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POSTER ABSTRACT BOOK

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National Collection of Pathogenic Viruses: A Repository for Well Characterised and Authenticated Viruses

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Abstract

The National Collection of Pathogenic Viruses (NCPV) is one of four repositories making up Public Health England Culture Collections. It was established two decades ago to offer safe and secure facilities for storage of patents and deposits as well as to supply standardised virus products to the wider scientific community. Within the collection are strains of both medical and veterinary importance, with representation from a wide variety of virus families, classified as Hazard Groups 2, 3 and 4 by the Advisory Committee for Dangerous Pathogens (ACDP). These include arboviruses such as Dengue, Yellow-fever, Oropouche and Sandfly fever Naples.

Throughout the year, the collection receives virus deposits. The depositing process is free and simple. The quality of every virus material received in the collection is assessed through a series of tests to confirm viability and absence of microbial contamination (including mycoplasmas). Virus identity is confirmed by nucleic acid profiling. Once standards are met, the virus products are made available to the wider scientific community. In addition to facilitating access to viruses and their associated products as well as secure storage for patents, NCPV also undertakes collaborative projects and contract research/developmental work with academic and commercial partners, by providing expertise in the handling of viral pathogens.

A repository for well-characterised, authenticated viruses is a useful resource for the scientific community, aiding in the understanding of circulating viral pathogens and providing access to quality-assured reference reagents for the identification and development of vaccines and therapeutics in the event of global viral outbreaks.

Application of Next Generation Phage Display technology to identify peptides that can be used to develop a new serological assay for louping ill virus

2

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Abstract

Louping ill virus (LIV) is a UK endemic flavivirus vectored by *Ixodes ricinus* ticks. LIV mainly affects sheep and red grouse usually with a fatal outcome in the latter. The disease in sheep was controlled by a vaccine that has recently became unavailable. This is likely to lead to a rise in new cases and economical loss, especially in lambs who would have been protected by maternal antibodies. There is a need for a rapid diagnostic test as current serology diagnostics require CL3 facilities and are time consuming.

Next Generation Phage Display is a technique that combines classic phage display with next generation sequencing. The main advantage of phage display is the linking of the phenotype (peptide binding properties) with genotype (the peptide gene displayed by the phagemid). Serum antibodies can be used to select specifically binding phage.

Serum from LIV infected and uninfected sheep have been biopanned against P8 linear random peptide phage library to generate a phage sub-library specific for LIV antibodies. The phage genomes will be sequenced using Ion Torrent sequencing and antigenic peptide regions ranked using Z-score analysis. The most antigenically potent peptides will be used to create a rapid diagnostic assay such as an ELISA.

DENV-captured plasmin enhances mosquito midgut infection and is inhibited by an endogenous Kazal-type inhibitor AaTI

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Abstract

Deciphering how dengue pathogenesis influences vector transmission will improve our understanding on virulence evolution, epidemiology and design of transmission-blocking tools. Here, we demonstrate that addition to blood meal of plasmin, the human fibrinolytic factor, increased permeability and DENV infectivity in mosquito midgut, resulting in higher infection rate and dissemination in whole mosquitoes. Further, we show that a plasmin-selective mosquito Kazal-type protease inhibitor, AaTI reverted this enhanced infection by inhibiting proteolysis. We also determined that DENV or E-protein, plasmin (not plasminogen) and AaTI can interact to form a tripartite complex using biolayer interferometry, suggesting physical interaction between DENV E-protein and kringle domain of plasmin. Our study suggests that (a) DENV recruit plasmin in solution to increase local proteolytic activity in midgut, thus enhancing DENV infection and (b) AaTI can act as a transmission-blocking agent, which could also alleviate hemorrhagic patients. By discovering that dengue pathogenesis can enhance DENV fitness by increasing mosquito infectivity, our results provide the first evidence of mosquito-based evolutionary pressures on dengue virulence in human. We are currently determining the effect of plasmin and AaTI on DENV infection in cellular and mouse models. H/D exchange mass spectrometry is being employed to identify which kringle domain of plasmin is interacting with DENV E-protein. The structure of the complex formed between the kringle domain of plasmin and DENV E-protein and between the catalytic domain of plasmin and AaTI are in progress.

Pan-antivirals to combat re-emerging alphaviruses

4

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Abstract

Mosquito-transmitted alphaviruses are distributed globally and include human pathogens that can cause severe long-term arthritogenic or neurological complications. There are currently no market-approved antivirals or vaccines to treat or prevent these infections. Outbreaks are difficult to predict as they can emerge spontaneously in susceptible human populations. Furthermore, it is challenging to determine the causative pathogen due to great similarities in clinical features of alphavirus-associated diseases. Therefore, drugs with broad-spectrum anti-alphavirus activity could serve as a fast first-line therapy in case of an outbreak. We have performed high-throughput screenings of broad-chemical space libraries and identified a series of small molecules with antiviral activity against different chikungunya virus lineages. Interestingly, we demonstrated that this series exerts broad-spectrum anti-alphavirus activity against a range of arthritogenic alphaviruses. Time-of-addition studies showed that this series has *in vitro* antiviral activity early in the viral RNA replication stage. Furthermore, these molecules were not cross-resistant with favipiravir-resistant chikungunya virus, a compound that inhibits the viral RNA polymerase. To investigate the mechanism of action in more detail, *in vitro* resistance selection is currently ongoing. Elucidation of the specific antiviral target of this newly identified series could reveal a new target in the alphavirus proteome, which could be valuable for the development of broad-spectrum anti-alphavirus drugs.

Pre-clinical protective efficacy of a virus-like particle-based vaccine against Zika virus

5

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Abstract

Zika virus is a mosquito-borne flavivirus which has recently caused outbreak in Africa, Americas and Asia. Severe neurological symptoms following Zika virus infection were reported. Currently, there is no specific treatment for Zika virus infection. Hence an effective Zika virus vaccine is urgently needed.

In this study, a subunit protein of Zika virus (cvD-ZIKV-sE) and a Zika virus-like particle (cvD-ZIKVLP) were studied for their ability to induce protective antibody. The two vaccine candidates were designed and engineered to express class of neutralising epitopes presented on dimeric envelope protein of Zika virus to animal immune system. Both candidates induced high level of antibody after three immunisation in immunodeficient A129KO mice. Mouse sera were shown to neutralise Zika virus *in vitro*. *In vivo* challenge test, in comparison with their wild-type antigens, showed that vaccinated mice survived through Zika virus infection where mice receiving mock immunisation exhibited clinical symptoms or died 8 days post-challenge. Serum viral load post-challenge further confirmed protection of the two engineered antigens compared to their wild-types and placebo. ADE test against panel of flaviviruses in K562 cells also indicated superiority of the designed candidates.

In conclusion, specially designed candidates (cvD-ZIKV-sE and cvD-ZIKVLP) showed their ability to induce protective immunity and protect mice from Zika virus infection inferring possibility of Zika virus vaccine development.

Species-specific restriction of Bluetongue virus replication correlates to host resilience

6

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Abstract

Bluetongue is a vector-borne disease of ruminants caused by bluetongue virus (BTV). BTV can infect essentially all domestic and wild ruminants but the clinical outcome of infection differs substantially between host species. Clinical disease induced by BTV, including haemorrhagic fever in severe cases, is normally evident only in sheep. Conversely, cattle are more resilient to BTV infection, as they develop high levels of viremia, but rarely show clinical signs. Here, we concentrated on BTV-host cell interactions using primary cells as an experimental system. First, we determined that BTV reaches higher titres in ovine cells, compared to bovine cells although it induces comparable levels of antiviral cytokines in both cell types. Importantly, these differences are abolished by inhibiting the Jak/Stat pathway. In addition, pre-treatment with interferon (IFN) severely hampers BTV replication in bovine, but not in ovine, primary cells. These data suggest that bovine, unlike ovine, IFN-stimulated genes (ISGs) are effective in controlling BTV replication. Using a high-throughput flow cytometry approach, we screened an expression library of bovine ISGs to identify genes with antiviral properties against BTV. We have successfully identified a subset of bovine ISGs which negatively impact BTV replication. We are currently investigating the ovine orthologues to the bovine ISGs with anti-BTV properties. Interestingly, the sheep ISGs showed either similar or decreased levels of restriction compared to their bovine counterparts. Our study provides an intellectual framework to better understand how host susceptibility and resilience to arbovirus infection correlates to species-specific differences of the type-I IFN response.

Flavivirus NS5 proteins – different inhibitory effects on innate immune pathways

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Abstract

Flaviviruses encode several nonstructural (NS) proteins of which NS5 proteins act as a RNA-dependent RNA-polymerase and methyl transferase. Despite the differences at amino acid level and sub-cellular localization, the functions of NS5 proteins are similar. Previously, many flavivirus NS5 proteins have been shown to inhibit innate immunity, but the data is inconclusive. We have analyzed the potential of flavivirus NS5 proteins to interfere with the RIG-I-induced interferon (IFN) promoter activation and IFN-a/b/l -induced Jak-STAT pathways.

HEK293 cells were transfected with expression plasmids for ZIKV, DENV1, TBEV, WNV, JEV and YFV NS5 proteins. The cells were co-transfected with luciferase constructs for IFN-l1 as well as IFN-inducible MxA1, Viperin, IFITM1 and IFITM3 promoters. RIG-I pathway, leading to activation of IFN-l1, was induced with constitutively active form of RIG-I. IFN-alpha1 was used to activate IFN-inducible promoters. Promoter activities were measured with a luciferase assay. The phosphorylation of IRF3 was analyzed by immunoblotting and the sub-cellular localization of NS5 proteins by immunofluorescense.

Sub-cellular localization of the NS5 proteins differed, DENV and ZIKV NS5 were nuclear and others mainly cytoplasmic. RIG-I pathway was strongly inhibited by ZIKV and YFV NS5, but not significantly by JEV, WNV, DENV and TBE NS5 proteins. IFN-induced pathways (MxA1 promoter activation) were strongly inhibited by ZIKV, DENV and TBE NS5 proteins. Results of the effect of NS5 proteins on IRF3 phosphorylation and other IFN-induced promoters will be presented.

NS5 proteins of flaviviruses act differently on innate immune pathways. The mechanisms of actions will be further studied.

Saving Private Toscana

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Abstract

Toscana virus (TOSV, *Phenuiviridae*, *Phlebovirus*), is largely distributed over the Mediterranean basin. It was first isolated in Italy from phlebotomine sandflies in 1971. In humans, TOSV causes febrile illness, which can evolve to meningoencephalitis in severe cases. Despite a constant expansion in the region, TOSV remains poorly characterized at the molecular level. Mutagenesis-based studies are however a prerequisite to better understand the cell biology of TOSV, from both virus-host cell interactions and replication perspectives. Here, we engineered a T7 RNA polymerase-driven reverse genetics system to recover infectious particles of TOSV lineage B strain H4906 (France, 2004). Our results show that the rescued virus (rTOSV) forms plaques in titration assays similar to those of our TOSV lab strain and is stable over more than 4 passages. When viral particles were amplified, semi-purified and analyzed by Coomassie, the typical bands corresponding to the virus envelope glycoproteins (G_N and G_C) and nucleoprotein were observed. Immunoblot against G_N, G_C, the nucleoprotein N, and the non-structural protein NSs expressed in cells exposed to the virus confirmed the TOSV infection. We then introduced random stop codons in the sequence coding for TOSV NSs and were able to rescue a rTOSV mutant impaired for NSs expression. Altogether, our study is the first demonstration that TOSV can be genetically engineered and recovered from plasmid DNAs. Our data therefore open a new avenue of research on this neglected emerging pathogen that represents a growing threat for human health in the Mediterranean region and neighboring countries.

Louping Ill Virus Outbreak in North-East Wales

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Abstract

The disease louping ill is an encephalitic viral infection that is fatal in sheep (*Oves aries*) and grouse (*Lagopus lagopus*). The disease is caused by the louping ill virus (LIV) and transmitted by sheep ticks (*Ixodes ricinus*) in upland areas of the United Kingdom. Reported outbreaks are sporadic and prevention is mainly targeted at controlling the vector. Despite the implications for animal welfare and the rural economy, research on the epidemiology and control of LIV has been neglected in recent years. In April of 2019, a group of 200 yearling ewes were moved onto hill grazing at a farm near Oswestry on the English/Welsh border. Within 2-3 weeks, ten had developed clinical signs suggestive of neurological impairment, including torticollis, fitting, head shaking and recumbency. A number of the affected ewes were later found dead. Following post mortem, histopathological investigation of brain tissue from an affected ewe, which had undergone euthanasia, detected glial nodules and perivascular cuffing indicating subacute non-suppurative encephalitis. The haemagglutination inhibition serological test was positive and provided evidence for infection with LIV. Reverse transcription polymerase chain reaction (RT-PCR) gave a positive result for samples of the hind brain and the resulting sequence confirmed the presence of LIV. Phylogenetic analysis indicated that the virus showed the highest identity (>99%) with LIV sequences from Aberystwyth in west Wales and was distinct from other LIV isolates found in the north of England. This case study highlights the ongoing threat to UK sheep from LIV.

Magnetic resonance imaging findings in Powasan virus, West Nile virus, Nipah virus, and Hendra virus encephaliditides.

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Abstract

Background-Powassan virus (POWV) is a tick-borne flavivirus that can cause encephalitis in North America and Northeast Asia. Here we compare magnetic resonance imaging (MRI) findings of POWV encephalitis with those caused by West Nile virus (WNV), Nipah virus (NiV), and Hendra virus (HeV).

Methods– We abstracted MRI findings of cases of POWV or WNV encephalitis seen at our institution and include previously published descriptions of MRI findings from the literature of POWV, WNV, NiV, and HeV to compare findings between these viral encephalidities and correlate these findings with differences in symptoms and mortality rates.

Results– We examined a total of 13 cases of POWV, 57 cases of WNV, 31 cases of NiV, and 3 cases of HeV encephalitis. In POWV cases, 15.4% had normal MRI findings while 84.6% demonstrated deep foci of increased T2/FLAIR signal intensity. In WNV cases, 43.9% had normal MRI findings, 12.3% had white matter only lesions, 36.8% had T2/FLAIR 11 deep gray matter/brain stem with a minority also including white matter lesions, and 7% had only meningeal involvement without encephalitis. In NiV cases, 100% had discrete lesions throughout the subcortical and deep white matter of the cerebral hemispheres on T2/FLAIR imaging. In HeV cases, 100% had cortical white matter involvement, while 66.7% progressed to have deep white matter involvement and both of these cases became fatal.

