adequate Public health education and sensitization on the VHFs, trainings in IDSR for focal persons and DSNOs, communication equipment, and adequate logistics.

Conclusion: In strengthening surveillance systems in Africa as a control strategy for VHFs, a simple and functional information pathway is essential for early reporting and early response. Involvement of the Communities and Primary health care facilities as 1st responders is critical to containing outbreaks in the future, whether it is Ebola virus disease or other diseases unknown at present.
Management challenges in a non-endemic region: German Public Health Experiences during “Ebola 2014-16”

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Public Health Response

IL 03
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N.N.
Public Health Response

IL 04
On the Ebola frontline — improve effective rapid response capacity

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IL 05
Antibodies against the Filoviruses

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Structural mechanisms of Filovirus immune evasion and replication

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Minigenome systems have been used as valuable tools to study various aspects of the filoviral replication cycle and to screen for antiviral compounds at biosafety level 2. I will present our recent work on how to improve minigenome systems to make them work in a wide range of cells and adapt them to a reliable and cost-effective high-throughput screening format, on the use of these systems to gain basic knowledge of the replication strategies of neglected Filoviruses, and how to use them for sophisticated molecular biological approaches to dissect the filoviral genome replication mechanisms.
Reverse genetics can be defined as the intentional genetic mutation of viral genomes or genome analogues, i.e. changes to a viral genotype, followed by assessing the resulting effects on the phenotype, and as such, it represents a powerful tool for studying virus biology. For negative-sense single-stranded RNA viruses, such as Filoviruses, reverse genetics systems are characterized by their reliance on either full-length cloned versions of virus genomes (full-length clone systems) or genome analogues (life cycle modelling systems). Full-length clone systems allow the rescue of recombinant viruses capable of independent replication, which restricts these systems to maximum containment laboratories. In contrast, life cycle modelling systems are reliant on an external source of some viral proteins, and therefore can be safely used outside of maximum containment laboratories; however, the necessity to provide these viral proteins in trans introduces artificial aspects to these systems when compared to an infection with actual Filoviruses.

Both types of systems have been used to successfully study the biology of Filoviruses. For example, recombinant viruses have been used to demonstrate a role of viral inclusion bodies in Filovirus genome replication and to assess the role of various genes in Filovirus pathogenesis. Life cycle modelling systems on the other hand have been used to extensively study genome replication and transcription, as well as transcriptional editing, nucleocapsid morphogenesis, and virus budding, to name just some examples. In addition, both of these systems can also be used for more applied purposes such as the screening and characterization of antivirals. Recent modifications of traditional lifecycle modelling systems (i.e. minigenome and transcription/replication competent virus-like particle systems) now also make it possible to model the complete virus life cycle over multiple infectious cycles, again in a non-infections context. In doing so these systems now have the potential to allow us to connect reverse genetics with classical forward genetics approaches (i.e. looking for phenotypic changes under selective conditions and analyzing the responsible genetic mutations).

This talk will highlight the various available reverse genetics systems, discuss their strengths and limitations, present recent improvements in their efficacy, and provide examples of their utility for the study of Filovirus biology.
IL 10
Ebola virus transcriptional RNA editing — normality and abnormalities

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Mechanism and inhibition of Ebolavirus entry into host cells

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A long-term goal has been to identify a synergistic pair of FDA-approved drugs to combat Ebola virus (EBOV) infections. In the wake of the 2013-2016 outbreak of EBOV in Western Africa, we (re-)screened twenty-eight drugs, twenty-two of which are currently FDA-approved and ten of which were on a WHO list of candidate drugs for the treatment of Ebola patients. Two independent blinded screens, in two different cell types, were conducted; one screen assessed inhibition of EBOV infection and the other assessed inhibition of replication of EBOV transcription-replication competent viral like particles. Nine drugs emerged as common hits from the two screens. These nine drugs plus four others (chosen for known or proposed mechanistic reasons) were then subjected to pairwise testing to search for combinations that block EBOV infection synergistically. Multiple synergistic combinations were identified including many pairs composed of two entry inhibitors. For several of the dual entry inhibitor pairs, synergy was demonstrated at the level of Ebola virus particle entry into the cytoplasm. Constituents of pairs of synergistic entry inhibitors include drugs that block different steps of the EBOV entry process: EBOV internalization from the cell surface, EBOV trafficking to NPC1⁺ endolysosomes (the site of EBOV fusion and entry into the cytoplasm), endosome acidification (required for priming and triggering the EBOV glycoprotein), and the fusion step, itself.
Virus Structure & Biology II

IL 12
Novel insights into Ebolavirus glycoprotein-driven cell entry and tetherin antagonism

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The Ebola virus (EBOV) glycoprotein (GP) mediates viral entry into host cells. The EBOV disease epidemic in West Africa raised the question whether the responsible virus (EBOV-2014) acquired mutations that altered the efficiency of host cell entry. Our results show that an amino acid exchange in the transmembrane domain of EBOV-GP-2014 markedly reduced viral entry into macrophages and dendritic cells as compared to EBOV-GP-1976. The implications of these findings will be discussed and are presented in poster P33. Apart from mediating viral entry, EBOV-GP also antagonizes human tetherin, an interferon (IFN)-induced antiviral host cell factor, which can restrict release of EBOV-like particles from cells. However, it is currently unclear whether EBOV-GP also counteracts tetherin in cells of fruit bats, a natural reservoir of EBOV. We cloned tetherin from fruit bats and show that the protein exhibits the same domain organisation as human tetherin but exerts increased antiviral activity and is largely resistant against EBOV-GP-mediated antagonism. Moreover, we demonstrate that tetherin expression in fruit bat cells is IFN inducible and essential for robust IFN-mediated inhibition of VSV infection. More importantly, we provide evidence that tetherin contributes to IFN-mediated inhibition of EBOV and Nipah virus infection in fruit bat cells. Collectively, our results identify fruit bat tetherin as a potent antiviral factor that may critically contribute to control of highly pathogenic viruses in their natural reservoir.
Like other viruses, Filoviruses are obligate parasites, relying on host proteins for infection, from the point of cell entry, through replication and finally egress from the cell. We are focused on identifying the host factors that control uptake of Ebolaviruses into cells. By using a combination of high throughput screens to identify small molecule inhibitors, molecular cellular biology and high resolution quantitative microscopy, we are developing a detailed understanding of how Ebola virus manipulates and takes advantage of host trafficking pathways to deliver its genome into the cell cytoplasm. Our earlier work demonstrated that Ebola virus is internalized predominantly by macropinocytosis. Macropinocytosis occurs by a large-scale extension of the cell plasma-membrane to surround and engulf extracellular fluid. The membrane then collapses back onto the cell body and closes over to form a large endocytic vesicle, termed the macropinosome. Despite being the earliest endocytic route observed, macropinocytosis remains poorly studied. From what we and others have shown, Ebola virus uptake fulfills most of the criteria for classical macropinocytosis. Our latest findings demonstrate that Ebola virus serves as a powerful tool to teach us more about macropinocytic mechanism from its earliest steps through to late stage endocytic trafficking. We will discuss these findings and how the knowledge of these new host factor dependencies can be exploited as drug targets.

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New insights into Marburg virus pathogenesis in nonhuman primates

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Previously there have been several studies performed to study the development and progression of Marburg virus in nonhuman primates. The aim of these studies was to characterize the evolution of the disease in this animal model ultimately understand the mechanisms / causes of severe disease. Thus, when scientists and pathologists reviewed the samples collected and data generated it was through the lens of “why did the animal succumb?” Following the 2014 Ebola virus outbreak there has been a reassessment of the available filovirus models to evaluate the utility of these models for testing candidate countermeasures and faithfully recapitulating human disease. The high lethality of the nonhuman primate models has raised doubts as to the ability of these models to provide meaningful data for the full spectrum of disease observed in humans. Of interest is the etiology of sequelae observed in humans that survived Ebola virus disease. In a recent study, we evaluated the progression of Marburg virus disease in nonhuman primates. Unlike previous studies there was a focus to look beyond the main contributors to severe morbidity and mortality and to evaluate if there was data that supported the potential for development of sequelae similar to what has been reported in human survivors of Ebola virus.
Pathology and Pathogenesis

IL 15
Same story, different versions — the interplay of Marburg and Ebolavirus with the unfolded protein response

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Too many unfolded proteins impose stress on the endoplasmic reticulum (ER) leading to the activation of three distinct signaling cascades, referred to as unfolded protein response (UPR). The most conserved UPR signaling pathway is activated by IRE1α. If ER stress is imposed, activated IRE1α splices XBP1 (X-Box binding protein 1) mRNA leading to the translation of the transcription factor XBP1s (XBP1 spliced). XBP1s translocates to the nucleus and binds cis-acting UPR elements (UPRE) enhancing the expression of genes that contribute to the restoration of ER homeostasis (e.g. chaperones). Under non-UPR conditions the XBP1 mRNA is not spliced, resulting in the translation of XBP1u (XBP1 unspliced). XBP1u recruits its own mRNA to the ER membrane to support IRE1α-mediated splicing and expression of XBP1u.

Published evidence show that UPR can either support or impair viral replication. The aim of our study was to examine how Marburg virus (MARV) and Ebola virus (EBOV) interact with the IRE1α-dependent UPR signaling.

To analyze if viral proteins regulate UPR signaling, we utilized luciferase assays to monitor UPRE activity. Interestingly, while MARV infection did not activate UPRE, the recombinant expression of MARV GP did. The situation was different with EBOV where the viral infection and the ectopic expression of EBOV NP or GP led to activation of UPRE signaling.

Using a tagged variant of XBP1, we demonstrated at single cell level that XBP1s protein was activated by MARV GP. The apparently contradictory results of MARV-infected versus MARV GP-transfected cells could be reconciled by showing that MARV VP30 counteracted the GP-activated UPR. Co-immunoprecipitation studies revealed that VP30 directly interacted with XBP1u protein, which might influence the availability of the XBP1s mRNA during MARV infection.

EBOV infection on the contrary to MARV infection induced XBP1 mRNA splicing as shown by RT-PCR. Additionally, EBOV infection of XBP1-overexpressing cells shifted the XBP1s/XBP1u protein ratio towards XBP1s. MARV infection shifted this ratio towards XBP1u.

We discovered a tight regulation of the IRE1α-dependent UPR by MARV VP30 and EBOV suggesting Filoviruses to balance the level of UPR according to the needs for efficient viral propagation.
Uncontrolled virus replication and accompanying inflammatory responses likely play major roles in the manifestations of Ebola virus disease. Ebola viral protein of 35kDa (VP35) is a potent suppressor of the RIG-I signaling pathway that detects dsRNAs with 5’-triphosphates and triggers type I interferon (IFN) responses. VP35 blocks RIG-I sensing of viral RNAs by several mechanisms, including sequestration of immune stimulatory dsRNA and through interaction with the cellular protein PACT, which facilitates activation of RIG-I by PACT. By blocking RIG-I signaling, VP35 is able to suppress production of IFN and prevent dendritic cell (DC) maturation, and mutations that disrupt VP35 dsRNA binding also disrupt PACT interaction. Such mutations are sufficient to largely abrogate IFN suppression and DC inhibition. To address the contribution of these VP35 functions to pathogenesis, recombinant Ebola viruses encoding mutant VP35s were constructed and tested in non-human primate (NHPs) models. Despite replicating with an efficiency comparable to wild-type virus in IFN-deficient cells, the VP35 mutant virus is highly attenuated in cells that mount an effective IFN response and are highly attenuated in vivo. Whereas a dose of 1 x 10^6 infectious units of wild-type Ebola virus administered intramuscularly is uniformly lethal in macaques, VP35 mutant virus (VP35mut) caused no measureable illness or death at 1 x 10^5 infectious units. Administration of 1 x 10^6 infectious units of VP35mut caused only a mild and transient fever in 2 of 5 animals and did not cause death, demonstrating a critical role for VP35 IFN-antagonist function for Ebola virus pathogenesis. Further, VP35mut effectively immunized 2 of 3 NHPs against challenge with wild-type Ebola virus. Transcriptome analyses of PBMC following VP35mut vaccination and challenge provide correlates for effective vaccination by VP35mut. The 2 surviving animals exhibited very weak systemic innate antiviral responses to VP35mut but fully blocked systemic innate inflammatory and antiviral innate immune responses to wild-type challenge. In contrast, the animal that succumbed to challenge exhibited more robust systemic innate immune responses to VP35mut and excessive innate inflammatory and antiviral responses to wild-type challenge. Overall, our data suggest that VP35 IFN-antagonist function is required for systemic replication and for triggering the excessive inflammation that underlies the severity of Ebola virus infection. Further comparisons of host responses to wild-type versus VP35mut infections are expected to enhance our understanding of VP35 as a determinant of pathogenesis and immune suppression.
Pathology and Pathogenesis

IL 17
Subversion of cell-mediated responses by Ebolavirus


Introduction: Fatal outcomes of Ebola virus (EBOV) infections are typically preceded by a ‘sepsis-like’ syndrome and lymphopenia despite T cells being resistant to Ebola infection. The degree of lymphopenia is highly correlative with fatalities.

Objectives: 1) To investigate the role of EBOV interferon inhibiting domains (IID) on induction of cell-mediated response; 2) To investigate consequences of interaction of the EBOV glycoprotein (GP) with T cells.

Materials and methods: We used a recombinant EBOV expressing GFP and it derivatives with disabled VP24 and VP24 IIDs and primary human PBMCs, CD4+ T cells, CD8+ T cells, B cells and NK cells.

Results: To determine the effects of IIDs on cell-mediated responses, we used a panel of recombinant strains of EBOVs with point mutations disabling the VP24 and/or VP35 IIDs. The viruses were used for infection of human PBMCs or dendritic cells (DCs), which were co-cultured with T cells. We found that IIDs block activation and proliferation of T cells as a result of their functional role in suppressing maturation of DCs and limiting the formation of immunological synapses. Similarly, IIDs were demonstrated to suppress activation and differentiation of B cells, and skew activation of NK cells. We also investigated whether the addition of EBOV or GP induces death of primary human CD4+ T cells. We observed a significant decrease in cell viability in a GP-dependent manner, which is suggestive of a direct role of GP in T cell death. Using immunoprecipitation assays and flow cytometry, we demonstrate that EBOV directly binds to CD4+ T cells through interaction of GP with TLR4. Transcriptome analysis revealed that the addition of EBOV to CD4+ T cells results in the significant upregulation of pathways associated with interferon signaling, pattern recognition receptors and intracellular activation of NFκB signaling pathway. Both transcriptome analysis and specific inhibitors allowed identification of apoptosis and necrosis as mechanisms associated with the observed T cell death following exposure to EBOV. The addition of the TLR4 inhibitor CLI-095 significantly reduced CD4+ T cell death induced by GP. Lastly, we show that TLR4 receptor antagonist Eritoran protects mice from lethal Filovirus challenge.

Conclusion: EBOV IIDs cause a global suppression of cell-mediated responses, including T, B and NK cells, as a consequence of the deficient DC maturation. In addition, GP directly subverts the host’s immune response by triggering lymphopenia through direct and indirect mechanisms.
Human Ebola virus disease (EVD) is complex, comprising high levels of virus replication and dissemination, dysregulated immune responses, extensive virus- and host-mediated tissue damage, and disordered coagulation. To clarify how host responses contribute to EVD pathophysiology, we performed multi-platform ‘omics analysis of peripheral blood mononuclear cells and plasma from EVD patients. Our results indicate that EVD signatures overlap with those of sepsis, suggest that pancreatic enzymes contribute to tissue damage in fatal EVD, and show that EBOV infection induces aberrant neutrophils whose activity may explain hallmarks of fatal EVD. Moreover, integrated biomarker prediction identified putative biomarkers from different data platforms that differentiated survivors and fatalities early after infection. This work reveals insight into EVD pathogenesis, suggests an effective approach for biomarker identification, and provides an important community resource for further analysis of human EVD severity.
Host responses to Filovirus infection provide presymptomatic and diagnostic transcriptional signatures

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The host immune response to filovirus infection is a critical aspect of pathogenesis. In my presentation I will discuss efforts to understand the host response to filovirus infection using a transcriptomics approach to 1) identify early stage host factors that are associated with acute illness and 2) differentiate patient survival from fatality. In collaboration with others, we have investigated how circulating immune cells temporally respond to EBOV or MARV infection in animal challenge models and we have examined the transcriptomes in peripheral blood from infected and convalescent recovering EBOV patients. Our analysis shows that in animal models of filovirus disease there is a multiphasic response to infection that begins with the accumulation of select innate immune response mRNAs, followed by at least two phases of cytokine mRNA accumulation as disease progresses. Through the selection of mRNAs that represent the early response to infection we have developed nanostring assays that predict infection by host-response at times prior to viral RNA detection, suggesting that the host response to infection provides tractable approach for early detection of filovirus disease. Similarly, in data collected from a single cohort of human patients infected with EBOV we could select genes whose expression properties discriminated between fatal cases and survivors. A small panel of responding genes acted as strong predictors of ultimate patient outcome, independent of viral load. These findings suggest that analysis of the host response to infection can provide valuable information on disease outcome and early detection of filoviral disease.
One of the most prominent clinical hallmarks in Ebola virus disease/Ebola hemorrhagic fever (EHF) patients is an uncontrolled, systemic pro-inflammatory response that leads to coagulation abnormalities, hemorrhagic manifestations, and multi-organ failure. While it is well known that this immune dysregulation contributes to EHF pathogenesis, the mechanism by which Ebola virus (EBOV) induces this response remains elusive. To obtain insights into the mechanism that underlies the EBOV-induced uncontrolled pro-inflammatory response, we first wondered whether any EBOV proteins were responsible for the activation of the NF-κB pathway, which is one of the major signaling pathways involved in driving inflammation. We demonstrated that the EBOV matrix protein VP40, but not other EBOV proteins, activated NF-κB and induced pro-inflammatory cytokines/chemokines in a dose- and time-dependent manner in vitro. Interestingly, we found that VP40 from Reston and Bundibugyo virus, which are thought to be apathogenic or less pathogenic than EBOV in humans, activated NF-κB less efficiently than did EBOV VP40. In conclusion, here we provide the first data indicating that EBOV VP40 has an ability to over-activate the NF-κB pathway and this ability of VP40 is different between EBOV species, suggesting that VP40 might be a novel virulence determinant that is responsible for the differential pathogenesis observed among EBOV species. Moreover, analyses for elucidating the mechanism of inflammatory activation suggested that VP40 probably does not activate the inflammatory response via the canonical NF-κB activation pathway. This study provides novel insights into the molecular mechanism underlying EBOV pathogenesis, and will facilitate the development of novel countermeasures against EHF that specifically target the host-virus interaction.
Immune Response to Filovirus Infection

IL21 Antibodies from a human survivor afford the development of a broadly protective anti-Ebolavirus cocktail with reduced susceptibility to viral escape


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Experimental monoclonal antibody (mAb) therapies have shown promise for treatment of lethal Ebola virus (EBOV) infections, but their species-specific recognition of the viral glycoprotein (GP) has limited their use against other divergent Ebolaviruses associated with human disease. Here, we mined the human immune response to natural EBOV infection, and identified mAbs with exceptionally potent pan-Ebolavirus neutralizing activity. These neutralizing mAbs (NAbs) recognize an inter-protomer epitope in the GP fusion loop, a critical and conserved element of the viral membrane fusion machinery, and neutralize viral entry by targeting a proteolytically primed, fusion-competent GP intermediate (GPCL) generated in host cell endosomes. Post-exposure challenge studies in mice showed that two fusion loop-targeting NAbs (ADI-15878 and ADI-15742) could protect against all three known virulent Ebolaviruses. However, antibody monotherapy afforded only partial protection in guinea pigs and ferrets, and one NAb (ADI-15742) appeared to elicit viral escape mutants, providing a strong rationale for the development of a pan-Ebolavirus protective cocktail. Accordingly, we screened two-antibody combinations containing ADI-15878 and other GP base-binding NAbs in vitro, and down-selected to combinations in which both mAbs could bind to GP without competitive inhibition. Viral escape analysis revealed a valuable property observed with only a single combination (ADI-15878/ADI-15946): escape mutants elicited by each NAb were more sensitive to neutralization by the other, and an escape mutant elicited by the combination was highly attenuated in tissue culture. To enhance the potency of ADI-15946 against SUDV, we next affinity-matured it against SUDV GP by yeast display, and identified an optimized variant, MBP047, which demonstrated increased antiviral breadth in vitro and in mice, but retained the desirable properties of its parent NAb when combined with ADI-15878. Taken together, these findings set the stage for the development of a therapeutic cocktail comprising fully human antibodies with the capacity to protect against all known Ebolaviruses.
The physiology of the host immune response to infection with Ebola viruses, and in general with Filoviruses, is poorly understood. One of the main reasons for this lack of insight into this important aspect of Filovirus pathogenesis, is the lack of immune competent mouse models susceptible to infection with non-adapted Ebola viruses. To address this issue, our laboratory has invested effort during the last years in the development of immune competent mice retaining susceptibility to wild type Filoviruses. Our approach has been the use of transplantation technology to modify the hematopoietic compartment of recipient mice thereby harnessing the function of Filovirus target cells. Here we will discuss how congenic as well as xenotransplantation methods allows identification of barriers for virus dissemination, target cells, and identification of correlates of host protection in vivo. In particular, we will show how these models underscore the importance of inflammatory dendritic cells and T cells as natural barriers for Ebola virus dissemination. We hypothesize that this transplantation technology may further help to dissect the role of the immune system on Ebola virus pathophysiology and may hopefully serve to identify targets for highly needed post-exposure immune-based therapy.
The emergence of novel infectious diseases with pandemic potential into the human population is on the increase. Most of these emerging infectious diseases (EIDs) are zoonotic in nature, being transmitted through direct or indirect human contact with agricultural and wildlife animal sources. The frequent emergence of EIDs of global human health significance within geographic ‘hot-spots’ in poorer, under-resourced countries represents a serious problem to the efficient control of these frequently highly virulent pathogens at source in the animal species involved in zoonotic emergence. Herpesvirus-based vaccines are a relatively new viral vaccine platform that has been shown to provide stringent immunological protection against a variety of viral and bacterial pathogens. These vaccines have been shown to induce high levels of durable T and B cell immunity, frequently after only a single dose. Herpesvirus-based vaccines also have potential for development as a ‘self-disseminating’ vaccine to target inaccessible animal species involved in zoonotic transmission whereby the vaccine spreads from animal to animal without the need for individual animal vaccination. Together, these characteristics may make the herpesvirus vaccine platform suited to the control of pathogens associated with hot spots of EID pathogen emergence in animals involved in zoonotic transmission. We will present recent innovations towards the development of herpesvirus-based vectors as a versatile, rapid response vaccine against Ebola virus and other zoonotic EIDs.
Therapy I

IL 25
Development of the pan-Ebolavirus therapeutic cocktail MBP134

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The 2014-2016 Ebola virus outbreak highlighted the absence of available medical countermeasures (MCMs) for Filoviruses. Fortunately, by the resolution of the outbreak, one vaccine (VSV-EBOV) and one therapeutic (ZMapp™) were shown in controlled clinical trials to have efficacy potentially supportive of licensure by the Food and Drug Administration (FDA) and development of both products continues to advance toward that goal. However, each of these products are specific for Ebola virus (EBOV; formerly known as Ebola Zaire), and have shown no efficacy against the related Sudan (SUDV) and Bundibugyo (BDBV) Ebolaviruses which together have caused nine sporadic and unpredictable deadly outbreaks responsible for 645 infections and 289 deaths since 2000. Further, because of these products’ specificity for EBOV, they are unlikely to have activity against any new Ebolavirus species or variants that may emerge in the future. Thus, there is a clear unmet need for a pan-Ebolavirus therapy to address the threat of biowarfare as well as future natural outbreaks.

Mapp’s approach to address this unmet need was to collaborate with Adimab, LLC (Lebanon, NH), Albert Einstein College of Medicine (New York, NY), Public Health Agency of Canada (Winnipeg, Canada), UNITED STATES OF AMERICARMIID (Frederick, MD) and the University of Texas Medical Branch at Galveston (Galveston, TX) to develop a therapeutic based on fully human monoclonal antibodies derived from a single survivor of the 2014 EBOV/Makona outbreak. A thorough down-selection was performed on a 349 mAb panel involving in vitro neutralization testing and in vivo testing in the mouse, guinea pig, and ferret models for EBOV, SUDV and BDBV. Two mAbs, MBP047 and MBP087, from this work were then combined into a pan-Ebolavirus immunotherapeutic cocktail, MBP134. MBP134 has proven to be extremely potent against EBOV, SUDV, and BDBV providing 100% protection in both the lethal guinea pig and ferret models for these viruses. MBP134 also conferred protection from EBOV, SUDV and BDBV challenge in non-human primates when administered therapeutically once symptoms were apparent. MBP134 is the first product to demonstrate protective efficacy post-infection with a single dose against every known pathogenic Ebolavirus species.
Therapy I

IL26

Antibody cocktails protect rhesus macaques against Sudan virus infection

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Although Ebola virus (EBOV) has dominated the Filovirus landscape due to the 2013-2016 outbreak in Western Africa, there have also been four Sudan virus (SUDV) outbreaks since 2000 with 444 cases and 223 deaths (50% case-fatality rate). All Ebolavirus antibody therapeutics assessed in animals and humans to date are specific for EBOV with no cross reactivity against SUDV. With this in mind, we set out to develop monoclonal antibody cocktails capable of protecting against SUDV disease in non-human primates (NHP) to offer treatment options in the event of an intentional or natural outbreak. To meet this goal, we worked in parallel to develop a cocktail of antibodies that would have cross reactivity against all Ebolaviruses as well as a cocktail of SUDV-specific antibodies.

In collaboration with Adimab, Albert Einstein College of Medicine, Public Health Agency of Canada, University of Texas Medical Branch at Galveston and Mapp Biopharmaceuticals, we developed a fully human, pan-Ebolavirus monoclonal antibody (mAb) cocktail by evaluating a panel of 349 mAbs isolated from a survivor of the 2014 EBOV/Makona outbreak for neutralizing activity in vitro and protective efficacy against SUDV in rodents. From these screens, two pan-ebolovirus mAbs were identified and the resulting cocktail (MB134) was selected for transition into NHP testing. SUDV-specific mAb cocktail development proceeded in collaboration with Biofactura and Fraunhofer USA. Panels of SUDV-specific mouse/human and NHP/human chimeric mAbs were evaluated for in vitro neutralization and in vivo protective efficacy against SUDV in mice. A cocktail consisting of two mAbs (16F6 and X10H2) was selected for testing in NHPs. In proof of concept studies, both MB134 and 16F6/X10H2 cocktails provided complete protection against lethal SUDV infection in rhesus macaques when administered several days after infection. These studies illustrate that both a SUDV-specific cocktail and a pan-Ebolavirus-specific cocktail are effective countermeasures against SUDV infection.
Therapy I

IL 27
Targeting the receptor binding and fusion for pan-Ebolavirus therapy with monoclonal antibodies

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The genus *Ebolavirus* consists of Ebola (EBOV), Sudan (SUDV), Bundibugyo (BDBV), Tai Forrest (TAFV) and Reston (RESTV) viruses. Current vaccines and immunotherapeutics are primarily focused on EBOV (Zaire) while progress towards countermeasures for other species has been lagging behind. While significant homology exists between *Ebolavirus* glycoproteins (GP) within the receptor binding site (RBS) as well as GP2, the virus has evolved mechanisms to conceal key cross protective epitopes. Consistently, vaccine mediated antibody responses are largely targeted to species-specific epitopes. Using vaccination with an immunogen cocktail in macaques we have generated several monoclonal antibodies (mAbs) that cross neutralize all pathogenic *Ebolaviruses*. In particular, an antibody targeting the RBS and a second mAb targeting the internal fusion loop were exceptionally potent. Efficacy of the antibodies was demonstrated in mouse, guinea pig, and ferret models of EBOV, SUDV, and BDBV. A combination of the two antibodies appeared superior protecting 100% of guinea pigs against EBOV and SUDV when administered in a single dose as late as 4 days post exposure and 100% of ferrets when administered on days 3 and 6 post challenge with BDBV. Efficacy studies are currently in progress in nonhuman primates and data will be presented. Furthermore, mechanistic studies on the nature of epitopes, evolution of the protective responses, and findings important for vaccine development will be discussed.
The Ebola virus (EBOV) epidemic in West Africa increased the focus on vaccine development against this hemorrhagic fever-causing pathogen. Consequently, human clinical trials for a few selected platforms were accelerated. One of these vaccines is VSV-EBOV (rVSV-ZEBOV), so far, the only vaccine with reported efficacy against EBOV infections in humans in phase III clinical trials (ring vaccination trial). Our previous work has shown that VSV-EBOV is a fast-acting prophylactic vaccine, but can also be used in post-exposure prophylaxis as it rapidly stimulates strong innate followed by critical adaptive immune responses. More recently, we have studied the fast-acting potential of the VSV-based Marburg virus vaccine in nonhuman primates resulting in similar pre- and post-exposure prophylactic efficacy as described for VSV-EBOV. Thus, both vaccine vectors have the potential to act as emergency vaccines and post-exposure treatment options.