Conclusion– Differences in the pattern of lesions occurred between the different vial encephalitides examined. In general, more extensive and/or progressive lesions were associated with increased morbidity and mortality.

Dengue Virus Non-Structural Protein 1 Disrupts the Interaction Between Endothelial Cells and Pericytes Causing Hyperpermeability

11

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Abstract

High levels of the secreted non-structural protein 1 (NS1) have been shown to contribute to dengue haemorrhagic fever (DHF), via multiple mechanisms affecting endothelial cells. However, previous studies have not considered the potential role of pericytes, which are perivascular cells wrapped around the endothelial monolayer that tightly regulate endothelial barrier integrity. Dysfunction of pericytes contributes to other haemorrhagic pathologies, such as diabetes retinopathy. This study aims to investigate whether interactions between endothelial cells and pericytes are affected by NS1. Transepithelial electrical resistance (TEER), an in vitro measure of permeability, was strongly affected by the addition of NS1 and the effect was markedly increased when endothelial cells were grown in co-culture with pericytes, compared to endothelial cells cultured alone. This suggests that NS1 impacts endothelial cell-pericyte interactions, amplifying its direct effect on endothelial cells alone. In a 3D angiogenesis assay, the co-culture of endothelial cells and pericytes increases the angiogenic potential of endothelial cells. Upon NS1 treatment this effect was blunted, as measured by the reduction of the width of vascular tubules formed. Importantly, these effects were observed at lower and more patient-relevant concentrations of NS1 than in the absence of pericytes. The observed results were not dependent upon cell viability, as AlamarBlue assay showed no significant change in pericyte or endothelial cell proliferation upon NS1 treatment. We conclude that pericytes play a significant role in maintaining endothelial monolayer integrity that is disrupted by NS1 during dengue-associated vascular hyperpermeability. Unravelling this pathway could provide new targets for dengue diagnosis and treatment.

The Using of Serum Free Media for the Production of J93-463-1-16-10 Monoclonal Antibody against Japanese encephalitis virus

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Abstract

The development of J93-463-1-16-10 cell culture by sequential adaptation method in serum-free media of Hybridoma-SFM medium and CD Hybridoma medium to produce the monoclonal antibody specific to *Japanese encephalitis virus* was shown that the viabilities of J93-463-1-16-10 were more than 70%. Then the supernatant from the cell culture was purified after precipitation with ammonium sulfate until the final concentration was 50% in order to separate the IgG. After that, the protein concentrations were measured by using the Bradford assay. Next, the proteins were separated, which made each protein purer with SDS-PAGE method in order to find the molecular weights of the separated proteins. The putative IgG and its heavy chains and light chains were divided in two sizes which were approximate 23-25 kDa and 50-53 kDa respectively. Later, the type of immunoglobulin will be identified by the Matrix-assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) method and its antibody characteristic to *Japanese encephalitis virus* which will be done further.

In the future, a monoclonal antibody in this research could be used to study the expression of the envelope protein of *Japanese encephalitis virus* in green cos and red cos, and to develop the vaccine for *Japanese encephalitis virus* in swine.

Keywords: *Japanese encephalitis virus*, J93-463-1-16-10, serum-free media, MALDI-TOF

The Cryopreservation of Cos Lettuce Callus by Using Liquid Nitrogen

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Abstract

The cryopreservation of cos lettuce (CL) callus by using liquid nitrogen (LN) was further study from the previous research on callus induction and micropropagation of CL. That was studied on seed culture and micropropagation of CL and its embryogenic growth. The result was shown that the most suitable Murashing and Skoog (MS) medium concentration was in 1/2 MS. The medium supplemented with 0.1 mg/l of 6-benzylaminopurine (BAP) and 0.5 mg/l of 2,4-dichlorophenoxyacetic acid (2,4-D) gave the best for leaf inducing callus. Then it was brought to study the effects of cryoprotectants with the different percentages of dimethyl sulfoxide (DMSO). The cryopreserved callus by using LN was brought to subculture for the best inducing shoot in MS medium supplemented with 4.0 mg/l of BAP. The results that the best cryoprotectant at the percentage of DMSO at 7.5 gave the highest average number of shoots at 2.1 shoots/culture and the average shoot length was 1.56 cm. The MS medium supplemented with 0.5 mg/l of 1-Naphthaleneacetic acid was the best inducing root. The results that the best cryoprotectant at the percentage of DMSO at 7.5 gave the highest average number of roots at 9.7 roots/culture and the average root length was 1.59 cm. Plant differentiation from callus of CL leaf was transferred to grow in soil and had the same morphology as normal CL that was grown in soil by seed germination.

CL will be the host of Japanese encephalitis virus expression for the developmental swine vaccine.

Toolkit for the study of yellow fever virus

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Abstract

Yellow fever virus (YFV) is a dangerous re-emerging mosquito borne flavivirus with a high lethality, which causes untreatable haemorrhagic fever in humans. Important disadvantages regarding safety and limited vaccine supply have been identified in the existing live attenuated vaccine. Alternative safer therapeutic strategies are needed to reduce the lethality of infections and protect a wider group of people. To this point, we created an array of essential tools for the study of YFV. We designed a novel YFV reporter virus to develop a highly sensitive high throughput neutralisation assay (Z'=0.65), which can screen virus inhibitors three times faster than the classical plaque assay. Moreover, we designed a site-specific biotinylated soluble YF envelope (E) protein by a cloning strategy involving the use of a biotin acceptor peptide and *E. coli* Biotin-protein birA ligase. The biotinylated E protein was used to develop a sensitive ELISA to screen antibodies that bind to the YFV E protein. Furthermore, we immunised mice with a soluble YFV E protein and generated anti-YFV E IgG secreting hybridoma cell lines. Monoclonal antibodies secreted by hybridomas were purified by affinity chromatography and are being characterised. The developed antibodies against YF E protein are a crucial tool for the molecular study of YF. Additionally, neutralising antibodies against YFV could potentially be developed into the first therapeutic treatment against YF infection. The toolkit developed in this project includes a neutralisation assay, a binding ELISA, and anti YFV E antibodies. These are essential instruments to expand the knowledge of YFV.

Sheep in wolves' clothing – benign mosquito-specific virus chimeras present authentic antigens of pathogenic flaviviruses for diagnostic and vaccine applications

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Abstract

Mosquito-borne flaviviruses cause a significant global health burden and have potential for pandemic emergence. Upscaling of vaccine and diagnostic capabilities during outbreaks can be slow and expensive, especially for developing countries. Here we describe a recombinant platform for the rapid manufacture of chimeric virus particles for a spectrum of major pathogenic flaviviruses using a new lineage II insect-specific flavivirus, named Binjari virus (BinJV). BinJV was found to be remarkably tolerant for exchange of its structural protein genes (prME) with those of pathogenic vertebrate-infecting flaviviruses (VIFs), including Zika, (ZIKV), dengue (DENV) and West Nile (WNV) viruses. To date, we have successfully produced different BinJV chimeras displaying the structural proteins of >25 VIFs. These chimeric BinJ/VIF-prME viruses are completely replication-deficient in vertebrate cells, but replicate with very high efficiency in mosquito cells (up to $10^{9.5}$ $CCID_{50}$/mL), a true sheep in wolves' clothing, to provide a safe and rapid means of antigen production. Structure reconstruction of chimeric ZIKV particles to 7.1 Å, as well as affinity binding profiling with monoclonal antibodies using BinJ/ZIKV-prME, BinJ/DENV-prME and BinJ/WNV-prME chimeric particles, revealed that the chimeric virions are structurally and antigenically indistinguishable from the parental pathogenic flaviviruses. BinJ/VIF-prME viruses showed utility in diagnostic (microsphere immunoassays, ELISAs and virus neutralization assays) and vaccine applications (protection against Zika virus challenge in murine models). BinJ/VIF-prME viruses thus represent a simple, non-infectious (to vertebrate cells), high-yield technology for generating chimeric flavivirus particles with low biocontainment requirements.

The Expression of Japanese Encephalitis Virus Envelope Gene in Green Cos 16

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Abstract

The expression of Japanese encephalitis virus (JEV) envelope gene in green cos leaf was a project undertaken at the John Innes Centre during 6 September to 20 October 2018 and is one of the projects of the research training program at the John Innes Centre during long vacation every year since 2010 until now.

The summary of this research is as follows. The production of the envelope protein of the JEV in green cos by using the envelope gene expression by 8 epitopes (MEP: multiepitope) of Japanese encephalitis virus vaccine strain SA14-14-2 that provides the highest immune response against JEV. After incorporated the envelope gene into pEAQ-HT and pEAQ-HT-HBcAg-tEL vectors, we cloned genes in *Escherichia coli* and *Agrobacterium tumefaciens*, respectively, then expressed the envelope protein of JEV in green cos leaves. The result can be seen in photographs of green cos leaves by both visible light camera and ultraviolet light camera.

Further research should include the analysis and identification by chemiluminescence immunoassay and by the Matrix-assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF). Further purification of the envelope protein expression of JEV in green cos that will be benefited for the production of the envelope protein of the JEV plant-based vaccine.

Key words: *Japanese encephalitis virus*, plant-based vaccine, chemiluminescence immunoassay, MALDI-TOF

Zika virus infection of glia leads to secondary injury to axons and dendrites

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Abstract

Zika virus infection was recently linked to microcephaly and peripheral neuropathy (GBS) in Zika virus epidemic areas. Building on our previous work (Cumberworth *et al,* 2017) we investigated, in a time-course study, how the viral infection and the injury of cell processes of oligodendrocytes and neurons are related to each other in the same *in vitro* model.

We generated CNS myelinating cultures from a reporter mouse (*Thy1-YFP*) on the *Ifnar1* -/- background. A proportion of neurons and their processes are positive for YFP in those cultures, which enabled us to visualise single neurons. Cultures were infected with the Brazilian Zika virus strain (PE243) at an MOI of 0.3 and cultured for up to 6 days post infection. We observed that the neuronal cell processes were affected as early as the appearance of the first clusters of infected glial cells.

To analyse the interrelation of neurons and myelin further, cultures were labeled with an antibody recognising proteolipid protein. We found that the myelin got injured as early as neuronal processes. These results suggest that the injury to neuronal processes might be a consequence of the infection of the primary target of Zika virus: oligodendroglia.

These data help us to understand disease pathogenesis of Zika virus infection of the CNS, and whether there is a time-window to intervene therapeutically. Furthermore, this gives an insight as to how viral infection of glial cells can affect neuronal processes such as axons.

Of Mice and Monkeys: Determining Protective Serological Titres in Model Zika Virus Infections

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Abstract

Developmental vaccines against emerging pathogens face many hurdles including determining what protective level of serological responses must be generated. Knowledge of a likely protective titre is critical where human challenge studies are not possible.

We have used an anti-zika plasma pool from convalescent patients (candidate serological reference reagent NIBSC16/320-14), infused into cynomolgus macaques and Type-1 IFN deficient mice to determine likely protective neutralising titres.

Anti-zika plasma was administered to a group of 4 macaques (single concentration) and groups of 8 A129 mice (4 group titration series) 24 hours prior to sub-cutaneous challenge with Zika virus PRVABC59. Plasma/sera samples were collected at regular intervals to track peripheral viremia and anti-zika antibody responses. FFPE tissues were collected at termination for histological analysis.

Immediately prior to challenge, human IgG was detectable in all infused animals. Within macaques the NT_{50} at this time was 250. All macaques that received plasma were protected against zika virus infection as determined by plasma/tissue qRT-PCR and IgM responses.

Titration within A129 mice gave a neutralisation titre of 110, above which mice were generally protected against zika infection. However this was not absolute as a small number of mice with high neutralisation levels were infected.

A protective NT50 of 250 has been identified for macaques and this further titrated in A129 mice to give a guide protective neutralisation titre of 110. The lack of protection in some mice with higher titres is currently unclear and studies are underway to compare their infection pathology with that of controls.

Lumpy skin disease virus: transmission to dipteran vectors using animal and ex-vivo models.

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Abstract

Lumpy skin disease (LSD) is a tropical neglected viral disease of cattle, characterised by numerous cutaneous lesions disseminated throughout the body. Historically endemic to the African continent, it has become a threat to Europe following the outbreaks of LSD in the Middle East and Eastern Europe.

LSD virus (LSDV) is a *Capripoxvirus* transmitted by insect vectors. Experimental and epidemiological studies have indicated a role for the stable fly (*Stomoxys calcintrans*) and the mosquito *Aedes aegypti*. Nevertheless the relative importance of these vector species and others is unclear. A study was designed to explore the risk of transmission of LSDV from cattle to different vector species including *Aedes aegypti*, *Culex quinquefasciatus*, *Stomoxys calcitrans* and *Culicoides nubeculosus*. Cattle was challenged with LSDV to produce a bovine experimental model used as a natural source of LSDV to the potential vectors. Cattle samples were taken to quantify LSDV in different tissues and characterise the disease. All insect species were allowed to feed on LSDV-challenged cattle at regular intervals and incubated for up to eight days. This data was then used to model the dynamics of LSDV infection and transmission. All four species were able to acquire and maintain LSDV for up to eight days post feeding, and the risk of transmission from bovine donor to insect was dependent on the severity of the disease. A model was then generated using ex-vivo skin lesions and infectious blood that will allow further studies of the role of these vectors.

Exposure to ultraviolet light modulates host susceptibility to arbovirus infection

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Abstract

Medically important viruses spread by arthropods (arboviruses) infect millions of people each year and include dengue (DENV), Zika (ZIKV) and chikungunya (CHIKV) viruses, for which the day-biting Aedes aegypti mosquito is the primary vector. The clinical course of these diseases is highly variable and unpredictable, ranging from asymptomatic to those that are severe and life-threatening. Factors that predispose the host to more severe arbovirus disease are poorly understood, but are most likely due to a combination of viral, host and environmental factors. One such factor that is pivotal in defining the outcome of infection is the activation of immune responses at the inoculation site, where mosquitoes deposit virus during biting. We have studied how one important environmental factor that influences these responses; exposure to ultraviolet (UV) light from the sun.

Infections are more common in regions that have high levels of ambient UV. However, the level of sun exposure that individuals receive varies considerably, as does their tolerance to this exposure. We hypothesised that prior exposure of the inoculation site to UV may modulate activation of inflammatory pathways to either the virus and/or mosquito-derived factors, and thereby alter host susceptibility to infection. We found that prior acute erythemal exposure to UV (sunburn) increased host susceptibility to arbovirus infection. In contrast, repeated prior non-burning exposure of UV protected from arbovirus infection in a type I IFN-dependent manner. We suggest that the ability of UV to modulate skin inflammatory responses underlie the observed changes in host susceptibility.