This work was funded by the Intramural Research Program of the NIAID, NIH.
IL 29
Efficacy of Multivalent rVSV vaccine vectors against Ebolaviruses in ferrets

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The filoviruses, Lake Victoria Marburgvirus (MARV), Zaire ebolavirus (EBOV), Sudan ebolavirus (SUDV), and Bundibugyo ebolavirus (BDBV) cause severe and often fatal hemorrhagic fever in humans and nonhuman primates (NHPs). Recombinant vesicular stomatitis virus (rVSV)-based vaccine vectors, which encode a filovirus glycoprotein (GP) in place of the VSV glycoprotein, have shown 100% efficacy against homologous filovirus challenge in rodent and NHP studies. Previously we observed 100% efficacy of a single-particle, single-injection trivalent rVSV vector expressing MARV, EBOV, and SUDV GPs to protect against MARV, EBOV, and SUDV induced disease in Hartley guinea pigs. However, the SUDV guinea pig model used in this study was not 100% lethal. Recent development of a lethal ferret model for EBOV, SUDV, and BDBV offers the opportunity to assess cross-protection countermeasures targeting the lethal ebolavirus species. Here we examined the utility of a single-particle, single-injection trivalent rVSV vector expressing MARV, EBOV, and SUDV GPs to protect against EBOV, SUDV, and BDBV lethal challenge in the newly developed ferret models.
Ecology & Epidemiology

IL 30
Marburgvirus transmission and immunity in Egyptian rousette bats

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The Egyptian rousette bat (ERB) is a natural reservoir host for Marburg virus (MARV). However, it is unclear 1) how MARV is transmitted bat-to-bat or to other animals, and 2) how long the host antibody response to infection lasts and if such immunity is long term. To address these questions, we co-housed MARV-inoculated donor ERBs with naïve contact ERBs. In donor bats inoculated with 10^4 TCID₅₀, MARV was shed in oral, rectal and urine specimens up to 19 days post-infection (p.i.). Simultaneously, MARV was detected in oral specimens from naïve contact bats, indicating oral exposure to the virus. Later, we demonstrated horizontal transmission of MARV by finding virus-specific RNA in naïve contact bats, soon followed by seroconversion. Interestingly, the anti-MARV IgG levels rapidly waned in both experimentally and “naturally” infected bats, and within 3 months of infection, the bats were seronegative. “Naturally” and experimentally infected ERBs bats were then followed for a total of roughly two years. To determine if reinfection might play a role in the overall natural MARV maintenance cycle in ERBs, we challenged a subset of these previously infected bats with 10^4 TCID₅₀ of MARV 17-24 months after their primary infection. No bats showed evidence of MARV replication or shedding and all bats quickly developed secondary immune responses. Overall, these studies demonstrate that MARV can be horizontally transmitted between ERBs, and that MARV infection induces long-term (life-long?) protective immunity against reinfection, indicating that other factors, such as host population dynamics, drive MARV maintenance in nature.
IL 32
Dissecting the immune response of EVD survivors — implications for sub-clinical infections and vaccine development

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Clinical Management

MSF and responding to future Filovirus outbreaks

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Médecins Sans Frontières has been responding to Filovirus disease outbreaks since 1995. For most of that time, the medical management of EVD patients has been our core intervention. While the patient outcomes achieved in the outbreak setting are not as favorable as those of patients evacuated to North America and Europe, the major determinants of outcome appear to be factors independent of care: patient age and level of viremia at presentation. To improve patient outcomes, therapeutics directly targeting specific disease processes would be of great utility. The future availability of such therapeutics is dependent on research on human EVD patients that may need to be carried out across multiple locations and likely across multiple outbreaks. Appropriate risk stratification of the human participants in these studies will require comparability of field proxy measures of patient viremia, which is currently troubled by the heterogeneity of assays and techniques of field laboratories. A means of standardized measurement of patient viremia will be necessary to conduct the research needed to provide the effective therapeutics that will allow Médecins Sans Frontières to carry out its mission of providing life saving care to EVD patients during future outbreaks.
Clinical Management

IL 35
Ebola – managing post outbreak and chronic complications

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Clinical Management

IL 36
The implications and results of the use of advanced ICU care for Ebolavirus disease in non-human primates

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Although supportive care is assumed to improve overall survival for patients with ebola virus disease (EVD), the lack of definitive evidence, as well as concern for health care provider safety in the field, has curtailed its use. Despite limited experience of treating EVD patients in non-African settings, using combinations of supportive care and experimental agents (e.g. ZMapp, convalescent plasma, antivirals and others), resulting in much lower mortality rates (approximately 18.5%), it remains unclear which aspects of the treatments provided are responsible for this reduced mortality. We have used the Rhesus macaque model of high challenge dose (1000 pfu) and route (IM) which is uniformly lethal and based on a typical human needle-stick dose of EVD to study intensive care unit (ICU) supportive care practices and their effects on EVD outcome. Using bacterial sepsis/septic shock as our guide, we evaluated ICU supportive care practices and their effects on physiology and outcome. Results show that like septic shock, supportive care without source control is insufficient to extend the survival. Through these studies we have demonstrated that supportive care alone is insufficient to lower the lethality of EVD.
Public Health Response

IL 37
CEPI: A new broad coalition for developing vaccines to stop future epidemics

C. Clark
Abstracts of Oral Presentation

Virus Structure and Biology I

**OP 01**

Interplay of VP30 and RNA secondary structures at the transcription start sites of the Ebolavirus (EBOV) genome

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Ebola virus (EBOV) genome replication is mediated by a protein complex comprising the viral RNA polymerase L, the nucleoprotein (NP) and viral protein 35 (VP35). To accomplish transcription of viral mRNAs, the replication complex needs to be supplemented with viral protein 30 (VP30), an EBOV-specific transcription factor. The transcriptional start regions of all EBOV genes are embedded in 5'-UTR secondary structures of varying stability on the genomic (-) RNA as well as (+) mRNA level. In this study, we used replication-proficient and -deficient EBOV minigenomes (either monocistronic or bicistronic in order to uncouple transcription from replication promoter elements) to investigate the role of these secondary structures in viral transcription and their dependency on VP30. First results indicate that the spacer between the two replication promoter elements PE1 and 2, which includes the transcription start of the first NP gene, has to be a multiple of 6 nt not only for efficient replication [1] but also efficient transcription initiation 56 nt upstream of the genome 3'-end. The “rule of six” applying also to the spacer length suggests that the replication promoter directs genome replication and mRNA transcription. This was inferred from the observation that the 5'-terminal hairpin structure of the NP 5'-UTR could be replaced with corresponding structural elements derived from internal genes as long as the replacement followed the “rule of six”. This principle was weakened when the VP24 5'-element was substituted for the NP element. As the VP24 element represented the longest insertion variant tested (14 x 6 nt), we reduced its size to 12 x 6 nt, which indeed rescued transcription. This confirms that transcriptional efficiency decreases upon exceeding a certain length limit of the spacer separating PE1 and PE2. Also, transcriptional start regions devoid of any RNA secondary structure are being tested (± VP30). With the bicistronic minigenome, we are investigating if the 5'-terminal RNA structures of internal mRNA transcripts determine the declining mRNA levels from the first to the last EBOV gene.

LC3B lipidation is required for Ebolavirus internalization into host cells by controlling macropinosome biogenesis

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Ebola virus (EBOV) uptake into host cells by macropinocytosis depends on plasma membrane rearrangement by a poorly characterized mechanism. Here, we demonstrate that lipidation of LC3B protein, originally described to be critical for autophagosome formation, is also required for EBOV cell entry by controlling biogenesis of macropinosomes. Depleting cells of LC3B or cellular machinery coordinating LC3B processing abolished internalization, but not cell binding, of EBO virions and uptake of the fluid-phase marker dextran known to require macropinocytosis for trafficking into the cell. The block of macropinosome formation appeared to be at the earliest detectable step when Ankfy1 protein and phospholipid PtdIns(3,4,5)P3 mark sites of macropinocytic cups. Both unprocessed and lipidated forms of LC3B interacted with Ankfy1 in the immunoprecipitation and colocalization assays at the cell surface, suggesting that although various moieties of LC3B possess an inherent ability to associate with forming macropinosomes, it is the lipidated form that drives closure of macropinocytic cups and therefore EBOV internalization. Based on these findings, we speculated that inhibition of LC3B lipidation would interfere with virus infection. Indeed, PIK-III, a selective inhibitor of LC3B processing machinery, efficiently blocked replication-competent EBOV infection in human immortalized cell culture and blood-derived primary macrophages. Our findings demonstrate a clear role for LC3B in controlling EBOV entry into cells as well as identify novel targets for disease treatment. This work was supported by NIH and DOD/DTRA grants.
Ebola virus requires a host scramblase for its efficient entry

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Cell surface receptors for phosphatidylserine (PtdSer), such as TIM (T-cell immunoglobulin and mucin domain)-1 and TAMs (TYRO3, AXL, and the MERTK family of receptor tyrosine kinases) contribute to the internalization of Ebola virus (EBOV) particles, indicating that binding of PtdSer likely in EBOVs envelop to PtdSer receptors is important for the internalization of EBOV particles in addition to the glycoprotein (GP) binding cell surface moieties. PtdSer typically distributes in the inner layer of the plasma membrane in normal cells. Progeny virions bud from the cell surface of infected cells, suggesting that PtdSer is likely flipped to the outer membrane of the plasma membrane in the infected cells for EBOV virions to acquire it. Currently no direct evidence demonstrates PtdSer to be in the viral envelop. Furthermore, the intracellular dynamics of PtdSer under EBOV infection is poorly understood. We have therefore examined the role of XK-related protein (Xkr) 8, which is a scramblase and responsible for externalization of PtdSer to the outer plasma membrane of apoptotic cells to understand its potential role in PtdSer-dependent entry of EBOV.

We observed that transiently expressed GP partially co-localized with endogenous Xkr8 in the cytoplasm and periphery of the plasma membrane, while neither NP nor VP40 co-localized with it. 293T cells lacking Xkr8 gene generated by CRISPR/Cas9, produced EBOV virus-like particles (VLPs) as do control cells and these viral particles retain a filamentous morphology. However, EBOV VLPs produced from Xkr8 knock out cells were internalized in recipient Vero-E6 cells with 10-20% the efficiency as VLPs derived from control cells.

Our findings indicate that Xkr8 is trafficked to the plasma membrane via GP-containing vesicles and confer on released EBOV the competency to enter recipient cells.
A non-infectious live-cell imaging system reveals the functions of viral proteins for the transport of Ebolavirus nucleocapsids

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The Filoviruses Marburg (MARV) and Ebola (EBOV) cause severe hemorrhagic fever with high case-fatality rates in humans and nonhuman primates. No approved specific therapy is available and therefore further understanding of the Filovirus life cycle is essential for the development of novel therapeutic options.

EBOV particles contain the non-segmented negative-sense RNA genome and seven viral proteins: NP, VP35, VP40, GP (glycoprotein), VP30, VP24, and the RNA-dependent RNA polymerase (L). Necessary for EBOV transcription and replication are the nucleocapsid proteins NP, VP30, VP35, and L. VP24 is an additional factor required for nucleocapsid (NC) assembly. A layer of the matrix protein VP40 connects the NC with the viral membrane in which GP is inserted. It is known that NP, VP24 and VP35 are the main structural components of the filoviral nucleocapsids. Recent live-cell imaging studies of MARV- and EBOV-infected cells revealed a long-distance actin-dependent transport of NCs from viral inclusions to the plasma membrane. In order to identify the essential viral proteins for the intracellular NC transport, we have constructed a set of fluorescently tagged viral proteins (e.g., VP30-GFP, VP35-GFP, VP24-TagRFP) and developed novel systems to visualize transport of NC-like particles in the background of Filovirus-specific virus-like particle systems. We detected that transport of NC-like particles was dependent on the polymerization of actin and proceeded with similar speed as NC in EBOV- or MARV-infected cells. Moreover, using this system, we identified the viral factors essential for the transport of NC-like particles. Also, we focused on matrix protein functions during EBOV NC-like particle transport. The newly developed non-infectious live-cell imaging system will further contribute to our understanding of molecular interactions between NCs and cellular proteins, and the development of anti-viral drugs.
Virus Structure and Biology II

OP 05

Hydrophobic loop region of Marburgvirus matrix protein-VP40 may indicate mechanism of protein trafficking to plasma membrane

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Marburg virus and Ebola virus are Filoviruses and can cause fatal hemorrhagic fever in humans. Viral protein-40 (VP40) underlies the lipid envelope of these viruses forming the viral matrix, providing structural stability to viral particle. Inside an infected host cell, VP40 interacts with host plasma membrane (PM) and facilitate the assembly of viral matrix and budding of virions. Ebola VP40 (eVP40) interacts with the inner leaflet of the PM through electrostatic interactions and it also penetrates the PM through a hydrophobic loop at its C-terminal domain establishing hydrophobic interactions. Marburg VP40 (mVP40) also interacts with anionic phospholipids at the inner leaflet of the PM but unlike eVP40, mVP40 does not penetrate the hydrocarbon core of the PM despite it having a structurally similar hydrophobic loop region to eVP40. To investigate the function of this hydrophobic loop region of mVP40, we created single, double and triple amino acid mutants of this region specifically targeting L201, F281, L283, and F286 residues and observed the phenotype of the cells transfected with these mutants using a GFP tag. Mutants L201A, F281A/F286A and F281A/L283A/F286A exhibited a change in the localization of VP40 by accumulating into intracellular vesicles, while all other mutants maintained the Wild Type phenotype by associating with the PM. In-vitro lipid binding assays were performed with the L201A mutant resulting in an insignificant difference in lipid binding when compared to the wild type mVP40. This indicated that L201A is still able to interact electrostatically with anionic phospholipid membranes. When these mutants were co-expressed with Early Endosome Antigen 1 (EEA1), it led to co-localization of the EEA-1 with the mutants. EEA1 is an early endosomal marker that interacts with phosphoipid-Pi3P and Rab5 protein on early endosomes. mVP40 is known to use retrograde late endosomal pathway accumulating at multivesicular bodies (MVBs) before reaching the PM. Therefore, these mutations likely to have caused a disruption in an early step of Marburg VP40 trafficking to PM and may provide valuable insight into Marburg VP40 membrane trafficking pathway.
Pathology and Pathogenesis

OP 06
E3-ubiquitin ligase TRIM25 inhibits Ebolavirus replication

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Introduction: RNA viruses are sensed by pattern recognition receptors which activation trigger a signalling cascade, leading to expression of type-I interferon (IFN), and ultimately resulting in the upregulation of IFN-stimulated genes (ISGs). ISGs target specific steps of virus life cycle limiting its spread and replication. Using a library of human ISGs we identified TRIM25 as inhibitory of Ebola virus (EBOV) replication (abstract by Wilson H). TRIM25 is an E3-ubiquitin ligase known to ubiquitinate the RNA sensor RIG-I to facilitate its interaction with MAVS, thus modulating downstream signalling of the IFN-response.

Objective: Characterise the molecular mechanisms underlying the antiviral effect of TRIM25 on EBOV replication.

Methods: A transcription-and replication-competent virus like particle (trVLP) system was used to study the cell biology of EBOV life cycle and associated innate immunity. Knockout cells for components of TRIM25-related cellular pathways were established by gene editing (CRISPR/Cas9), and used to characterise their role in TRIM25-mediated restriction of EBOV. TRIM25 interaction with viral RNA and proteins are being presently addressed.

Results: Although EBOV have evolved strategies to counteract the host innate response, we observed that treatment of target cells with type-I IFN limits the propagation of EBOV trVLP. Furthermore, we identified TRIM25 as inhibitor of EBOV replication and demonstrated that its antiviral mechanism is strictly MDA5- and MAVS-dependent, and potentially linked with TRIM25 modulation of Zinc-finger antiviral protein (ZAP) inhibition of EBOV. This antiviral activity is independent of RIG-I and signalling events downstream of TBK1. Finally, we showed that TRIM25 interacts and ubiquitinates components of EBOV-ribonucleoprotein complex, and blocks viral RNA synthesis.

Conclusion: The uncovered capacity of TRIM25 to ubiquitinate viral proteins suggests that the full scope of its antiviral functions is still to be defined. Furthermore, these findings present a novel mechanism for TRIM25/ZAP involvement with MDA5/MAVS RNA sensing pathway, that might be a general antiviral mechanism coupled to sensing of negative strain RNA viruses.
OP 07
The role of viral replication in macrophages in the pathogenesis of Ebolavirus infection

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Introduction: Cells of the mononuclear phagocyte system (MPS) such as macrophages/monocytes and dendritic cells are the primary targets for Ebola virus (EBOV) infection. However, the pathobiological significance of EBOV replication and host responses in these cells is not fully understood.

Objectives: The cellular tropism of viruses can be engineered through the insertion of the target sequence for microRNA that regulate host gene expression. In this study, we generated recombinant EBOV (rEBOV) encoding the target sequences of microRNA (rEBOV-miRt) expressed predominantly in the MPS, to inhibit replication specifically in these cells. To determine the pathological significance of EBOV replication in macrophages/monocytes, we analyzed viral replication and pathogenicity of rEBOV-miRt.

Materials and methods: We generated the rEBOV possessing the miRt sequence within the 3′untranslated region of the EBOV L gene. Viral replication of rEBOV-miRt in human hepatoma cell line (Huh7) and human macrophage-derived cell line (THP-1) was evaluated. We also generated mouse-adapted EBOV with miRt (rMAEBOV-miRt) and investigated its pathogenicity in the mouse. Mice were inoculated with rMAEBOV-miRt or rMAEBOV-control and monitored for disease progression.

Results: Compared with parental rEBOV, rEBOV-miRt showed reduced replication in THP-1 cells despite both viruses having similar growth kinetics in Huh7 cells, suggesting that rEBOV-miRt replication was inhibited in macrophages. Mice infected with 1 FFU of rMAEBOV-control succumbed to infection by 7 days post-infection. In contrast, only one mouse out of six infected with rMAEBOV-miRt succumbed to disease. Thus, rMAEBOV-miRt showed attenuated pathogenicity in mice compared with the rMAEBOV-control.

Conclusion: Our results suggest that viral replication in macrophages is an important factor for lethal outcome associated with EBOV disease. We will discuss how this phenotype relates to viral replication, tissue tropism and host immune responses. Our study demonstrated direct involvement of the MPC cells in the EBOV pathogenesis in vivo.

This research was supported by the Intramural Research Program of the NIH, NIAID.
Ebola virus and other emerging RNA viruses are significant but unpredictable public health threats. Therapeutic approaches with broad-spectrum activity could provide an attractive response to such infections. Ebola virus (EBOV) protein VP35 inhibits production of interferon-α/β (IFN) by blocking RIG-I-like receptor signaling pathways, thereby promoting virus replication and pathogenesis. A high-throughput screening assay, developed to identify compounds that either inhibit or bypass VP35 IFN-antagonist function. This assay identified as hits cancer chemotherapeutic drugs, including doxorubicin. Follow-up studies provide new insight into how doxorubicin induces interferon (IFN) responses, revealing activation of both the DNA damage response kinase ATM and the DNA sensor cGAS and its partner signaling protein STING. In vitro treatment with these compounds led to activation of the ATM signaling pathway; furthermore knock down of ATM kinase led to inhibition of IFN activation, suggesting ATM pathway is important for IFN activation by these compounds. Additionally, these compounds were demonstrated to trigger the DNA-sensing cGAS-STING pathway of IFN induction. Interestingly, VP35 was not able to block IFN activation by these compounds but suppressed Sendai virus (a known activator of RIG-I signaling pathway) mediated inducer of IFN, suggesting that ATM- and cGAS-STING-dependent IFN responses are insensitive to inhibition by VP35. These compounds also suppress EBOV replication in vitro and induce IFN in the presence of IFN-antagonist proteins from multiple negative-sense RNA viruses. This work provides new insight into signaling pathways activated by important chemotherapy drugs, identifies how limits of VP35 function can be exploited for therapeutic development and defines a novel avenue for therapeutic intervention against emerging RNA viruses.
Ebola virus (EBOV) persistence in asymptomatic humans and Ebola virus disease (EVD) sequelae have emerged as significant public health concerns since the 2013–2016 EVD outbreak in Western Africa. Until now, studying how EBOV disseminates into and persists in immune-privileged sites was impossible due to the absence of a suitable animal model. Here we detect persistent EBOV replication coinciding with systematic inflammatory responses in otherwise asymptomatic rhesus monkeys that had survived infection in the absence of or after treatment with candidate medical countermeasures. We document progressive EBOV dissemination into eyes, brain, and testes through vascular structures similar to observations in humans. We identify CD68+ cells (macrophages/monocytes) as the cryptic EBOV reservoir cells in the vitreous humor and its immediately adjacent tissue, in the tubular lumina of the epididymides, and in foci of histiocytic inflammation in the brain but not in organs typically affected during acute infection. In conclusion, our data suggest that persistent EBOV infection in rhesus monkeys could serve as a model for persistent EBOV infection in humans, and we demonstrate that promising candidate medical countermeasures may not completely clear EBOV infection. A rhesus monkey model may lay the foundation to study EVD sequelae and to develop therapies to abolish EBOV persistence.
Virus Structure and Biology III

**OP 10**

**Comparative transcriptomics of Ebolavirus Makona strain infected ferrets (Mustela putorius furo), non-human primates, and humans**

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Small animal models have been developed for several Filoviridae species, yet most all require serial adaptation to achieve uniform lethality. Using outbred domestic ferrets, we recently described lethal models of disease for Bundibugyo, Sudan, and Zaire species of *Ebolavirus*, using wild-type, nonadapted viruses. Pathologic features were consistent with disease in humans and non-human primates. While there is great advantage for the development of medical countermeasures using a model that does not require adaptation, a comprehensive characterization of the host response is difficult for the ferret given the dearth of available reagents to characterize the response to infection. With the recent publication of the ferret draft genome, comprehensive transcriptomic analysis is now possible. In this work, we present RNAseq data of Ebola virus-Makona infected ferrets taken from whole blood over the course of disease. Our analysis includes direct comparisons with longitudinal RNAseq data taken from humans and non-human primates and provide further support for the use of ferrets as an excellent model for the development of vaccines and therapeutics for *Ebolavirus* infections.
Immune Response to Filovirus Infection

OP 11
Immunogenetics of Ebola virus disease

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The HLA class I and II genes play a very important role in the initiation of the adaptive immune responses. These molecules are highly polymorphic in order to present a wide array of antigenic peptides and different levels of this diversity as well as the expression of specific alleles have been associated to improved immune responses to viral infections. Facing the HLA-peptide complex during the immunological synapse, the CDR3 sequence in the T cell receptor (TCR) determines antigen specificity of the T cell responses. In order to be able to recognize the infinite possibility of peptides presented, the TCR display a highly diverse repertoire which can also be correlated to an improved capacity of the host to clear viral infections. In this study we genotyped the HLA class I and II alleles from fatal cases and survivors in order to evaluate the potential influence of these alleles polymorphism in disease outcome. Heterozygosity at the HLA-A locus was significantly associated with survival supporting the important role of CD8 T cell responses during EVD disease. We also sequenced the TCR of Ebola virus disease (EVD) patients who succumbed or survived the disease and evaluated the possible influence of the TCR repertoire diversity in the clearance of Ebola virus (EBOV) and in recovery. Our results indicated that survival is significantly associated with an elevated TCR diversity as well as with a lower amount of shared TCR sequences between individuals. Our findings support the importance of HLA and TCR polymorphisms for the activation of a broad and effective adaptive immune response.
Introduction: For several decades after the discovery of Filoviruses, immune responses that arose in survivors remained poorly understood. Early studies revealed IgM and IgG antibody responses to infection with various Filoviruses, but recent years have seen an expansion in our understanding of Filovirus immune responses due to recent outbreaks. Immune responses in survivors of EBOV and SUDV infections have provided the most insight, with CD4+ and CD8+ T cell responses as well as detailed antibody responses having been described. Immune responses to MARV, however, remain almost entirely uncharacterized.

Objectives: We have undertaken the characterization of immune responses in survivors of MARV infection.

Patients and methods: Our studies included confirmed survivors, according to patient PCR and ELISA results, from the MARV outbreak of 2012 in the Ibanda and Kabale districts of Uganda, as well as healthy local community members that were not infected. Flow cytometry, ELISA and PRNT assays were used to characterize anti-MARV immune responses.

Results: MARV survivors developed multivariate CD4+ T cell responses but limited CD8+ T cell responses, more in keeping with responses seen in SUDV survivors than EBOV survivors. In stark contrast to SUDV survivors, rare neutralizing antibody responses in MARV survivors diminished rapidly after the outbreak.

Conclusions: We demonstrate that immune responses in MARV survivors share characteristics with EBOV and SUDV infections; however, there are distinct differences. These results warrant serious consideration for any vaccine or therapeutic that seeks to be broadly protective as different Filoviruses may require different immune responses to achieve immunity.
Immune Response to Filovirus Infection

OP 13
Immune responses and clinical outcomes in Ebolavirus (Makona)-infected patients

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In addition to the diagnosis of EBOV infection performed in patients admitted into the Ebola Treatment Center in Macenta (Guinea), we studied virological and immune parameters associated with fatal and non-fatal outcomes in a cohort of patients who did not receive experimental specific EBOV therapy and observed different responses according to the outcome. Indeed, fatal issue was associated with a significantly higher infectious and RNA viral load, whereas weak, even absent, humoral immune responses characterized these patients. In contrast, early and robust IgG responses directed against the different proteins of EBOV were detected in survivors. To further characterize the immune responses induced during the course of EBOV disease (EVD), 96 analytes were analyzed in 120 patients plasma samples thanks to the Luminex technology and a microarray analysis was performed in 60 RNA samples obtained in leucocytes from patients to describe the transcriptomic responses. Interestingly, specific immunological patterns were associated with each outcome from the very first days after symptom onset. Together with the virological and humoral response parameters, these results suggest that the outcome during EVD is early determined in the absence of specific antiviral therapy. Further studies are needed to understand the mechanisms responsible for these different responses.
Characterization of memory immunity in long recovered Sudan virus survivors sheds light on the role of individual viral proteins in triggering memory immune activation


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Question: Robust humoral and cellular immunity are critical for survival in humans during an Ebola virus infection. However, the interplay between these two arms of the immune response in long recovered Ebola survivors is poorly understood.

Methods: We examined residual immune responses in survivors of the Sudan virus (SUDV) outbreak in Gulu, Uganda (2000–2001). Cytokine and chemokine expression levels in SUDV PBMC cultures were assessed by multiplex ELISA and flow cytometry following SUDV viral protein GP, NP, VP30, VP40 and irradiated whole virus stimulation. Antibody and corresponding neutralization titers were also determined.

Results: Flow cytometry and multiplex ELISA results demonstrated significantly higher levels of CD4 T cell response following NP, VP40 and irradiated whole virus stimulation in survivors with serological neutralizing activity. These survivors also demonstrated the capacity to strongly trigger an ADCC response using FcγRI and FcγRIIIA reporters with an antigen specific binding ELISA.

Conclusions: The results of our study indicate that long-lasting Sudan virus-specific memory immunity persist years after recovery in some survivors and, importantly, this memory immunity could be triggered by individual viral proteins. The strong interplay relationship between memory CD4 T cell responses, serological neutralizing capacity and ADCC immunity in this cohort of SUDV survivors is key for understanding long lasting memory immunity in survivors of Filovirus infections.
Immune Response to Filovirus Infection

OP 15
Dissecting the Ebolavirus specific humoral response at the immunoglobulin isotype level using domain programmable arrays

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Hemorrhagic fever disease outbreaks caused by Filoviruses were considered sporadic and limited to certain regions of Africa, until the recent Ebola virus (EBOV) disease outbreak in Western Africa. The last event has severely impacted 3 countries (Guinea, Sierra Leone and Liberia) and affected the United States of America of individuals (28,610 confirmed cases and 11,308 deaths). Although several vaccine candidates had significantly advanced their development as a response to the outbreak, a comprehensive dissection of the immune response generated by the vaccines and its comparison with human infection, were identified as significant gaps of knowledge to identify correlates of immunity and protection, which are crucial for the approval of these vaccine candidates. We have applied next-generation sequencing based approaches to dissect the humoral and cellular immune response after vaccination or infection with EBOV. Characterization of the humoral immune response include deep sequencing of the B cell receptor (Ig-Seq) and the utilization of a phage display system (Domain Programmable Arrays) to identify the epitopes targeted by the antibodies present in the serum of VSV-DG EBOV-GP vaccinated NHPs (Macaca fascicularis) or infected individuals. Notably, we observe that immunoglobulin isotypes responses in the vaccinated NHPs presented different patterns of epitope recognition. IgM and IgA response were broader; detecting epitopes throughout all the ORF of the EBOV GP, while IgG responses were more focused, mainly targeting the mucin-like domain of the EBOV GP. Although neutralizing antibodies that target the mucin-like domain (6D8 and 13F6) were described, most of the "broadly neutralizing antibodies" described until today against Filovirus had been identified against the GP base domain, which is the more conserved area of the GP. Understanding the mechanisms that promote IgG isotype switching in B cell clones that preferentially bias the response towards epitopes at the mucin-like domain of EBOV GP might have an important impact in vaccine design. Even when "protective" epitopes were described in this region, ideally, vaccines should promote broad responses to minimize the chances of generation of escape mutants.
Filovirus Vaccines I

OP 16
Human antibody repertoire following VSV-Ebola vaccination identifies novel targets and virus neutralizing IgM antibodies

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Development of an effective vaccine against Ebola virus is of high priority. However, knowledge about potential correlates of protection and the durability of immune response after vaccination is limited. We elucidated the human antibody repertoire after administration of vesicular stomatitis virus (VSV)-Ebola vaccine at 3 million, 20 million and 100 million plaque-forming units (PFU) and homologous VSV-Ebola vaccine boost in healthy adult volunteers. Whole genome-fragment phage display libraries, expressing linear and conformational epitopes of Ebola glycoprotein (GP), showed higher diversity of antibody epitopes in individuals vaccinated with 20 million PFU than in those vaccinated with 3 million or 100 million PFU. Surface plasmon resonance kinetics showed higher levels of GP-binding antibodies after a single vaccination with 20 million or 100 million PFU than with 3 million PFU, and these correlated strongly with neutralization titers. A second vaccination did not boost antibody or virus neutralization titers, which declined rapidly, and induced only minimal antibody affinity maturation. Isotype analysis revealed a predominant IgM response even after the second vaccination, which contributed substantially to virus neutralization in vitro. These findings may help identify new vaccine targets and aid development and evaluation of effective countermeasures against Ebola.

Recombinant vesicular stomatitis virus (VSV) expressing the Ebola virus glycoprotein (EBOV-GP) in place of the VSV glycoprotein (VSV-G) is a promising vaccine candidate for protection from Ebola virus disease. Although chimeric VSV/EBOV-GP was tolerated by the majority of vaccinated volunteers it frequently caused adverse effects such as fever and arthritis suggesting that it might not be sufficiently attenuated. In this study, we present VSV/EBOV-GP vectors which have been modified to achieve higher attenuation while maintaining immunogenicity of the antigen. VSVMq/EBOV-GP expressing a mutant VSV matrix protein was unable to antagonize the cellular innate immune response and showed limited spread in interferon-competent cells as well as lower antigen expression levels. Immunization of guinea pigs with this construct resulted in lower titers of neutralizing antibodies than immunization with VSV/EBOV-GP, which was analysed by measuring antibody-mediated inhibition of virus spread in vitro taking advantage of a chimeric VSV/EBOV-GP expressing secreted Nano luciferase. Likewise, VSV expressing either a mutant EBOV-GP devoid of the mucin-like domain or secreted versions of EBOV-GP turned out to be less immunogenic than VSV/EBOV-GP. In contrast, VSV expressing the EBOV-GP F88A mutant or modified vaccinia virus Ankara (MVA) vector expressing wild-type EBOV-GP were unable to propagate autonomously but induced high levels of neutralizing antibodies in guinea pigs. These findings indicate that VSV vectors do not need to propagate in order to induce strong humoral immune responses to EBOV-GP. Propagation-incompetent viral vectors are expected to cause less adverse effects, which has already been clinically proven for MVA.
Filovirus Vaccines I

OP 18
Development, manufacturing and clinical evaluation of a multivalent vaccine targeting the viral hemorrhagic diseases Ebola, Sudan, Marburg and Lassa fever

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There is a significant unmet need for an effective hemorrhagic fever vaccine that would be safe for all at risk groups including pregnant women, infants and immune compromised individuals. Based on our ongoing research, we continue to further develop a safe, and immunogenic tetravalent vaccine containing four inactivated rabies virus chimeras expressing the glycoproteins of Ebola Zaire, Ebola Sudan, Marburg and Lassa viruses and a potent, clinically tested adjuvant (TLR-4 agonist) to address that need. The tetravalent vaccine as well as selected monovalent and trivalent components are tested for safety, immunogenicity and efficacy in non-human primate (NHP) studies and for safety in pivotal GLP toxicology studies. GMP compliant vaccines will be tested in a Phase 1 clinical trial to establish safety and demonstrate immunogenicity of the combined components.
Preventing future Ebola outbreaks — candidate Ebola vaccines delivered using a ring vaccination strategy

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Ebola outbreaks will inevitably occur in the future. A dozen candidate vaccines (including monovalent, bivalent or multivalent candidates) underwent or are actively undergoing clinical development at different trial phases. Seven vaccines have completed or are in trials up to Phase 1 stage, four vaccines up to or in Phase 2 stage, and one vaccine has completed Phase 3 stage. The Phase III trial for an rVSV-vectored candidate vaccine (rVSVΔG-ZEBOV-GP) was undertaken in Guinea and is the only study that has reported clinical efficacy and effectiveness for any candidate Ebola vaccine. Subsequently to the Phase 3 trial, ring vaccination of adults and children with rVSV-ZEBOV under Expanded Access was safely and effectively implemented in response to an outbreak of Ebola Virus Disease in Guinée Forestière (2016).

Should an Ebola virus disease outbreak occur before any candidate vaccine is licensed, the Strategic Advisory Group of Experts (SAGE) on Immunization recommends that the rVSVΔG-ZEBOV-GP vaccine be promptly deployed under the Expanded Access framework, with informed consent and in compliance with Good Clinical Practice. If the outbreak is caused by an Ebola virus species other than Zaire, consideration should be given to the use of other candidate vaccines that target the putative viral species. Ring vaccination, as used in the Phase 3 study in Guinea, is the recommended delivery strategy. This should be adapted to the social and geographic conditions of the outbreak areas and include people at risk including but not limited to: (i) contacts and contacts of contacts; (ii) local and international health-care and front-line workers in the affected areas and (iii) health-care and front-line workers in areas at risk of expansion of the outbreak.

We will present an overview of the above, together with the data on effectiveness and safety and lessons learned from implementing ring vaccination under the Expanded Access framework in response of the Ebola outbreaks (in Guinée Forestière, 2016 and as planned in DRC, 2017) and will discuss how WHO is working with partners and Member States improve our level of preparedness for deployment of candidate Ebola vaccines using ring vaccination in future outbreaks.
Filovirus Vaccines I

OP 33
Microneedle patch delivery of EBOV GP nanoparticles and sGP protein subunit vaccines protects mice against lethal EBOV challenge

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Introduction: The 2014 EBOV outbreak in West Africa caused over 28000 human infections with over 11000 fatalities. Presently, a new outbreak is occurring in DRC and the number of suspected cases has risen to 29 as of May 20, 2017. While a recombinant VSV EBOV vaccine demonstrated high efficacy during the 2014 outbreak, it is still unlicensed. Thus, the development of new vaccine technologies that are safe, stable, and efficacious is still of high interest.

Objectives: To investigate immunogenicity of EBOV GP and sGP protein subunit vaccines delivered to skin by MN and their protective efficacy against challenge.

Materials and methods: EBOV GP nanoparticles were produced from SF9 insect cells by Novavax. The sGP was produced in HeLa cells and purified through a his-tag column. GP nanoparticles and sGP were coated onto MN with or without an adjuvant (Matrix-M1), and used to immunize mice (groups of 5) via intradermal (ID) delivery. For comparison, groups of mice were also immunized by IM injection of the same dose of GP or sGP with or without the adjuvant. The control mouse group received IM injection of PBS. Mice were vaccinated twice at weeks 0 and 4, and challenged with 100 pfu EBOV at week 12. Blood samples were collected at week 6 for analysis of antibody responses.

Results: ID delivery of GP nanoparticles or purified sGP by MN induced higher levels of antibody responses against GP than IM injection of the same vaccine at the same dose. Further, co-delivery of an adjuvant with these vaccines enhanced the levels of antibody responses induced by ID delivery or IM injection. All mice that received GP or sGP vaccines together with the adjuvant by ID or IM route were protected from lethal challenge. Further, 4 out of 5 mice that received MN delivery of GP without adjuvant also survived the challenge, but only 1 out of 5 mice survived challenge after IM injection of GP without adjuvant.

Conclusions: ID delivery of EBOV GP and sGP subunit vaccines using MN, which offers ease of administration, safety and stability, can confer effective protection against EBOV challenge. Successful protection against EBOV challenge correlated with the level of antibodies to GP.
Monoclonal antibodies (mAbs) targeting the Ebola virus glycoprotein (GP) are an important approach for treatment of Ebola virus disease (EVD). Several potent mAb clones have demonstrated successful protection against Ebola virus infection in animal challenge models and an anti-GP mAb cocktail, ZMapp, was associated with favourable recovery in confirmed human patients. Unfortunately, protein IgG mAb delivery for treatment of EVD is met with significant hurdles including the requirement for several high-dose administrations and manufacturing costs that could be limitations for wide-spread delivery during a potential outbreak. We recently described the engineering and delivery of synthetic DNA-plasmid encoded antibody (DMAb) as an alternative approach for \emph{in vivo} administration of potent mAb clones. This strategy employs skeletal muscle cells as biological factories to secrete functional antibody that is detectable in systemic circulation, bypassing the need for protein IgG delivery. We have designed and evaluated >40 DMAbs encoding anti-GP mAbs that target the GP glycan cap, fusion loop, chalice base, and HR2 region. EVD DMAbs can be detectable in mouse serum for >100 days and achieve 20-80μg/mL Cmax expression levels. These have similar binding ability to protein IgG and map to the same epitope, with similar dropout mutations. BALB/c mice were injected with DMAbs and challenged with 1000LD50 of mouse-adapted EBOV (Mayinga). We observed 100% protection with two different DMAbs in mice when delivered 28 days prior to challenge. Experiments evaluating delivery of multiple DMAbs as a cocktail approach are ongoing. Additionally, we are studying non-traditional engineered bispecific IgG isoforms that have affinity for multiple GP targets. This new technology has (DMAbs) significant advantages for the generation of rapid protection from EID pathogens.
Therapy I

OP 21

Anti-glycoprotein immune responses of non-human primates following the treatment of Ebolavirus infection with monoclonal antibodies

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Introduction: The 2013-16 outbreak of Ebola virus disease in West Africa forced the international and scientific communities to work together in accelerating the development of post-exposure treatments for the infection. One of the more effective treatment approaches, cocktails of a few monoclonal antibodies directed against the glycoprotein, has very little history in being used for the treatment of infectious diseases. Vaccine studies have suggested that the anti-glycoprotein immune response is sufficient and necessary for protection. Given that the antibodies in the current therapies are directed at the glycoprotein, they may impact the anti-glycoprotein response; whether they do so positively or negatively remains unknown. With some treatments already under review for licensing by the FDA, the nature and intensity of the immune response in survivors of Ebola virus disease (or other Filovirus infection) will be important in understanding and predicting the development of future outbreaks, and managing the response to these outbreaks.

Methods: Here, we use IgG ELISA, flow cytometry, and serum cytokine measurement to look at the non-human primates that survived a lethal exposure to Ebola virus after receiving the treatments ZMAb, ZMappTM, or MIL77. We are interested in potential changes in the nature and the intensity of the overall and glycoprotein-specific immune responses. Some of the survivors have been re-challenged to assess whether their immune response would be protective on its own.

Results: There is considerable variation in the immune responses following treatment with ZMAb/ZMappTM/MIL77, possibly due, in part, to the different nature of the antibodies composing them and variation in the timing of the treatment. Re-challenged animals showed correlations between survival and both IgG responses and interferon γ production patterns in T cells.

Conclusion: With many more therapies in development, it will become more important to understand the immunological effects of those treatments for survivors. We highlight some patterns which may be of interest in future animal and human trials as new treatments are being developed.
Therapy I

OP 22
Protection from Sudan virus disease by a pan-Ebolavirus and pan-Filovirus antibody cocktail

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Introduction: Monoclonal antibodies (mAbs) have emerged as a promising candidate for treating Ebola virus disease. The majority of therapeutics in development thus far, however, are species-specific. We have previously described two antibodies, FVM04 and CA45, which can neutralize multiple species of Ebolavirus. Objectives: We evaluated the efficacy of a pan-ebola cocktail (FVM04+CA45) and a pan-filo cocktail (FVM04+CA45+MR191) in the guinea pig and NHP models of Sudan virus (SUDV) disease.

Methods: Guinea pigs were exposed to a guinea pig-adapted variant of Sudan virus (SUDV-GA) via the intraperitoneal route (IP). On day 3 or 4 following infection, guinea pigs were treated with the pan-ebola or pan-filo cocktail at a dose of 2.5 mg each mAb/animal of the anti-ebola antibodies, and 10 mg/kg/animal of the anti-marburg antibody. Eight rhesus macaques were challenged with 1000 plaque forming units of SUDV. Four days after infection, 3 animals were treated with the pan-ebola cocktail, and 3 were treated with the pan-filo cocktail. Two control animals were given vehicle. Animals were treated again on day 6 post infection with a lower dose of each antibody, or vehicle.

Results: While 5/6 PBS-treated guinea pigs succumbed to infection, all animals treated with the pan-ebola or pan-filo cocktail on day 3 post infection survived. There was no difference between guinea pigs treated on day 4 and control animals. In the NHP study, all animals treated with either the pan-ebola or pan-filo cocktail survived infection with minimal to no clinical signs of disease. One of two control animals succumbed on day 11 post infection, and both displayed signs of clinical disease over the course of the study.

Conclusion: These data are the first to demonstrate protective efficacy of mAbs against SUDV in the NHP model. Further studies to evaluate the efficacy of the pan-ebola and pan-filo cocktails against other species of Filovirus are ongoing. Monoclonal antibody cocktails that can protect against multiple species of Filovirus disease offer the best hope protection against multiple viruses, including species that may yet emerge.
Filovirus Vaccines II and Therapy II

OP 23
Different repertoires of antibodies induced by eight protective paramyxovirus-derived vaccines against Ebolavirus

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We have previously shown that a human parainfluenza virus type 3 (HPIV3)-vectored vaccine expressing the glycoprotein (GP) of EBOV (HPIV3/EboGP) administered as one aerosol dose to the respiratory tract conferred 100% protection in rhesus macaques exposed to EBOV (Meyer et al., 2015, Journal of Clinical Investigation). Here we generated a repertoire of nine replication competent mucosal vaccines derived from human (HPIV3 and HPIV1) or several serotypes of avian paramyxoviruses (AMPVs) expressing full-length GP to circumvent vector immunity and improve potency. Groups of guinea pigs received a single intranasal vaccine dose and were subsequently infected with lethal guinea-pig adapted EBOV. We show that the HPIV3, HPIV1, and several of the AMPV constructs were protective and generated different GP-specific IgG and neutralizing antibody levels. With Biolayer interferometry (BLI), we found differences between the vaccine groups sera interaction with the following GP forms: GPΔTM (transmembrane-deleted), sGP, GPΔmuc (lacking the mucin-like domain (MLD)), and GPcl (truncated GP1 bound to GP2). The GP epitope diversity of the sera was mapped by competition BLI assays. Sera preadsorped with the different GP forms revealed that a substantial portion of the response targets regions within the GPΔmuc, not shared with sGP or GPcl, which was somewhat vaccine vector dependent. The magnitude of serum competition with known neutralizing monoclonal antibodies targeting key GP epitopes correlated with neutralizing titers. Peptide microarrays identified three regions within the MLD and the C-terminal region of GP1 recognized by sera from most vaccine groups. Preadsorption of sera with GPΔmuc, but not sGP, led to a vaccine vector-dependent loss of neutralization activity indicating that the MLD and epitopes shared between sGP and the GPΔTM may not be responsible for virus-neutralizing activity. These findings indicate that different mucosal vaccines expressing the same GP antigen evoke a different repertoire of host antibodies, which may influence the protective outcome. The vaccine vectors successful in guinea pigs are being tested for protective efficacy in non-human primates.
Filovirus Vaccines II and Therapy II

OP 24

Preclinical development of an Ebola virus vaccine based on recombinant subunits expressed in insect cells

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Introduction: The 2013-2016 Zaire Ebola virus (EBOV) outbreak in several West African countries with more than 28,000 reported cases revealed the true epidemic potential of Filovirus infections. Export of cases outside the outbreak region and subsequent limited spreading highlighted to the global health community the potential threat posed by a Filovirus outbreak that cannot be contained at its source.

Despite significant progress with the clinical development of EBOV vaccine candidates during and after the West African outbreak, no EBOV specific vaccines have yet received regulatory approval.

Objectives: Our objectives are to initially develop a safe and effective subunit EBOV vaccine and to define the mechanism and immune correlates of protection for EBOV and subsequently to other Filoviruses.

Methods: We produced soluble recombinant glycoprotein (GP), as well as VP24 and VP40 from EBOV, SUDV and MARV using the Drosophila S2 cell expression system. Immunogenicity and protective efficacy of highly purified recombinant subunits and admixtures formulated with or without adjuvants were evaluated in rodents and macaques.

Results: Strong antigen-specific IgG and virus neutralizing titers were observed after administering two or three doses of adjuvanted formulations.

Vaccine candidates showed that both humoral and cell-mediated immunity can be raised by recombinant subunits and contribute to protection in the mouse model of EBOV. Guinea pig studies helped to optimize antigen dosing, antigen balance and adjuvant selection leading to 100% protective efficacy in the EBOV guinea pig model.

Non-human primate studies demonstrated that vaccination with formulations of recombinant EBOV subunits and an emulsion-based adjuvant consistently produced high anti-EBOV IgG and virus neutralizing titers. These formulations significantly reduce viremia subsequent to challenge with 100 or 1000 pfu of low-passage EBOV Kikwit strain (7U) and completely protect cynomolgous macaques from terminal EBOV disease.

Conclusion: Our studies suggest that we have defined a safe and immunogenic EBOV vaccine candidate based on non-replicating viral subunits with a desirable efficacy profile.
We have previously shown that macropinocytosis is the primary cellular entry route taken by Filoviruses. Blocking this pathway profoundly inhibits infection. Unfortunately, only a few small molecule inhibitors of macropinocytosis, with the exception of amilorides (which suffer from high toxicity) are available. We previously reported a high-throughput screen of 319,855 small molecules against Ebola and Marburg viruses. An analysis of the mechanism of action of these compounds revealed novel macropinocytosis inhibitors. These inhibitors were shown to prevent Ebola virus and Marburg virus infection of cell lines as well as primary human macrophages, and had lower cytotoxicity than the most potent amiloride, EIPA; thereby demonstrating their potential for development into therapeutic drugs.

MLS000733230 (EC50-16 µM) was a more promising compound due to its chemical structure, ease of synthesis and derivatization potential. We have been using medicinal chemistry approaches to improve potency of this compound, and to evaluate structure activity relationships of different derivatives. This effort has identified a derivative that has improved the EC50 to 1.5 µM. We have also been able to identify the core structure required for activity. Evaluation of dose tolerance in mice showed that this derivative can be administered without adverse side effects and is now being evaluated for treatment of disease. Along with developing more potent derivatives, we are using this compound to better understand Ebola virus trafficking into cells and to identify its molecular target. We will present the data from this work including the latest developments.
Filovirus Vaccines II and Therapy II

OP 26
Advanced development of immuno-therapeutics against Ebolavirus infection

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Introduction: Ebola virus causes a severe haemorrhagic fever in humans with a case fatality rate from 50-90%. The 2013-16 Ebola outbreak in West Africa has claimed >28,000 infections and 11,300 deaths. This historical outbreak has forced us to expedite the clinical evaluation of several Ebola vaccine and therapeutic candidates. ZMapp was one of such therapeutic based on its strong preclinical and clinical trial data. These promising results have stimulated the scientific field to develop more antibody (Ab) therapeutics options against Ebola and other Filovirus infections.

Objectives: to developed more Ab products which include: 1) more potent and broad-spectrum/cross-protective monoclonal antibodies (mAbs) against all species of Ebola; 2) alternative mAb products such as hyper-immune horse F(ab)2"; 3) reformulation of ZMapp/MIL77 with a nanoparticle to develop thermo-resistant Abs which would avoid the cold chain storage and transportation requirement.

Methods: ELISA and neutralization assay were used to evaluate the binding affinity and the neutralization potency of the Ab products; the protective efficacy of the Ab products were evaluated in guinea pigs, ferrets and Rhesus macaques.

Results: 1) two Pan-Ebola mAb cocktails were identified and they are cross protective against Ebola, Sudan and Bundibugyo virus infection in guinea pigs and ferrets, respectively. 2) Administration of equine F(ab')2 resulted in 100% protection in Rhesus macaques, and the treated animals showed virtually no observable signs of disease throughout the course of the experiment; 3) In comparison to the fresh MIL77, Nanoparticle reformulated MIL77 which was heated at 70oC for 7 and 14 days, similar level of protection in guinea pigs infected with Ebola virus was observed; even the nano-MIL77 heated for 21 days group showed partial protection.

Conclusions: These products will be great supplements to the existing immuno-therapeutics pool for Ebola virus. The protective efficacy of these products in animal models is very promising and further investigation is warranted.
Evaluation of GS-5734 as a therapeutic treatment for Filovirus infections

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Introduction: The 2013-16 Ebola virus (EBOV) outbreak in West Africa highlighted the urgent need for development of agents for the treatment and prevention of Ebola virus disease (EVD). Currently, there are no approved therapeutics for any Filovirus infection. Developed by Gilead Sciences in collaboration with UNITED STATES OF AMERICA MRIID and US CDC, GS-5734 is a monophosphoramidate prodrug of a modified adenosine nucleoside analog with a potent in vitro antiviral activity against multiple RNA viruses. In cell-based virus replication assays in multiple human cell types, GS-5734 exhibited antiviral activity against a broad-spectrum of Filoviruses with EC50 values ranging from 14 to 240 nM. Objectives: Research objectives were to characterize the post-exposure efficacy of GS-5734 in nonhuman primate (NHP) Filovirus disease models.

Materials and methods: GS-5734 was administered once daily by IV injection to NHPs beginning 3 to 5 days following exposure to either EBOV Kikwit/1995, EBOV Makona/2014, or MARV Angola/2005.

Results: In rhesus monkeys infected with EBOV Makona/2014, injection of GS-5734 (10 mg/kg) for 12 days initiated 3 days after challenge resulted in 100% survival accompanied by > 5 log10 plasma viral load reduction. In rhesus monkeys infected with EBOV Kikwit/1995, administrations of 5 mg/kg GS-5734 beginning 4 days (with 10 mg/kg loading dose of) and 5 days (without loading dose) after challenge, when systemic viral RNA was detectable in nearly all monkeys, conferred survival to 100% and 50% of treated animals, respectively. The in vivo efficacy of GS-5734 was further demonstrated in cynomolgus monkeys infected with Marburg virus (Angola/2005) where GS-5734 treatment starting at 5 days post-infection resulted in > 80% survival.

Conclusion: GS-5734 exhibits broad-spectrum efficacy against Filoviruses in multiple NHPs disease models. Phase 1 single and multiple dose studies with GS-5734 in healthy humans have seen completed. The clinical safety and efficacy of GS-5734 is being tested in West African male EVD survivors with persistent virus shedding in a phase 2 clinical study (PREVAIL IV).
The role of passive and acquired humoral immunity in the maintenance of Marburgvirus in Rousettus aegyptiacus bat colonies

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The Egyptian fruit bat, \textit{Rousettus aegyptiacus}, is a reservoir host for Marburg virus (MARV), but the role of naturally acquired humoral immunity in the dynamics of MARV transmission and maintenance in bat populations is poorly understood. Determining the duration of maternal immunity in bats, and whether infection with MARV induces long-term, sterilizing or transient immunity after primary infection may therefore assist in understanding how herd immunity impacts on MARV population transmission dynamics. Maternal anti-glycoprotein (GP) and anti-nucleoprotein (NP) antibodies were lost within 4 months after birth, making juveniles susceptible to MARV infection around 4 to 5 months of age. Antibodies against the GP and the NP in experimentally MARV-infected \textit{Rousettus aegyptiacus} peaked at 12 days post-infection, and then declined towards day 110, suggesting that bats eventually lose humoral immunity following primary infection and may become susceptible to re-infection with MARV. To test if naturally immune bats are fully protected, we infected 18 \textit{Rousettus aegyptiacus} bats with different levels of virus-specific IgG antibodies, with MARV. Blood of 14 (77.7\%) bats was positive for MARV by PCR. Viral RNA was also detected in spleen (50\% of bats), liver (27.7\%) and lung (5.5\%) at different days post infection. Levels of anti-MARV IgG antibodies also increased rapidly from day 5 post re-infection. Our results suggest that infection of \textit{Rousettus aegyptiacus} bats with MARV does not induce long-term and sterilizing immunity, which might be one of the important factors driving virus maintenance in nature.
Non-retroviral integrated RNA viral sequences (NIRVs) are thought to reflect rare events where sequences of RNA viruses were reverse transcribed through co-option of reverse transcriptase and integrated into the germlines of select species. Among the identified NIRVs are Filovirus VP35-like genes present in the genomes of a number of mammals, including mouse-eared bats (Myotis). The syntenic integration of a VP35-like gene in Myotis bats signifies a single integration event that occurred an estimated 13.4 million years ago. Strikingly, the open reading frame has been preserved across this time, suggesting functional benefit of the VP35-like protein-coding region. Filovirus VP35 proteins are multi-functional with essential roles in the viral RNA polymerase complex, as structural components of the viral particle and as an interferon (IFN) antagonist. To better understand this apparent vestige of an ancient virus-host interaction, we sought to define the structural and functional conservation and to define how selective pressures have shaped the evolution of Myotis VP35-like genes. A panel of Myotis-derived VP35s were expressed in 293T cells and demonstrated to retain some IFN-antagonist function. However, this antagonism was substantially less efficient than that seen with viral VP35s. Consistent with this observation, batVP35s lacked dsRNA binding activity. We further demonstrated that batVP35s are unable to functionally substitute for Ebola virus or Marburg virus VP35s in minigenome assays of viral polymerase function and fail to interfere with these systems. Despite these findings, the N-terminus of batVP35 has retained the ability to form tetramers, like Ebola virus VP35, properties important for anti-IFN and RNA synthesis roles of viral VP35. A solved crystal structure of the C-terminal domain of batVP35, which corresponds to the interferon inhibitory domain (IID) of viral VP35s, exhibits striking structural homology to viral VP35s. Together, these data suggest a model where evolution has preserved the structure of batVP35 but substantially altered its function, suggesting evolution toward functions that may differ from VP35s present in extant Filoviruses.
Ecology and Epidemiology

OP 30
Epidemiological and ecological investigations surrounding the 2017 Ebola outbreak in the Democratic Republic of the Congo

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Clinical Management

OP 31
Management of an Ebola virus infection in a specialized treatment center in Hamburg, Germany

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Depending on the setting (outbreak in a resource limited area in Sub-Saharan Africa, cases imported to developed countries, voluntary evacuation of patients to specialized treatment centers) treatment options of patients with Ebola virus infections vary vastly.

In this presentation we would like to share our experience from the 2013-2015 Ebola-outbreak in West-Africa where our center was involved in the treatment of an infected WHO employee. In Hamburg, patients like this are treated in the unit for the treatment of highly contagious infections (UTHCI) at the University Medical Center Hamburg-Eppendorf. Inside the UTHCI an autonomous intensive care unit equipped with state of the art point-of-care diagnostics and treatment options, including extended monitoring for vital parameters, laboratory testing, radiology, ultrasound and extracorporeal organ support, like hemodialysis and mechanical ventilation is located.

Finally, we demonstrate that rigorous training of all professions involved (nurses, infectious diseases physicians, intensivists, radiologists, hematologists, virologists, microbiologists, facility managers, public relation and media specialists) is necessary to successfully run an operation like this.
Clinical Management

OP 32
T cell analysis amongst survivors of the 2014 – 16 EBOLA outbreak in West Africa

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Introduction: The recent outbreak of Ebola virus disease (EVD) in West Africa resulted in ~30,000 infections and ~11,000 fatalities, thus highlighting the need for effective vaccines and therapeutics. Vaccines to EVD include the ChAd3 and rVSV-ZEBOV candidates, both presenting the Zaire Ebola glycoprotein (GP) and both showing promise in in vivo experiments and clinical trials. However, there is limited information on how these vaccines correlate with the natural immune response elicited by EVD. Objectives: To aid in the understanding of these vaccine candidates, and to help inform on the natural immune response to EVD we investigated the T cell profile among survivors. Previous work has demonstrated a role for T cells in protection amongst those contracting EVD disease. However, little is known with regards to their involvement in naturally acquired immunity, for which dogma suggests provides protection from re-infection. Therefore we analysed the T cell response to a GP peptide pool using intracellular cytokine staining amongst two cohorts of survivors in Guinea. The objective was to identify the type and nature of polyfunctional T cell activated by our GP peptide pool.

Patients and methods: Peripheral blood mononuclear cells (PBMCs) from 50 EVD survivors and 20 EVD negative nationals were obtained with consent in 2016. Samples were obtained from two regions within Guinea, Coyah and Guéckédou, samples were tested by flow cytometry for their polyfunctional secretion of IFNγ, TNFα and/or IL2 amongst CD4+ or CD8+ T cells. Cells were stimulated overnight with a pool overlapping peptides which make up the GP.

Results: We found that up to 2 years after the event responses to GP peptide were detectable and that the polyfunctional T cell profile was similar to that reported for ChAd3 vaccination. Whereby, within the CD8+ and CD4+ T cell populations there were specific polyfunctional T cells showing either a memory or effector phenotype.

Conclusions: The results we obtained from Ebola survivors correlate well with ChAd3 vaccine data and should be used to help inform on vaccine efficacy and potential correlates of protection.
Public Health Response to Filovirus-Outbreaks

P 1
Stability, persistence, and disinfection of Ebolavirus in human blood

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We investigated the persistence and disinfection of two Ebola virus (EBOV) variants (EBOV/Makona-C05 and EBOV/Yam-May) in clinical matrices (human blood, simulated vomit or feces). Tests were performed on non-porous surfaces representative of hospitals, patient air-transport units, and personal protective equipment utilized by individuals treating Ebola patients. During the persistence study, both EBOV/Makona-C05 and EBOV/Yam-May persisted longest in dried blood in an environment representative of exterior West Africa, with short persistence in simulated vomit, and no persistence in feces. Persistence was not affected by non-porous surface type. EBOV/Makona-C05 was more resistant to the effects of drying in blood than EBOV/Yam-May, and therefore may persist longer in dried blood when starting virus titers are the same. We also examined the efficacy of common disinfectants (bleach, acidified bleach, Micro-Chem PlusTM, SteriplexTM SD, PurellTM Advanced, and peracetic acid) against EBOV in human blood and cell culture media matrices. Five percent peracetic acid was the only disinfectant that consistently reduced EBOV titers to the assay limit of quantification in dried blood. These data represent the first examination of the susceptibility of EBOV in dried blood to a broad range of disinfectants and demonstrates the difficulty associated with disinfecting EBOV in a dried blood matrix. These findings can be used to bolster public health efforts by informing risk assessments, informing preparedness and response procedures, and structuring remediation decisions during future Ebola virus disease (EVD) outbreaks. The resistance of EBOV/Makona-C05 to the stresses of drying in blood and the observed difficulty of disinfecting EBOV in dried blood may partially explain the magnitude of the 2014 outbreak in West Africa.

Figure 1
Transcriptomic and proteomic analysis of THP-1 macrophages infected with the Ebolavirus, Makona variant compared with Reston virus

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The host response to the Ebola virus, Makona variant was compared with that induced by Reston virus using a differentiated THP-1 cell model. High resolution RNASeq was used to profile the transcriptomic changes during infection and this data was then combined with SILAC proteomic data to identify significantly changing host factors. Validation experiments were performed in THP-1, A549 and HEPG2 cell lines to confirm observed transcript and protein abundance changes. Analysis identified networks of host factors regulated by nuclear factor kappa-beta, tumour necrosis factor and toll-like receptor 4. Subnetworks of genes showing significant differences in Reston virus and Ebola virus infected cells were also identified, which were involved in the antiviral response. Upstream regulator activity was manipulated using inhibitory compounds and the effects on Ebola virus and Reston virus lifecycle assessed by mini-replicon.
Public Health Response to Filovirus-Outbreaks

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The Ebola epidemic in West Africa proved to be the largest in the history of Filovirus outbreaks, causing the World Health Organization to declare a public health emergency of international concern in August of 2014. In collaboration with domestic and international partners, the Biomedical Advanced Research and Development Authority (BARDA) initiated investments in vaccines against Ebola in support of the overall response efforts. The urgency associated with the epidemic triggered the clinical evaluation of lead vaccine candidates starting in late 2014, with candidates rapidly progressing into Phase 2/3 studies in West Africa. BARDA has continued to support vaccines against Zaire Ebolavirus, focusing on the last remaining gaps to get at least one such vaccine to FDA licensure. As the Zaire Ebolavirus programs continue to mature, BARDA will begin to seek effective vaccine(s) against a broader range of Filoviruses.
Public Health Response to Filovirus-Outbreaks

P 5
Use of reverse genetics to recover virus strains solely relying on field-collected sequence information

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In recent years, the growth of deployable high-throughput sequencing capacity has enabled the rapid generation of complete viral genomes during outbreaks, facilitating near real-time molecular epidemiology. These genome sequences are usually made publicly available weeks to months before natural virus isolates are available to researchers. Not only there have been many cases where natural isolates were not widely available, but there have also been instances where they are not available at all, hindering progress in the development of diagnostics and medical countermeasures. Reverse genetics, the process of using cloned DNA to generate replicating viruses, can be used to generate virus stocks when natural isolates are unavailable. The "rescued" virus stocks can be used to study viral biology, and can also support regulatory requirements for the development of diagnostics. We have developed an Ebola Makona reverse genetics system starting from a commercially synthesized genome, and have used it to rescue variants of interest identified through our genomic studies for use in in vitro and in vivo assays of replication fitness and pathogenicity. We will describe our efforts to optimize rescue efficiency and present a comparison of the behavior of the rescued Ebola Makona variants in in vitro and in vivo assays. By extending this capability to other biodefense related pathogens and coupling this capability to fieldable sequencing and local gene synthesis capabilities, we have been able to significantly reduce the time to acquire critical reagents for biodefense related countermeasure development.
Public Health Response to Filovirus-Outbreaks

Comprehensive mapping of antibody epitopes and binding escape mutants using saturating mutagenesis and high throughput sequencing

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Defining viral mutations that allow antibody escape is important to our understanding of virus evolution in response to host immunity and critical to the development of effective medical immune-based countermeasures (MCMs) against infection. We have developed an approach to comprehensively map both linear and conformational antibody epitopes with a virus-free, mammalian cell-based, display system. Using a virus-free system allows antibody epitope mapping of high consequence pathogens outside of biocontainment; while the use of a mammalian cell-based system ensures the retention post-translational modifications. Additionally, we posit that a similar platform could be applied to any virus of interest, even those without a robust reverse genetics systems that would be needed to create a diverse library of replication competent viruses in alternative strategies. Moreover, our approach maps antibody epitopes with much greater resolution than traditional alanine scanning methods by representing every possible alternate amino acid at each position along the full-length target protein. Further, by using a mammalian cell display system, where the protein can be displayed in its natural conformation, this approach can map complex conformational epitopes.

We created a defined diversity library (DDL) of Ebola glycoprotein (GP) mutants using a high-throughput methods of saturating mutagenesis such that every possible "1aa-to-wild type" mutant is represented at each position across the full-length protein. The expression-controlled plasmid DDL of ~15,000 mutants was maintained in eukaryotic cells, where each cell expresses (after induction) copies of an individual "1aa-to-wild type" defined mutation. These cells were then sorted via fluorescence activated cell sorting (FACS) or magnetic beads to separate GP mutant expressing cells that bind or don't bind the antibody of interest. Deep sequencing of the separated GP mutant pools identified mutations enriched in the non-binding pool that are critical to antibody binding. This approach determined not only which residues are required for antibody binding, but also which specific amino acids at each residue are important. Interestingly, some mutations at a specific residue were strongly enriched in the non-binding pool while others were not enriched, indicating that only specific mutations at each position alter the binding capability of the antibody. Mapping antibody epitopes with DDLs will enhance our understanding of virus evolution within the host and define the evolutionary potential for the virus to escape antibody binding. This approach can likely be applied widely across virus families to inform more efficient and effective MCM development.