First report of L1014F-*kdr* mutation in *Culex pipiens* complex from Morocco

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Abstract

Background: The intensive application of chemical insecticides led to the development of resistance in many insects including *Cx. pipiens* mosquitoes. The absence of data on resistance mechanisms in Morocco allow us to assess the levels of lambdacyhalothrin resistance and the frequency of the mutated gene L1014F *kdr* in different forms of this complex from three regions of Morocco.

Methods: Mosquito adults were reared from immature stages collected in Tangier, Casablanca and Marrakech (Morocco). Standard WHO insecticide susceptibility tests were conducted on adults emerged from collected larvae. Identified mosquitoes were then tested for the presence of the L1014F *kdr* mutation using PCR assay.

Results: Our results showed that 21% of the tested population has a resistance to lambda-cyhalothrin. The molecular identification of survivors shows that 43% belonged to the *Cx. pipiens pipiens* and only 9.5% to the *Cx. pipiens molestus* form. On the other hand, 416 specimens were screened for the L1014F *kdr* mutation. L1014F mutation was detected in different forms of *Cx. pipiens* in different sites. The frequency of L1014F mutation was similar between *pipiens* form and hybrid form, while it was lower in *molestus* form. The presence of the L1014F *kdr* allele was significantly associated with resistance to lambdacyhalothrin in *pipiens* ($P < 0.0001$) and hybrid form ($P < 0.0001$).

Conclusion: Resistance to lambdacyhalothrin of *Cx. pipiens* populations appears to be largely due to the L1014F *kdr* mutation. The frequencies of this mutation are examined for the first time in natural populations of the *Cx. pipiens* complex in Morocco.

G119S *ace-1* mutation conferring insecticide resistance detected in the *Culex pipiens* complex in Morocco

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Abstract

BACKGROUND: Arboviruses are controlled through insecticide control of their mosquito vector. However, inconsiderate use of insecticides often results in the selection of resistance in treated populations, so that monitoring is required to optimize their usage. Here, *Culex pipiens* (West Nile and Rift Valley Fever virus vector) specimens were collected from four Moroccan cities. Levels of susceptibility to the organophosphate (OP) insecticide malathion were assessed using World Health Organization (WHO)-recommended bioassays. Individual mosquitoes were tested for the presence of the G119S mutation in the *ace-1* gene, the main OP-target resistance mutation.

RESULTS: Bioassays showed that mosquitoes from Mohammedia were significantly more resistant to malathion than those from Marrakech. Analyzing the *ace-1* genotypes in dead and surviving individuals suggested that other resistance mechanisms may be present in Mohammedia. The *ace-1* resistance allele frequencies were relatively moderate (< 0.4). Their analyses in three Moroccan cities (Tangier, Casablanca and Marrakech) however showed disparities between two coexisting *Cx. pipiens* forms and revealed that the G119S mutation tends to be more frequent in urban than in rural collection sites.

CONCLUSION: These findings provide a reference assessment of OP resistance in Morocco and should help the health authorities to develop informed and sustainable vector control programs.

A salivary protein of Aedes aegypti promotes dengue-2 virus replication and transmission

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Abstract

Although dengue is the most prevalent arthropod-borne viral disease in humans, no effective medication or vaccine is presently available. Previous studies suggested that mosquito salivary proteins influence infection by the dengue virus (DENV) in the mammalian host. However, the effects of salivary proteins on DENV replication within the *Aedes aegypti* mosquito remain largely unknown. In this study, we investigated the effect of a specific salivary protein (named AaSG34) on DENV serotype 2 (DENV2) replication and transmission. We showed that transcripts of AaSG34 were upregulated in the salivary glands of *Aedes aegypti* mosquitoes after a meal of blood infected with DENV2. Transcripts of the dengue viral genome and envelop protein in the salivary glands were significantly diminished after an infectious blood meal when AaSG34 was silenced. The effect of AaSG34 on DENV2 transmission was investigated in *Stat1*-deficient mice. The intradermal inoculation of infectious mosquito saliva induced hemorrhaging in the *Stat1*-deficient mice; however, saliva from the AaSG34-silenced mosquitoes did not induce hemorrhaging, suggesting that AaSG34 enhances DENV2 transmission. This is the first report to demonstrate that the protein AaSG34 promotes DENV2 replication in mosquito salivary glands and enhances the transmission of the virus to the mammalian host.

Discovery of novel endogenous viral elements in Aedes spp. mosquitoes

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Abstract

The genomes of *Aedes* spp. mosquitoes contain integrated sequences from nonretroviral RNA viruses that are enriched in piRNA clusters, are embedded next to transposable elements (TEs) and produce piRNAs. The parallelism between TEs and viral integrations led to the hypothesis that viral integrations may constitute an archive of past viral infections and potentially have an immunity impact on novel infection with cognate viruses, similarly to how the piRNA pathway interacts with TEs. A corollary of this hypothesis is that the landscape of viral integrations should be variable across populations depending on their viral exposure. The highly repetitive nature of *Aedes* spp. genomes make the discovery of viral integrations from whole genome sequencing data of wild mosquitoes a daunting task.

Here we describe a novel bioinformatic pipeline to rigorously identify viral integrations using Next Generation Sequencing (NGS) data. Libraries from single or pools of mosquitoes, reference genome statistics, the landscape of TEs and the geographic origin of the analyzed samples are the actors of the analysis.

This pipeline has been tested in *Ae. aegypti* and *Ae. albopictus* mosquitoes, allowing to compare the performance of the analyses on genome assemblies of different completeness. We identified novel viral integrations in both genomes. Additionally, we show that the landscape of viral integrations is dynamic, with a population-specific behavior that we can leverage to formulate hypothesis on mechanisms of integration and the biological role of viral integrations.

Biotyping of TBEV-infected IRE/CTVM19 tick cell line

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Abstract

Background

Ticks have developed defense mechanisms and pathways against transmitted infections, including tick-borne encephalitis virus (TBEV). An important issue is to reveal mechanisms allowing them to control the virus at a level which does not hinder ticks' fitness and development.

Methods

Biotyping was performed on an Autoflex Speed MALDI-TOF/TOF (Bruker Daltonik). Protein digests were analyzed using Synapt G2-Si High Definition mass spectrometer (Waters).

Results

MS profiles of TBEV-infected and non-infected IRE/CTVM19 cells were analyzed using principal component analysis. Obtained spectra were clustered based on the cultivation time, but not the infection status. Nevertheless, analysis of loading plots revealed different factors to be important for clustering of infected and non-infected cells. Out of them, nine were assigned with proteins: five and four for non-infected and infected cells, respectively. Peak with m/z 8565 was found to be of interest from viewpoint of tick-virus interaction and assigned to proteasome subunit alpha type (B7QE67)

Conclusion

MALDI-TOF MS was shown to be useful for characterization of tick cell lines and studying tick-virus interactions. Signals in MS profiles discriminating cell aging and those affected by TBEV were revealed, and matched with proteins.

We thank Dr Lesley Bell-Sakyi and the Tick Cell Biobank for provision of IRE/CTVM19 cells.

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Karyotype changes in cultivated tick cell lines

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Abstract

Background. Tick cell lines are an easy-to-handle system for the study of viral and bacterial infections and different aspects of tick physiology. However, long-term cultivation of tick cells can influence genome stability. **The aim** of our work was to analyze and compare the karyotypes of *Ixodes scapularis*, *I. ricinus*, and *Ornithodoros moubata* tick cell lines after long-term been in culture. **Methods.** Mitotic spreads were prepared to count the number of chromosomes in ISE6, ISE18, IRE11, IRE/CTVM19, IRE/CTVM20, and OME/CTVM22 cell lines. The genome size of tick cell cultures was estimated by flow cytometry using propidium iodide staining. **Results.** The modal chromosome numbers around 22 and around 48 were typical for both *I. ricinus* and *I. scapularis* cell lines and differed from the diploid chromosome number in *Ixodes* ticks – 28. In the OME/CTVM22 cell line, the modal chromosome number was 33, instead of 20 in *Ornithodoros* ticks. All tick cell lines had a larger genome size in compare to genomes of parental ticks. **Conclusions.** Tick cell lines can be used for research purposes, however, differences in the internal processes between different cell populations should be taken into account.

Acknowledgments. We thank Lesley Bell-Sakyi and the Tick Cell Biobank for provision of the tick cell lines.

Characterization of PIWI proteins and viral piRNA biogenesis in the mosquito vector, *Aedes albopictus*

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Abstract

Multiple arboviruses are transmitted by *Aedes* mosquitoes, where the siRNA pathway is the chief mediator of antiviral immunity. Recently, the PIWI-interacting RNA (piRNA) pathway was also shown to have a similar role. As compared to 3 PIWI-family proteins in *Drosophila*, the PIWI gene family has undergone expansion to 8 proteins (PIWI 1-7 and Argonaute 3) in *Aedes* mosquitoes. Earlier, PIWI5 and Ago3 were shown to be responsible for biogenesis of viral piRNAs (vpiRNAs) in *Ae. aegypti*. We sought to elucidate piRNA biogenesis and PIWI dependence in another important mosquito vector, *Ae. albopictus*. Using the recently annotated *Ae. albopictus* genome, orthologs corresponding to the *Ae. aegypti* PIWI proteins were identified. Surprisingly, the *Ae. albopictus* genome possesses multiple seemingly redundant copies of Ago3, PIWI4, PIWI5 and PIWI7. PIWI protein knockdown in *Ae. albopictus* U4.4 cells through dsRNA-mediated silencing revealed that production of Sindbis virus (SINV)-derived vpiRNAs is also dependent on PIWI5 and Ago3. Unlike that observed for *Ae. aegypti*-derived Aag2 cells, no antiviral effect of PIWI4 knockdown was seen against two different alphaviruses. *Drosophila* CRISPR-Cas9 reagents were optimized for use in U4.4 cells and functional knockouts were generated for PIWI5 and PIWI6. However, knocking out either Ago3 or PIWI4 seemed to be lethal. In the context of a PIWI5 knockout, vpiRNAs derived from SINV or dengue virus infections were reduced to negligible levels and a putative antiviral function could be uncovered. *Ae. albopictus* PIWI5 therefore seems to have an important role, not only in vpiRNA biogenesis, but also as an antiviral effector.

Understanding the recent emergence of TBEV in the Netherlands

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Abstract

One of the most important arboviral diseases in Eurasia is caused by tick-borne encephalitis virus (TBEV), transmitted by *Ixodes* ticks endemic to large parts of Eurasia. TBEV has recently emerged in the Netherlands but the ecological and molecular factors underlying this emergence remain poorly understood. The TBEV-NL strain isolated in the Netherlands is a phylogenetically unique strain with different replication dynamics compared to the known European TBEV strains. Because of the recent emergence and unique characteristics of this distant TBEV strain, important issues need to be addressed, namely: 1) the vector competence of ticks, 2) the identification of host species that are reservoir hosts of TBEV, and 3) the influence of TBEV on tick behaviour. Using *in vitro* and *in vivo* models of *Ixodes ricinus* ticks, we study these three issues in our Biosafety Level 3 facilities. We have set-up an artificial membrane system to blood-feed and infect nymphs with TBEV via a natural route. Besides vector competence and co-infection interactions with other tick-borne pathogens (e.g. *Borrelia burgdorferi*), we will experimentally test whether and how TBEV manipulates tick behaviour, which affects virus transmission. The main route of TBEV transmission is assumed to be via co-feeding ticks on rodent species (non-viraemic transmission). However, recent studies have challenged the current dogma. We hypothesize that viraemic transmission is equally or even more important than non-viraemic transmission and we will test this using a mice-tick TBEV transmission model. Studying both ecological and molecular factors influencing TBEV transmission is important for our understanding of TBEV emergence in the field.

Monitoring of Insecticide Resistance on Aedes sp Mosquitoes in Banyumas Regency, Indonesia

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Abstract

Background. The continuous use of insecticides for mosquito-borne disease control can cause insecticide resistance, and if left unchecked, this could lead to a substantial increase in disease incidence. The aims of this study are to monitor insecticide insecticides against Malathion and Cypermethrin and identifying the mechanisms underlying the resistance in the area of study.

Method This is a descriptive study located in Banyumas Regency. *Aedes sp* mosquitoes were collected from three endemic areas (Arcawinangun, Karangpucung, and Purwanegara) by the ovitrap installation to 100 houses each village (total 300 houses). Filial 1 of Aedes were tested their insecticide resistance to Malathion and Cypermethrin by susceptibility test, biochemical assay and molecular by PCR.

Results The results of the susceptibility test showed the average percentage of mosquito mortality from three villages was 30,67% which were included in the resistance category. However, the results of the biochemical assay showed that 70% of mosquitoes are still very susceptible (AV<0,7). Molecular tests are underway and the results are likely to be obtained in August 2019

Conclusion The population of *Aedes sp*. in the study area has been resistant to malathion and cypermethrin, and the mechanism underlying this resistance was not based on a biochemical mechanism. It is necessary to rotate the use of insecticide active substances in DHF vector control by selecting insecticides that have a different mode of action.

Prevalence of tick-borne encephalitis virus in questing ticks in northern Italy

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Abstract

Tick-borne encephalitis (TBE) is a severe disease that has been endemic in northeast Italy since 1992. Over the last decade, there has been an increase in the number of human cases reported in many European countries, including Italy. To assess the current TBE infection risk in northeast Italy, questing ticks were collected from suspected TBE foci. These sites were selected based on the distribution of human cases and the results of a screening for TBE virus (TBEV) specific antibodies of goat sera samples collected during ordinary surveillance activities by the local Veterinary Hygiene and Public Health Agency. Samples were tested by ELISA and confirmed by neutralization assay. Ninetheen sites were selected based on these evidences, from which a total of 2513 *Ixodes ricinus* (2116 nymphs and 397 adults) were collected and analyzed for TBEV prevalence by a real-time RT-PCR targeting the 3' non-coding region. Four tick samples from 3 sites were found positive and confirmed by nested RT-PCR targeting a fragment of the E gene. The overall prevalence of TBEV in the region was 0.16%, but locally it reached 1% and 1.2% in 2 of the 3 sites. A phylogenetic analysis of the partial E gene confirmed a previous report that the European TBEV subtype is the only one circulating within the TBE foci in northeast Italy.