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Public Health Response to Filovirus-Outbreaks

P 7
Two tales of Ebolavirus genomics – near-real time epidemiology and viral persistence as a new focus on EVD clinical research


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Western Africa recently experienced the largest Ebola virus disease (EVD) outbreak ever recorded, with >28,000 reported cases and >17,000 EVD survivors. Using near real-time genomic surveillance, we were able to monitor viral evolution and track the spread of the virus through space and time. By establishing a local sequencing capability, we were able to obtain genomic data on timescales relevant to influence the public health response. At a broad level, the consolidated analysis of genomic information obtained by multiple Centers throughout Western Africa highlighted important roles for high-density population centers and frequent human migration in determining the magnitude and duration of the outbreak. Additionally, our investigation of local EVD flare-ups played a key role in shaping the World Health Organization’s recommendations for sexual contact among EVD survivors. We were able to document multiple instances in which EBOV was transmitted from persistently-infected survivors, even several months following acute infection. We also observed that these reemerged transmission chains presented a reduced evolutionary rate in comparison with the acute rate observed during the outbreak, thus indicating distinct replication dynamics during persistence. These persistent infections represent an important challenge for outbreak control and therapeutic treatment, and therefore, we have focused on understanding the mechanisms of EBOV persistence using a combination of in vitro and in vivo models with state-of-the-art viral population genomics and other "omics" technologies. Here, we will present our preliminary findings analyzing the persistence of EBOV in human and NHP immunopriviliged sites; as well as our "in vitro" studies of persistence in bat, human and NHP primary cell lines.
Filoviruses are filamentous viruses and include Ebola (EBOV) and Marburg (MARV), which are morphologically identical but antigenically distinct. Mortality rates can be as high as 90% and to date there are no FDA approved vaccines or small molecules for treatment outside the realm of emergency situations under the compassionate use clauses of FDA policy. EBOV harbors a genome of 7 proteins, the most abundantly expressed in mature virions is the matrix protein, Viral Protein 40 (VP40). VP40 is required for assembly and budding of EBOV and alone VP40 can form virus like particles (VLPs) from the plasma membrane of host cells. Recent work by the Stahelin and Ollmann Saphire labs indicates that VP40 adopts multiple different structures to elicit different functions in the viral life cycle. Cellular data demonstrates that each structure adopts a specific function, one for budding from the plasma membrane of human cells and one for regulation of viral transcription. This work investigates how distinct VP40 structures assemble in the presence of synthetic lipid vesicles and at the inner leaflet of the plasma membrane in live cells. This project aims to determine lipid composition requirements for functionality of VP40 mutants vs. wild-type VP40, and to elucidate the function of VP40 oligomerization with site-specific mutants. The C-terminal domains of the VP40 homodimer exhibit a highly conserved basic patch which is thought to promote interaction with anionic lipids. Preliminary data confirms VP40 binds with nanomolar affinity to liposomes containing phosphatidylserine (PS), an anionic lipid found within the inner leaflet of the plasma membrane, and has particular specificity for PS. Cellular data from the Stahelin lab provides precedent that VP40 requires PS in order to bud from the plasma membrane. Lipidomics analysis of mammalian cells transfected with VP40 has aided in the investigation of the species and saturation of lipid populations of in the plasma membrane versus lipid populations in VLPs. Preliminary data suggest that VP40 selects for not only specific species of lipids, but for particular saturation states of lipids within the plasma membrane.
Virus Structure

P 9
Ebolavirus and Marburgvirus use different modes of lipid gymnastics to assemble and exit the host cell

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Lipid enveloped viruses replicate and bud from the host cell where they acquire their lipid coat. Filoviruses, which include Ebola and Marburg viruses, bud from the plasma membrane of the host cell. These viruses cause viral hemorrhagic fevers and have a high rate of fatality. To date little is known about how these viruses use plasma membrane lipids to facilitate assembly and budding. This study has investigated the molecular basis of the plasma membrane assembly, budding and egress of these viruses, which is regulated by their matrix protein, VP40. Biochemical and biophysical tools along with cellular imaging and viral replication assays have been used to investigate how VP40 interacts with plasma membrane lipids to regulate viral replication. This presentation will outline the molecular basis of VP40 association with plasma membrane lipids and how lipid-protein interactions regulate VP40 oligomerization and plasma membrane bending. Furthermore, VP40 plasma membrane binding displays sensitivity to the lipid composition in the plasma membrane, which can be altered to inhibit Filovirus assembly and egress.
**Introduction and objectives:** A typical pipeline in our field has been antibody discovery, followed by cell culture neutralization and animal model testing. What particular antibody features, however, lead to ultimate in vivo protection and why have not been clear. Certainly neutralizing antibodies are known that do not protect animals and protective antibodies that do not neutralize in cell culture.

**Methods and procedures:** The Viral Hemorrhagic Fever Immunotherapeutic Consortium is a 40–laboratory, 5-continent academic, industrial and government collaboration aimed to understand which antibody features, measurable in vitro, best correlate with in vivo protection, and how we can use this information to improve antibody therapeutics and evaluate candidate vaccines.

**Results:** The statistical analysis of our collaborative, four-year Ebola virus antibody project indicates multiple paths to protection. Mechanical neutralization leads to protection as expected, but particular immune effector functions and polyfunctionality in particular also provide protection, indicating ways of engineering antibodies and combining antibodies for improved efficacy. A focus of our lab within this consortium is structural analysis to understand and improve function. New molecular structures of candidate immunotherapeutics against Marburg virus and Lassa virus illustrate their mechanism of action and propensity for escape. We will also describe structure-based rational engineering of a human antibody for pan-Ebolavirus activity.
Exploitation of the host cell SUMOylation machinery by Ebolavirus

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The conjugation of the small ubiquitin-like modifier (SUMO) molecules to target proteins has an important role in the modulation of a wide range of cellular processes by regulating different properties of cellular components, including their subcellular localization, stability, or activity. In addition, an increasing number of SUMOylated proteins can also interact with SUMO in a non-covalent manner through a SUMO interacting motif (SIM), an event that has been shown to be important for the formation of SUMO-dependent protein networks. Viruses have evolved to exploit the cellular SUMOylation system in order to improve their replication. In the case of Ebola virus, the VP35 protein induces the SUMOylation of IRF7 to disrupt antiviral responses. Moreover, the Ebola virus matrix protein, VP40, is modified by SUMO and this modified protein is included into the viral-like particles generated by VP40. Here we show that, in addition to VP40, SUMO can be also covalently conjugated to other structural proteins: GP, NP, VP35, VP24, and VP30; and that VP24 can also interact with SUMO in a non-covalent manner through a SIM domain. Regulation of the Ebola virus proteins by SUMO is accompanied by an increase in the abundance of proteins modified by SUMO and of unconjugated SUMO in the infected cells. Finally, we show that the VP40 protein is a key contributor to this SUMO response. In summary, our results indicate that Ebola virus interacts extensively with the cellular SUMOylation machinery suggesting that the SUMO pathway may be a promising target for therapeutic treatment of Ebola virus infections.
New infectious Ebola virus (EBOV) particles are released by budding from the host cell in a process that is driven by the viral matrix protein VP40. To this end VP40 encodes two overlapping late domain motifs at amino acid position 7 to 13 (PTAPPEY), which are also crucial for the formation of virus-like particles (VLPs). However, experiments with recombinant viruses have shown that neither of these late domain motifs is absolutely essential for viral replication in cell culture, indicating that alternative budding mechanisms must also exist. Recently, a potential third late domain motif (YPx(6)I) was proposed at amino acid position 18 to 26 of VP40. To analyze the importance of this motif in viral budding and (EBOV) particle infectivity, we used a transcription and replication-competent virus-like particle (trVLP) system. This system is based on a tetracistronic minigenome that encodes a reporter, as well as VP40, the EBOV nucleocapsid-associated protein VP24, and the viral glycoprotein GP1,2. Coexpression of this minigenome together with the viral proteins driving genome replication and transcription leads to the production of these minigenome-encoded proteins, and thus the formation of trVLPs, which incorporate minigenomes and can infect target cells. The advantage of this system is that the minigenome-encoded viral proteins are expressed in a regulated fashion rather than overexpressed. Thus, the study of viral morphogenesis, budding, and entry can be modeled in a more authentic way compared to conventional VLP assays. Using this system and introducing mutations to specifically disrupt the new potential late domain motif (YPx(6)I) we show that this motif does not appear to contribute to trVLP infectivity and budding. Further work with recombinant viruses will now be required to definitively clarify the role of this motif in EBOV budding. This finding has significance for our understanding of the budding process of EBOV, and also for the identification and prioritization of molecular targets for the development of novel therapeutics against these viruses.
Bats are considered the natural host for Filoviruses, but other animal species including pigs, duikers, non-human primates, and humans are also susceptible to infection with Filoviruses. The factors that govern host-susceptibility to infection and disease are only partially understood. Investigating such questions with infectious virus requires a maximum containment laboratory. However, tetracistronic transcription and replication-competent virus-like particle (trVLP) systems allow modelling of the complete Filovirus life cycle under biosafety level 1 or 2 conditions (depending on local biosafety regulations). These systems utilize tetracistronic minigenomes encoding a reporter protein, the matrix protein VP40, the glycoprotein GP1,2, and the nucleocapsid-associated protein VP24, flanked by non-coding Filovirus leader and trailer regions. Replication and transcription of these minigenomes results in formation of minigenome-containing trVLPs that can infect target cells, which are usually pretransfected to express the viral proteins needed for further minigenome replication and transcription, resulting in reporter activity reflecting infection. As such, the trVLP system is extremely well suited to studying virus entry, and thus also the susceptibility of different species to infection at the cellular level.

In order to investigate the potential host tropism of Filoviruses we have produced trVLPs carrying GP1,2 from different Ebolavirus species as well as from Marburg and Lloviu virus. Initial characterization in human cell lines showed that in all cases infectious particles are produced, and thus that functional GP1,2 proteins are incorporated into particles. However, there were pronounced differences in infectivity, which was not correlated to a phylogenetic relation of GP1,2 and other trVLP components. We further optimized these systems for detectable infection of naïve (i.e. untransfected) target cells, and are now in the process of screening a number of cell lines from various animal species in order to better understand the potential host range of different Filoviruses and the cellular requirements for susceptibility to Filovirus infection.
Ebola virus (EBOV) infection results in severe disease and in some cases lethal haemorrhagic fever. The infection is directed by seven viral genes that encode nine viral proteins. By definition, viruses are obligate intracellular parasites and require aspects of host cell biology in order to replicate their genetic material, assemble new virus particles, and subvert host cell antiviral responses.

An emerging area in virology is to transiently target host cell proteins that play critical proviral roles in virus biology, especially for acute infections. Proteomics can aid in discovery biology and identify cellular proteins that may be utilized by the virus to facilitate infection.

This work focuses on using high resolution approaches to characterize the interactome of the EBOV nucleoprotein (NP) in order to better understand the function of NP in the virus biology and also provide potential new targets for antiviral therapy.

To determine which cellular proteins or complexes interact with NP and to predict function, high-affinity purification coupled with a label-free mass-spectrometry-based approach was used. After the detection of the potential cellular interacting partners, inhibitors were used to ablate cellular protein function and monitor the effect on viral biology.

Using conservative selection criteria, approximately 120 cellular proteins were identified to have a high probability of interacting with NP. Some of the candidate proteins were cellular chaperones, including HSP70, which might associate with NP to promote stability.

A small molecule inhibitor, VER155008, was used to see the effect of the inhibition of the function of HSP70 for the EBOV biology. Utilization of a mini-genome replication system based on a recent Makona isolate demonstrated that disrupting the stability of NP through inhibition of HSP70 had an adverse effect on viral RNA synthesis, suggesting that the chaperone activity of HSP70 is responsible for maintaining the stability of NP.
Ebola virus (EBOV) causes severe hemorrhagic fever, and the mortality rate is up to 90%. There are no approved vaccines or antivirals against EBOV infection. To understand the molecular mechanism of EBOV replication facilitates to develop anti-EBOV drugs. Among seven genes which are encoded by Ebola virus genome, VP40 is known as a matrix protein. Therefore, sole VP40 expression in cells produces virus-like particle (VLP), which allowed us to investigate the molecular mechanism of virus particle production in Bio Safety Level (BSL)-2. So far, several domains and amino acids in EBOV VP40 were reported to be involved in the virion production procedure. C-terminus of VP40 is known to be important for membrane interaction and for interacting with COP-I vesicular trafficking machinery.

In this study, we focused YIGL motif which resides at the surface of the VP40 C-terminus on VLP production. Replacement of YIGL to AAAA (VP40-Mut) in VP40 significantly reduced VLP production compared to that of Wild type (WT). There were no obvious difference between VP40-WT and VP40-Mut cellular localization using immunofluorescence assay. We then utilized biochemical method to prepare subcellular fractions (membrane, cytoplasm, soluble nucleus, chromatin-bound nucleus, and cytoskeleton) to examine both VP40s cellular distribution. VP40-Mut localized in cytoplasm fraction with much higher degree compared to that of VP40-WT. Finally, we aimed to determine specific amino acid which is critical for reducing VLP production.

We introduced single alanine mutation to each YIGL amino acids, and then performed VLP assay. Our result showed that mutation in second I and forth L significantly reduced VLP production, while mutation in first Y and third G did not affect to the VLP production compared to that of VP40-WT. In silico study suggested that these amino acids affect to VP40 intramolecular interaction which might be important for the natural structure and function.

Overall, our results showed that YIGL motif in EBOV VP40 regulates VLP production, most likely through affecting cytoplasmic distribution.
In the year of 2016, a new state-of-the-art Biosafety Level 4 (BSL-4) facility was established in the Szentágothai Research Centre (SzRC) of the University of Pécs (UP). The SzRC is a new research institute established on the basis of modern international science organizational and management normatives. It covers all aspects of education, research and innovation in the fields of biomedical, natural and environmental sciences. SzRC provide an excellent basis to become a well-known, leading research facility in Hungary, as well as in Central Europe with an extensive collaboration network.

The BSL-4 virological laboratory is well equipped, designed properly and meets the requirements of the laboratory protocol declared by the Center for Disease Control and Prevention, Biosafety in Microbiological and Biomedical Laboratories (CDC-BMBL), ensuring the safe working conditions with highly infectious materials. The BSL-4 laboratory has official certificates for Occupational Health and Safety Management System (OHSAS 18001:2007) and for safety BSL-4 requirements. Besides the BSL-4 facility, our research group has an already suitably running molecular virological laboratory, sequencing and microscopic facility which provides all the necessary instruments. Moreover, SzRC is prepared and well equipped with laboratories for other molecular studies of high standards.

The BSL-4 laboratory belongs to the Virological Research Group (VRG), which is one of the leader group with outstanding research activity. The main profile of the VRG is the research of viral zoonoses. One of the main research aims is to identify well known and new pathogens in samples (primarily rodents, bats, mosquitoes, ticks and additional arthropod species) originating from any places of the World thanks to the extensive international collaboration network. Due to the BSL-4 facility, basic research activities just started recently, focusing mainly on the molecular pathomechanism of viruses, the molecular mechanism of host-parasite interactions, as well as the mechanism of action of different antivirals.

Web page: szkk.pte.hu/en/research_groups/molecular_biology_cluster/virological_research_group_0
Investigating the biogenesis of EBOV-encoded non-coding RNAs during infection

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The generation of viral non-coding RNAs (ncRNAs) has been a topic of interest in recent years. Virally encoded ncRNAs have been shown to play a role in viral pathogenesis and can act in a similar manner as microRNAs (miRNAs) by downregulating expression of mRNA targets. However, the biogenesis and mechanism of action of small ncRNAs by negative sense viruses such as Ebola virus (EBOV) are often overlooked. Previous studies have predicted EBOV-encoded miRNA sequences and potential host mRNA targets. However, the miRNAs and targets were not validated in the context of infected cells. Therefore, it is probably more appropriate to refer to them as ncRNAs until their biogenesis and functions during infection are determined. Since it is unlikely that EBOV, which replicates in the cytoplasm, generates small ncRNAs through a canonical mechanism involving nucleus-resident proteins, we have explored the biogenesis of these small ncRNAs. We have confirmed by small RNA sequencing and qPCR that one of the predicted small ncRNAs, located in the 5' UTR of VP40, is generated in infected cells. 5' RACE of RNA from infected cells demonstrated an uncapped RNA that we hypothesize corresponds to the cleavage product that yields the small ncRNA. Future experiments will address the involvement of Drosha/Dicer in the processing of the small ncRNA during infection.
Lentiviral- and filoviral-based platforms for studying the cell biology of Ebolavirus entry

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Introduction: Ebola virus (EBOV) is a negative-sense single-stranded RNA virus that enters target cells via macropinocytosis, which is induced by binding of the virus to cell surface components. Previous publications have identified T-cell immunoglobulin and mucin domain 1 (TIM-1), the Axl/Tyro3 TAM family, and Niemann-Pick C1 (NPC 1) as receptor candidates for Filoviruses entry.

Objectives: The aim of this project is to use both lentiviral and filoviral virus-like particle systems to study the cell biology of Ebola virus entry and tropism.

Material and methods: We use pseudotyped lentiviral particles to measure virus like particle (VLP) entry. To study EBOV entry specifically, we established an enzymatic-assay using VLPs containing β-lactamase tagged to the major viral matrix protein VP40 and the EBOV glycoprotein.

Results: Using pseudotyped lentiviral and Ebola VLP systems, we confirm that NPC1 is an absolute requirement for Filovirus entry, but that TIM-1 is necessary only in some cell lines. Macropinocytosis inhibitors block the entry of all filoviral pseudotyped particles, regardless of viral background. Furthermore, TIM-1 requirement in entry can be circumvented by spinoculation, implying that it is only necessary for initial attachment. Epidemic-associated changes in EBOV GP confer enhanced titre, but the magnitude of these effects is more pronounced on a filoviral-like particle background. While examining different cell lines, we have found evidence of clonal differences in human tumour cells that differentially effect the permissivity for Ebola vs Marburg entry. This appears independent of known entry factors and is not rescued by in vitro GP cleavage. Moreover, heterokaryon analysis suggest the existence of an unidentified EBOV entry cofactor.

Conclusion: The systems we established give us the possibility to investigate the EBOV entry. We will adapt these systems to perform a targeted CRISPR screen against cytoskeleton regulators to uncover novel cellular cofactors. This will yield further information on EBOV entry, detailing the important mechanism of macropinocytosis and cytoskeletal rearrangement.
Establishment of primary cell cultures from a microbat and study of their host cell factors for Filovirus entry

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The Ebola virus (Ebov) entry process involves many host cell factors including the C-type lectin (DC-SIGN), Niemann-Pick C1 (NPC1), T cell immunoglobulin and mucin domain (TIM) and TAM receptor tyrosine kinases (Axl, Mer and Tyro3). The significance of these factors for EBOV entry, a process predominantly mediated by the viral glycoprotein (GP), has been determined using various cell lines derived from humans, Ebov spill-over hosts, and potential Ebov reservoir-host including fruit bats. The host cell factors potentiating Ebov entry into cells derived from microbats, a potential reservoir host, remain unstudied.

In order to elucidate Ebov entry in microbats, we have derived primary cell cultures from numerous organs from M. condylurus, which has been implicated in spill-over during the 2014 West African Ebola epidemic and it has been shown as a susceptible species to experimental infection in a prior study. Primary cell cultures from spleen, liver, kidney, lung, trachea and heart were generated to provide diverse cell types for receptor expression characterization, as various bat cell types have been shown to take up Ebov-GP with different efficiencies. We have characterized receptor expression using immunofluorescence in early-passage and late-passage cells in an attempt to correlate receptor expression with cell permissiveness to Ebov infection. Susceptibility of primary cells to Ebov infection was determined by the presence of cytopathic effect and Ebov growth kinetics. Overall, our findings elucidate the relationships between host cell receptor-expression and Ebov replication efficiency in vitro in a potential Ebov reservoir-species.
Ebola virus inhibits cellular Nrf2-dependent anti-oxidant signaling pathway via expression of structural protein VP24

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Ebola virus (EBOV) causes hemorrhagic fever in man characterized by massive virus replication, dysregulated inflammation and an extremely high fatality rate. The VP24 protein of EBOV impairs the cellular response to IFN α/β and IFN γ, thus disrupting a key cellular antiviral pathway. Earlier we have demonstrated that Marburg virus (MARV) manipulate cellular Nrf2-dependent oxidative stress response via structural protein VP24. MARV VP24 was shown to bind Kelch-like-ECH-associated protein-1 (Keap1), a negative regulator of Nrf2. In this study we present evidence that, unlike MARV, EBOV affects oxidative stress response system in a different, inverse manner. EBOV VP24 appears to block translocation of Nrf2 into the nuclei strongly inhibiting transcription of cell-protective genes controlled by Nrf2. Repression of the genes controlled by Nrf2 results in increased production of pro-inflammatory mediators by infected cells, which likely to contribute to the pathogenesis of EBOV hemorrhagic fever. Recombinant EBOV carrying mutations that dramatically alter virus inhibition of the Nrf2 dependent pathway have been generated and assayed in this study.
The Ebola virus nucleocapsid protein VP30 is an essential factor for viral transcription but is not needed for replication of the viral genome. The transcription support activity of VP30 is regulated via phosphorylation at two N-terminal clusters (S29-S31 and S42, S44, S46). Other phosphorylation sites are threonine 52 and threonine 143 and 146. Phosphatase 1 and Phosphatase 2A (PP1 and PP2A) were identified to dephosphorylate VP30 in vitro, while the responsible VP30-specific kinase is unknown. Recently, it was demonstrated that dynamic phosphorylation of VP30 is essential for the viral replication cycle most likely because during early timepoints of infection only phosphorylated VP30 is transported along with the intruding nucleocapsids to the sites of primary transcription. Reverse genetics showed that a recombinant EBOV containing only serine 29, while the other five phosphorylatable serine residues of the VP30 N-terminal clusters were exchanged by alanine, was viable and had growth characteristics as wild type EBOV.

To further understand the dynamics of VP30 phosphorylation during the EBOV lifecycle, we generated a polyclonal phosphospecific antibody recognizing VP30 which is phosphorylated at serine 29. Here we demonstrated the specificity of this antibody for phosphorylated VP30 using transfection-, infection- and in vitro-studies. We show that during EBOV infection, VP30 is mainly dephosphorylated and phosphorylation of VP30 is largely influenced by the presence of other viral proteins, especially the nucleoprotein NP. The coexpression of NP resulted in a mainly dephosphorylated VP30. Using an in vitro phosphorylation assay, we could demonstrate that both phosphorylation and dephosphorylation of VP30 take place in NP-induced inclusion bodies suggesting both, phosphatases and kinases, to be recruited by viral proteins.

These results give insight into the spatio/temporal dynamics of VP30 phosphorylation during the EBOV lifecycle and contribute to the understanding of how EBOV transcription and replication are regulated.
Marburg virus (MARV) and Ebola virus (EBOV) belong to the family Filoviridae and cause severe, often fatal illness in humans and non-human primates. The recent epidemic of Ebola virus in West Africa with over 28,000 cases revealed the need for a deeper understanding of Filovirus infections in order to develop novel vaccines and antivirals. The latter might be achieved by identifying new cellular targets that play crucial roles during Filovirus infections. Here we present the results of a study to identify cellular interaction partners of MARV VP30, which modulates MARV transcription. A high-throughput yeast two-hybrid assay was performed with VP30 as the bait protein and the identified interactors were confirmed by co-immunoprecipitation. Using reverse genetics-based filoviral minigenome assays the function of the identified MARV VP30 interactors for filoviral transcription and replication was tested. We were able to show that the host cell proteins Protein inhibitor of activated stat 2 (PIAS2) and PIAS1 interact with MARV VP30 as well as EBOV VP30. The interaction site could be narrowed down to a conserved region in the N-Terminus of VP30. The PIAS proteins are E3 SUMO-protein ligases that play a role in different cellular pathways including interferon inhibition and cellular transcription regulation. We were able to show that PIAS2 and PIAS1 furthermore negatively regulate the transcription and replication in Filovirus minigenome assays. For PIAS2 a dose-dependent effect was shown. In contrast when PIAS2 function was abrogated using CRISPR/Cas9 technology, filoviral transcription and replication were elevated. Our study indicates PIAS2 and PIAS1 play a role as cellular restriction factors during Filovirus infections.
Emerging role of IQGAPs in Marburgvirus infection

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IQGAPs are evolutionary conserved proteins involved in the regulation of many cellular processes including cytokinesis, cell migration and proliferation, intracellular signaling, vesicle trafficking and cytoskeletal dynamics. Humans express three isoforms IQGAP1, IQGAP2 and IQGAP3. IQGAP1 is expressed ubiquitously, whereas IQGAP2 is mainly expressed in the liver and IQGAP3 in the brain. We could show earlier that IQGAP1 forms a comet-like structure at the rear end of Marburg virus (MARV) nucleocapsids during their transport in infected Huh-7 cells and siRNA down-regulation of IQGAP1 resulted in decreased MARV release. To further characterize the role of IQGAP1 in MARV infection we analyzed the release of infectious MARV from IQGAP1 knockout HAP1 cells. Here we observed almost no differences or even slightly increased titers in comparison to the parenteral HAP1 cells. Interestingly, we detected an upregulation of IQGAP2 expression in the IQGAP1 knockout cells in comparison to the HAP1 cell line. Together this data showed reciprocal regulation of IQGAP1 and IQGAP2 in knockout cells which in turn could explain minor effects on the release of MARV. To figure out the function of IQGAP1 and IQGAP2 in MARV infection we currently analyze the replication of MARV in IQGAP1 and IQGAP2 double knockout cells.
A single amino acid change in the active site of the L protein increases the polymerase activity

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The Marburg virus (MARV) causes a severe, often fatal, disease in humans and only a transient illness in rodents. Sequential passaging of MARV in guinea pigs resulted in selection of a guinea pig-lethal virus containing only 4 amino acid changes. A D184N mutation in VP40 (VP40D184N), which leads to a species-specific gain of viral fitness, and three mutations in the active site of viral RNA-dependent RNA polymerase L, which were investigated in the present study regarding their functional significance in human and guinea pig cells. The transcription/replication activity of the L mutants was strongly enhanced by a substitution at position 741 (S741C), and strongly inhibited by the other substitutions (D758A and A759D) in both tested species. The polymerase activity of L carrying the S741C substitution was eightfold higher in guinea pig cells than in human cells upon co-expression with VP40(D184N), suggesting that the additive effect of the two mutations provides MARV a replicative advantage in the new host.
Development of a novel stable Ebolavirus minigenome replicon reveals remarkable stability of the viral genome

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Introduction: Studies on Ebola virus (EBOV) have been greatly hampered since experimentations on infectious EBOV are restricted to a few biosafety level 4 (BSL-4) labs. EBOV minigenome system has provided researchers with the opportunity to study EBOV under BSL-2 condition. However, transient transfection of multiple plasmids each time to make this system work brings out problems such as hard manipulation, low efficiency and low reproducibility.

Objectives: Development of a more convenient and highly efficient EBOV minigenome system that allows for the investigation of viral replication and screen for antivirals under BSL-2 condition.

Materials and methods: Based on Huh7 cells, we generated the Huh7-4P cell line which stably expressed the viral RNP proteins (NP, VP35, VP30 and L). We then transfected EBOV minigenome EBOV-GLuc-Hyg containing Gaussia Luciferase (GLuc) reporter and hygromycin (Hyg) selection marker into Huh7-4P cells to generate the stable minigenome cell line. Employing this system, we investigated the replication of EBOV and property of EBOV genome by measuring GLuc activity and RNA levels.

Results: Under hygromycin selection, EBOV minigenome can replicate for more than 4 months. Compared to the previously developed transient transfection-based EBOV minigenome system, the stable minigenome replicon had more active genome replication but less active transcription. Interestingly, EBOV replication in the stable system was insensitive to interferon treatment or RNA interference. Moreover, RNase digestion of the replicon cell lysates revealed the remarkably stable nature of EBOV minigenomic vRNA ribonucleoprotein complex.

Conclusions: In summary, we established a novel EBOV minigenome replicon system with robust and stable viral genome replication, which provides an important tool for studying EBOV replication and antiviral development.
The methyltransferase domain of Sudan Ebolavirus L protein catalyses RNA-cap methylations and unconventional internal 2’O methylations

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In the Mononegavirales order, viruses encode a large protein (L), which is responsible of replication/transcription and RNA modifications. This protein harbours six conserved domains accountable of these different activities (Poch et al., 1990; Liang et al., 2015). Among these domains, the conserved region VI (CRVI) has been predicted to support cap-methyltransferase (MTase) activity (Ferron et al., 2002). The cap consists in a N7-methylated guanosine linked to the first nucleotide at the mRNA 5’-end by a 5’-5’ triphosphate bond. This structure is also methylated at the 2’O position of N1 ribose. These methylations play a critical role in virus survival as N7 methylation triggers efficient viral RNA translation and 2’O methylation limits the detection of viral RNA by the host innate immunity (reviewed in Decroly et al., 2011). Thus, the characterization of this domain in Ebola virus is a key point to understand replication of mononegaviruses and design new antiviral strategies. We produced the MTase of Sudan Ebolavirus (SUDV) extended or not by the L C-terminal domain (CTD) and we characterized their MTase activity and RNA interaction properties. We demonstrated that the CTD is essential for MTase activity as this domain is key for the RNA recognition. The MTase activity has been further characterized using synthetic short RNAs holding different cap structures. Surprisingly, we discovered that SUDV MTase harbours an unconventional activity. Besides this MTase domain is able to methylate the cap structure at N7 and 2’O positions, it also catalyses 2’O methylations specifically on A residues in the inner RNA sequence. Even the role of such methylations is not yet understood, we hypostatized that such epitranscritomic RNA modifications may be involved in host immunity escape mechanisms or may regulates translation or RNA packaging by NP. Finally, we identified compounds limiting the Ebola virus MTase in the micromolar range. Further biochemistry and compounds characterization results will thus pave the way towards the development of an innovative antiviral strategy.

Figure 1:
The Ebolavirus protein VP24 antagonises the host immune response by binding to both karyopherins and STAT1. Here, we used molecular dynamics simulations to compare the interaction of Ebola virus or Reston VP24 with human karyopherin a5 (KPNAS), including the effects of point mutations that have been experimentally shown to affect VP24 function.

**Methods:** Based on the crystal structure of the complex between Ebola virus VP24 and human KPNAS, a model of the Reston virus VP24 complex with KPNAS was formed. 500ns simulations were performed for the VP24/ KPNAS complexes. In addition, 100ns simulations were run for the Ebola virus VP24/ KPNAS complex with point mutations. The number of hydrogen bonds in the interface, the correlation of protein movement, and the ability of water to penetrate the interface were used to analyse the VP24/ KPNAS interactions.

**Results:** In agreement with pre-existing experimental data, the Ebola virus VP24 mutation R137A, which is known to largely abrogate binding, reduced the VP24/ KPNAS binding with a large cavity opening between them, very few hydrogen bonds between them, and water molecules able to access the interface area.

The analysis of the movement of water molecules into the complex interface identified did not reveal substantial differences between Reston virus and Ebola virus VP24. However, comparison of the Ebola virus and Reston virus VP24 complexes with KPNAS revealed that the Reston virus VP24 complex forms fewer hydrogen bonds to KPNAS and results in fewer correlated motions indicating reduced complex stability.

**Conclusions:** Our results suggest that the interaction of Reston VP24 with KPNAS is different to that of Ebola VP24. This difference could contribute to the differences in human pathogenicity observed between Reston viruses and Ebola viruses.
Question: Among the Ebolaviruses, only Reston viruses do not cause disease in humans. Here, we set out to identify the molecular determinants of Ebolavirus pathogenicity in an in silico approach that analysed the differences between the genomes of Reston viruses and the other Ebolaviruses. We combined this approach with an analysis of the mutations required for Ebolaviruses to adapt to and cause disease in novel species, based on genomic data from rodent-adapted Ebola virus strains.

Methods: Ebolavirus genomes were compared to identify specificity determining positions (SDPs), positions that are differentially conserved between different groups. SDPs were mapped onto protein structures or models and analysed for their effect on protein structure and function.

Results: Our analysis of 196 Ebolavirus genomes identified 189 SDPs spread across all 7 Ebolavirus proteins, 47 SDPs of which could be mapped onto protein structures. For eight SDPs we found strong evidence that they alter protein structure/function. Four were present in VP24, two in VP40 and one in each of VP30 and VP35. The effects of the SDPs in VP24 and VP40 were most striking. The changes in VP24 may alter the interaction with human karyopherins as three SDPs are located in the interface between the two proteins, which could alter the effects of VP24 on the interferon response. The analysis of the genomes of rodent-adapted Ebola viruses resulted in very similar findings further indicating a role of VP24 in determining Ebolavirus pathogenicity (Figure 1).

Conclusions: Our analyses suggest that VP24 is a central determinant of Ebolavirus pathogenicity. Fewer than five mutations seem to be required for the adaptation of Ebolaviruses to new hosts, which may include Reston virus adaptation to humans.

Figure 1: Mutations in VP24. The complex of Ebola virus VP24 (grey) and human KPNA5 (cyan) is shown, SDPs (red) and adaptation mutations (blue).
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Functional analyses of endogenous Ebolavirus-like element

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Introduction: It has been proposed that some non-retroviral RNA virus genes are integrated into vertebrate genomes. An Ebolavirus VP35-like element (mlEEL35) found in the little brown bat genome contains nearly full-length open reading frame corresponding to Ebolavirus VP35, which has been shown to inhibit type-I interferon (IFN) production and also known as a viral polymerase cofactor that is essential for viral RNA transcription/replication. However, potential roles of mlEEL35 in Ebolavirus infection is unclear. In this study, we focused on the biological property of the putative mlEEL35-derived protein (mlEEEL35p) in cultured cells.

Objectives: The objective of this study was to investigate homo-oligomerization potential of mlEEL35p and its potential function as an IFN antagonist and/or a viral polymerase cofactor.

Material and methods: Human embryonic kidney cells were transfected with the plasmids expressing mlEEL35p and/or Ebolavirus VP35 along with the plasmids for immunoprecipitation, human IFN-β promoter reporter, and mini-genome reporter assays. Expressed proteins were detected by western blotting and luciferase activities were measured in reporter assays.

Results: Comparison of amino acid sequences among mlEEL35p and VP35s revealed that the primary structure of mlEEL35p showed high similarity to Ebolavirus VP35s. We found that mlEEL35p lacked the nucleoprotein (NP) binding peptide, whereas several amino acid residues important for VP35 homo-oligomerization and the IFN antagonist function were conserved among mlEEL35p and VP35s. Accordingly, mlEEEL35p was immunoprecipitated with itself and Ebolavirus VP35s but not with NP. We then found that mlEEL35p inhibited the human IFN-β promoter activity as well as VP35 whereas mlEEL35p did not support viral RNA transcription/replication.

Conclusion: The bat-derived mlEEL35p potentially acts an IFN antagonist but not a polymerase cofactor.
Resistance of the non-lymphocytic cell line SH-SYSY to filoviral cell entry is likely due to a currently unknown host factor

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Ebola virus disease (EVD) was the cause of the infamous 2013-2016 West African outbreak. Although in early stages of infection macrophages, monocytes, and dendritic cells are the primary target, Filoviruses have a wide cell tropism. They are able to infect virtually any cell type with the exemption of cell lines of lymphocytic origin (Wool-Levis and Bates. 1998) and later confirmed in vivo (Geisbert et al. 2000). Moreover, Dube et al. demonstrated that EBOV refractory suspension cells (293F and lymphocytic cell lines) contain an intracellular pool of putative EBOV RBR binding partners that become translocated to the surface upon cell attachment. An unbiased lentiviral glycoprotein (GP)-driven Filovirus susceptibility cell screening identified SH-SYSY, a neuroblastic adherent non-lymphocytic cell line, as a novel cell line refractory to Filovirus entry. These results were later validated by rVSV-EBOV-GP and authentic Filovirus infection. Here, we set out to characterize the Filovirus resistance nature of SH-SYSY. Firstly, clustering analysis of gene array of a panel of cell lines failed to correlate Filovirus entry factor gene expression with susceptibility/resistance. Next, protein expression and functionality studies suggested that Filovirus entry surface factors (ITGVA, ITGB1, FOLR1, Axl, TIM-1) were heterogeneously expressed among resistant and susceptible cell lines, and intracellular factors (NPC1, TPC1/2, CTSL, CSTB) were present in most of the cell lines and functional regardless the cells susceptibility phenotype. In order to rule out the possibility of a dominant restriction factor as the cause of resistance, cell fusion assays were conducted. Strikingly, cell fusion between the resistant cell line SH-SYSY and the permissive cell line 293T rendered heterokaryons susceptible to EBOV and MARV pseudoparticles. Importantly, lentiviral particles lacking envelope GP failed to transduce heterokaryons, suggesting that lentiviral entry is GP-specific. In conclusion, all these data suggest that expression of previously reported entry factors are not sufficient to explain Filovirus resistance on SH-SYSY and yet to be discovered essential/s entry factor likely exist.
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The significance of virus amplification on Marburg- and Ebolavirus genotype and phenotype

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The 2014 West Africa Ebola virus outbreak emphasized the need for vaccines and therapeutics to treat Ebola virus disease. One path to licensure is via the "Animal Rule" that relies on efficacy data from a well-characterized animal model. A critical component of these studies is challenge material that closely resembles the naturally occurring pathogen. Therefore, there needs to be a balance between generating sufficient virus for multiple studies and avoiding the introduction of mutations that may result in a changed phenotype. During in vitro amplification, RNA viruses can accumulate mutations as a consequence of their error prone RNA dependent RNA polymerase. To understand the impact of these mutations on virus genotype and phenotype, we serially passaged Ebola virus (EBOV) and Marburg virus (MARV) using different amplification conditions in VeroE6 cells and characterized the virus at each passage. We varied the multiplicity of infection (MOI), media volume, removal of media daily, and time to harvest during amplification. Using plaque assay and transmission electron microscopy, we determined the particle:plaque forming unit ratio of MARV and EBOV increased following passages. Whole genome sequencing showed that changes accumulated across the EBOV genome. Interestingly, only one non-synonymous mutation occurred in the MARV genome. This change was in the signal peptide of GP. In EBOV, amplification at a higher MOI and daily media removal resulted in reduced change at glycoprotein-editing site. Our data provides evidence that manipulating amplification conditions impacts virus genotype and phenotype, specifically the EBOV GP editing site and the specific infectivity of MARV and EBOV. These observations are important as they may affect how future studies are designed to model EBOV and MARV disease and test countermeasures.
Filovirus entry comprises stepwise interactions of the viral surface glycoprotein GP with multiple host factors. Following trafficking of virus particles to late endosomal/lysosomal fusion compartments, GP is proteolytically processed, and a cleaved form of GP (GP\textsubscript{CL}) engages with the critical intracellular receptor Niemann-Pick C1 (NPC1). Mechanistic delineation of this indispensable binding step has been severely hampered by the unavailability of a robust cell-based assay monitoring interaction of GP\textsubscript{CL} with NPC1. Current assays are based on a soluble form of the GP-interacting domain C of NPC1, and not the authentic full-length endosomal transmembrane protein. Further, results with NPC1-targeting inhibitors suggest that these \textit{in vitro} assays do not fully recapitulate the authentic endosomal GP\textsubscript{CL}-NPC1 domain C interaction. Accordingly, we developed a novel \textit{in situ} assay to monitor GP\textsubscript{CL}-NPC1 engagement in infected cells. Visualization of the subcellular localization of the binding complexes at single molecule resolution is based on the principle of DNA-assisted, antibody-mediated proximity ligation. As reported in previous studies, we detected GP\textsubscript{CL}-NPC1 domain C complex formation on the luminal site of late endosomes; this interaction was blocked efficiently by perturbing proteolytic cathepsin B/L-mediated priming of GP and hence unmasking of its receptor-binding site. Upon proteolytic cleavage, amino acids forming a putative interface with NPC1 domain C are exposed; mutation of those residues in GP disrupted NPC1 binding, displayed by blocked proximity ligation. We also examined the effect of FDA-approved small molecule inhibitors on the establishment of GP\textsubscript{CL}-NPC1 domain C complexes using the proximity ligation assay. Our findings revealed that inhibitor treatment significantly disrupted Filovirus entry and GP\textsubscript{CL}-NPC1 domain C binding by distinct mechanisms. In summary, we describe an \textit{in situ} proximity ligation assay allowing us to monitor GP\textsubscript{CL}-NPC1 domain C engagement, as well as to study host factors and inhibitors impacting GP\textsubscript{CL}-receptor interaction.
Introduction: Host cell entry of Ebola virus (EBOV) is orchestrated by the viral glycoprotein (GP), which mediates attachment to target cells and membrane fusion. The latter is achieved following virus uptake into endosomal vesicles, where GP is triggered through proteolysis by host cell proteases and subsequent engagement with its cellular receptor NPC1 (Niemann Pick-C1).

Objectives: The magnitude of the Ebola virus disease (EVD) epidemic in West Africa in 2013-2016 led to the question whether host cell interactions of the responsible EBOV strain during the entry process is different compared to other Ebolaviruses.

Materials and methods: We employed rhabdoviral vectors, virus-like particles and authentic EBOV to compare the efficiency of host cell entry driven by the GP of the viruses responsible for the West African EVD epidemic (EBOV2014-GP) and the outbreak in Zaire in 1976 (EBOV1976-GP).

Results: It was observed, that EBOV2014-GP mediated entry into cells of nonhuman primate origin and primary human macrophages and dendritic cells with less efficiency than EBOV1976-GP. Further, using mutant GPs we could demonstrate, that single amino acid polymorphisms in the receptor binding domain (RBD) and internal fusion loop (IFL) between the two GPs affect host cell entry, with the latter being responsible for the aforementioned phenotype.

Conclusion: In sum, we show that entry driven by EBOV2014-GP is reduced compared to EBOV1976-GP for certain target cells, and that this phenotype results from a single polymorphism within the IFL.
Marburg virus (MARV; family Filoviridae) is a close relative of Ebola virus, and can cause sporadic outbreaks of severe disease known as Marburg hemorrhagic fever (MHF) in humans in sub-Saharan Africa. Case fatality rates can be as high as 90%, and human-to-human transmission has been documented. Wild-type Filoviruses are likely unable to suppress the type I interferon response in rodents, such as mice, hamster and guinea pigs, and therefore require adaptation of the viruses to cause disease in immune-competent rodents. Here, we describe the development of a novel small rodent model for wild-type MARV and show that Syrian hamsters in which the STAT2 gene was knocked out (KO) are susceptible to infection with different wild-type MARV variants, with higher lethality for MARV Musoke, Voege, and Angola compared to Ravn. In a serial dosing study with MARV Musoke, one hundred percent mortality was observed in STAT2 KO hamsters after infection with a range of challenge doses via the intraperitoneal route. The LD50 was determined to be about 10 plaque forming units. Infection with MARV Musoke caused widespread infection with virus titers detectable in serum and multiple organs including brain, lung, liver, spleen, heart and kidney within 5 days of infection. Virus was also observed in physically disparate lymph nodes (mediastinal, axillary, mesenteric, superficial and deep cervical, and inguinal). Analysis of histopathology and immunohistochemistry, as well immune responses, is currently ongoing. The STAT2 KO hamster may provide a useful small animal model to study MARV pathogenesis and to evaluate potential antiviral therapeutics and vaccine interventions against wild-type MARV.
Functional mutations in spike glycoprotein of Ebolavirus associated with alterations in infection efficiency

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Ebola virus (EBOV) is extremely virulent, and its glycoprotein (GP) is necessary for viral entry. EBOV may adapt to its new host humans during outbreaks by acquiring mutations, especially in GP, which allows EBOV to spread more efficiently. To identify these evolutionary selected mutations and examine their effects on viral infectivity, we adopted experimental–phylogenetic–structural interdisciplinary approaches. In an evolutionary analysis of all available EBOV GP sequences, we detected two codon sites under positive selection, which are located near/within the region critical for the host-viral membrane fusion, namely alanine-to-valine and threonine-to-isoleucine mutations at 82 (A82V) and 544 (T544I), respectively. The fine-scale transmission dynamics of EBOV Makona variants that caused the 2013-2016 outbreak revealed that A82V mutant was fixed in the population while T544I was not. Using replication-incompetent VSVs pseudotyped with Makona GP and its mutants, we demonstrated that the A82V mutation caused a small increase in viral infectivity compared with the T544I mutation in Huh7 and several human cell lines. These findings suggest that mutation fixation in EBOV GP is closely associated with their increased infectivity levels; the mutant with a moderate increase in infectivity will fix. Furthermore, the A82V mutation in another EBOV isolates, Mayinga, and Reston virus caused a significant decrease in viral infectivity in human and non-human primate cells, suggesting that the increased infectivity with A82V might be a distinct feature in Makona variant. Our findings demonstrated that a driving force for EBOV evolution via GP may be a balance between costs and benefits of its virulence.
Pathology and Pathogenesis

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Haemostatic changes in patients infected with Ebolavirus

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Introduction: There is little information on coagulopathy in humans infected with Ebola virus. Understanding haemostatic changes that occur when a person is infected with Ebola virus may enable improved management and targeted treatment of patients and improve patient outcome. As medical treatments can be beneficial or harmful depending on the underlying pathology, it is important to understand physiological changes during infection with Ebola virus.

Objective: To determine pathological changes in blood clotting using plasma samples taken from patients during the Ebola virus outbreak in West Africa.

Patients and methods: This study was reviewed and received a favourable opinion by the UK Ministry of Defence Research Ethics Committee. Samples from adults with confirmed Ebola virus disease were obtained from patients at the Kerry Town Treatment Facility from January to June 2015. Plasma was obtained after blood tubes were left to stand for 3 hours at 4°C. Plasma was aliquoted and stored at -20°C until transported back to the UK when it was stored at -80°C prior to analysis. Analysis of samples was performed on the CA-660 analyser (Sysmex, UK) held within a rigid-walled double half-suit isolator within a Containment Level 4 laboratory at Dstl. Four tests were carried out on all UK healthy donor samples and samples from Ebola virus infected patients. Each test was performed in duplicate.

Results: Samples were acquired from 5 patients. Each sample was analysed for Prothrombin Time, Activated Partial Thromboplastin Time, fibrinogen and D-dimers. Testing of samples occurred only if the QC for each test passed each day. All tests were performed in at least duplicate. Analysis of samples from patients infected with Ebola virus showed a much wider (including abnormal) range of values that fell outside the normal range. Specifically, clotting times were prolonged, fibrinogen levels were low and D-dimer levels were elevated in patients with Ebola virus disease.

Conclusion: Plasma samples from patients suffering from Ebola disease have changed, and in some cases abnormal, haemostatic properties.
Pathology and Pathogenesis

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Severity of disease in Ebolavirus- and Restonvirus-infected humanized mice associated with magnitude of early viral replication in liver

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Ebola virus (EBOV) causes severe disease in non-human primates (NHPs) and humans. RESTV causes high mortality in NHPs, but is asymptomatic in humans despite evidence of infection. To investigate differences in pathogenicity between EBOV and RESTV, we inoculated humanized mice (hu-NSG™-SGM3) with EBOV-Makona or RESTV-Pennsylvania. A serial euthanasia study was performed to monitor clinical signs and compare viral load, histopathology, and clinical chemistry at 6, 10, and 14 days post infection (DPI). Consistent with human infection, EBOV caused severe illness, but RESTV infection was largely asymptomatic. Alterations in clinical chemistry analytes were observed in EBOV-inoculated mice beginning at 10 DPI, whereas none of the RESTV-inoculated mice had significant changes in analyte values compared to control mice. At 10 DPI and/or 14 DPI time points, significant differences were observed between RESTV- and EBOV-inoculated mice in AST, ALT, ALP, ALB, TP, and GLU levels. EBOV and RESTV replicated over time to similar levels in the spleen. However, in the liver, EBOV replication was significantly higher at early time points post infection. These data suggest that efficiency of viral replication early in infection in specific tissues, such as the liver, may contribute to differences in viral pathogenicity.

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The Makona variant of Ebolavirus is highly lethal to immunocompromised mice and immunocompetent ferrets

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**Introduction:** During 2014-16, a novel isolate of Ebola virus (EBOV-Makona) caused an epidemic in Western Africa, with over 28,000 infected and 11,000 deaths. The virus was distinct from previously known EBOV strains (EBOV-Kikwit and EBOV-Mayinga) that were responsible for outbreaks in Central Africa and causes at most hundreds of cases and deaths during an outbreak.

**Objective:** To investigate the pathogenicity of EBOV-Makona, we engineered and rescued an early isolate from the 2014-16 epidemic (H.sapiens-wt/GIN/2014/Makona-Gueckedou-C07, EBOV-C07) using an updated reverse genetics system, and then determined the LD50 values of this virus in immunocompromised mice and immunocompetent ferrets.

**Materials and methods:** The pSP72 cloning vector was modified to include the full length EBOV-C07 viral genome under control of the eukaryotic human cytomegalovirus promoter, as well as hammerhead ribozyme and hepatitis delta virus ribozyme/β-globin transcription terminator at the 5' and 3' ends, respectively. Groups of three Type I interferon receptor knockout mice (Ifnar1⁻/⁻) or domestic ferrets were used to test the virulence of EBOV-C07 via intraperitoneal or intramuscular challenge, respectively, using 10-fold serial dilutions of the virus from 100 to 0.01 plaque forming units (PFU).

**Results:** EBOV-C07 was found to be highly pathogenic in both knockout mice and ferrets. All animals died between 6-8 days post-infection, accompanied by rapid weight loss and increase in clinical scores at challenge doses over 1 PFU, and the LD50 was calculated to be 0.078 PFU for knockout mice and 0.015 PFU for ferrets. Partial or complete survival was observed in the 0.1 or 0.01 PFU groups.

**Conclusions:** The results showed that the EBOV-C07 rescued from our reverse genetics system is extremely pathogenic in both the knockout mice and ferret animal models with low LD50 values, demonstrating that specific medical countermeasures can be effectively screened in these two species before further studies in nonhuman primates.
A retrospective analysis of data from 56 Ebola virus infection-control rhesus macaques from 11 independent studies

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A natural history of Ebola virus disease will be required for any therapeutics that seek licensure under the animal rule. In order to inform, or perhaps substitute for, the natural history study, a retrospective analysis of 56 rhesus macaques was conducted. Each of the 11 studies from which these data were compiled had similar observations, euthanasia criteria, target dose, and secondary endpoints. The mortality rate in the 56 animals described in this report was 92.8%. Four animals spontaneously survived the challenge. The mean time to death calculated for nonsurvivors was 210.3 ± 70.97 h (8.76 ± 2.96 days), and the median was 193.0 h (8.04 days). Plasma viral RNA was detectable in most animals beginning Day 3, and maximum plasma viral RNA (9.3 log10 genome equivalents/mL) was observed on Day 6. In animals with real-time biotelemetric monitoring, average time of fever occurred on Day 3.63. Alterations in clinical pathology parameters consistent with inflammation were noted as early as Day 3. All animals that succumbed to EBOV prior to the end of study exhibited gross necropsy changes consistent with intramuscular EBOV infection in rhesus macaques. The most commonly affected tissues upon histologic evaluation included liver, spleen, and other lymphoid tissues, adrenal gland, kidney, and the gastrointestinal tract. Disease manifestations and viral load observed in rhesus macaques exposed to EBOV by IM route recapitulated findings of fever, high viral load, and key clinical pathology findings reported in humans from historical outbreaks. When comparing the time course of EVD in rhesus to humans, it is noteworthy that the time course is shortened with regard to incubation period, time to peak viremia, time course of fatal disease, and mean time from infection to death. It was not possible to definitively determine the impact of supportive treatments to survival, apparent disease progression, or secondary euthanasia endpoints. Although statistically significant associations were noted, many of the statistically significant results were driven by a small handful of survivors and should be interpreted with caution.
Introduction: Currently, there are no FDA-approved vaccines or treatments available for Ebola virus disease (EVD) and therapy remains largely supportive. A greater understanding of virus biology will facilitate development of novel approaches for treatment. Ebola virus has broad tissue tropism and can infect a wide variety of cells including immune cells, epithelial cells and endothelial cells. Epithelial cells differ from most other cell types in their polarized phenotype and barrier function. The most important feature is their apical and basolateral membrane domains which are strictly separated by tight junctions (TJs). Due to specialized protein-sorting machineries in these cells, the two membrane domains differ substantially in their compositions. Viral entry and egress both involve interactions with the host cell membrane. Thus, the polarized distribution of the viral receptor can potentially restrict virus entry to one surface domain, sorting of viral proteins can lead to a vectorial virus release.

Objective: The objective of this study is to elucidate the mechanism of EBOV entry and egress in polarized cells.

Materials and methods: We are using Caco-2 (human colorectal adenocarcinoma) cells as a model to examine the impact of Ebola virus (EBOV) infection on polarized epithelial cells. Polarized epithelial cells are, by nature, asymmetrical, and their plasma membranes are divided into distinct apical and basolateral parts.

Results: Our data indicate that in polarized cells, the route of infection (apical or basolateral) may have an effect on infection. We have found that EBOV preferentially infects from the basolateral route, and this preference may be influenced by the extent of polarity. Our experiments to examine cell monolayer integrity and viral egress show that EBOV is released from polarized cells preferentially through the Basolateral cell surface without changes disruption in cellular permeability. Our ongoing experiments are examining the involvement of various entry factors for causing the basolateral preference shown by the virus during entry in Caco-2 cells.
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Ebola VP40 in exosomes can cause immune cell dysfunction

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Introduction: Ebola virus can result in severe hemorrhagic fever with up to 80-90% mortality; however, long-lasting persistence and recurrence in survivors has been documented, potentially leading to further transmission of the virus. We have previously shown that exosomes from cells infected with HIV, HTLV and RVFV are able to transfer viral proteins and noncoding RNAs to naïve recipient cells, resulting in altered cellular activity.

Objectives: The purpose of our study was to elucidate the role of Ebola structural proteins and exosomes in Ebola virus pathogenesis.

Materials and methods: Here, we examined the effect of Ebola structural proteins VP40, GP, and NP on recipient immune cells, as well as the effect of exosomes containing these proteins on naïve immune cells.

Results: We found that VP40-transfected cells packaged VP40 into exosomes which were then capable of inducing apoptosis in recipient immune cells. Additionally, we show that VP40 within parental cells or in exosomes delivered to naïve cells could regulate host RNAi machinery, which may contribute to the induction of cell death in recipient cells. Exosome biogenesis was regulated by VP40 in transfected cells by increasing levels of ESCRT-II proteins EAP20 and EAP45 and exosomal markers CD63 and Alix. VP40 was phosphorylated at Serine 233 by Cdk2/Cyclin A/E complexes, which could be reversed with r-Roscovitine treatment. Additionally, we used novel nanoparticles to capture VP40 from human samples spiked with Ebola VLPs using SDS/reducing agents, minimizing the need for BSL-4 conditions for downstream assays. Finally, we have new data showing that VP40 alone is sufficient to regulate cell cycle progression in donor cells, pointing to its control of cell cycle checkpoints.

Conclusion: Collectively, our data indicates that VP40 is packaged into exosomes which may be responsible for the deregulation and eventual destruction of the T-cell and myeloid arms of the immune system, allowing for the virus to replicate to high titers in the immunocompromised host.
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Hamster-adapted Ebola virus in hamsters as model for the haemorrhagic disease in people

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Ebola virus infection causes spontaneous haemorrhages in about 18% of the people infected, and are associated with a fatal outcome. The pathogenesis of this haemorrhage is not well understood. This is partly because currently used rodent models fail to show spontaneous haemorrhages. Syrian hamsters (Mesocricetus auratus) are promising candidates for the study of spontaneous haemorrhages in Ebola virus infection, as has been previously shown for Marburg virus infection. For the development of a Syrian hamster model for Ebola virus infection, which better reflects the human disease, Mayinga Zaire strain of Ebola virus was adapted to Syrian hamsters by seven passages in this species. Next, hamsters (six per group) were inoculated intraperitoneally with either a high (1000 TCID50/ml) or low dose (100 TCID50/ml) of this adapted virus, or with DMEM, to determine mortality rate, virus antigen distribution, and associated lesions. The mortality rate was similar in the high dose group (6/6) and low dose group (5/6). All control hamsters survived. Overall, virus antigen distribution and microscopic lesions were similar for hamsters in the high and low dose groups. The hamster-adapted Ebola virus had a clear tropism for cells of the monocyte lineage, hepatocytes and endothelial cells. Livers showed midzonal to diffuse necrosis of hepatocytes, associated with neutrophilic infiltrates. Lymphoid organs showed lymphoid necrosis. Strikingly, one out of six hamsters in the low dose group showed macroscopically (Figure 1) and microscopically (Figure 2A) visible haemorrhages in the skin, associated with virus antigen (Figure 2B). No virus antigen or lesions were detected in the control hamsters. Our results suggest that hamster-adapted Ebola virus infection in hamsters is a good animal model for Ebola virus infection in people. If the occurrence of spontaneous haemorrhages as seen in one of our hamsters is reproducible in future studies, it could be used to study the mechanism underlying the spontaneous haemorrhages in human Ebola virus infections.

Figure 1:

Figure 2:
Filoviruses are important etiological agents of emergent diseases with high mortality rates. Traditionally, Filovirus fever diseases have been primarily a burden of African countries. However, global interconnection has increased the probability of the worldwide spread of Filoviruses. Therefore, national healthcare organizations need tools for the management of Filovirus risks. These tools should definitely include diagnostic kits based on real-time reverse transcription PCR (RT-PCR), because it is the most suitable method for the diagnosis of Filovirus fever diseases. Here, we describe a real-time RT-PCR assay for the detection of Filoviruses. This assay is a further development of our earlier reported EBOV (Zaire)-Fl kit.

All sequences of SUDV, MARV, BDBV, RESTV and TAFV available in GenBank (NCBI) were aligned to identify conserved sites suitable for targeting using real-time RT-PCR. Next, a 135 nucleotide fragment of the NP gene of MARV, a 130 nucleotide fragment of the VP30 gene of SUDV, a 97 nucleotide fragment of the VP40 gene of TAFV, an 85 nucleotide fragment of the NP gene of BDBV, and a 86 nucleotide fragment of the L gene of RESTV were manually selected as targets for amplification. Additionally, a 113 nucleotide fragment of the L gene, described previously, was used for EBOV targeting.

We divided our assay into two sets due to limitations on the number of fluorescence channels in the real-time PCR machine. The first FiloA-Fl set was designed to detect the Filoviruses EBOV, SUDV and MARV. The second FiloB-Fl set was designed to detect BDBV, TAIFV and non-pathogenic for humans RESTV.

Recombinant plasmids and armored RNA phage particles (ARs) were prepared as positive DNA and RNA controls for real-time RT-PCR detection of Filoviruses. A reagent consisting of armored phage particles containing an artificial sequence, STI-87rec (AmpliSens, Russia), was used as the internal control.

Two sets (FiloA-Fl and FiloB-Fl) of real-time RT-PCR assays for the detection of Filoviruses were developed and evaluated. Assay specificity was studied using a representative sampling of viral and human RNA/DNA. No cross-reactions were observed. The LOD of the assay was 500 copies/ml of the AR-positive control for the FiloA-Fl set and 5000 copies/ml of the AR-positive control for the FiloB-Fl set.

The assay contains all of the necessary components to perform the analysis. The high specificity and sensitivity of the assay makes it useful for clinical and epidemiological investigations in the field of FFDs.
Filovirus Diagnostics

P 44
Establishment of serological assay for the detection of Zaire Ebolavirus in swine

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Since their first discovery in 1976, infections with Ebolaviruses have resulted in numerous outbreaks of severe hemorrhagic fevers in humans and other primates. The recent epidemic in West Africa (2013-2016) caused by Ebola virus (EBOV) mainly affected three countries: Guinea, Sierra Leone and Liberia. The outbreak was of unprecedented size and resulted in more than 11,000 recorded deaths. There is molecular and serological evidence for a zoonotic origin of Ebolaviruses in wildlife, including bats which serve as the host species. Concerning livestock, pigs were identified as a susceptible host for Reston virus (species *Reston Ebolavirus*), with reported transmission to humans in the Philippines. Under experimental conditions, pigs were also shown to be susceptible to EBOV infection. However, there is no evidence of natural EBOV infection in pigs to date. To investigate the potential role of pigs in the biology of EBOV, porcine serum samples were collected from different districts in Sierra Leone. Areas that were heavily affected by the recent Ebola epidemic were especially targeted, as well as communities where close contact between livestock and the surrounding wildlife population is known to occur. To test the porcine sera for the presence of Ebolavirus specific antibodies, a full-length recombinant EBOV nucleoprotein was expressed in *E. coli* and purified for further use for indirect ELISA development. From one liter of *E. coli* culture, we obtained protein yields with a concentration of up to 1 mg/ml. A panel of sera from naïve pigs from Germany was used as a negative control, while sera from pigs immunized with EBOV-like particles served as positive controls and reacted clearly with the recombinant antigen. Furthermore, these sera displayed neutralizing activities in a transcription and replication-competent virus-like particle (trVLP)-based neutralization test, which allows modeling of EBOV infection under biosafety level 1/2 conditions. Porcine sera from Sierra Leone will now be screened in the established ELISA and potentially reactive samples will then be confirmed in a second assay, such as the newly established neutralization test.
Filovirus Diagnostics

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A Single-tube assay for detection of Ebola, Marburg or Lassa virus from blood

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Easy to operate assays that require a minimum of sample preparation and offer maximum biosafety are a continuing goal for hemorrhagic fever diagnostics. We have sought to develop assays for Ebola, Marburg, and Lassa virus using a nanotechnology-based point-of-care (nanoPOC) diagnostic platform that combines the high sensitivity of instrumented immunoassays with the low cost and simple workflow of a rapid diagnostic test. Our approach uses a capture antibody linked to a magnetic bead and a detector antibody that is linked to an Enhanced Raman Spectroscopy "beacon" to detect the presence of viral antigen from a whole blood sample using an automated reader. Using this technology, we have developed assays that are capable of identifying Ebola virus, Marburg virus, and Lassa virus glycoproteins in blood. However, a challenge of Filovirus glycoprotein assays is the relatively low concentrations of glycoproteins present in clinical specimens, resulting in assays that lack the desired analytical sensitivity. By selecting alternate antigen targets, we have developed highly sensitive assays for Ebola, Marburg and Lassa viruses with Limits of Detection (LODs) ranging from 10^4 to 10^6 PFU/ml. These assays show good sensitivity and specificity in spiked whole blood samples using both surrogate antigens and authentic virus. Additionally, one of the benefits of the nanoPOC technology is the ability to combine these assays to form a multiplexed assay that can be used to test a single blood sample for multiple potential infectious agents simultaneously. These results suggest that the nanoPOC technology is a promising approach for developing a multiplexed one-tube assay for the sensitive detection of multiple viruses at the point of need.
Ebola virus disease causes widespread and highly fatal epidemics in human populations. Today, there is still much need for point-of-care tests for diagnostics, patient management and monitoring, during and post outbreaks. We present a semi-quantitative point-of-care assay to detect Ebola-specific antibodies in human survivors. A Sudan Ebolavirus glycoprotein monoplex platform was developed and validated using sera from 90 human survivors and 31 closely related non-infected controls. The results showed 100% sensitivity, 98% specificity compare to standard whole antigen ELISA. In addition, a multiplex test was constructed for simultaneous detection of antibodies against three recombinant viral Sudan Ebolavirus proteins, including viral protein 40, nuclear protein and glycoprotein. A pilot study comprised of 15 survivors and 5 non-infected controls was carried out, demonstrating sensitivity and specificity of 100% compare to whole antigen ELISA and identification of higher number of survivors by using this multiplex test. Finally, a second multiplex subtype assay test for the identification of three Ebola virus species Sudan, Bundibugyo and Zaire Ebolavirus was also developed using recombinant viral glycoprotein protein. This multiplex assay was able to distinguish between the hosts immunity to specific viral species and to identify cross-reactive immunity. These newly developed assays consisted of novel capture ligands, a lateral flow format with a custom smartphone application, exhibited high specificity and sensitivity, enabling rapid testing, data storage and share and geological tagging. Such test could have a great potential as a field tool for diagnosis, vaccine development and therapeutic evaluation.
Introduction: The animal reservoir and ecology of Ebola viruses (EBV) remain largely unknown, but previous detection of viral RNA and anti-EBV antibodies in bats suggests that they play a role in EBV zoonotic transmission. Today only little information is available on interactions between humans and bats.

Objectives: Conduct an exploratory study on extent and modes of contacts between humans and bats in southern Cameroon, an area at risk for EBV outbreaks, in order to obtain more information on risk of potential transmissions of bat viruses to humans.

Methods: The study was conducted in 11 villages in 4 rural areas in southern Cameroun. Semi-structured questionnaires were proposed between February and May 2017 on bat bush-meat practices, children-bat interactions, indirect contact, perception of bat-related disease. Responses were summarized using descriptive statistics, fisher’s exact test and generalized linear model.

Results: 135 villagers from 16 different ethnic groups participated. Majority were subsistence cultivators and relied on self-hunted bush-meat. Preliminary results showed that consumption of bats and thus direct contact, varied significantly between regions from 0% (0/22) to 87% (34/39), (p<10-10) and ethnic origin. Bat bush-meat appears to be an occasional meat resource with a median yearly consumption of 3 (3rd quantile=11). Both frugivorous and insectivorous bats are consumed, with a preference for frugivorous bats (42/54). 30/135 (22%) respondents reported children catching bats, especially if respondent consume bats. Indirect contact is also common; 57% of hunters using caves as shelters and 67% of interviewees eat fruits munched by bats. Between 11% (4/35) and 64% (14/22) of respondents according to the site consider that bat consumption may be risky.

Conclusion: Geographical and cultural diversity of contacts and perceptions regarding bats in Cameroun points towards a high variability in potential transmission risks of bat pathogens. This emphasizes the need to lead large-scale surveys of this type in order to identify high risk sites and population to target for more efficient health and education campaigns.
Virus Ecology and Epidemiology

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Filovirus surveillance in Rousettus aegyptiacus bats in Zambia

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Introduction: Rousettus aegyptiacus bats have been shown to be a reservoir species for Marburg virus (MARV) and Ravn virus. They have also been found to be seropositive for Ebola virus (EBOV). These bats are widely distributed in Africa, including Zambia. This study presents preliminary results of a genomic- and sero-survey for Filoviruses in these bats in Zambia.