Salivary gland RNA-seq from arbovirus-infected *Aedes aegypti* and *Aedes albopictus* provides insights into virus transmission

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Abstract

Yellow fever mosquitoes (*Aedes aegypti*) and Asian tiger mosquitoes (*Aedes albopictus*) are the primary vectors of dengue virus (DENV), Zika virus (ZIKV) and chikungunya virus (CHIKV). These viruses are transmitted to humans through mosquito saliva, making the vector salivary gland (SG) a critical tissue to identify transmission-blocking targets. We examined gene expression in infected SGs for both vector species and for three different virus infections. *Aedes aegypti* SGs were infected separately with DENV, ZIKV and CHIKV, and *Ae. albopictus* with CHIKV. RNA-sequencing identified differentially expressed coding and long non-coding RNAs (lncRNAs). Differentially expressed genes determined from genome annotations were greater in number and functional diversity in comparison to differentially expressed transcripts from *de novo* transcriptome assemblies. Salivary protein transcripts were the most abundant, but were downregulated in all three virus infections. Commonly upregulated genes were associated with apoptosis, cytoskeletal proteins, replication/transcription/translation, redox/stress and immunity. An enrichment of upregulated genes related to apoptosis were observed in CHIKV infection in comparison to DENV and ZIKV infections. Upregulation of serine proteases and other genes associated with immunity and cellular stress responses (cytochrome P450 genes) varied between vectors. There were also immune response commonalities between vectors, for instance RNA-interference was observed to be a non-specific antiviral defense. The number of lncRNA transcripts differentially expressed were few and none were common to all infections, likely having minor roles, unlike the lncRNA antiviral effects proposed for mosquito midgut. Determining common infection patterns for different viruses and vectors has applications in refractory vector engineering.

Conversion of forest to oil palm plantation in Borneo reduces mosquito diversity and abundance and favors a vector of arboviruses

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Abstract

Land cover and land use change (LCLUC) are important drivers of arbovirus spillover from ancestral sylvatic cycles maintained in wildlife reservoirs into the human population, but specific mechanisms for these effects are incompletely understood. We investigated the impact of oil palm cultivation on the potential for arbovirus spillover in Sarawak, Borneo, a focus of sylvatic dengue virus (DENV) transmission. We tested the hypothesis that conversion of forest to oil palm would result in decreased mosquito diversity. A total of 883 mosquitoes were collected from 340 sites distributed among 10 distances from interior oil palm (-100, -50, -20, -10 m), edge (0 m), and interior forests (+10, +20, +50, +100, +500 m). These distance categories captured significant differences in microclimate and vegetation. Mosquito abundance (P = < 0.0001) and diversity (P = < 0.0001) decreased significantly from interior forest to interior plantation. *Aedes albopictus*, a generalist species and likely bridge vector for sylvatic DENV, was the only species detected at the deepest distance into plantations; this species was equally abundant at all distances sampled in both land covers (P = 0.09). The only known vector of sylvatic DENV in Asia, *Ae. niveus*, was found only in forests. Analysis of bloodmeals revealed that *Ae. albopictus* fed on humans in both plantations and forest. These data indicate that the risk of spillover of mosquito-borne pathogens is greatest during the initial conversion of forest to plantation but is lower in established plantations and at planation-forest edges than in interior forests.

Impacts of microclimate on mosquito communities across levels of urbanization in Manaus, Brazil

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Abstract

Urbanization is accelerating and there is considerable concern about its impact on vector-borne disease. Particularly, changes in microclimate associated with "urban heat islands" may affect the ecology of vector mosquitoes. Our study characterized microclimate (via iButton hygrochrons) as well as abundance and diversity of mosquitoes (collected with BG sentinel traps) across three levels of urbanization (according to the Normalized Difference Built-up Index, NDBI) in Manaus, Brazil. Manaus is a growing city that experiences high levels of arbovirus transmission mediated by *Aedes aegypti,* and potentially by *Ae. albopictus*. Mean humidity, maximum humidity, and day-time mean humidity differed significantly among NDBI levels (Kruskal-Wallis, DF=2, $P<0.02$ for all comparisons), with high NDBI showing lower values than the other categories (Wilcoxon each-pair comparison, $P<0.03$). Temperature was not significantly different among NDBI levels, but the highest NDBI level had a maximum daily temperature that was 1.2 ˚C hotter than the other two categories. Total mosquito abundance and diversity did not differ among NDBI levels and mosquito communities were 98-99% similar across levels according to the Morisita overlap index. However, when analyzing target vector species, *Ae. aegypti* presence per site differed significantly across categories (Pearson Chi-square, DF=2, $P=0.0003$), with percent sites positive for *Ae. aegypti* increasing with NDBI. *Ae. albopictus* presence did not differ significantly among NDBI levels (Pearson Chi-square, DF=2, $P=0.06$). Additionally, linear regression revealed significant associations of urbanization, temperature, and humidity with the abundance of *Ae. aegypti* but not *Ae. albopictus*. These data advance our understanding of the relationships between vectors, urbanization, and microclimate.

The Tick Cell Biobank: new arthropod cell lines for arbovirus research

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Abstract

The Tick Cell Biobank is the world's only dedicated culture collection for cell lines derived from ticks and other arthropod vectors. As well as storing and distributing arthropod cell lines and training in their maintenance to UK and international researchers, the Tick Cell Biobank generates novel cell lines from arthropod species and geographic strains not already represented in the collection. Currently, efforts are focussed on European *Argas*, *Dermacentor*, *Hyalomma*, *Rhipicephalus* and *Ixodes* spp. ticks, *Lutzomyia* and *Phlebotomus* spp. sandflies, *Culicoides* spp. biting midges, *Rhodnius prolixus* kissing bugs and *Glossina morsitans* tsetse flies. Techniques used previously for ticks and insects are applied or adapted for use with embryonic or larval arthropods to generate primary cell and tissue cultures; these primary cultures are then maintained until significant cell multiplication commences and subculture can be attempted, which may take several years. This approach has yielded new cell lines from the soft tick *Argas reflexus*, the hard tick *Hyalomma lusitanicum*, the New World sand fly *Lutzomyia longipalpis* and the UK midge *Culicoides nubeculosus*. Most of these novel cell lines are now available for arbovirus research through the Tick Cell Biobank.

IMPLICATIONS OF VERTICAL TRANSMISSION OF ALPHAVIRUSES IN *AEDES AEGYPTI* MOSQUITOES

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Abstract

Mosquitoes are vectors for epidemic transmission of viruses of public and veterinary health. The mosquito vector is generally infected for life although, unlike the vertebrate counterpart, does not suffer a high fitness cost. Having a vector infected for the length of its life enables routes of viral transmission other than the classic infected bite (horizontal), including sexual (horizontal) and to the progeny (vertical). Vertical transmission is considered a route of transmission that allows for the persistence of the virus during adverse environmental periods (e.g., droughts, cold periods). Because *Aedes aegypti* and *Aedes albopictus* eggs are resistant to dessication, it is hypothesised that this attribute could promote arbovirus survival between transmission cycles, playing an important role in maintaining the pathogen.

Vertical transmission of arboviruses has been extensively documented for flaviviruses and bunyaviruses. However, there is very little and contradictory reports of vertical transmission of alphaviruses. In this research we establish the mechanisms of vertical transmission of the alphaviruses Semiliki Forest virus (SFV) and Ross river virus (RRV) and its implications in pathogen transmission of future generations.

Aedes aegypti mosquitoes were infected with SFV virus in different gonotrophic cycles. Their offspring was then reared and challenged with SFV, RRV and dengue virus (DENV). Offspring from infected parents showed significant reduction in viral load if infected with SFV or RRV but not with DENV.

Findings of this research highlight the importance of vertical transmission of alphaviruses in the general arbovirus infectious cycle.

JNK pathway-a key mediator of antiviral immunity in mosquito salivary glands

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Abstract

Background: Mosquito salivary glands play crucial role in transmission of arboviral diseases like Dengue (DENV), Zika (ZIKV) and Chikungunya (CHIKV). We aimed to characterize virus responsive gene expression in *Aedes aegypti* salivary glands against these pathogenic arboviruses.

Methods: We performed high throughput RNA-sequencing on uninfected and virus-infected (DENV, ZIKV, CHIKV) female *Ae. aegypti* salivary glands to elucidate differential expression of genes at the transcript level. We validated the transcriptomic analysis by qPCR and performed RNA-i based functional characterization of virus-induced immune genes.

Results: DENV, ZIKV or CHIKV infected salivary gland transcriptome revealed regulations of genes related to blood feeding, metabolism, apoptosis, and immunity; the latter including Toll, IMD, and JNK pathway components. Silencing of Toll and IMD pathway components did not increase replication of all three viruses. However, depletion of infection induced JNK pathway activator and repressor showed conserved antiviral response of this pathway against the viral infections. We further showed that JNK activation by arboviruses is mediated by antiviral complement and apoptosis activation.

Conclusion: This study determined the previously unknown antiviral mechanism of JNK pathway in mosquito salivary glands against important pathogenic arboviruses. This pathway shows potential to be utilized for developing effective transmission blocking tools.

De novo assembly of the midgut transcriptomes of blood-fed Culex pipiens and Aedes vexans mosquitoes, important arbovirus vectors.

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Abstract

Culex pipiens and Aedes vexans mosquitoes are competent vectors for many arboviruses of major human and veterinary importance. Conversely, little genomic information is available for these species, significantly hindering research efforts to better understand their biology and interactions with arboviruses.

In this study we performed next generation mRNA sequencing on midgut samples of blood-fed Cx. pipiens and Ae. vexans mosquitoes to gain an insight into the cellular environment arboviruses face at early stages during midgut infection.

In the absence of reference genomes, transcripts were de novo assembled using Trinity. We obtained 82,282 contigs for the Cx. pipiens and 77,147 contigs for the Ae. vexans data sets. Translated cDNA sequences were then aligned using DIAMOND. About 40% of translated contigs derived from both midgut samples mapped uniquely to known sequences, the majority of which showed the highest degree of homology to Cx. quinquefasciatus or Ae. albopictus and Ae. aegypti sequences, respectively. The midgut microbiomes and viromes were also characterised. Contigs with unique protein identifiers were then subjected to GO term and pathway analyses using either combined UniProt and g:Profiler or Blast2Go workflows. Both multi-step workflows resulted in some loss of information as a result of data conversion. Molecular functions and biological processes reflecting digestion of the blood meal and responses to the resulting oxidative stress were significantly overrepresented in both data sets.

The generated midgut transcriptomes will be shared with the research community as a valuable resource for genomic information of Cx. pipiens and Ae. vexans.

A Viral Metagenomic Analysis Reveals Rich Viral Abundance and Diversity in Mosquitoes from Pig Farms

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Abstract

Mosquitoes harbor a diversity of viruses and are responsible for several mosquito-borne viral diseases of humans and animals, thereby leading to major public health concerns and significant economic losses across the globe. The viral metagenomics offers a great opportunity for bulk analysis of viral genomes retrieved directly from environmental samples. In this study, we performed a viral metagenomic analysis of five pools of mosquitoes belonging to Aedes, Anopheles and Culex species, collected from different pig farms in the vicinity of Shanghai, China to explore the viral community carried by mosquitoes. The resulting metagenomic data revealed that viral community in the mosquitoes was highly diverse and varied in abundance among pig farms, which comprised of more than 48 viral taxonomic families, specific to vertebrates, invertebrates, plants, fungi, bacteria, and protozoa. The read sequences related to animal viruses included parvoviruses, anelloviruses, circoviruses, flavivirus, rhabdovirus, and seadornaviruses, which might be taken by mosquitoes from viremic animal hosts during blood feeding. Notably, sample G1 contained the most abundant sequence related to Banna virus, which is of public health interest because it causes encephalitis in humans. Furthermore, non-classified viruses also shared considerable virus sequences in all the samples, presumably belonging to unexplored virus category. Overall, the present study provides a comprehensive knowledge of diverse viral populations carried by mosquitoes at pig farms, which is a potential source of diseases for mammals including humans and animals. These viral metagenomic data are valuable for assessment of emerging and re-emerging viral epidemics.

Stage specific transcriptome profiling of castor bean tick *Ixodes ricinus*

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Abstract

Arthropod borne diseases are ubiquitously discussed topic whose relevance increases with ongoing changing climate, which extends the area of their incidence and affects profoundly the size of vector population, as well as its reproductive capacity, the abundance and spread of reservoir hosts and other variables that are generally tightly correlated with the spread of zoonoses.

The life cycle of arthropod borne pathogens are tightly bound to the life cycle of their host as well as to their vector organism. Therefore, the description of the vector life cycle should elucidate some questions related to the vector-pathogen dynamics including the factors important for successful disease transmission.

Tick has complex life cycle and for its completion it requires feeding on several host organisms, which is abused by the pathogen for its spreading within reservoir and host organisms.

Thus, more thorough description of factors driving tick developmental processes controlling its life cycle will be instrumental in understanding the nature of *I. ricinus* and its pathogens interactions and may also shed light on the process of blood feeding as an integral event in tick development as well as in potential pathogen transmission.

In our study we perform transcriptional profiling of all life stages of *I. ricinus* and provide a list of genes associated with particular life stages of *I. ricinus* and hence extend our knowledge in pursuit of potential acaricidal strategies.

Antagonistic action of blood-feeding and mating on the gut immunity in the female mosquito *Aedes aegypti*

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Abstract

The female mosquito *Aedes aegypti* needs a blood meal to reproduce and is therefore a vector of arthropod borne viruses (arboviruses) such as Zika (ZIKV) or chikungunya (CHIKV) viruses. Even though mosquitoes transmit viruses, they possess different immune pathways to fight against pathogens. Surprisingly, the analysis of immune gene expression in different tissues from non blood fed (NBF) or blood fed (BF) females revealed that these genes were repressed in the digestive tract from 2 to 30 h following blood meal. As the digestive tract is the first barrier to be overcome by an arbovirus during the first few hours/days after blood meal, our findings could explain the high susceptibility of *Ae. aegypti* mosquitoes to arboviruses. To investigate this hypothesis, it is required to identify the mechanism by which gut immunodeficiency is triggered. We would then be able to inhibit it and analyse the impact on mosquito infection/transmission. In addition to the acquisition of a blood meal, the female must mate before laying fertilized eggs. Mating has been shown to cause changes in the female physiology and behaviour such as refractoriness to further mating in many insects but also, mating has been shown to influence immunity in *Drosophila*. We found that mating transiently antagonizes the immune gene repression in the digestive tract of BF females compared to virgin BF females. The mechanisms by which blood-feeding and mating modulate gut immunity are under investigation and will be presented during the conference.