Objectives: The general objective of this study was to determine the presence of Filoviruses in cave-dwelling R. aegyptiacus bats in Zambia.

Materials and methods: Healthy R. aegyptiacus bats were captured in LUnited States of Americaka province, at Leopards Hill cave in Chongwe and Chipongwe cave in Shimabala using a harp trap. Blood samples were collected for filoviral antibody detection with an enzyme-linked immunosorbent assay based on the viral glycoprotein antigens. Tissue samples were collected for detection of nucleoprotein and viral protein 35 genes using reverse-transcription polymerase chain reaction (RT-PCR) assays. Results: From a total of 147 bats, the seroprevalence of Filovirus-specific immunoglobulin G antibodies was 50.3%. The highest prevalence was seen against MARV (38.8%). The seroprevalences of Sudan virus, Tai Forest virus and Reston virus (RESTV) were 3.4% each while 0.7% were reactive to Bundibugyo virus and Lloviu virus (LLOV) each. Some of the positive samples showed cross-reactivity to multiple Filovirus species. There was no significant difference between male and female bats for either Filovirus- or species-specific seropositivity. There was no association between seropositivity and body weight, wing span or body length. RT-PCR assays to detect the filoviral genome were all negative.

Conclusion: The seroprevalence of MARV was found to be much higher than those from previous studies on R. aegyptiacus in areas that have been associated with outbreaks of Marburg virus disease (MVD). The high seroprevalence of MARV in this study, as well as the fact that Zambia lies within the zoonotic niche of Marburgviruses, suggest that Zambia is at risk for an outbreak of MVD. The seroprevalence of MARV was also much higher, while that of EBOV was lower, than that found in a similar study of Eidolon helvum bats in Zambia, confirming the strong preference of MARV to R. aegyptiacus. The presence of antibodies to RESTV and LLOV suggests either a wider circulation of these Filoviruses than has been previously thought, or presence of unknown closely related Filovirus species.
Aims: Since the 2014 outbreak of Ebola virus disease (EVD), there has been a significant effort to develop a vaccine eliciting protective immunity for population at greater risk of disease during outbreaks. The objective of this study is to establish an optimized assay to measure neutralizing antibodies that are an important immunological correlate of vaccine-mediated protection against viral diseases.

Methods: As with other enveloped viruses, EBOV entry into host cells requires virus-cell attachment, followed by the fusion of the viral and host cell membranes, a process that is mediated by the viral glycoprotein (GP). We have therefore produced a replication-deficient reporter virus bearing EBOV GP in its envelope that represents a powerful surrogate system for viral entry studies. By the process of pseudotyping, the viral GP, provided in trans, was incorporated into a replication-deficient vesicular stomatitis virus (VSV) recombinant in which the glycoprotein gene (G) was deleted and replaced with genes encoding fluorescent reporter protein (EGFP) and luciferase (Luc).

Results: Pseudotypes were produced, carrying the GPs from EBOV variants Mayinga, Kikwit and Makona at a titer of $10^6$ to $10^7$ infectious units (IU) /mL. The specificity of the pseudoviruses was assessed using monoclonal antibody I1 directed against VS VG and monoclonal antibody KZ52, originally derived from an EVD survivor. The assay was further implemented using a set of International Reference Reagents established under the auspices of WHO. The results were compared to neutralization assays with authentic Ebola virus.

Conclusion: A major obstacle for the assessment of the potency of EVD antibody therapies and vaccines is the absence of standardized methods to measure neutralizing antibody levels. We successfully established a pseudotype platform for EBOV which is suitable for the detection of neutralizing antibodies elicited by vaccination and that can be rapidly adapted to novel emerging viruses.
Sierra Leone in West Africa is located in a Lassa fever hyperendemic region that encompasses Guinea, Sierra Leone, and Liberia. Each year suspected Lassa fever infections result in approximately 500-700 samples being submitted to the Kenema Government Hospital (KGH) Lassa Diagnostic Laboratory located in eastern Sierra Leone. Of the samples tested, generally only 30-40% are positive for Lassa virus antigen and/or Lassa-specific IgM antibodies; therefore 60-70% of the patients are presenting with acute diseases of unknown origin. Using IgM capture enzyme-linked immunosorbent assays (ELISAs) we evaluated the patient samples for antibodies to arthropod-borne and hemorrhagic fever virus pathogens that could mimic Lassa fever presentation and occur in the region. We found evidence for IgM antibodies to dengue, West Nile, yellow fever, Rift Valley fever, Chikungunya, Ebola, and Marburg viruses, but not Crimean-Congo hemorrhagic fever virus. The highest antibody prevalence was to Ebola Zaire virus, the cause of the Ebola outbreak 2014-2016. To further understand viruses circulating in Sierra Leone, we developed a multiplexed MAGPIX® assay to detect IgG antibodies against Lassa, Ebola, Marburg, Rift Valley fever, and Crimean-Congo hemorrhagic fever viruses as well as pan-assays for flaviviruses and alphaviruses. This assay was used to survey 675 human serum samples submitted to the Lassa Diagnostic Laboratory between 2007 and 2014. In the study population, 50.2% were positive for Lassa virus, 5.2% for Ebola virus, 10.7% for Marburg virus, 1.8% for Rift Valley fever virus, 2.0% for Crimean-Congo hemorrhagic fever virus, 52.9% for flaviviruses and 55.8% for alphaviruses. These data exemplify the importance of disease surveillance and differential diagnosis for viral diseases in Sierra Leone. We demonstrate the endemic nature of some of these viral pathogens in the region and suggest that unrecognized outbreaks of viral infection have occurred.
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**Extensive serological survey suggests that Ebolaviruses are not widespread in monkeys in Central Africa**

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**Introduction:** Bats are considered to be a reservoir for Ebola viruses (EBV) but non-human primates (NHP) represent an important source of infection for humans in EBV outbreaks. Great apes play a role as amplifying hosts, but few information is available on the role of monkeys.

**Objective:** Study the presence of EBV antibodies in monkeys in Central Africa to evaluate whether EBV circulates in NHP.

**Materials and methods:** We used a highly-specific and sensitive serological assay based on the luminex (xMAP) technology to detect EBV antibodies in monkeys from Cameroon and DRC. We included recombinant proteins (NP and/or GP and/or VP40) from Zaire (EBOV), Sudan (SUDV), Bundibugyo (BDBV) and Reston (RESTV) Ebola viruses. Samples are considered positive when simultaneous reactivity against NP and GP proteins is present.

**Results:** 2098 samples from 26 monkey species, collected as pets or bushmeat at 8 different forest sites in southern Cameroon (n=1454) and 6 sites in DRC (n=644) were analyzed. In Cameroon, the predominant species were *C.cephus* (35.8%), *C.nictitans* (25.2%), *C.pogonias* (12.0%), *C.agilis* (7.5%) and *L.albigena* (6.7%). In DRC, *C.ascanius* (37.4%), *P.tholloni* (13.4%), *C.wolfi* (11.2%), *C.mitis* (7.9%) and *A.nigroviridis* (7.3%) predominated. A significant proportion (15 to >50%) of certain arboreal Cercopithecus species were reactive with GP proteins from EBOV and SUDV, reactivity with NP or VP40 proteins was rare. When applying the algorithm (i.e. positivity to at least NP and GP from an EBV species), we observed two positive samples, one baboon and one mustached monkey (*C.cephus*). GP antibody reactivity is highest in *C.cephus* in Cameroon and *C.ascanius* in DRC; two ecologically equivalent and phylogenetically closely related species.

**Conclusion:** This is the first study to evaluate a large number of frequently-hunted monkeys, especially from the Cercopithecus genus. EBV seems not to be widespread in monkeys, we found only two (0.1%) potential EBV antibody positive animals. However, more samples should be tested from premates as well as from other animal species in order to define their role in the ecology of Ebola viruses and their outbreaks.
Introduction: The reservoir(s) and ecology of Ebola viruses (EBV) remains largely unknown, but previous detection of viral RNA and anti-EBV antibodies in bats suggests that they may play a role in zoonotic transmission.

Objectives: Gain insight into the circulation of EBV in bat populations in West and Central Africa by testing for the presence of antibodies against different EBV, using high throughput technology.

Materials and methods: Bats were captured across 7 regions in Cameroon and 4 in Guinea, and released immediately after collection of dried blood spots and biological data. Here we used a multiplex immunoassay with Luminex® technology for antibody detection against NP, GP and VP40 antigens for Zaire (EBOV), Sudan (SUDV), Bundibugyo (BDBV) and Reston (RESTV) EBV. In the absence of positive controls, cut-off values were determined using the change-point analysis method with bootstrapping (10 000 times). A sample was considered positive if the detected antibodies level was over the estimated cut-off for both NP and GP antigens.

Results: We studied 1796 bats (Cameroon, n=1365 and Guinea, n=431) belonging to 10 genera of the frugivorous family Pteropodidae (n=641) and 12 genera of 6 insectivorous families (n=1155). Based on the change-point analysis, 0,2% (3/1796) of bats were positive for EBOV (E. helvum, n=1; M. angolensis, n=1 and Mops sp., n=1) and 0,1% (1/1796) for SUDV (R. aegyptiacus). A total of 7,9% (142/1796) reacted to at least one EBV antigen, mainly GP. These bats belonged mainly (97%) to 8 frugivorous species and one insectivorous genus (Mops).

Conclusion: we confirm the presence of antibodies in 2 frugivorous bat species and 1 insectivorous genus previously found to be seropositive, as well as for the first time in M. angolensis, a frugivorous species. Using a stringent method of interpretation (change-point analysis), prevalence of EBV antibodies can be underestimated. More studies are needed to evaluate the extend of EBV in bats in areas at risk for EBV outbreaks in Africa and complementary less conservative methods to define cut-offs could be used for comparison in order to reflect natural circulation or exposure to Filoviruses.
Molecular tracing of Ebolavirus disease cases in the Western Area region of Sierra Leone, August 2014 to March 2015

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Of the 12,275 Ebola virus disease (EVD) cases reported from Sierra Leone (SLE), 4,955 occurred in the smallest but most densely populated Western Area (WA) region. The first EVD cases in the WA were reported in June (WAU) and July (WAR), and the number of cases increased dramatically in August and September, and peaked from October to December 2014. Recently published studies provide some understanding of the transmission and molecular epidemiology of Ebolavirus (EBOV) in Sierra Leone but gaps exist in available sequence data, particularly for the early cases in WA \cite{1}. We aim to provide further data on the molecular epidemiology of EBOV for the period August 2014 to March 2015. Clinical material from EVD cases confirmed by the SA field Ebola diagnostic laboratory in Freetown were subjected to next-generation sequencing after cDNA preparation by host rRNA depletion and SISPA amplification. Of 375 specimens sequenced, 218 yielded high quality sequence with > 90% genome coverage (mean 97.3%). The 218 new sequences represent 16 sequences from WA for the period Aug-Sept 2014, 15 from October, 38 from November, 45 from December 2014, and 29 from January-March 2015. In addition we sequenced 75 genomes of cases confirmed between August–November 2014 from other districts in the western part of SLE (PortLoko, Kambia, Bombali, Tonkolili and Moyamba). The new sequences were aligned with genomes from SLE available on Genbank \cite{1}. Metadata for all new sequences were analyzed with sequence data, including dates, district and town. These new sequence data from our study provide additional insights into the transmission of EBOV in the highly affected Western Area of Sierra Leone. The data confirms that multiple lineages and sub-lineages of EBOV Makona were introduced into, and circulated in WA of SLE.

Reference:

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Application of a luminex-based multiplex assay for serological surveillance of Ebolaviruses and Marburgviruses in Southeast Asian bat populations

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The mass of evidence has implicated that bats, Order Chiroptera, are the natural hosts of Ebolaviruses and Marburgviruses, Family Filoviridae. Zoonotic outbreaks in human populations caused by Ebolaviruses and Marburgviruses has occurred in West, Central, and East African countries. Ecological niche mapping of these viruses and the geographic distribution of potential bat hosts suggest that the global distribution of Ebolaviruses and Marburgviruses extends from Africa throughout Asia. To enhance virus surveillance efforts, our lab has created a Luminex-based multiplex binding assay using soluble versions of the virus attachment glycoprotein (GP) from all presently described Ebolavirus and Marburgvirus species. Sera samples from three fruit bat species endemic to Singapore, where samples were collected over five years, and widely distributed throughout Southeast Asia, were simultaneously screened for the presence of antibodies reactive to Ebolaviruses and Marburgviruses GPs. Sera from the fruit bat species: Eonycteris spelaea, Cynopterus brachyotis, and Penthetor lucasi, reacted to GPs from Ebola virus, Sudan virus, Bundibugyo virus, and Tai Forest virus; however, we detected no reactivity to Reston virus, Marburg virus or Ravn virus. Seroprevalence was not affected by the age of the bats (adult versus juvenile); although, we observed a sex-related seroprevalence increase in female Cynopterus brachyotis. In the absence of Ebola virus disease in Singapore, we concluded that Filovirus(es) antigenically related to EBOV, SUDV, and BDBV persist in these three fruit bat populations. Furthermore, novel Filovirus sequences have been identified in bat species endemic to China, and it is plausible that novel, antigenically cross-reactive Filoviruses were detected in our study. Presently, we are in the process of expanding the multiplex assay to include the GP from Lloviu virus, the first Filovirus endemic in Europe. Ongoing experiments are focused on defining assay specificity, sensitivity, and limit of detection, and utility of the multiplex assay in surveillance projects focused on Ebolavirus or Marburgvirus transmission from wildlife to livestock or human populations.
Development and optimization of a GFP-expressing tetracistronic trVLP system for Ebolaviruses

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During recent years, there has been tremendous progress in developing countermeasures against Ebolaviruses, although still no approved therapeutics exist. However, progress continues to be hampered due to safety constraints, restricting work with infectious Ebolaviruses to biosafety level (BSL) 4 laboratories. Life-cycle-modelling systems, which model aspects of the Ebolavirus life cycle at lower BSLs, mitigate this problem. These systems use minigenomes, i.e. miniature versions of the viral genome, in which some or all viral genes are removed and replaced with a reporter gene (most commonly a luciferase). These minigenomes are expressed in mammalian cells and replicated and transcribed by plasmid-expressed viral proteins.

The newest life-cycle-modelling system, i.e. the tetracistronic transcription and replication-competent virus-like particle (trVLP) system, which we recently developed, uses infectious minigenome-containing trVLPs as surrogates for Ebolaviruses to safely model virtually every aspect of the Ebolavirus life cycle under BSL1 or BSL2 conditions (depending on country-specific biosafety regulations). We have now created a new variant of this system using green fluorescent protein (GFP) instead of luciferase as a reporter, resulting in several advantages: 1) Information regarding the absolute number of infected cells is acquired, while luciferase only provides a relative measure of infection within an experimental sample, 2) the system is compatible with the high-content-imaging approaches most often used for antiviral screening, 3) measurement is possible in living cells, facilitating multiple measurements of infection over time without costly reagents, and 4) this system can very easily be used for prolonged passaging of trVLPs, and thus can be used for forward genetics studies.

We report details of the development of this system as well as its optimization, resulting in a significant improvement in the rate of infection, which is particularly relevant for high-content-imaging, but also advantageous for other experimental approaches.
Ebola virus disease (EVD) is a deadly disease in humans characterized by severe immunosuppression, high virus replication and a case-fatality rate of up to 90%. With more than 11,000 deaths, the 2013-2016 West African Ebola virus (EBOV) outbreak highlighted the need to find efficient vaccines and therapeutics, which are currently unavailable. Viral genomes require the host cell machinery for RNA synthesis such as transcription and replication and also translation. These involve virus-host protein/protein interactions. The use of the specific virus-host protein-protein interactions as drug-targets is an attractive alternative to directly targeting viral proteins since RNA viruses are more prone to accumulate mutations. Responding to the urgent need to find efficacious antivirals, our laboratory has developed a rapid screening method to detect these interactions and to test the effect of their inhibition on the replication and transcription processes in EBOV.

The interactome of ZEBOV VP35, a viral component of the nucleocapsid complex that is required for the virus transcription, replication and antagonism of the type-I IFN response of the host, was elucidated. Two vectors encoding Zaire EBOV VP35 fused with either an N-Terminal or a C-Terminal GFP protein were transfected, co-immunoprecipitated and analysed by LC-MS/MS.

The cellular interactome of VP35 in human cells (HEK293T) showed the viral protein to associate with 28 host proteins, including nucleic acid binding proteins, transporter and cytoskeletal proteins, transfer/carrier proteins, enzyme modulators, hydrolases and membrane traffic proteins were the most abundant. As seen in other studies, the cytoplasmic dynein light chain 1 (DYNLL1/CD1L1) was found to have the highest potential association with VP35. This makes it a good candidate for drug-targeting antiviral therapies with siRNA and small molecule inhibitors in cells where a reverse genetics system that mimics the transcription and replication of Ebola virus can be used as part of high throughput screening assays.
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Antiviral potency of an extract from *Nerium oleander*

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Introduction: Targeting of multiple Filovirus species with a single therapeutic is a highly coveted goal. Recent approaches using chimeric antibodies with dual specificities have demonstrated therapeutic efficacy in murine models against EBOV and SUDV, with extended efficacy against BDBV, TAFV and RESTV *in vitro*. The breadth of efficacy, however, is still constrained by genus as efficacy against *Ebolavirus* members was not enjoyed with *Marburgvirus* members. PBI-05204 is a cardioglycoside-containing extract from *Nerium oleander* that has demonstrated antiviral efficacy and is a cancer therapeutic in clinical trials. PBI-05204 and oleandrin, the cardioglycoside that is the putative therapeutic agent in the PBI-05402 extract, are assessed for antiviral efficacy.

Objectives: Assess broad spectrum potency of extract from *Nerium oleander*.

Materials and methods: An extract from *Nerium oleander* and purified oleandrin were used to pretreat Vero cells prior to and post-infection with MARV and EBOV. An immunofluorescence-based assay was used to determine antiviral efficacy 48hr post-infection. For passaging experiments, Vero cells were infected in the presence of PBI-05204 or oleandrin then supernatant was collected 24hr or 48hr later. The supernatants were then assayed for the presence of infectious virus. An EBOV minigenome was used to assess viral transcription in the presence of PBI-05204 or oleandrin.

Results: PBI-05204 and oleandrin inhibited infection rates in Vero cells. Reduced progeny virus was recovered from supernatants of cells infected with EBOV or MARV when treated with PBI-05204 or oleandrin. Virus transcription was not inhibited by treatment with PBI-05204 or oleandrin, indicating the inhibition may not be related to virus transcription. Preliminary results also indicate PBI-05204 and oleandrin have antiviral efficacy against other enveloped viruses, demonstrating a broad antiviral profile.

Conclusion: This broad spectrum efficacy we've presented may be especially critical as PBI-05204 can be found to accumulate in the CNS, which is essential for viruses that have demonstrated neuropathic effects.
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Selective AAK1 and GAK inhibitors demonstrate activity against multiple RNA viruses

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Introduction: Filoviruses are highly pathogenic and are of great priority to public health. Yet there remains an unmet need for broad spectrum, pan-filovirus therapeutics that may be dispensed quickly, without species identification. Our group has reported the appropriation of host AP2-associated protein kinase 1 (AAK1) and cyclin G-associated kinase (GAK) for viral entry, assembly, and/or egress by multiple RNA viruses, across several unrelated viral families. This shared characteristic makes AAK1 and GAK appealing broad spectrum targets for the potential treatment of multiple viral infections. We have demonstrated that treatment with sunitinib and erlotinib, approved anticancer drugs with anti-AAK1 or GAK activity, is effective in inhibiting replication of Ebola, hepatitis C and dengue viruses in vitro. In order to improve safety and toxicity, we are developing novel, chemically distinct next-generation inhibitors specific for AAK1 and GAK.

Objective: Demonstrate the inhibitory effect of next-generation AAK1 and GAK inhibitors on infection with RNA viruses, to include Ebola virus and Marburg virus.

Materials and methods: Inhibitors were screened in vitro in several biologically relevant cell types. Cells received pre-treatment with inhibitors, followed by inoculation with virus 1 hour. Following infection, virus was washed from cells, and inhibitors were re-added for 24-96 hours, based on growth kinetics of each virus evaluated. Supernatants were saved and evaluated by plaque assay to evaluate viral budding. Cells were fixed then stained with virus specific monoclonal antibodies. Data was acquired with a PerkinElmer Operetta® High Content Imaging System to evaluate viral spread.

Results: Here, we describe the inhibitory effect of selective, next-generation AAK1 and GAK inhibitors on infection with 3 RNA viruses: Ebola virus, Marburg virus, and Chikungunya Virus.

Conclusion: By developing broad-spectrum, host-targeted post exposure therapeutics, we have the potential to treat infected individuals before a definitive diagnosis has been made, saving valuable time during critical early days of infection.
Ebolavirus (EBOV) enters cells via low pH-dependent pathways similar to the majority of category A-C enveloped viral pathogens, such as other Filoviruses (Marburg), flaviviruses (Dengue), the alphavirus Chikungunya, or coronaviruses (SARS-CoV, MERS-CoV). Although specific factors involved in the fusion process of EBOV have been identified in the last years, the repertoire of cellular factors common to pH-dependent virus entry is not known. Identifying and characterizing these factors would provide information that will be useful for identifying novel cellular targets for broad spectrum antiviral drug-development.

We used a comparative approach (similar to our recent publication*) on nine published and four so far unpublished genome-wide siRNA screening data sets to identify cellular factors that are commonly involved in low pH-dependent virus entry mechanisms. We used a comparative approach to identify targets for already approved drugs or drugs currently in clinical trials for possible drug repurposing. 173 candidate genes were selected and a library of siRNAs targeting these genes was assembled. An siRNA screen was performed using lentiviral vectors pseudotyped with Ebolavirus envelope or other pH dependent or pH independent viral envelopes and promising candidates were identified for validation.

The proposed studies will provide a comprehensive view of cellular factors involvement in the low pH-dependent mechanisms of entry used by Ebola or other category A-C viruses. This information will significantly advance our understanding of the cellular processes involved in virus entry and will identify novel candidate targets and potential novel therapeutics.

*Tripathi et al., Cell Host Microbe, 2015
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Immunization-elicited neutralizing antibody against Sudan Ebolavirus

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The severe death toll caused by the outbreak of Ebola virus disease epidemic during 2013-2016 strongly reinforces the importance of Ebolavirus prevention and treatment. As a member of Ebolavirus family, Sudan virus (SUDV) causes severe disease with high mortality, yet no approved therapeutic antibody or vaccine is available. Here, we isolated a panel of monoclonal antibodies (mAbs) targeting SUDV glycoprotein (GP) from the peripheral B cells of two macaques immunized with VSV-GP pseudotype virus particles followed by 2 booster immunizations with GPΔMuc protein, using multicolor antigen-specific single-cell sorting technology. Through single B cell RT-PCR and recombinant mAb cloning technologies, we were able to dissect the GP-specific antibody Ig repertoire at high resolution. Genetic analysis of GP-specific Ig repertoire revealed that: 1) macaque Ig heavy chain VH4 gene families were heavily used by GP-specific memory B cells in this study; 2) the repertoire was diversified with most of the B cell lineages having single expansion; and 3) most of the GP-specific antibodies possessed modest somatic hypermutation level (SHM) and CDRH3 length with average 13.4 amino acids. Interestingly, we found that 17% (22/128) clones bound to both SUDV and Zaire Ebolavirus GPΔMuc protein. Furthermore, three SUDV GP-specific mAbs potently neutralized VSV- SUDV GP pseudotype viruses, with two mAbs originated from the same clonal lineage. In vivo protection study is underway to evaluate the therapeutic efficacy of these neutralizing mAbs.
Question: Recent successes have provided convincing evidence that antibody-based immunotherapies can be an effective countermeasure against Filovirus infection. However, antibody therapies developed thus far specifically target Ebola virus, leaving no effective treatment options for Sudan virus (SUDV) infection. Here we report on our efforts to develop an antibody-based immunotherapy for the treatment of SUDV infection.

Methods: We engineered murine-human chimeric IgG monoclonal antibodies (mAbs) from a library of SUDV GP-specific murine mAbs and nonhuman primate scFvs and produced these mAbs in both plant and mammalian-based expression systems. Antibodies generated in both systems were then compared head-to-head to determine antigen specificity by ELISA and neutralization activity by microneutralization assay. Competition groups were determined by competitive ELISA prior to in vivo testing. Protective efficacy of individual mAbs, produced in both plants and mammalian cells, was determined using the IFNAR-/SUDV mouse model.

Results: In total, 8 SUDV GP-specific chimeric mAbs were evaluated in vitro for antigen specificity and neutralization. All 8 chimeric mAbs produced in either expression system retained SUDV GP-specificity. Five competition groups were identified; one group containing 3 mAbs (X10H2 group), one group containing 2 mAbs (16F6 group) and three groups containing 1 mAb each (17F6, 19F10, 5G2). h19F10, h5G2, and h17F6 had little or no detectable SUDV neutralizing activity while antibodies in the hX10H2 and h16F6 groups had SUDV neutralizing IC50 values of ~30nM and <1nM, respectively. In vivo efficacy studies determined that only mAbs from the hX10H2 and h16F6 groups provided any protection against SUDV infection in mice. hX10H2 consistently outperformed other competing antibodies and no statistical difference between h16F6 and its competitor, hX10B1, was observed.

Conclusions: Through a series of in vitro and in vivo evaluations, we have selected a cocktail of two SUDV GP-specific mAbs that have independently demonstrated in vivo efficacy against SUDV infection in mice. This cocktail is now being tested in macaque models of SUDV disease.
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Characterization of a nanoparticle formulation containing MIL77 against in vitro and in vivo Ebola virus infection

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Introduction: Ebola virus is a zoonotic pathogen which can cause severe hemorrhagic fever in humans with no approved therapeutics. MIL77 is a monoclonal antibody (mAb)-based cocktail based on ZMapp, which has been shown to have beneficial therapeutic effects in animal studies and compassionately treated Ebola infected patients during the West Africa Ebola outbreak. Despite improvements in the production of MIL77 compared to ZMapp, another logistical obstacle to overcome is the stability and activity of these compounds during long-distance transportation and long-term storage in areas without conditions for cold-chain.

Objective: In this study, we examined the in vitro and in vivo effects of nanoparticle formulation on MIL77 (Nano-MIL77).

Materials and methods: Nano-MIL77 was stored at room temperature or heated at 70°C for 7, 14 or 21 days. The in vitro activity was assayed using ELISA with Ebola glycoprotein (GP) as the capture antigen as well as neutralizing antibody assays with Ebola GP-pseudotyped virus. The in vivo activity was characterized using guinea pigs (n=6 per group), in which one dose of 5 mg Nano-MIL77 was given intraperitoneally per animal at 3 days post-infection. Control animals were given MIL77 at the same regimen.

Results: Compared to MIL77, Nano-MIL77 stored at room temperature or heated at 70°C for 7, 14 and 21 days exhibited slightly decreased binding affinities by ELISA and a trend of reduced neutralizing effects against Ebola pseudoviruses. In guinea pigs, Nano-MIL77 stored at room temperature or heated at 70°C for 7 days provided protection at a level comparable to MIL-77 (67% survival), whereas Nano-MIL77 heated at 70°C for 14 days showed reduced protection (50%), and partial protection (17%) was observed even in the 21-day group.

Conclusions: Our results indicate that nanoparticle formulation can reduce the sensitivity of MIL77 to substantial temperature changes and preserve in vivo activities, indicating Nano-MIL77 is suitable for long-term storage and use even in areas without cold-chain.
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Hyperimmune equine immunoglobulin fragments confers post-exposure protection to Ebolavirus infection in nonhuman primates

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Introduction: During 2014-16, an outbreak of Ebola virus (EBOV) disease in Western Africa claimed the lives of over 11,000 people. Monoclonal antibody (mAb)-based candidates, such as ZMapp and MIL, are especially promising as treatments due to their ability to reverse advanced EBOV disease in humans and nonhuman primates (NHPs), but can be expensive and time-consuming to manufacture in large quantities, thus restricting their usefulness in areas with limited resources.

Objective: Our previous work showed that hyperimmune equine immunoglobulin fragments F(ab’2) strongly neutralize EBOV infection in vitro, and completely protect mice and guinea pigs against EBOV infection in vivo. The current aim is to characterize the protective efficacy of equine F(ab’2) in NHPs.

Materials and methods: Rhesus macaques were infected with 1000 plaque forming units of an EBOV strain isolated from the 2014-16 outbreak (EBOV-Makona-C07). Beginning at 3 days post-infection (dpi), animals (n=4) were treated with 100mg/kg of equine F(ab’2) once daily until 7 dpi (5 doses), with identical doses also given at 9 and 11 dpi. A control animal (n=1) was given identical volumes of PBS in place of equine F(ab’2) until it succumbed to infection. Another control animal (n=1) was untreated. Survival, in addition to changes in clinical, hematological, biochemical, immunological and virological parameters, was monitored for 28 dpi.

Results: Administration of equine F(ab’2) resulted in 100% protection, and the treated NHPs showed virtually no observable signs of disease throughout the course of the experiment. Measureable parameters indicate that the animals remained healthy and did not shed virus, which gave them time to develop a robust immune response against the infection.

Conclusions: Equine F(ab’2) is effective as a post-exposure treatment in NHPs and the next steps include characterizing the efficacy of the product at later times post-infection, as well as clinical trials. Due to well-established production protocols and good safety record, equine F(ab’2) should be considered as an alternative to mAbs for large-scale treatment of patients in the event of another EBOV epidemic.
Antivirals

P 65
Intravenous administration of favipiravir provides protection against lethal marv infection in cynomolgus macaques

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Introduction: Marburg virus is a highly pathogenic, lethal virus that causes hemorrhagic fever in humans. While medical countermeasures have been evaluated, there is still no approved vaccine or therapeutic for Filoviruses. Favipiravir (T705) is a nucleobase analog with potent antiviral activity against many families of RNA viruses. The efficacy of favipiravir against Filoviruses such as EBOV has been evaluated in rodent models and in the West Africa Ebola outbreak. However, there is no published data on the efficacy of favipiravir against Filoviruses in nonhuman primate (NHP) model.

Objectives: The objective of this study was to determine if favipiravir afforded protection against lethal MARV challenge in NHPs. Materials/Methods: Ten cynomolgus macaques were infected intramuscularly with 1000 pfu MARV Angola. All animals received intravenous (IV) treatment with either vehicle (n=4) or favipiravir in vehicle (n=6) administered via catheter. Favipiravir was administered BID at a loading dose of 250 mg/kg on Day 0 and 150 mg/kg on Days 1 through 13. Animals were assessed for signs of clinical disease, and blood was collected for analysis of viral loads, serum chemistry, hematology, coagulation, and favipiravir levels. Animals were humanely euthanized upon meeting endpoint criteria, or at the end of the study. Gross pathology, histopathology, and immunohistochemistry for viral antigen were performed on all animals following necropsy.

Results: All control animals succumbed to MARV infection between Days 7 and 9 with typical MARV clinical signs and changes in blood parameters. Five of the six favipiravir-treated animals survived until the end of the study. The favipiravir-treated animal that succumbed had a delayed time-to-death, lower viral loads, and fewer alterations in blood parameters as compared to controls. The treated survivors had reduced levels of viral RNA at Day 6 as compared to the controls, with undetectable levels by Day 14. Clinical signs in the treated survivors were also milder than those in the non-survivors, and were generally limited to anorexia.

Conclusion: Intravenous administration of favipiravir protects NHPs against lethal MARV infection.
Antivirals

P 66
Identification of entry inhibitors of Ebolavirus pseudotyped vectors from a myxobacterial compound library

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Myxobacteria produce secondary metabolites many of which were described to have various biological effects including antifungal, anti-bacterial and anti-viral activity. The majority of these metabolites are novel scaffolds with unique modes-of-action and hence might be potential leads for drug discovery. Here, we tested a myxobacterial natural product library for compounds with inhibitory activity against Ebola virus (EBOV). The assay was performed with a surrogate system using Ebola envelope glycoprotein (GP) pseudotyped lentiviral vectors. EBOV specificity was proven by counter-screening with vesicular stomatitis virus G protein pseudotyped vectors. Two compounds were identified that preferentially inhibited EBOV GP mediated cell entry: Chondramides that act on the actin skeleton but might be too toxic and noricumazole A, a potassium channel inhibitor, which might constitute a novel pathway to inhibit Ebola virus cell entry.
Antivirals

P 67
Discovery of anti-Ebolavirus drugs using an optimized minigenome system for high-throughput screening

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Ebolavirus is the causative agent of Ebola viral disease, a viral hemorrhagic fever characterized by sporadic outbreaks in different parts of Africa and with a high case fatality ratio. Currently, there is no approved drug for the specific treatment of Ebola viral disease. Given the aforementioned, and coupled with the absence of a vaccine for prevention of the disease, Ebolavirus is classified as a biosafety level 4 (BSL-4) agent. With a small number of BSL-4 containment facility available worldwide, there is a limitation to the research that can be done on the live virus. Using a reverse genetics system compatible with BSL-2 facility, we adapted a minigenome system as a high throughput screen for discovering inhibitors of the replication and transcription of Ebolavirus. With a Z factor of 0.84 measured in a 96 well plate, our minigenome system is suitable for use as a high throughput screen. Following the testing of 76 compound library, we discovered two candidate compounds - #25 and #44 - with potent activity in inhibiting the replication and transcription of Ebolavirus. The IC50 for compounds #25 and #44 were 2.0 μM and 2.7 μM, respectively. The cytotoxicity of both compounds were also evaluated and were less than 20% at the maximum concentrations tested. We chose compound #25 for further testing using a live Ebola virus screening system based on its better IC50 and cytotoxicity result. Compound #25 showed a 72% inhibition of live Ebola virus replication.

Figure 1

![Ebolavirus minigenome assay system. A) EBOV RNA genome B) Architecture of p3E5EnLUC plasmid. C) Schematic representation of EBOV minigenome assay system. D) High throughput EBOV minigenome assay system.](image-url)
Figure 2

High throughput screen of 76 compounds using EBOV minigenome assay.
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P 68
Development and screening of a high throughput screening assay using the Ebolavirus transcription- and replication-competent virus-like particle system


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Ebola virus (EBOV) is the causative agent of a severe form of viral hemorrhagic fever with high case-fatality rates, as high as 90%. To date, neither virus-specific therapies nor vaccines are available, mainly because this pathogen has to be manipulated under BSL4 containment. Recently, a transient transfection-based transcription- and replication competent virus-like particle (trVLP) system was described, enabling to model the entire EBOV life cycle under BSL2 condition. Using this system, we optimized the condition for bulk co-transfection of multiple plasmids, developed a luciferase reporter-based assay in 384-well microtiter plate and performed a high-throughput screening (HTS) campaign of an 8,354 compound collection consistent of FDA-approved drugs, bioactives, kinase inhibitors, and natural products in duplicates. Applying stringent hit selection criteria of ≥70% EBOV trVLP inhibition and ≥70% cell viability, 381 hits were selected targeting early steps and 49 compounds target late steps in the viral life cycle. Of the total 430 hits, 220 were confirmed by dose-response analysis in the primary HTS assay, and were clustered and ranked according to their chemical structures and therapeutic indices (CC50/EC50), respectively. Currently, hits are being triaged to discriminate between entry and replication inhibitors, and favorable hits will be evaluated by drug-drug combination studies to select for synergistic therapeutics. Taken together, we developed and screened an HTS assay using the novel EBOV trVLP system. Newly identified inhibitors are tools to study the poorly understood EBOV life cycle and present an opportunity to either repurpose FDA-approved drugs to Ebola disease or are opportunities for the development of novel viral interventions.
Filoviruses are highly pathogenic viruses, causing rapidly progressing and lethal diseases in humans. However, currently there is no approved therapy. The recent large outbreak of Ebola virus disease in West Africa suggested an urgent need of antiviral drug development. Our previous work demonstrated that tetrandrine, a plant alkaloid, potently blocked Ebola virus infection by targeting host two pore channels (TPCs). Tetrandrine is classified as a bis-benzylisoquinoline compound. Here we tested other existing bis-benzylisoquinolines and found that all of them potently blocked Ebola virus infection as well as other Filovirus infections. In contrast, mono-benzylisoquinolines did not block the infection, suggesting that benzylisoquinoline compounds require two isoquinoline nuclei in one molecule in order to show the antiviral activity. Only active compounds also inhibited infection of VSV-based pseudotyped viruses bearing Ebola virus glycoproteins, but not minigenome activity, suggesting an inhibition at the entry step. Moreover, consistent with their expected function, patch-clamp analysis revealed that bis-benzylisoquinolines, but not mono-benzylisoquinolines, suppressed TPC activity, indicating a tight relationship between TPC inhibition and blocking Filovirus entry. Finally, cepharanthine, the most potent compound in vitro, saved mice from the lethal disease. These results indicate that bis-benzylisoquinolines constitute a group of potent Filovirus entry inhibitors targeting host ion channels. We are now synthesizing novel bis-benzylisoquinolines to maximize potency and testing them against Filovirus infections. This study has been supported by funds from National Institute of Health, and William and Ella Owens Medical Research Foundation.
Antivirals

P 71
Effectiveness of sub-therapeutic staurosporine on inhibition of budding and replication of lipid-enveloped viruses

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Infectious Diseases are a major cause of morbidity and mortality internationally, disproportionately affecting developing countries. Many of the deficits caused by infectious diseases are due to viruses, specifically lipid-enveloped viruses. By identifying a common infectious pathway via viral phosphatidylserine (PS) to host TIM protein receptor and a class of drug that was recently found to potentially interfere with this common pathway, it was hypothesized that staurosporines, specifically UCN-01, can inhibit viral infection and replication of lipid-enveloped viruses dependent on viral PS and host TIM receptor interactions, including but not limited to Ebola virus, Marburg virus, HIV-1, Dengue virus, Chikungunya virus, and Zika virus. The effects of UCN-01 on the effects of PS localization in mammalian cells, the effects of UCN-01 on GFP tagged viral matrix proteins in cell culture, and the efficacy of UCN-01 on live virus infected cells are being studied. It was shown using confocal microscopy that UCN-01 is able to significantly reduce plasma membrane localization of both ebola VP40 and HIV-gag proteins in HEK cells. Flow cytometry was also performed on samples under the same experimental conditions showing alterations in external cell membrane PS expression and drug toxicity over a range of concentrations. This pathway was further supported by previous work done in this lab by showing that Ebola VP40 protein induces PS expression in a cell culture model by using confocal microscopy and flow cytometry. By testing UCN-01 to determine cell toxicity, how UCN-01 affects cell lipidomics using mass spectrometry, and how effective UCN-01 is at interfering with virus like particle production through virus like particle assays. After showing efficacy at sub-toxic levels in the matrix protein model, live virus studies were initiated. These studies, if successful at decreasing infectivity or viral replication, could lead into future animal model studies that may be taken into clinical trials, producing a broad-spectrum anti-viral therapy that would have the potential to eliminate a large portion of the global health burden.
Filovirus Vaccines

P 73
Recovery of the Chinese hog cholera lapinized virus from an infectious cDNA clone

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Classical swine fever virus (CSFV), the etiological agent of classical swine fever, is one of the economically important infectious diseases in the China swine industry. Until now, the control of the disease has been relied on vaccination by using the Chinese hog cholera lapinized virus (HCLV) (also known as. C-strain) vaccine. Here, we reported the construction of a full-length infectious cDNA clone of the HCLV genome in a bacterial artificial chromosome (BAC) vector, which provide a stable and convenient reverse genetics platform for CSFV genome manipulation and study of CSFV genes. The BAC clone, designed BAV-HCLV, was rapidly and precisely assembled using a conventional restriction-ligation cloning in a step-wise strategy. The CMV promoter was introduced to the 5' end of HCLV and the hepatitis delta virus ribozyme (HDV) and the bovine growth hormone termination and polyadenylation sequences (BGH) were included at the 3' end of HCLV. The rescued viruses virus showed similar growth kinetics with those of the wild-type virus in the infected PK-15 cells. This BAC clone provides a reverse genetics system to study the molecular biology of the CSFV and to develop the gene deleted live CSFV vaccine as vaccine candidates.

Figure 1

![Diagram 1]

Figure 2

![Diagram 2]
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Efficacy of mosaic Glycoprotein as an immunogen for Ebolavirus vaccine

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Question: Current Filovirus vaccines platforms rely on two species-specific glycoprotein (GP) immunogens to elicit protective immunity against two of the most deadly Ebolavirus species, Ebola virus (EBOV) and Sudan virus (SUDV). Identifying a single immunogen capable of generating cross-protective immunity against multiple Ebolavirus species would significantly reduce advance development costs and potentially streamline vaccination campaigns. Borrowing technologies used by the HIV vaccine research field, we employed an algorithm specially developed by Los Alamos Laboratories to create “Mosaic” GP amino acid sequences designed to maximize homology across all known Ebolavirus GPs.

Methods: Utilizing routine cloning methods, the mosaic GP amino acid sequences were inserted into rVSV∆G genome vectors, as described for other rVSV∆G vaccines. rVSV expressing wild-type (WT) EBOV GP, WT SUDV GP, or candidate mosaic GPs were rescued, plaque purified, and sequenced. Individual rVSV vaccine lots were then produced and purified from infected Vero cell cultures. Mice were vaccinated with rVSV vaccines and virus-specific IgG titers were determined by ELISA prior to challenge with either mouse adapted-EBOV (ma-EBOV) or SUDV.

Results: Protective dose 50 studies for rVSV-EBOV GP and rVSV-SUDV GP determined that a single vaccination with as little as 102 pfu provided significant protection against ma-EBOV challenge in WT mice, as reported by others, and SUDV challenge in IFNAR−/− mice. Cross-protective efficacy testing of candidate rVSV-Mosaic GP vaccines in rodents is currently ongoing and results from these studies will be presented.

Conclusion: Rescue of infectious rVSV-Mosaic GP clones suggests that mosaic GPs fold similarly to WT GPs, similarly enough to at least mediate entry of rVSV particles. In addition, mosaic GPs tested thus far maintain epitopes required for several EBOV and SUDV GP-specific antibodies to bind. Whether these mosaic GP immunogens can elicit cross-protective efficacy against both EBOV and SUDV remains the focus of ongoing research efforts.
Filovirus Vaccines

P 75
Enhancement of DNA vaccines for Ebolavirus with genetic adjuvants and improved plasmid designs

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We previously showed that DNA vaccines expressing the codon-optimized GP genes of Ebola (EBOV) or Marburg (MARV) viruses protect both mice and nonhuman primates from viral challenge when delivered by intramuscular electroporation (EP). To determine if we could achieve equivalent immunogenicity and protective efficacy in the absence of electroporation by using improved DNA vaccines, we tested co-expression of the EBOV DNA vaccine and molecular adjuvants designed to potentiate immune responses. The molecular adjuvant genes evaluated included those for the Th1-inducing cytokine IL-12 and the granulocyte growth factor GM-CSF, both of which have demonstrated significant adjuvant effect when included in clinical DNA vaccine formulations. Additionally, we tested enhancement of IFN-α/β production by a plasmid encoding the cytosolic RNA innate immune sensor retinoic acid-inducible gene 1 (RIG-I), which has been shown to be required for clearance of Filovirus infections. We also compared EBOV GP DNA vaccines constructed in minimalized plasmids (Nanoplasmid™ vectors, Nature Technologies, Corp), which are smaller than traditional DNA vaccine plasmids and have been shown to have improved uptake, persist longer in transfected cells leading to increased transgene expression, and result in enhanced immune responses and improvements in immunological memory. Nanoplasmids expressing EBOV GP were designed with or without the RIG-I agonist and CpG motifs. Studies in mice demonstrated that intramuscular injection of the standard EBOV DNA vaccine along with molecular adjuvants enhanced immunogenicity and resulted in skewing toward Th1- or Th2-based responses when compared to responses obtained with the EBOV GP DNA vaccine alone. We found that mice vaccinated by IM injection of the nanoplasmid DNA vaccines developed increased virus-specific antibody and cellular immune responses as compared to mice vaccinated with the standard EBOV GP DNA vaccine. Challenge studies are currently in progress to determine if the enhanced immunogenicity that we observed correlates with increased protection from EBOV infection and if that protection equals that obtained previously using EP delivery.
Filovirus Vaccines

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Marburgvirus glycoprotein antibody isolation by antigen-specific single B Cell sorting in vaccinated macaques

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Introduction: In contrast to the progress towards the vaccine and therapeutics development against Ebolaviruses, limited treatment option is available for Marburgvirus (MARV). A set of neutralizing antibodies (nAbs) were recently isolated from a MARV survivor by hybridoma technology. MR191, one of these nAbs targeting the GP receptor binding site (RBS) showed full efficacy in NHPs challenged by Marburg (MARV) or Ravn (RAVN) viruses.

Objectives: The objective of this study is to identify more potent broadly protective antibodies with natively paired heavy and light chain (HC and LC) in GP immunized NHPs and explore possible protective epitopes besides the RBS.

Materials and Methods: We sequentially immunized a macaque with MARV GP virus-like particle as priming immunogen followed by a cocktail of Filovirus GPs as boosting immunogen. We then isolated memory B cells recognizing RAVN GP or cross-reactive to both MARV and RAVN GPs and sorted single cells by flow cytometry. Antibodies were cloned by single cell PCR amplification and sequencing of the HC and LC variable domains, expressed in mammalian cells as macaque-human chimeric antibodies, and used for in vitro characterization and efficacy studies.

Results: About 0.08% of the memory B cells of this macaque were RAVN GP positive and ~0.03% was cross-reactive with RAVN and MARV GPs. Among 76 GP-reactive memory B cells, 25 had paired HC/LCs. Four clonal lineages with multiple expansions were identified covering 13 of 25 clones. Interestingly, they exclusively used Ig VH3 gene families. 21/25 (84%) of the HC/LC pairs use kappa chains. Most of the antibodies possessed modest somatic hypermutation level and CDRH3 length with average 11.4 amino acids. In total, we expressed 21 MARV/RAVN GP cross reactive monoclonal antibodies. Epitope mapping and in vivo efficacy study of selected mAbs is underway.

Conclusions: The data provide insights of the immune response to a Marburgvirus GP VLP and protein cocktail immunization in macaque. Although the neutralizing antibody frequency is low it is possible that optimized immunogen and immunization regimen can elicit broadly neutralizing antibody against all Marburgviruses.
**Filovirus Vaccines**

**P 77**

**Effects of differential glycosylation on Filovirus glycoprotein immunogenicity**

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**Question:** All current Filovirus vaccine platforms utilize the highly glycosylated Filovirus attachment protein (glycoprotein, or GP1,2) as an antigen, but the specific glycan patterns and their effects on immunogenicity are not well-described.

**Methods:** Here, we report glycosylation of GP1,2 of the pathogenic Filoviruses Ebola (EBOV) Makona and Mayinga; Sudan (SUDV); Tai Forest (TAFV); Bundibugyo (BDBV); and Marburg (MARV) Angola produced in mammalian 293T cells and insect Sf9 cells, two cell systems used to generate virus-like particles (VLPs) for vaccine development.

**Results:** Our data showed similarities in the N-linked glycan profiles between Ebolavirus GP1,2s, but significant differences in glycosylation were imparted between the cell types. The O-linked glycans demonstrated more variation between Ebolaviruses, including between two EBOV strains, Mayinga and Makona. There was a lack of O-linked glycans on the Sf9-produced GPs, compared to extensive O-glycans imparted by 293T cells. Finally, we report significantly higher activation of specific dendritic cell subsets in vitro using insect cell-derived VLPs compared to mammalian cell-derived VLPs, which correlated with increased in vivo immunogenicity of insect cell-derived GP1,2.

**Conclusions:** The data presented here support a hypothesis that differential Filovirus GP1,2 glycosylation imparted by vaccine production in various cell types can affect subsequent immunogenicity. Further studies are being pursued to explore these findings.

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**Filovirus Vaccines**

**P 78**

**Mechanism of action of a broadly neutralizing pan-Ebolavirus antibody that recognizes a key site of vulnerability within the glycoprotein internal fusion loop**

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**Introduction:** Despite great progress towards development of vaccines for Zaire EBOV, little is known about the structural requirements and conserved epitopes for broad neutralization of Ebolaviruses.

**Objective:** To identify vaccine-elicited potent broadly protective antibodies and characterize the structural requirements for broad neutralization.

**Materials and methods:** A rhesus macaque was immunized with a Filovirus GP cocktail and boosted with VLPs. EBOV and SUDV cross-reactive memory B cells were isolated by single cell flow cytometry, and the corresponding mAbs identified, expressed in 293T cells, and characterized.

**Results:** We identified a broadly neutralizing antibody (bNAb) called CA45, that neutralizes and protects against EBOV, SUDV and BDBV. CA45 recognizes a conserved site in the internal fusion loop involving R64, Y517 and G546. CA45 partially blocks the cleavage of GP, and is more effective in neutralizing pre-cleaved Ebolaviruses, indicating a two-punch mechanism of action at early and late stages of entry. Consistently, CA45 bound cathepsin cleaved GP (GPCL) with >1000-fold higher affinity than full length GP. The CA45 epitope is partially occluded by residues D192, F193, and F194 (DFF lid) in the cathepsin cleavage loop. Mutation of these residues increased CA45 binding indicating the lid partially occludes CA45 access to this site. The DFF lid also interferes with binding of toremifene to EBOV GP, suggesting that the virus may have developed a protection mechanism for this key site of vulnerability. Interestingly, germline reverted CA45 (CA45GL) showed mM affinity for uncleaved GP but bound GPCL with pM affinity. However, CA45GL failed to neutralize pre-cleaved virus and this was related to its inability to bind GPCL at acidic pH, indicating that evolution was required for endosomal binding.

**Conclusion:** Lack of evolutionary pressure for affinity maturation toward binding at acidic pH may explain the low frequency of bNAbs. Our findings indicate that GPCL is a germline-targeting antigen that may be used to stimulate bNAb B cell precursors. Our data bear important implications for pan-Ebolavirus vaccine design.
Filovirus Vaccines

P 80
Recombinant modified vaccinia virus Ankara generating Ebolavirus-like particles

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There are currently no approved therapeutics or vaccines to treat or protect against the severe hemorrhagic fever and death caused by Ebola virus (EBOV). Ebolavirus-like particles (EBOV-VLPs) consisting of the matrix protein VP40, the glycoprotein (GP), and the nucleoprotein (NP) are highly immunogenic and protective in non-human primates against Ebola virus disease (EVD).

We have constructed a modified vaccinia virus Ankara-Bavarian Nordic® (MVA-BN®) recombinant co-expressing VP40 and glycoprotein (GP) of EBOV Mayinga and the nucleoprotein (NP) of Tai Forest virus (TAFV) (MVA-BN-EBOV-VLP) to launch non-infectious EBOV-VLPs as a second vaccine modality in the MVA-BN-EBOV-VLP-vaccinated organism. Human cells infected with either MVA-BN-EBOV-VLP or MVA-BN-EBOV-GP showed comparable GP expression levels and transport of complex N-glycosylated GP to the cell surface. Human cells infected with MVA-BN-EBOV-VLP produced large amounts of EBOV-VLPs that were decorated with GP spikes but excluded the poxviral membrane protein B5, thus resembling authentic EBOV particles. The heterologous TAFV-NP enhanced EBOV-VP40-driven VLP formation with comparable efficiency as the homologous EBOV-NP in a transient expression assay, and both NPs were incorporated into EBOV-VLPs. The levels of EBOV-GP-specific neutralizing and binding antibodies induced by MVA-BN-EBOV-VLP and MVA-BN-EBOV-GP in mice were similar, raising the question whether the quality rather than the quantity of the GP-specific antibody response might be altered by an EBOV-VLP-generating MVA recombinant.
Filovirus Vaccines

P 81
A recombinant vesicular stomatitis virus-based vaccine mediates post-exposure protection against Marburg virus Angola in rhesus macaques

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Previously, a recombinant vesicular stomatitis virus (rVSV) vaccine expressing the MARV Musoke strain glycoprotein (GP) was reported to fully protect macaques against several Marburg virus strains [1]. The vaccine was also effective against a lethal homologous challenge when treated post-exposure [2]; however, no cross-protection was afforded against the most pathogenic strain, Angola [3].

To increase target cell and/or antibody specificity, we engineered an rVSV expressing the MARV Angola strain GP (rVSVΔG/AMARV GP) for use as a therapeutic. Though treated macaques had a delayed time-to-death (TDD), only partial protection was observed (25%) against a 1000 plaque-forming unit (pfu) MARV Angola challenge. We hypothesized the rapid disease progression of MARV Angola compared to other strains [4] may account for the failure of our rVSV as a therapeutic. To test this, we challenged rhesus macaques with a low, uniformly lethal (50 pfu) dose of the virus to extend the disease course. All but one of the treated monkeys survived (8/9) and the treated non-survivor had a 2-3 log reduction in viremia and delayed time-to-death. To explore mechanisms of protection, we performed IgM and IgG ELISAs, cytometric bead arrays, flow cytometry, and RNAseq. Our results suggest rVSVΔG/AMARV GP slows MARV Angola disease progression and leads to altered gene expression resulting in a protective adaptive response.

3. TW Geisbert personal communication.
Filovirus Vaccines

P 82
Single low dose of VSV-EBOV protects macaques from lethal homologous challenge


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Ebola virus (EBOV) Makona was the causative agent of the 2013-2016 West African Ebola epidemic responsible for over 28,000 human infections and over 11,000 fatalities. During the epidemic, the development of several experimental vaccine platforms was accelerated through clinical trials. One of them, the vesicular stomatitis virus (VSV)-based vaccine VSV-EBOV, was shown to be effective in a phase III clinical trial in Guinea. This vaccine expresses the EBOV-Kikwit glycoprotein from the 1995 outbreak as the immunogen. We generated a VSV-based vaccine expressing the EBOV-Makona glycoprotein and determined the dose-dependent protective efficacy in the macaque model. We observed a dose-dependent decrease in antibody responses to vaccination with doses ranging from 10,000,000 to 100 plaque-forming units (pfu), but all vaccinated animals survived the lethal EBOV-Makona challenge. Some animals receiving lower vaccine doses developed low level EBOV viremia with clearance of the virus from the blood by day 14 after challenge. Control animals developed classical Ebola hemorrhagic fever and were euthanized within one week after EBOV-Makona challenge. This study demonstrates that even a low dose of VSV-EBOV (100 pfu) protects macaques against lethal homologous EBOV challenge.

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Filovirus Vaccines

P 83
T-cell dependent mechanisms promote Ebola VLP-induced antibody responses, but are dispensable for vaccine-mediated protection

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Introduction: Humoral responses are essential for protective efficacy of most Ebola virus (EBOV) candidate vaccines; however, in vivo studies defining 1) how EBOV B-cell immunity is established, 2) the relative contributions of T-cell-independent and T-cell-dependent (TD) B-cell mechanisms and 3) the direct requirement of B-cell responses for protection from EBOV infection have been limited.

Objective: Vaccination with viral-like particles (VLP) consisting of the EBOV matrix protein (VP40) and glycoprotein (GP₁,₂) can elicit protection from EBOV infection within laboratory mice, guinea pigs, and non-human primates. Recently, we identified that inclusion of a clinical grade dsRNA adjuvant, Poly-ICLC, during VLP vaccination increased EBOV GP₁,₂-specific antibody titers and durable immunity against EBOV infection. Here, we use the EBOV VLP as a model system to further examine the contribution of B-cell immunity in protection from EBOV lethality and the impact of adjuvant signaling on VLP-mediated B-cell responses.

Methods: Using the well-defined mouse-adapted EBOV model, we delineate the in vivo contribution of VLP-mediated B-cell immunity through a series of cellular and humoral immunogenicity and efficacy studies utilizing mice deficient in either TD antibody responses, antibody class-switching or having a complete lack of B cells.

Results: We demonstrate 1) humoral responses induced by both VLP vaccination and EBOV infection are established through TD mechanisms, 2) inclusion of adjuvant with VLP vaccinations both augments and sustains germinal center B-cell reactions, T-cell follicular help and antigen-specific B-cell frequencies, 3) antibodies, but not B cells, are dispensable for protection and 4) adjuvant signaling could circumvent the complete requirement for B-cell immunity in protection against EBOV.

Conclusion: Here, we propose a division of labor for humoral immunity against EBOV in which B-cell autonomous and antibody-dependent responses collectively promote protection. Further, we define unappreciated redundancies for EBOV vaccine-mediated immunity within the murine model which may prove valuable for future vaccine candidate development.
Filovirus Vaccines

P 84
Safety, immunogenicity, and efficacy of the msd rvsvδg-zebov-gp Ebola vaccine

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The 2013-2016 Ebola outbreak has caused over 28,000 cases and 11,000 deaths. MSD is working with private and public partners to develop an Ebola vaccine that has demonstrated efficacy during this outbreak. The vaccine is a live recombinant vesicular stomatitis virus (VSV) with complete substitution of the VSV-G envelope glycoprotein (GP) with Zaire Ebolavirus GP. Phase 1-3 clinical trials have been conducted to assess safety, immunogenicity, and/or efficacy of rVSVΔG-ZEBOV-GP in humans.

One IM dose of $2 \times 10^7$ PFU is well-tolerated when administered to healthy adults. Injection site reactions following vaccination are typically mild or moderate and self-limited. There is a predictable period of generally mild reactogenicity, including fever and a flu-like syndrome typically lasting 1-3 days. Joint pain is a common part of the early flu-like syndrome. In a small proportion of subjects (<5% in most studies), joint swelling (arthritis) may develop in the weeks following vaccination. Arthritis is generally mild to moderate in severity and is likely mediated by direct viral infection of joint tissues; the vast majority of arthritis events resolve spontaneously, though persistent and recurrent symptoms have also been reported. An open label cluster-randomized ring vaccination trial in Guinea enrolled over 11,000 subjects to receive immediate (day 0) or delayed vaccination (day 21). The primary analysis to estimate vaccine efficacy (VE) against laboratory-confirmed EVD among subjects randomized to the immediate arm and vaccinated compared to subjects randomized to the delayed arm who were eligible and consented on day 0 demonstrated VE of 100% (95% CI 63.5% to 100%); $p=0.0471$. No EVD developed in any subject that received study vaccine 10 or more days after vaccination. A phase 3 randomized placebo-controlled trial evaluated the safety and immunogenicity of three consistency lots and a high dose lot of rVSVΔG-ZEBOV-GP in healthy adults. Lot-to-lot consistency was met as shown by the equivalent immune responses, measured by GP-ELISA 28 days after vaccination, across the three lots. In this presentation we will provide the current status of the development program.
The urgent need for a vaccine against Ebola virus (EBOV) was underscored by the large outbreak in West Africa in 2014 with more than 28,000 cases and 11,000 fatalities. Although several promising vaccine candidates have been tested successfully in animal models and in recent clinical trials in humans, none of those is licensed yet. Based on the recombinant Modified Vaccinia virus Ankara (MVA), new vaccine candidates encoding the EBOV glycoprotein (MVA-GP) or nucleoprotein (MVA-NP) were developed and tested for efficacy in a mouse model. Another objective was to assess the correlates of protection of both vaccine candidates which include neutralizing antibodies and/or CD8+ T cells. Type I interferon receptor knockout (IFNAR-/-) mice were immunized with the different vaccine candidates in a prime-boost regimen and mock-vaccinated mice served as controls. Mice were infected with EBOV and monitored for clinical signs. Serum and organ samples were evaluated for viral load by qPCR and immunoplaque assay. Sera were used for clinical chemistry and organ samples were examined histopathologically. Antibody-based depletion of CD 8+ T cells in MVA-NP vaccinated mice was performed to evaluate their role for protective immunity. Mice vaccinated with MVA-GP and MVA-NP developed high titers of EBOV-specific antibodies which were partially virus-neutralizing. In contrast to the mock-vaccinated mice, all vaccinated mice survived and showed decreased viral loads in serum and organs, reduced organ pathology and clinical chemistry was within physiological range. Depletion of CD 8+ cells of MVA-NP vaccinated mice resulted in susceptibility of the otherwise protected mice, indicating the importance of these cells for the protective immune response against EBOV. The MVA-based vaccines against EBOV GP and NP induced a protective immunity in mice. While for MVA-GP the virus-neutralizing antibodies seem to be the most important correlate of protection, the cytotoxic CD 8+ T cells seem to play the major role in the protective immune response for the MVA-NP vaccine candidate. Both vaccines are therefore efficient in the tested mouse model and promising candidates for further analyses.
Filovirus Vaccines

P 87
Protection and long-lasting immunity in cynomolgus macaques with a pre-exposure prophylactic Zaire Ebolavirus (EBOV) GP DNA vaccine

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Although there are currently no licensed vaccines or therapeutics for treatment of Ebola virus disease (EVD), there are several promising experimental vaccine candidates including the live-attenuated rVSV-ZEBOV vaccine which demonstrated efficacy in a ring-vaccination trial. However, the establishment of robust memory responses is may be limited by potential anti-vector immunity and some of these platforms may not be suitable for certain populations. We designed a novel synthetic Zaire Ebola virus (EBOV) GP DNA vaccine as an alternative pre-exposure candidate that induces strong anti-GP immune responses, in a serology independent fashion allowing for homologous vector re-administration, and has a clean safety profile. The EBOV-GP DNA vaccine was administered to cynomolgus macaques (n=4-5/group) as a homologous prime-boost (2 dose) regimen by intramuscular (IM-EP) or intradermal (ID-EP) delivery, followed by electroporation. We observed 100% protected against lethal EBOV Makona C07 challenge by the ID-EP route and 75-100% protection by IM-EP route. A parallel group of animals (n=5/group) with different IM-EP injection regimens were followed over the course of 1 year to monitor long-term immunogenicity following DNA immunization. NHPs rapidly seroconverted and had long-lasting total IgG antibody titers and T cell responses to EBOV GP antigen. Twelve months post-final vaccination, all NHPs received a 1 year homologous vaccine boost. The magnitude of the recall response was significant for all groups. An ID-EP memory study is currently in progress. Together, the data support that this EBOV-GP synthetic DNA vaccine for protection against EBOV as well as robust memory immune responses.
Immune Response to Filovirus Infection

P 88

Xenotransplanted animal models to study Ebola virus pathogenesis

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Filoviruses such as Ebola virus (EBOV) and Marburg virus cause severe disease in humans and non-human primates but cause sub-clinical infection in other species such as laboratory mice and bats. We have recently established a humanized mouse model (huNSG) that is susceptible to non-adapted EBOV and recapitulates many aspects of human Ebola virus disease including viremia and hepatic failure. However, NSG mice transplanted with mouse bone marrow progenitor cells were entirely resistant to EBOV. These findings strongly suggested that xenotransplantation of bone marrow into NSG mice could serve to study species-specific EBOV pathogenesis and would allow for comparative studies across species. Based on this rationale, we have generated preliminary data, which compares EBOV infection in NSG mice transplanted with mouse bone marrow (moNSG), human bone marrow (huNSG) and bat bone marrow (batNSG). BatNSG transplanted with bone marrow progenitor cells from Rousettus aegyptiacus, and moNSG displayed viremia and transitional elevation of serum aminotransferases but cleared the virus and survived infection. Conversely, huNSG did not resolve viremia and died between days 10-20 post-infection.

Although preliminary, our data supports that xenotransplantation of NSG mice may be an important tool to identify correlates of EVD survival in humans and other species and warrant further investigations.

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Immune Response to Filovirus Infection

P 89
Transcriptomic signatures differentiate survival from fatal outcome in humans infected with Ebolavirus

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The recent outbreak of Zaire Ebolavirus in West Africa was unprecedented in scale and caused an explosion in the cases of Ebola virus infections. Early symptoms are non-specific and flu-like, but these rapidly dissolve into systemic issues, frequently resulting in death. The distinguishing features of fatal and non-fatal infections have been difficult to elucidate, though an increased viral load is indicative of a poor prognosis. Using transcriptome data from blood samples of both acute and convalescent patients we have identified host factors that are associated with acute illness and those that differentiate patient survival from fatality. These data reveal that individuals who succumbed to the virus experience an upregulation in interferon signalling and that of their acute phase response in comparison to their surviving counterparts. These upregulated genes suggest an increase in inflammation and liver pathology.