A novel class of small molecule inhibitors targeting the chikungunya virus capping machinery with a high barrier to resistance

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Abstract

Background: Despite the worldwide re-emergence of the chikungunya virus (CHIKV) and the high morbidity associated with CHIKV infections, there is no approved vaccine or antiviral treatment available. Here, we identified a novel class of CHIKV inhibitors i.e. the CHVB series. **Methods:** CPE-reduction and virus yield assays were performed in Vero cells. Drug-resistant variants were selected using clonal resistance selection. The enzymatic assays for alphavirus capping were done using the non-structural protein 1 (nsP1) of Semliki Forest virus (SFV) and Venezuelan equine encephalitis (VEEV). **Results:** CHVB compounds inhibited the *in vitro* replication of CHIKV isolates with EC_{50} values in the low µM range. In virus yield assays, the most potent analogues reduced the viral load with 4-5 log10. CHVB-resistant variants were selected and found to carry (i) two mutations in the gene encoding nsP1 (responsible for viral RNA capping), (ii) one mutation in nsP2 and (iii) one mutation in nsP3. Reverse-engineering suggested that nsP1 is the target of CHVB, since both nsP1 mutations were needed to achieve 10-fold resistance. Interestingly, the $CHVB^{res}$ virus proved cross-resistant to the MADTP series, a class of CHIKV capping inhibitors that we described earlier, suggesting a similar mode of action. In enzymatic assays, CHVB proved a potent inhibitor of the methyltransferase and guanylyltransferase activities of nsP1 of SFV and VEEV. **Conclusion:** We identified a class of CHIKV inhibitors that targets the viral capping machinery. The potent anti-CHIKV activity and the high barrier to resistance make this chemical scaffold a potential candidate for CHIKV drug development.

Profiling RNAs binding with nucleoprotein of severe fever with thrombocytopenia syndrome virus revealed orchestrated replication of a segmented RNA virus

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Abstract

Background. Virus nucleoprotein (N) binds to virus genomic RNA to coordinate RNA replication and transcription together with RNA polymerase. Here, to reveal the dynamics of virus RNA and host RNA contributions during virus replication, we studied a number of virus and host RNAs binding with severe fever with thrombocytopenia syndrome virus (SFTSV) N. **Methods**. Human hepatoma (Huh-7) cells infected with SFTSV were lysed and co-immunoprecipitated RNAs with N (IPed RNA) and RNAs from cell lysate (total RNA) were sequenced using HiSeq 3000 (Illumina) using a strand-specific method. N-binding host RNAs were defined as transcripts with 50 times larger numbers of sequencing reads in IPed RNA than thosein total RNA. Titer of SFTSV was monitored in Huh-7 cells transfected with siRNA targeting N-binding host RNAs. **Results**. The amounts of genomic sense viral RNAs binding to N were significantly different among three segments in IPed RNA while almost identical in the supernatant. Twenty-nine host RNA transcripts binding with N were identified in the cells, while no N-binding host RNAs were found in supernatant. In the cells transfected with siRNA targeting one of the 29 N-binding RNAs, MAX-gene associated protein gene (*MGA*), the titer of SFTSV at 1 day post infection was significantly lower than control cells. **Discussion**. The present study suggested that each RNA segment might be replicated or transcribed in a different manner to control virus protein expression. And we also demonstrated that various N-binding host RNAs may play a pivotal role during SFTSV replication.

Potential New Class of Antivirals: A Macrodomain Inhibitor

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Abstract

Macrodomain is a small protein domain of ~160 amino acid residues. Members of the macrodomain family can be found in all domains of life, and they are often active in binding and/or hydrolysis of ADP-ribose-metabolites, e.g. by removing ADP-ribosyl marks from modified proteins. The activities of the alphavirus macrodomain (located in replicase protein nsP3) are critically important for viral RNA replication, whereas the coronavirus-encoded macrodomain seems to be important for pathogenesis. After an initial computational screen for potential macrodomain binders, we tested a set of commercially available compounds for antiviral activity against Semliki Forest virus (SFV) replication in cell culture. One of the compounds, MB1 (for macro blocker 1) strongly reduced viral protein and RNA levels, as well as the formation of infectious particles. MB1 apparently acted against the RNA replication stage of the viral life cycle, since it also inhibited the plasmid-based trans-replication systems of SFV and chikungunya (CHIKV) viruses, with IC50 values of 5.8 µM and 8.1µM, respectively. At higher concentrations of 50-100 µM, MB1 exhibited some toxic properties, and interestingly, appeared to decrease overall protein synthesis both in infected and uninfected cells, as measured by puromycin incorporation. Finally, MB1 inhibited the enzymatic activity of purified macrodomains. Therefore, we have potentially discovered a macrodomain inhibitor, which exhibits antiviral properties in cell culture. Several areas require further study, including the toxic effects of MB1 in cell culture, and its selectivity for viral versus cellular macrodomain proteins.

In-Gel Chemical Probing Reveals Structure of Dengue Virus RNA Untranslated Regions.

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Abstract

Dengue virus RNA 5' and 3' untranslated regions (UTRs) exist as a mixed population of conformers in solution, since they can hybridise, as occurs in genome circularisation. Our in-gel SHAPE (selective 2'OH acylation analysed by primer extension) technique was used to structurally probe individual conformers within this mixed population, in order to characterise the structures that exist before and after genome circularisation. In-gel SHAPE involves native gel electrophoresis to separate the RNA conformers, followed by treatment of each conformer with NMIA, an electrophile that preferentially reacts with single-stranded RNA. Reverse transcription and capillary electrophoresis of fluorophore-labelled cDNA allows quantification of electrophile reactivity at each nucleotide position. Free energy minimising software was used to model the secondary structures of both the 5' and 3' UTRs as monomers, and of the 5'-3' UTR heterodimer. The predicted structures show similarities to and some significant differences from previously published work. In particular, hybridisation of the UTRs induced conformational changes that have not previously been reported involving extensive novel interactions. Further investigation of these RNA structures and RNA-protein interactions is ongoing to understand this aspect of the dengue lifecycle with the aim of revealing novel targets for the future development of antiviral agents.

Targeting the conserved cap-snatching region in a pan-bunya antiviral approach

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Abstract

A wide variety of bunyaviruses cause pathologies in humans, three of which are in the top-10 'priority list of infectious diseases' of the WHO, i.e. Rift Valley fever virus (RVFV), Crimean Congo hemorrhagic fever virus (CCHFV) and Lassa virus. Their ubiquity, variety of hosts and pandemic potential, together with a lack of prophylaxis and treatment options makes the bunyaviruses a serious public health threat. Their great diversity makes drug development under the 'one-drug-for-one virus' paradigm particularly challenging. Therefore, we explored highly conserved domains among bunyaviruses that could serve as targets in a pan-bunya antiviral strategy. Through comparative genomic analysis, we identified the L-protein endonuclease domain as the most conserved among bunyavirus species. As starting point, a selection of known influenza virus inhibitors targeting the endonuclease was tested in our high-content imaging-based antiviral assays using fluorescently labelled Bunyamwera virus (orthobunyavirus), and RVFV (phlebovirus). Two compounds (L-742,001 hydrochloride and L311227) were found to inhibit Bunyamwera replication [EC_{50} 11±2µM and 7±3µM, respectively]. Additionally, we have constructed homology models for RVFV and CCHFV endonucleases based on the endonuclease crystal structures of La Crosse and Hantaan virus. A database of ~3.5 million commercial compounds was screened in order to select small molecules containing metal-binding groups. These molecules were further screened by docking simulations using the endonuclease homology models and crystal structures. After final rounds of visual inspection 292 small-molecules were selected, which are currently being assessed in our *in vitro* assays against different bunyaviruses.

Structural and phenotypic analysis of Chikungunya virus RNA replication elements

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Abstract

Chikungunya virus (CHIKV) is a re-emerging, pathogenic *Alphavirus* transmitted to humans by *Aedes spp* mosquitoes. We have mapped the RNA structure of the 5' region of the CHIKV genome using selective 2'-hydroxyl acylation analysed by primer extension (SHAPE) to investigate intramolecular base-pairing at single-nucleotide resolution. Taking a structure-led reverse genetic approach, in both infectious virus and sub-genomic replicon systems, we identified six RNA replication elements essential to efficient CHIKV genome replication - including novel elements, either not previously analysed in other alphaviruses or specific to CHIKV. Importantly, through a reverse genetic approach we demonstrate that all the replication elements function within the positive-strand genomic copy of the virus genome, in predominantly structure-dependent mechanisms during efficient replication of the CHIKV genome. Comparative analysis in human and mosquito-derived cell lines reveal that a novel element within the 5'UTR is essential for efficient replication in both host systems, while those in the adjacent nsP1 encoding region are specific to either vertebrate or invertebrate host cells. In addition to furthering our knowledge of fundamental aspects of the molecular virology of this important human pathogen, we foresee that results from this study will be important for rational design of a genetically stable attenuated vaccine.

Targeting functional RNA structures in CHIKV and ZIKV

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Abstract

Emerging arboviruses such as Zika virus (ZIKV) and Chikungunya virus (CHIKV) represent a significant threat to human health and have a high potential to cause outbreaks in the near future. At present, there are no specific antivirals available for either of these important pathogens, despite the wide global prevalence of their vector, Aedes spp. mosquitos.

The positive-strand genomes of ZIKV and CHIKV, members of the *flavivirus* and *alphavirus* genera respectively, contain functional, structured *cis*-acting RNA elements which are essential for virus replication. By specifically targeting such RNA elements using antisense locked nucleic acid oligonucleotides (antisense-LNA), we aim to disrupt their function, and analyse the effect this has on virus replication at different life cycle stages.

Obtaining high quality, single-nucleotide-resolution structural data is essential prior to targeting RNA structures. Consequently, we mapped RNA structural elements within the ZIKV 5' genome region using a combination of biochemical SHAPE probing, thermodynamic models and phylogenetic analysis. We are currently validating our structural data by analysis of mutant phenotypes in a reverse genetic system.

We demonstrate that functional RNA elements in CHIKV can be specifically targeted - inhibiting replication in both sub-genomic replicon and infectious virus systems. Surface plasmon resonance confirmed that an antisense-LNA binds to a specific stem-loop target with a K_d of 310nM and has an IC_{50} of 35nM in a sub-genomic replicon system. In future work, we aim to investigate selection of RNA-aptamers against CHIKV and target ZIKV genomic stem-loops using antisense-LNAs.

Assessing co-replication and RNA recombination between alphavirus replicon vectors and wildtype alphaviruses

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Abstract

Alphavirus replicon vectors form an innovative vaccine platform due to rapid and straightforward adaptation of the expressed heterologous antigens to new viral outbreaks. To allow global implementation of this platform technology a comprehensive environmental safety evaluation is required. We currently evaluate the possibility of RNA recombination between alphavirus replicons and wildtype alphaviruses. Fluorescence microscopy and flow cytometry analysis of co-infected and sequential infected Venezuelan equine encephalitis virus (VEEV) replicon particles expressing eGFP and mCherry showed that replication of two replicons within the same cell is possible, but that the efficiency is greatly influenced by the timing of the secondary infection. Furthermore, several heterologous wildtype alphavirus infections of cells that underwent a primary infection with VEEV replicon particles, demonstrated that the replicon can inhibit replication of the superinfecting alphavirus. Nevertheless, this exclusion can be overcome by increasing the multiplicity of the secondary infection. Finally, a novel CRISPR-Cas9 based cell assay has been designed to detect potential alphavirus recombinants with very high sensitivity. We now use this system to quantify the incidence of RNA recombination with different replicon-wildtype alphavirus combinations and to analyze recombination events at the molecular level. Our results will provide input for proper risk assessment of the environmental safety of the replicon particle vaccine platform.

Development of reverse genetics for Toscana virus

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Abstract

Toscana virus (TOSV) is a *Phlebovirus* in the *Phenuiviridae* family, found in the countries surrounding the Mediterranean. It is unusual within the *Phlebovirus* genus for exhibiting tropism for the central nervous system: TOSV is one of the top causes of seasonal acute meningitis/encephalitis within its range. However, little progress has been made in the study of TOSV, largely due to the lack of a reliable reverse genetics system.We used RNA sequencing to determine the sequence of Toscana strain 1500590, a lineage A virus obtained from an infected patient (Marseille, 2007). This data was used to construct cDNA plasmids encoding the viral L, M and S antigenomic sequences under control of the T7 RNA promoter to recover recombinant viruses when expressed in BSR-T7/5 CL21 cells. By sequencing amplified viral cDNA, we identified two single base pair mismatches in the original TOSV reference genome (NC_006318, 19, 20), which when corrected restored functionality to the polymerase. We were then able to recover infectious recombinant TOSV (rTOSV) from cDNA clones, as well as establishing a minigenome system. Using reverse genetics, it has been possible to produce the non-structural gene (NSs) deletion mutants (a known interferon antagonist). These strains are in the process of characterisation and are to be used in infection studies in *Phlebotomine* flies.TOSV vaccine development has been severely hindered by the failure of previous efforts to develop reverse genetics systems for this virus. With a system now in place, it will hopefully be possible to design novel attenuated vaccine candidates.

Cas13b induced knockdown of Chikungunya

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Abstract

Chikungunya (CHIKV) is a re-emerging alphavirus that has caused severe outbreaks in tropical and sub-tropical regions in the last decade. As there is no effective drug treatment, disease control is predominantly through vector control. Targeted genetics-based methods may allow us to reduce the vectors' CHIKV transmission capacity.

We assessed the ability of the RNA-targeting CRISPR–Cas protein Cas13b to target CHIKV RNA, comparing *in vitro* transcribed and U6 promoter driven RNA guides (U6-guides) targeting two sequences. A synthetic luciferase reporter (CHIKV-Luc) and a luciferase tagged CHIKV trans-replicon system (CHIKV-Rep) were used to measure target knockdown in *Aedes aegypti* (Aag2) and *Aedes albopictus* (C6/36) cells.

Cas13b dependent knockdown of CHIKV-Luc was observed with U6-guides in both cell lines (two-way ANOVA, $p<0.001$) but knockdown observed with *in vitro* transcribed guides was Cas13b independent. For U6-guides, Cas13b dependent knockdown levels above 70% for CHIKV-Luc were measured with both guides (Tukey's test, $p<0.001$), and less efficient but still significant Cas13b dependent knockdown above 40% was observed with CHIKV-Rep (Tukey's Test, $p<0.0005$) in Aag2 and C6/36 cells.