Our RNA-sequencing data allowed the employment of a machine learning technique in order to create a genetic profile for an average survivor. These host biomarkers proved to be highly predictive and with the expression levels of ten determined genes we are able to predict patient outcome with 85% accuracy. This is particularly useful in cases of intermediate viral load when this variable is a poor prognostic indicator. The data suggested that rapid analysis of the host response to infection in an outbreak situation can provide valuable information to guide an understanding of disease outcome and mechanisms of disease, and the importance of host response in Ebola virus infections.
**Immune Response to Filovirus Infection**

**P 90**

**Development of an Ebolavirus pathogenesis model using iPSC-derived hepatocytes**

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**Background:** Ebola virus (EBOV) infection causes a severe disease (EVD) in humans which causes liver damage. The liver is an early target organ of EBOV infection, at which time viremia spikes and symptoms worsen. Therefore, it is thought that hepatocytes are a key site of viral replication in vivo and potentially a promising target for therapeutic interventions. Primary human liver samples are difficult to acquire, and animal models of EBOV infection either incompletely recapitulate disease or are costly. Our goal is to use induced pluripotent stem cell (iPSC)-derived hepatocytes to develop a disease-relevant infection platform for modeling EBOV pathogenesis.

**Methods:** We differentiated hepatocytes from human iPSCs using a step-wise directed differentiation protocol which follows normal embryonic development. iPSC-derived hepatocytes were characterized using flow cytometry, intracellular staining, qRT-PCR, and functional assays. iPSC-derived hepatocytes and immortalized hepatic carcinoma cells (Huh7) were then infected with recombinant vesicular stomatitis virus expressing the EBOV surface glycoprotein (VSV-Z76-GFP) or EBOV. Interferon production was analyzed by qRT-PCR.

**Results:** iPSC-derived hepatocytes expressed mature hepatic markers and key hepatic enzymes were active. In addition, these cells store low density lipoprotein in vacuoles. iPSC-derived hepatocytes were susceptible to infection with both VSV-Z76-eGFP and EBOV. Upon VSV-Z76-eGFP infection, iPSC-derived hepatocytes expressed significantly higher levels of IFNβ than Huh7 cells. Infection with VSV-Z76-eGFP at a high MOI did not lead to cell death whereas Huh7 cells died within 24 hours, indicating significant differences between the antiviral response in primary-like and immortalized hepatocytes. iPSC-derived hepatocytes supported EBOV infection as indicated by the presence of cytoplasmic viral inclusions.

**Conclusions:** Our iPSC-derived hepatocytes are a suitable in vitro model for EBOV infection of hepatocytes. We propose to now use this model to better understand the molecular mechanisms leading to liver damage during EBOV infection.
Immune Response to Filovirus Infection

P 91
Assessment of a pseudotyped virus neutralisation assay for Ebolavirus

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Introduction: Ebola virus (EBOV) is an enveloped, single stranded RNA virus that can cause Ebola virus disease (EVD), a highly lethal illness with up to 90% mortality. The viral surface glycoprotein (GP1,2) mediates host cell attachment and fusion, and is the primary target for neutralising antibodies. Serological studies are vital to assess the neutralising ability of antibodies targeted to GP1,2, however handling of EBOV is limited to containment level 4 laboratories. Pseudotyped viruses (PVs) are increasingly being used for the investigation of viral infection or vaccine seroconversion.

Objectives: The aim of this study was to assess the suitability of an EBOV GP PV system to measure neutralising antibody titres in plasma derived from EVD convalescent volunteers.

Materials and methods: EBOV GP PVs were generated by transfection using plasmid DNA encoding EBOV GP, HIV-1 gag-pol, and firefly luciferase reporter. The PVs were titrated using a range of cell lines and relative luminescence units per ml (RLU/ml) were determined. Neutralisation of the EBOV GP PVs by plasma from EVD survivors of the 2013-2016 EBOV outbreak was evaluated. Percentage infectivity was determined relative to infectivity of EBOV GP PV alone and 50% inhibitory concentration (IC50) of PV neutralisation were estimated by model of nonlinear regression fit. For each sample, pre-existing data were available for live EBOV neutralisation.

Results: Differences in infectivity were observed depending on the target cell line used for infection, with 293T/17 generating the highest RLU/ml. There was a significant correlation when IC50 values of EBOV GP PV neutralisation were compared with geometric mean titre values for a live EBOV neutralisation assay.

Conclusion: The EBOV GP PV neutralisation assay can be used to test plasma from EVD convalescent patients. Assay optimisation has the potential to improve the discriminatory power of the assay with regards to differing antibody titres, which has value for vaccine evaluation and assessment of convalescent blood products for use as immunotherapeutics. This research could provide a better understanding of the correlates of protection against EBOV.
Immune Response to Filovirus Infection

P 92

Molecular and functional diversity of antibody repertoire from a survivor of Ebolavirus infection

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Introduction: The genus Ebolavirus contains diverse viral species known to cause large deadly outbreaks in Africa. The Ebolavirus glycoprotein (GP) is a key target for the neutralizing antibodies (Abs). This study was focused on clonal and functional diversity of a large GP-reactive Ab repertoire containing over 1,000 new mAbs from a single Ebolavirus survivor, which could inform principal Abs specificities and molecular mechanisms contributing to virus clearance and cross-protective immunity.

Objectives: Elucidate molecular and functional diversity of mAbs within a large GP-reactive Ab repertoire.

Patients and methods: PBMCs were isolated from a survivor of the 2014 Ebolavirus infection outbreak in Nigeria. Ebola virus Zaire (EBOV) GP-reactive B cells were purified by FACS and subjected to bulk next-generation sequencing (NGS) analysis, or high-throughput generation of hybridoma cell lines. The hybridoma lines isolated were assessed for Ab subclass, clonal diversity, and cross-reactivity to the GP of three Ebolavirus species, Zaire, Bundibugyo (BDBV), and Sudan (SUDV). Culture supernatants with broadly-reactive Abs were assessed further for neutralizing activity and capacity to induce cellular phagocytosis.

Results: Over 11,000 of EBOV GP-reactive B cells were sorted, ~5,000 of those were sequenced, and 1,017 hybridoma cell lines were isolated. NGS sequencing identified 3,071 heavy, and 4,174 unique light chains, and showed a prevalence of BVH3/JH6 germline gene recombination. Abs produced by cell lines were mostly of IgG isotype and of diverse subclasses. Remarkably, a large fraction (~44%) of Abs from the panel were reactive to GP from all three Ebolavirus species. However, only a minor fraction of broadly-reactive Abs (four of the ~200 assessed Abs) possessed cross-neutralizing activity. In addition, both neutralizing and non-neutralizing Abs from the immune repertoire exhibited functional activity through their Fc region to induce cellular phagocytosis.

Conclusions: Our study unveiled a high complexity of GP-reactive Abs repertoire from a single survivor, and suggested diverse mechanisms for cross-protective immunity against Ebolavirus infections.
Immune Response to Filovirus Infection

**P 93**
**Analysis of Ebolavirus glycoprotein interactions with human myeloid cells**

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**Introduction:** EBOV infection, which is permissive in dendritic cells (DCs) and macrophages, results in hemorrhagic fever, characterized by immune dysfunction and T cell apoptosis. We hypothesize that EBOV induced changes in myeloid gene expression result in dysregulation of innate immune activation, processing and presentation of antigen, thus affecting viral recognition and clearance.

**Objectives:** We aim to characterize changes in gene expression of myeloid cells exposed to EBOV virus-like particles (VLPs) and address their impact on myeloid cell function and cross talk of myeloid cells with T cells.

**Methods:** Immature DCs (iDCs) were differentiated with IL-4 and GM-CSF from healthy donor blood derived CD14+ monocytes. iDCs were characterized by relative expression of MHC-II, CD86, CD80, CD83, DC-SIGN, CD14 and CD1a. To study virus entry, EBOV VLPs were produced with different EBOV glycoproteins (GPs) and the viral matrix protein VP40 tagged to b-lactamase (BLAM-VP40). Macrophage-like cells were differentiated using PMA from the monocyte like cell line, THP-1 and characterized by their relative expression of CD206, CD36, DC-SIGN, CD16 and CD11b.

**Results:** Entry of EBOV VLP’s was demonstrated in human DCs and inhibited in the presence of an anti-EBOV GP MoAb. Analysis of DC maturation markers (MHC-II, CD86, CD80, CD83, DC-SIGN) after EBOV VLP entry demonstrated a trend towards an increase in cell surface expression as compared with mock treated control cultures. Furthermore, chemokine secretion (IL-8 and MCP-1) was increased post EBOV VLP entry. Strikingly, EBOV VLP entry levels were highly variable among blood donors. In contrast to EBOV VLP entry in primary cells, conditions for differentiation of THP-1 cells were identified which provide a robust in vitro myeloid cell model for analysis of entry by EBOV VLP’s containing GPs from stains of different pathogenicity.

**Conclusion:** Primary DCs and a transformed macrophage-like cell line provide complementary model systems for dissecting the early host response to pathogenic and non-pathogenic Ebola viruses and an insight into the underlying mechanisms determining the pathogenicity of different Ebola virus species.
Immune Response to Filovirus Infection

P 94
Establishment of bone marrow-derived dendritic cells from microbats for the study of Ebolavirus infection

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In vivo models have established that antigen presenting cells (APCs), specifically dendritic cells (DCs) are targets of Ebola virus (Ebov) infection. Following viral entry, DC maturation is impaired and minimal co-stimulatory molecules are expressed, low-levels of cytokines are produced and high levels of co-inhibitory T-cell molecules such as B7H1 are upregulated, all the while supporting high-titer Ebov replication. Dysregulation of DC function hinders the induction of Ebov-specific adaptive immune responses, which, when induced, can set apart surviving versus fatal Ebov cases.

Interaction between human, mouse or non-human primate DCs and Ebov has been characterised, however the response of reservoir-host DCs to Ebov infection remains unstudied due to lack of in vitro DC culture methods and bone fide reservoir-host infection models. Currently, only one method for bone-marrow derived DC (BM-DC) culture for P. alecto, the natural host of Henipaviruses has been published. To date specific fruit bats and few microbats have been postulated as Ebov reservoir hosts.

Understanding interaction between reservoir-host DC and Ebov may elucidate pathogenic mechanisms, whereas epithelial and fibroblast cells from reservoir hosts may not, as these cell types are not always predictive of host susceptibility.

We have developed a culture system for BMDCs of Mops condylurus (Moco), an Angolan Free-tailed Bat, suspected to be involved in spill-over during the 2014 West African outbreak. BM cells harvested from Moco were cultured with recombinant equine GM-CSF and IL-4, which share 63% and 66% sequence identity to equivalent cytokines of microbats, respectively. BMDCs appeared as semi-adherent clusters after 5 days in culture, and after 11-12 days, exhibited prominent DC-like veiled morphology. Their morphology was further imaged by EM and phenotype examined using cross-reactive antibodies targeting conventional DC surface markers. The presence of Ebov receptors was determined and permissiveness of BMDC to Ebov-Zaire was also assessed to determine the suitability of these cultures for studying mechanisms associated with viral-reservoir-host DC interactions.
Immune Response to Filovirus Infection

P 95
Broad neutralizing activity in plasma from Ebolavirus disease convalescent patients against Ebolavirus lacking the mucin-like domain

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Introduction: Kinetics and specificity of the neutralizing antibody response in Ebola virus infected individuals are not completely clear. During cellular infection, cleavage of virion-associated GP by host cell cathepsins removes the glycan cap and mucin-like domains producing another functionally distinct form of GP.

Objectives: To analyze the breadth of cross-neutralizing response in Ebola virus disease convalescent patients (CP) from the recent outbreak in West Africa (2013-2016) caused by Ebolavirus Makona variant.

Materials and methods: Ebolavirus GP-pseudotyped lentiviruses (Zaire, Sudan, Bundibugyo and Reston viruses) were produced to measure infectivity and antibody-neutralization response in plasma from three EVD-CP from the recent outbreak in West Africa.

Viral particles were treated with thermolysin to remove the glycan cap and mucin-like domains and infection results were compared with those obtained with the native form on HeLa cells.

Results: After cleavage with thermolysin Ebolaviruses showed an increased infectivity capacity as compared with the native. The fold increase of infectivity differed between specific agents from 3 in the case of EBOV/May and RESTV to 28 in the case of SUDV.

Specific neutralizing titer of plasma from EVD CP was significantly higher against EBOV/Mak as compared to other Ebolaviruses including Zaire strains. This neutralizing titer increased significantly upon thermolysin treatment and the breadth of activity expanded to other members of the Ebolavirus genus such as Sudan, Bundibugyo and Reston viruses.

Conclusions: Natural humoral response after Ebolavirus infection is variant specific. However broad neutralizing antibodies against different viruses within the Ebolavirus genus are present in plasma from EVD CP being only evident after removal of the glycan cap and mucin-like domains. The mechanism involved in this neutralization needs to be further elucidated, but probably the target of those broad neutralizing antibodies could be the RBD that is only exposed after cathepsine cleavage and highlights the biological properties of these domains on shielding conserved epitopes on Ebola glycoproteins.
Immune Response to Filovirus Infection

P 96
Biomarker correlates of clinical outcome in patients with Ebolavirus disease

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Background: The Ebola virus disease (EVD) outbreak from 2014 to 2016 in West Africa has been the largest recorded Filovirus outbreak in human history. Diagnostic activities were performed by the European Mobile Laboratory during the outbreak response, and under the umbrella of the WHO. Leftover plasma samples had been preserved and were available for further analysis.

Methods: Multiplex assays were used to determine the concentration of 63 soluble cytokines and chemokines in 398 plasma samples of 179 hospitalised EVD patients with different disease outcome.

Results: Our results indicate that fatal EVD is characterized by high levels of circulating pro-inflammatory mediators such as TNF, IFN-gamma, MIP-1-alpha or MIP-1-beta. The plasma levels of these mediators were significantly higher in fatal EVD patients compared to surviving patients at the time of admission at the Ebola Treatment Center.

Conclusion: These results substantiated the notion that human EVD is associated to overwhelming immune activation rather than immune suppression, and pointed out at a discrete subset of cytokines as early markers of disease outcome.
Immune Response to Filovirus Infection

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Comparison of differentially expressed genes in two human cell lines infected with Zaire and Reston Ebolaviruses

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Ebolaviruses are negative single stranded RNA viruses, member of the family Filoviridae, comprising five known species: Bundibugyo Ebolavirus, Reston Ebolavirus (REBOV), Sudan Ebolavirus, TaiForest Ebolavirus and Zaire Ebolavirus (ZEBOV). ZEBOV is among the most lethal viruses for human. REBOV is known to be able to infect humans however, with no reported disease. It is not known which are the molecular mechanisms that lead to the different infection outcomes of ZEVOV and REBOV in humans.

To address this question, the human hepatoma cell line (HuH7) and THP1, a human monocyte cell line which was differentiated to show a macrophage phenotype, were infected with ZEBOV, REBOV, or were left non-infected (Mock). Total RNA was extracted at 3 hours and 24 hours post infection. We performed differential expression analyses on microarray and small RNA-Seq level and compared significantly expressed protein- and non-coding genes between ZEBOV-, REBOV- and Mock-infected cells. Analysis of the microarray results showed no common differentially expressed (DE) genes between the two cell lines. However, DE genes encoding proteins mapping to the same cellular process were enriched in both HuH7 and THP1 cells. Additionally, a PCA analysis showed REBOV and MOCK clustering together, while being clearly separated from ZEBOV, at 3 h p.i. A similar pattern was observed in a gene expression heatmap, suggesting the infection response to REBOV was slower than to ZEBOV. Also, at 24 h p.i., several genes show the same DE pattern between REBOV and ZEBOV compared to Mock. Furthermore, the read profiles from small RNA-Seq showed coding and noncoding regions being covered which would imply the presence of novel ncRNA molecules. This profile was also found in the viral genomes, suggesting the existence of ncRNAs in REBOV and ZEBOV.

To conclude, all the data shows promising differences to elucidate the host response against REBOV and ZEBOV, which would give a hint on the viral pathogenesis. Currently, the results are being validated and further analysed.
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FANG primate assays for human and nonhuman primate Filovirus vaccine studies

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A FDA/EMA licensed vaccine for prevention of Filovirus hemorrhagic fever will significantly reduce the morbidity/mortality in the event of an outbreak of disease caused by EBOV, SUDV, or MARV, in addition to providing protection for laboratory workers. A correlate or surrogate markers of immunity/protection will likely be required to achieve licensure via the FDA Animal Rule. Well characterized humoral and cellular immune response assays are required to bridge the relationship between the NHP immune response and protection from Filovirus challenge to the human immune response. We have developed a suite of immunological assays to evaluate the immune response to Filovirus vaccines for identification of the surrogate measure(s) to establish vaccine effectiveness/efficacy. To evaluate humoral immune responses, IgG is assessed by Filovirus anti-GP IgG and IgG subclass ELISAs. Neutralizing antibody activity is measured using a pseudovirion neutralization assay. To evaluate cellular immune responses, intracellular cytokines are assessed by flow cytometry, secreted cytokines by Luminex based assay, and ADCC using a GP-specific luciferase reporter bioassay. The EBOV-specific GP IgG ELISA was validated for use on GLP studies and reagents (coating antigen, reference standard, QCs) were established for long-term use. Statistical process control charting is being implemented to monitor tracking/trending over time. The EBOV ELISA was transferred to multiple labs and blinded proficiency panels were established for monitoring assay performance. SUDV and MARV GP IgG ELISAs were partially qualified and when sufficient reagents are available will be fully qualified/validated. The pseudovirion neutralization assay was optimized and will be qualified/validated. EBOV GP-specific ADCC and cytokine responses were detected in a subset of samples post-immunization. All assays were optimized for use with standard operating procedures and established reagents. The assays are well-characterized for testing NHP and human clinical samples from multiple vaccine platforms. The advancement of these assays will play an important role in immune bridging between NHPs and humans.
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*Ebola virus glycoprotein-mediated inflammatory responses*

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**Background:** Ebola virus (EBOV) infection of human macrophages leads to a profound proinflammatory response. This response is triggered by the EBOV glycoprotein (GP) via activation of Toll-like receptor 4 (TLR4) and can be inhibited by TLR4 antagonists. In contrast, Reston virus (RESTV), which is thought to be nonpathogenic in humans, fails to stimulate a strong host response in macrophages due to the inability of RESTV GP to stimulate TLR4 (Olejnik et al. 2017). Therefore we aim to further characterize the interaction of different Ebolavirus GPs and TLR4.

**Methods:** We have employed virus-like particles (VLPs) to further dissect the GP-mediated host response using wildtype and mutant versions of EBOV and RESTV GP. In addition to human macrophages, we have tested mouse macrophages to determine potential species-specific effects. Further, we have explored if inhibitors of inflammation can be used to suppress the EBOV GP-mediated cytokine response.

**Results and conclusions:** We will present data defining the regions in EBOV GP required for activation of TLR4 signaling. In addition, we will demonstrate the use of anti-inflammatory treatments to inhibit EBOV GP-mediated immune signaling. We will further provide data on the species specificity of EBOV GP-triggered TLR4 activation. This work will provide the basis for future in vivo studies targeting the EBOV GP-induced immune response that seems to be detrimental to the infected host during severe EBOV disease.

**References:**

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Cell type-specific roles of mitochondrial antiviral signaling protein (MAVS) during Ebola virus infection

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Introduction: Ebola virus (EBOV) is sensed by the cytoplasmic RNA helicases, RIG-I and MDA-5, known as the RIG-I-like receptors (RLRs). Following dsRNA binding, RLRs signal through a single critical adaptor, mitochondrial antiviral signaling protein (MAVS), to induce type I interferon (IFN) expression. EBOV VP35 antagonizes RLR-MAVS signaling in vitro but its impact in vivo on viral anti-EBOV responses are unknown.

Objective: To understand the role of MAVS signaling in cellular responses leading to the control of EBOV replication in vivo.

Methods: C57BL/6, MAVS\(^{-/-}\) mice, and mice conditionally deleted for MAVS in macrophages (LysM-Cre+MAVSfl/fl) or dendritic cells (DCs; CD11c-Cre+ MAVSfl/fl) were infected with 100 ffu of WT- or mouse-adapted EBOV (MA-EBOV). Mice were monitored for survival and weight loss or underwent serial necropsy to collect samples for analysis of host responses.

Results: MAVS deficiency rendered mice susceptible to lethal disease following MA-EBOV infection and increased morbidity during WT-EBOV infection compared to control mice. MAVS\(^{-/-}\) mice had increased viral titers, reduced serum IFN, and altered transcriptional responses indicating reduced RLR signaling, increased inflammatory gene expression and increased cell death in the liver. Specific cellular responses including macrophages were implicated as differentially regulated in the absence of MAVS. To determine cell-type specific roles of MAVS, we conditionally deleted MAVS from macrophage populations (LysM-Cre+MAVSfl/fl) or DCs (CD11c-Cre MAVSfl/fl) while leaving MAVS expression in other cell types intact. LysM-Cre+MAVSfl/fl or CD11c-Cre MAVSfl/fl mice were uniformly susceptible to lethal disease similar to MAVS\(^{-/-}\) mice. Surprisingly, the role of macrophages was not due to intrinsic control of virus replication or IFN production, likely due to efficient viral antagonism of these pathways.

Conclusion: Impaired MAVS signaling in macrophages or myeloid-lineage DCs dominantly determines disease severity and outcome. The role of MAVS-dependent macrophage responses is likely indirect and may coordinate critical cellular innate and adaptive responses that ultimately control infection.
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A systematic approach to identify Interferon Stimulated Genes (ISGs) with antiviral potential against Ebolavirus

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Introduction: Significant progress has been made characterising mechanisms of host interferon (IFN) antagonism employed by Ebola. However antiviral effects of IFN, via downstream induction of interferon-stimulated genes (ISGs), are poorly understood. These ISGs, upregulated by signalling cascades triggered by activation of pattern recognition receptors (PRRs), can target steps throughout the virus life cycle limiting viral spread, replication and activation of adaptive immune responses.

Objective: Further characterise IFN’s antiviral effect on Ebola and identify specific ISGs with antiviral potential by screening through a gene library.

Materials and methods: A previously characterised ISG library of approximately 400 Human ISGs (Schoggins et al, Nature, 2011) was screened for anti-Ebola effect using a transcription- and replication-competent virus-like particle (trVLP) system (Hoenen et al, JVI, 2014). Selected ISG candidates then were validated and ectopic expression characterised in terms of: effect on minigenome RNA (via qPCR), transfected Ebola proteins (via Western blots) and activation of NFkB and IFNb reporter assays.

Results: Type I IFN treatment of target cells resulted in decreased trVLP production in target U87 glioblastoma and HEK 293 cells (1.5 and 0.5 logs lower respectively). An ISG library screen was then conducted where 30-40 putative candidate genes were identified as inhibitors of Ebola. The candidates were further validated and those with strongest antiviral phenotype had a considerable impact on viral genome replication/ transcription. Ebola NP and VP35 protein levels were not affected by ectopic ISG expression, though a few impacted VP30 expression. No direct correlation was observed between antiviral effect and NFkB- and IFNb-reporter activation mediated by specific ISGs.

Conclusion: Both IFN and selected ISGs (notably TRIM25) were observed to be antiviral against Ebola. Knowledge on which ISGs and where they target in the lifecycle may prove invaluable to understanding more about Ebola virus. This abstract is further carried on with the characterisation of TRIM25 antiviral mechanism (see Rui Galao’s abstract).
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PKR and HuR repress Ebolavirus replication in a trVLP system

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Introduction: Ebola virus (EBOV) is a nonsegmented, negative-strand RNA virus that replicates in the cytoplasm of infected cells. EBOV RNA can trigger host antiviral mechanisms by activating the RNA sensors RIG-I and PKR. PKR activation leads to translation inhibition resulting in the formation of stress granules (SGs). SGs are non-membrane-bound cytosolic organelles that assemble due to different stress conditions, such as viral infection. Viral protein 35 (VP35) has been shown to inhibit PKR and SG formation. However, whether SG proteins have antiviral effects on EBOV replication remains unclear.

Objectives: We are investigating how SG proteins regulate EBOV replication.

Methods: Using a transcription- and replication-competent virus-like particle (trVLP) system, we analysed the effect of overexpression and depletion of SG proteins on viral transcription, replication and virus production.

Results: To validate the trVLP system for analysis of SGs, we first analysed the role of PKR. Overexpression of PKR inhibited trVLP replication by 90%. Using a triple amino acid mutant of VP35 (VP35-3A) that is unable to inhibit PKR, we showed that trVLP virus production is decreased by 80% compared to wild type VP35. Interestingly, there was a large increase in virus production in PKR knockout cells compared to wild type cells and VP35-3A mutant virus production was fully rescued in PKR knockout cells. Then, we performed an overexpression screen for a subset of SG proteins to determine their effect on EBOV replication. We identified several proteins that inhibit trVLP replication by >70% in a dose-dependent manner that cannot be overcome by high virus input. HuR overexpression inhibited replication by >90% and HuR depletion increased trVLP replication by 2.5-fold. We are currently analysing the effect of depleting other SG proteins on trVLP replication and are investigating the mechanism by which specific SG proteins inhibit replication.

Conclusion: Overexpression of specific SG proteins inhibits EBOV trVLP replication. Moreover, knockout of PKR or HuR increases trVLP replication. Therefore, we hypothesise that SGs may inhibit EBOV replication.
Adaptive immune responses to Ebola virus infection is complex. There are conflicting reports of differential correlates of immunity for protection against Ebola virus in primary infection or in vaccinated populations. Several pieces of evidence have supported a hypothesis that lethal Ebola virus infection is caused by dampened antibody and cell-mediated immune responses. For example, lethal infection is correlated with extensive lymphocyte apoptosis and higher levels of viremia, and some studies have found higher levels of antibody and T cell responses in survivors. Recent data from our group and others have expanded on these findings, suggesting that adaptive immune responses may actually be robust in some lethal Ebola virus infections, and that lymphocyte apoptosis is not required for Ebola virus pathogenesis. These data have been supported by additional findings in human patients. Furthermore, we have found that inhibition of type I interferon responses leads directly to uncontrolled pro-inflammatory cytokine responses, which could have negative effects on the development of adaptive immune responses. We propose a revised model, based on extensive work from multiple groups, in which adaptive responses are developed in lethal Ebola virus infection, but at a slower rate due to hyperinflammation caused by inhibition of type I interferon responses.
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Characterisation of convalescent plasma using an EBOV GP HIV-1 pseudo-typed assay

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Introduction: Studying survivors of the West African EBOV 2014-16 epidemic provides a unique opportunity to delineate immune responses controlling viral replication. These individuals provide a bank of convalescent plasmas (CP) that can be used to treat future outbreaks. Understanding which immune responses associate with survival will indicate what an effective EBOV vaccine has to induce.

Objectives: We sought to develop an HIV-1 EBOV GP pseudo-typed assay which could be used to analyse antibody (Ab) neutralisation of CP.

Materials and methods: Single-round infectious EBOV GP pseudo-typed viral particles were produced by transfection of an HIV-1 envelope deficient backbone (either pSG3 or pNL4.3 expressing Luciferase) in conjunction with a plasmid expressing the 2015 GEBOV GP envelope into 293T cells. Produced virus was quantified via measuring HIV-1 p24 levels and infection of single-round infectious particles was monitored by measuring luciferase activity within infected cells. The generated viruses were tested for stability and infection on different cell-types, including TZM-bl and Huh7 cells. We utilised our pseudo-typed assay in inhibition assays where limiting dilutions of CP were tested for the capacity to restrict viral entry. A total of forty-five CP samples were selected for analysis, where total EBOV GP IgG binding responses had been characterised using the DABA assay.

Results: CP samples were shown to have the capacity to broadly neutralise EBOV with an inhibitory trend ranging from low to high. Ebola IgG titres were used to select longitudinal CP samples for further characterisation; samples with high IgG titres were shown to neutralise EBOV to a greater capacity as compared to lower titres (P<0.005).

Conclusion: We have developed a sensitive and robust EBOV neutralisation assay that has shown to correlate with total IgG binding. This assay can be used in future characterisation of EBOV Ab responses in both survivors as well as vaccine recipients.
Introduction: Filoviruses are RNA viruses with mortality rates of up to 90%. Pseudotyped viruses (PVs) are chimeric, replication deficient viruses with a core of one virus and the envelope glycoprotein of the virus of interest. Their genome is replaced by a reporter gene (luciferase, GFP) allowing viral entry and serological studies to be conducted in low containment facilities.

Objectives: To produce high titre pseudotyped viruses bearing the receptor glycoproteins of different Ebola and Marburg viruses and to assess their viability after lyophilisation, for transportation to resource limited countries as diagnostic and serological study tools.

Materials and methods: A 3-plasmid system was used to generate PVs by transfection (using various agents) into HEK293T/17 producer cells (in different culture vessels), expressing HIV-1 gag-pol, a luciferase reporter and the envelope glycoprotein of Marburg virus or the Zaire, Sudan and Bundibugyo species of Ebola virus. PVs were titrated by luminometry. Lyophilisation was performed by pre-freezing supernatants in sucrose-DPBS solution at 0.5M before freeze-drying overnight. Dried PV pellets were reconstituted in complete DMEM before re-titration.

Results: High titre, functional PVs were produced yielding from $1 \times 10^8$ Relative Light Units/mL for Ebola virus PVs and $1 \times 10^{10}$ RLU/mL for Marburg virus when using 5 cm dishes or T25 flasks. FugeneHD and PEI transfection agents produced the highest PVs titres. Production of Ebola PVs was also upscaled to T75 flasks. Lyophilisation of Ebola and Marburg pseudotyped viruses resulted in titres being constant when stored at -20°C and 4°C for up to a month, though in PVs kept at 22.5°C, less than 1log10 titre decrease in the same period was observed.

Conclusions: Functional high titre Ebola and Marburg PVs were produced in culture dishes and flasks from different manufacturers. FugeneHD performed better than PEI for Ebola virus, though it may be expensive for resource limited laboratories. Lyophilisation of Ebola and Marburg PVs was accomplished and titres were maintained over a month. Longer time scales are being assessed with a view to robust use as a future serological kit.
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Assessment of exposure and serostatus of contacts persons to Ebolavirus disease cases in guinea (contactebogui study)

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**Question:** The West African Ebola virus disease (EVD) outbreak has resulted in 28616 confirmed or suspected cases. Contacts tracing and quarantine for 21 days were key actions. However, data on exposure to EVD cases, the proportion of antibodies to EBOV and of a- or pauci-symptomatic infection, as well as the presence of virus in body fluids of seropositive contact persons, remain scarce. The study aimed i) to quantify individual risk of exposure of contact persons to EVD cases; ii) to measure the level of antibodies to EBOV; iii) to look for EBOV RNA in semen of adult seropositive men.

**Methods:** Persons living in the same compounds than patients enrolled in a survivors's cohort in Guinea (Postebogui) in Conakry and Forecariah were invited to participate. After consent, a questionnaire detailing exposure to EVD cases was passed to establish a score of exposure and an history of vaccination against EBOV was recalled. Antibodies to GP, NP and VP40 proteins were assessed using Luminex® and EBOV RNA in semen was analysed by PCR.

**Results:** As of April 30, 2017, 1072 individuals were enrolled (54% males, median age 20 years [16-27]). Overall, 29% declared to have been vaccinated and 80% were exposed to less than 3 EVD cases (median =1 [1-2]). Zero or low-risk exposure and 2+ high-risk exposures were estimated at 4%, 59% and 37%, respectively. Complete serology was available for 823 contact persons: 17 (2.0%) had antibodies to GP+NP or GP+NP+VP, and are strongly suspected to have been infected with EBV. In addition, 44 (5.3%) patients had antibodies to NP+VP40 or GP+VP40 and 168 (20%) to a single antigen, GP (n=127, 15.4%), NP (n=23, 2.8%) or VP40 (n=79, 9.6%). An history of vaccination was associated with antibodies to GP (24.7% vs 12.3%, p<10⁻³). There was a trend with exposure, both in NP+VP40 antibodies (p=0.016) and GP+NP antibodies (p=0.08). EBOV RT-PCR was negative in semen of the 11 tested men.

**Conclusion:** Only 2.0% had an antibody profile corresponding to an EVD infection suggesting a-/paucisymptomatic infections but various other antibodies profiles were observed, warranting complementary investigation.