In summary, knockdown of CHIKV is possible with Cas13b and its associated U6-guides in mosquito cells. It may be possible to use Cas13b, with effective guides, to develop a strain of transgenic mosquitoes with greater resistance to CHIKV to help reduce the rate of disease transmission.

The translational landscape of Zika virus infection reveals novel open reading frames

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Abstract

Zika virus (ZIKV) is an emerging Aedes mosquito-borne flavivirus isolated in Uganda in 1947 but not viewed as a particularly important pathogen until the Latin-American outbreak in 2016 when associated with congenital diseases. We applied ribosome profiling to mammalian and mosquito cells infected with ZIKV Brazilian isolate PE243. Ribosome profiling exploits the capacity of the translating ribosome to protect ~30 nucleotides of mRNA from ribonuclease digestion to give a global “snap-shot” of translation on host and viral mRNAs. Data obtained have provided the first high-resolution map of flavivirus translation in mammalian and mosquito cells. Strikingly, we found ribosome protected fragments mapping in-frame to two previously overlooked non-AUG upstream open reading frames (uORF1 and uORF2). In Old World isolates, uORF1 and uORF2 are fused into a single uORF, suggesting a potential virulence factor. To evaluate the significance of these uORFs in the context of virus infection, a set of mutant virus genomes was created and tested for RNA infectivity, virus titer, plaque size, stability of the introduced mutations and competitive growth with wild-type virus (wt). Knockout of uORF2 expression resulted in a smaller plaque size phenotype and a 10-fold reduction in titre compared to wt indicating that this uORF might encode for a protein involved in viral growth or increased virulence. However, knockout of uORF1 resulted in a fitter phenotype than wt virus as judged in a competition assay, suggesting that uORF1 may influence ZIKV replication. Therefore, we hypothesize that ZIKV uORFs have a strain-dependent impact on viral virulence and pathogenesis.

Teaching an old drug new tricks: Efficacy of fluoroquinolones to suppress replication of flaviviruses

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Abstract

Safe and effective treatments for infections with flaviviruses such as dengue (DENV) and Zika virus (ZIKV) are desperately needed. We investigated the potential for fluoroquinolones, a class of FDA-approved antibiotics, to be repurposed as antiflaviviral drugs. We demonstrated that ciprofloxacin, enoxacin and difloxacin suppress replication of six different flaviviruses (DENV-1,2 and 4, ZIKV, Langat and Modoc virus) in HEK293 cells at low micromolar concentrations. Moreover, time-of-addition assays revealed that enoxacin suppressed ZIKV replication when added at 2-8 hours post-infection, suggesting that it inhibits intermediate viral life cycle stages such as translation and replication, whereas ciprofloxacin and difloxacin suppressed ZIKV at 0-8 hours post-infection, suggesting inhibition of both entry and later life cycle steps. Efficacy of FQs *in vivo* was tested in two experiments. First, A129 mice were infected with $1x10^5$pfu ZIKV (n=20) or PBS (n=11) on day zero and subsets of infected and uninfected mice were treated with oral enoxacin at 10mg/kg or 15mg/kg or diluent on days 1-5. Second, mice were infected with $1x10^2$pfu ZIKV (n=13) or PBS (n=13) on day zero and a subset of each group were treated with 15mg/kg enoxacin or diluent on days zero (pre-treatment) and 1-5. No significant differences were detected, between enoxacin-treated and control mice in weight, virus titer in the serum, brain, or liver in either experiment however, viral load in the testes of treated mice was significantly lower than control mice (P=0.03, P=0.0004). ZIKV can be sexually transmitted, so suppression of ZIKV in the testes by enoxacin should be further investigated.

The emerged genotype I of Japanese encephalitis virus shows an infectivity similar to genotype III in Culex pipiens mosquitoes

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Abstract

Japanese Encephalitis virus (JEV) is a zoonotic flavivirus that cause childhood viral neurological infections. During last 20 years, JEV genotype dominance has shifted from genotype III (GIII) to genotype I (GI). To date, the exact mechanism of this replacement is still not known. In this study, we experimentally orally exposed female *Cx. pipiens* mosquitoes with either GI or GIII JEV strains and subjected to measure the infection rate, replication kinetics, dissemination rate and transmission potential by 50% tissue culture infective dose assay, to explore if mosquitoes played a potential role in JEV genotype shift. We found that *Cx. pipiens* mosquito was competent vector for both GI and GIII JEV infection, with similar infection rates, growth kinetics, dissemination rate and transmission rates. Our experiment data demonstrated that GI and GIII viruses have similar infectivity in *Cx. pipiens* mosquitoes, suggesting that mosquitoes may not play an essential role in JEV genotype shift.

Un-clipping the truth: Using iCLIP to resolve RNA binding sites of chikungunya virus non-structural protein 3

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Abstract

Chikungunya virus (CHIKV) is an alphavirus, transmitted to humans by mosquitoes of the *Aedes* genus. Infection with CHIKV causes chikungunya fever, in many cases leading to chronic joint disease. Despite its rising potential as a threat to global health, no effective vaccine or antiviral agents for protection or treatment are available. The CHIKV non-structural protein 3 (nsP3) is essential to the virus lifecycle and is believed to be a component of the genome replication complex. However, to date, the exact role of this protein has yet been determined. Our previous studies have demonstrated that nsP3 binds *in vitro* to RNAs corresponding to the untranslated sequences within the CHIKV genome but the precise binding sites have not been determined. In addition, it is not known whether nsP3 also binds to any cellular RNAs. To address this gap in our knowledge we employed individual nucleotide resolution UV crosslinking and immunoprecipitation (iCLIP) to identify precise nsP3-RNA binding sites in cells infected with CHIKV. Our data show that during virus infection, nsP3 binds directly to specific sites on the CHIKV genome RNA and that secondary RNA structures may be important for binding recognition. Additionally, nsP3 also binds to the host transcriptome. Further experiments are ongoing to determine the precise nature of the nsP3 bound RNA species and to dissect the functional relevance of these RNA interactions in both mammalian and mosquito cells. We expect that the results of these studies will yield insights into the molecular functions of nsP3 during the CHIKV lifecycle.

Investigating the role of the nsP3 alphavirus unique domain (AUD) in chikungunya virus replication and virulence

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Abstract

Chikungunya virus (CHIKV) is a re-emerging Alphavirus transmitted by *Aedes* mosquitoes and causing fever, rash and arthralgia. Currently there are no vaccines or antiviral agents against CHIKV, therefore it is important to understand the molecular details of CHIKV replication. Building on our previous studies (Gao et al, 2019) we generated a further panel of mutants in a conserved, surface exposed cluster in the nsP3 AUD, and tested their replication phenotype using a subgenomic replicon in a variety of mammalian and mosquito (*Ae. albopictus*) cells. We identified three mutants that replicated poorly in mammalian cells but showed no defect in mosquito cells. This was not due to reversion as shown by sequencing. As mosquito cells are cultured at 28°C, we postulated that these mutants might be temperature sensitive, rather than cell species specific. This was indeed the case and the mutant phenotype was lost in Huh7 cells propagated at 28°C. Intriguingly, both wildtype and all mutants replicated 1000-fold higher in Huh7 cells at 28°C compared to 37°C. To further investigate this effect of temperature on replication of both the mutants and wildtype, other cell lines including C2C12 murine myoblasts and Vero monkey kidney cells are currently being evaluated. In addition we are extrapolating our data to the BSL2 pathogen O'Nyong-Nyong virus (ONNV) in order to evaluate the role of the AUD both in vitro and in a mouse model.

Mapping the virome in lab-reared and wild-caught Aedes albopictusmosquitoes

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Abstract

Mosquitoes are known vectors that can harbor a wide array of viruses that range from classical arboviruses that can infect vertebrates to insect-specific viruses that are restricted only in mosquito. Recent advances in the field of metagenomics and bioinformatics have strongly supported studies on mosquito-associated viruses. However, the study for mosquito virome profile isstill inadequate. In the present study, we aimed to investigate and compare the virome structure and profile between laboratory reared and wild-caught *Aedes albopictus* mosquito. Four mosquito samples (egg, larvae, pupae, adult) of laboratory rearedand two (larvae and adult)of field collected from Guangzhou of China were prepared for next generation sequencing (NGS) and analyzed. The results indicated that the common viruses presented in all samples were High Island virus, Aedes flavivirus, and Guato virus, whereas the Wenzhou sobemo-like virus 4 and Hubei mosquito virus 2 were the most dominant virus and presented only in wild mosquito samples.Additionally, unclassified *Papillomaviridae* viruses was the most dominant vertebrate virus, which was significantly detected only in wild adult mosquitoes. Important to note, the viral composition of larvae obtained from the wild was the most diverse group with many viruses belonging to virus families including *Flaviviridae*, *Totiviridae*, *Tymoviridae*, *Rhabdoviridae*, *Orthomyxoviridae*, *Iflaviridae* and unclassified viruses. This observed diverse nature can be attributed to influence of environment substrates and water in which the mosquito larvae inhabit. In conclusion, our findings provide essential information necessary in understanding the structure and diversity of *Aedes* virome.

Keywords: mosquito, virome, *Aedes albopictus,* next generation sequencing

Uncovering worldwide diversity and evolution of insect-specific viruses associated with *Aedes aegypti*, the yellow fever mosquito and *Aedes albopictus*, the Asian tiger mosquito

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Abstract

Aedes aegypti and *Aedes albopictus* are the two most significant vectors of the arthropod-borne viruses dengue, yellow fever virus, Zika virus and Chikungunya virus. Both mosquitoes have expanded from historical forest niches to domestic and peri-domestic settings globally within the tropical and subtropical zones. Considerable progress has been made to determine the intrinsic and extrinsic host factors that influence the vector competence of these mosquitoes to transmit arboviruses. Recent studies have demonstrated that insect-specific viruses (ISVs) can modulate refractoriness of *Ae. aegypti* and *Ae. albopictus* to arboviruses *in vitro* and *in vivo*. However, significant gaps in our understanding of the composition and diversity of viruses of both of these mosquitoes exists. To address this gap, we uncovered previously unknown virome diversity using a metagenomics survey approach to analyse ~2500 published RNA-Seq libraries representing 3TB of data from wild-caught, laboratory colonies and cell lines of these mosquitoes. Our results indicate that common laboratory colonies and cell lines harbour numerous mononegaviruses, orthomyxoviruses, bunyaviruses, negeviruses, totiviruses, and tombusviruses, in addition to well-characterised flaviviruses and densoviruses. Remarkable inter-host variation of ISVs exists between individual mosquitoes as well as heterogeneity between different laboratory strains. Assembled viruses were additionally subjected to phylogenetic analyses, the outcomes of which suggest a pattern of evolution congruent with the geographical and temporal expansion of these mosquitoes. This study expands our understanding of the virome of the two important arbovirus vectors and provides a resource for further assessment of the diversity, evolution and interaction of ISVs with their mosquito hosts and arboviruses they transmit.

The piRNA pathway in host-pathogen interaction: *Aedes albopictus* and arboviruses

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Abstract

Aedes albopictus is an aggressive invasive species and a competent vector for over 20 arboviruses, including Chikungunya, Dengue and Zika viruses. Understanding the molecular and cellular interactions between viruses and vectors is key to implement transmission-blocking strategies to prevent viral outbreaks. However, the mechanisms that shape vector competence are poorly understood. Recent evidence reveals that the genomes of *Aedes* spp. harbour fragmented viral sequences which produce PIWI-interacting RNAs (piRNAs), suggesting a role in vector competence. Current knowledge of the piRNA pathway in *Ae. albopictus* is limited, and its possible role in the establishment of persistent infections widely unknown.

We combined cutting-edge bioinformatic analyses based on next-generation sequencing data with molecular biology and virology techniques to characterise the main genes of the piRNA pathway in this mosquito species, assess their polymorphisms and analyse their expression throughout mosquito development and following infection with the Chikungunya and Dengue-1 viruses.

We identified seven piwi genes which displayed high levels of polymorphism across populations and signs of adaptive evolution. Superposition of protein homology models indicate high structure similarity among all Piwi proteins, with high levels of amino acid conservation in the inner regions devoted to RNA binding. On the contrary, solvent-exposed surfaces showed low conservation, with sites under positive selection. Infection experiments indicated specific responses depending on viral species, time of infection and mosquito tissue, highlighting distinct roles for specific Piwi proteins.

In conclusion, this work helps define the role of the piRNA pathway in persistent arboviral infections and understand the evolutionary divergence among piwi proteins.

Assessment of tick-borne flavivirus host factors through genome-scale screens

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Abstract

Powassan virus (POWV) is the only tick-borne flavivirus (TBFV) known to circulate in North America. Although there are relatively few documented cases of POWV disease, investigations into POWV are justified due to the increasing incidence of infection and significant case fatality rate associated with this virus. To better describe the molecular biology of the POWV replication cycle in mammalian cells, we performed genome-scale screens to uncover host factors required for viral replication. Putative proviral host factors were identified by infecting pools of cells containing knockout mutations in non-essential genes with POWV, followed by analysis of cells resistant to virus-induced cell death. Many endoplasmic reticulum membrane complex proteins were revealed in these screens, suggesting that TBFVs share some common host cell hijacking strategies with mosquito-borne flaviviruses. Candidate proteins that function in cell-matrix adhesion, glycosylation, or RNA binding in uninfected cells were the focus of validation studies. We used single-gene knockout cell lines to investigate possible proviral roles for specific proteins in the replication cycle of either POWV or Langat virus (LGTV), a non-pathogenic and model TBFV. Proteins identified in the POWV screens were not necessarily critical for LGTV replication. These results suggest that our screens were able to identify both pan-flaviviral, as well as POWV-specific, host gene products exploited during virus replication. Ongoing work is focused on characterizing distinct host-cell requirements of diverse flaviviruses. This genetic assessment of POWV replication factors, in combination with ensuing mechanistic studies, will provide possible avenues for the development of host-targeting countermeasures.

Molecular determinants of dengue virus infection in *Aedes aegypti* midgut.

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Abstract

To be successfully transmitted to another susceptible human host by *Aedes aegypti*, dengue virus (DENV) must first successfully infect the mosquito midgut. The virus-host interactions that enable successful midgut infection, however, is not well understood. To understand the important interactions for successful midgut infection, we took advantage of the wild-type DENV2 16681 and its attenuated derivative PDK53, which has been shown to be refractory in mosquito infection. Using oral infectious-blood feeding, we observed that PDK53 failed to produce infectious progenies in the midgut, despite detectable viral genome replication. Furthermore, we found that the foci of PDK53 infection in the midgut, detected by immunofluorescence staining, was limited in both size and number, compared to its parent, 16681. Transcriptional analysis of the mosquito midgut revealed increased expression of genes in multiple innate immune pathways upon PDK53 infection but not 16681. To pinpoint the mutation responsible for this phenotype, we constructed an infectious clone of 16681 and used site-directed mutagenesis to substitute each of the known mutations in PDK53 into the 16681 genomic backbone. This approach pinpointed the NS1-53 (Gly-to-Asp) mutation as the single most important attenuating mutation in PDK53 in engendering refractoriness to mosquito midgut infection. Mechanistically, our data also suggests that this mutation affected the virus-ER-resident protein interactions that impacted the efficiency of DENV replication and hence induction of the innate immune response. Our findings reveal insights into the pathogenesis of dengue and adds to the body of knowledge on critical virus-host interactions that govern epidemiological fitness of DENV.

Crimean-Congo hemorrhagic fever in Yazd Province of Iran, a case-control study on epidemiological characteristics

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tums, Tehran, Iran, Islamic Republic of

Abstract

Abstract

Objectives: Several cases of Crimean-Congo hemorrhagic fever (CCHF), an arboviral disease, have been reported in different areas of Iran. The main objectives of this research were to determine the most important means and patterns of transmission and the epidemiologic characteristics of this disease.

Results: Variables which increased the chance of disease include: history of slaughtering (OR = 7.57, CI: 2.21–25.91), high-risk occupations (OR = 4.97, CI: 0.97–25.43), history of tick bite (OR = 105.89, CI: 9.32–1202.44), age above 40 years (OR = 7.32, CI: 1.06–50.26).

Conclusion: The results of this study confirm that the scheme of risk factors and risk groups for Crimean-Congo hemorrhagic fever (CCHF) in Iran do not differ substantially from the other parts of the world.

Key word: Arboviral disease, Crimean-Congo hemorrhagic fever (CCHF), *Yazd*

Agua Salud alphavirus defines a novel lineage of insect-specific alphaviruses discovered in the New World

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Abstract

Most alphaviruses are transmitted by arthropods and infect a broad range of vertebrate hosts. So far, only three mosquito-specific alphaviruses have been detected in the Old World. Mosquito-specific negeviruses are distantly related to alphaviruses and have been co-detected with mosquito-specific alphaviruses. The aim of this study was to analyze the genetic diversity of alphaviruses in mosquitoes of the New World and to test for putative interactions between alpha- and negeviruses.

Mosquitoes were collected in Panama. Mosquito pools were screened with a generic alphavirus RT-PCR. Full genomes were sequenced and analyzed. Virus growth kinetics were performed in insect and vertebrate cell lines.

In total 13,806 mosquitoes (1,414 pools) were tested for alphaviruses. One pool was found positive for a previously unknown alphavirus, tentatively named Agua Salud alphavirus (ASALV). Phylogenetic analyses placed ASALV basal to the mosquito-specific alphaviruses and the WEE clade. ASALV was successfully isolated in insect cells but vertebrate cells did not support ASALV infection. Replication in insect cells was blocked at temperatures above 31°C suggesting that ASALV is a novel mosquito-specific alphavirus. In addition, an unknown negevirus was detected in the ASALV-positive mosquito. Co- and superinfections of mosquito cells with ASALV and this negevirus revealed neither inhibitory nor synergistic effects but an increased cytopathogenicity.

We discovered and characterized the first mosquito-specific alphavirus of the New World. Our data suggest that mosquito-specific alphaviruses are more divergent than previously observed and may interact with negeviruses. Understanding of such interactions may provide novel targets to control alphavirus infections in mosquitoes.

Establishment of a stable subgenomic dengue virus type 1 replicon system in *Aedes albopictus* mosquito cells for identification of DENV transmission blocking molecules

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Abstract

To combat DENV transmission, we aim to screen for molecules that target mosquito host cell factors playing a role in the viral replication, consequently inhibiting the replication of virus in the mosquito. Use case scenarios for such compounds include human mass drug administration in endemic populations, application in sugar baits or bed-nets.

In order to accomplish this, we have set up high throughput screening (HTS) using viral replication inhibition assays. We describe here the generation of a novel stable Nanoluciferase-reporter based dengue replicon system in U4.4 mosquito cells. The U4.4-DENV1 replicon cell line has been stably and successfully maintained for over 30 passages without significant loss of reporter signal. In order to characterize the cell line further, the cells were subjected to treatment with antiviral compound and viral inhibition was observed with ribavirin (IC_{50} =1.69 x 10^{-6} M) and siRNA against NS3. For the purpose of HTS, viral inhibition, cytotoxicity and luciferase interference assays were established on a 384-well plate using the cryopreserved U4.4_DENV1 replicon cells. The latter two were used as deselection assays to differentiate between false- and true- positives. To conclude, we have developed a robust screening cascade to identify small molecules that may act as transmission blocking compounds. Screening of a chemical diversity library is ongoing and the results will be presented at the meeting.

RNAi response of vector mosquitoes against mosquito-specific viruses

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Abstract

In contrast to arboviruses, mosquito-specific viruses (MSVs) are characterized by their restriction to mosquito hosts. Although not fully investigated for many MSVs, it is suspected that these viruses are distributed worldwide and also infect mosquitoes transmitting arboviruses of medical importance.

It has been shown previously that RNAi is the main defense mechanism of mosquitoes against arboviruses and that some MSVs trigger and interfere with these pathways. It is unclear, whether this is universally the case for MSVs and whether this interaction could in turn affect co-infections with arboviruses.

Here, we performed knock-downs of key proteins of the different RNAi pathways (siRNA, miRNA, piRNA) and determined the effect on viral replication of the insect-specific Agua Salud alphavirus (ASALV) *in vitro*. ASALV has recently been isolated from a pool of *Culex* mosquitoes and remains one of few identified insect-specific alphaviruses.

Moreover, small RNA sequencing of ASALV-infected cells was performed to investigate the production of different small RNAs, comparing this to the RNAi response induced by arboviral alphaviruses.

We have found that the siRNA pathway, similarly to arboviral infections, seems to act antiviral with ASALV *in vitro*. Knock-down of some key proteins of the piRNA pathway resulted in increased viral RNA replication. However the extent to which piRNAs are involved in viral control remains unclear. Interestingly, the miRNA pathway also seems to act antiviral.

Gaining insights into the interaction of MSVs with the RNAi pathways is a crucial first step to judge potential effects on vector competence of mosquitoes for arboviruses.

Subgenomic flavivirus RNA binds the antiviral mosquito DEAD/H-box helicase ME31B and determines Zika virus transmission by Aedes aegypti

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Abstract

Zika virus (ZIKV) and dengue virus are transmitted by *Aedes aegypti* mosquitoes and pose a global human health threat. All flaviviruses, including those exclusively replicating in mosquitoes, produce a highly abundant, non-coding subgenomic flavivirus RNA (sfRNA) in infected cells, which implies an important function of sfRNA during mosquito infection. However, the functional role of sfRNA in flavivirus transmission by mosquitoes is not well understood. Here, we demonstrate that an sfRNA-deficient ZIKV (ZIKVΔSF1) replicates similar to wild type ZIKV in mosquito cell culture, but is severely attenuated in *Ae. aegypti*, with 5% saliva-positive mosquitoes for ZIKVΔSF1 vs. 31% for ZIKV. Thus, sfRNA is a key determinant of ZIKV transmission by *Ae. aegypti*. Comparison of mosquito infection via blood meals or intrathoracic injections shows that sfRNA is important for ZIKV to overcome the mosquito midgut barrier. Next-generation sequencing of small RNAs from mosquitoes infected with ZIKV or ZIKVΔSF1 reveals that sfRNA production correlates with decreased viral small-interfering RNA levels, suggesting that sfRNA suppresses RNA interference (RNAi) *in vivo* to facilitate virus transmission. RNA-affinity purification followed by mass spectrometry analysis uncovered that sfRNA specifically interacts with a limited set of *Ae. aegypti* proteins normally associated with RNA turnover and protein translation. The DEAD-box helicase ME31B showed the highest affinity to sfRNA and displayed potent antiviral activity against ZIKV in *Ae. aegypti* cells. We present a mechanistic model in which sfRNA suppresses RNAi and sequesters ME31B to promote flavivirus replication and facilitate transmission by mosquitoes.

Glucose regulated protein 78 (GRP78) interacts with Zika virus envelope and is required for a productive infection.

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Abstract

Zika virus (ZIKV) is a member of the *Flaviviridae* family and was until recently a relatively obscure tropical disease. Subsequently, ZIKV has been shown to be the causative agent of fetal abnormalities and Guillain-Barré syndrome in outbreaks across the Americas and so efforts towards delineating important factors in the viral lifecycle have increased. Combining protein pull-down with mass spectrometry, it was found that ZIKV envelope (Env) interacts with the endoplasmic reticulum (ER) resident chaperone, glucose regulated protein 78 (GRP78) in A549 cells. Flaviviruses such as Japanese encephalitis virus and dengue virus are known to co-opt ER resident proteins and members of the unfolded protein response, including GRP78, to enhance viral infectivity and propagation. The role these proteins play during the ZIKV lifecycle has yet to be elucidated.

To determine the importance of this interaction during ZIKV infection, A549 cells were treated with GRP78-specific siRNAs prior to infection with a NanoLuc expressing reporter virus or a wild-type virus. Depletion of GRP78 significantly reduced both virus luciferase readings and viral titres, indicating that GRP78 is necessary for efficient infection of mammalian cell culture. In contrast, inhibition of GRP78 with small molecule inhibitors did not reduce ZIKV infection. Interestingly, immunofluorescence of ZIKV infected cells reveal that GRP78 re-localises following infection and co-localises with Env. Depletion of GRP78 abrogated localisation of viral replication factories. Further experiments have shown that GRP78 is important for infection post entry and replication, and that putative GRP78 interactions partners are also required during infection.

Chikungunya virus resistant to the antiviral favipiravir is severely attenuated in mosquitoes

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Abstract

It is currently unclear whether antiviral drug-resistant arboviruses can be transmitted by their mosquito vectors. We showed previously that the dissemination and the transmission of a chikungunya virus (CHIKV) resistant to the antiviral favipiravir was markedly decreased as compared to WT (Delang et al, mSphere 2018). The attenuated phenotype of this resistant virus was confirmed in Aag2 and C6/36 mosquito cells. Here, we aimed to study the mechanism of the attenuated phenotype in more detail.

First, replication kinetics studies at 32°C for both Vero and mosquito cells confirmed that the attenuated fitness in mosquito cells is associated with the cell line and not with temperature. A passaging experiment of WT CHIKV on Vero cells in the absence of favipiravir showed that the observed attenuation of the resistant CHIKV was not due to passaging on Vero cells during the resistance selection. To identify the molecular mechanism of the attenuated phenotype, the genes encoding for the non-structural proteins (nsP) of the favipiravir^{res} CHIKV were swapped into a WT CHIKV backbone. The replication fitness of these nsP2, nsP3 or nsP4 single swap variants did not differ significantly from the fitness of WT CHIKV in Vero and C6/36 cells, indicating that a combination of mutations in multiple non-structural proteins is responsible for the attenuated phenotype. Double nsP swap variants are therefore constructed and these will provide additional information on the molecular mechanism of the attenuation. Together, our results may provide interesting insights in the mosquito tropism of CHIKV.

Massilia virus circulation in sand flies: the role of co-feeding on sugar meal

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Abstract

Background: Massilia virus (MASV), isolated from *Phlebotomus perniciosus*, is a phlebovirus closely related to human pathogens such as Toscana virus. The natural cycle of phleboviruses is poorly understood; transovarial and sexual transmission are not efficient enough for the maintenance of the virus in nature and to date there is no convincing evidence that a species of vertebrates is the reservoir of the virus.

Methods: In *P. perniciosus* 4 types of MASV infection were compared: by larval food to the first instar or to the fourth instar larvae, by blood meal to adult females and by sugar meal to adults of both sexes. Moreover, sugar meal infection was also tested with six other species of sand flies.

Results: From 875 adults emerged from infected larvae, only three were positive. In females infected by bloodmeal the infection rate was high before defecation, and then it decreased drastically. Surprisingly, the most efficient route of infection was through sugar meal: 72% of females (79/110) and 52% of males (51/99) became MASV RNA-positive. Moreover, MASV-infected sand flies regurgitated virus particles into the sugar drop and almost 30% of *P. perniciosus* get MASV from the sugar with expectorated virus.

Conclusions: We present the first description of arboviral infection of a dipteran vector using sugar meal. Our results showed that MASV can be transmitted between *P. perniciosus* either through co-feeding or via an infected sugar source such as plant sap, which may play an important role in the circulation of phleboviruses in nature.

Lumpy skin disease virus does not replicate productively in insect cell lines

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Abstract

Lumpy skin disease virus (LSDV) is a capripoxvirus of cattle causing a severe disease that results in substantial economic impact to affected communities. The virus is endemic across sub-Saharan Africa, and has recently entered Europe and the Balkans. The virus is thought to be transmitted by an insect vector, but little is known about the role of the vector in the LSDV cycle.

In order to investigate interactions between LSDV and insect vectors this work studied the permissivity of insect cell lines for LSDV. Insect cell lines were inoculated with LSDV strain Cameroon at a multiplicity of infection (MOI) of 5. The mammalian cell line MDBK was infected as a positive control. Samples were collected up to a week after infection and virus amounts measured using titrated plaque assays in order to construct a growth curve.

Lumpy skin disease virus replicated in the MDBK mammalian cell line, increasing by $3\log_{10}$ over the 7 day incubation from $2x10^4$ pfu/ml to $2x10^7$ pfu/ml. In contrast, no replication of LSDV was detected in the insect cell lines.

This work shows that LSDV does not productively replicate in insect cell lines and supports the current hypothesis that the insects act as mechanical rather than biological vectors of the virus.

Novel mosquito insect-specific flavivirus from the Czech republic

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Abstract

Background: Insect-specific flaviviruses (ISFs) are described almost all over the world, however, the majority of ISFs have not been discovered yet. There is only limited information about their ecology but several studies demonstrate that some ISFs can influence replication of other medically important flaviviruses and probably represent an ancestral lineage of the family *Flaviviridae*.

Methods: In our surveillance study we investigated the presence of ISFs in mosquitoes captured in various localities in Czechia. Collected mosquitoes were tested by RNA isolation, reverse transcription and specific PCR with *Flavivirus* universal primer pairs. We succeeded one novel ISF isolation on C6/36 mosquito cell culture and experimental infections with *Culex* mosquitoes were performed.

Results: Several interesting observations were noticed about the novel ISF from Czechia. In contrast to the majority of ISFs described till now, no cytopathic effect was detected and quantification of virus particles was impossible by most regular methods. The infection of cell culture was confirmed by PCR, with *Flavivirus* universal primer pairs, as late as 19 days post inoculation, not earlier. Four different methods were tested to experimentally infect laboratory reared *Culex* mosquitoes, but none of them led to persisting infection.

Conclusions: In our surveillance study the ISFs presence in mosquitoes captured in Czechia was investigated and RNA sequences representing four novel ISFs related to previously described ISFs were detected in almost all mosquito species. One of these viruses was isolated on C6/36 mosquito cell culture. The combination of NGS and conventional PCRs with specifically designed primer pairs provides the complete genome of this ISF.

Posaconazole is a novel inhibitor for alphavirus viral entry

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Abstract

Chikungunya virus (CHIKV), a mosquito-borne alphavirus, causes millions of infection globally. Posaconazole (PCZ) is an antifungal drug, which we and others have previously found to inhibit replication of a number of viruses, including dengue virus, a member of the *Flaviviridae* family. In this study, we analyzed the antiviral activity of PCZ against alphaviruses. We found that PCZ potently inhibits a number of alphaviruses, including Semliki forest virus (SFV), Sindbis virus (SINV) and CHIKV with half maximal effective concentration (EC_{50}) of 2.3 µM, 4.0 µM and 0.8 µM, respectively. Time-of-addition assays indicated that PCZ treatment before and at the time of SFV infection showed potent inhibition, whereas addition of PCZ at later time points post infection showed minor to no inhibition, suggesting inhibition at an early stage of the replication cycle. In accordance, PCZ treatment of a temperature sensitive mutant of SFV that is capable of cell entry and translation, but not RNA replication, resulted in an almost 90% reduction in luciferase activity. To confirm these findings, PCZ resistant mutant virus were generated and we identified mutations in E1 (V148A) and E2 (H255R) viral glycoproteins, of which the E2 mutation confers partial resistance to PCZ when introduced into wild-type SFV. To see whether PCZ alters clathrin-mediated endocytosis, we analyzed the uptake of fluorescence-tagged transferrin and found that PCZ reduced transferrin uptake by 50% compared to DMSO-treated cells. Together, these results establish PCZ as a novel inhibitor of alphaviruses and identify viral entry as its target.

The effect of tick saliva on oxidative stress responses in TBEV infected macrophages

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Abstract

Tick borne encephalitis virus (TBEV) is transmitted to human generally by the bite of an infected tick. Virus is transferred via tick saliva that influences host defence mechanisms by anti-haemostatic, anti-inflammatory, and immunosuppressive effects and creates a unique environment for pathogen transmission. Oxidative stress can affect antiviral and apoptotic responses of cells. We investigate whether TBEV infection of macrophages leads to oxidative stress and how it is affected by tick saliva.

The primary macrophages from C57BL/6N mice were infected with TBEV (strain Hypr) in the presence or absence of tick saliva. The production of ROS was determined by staining with fluorescence dye H_2DCFDA followed by fluorimetric analysis. Mitochondrial membrane potential (MMP) was determined by staining with fluorescence dye JC-1. The induction of Nrf2/ARE antioxidant pathway was assessed by gene expression levels of selected Nrf2-regulated genes by qPCR.

We found that ROS production was increased in TBEV-infected macrophages. Although ROS are essential for activation of Nrf2/ARE pathway, no induction of Nrf2-regulated genes was observed in infected cells. We assume that Hypr-induced increase of ROS was not sufficient to induce Nrf2/ARE pathway. The effect of tick saliva was determined by MMP changes and Nrf2/ARE pathway activation. Tick saliva led to both, the MMP depolarization and the induction of Nrf2/ARE pathway shortly upon infection.

In conclusion, tick saliva potentiates antioxidant Nrf2/ARE pathway likely through brief increase of mitochondrial ROS. Antioxidant enzymes may consequently increase a resistance to oxidative stress and thus influence cellular immune response.

Interrogation of the CHIKV nsP-host interactome in human and mosquito cells.

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Abstract

Chikungunya virus is a human pathogen transmitted by mosquitos. After a genetic adaptation of the virus that allows increased spreading by Aedes albopictus mosquitos, the past decade, Chikungunya virus has spread across the globe. In a significant number of patients, Chikungunya virus causes a chronic, debilitating, arthritic joint pain.
Chikungunya virus replicates in cells of both its vertebrate host and insect vector. To identify cellular pathways that the virus engages to allow optimal replication in these evolutionary distinct organisms we performed AP-MS to identify interaction partners of the viral non-structural proteins (nsPs) in both human and mosquito cells. Mass spectrometric analysis of on-bead-digestions of affinity purifications coupled to MiST analysis allowed sensitive and reproducible identification of a significant number of cellular protein interaction partners of nsP1, -3 and -4. The retrieval of well-established nsP3 interactors, G3BP and Bin1, in both human and mosquito cells validated our approach. Separate nsPs were associated with both shared and unique interaction partners, the latter belonging to different cellular pathways. Comparison of high-confidence interactors of nsP3 in human and mosquito cells identified 25 proteins that associate with nsP3 in both organisms. Functional classification of these shared nsP3 interactors using GO annotation showed engagement of cell-cell adhesion-, Hippo signaling-, ribosomal function- and innate immune signaling pathways by nsP3 in both human and mosquito cells. Interaction of members of each functional group with nsP3 were validated in AP-WB experiments. Functional roles in viral replication of several of these interactors are evaluated using knockouts.

The Basigin (CD147)-CD98 protein complex is involved in Chikungunya virus attachment and entry in human cells

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Abstract

Chikungunya virus (CHIKV), a positive stranded RNA alphavirus, recently reemerged causing multiple outbreaks around the world. Generally, alphaviruses enter the cell via clathrin-mediated endocytosis. Entry is supported by the structural envelope proteins E2 and E1. Different experimental approaches have been applied previously to identify receptor(s) molecules responsible for CHIKV binding and entry in human and mosquito cells. However they cannot account for all CHIKV entry events in all susceptible cell types.

We performed affinity purification coupled to mass spectrometry to identify entry factors of CHIKV in both human and mosquito cells. We transiently expressed the N-terminally Strep-tagged, full-length envelope gene in 293T and C6/36 cells. Affinity purifications were digested on-bead and analyzed by mass spectrometry. MiST analysis allowed the identification of 39 human proteins with a confidence score above 0.8. Twelve proteins were selected for validation with CRISPR/Cas9 knock-out cells. Three separate AP-MS experiments in C6/36 cells led to the identification of 31, 58 and 11 proteins with a MiST score higher than 0.7, of which 19 proteins were chosen for further analysis.

Using knock-out experiments in 293T cells and a reporter CHIKV (ECSA strain) resulted in the identification of the CD147-CD98 protein complex on human cells as possible entry factor. Repetition of this knock-out experiment using an Asian CHIKV strain combined with E2 staining, confirmed these results. CD147 contains 2 immunoglobulin domains which is similar to MXRA8, a previously identified alphavirus entry factor. The interaction of CD147 with E2 was validated on Western Blot after affinity purification.

Mosquito vector factors driving arbovirus infection in Anopheles

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Abstract

Anopheles mosquitoes are efficient vectors of human malaria, but they are the primary vector of just one arbovirus, O'nyong nyong virus (ONNV). Consequently Anopheles-virus interactions have been relatively unexamined. It is puzzling that almost all arbovirus transmission is mediated by Aedes and its relatives while Anopheles transmit just a single virus. One hypothesis is that antiviral mechanisms are more efficient in Anopheles than Aedes, but ONNV can circumvent them. Here, we combined empirical data and bioinformatic analysis to generate a high-value set of Anopheles candidate genes likely involved in host-virus interactions. Many of the candidate genes are unannotated. We screened our panel of genes through in vitro gene silencing and identified both proviral and antiviral factors in Anopheles mosquitos. So far, the many genes tested have shown an effect on ONNV replication. For instance, the silencing of the unannotated gene AGAP00570 limited 80% of viral replication suggesting its proviral role in the ONNV-Anopheles cells system. Similarly, silencing of the Leucine-Rich repeat IMmune 4 (LRIM4) reduced the viral replication in 78%. On the contrary, preliminary data in another leucine-rich repeat protein, the APL1C, indicates the antiviral activity of this gene in Anopheles cells. ONNV is an emergent virus in Africa with epidemic potential, and these results reveal the host vector factors that influence mosquito-ONNV infection.

Mosquito co-infection with Usutu virus and West Nile virus decreases vector competence for both viruses

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Abstract

Usutu virus (USUV) and West Nile virus (WNV) are mosquito-borne flaviviruses that are currently co-circulating in Europe. Both viruses maintain a similar transmission cycle involving mosquitoes and bird, whereas human infection is thought to be incidental. However, during the summer of 2018, an increase in human infections of both USUV and WNV was reported, which also includes a co-infection case. So far, the interaction between USUV and WNV during their transmission cycle and to what extent co-infection will affect transmission outcomes remains unclear. Here we investigated the impact of co-infection on vector competence of *Culex pipiens*, the main vector for both USUV and WNV. A dual-colour Taqman qPCR assay was used to simultaneously determine the infection and transmission rate of both viruses in the co-infected mosquitoes. We observed a decreased infection rate of both USUV (62% to 29%) and WNV (56% to 38%) in a co-infection scenario compared to single infection. The transmission rates also decreased, in particular for USUV (18% to 3%) but less for WNV (39% to 26%). This suggests that USUV is outcompeted by WNV during mosquito infection, a result which we could confirm in cell lines of different origin (mosquito, bird and primate). Surprisingly, only one mosquito saliva sample (1 out of 89) was positive for both WNV and USUV, indicating that the chance of transmission of both viruses via a single mosquito bite is limited. Altogether, the current study shows co-infection of USUV and WNV alters the vector competence of *Culex pipiens* for both viruses.

Identification of candidate molecular determinants of the vector competence of *Ixodes ricinus* for members of the tick-borne encephalitis complex

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Abstract

In Europe, tick-borne encephalitis virus (TBEV) and louping ill virus (LIV) are two flaviviruses both transmitted by the tick *Ixodes ricinus*. While the mechanisms of viral replication and transmission in this vector are incompletely understood, they are presumed to be largely governed by protein-protein interactions established between viruses and cells.

To elucidate the molecular determinants involved in vector competence, we have mapped the network of protein-protein interactions established between viral proteins of both TBEV and LIV and tick proteins encoded by a cDNA library of *I. ricinus*, by using yeast two-hybrid methodology. Twenty-two cellular partners from *I. ricinus* have been identified and all evidenced to interact with both viruses. Upon functional annotation, some of these tick proteins seem to be involved in such biological processes as the immune response or ribosomal maturation. To gain insight into the role of each tick protein in viral replication, the impact of gene silencing will be assessed by dsRNA knockdown. In parallel, *I. ricinus* cell lines have been persistently infected with TBEV or LIV and the level of expression of selected antiviral effectors monitored over time, in an effort to characterize the antiviral response of this arthropod.

This work represents the first description of the protein-protein interaction network for TBEV, LIV and *I. ricinus*. Certain tick partners may well represent molecular determinants of vector competence of *I. ricinus* for TBEV and LIV and potentially other flaviviruses, which we will ascertain by *in vivo* silencing of selected tick partners.

To be fit or not to be fit: mechanisms and fitness consequences of dengue virus resistance to ciprofloxacin

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Abstract

There are no licensed antiviral drugs to treat dengue virus (DENV) infection, but fluoroquinolones have been shown to suppress flavivirus replication *in vitro* and in animal models. To investigate mechanisms and potential fitness costs associated with evolution of resistance to fluoroquinolones, DENV was passaged in triplicate in human embryonic kidney (HEK293) cells in the presence of the fluoroquinolone ciprofloxacin until resistance was detected at passage 7. Triplicate control DENV lines were passaged in media. The ORF of all six lines as well as the parent were sequenced, revealing numerous mutations in both control and ciprofloxacin-resistant lines known to confer adaptation to cultured mammalian cells. Additionally, V15L was detected in the envelope gene of two of the three ciprofloxacin-resistant lines and E417A was detected in the envelope gene of the third resistant line, suggesting that ciprofloxacin may target viral entry. Multicycle replication curves in designated cell lines were used to assess fitness. In HEK293 cells, as expected, both resistant and control lines were considerably more fit than parent virus, and, on days 3, 4, and 6 post infection (p.i), resistant lines replicated to significantly higher levels than control lines (rmANOVA, DF (6, 24)=6.1, $P < 0.001$). However, replication of resistant and control lines were indistinguishable in *Ae. albopictus* C6/36 cells (rmANOVA, DF(6, 28)=0.6, P=0.7). These data suggest that ciprofloxacin resistance was gained through a general increase in fitness in mammalian cells that did not extend to mosquito cells. Ciprofloxacin-resistant lines were cross-resistant to difloxacin but not enoxacin.

Dissecting the genetic basis of Zika virus susceptibility in Aedes aegypti

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Abstract

Zika virus (ZIKV) is an emerging arbovirus mainly transmitted among humans by Aedes aegypti mosquitoes. We surveyed natural variation in ZIKV susceptibility among eight field-derived Ae. aegypti populations representing the global range of the species, following artificial oral exposure to six low-passage ZIKV strains spanning the current viral genetic diversity. Our results reveal that African Ae. aegypti are significantly less susceptible than non-African Ae. aegypti across all ZIKV strains tested. This difference translates into significantly lower vector competence in a mouse model of ZIKV infection. We are in the process of mapping genes underlying the striking difference in ZIKV susceptibility between African and non-African Ae. aegypti populations using a genetic cross and advanced methods of quantitative genetics. Unraveling the genetic basis of ZIKV susceptibility in Ae. aegypti will contribute to shed light on epidemiological patterns and support the development of novel disease control strategies

Transcriptomic analysis of human neurons and astrocytes infected with TBEV strains of different virulence

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Abstract

Tick-borne encephalitis virus (TBEV; *Flaviviridae*) can cause serious infections in humans which may result in encephalitis/meningoencephalitis. It has been previously reported that TBEV infects both, neurons and astrocytes, however, with a different outcome. So far, the principle of this cell type-specific response to TBEV is not fully understood.

In order to gain more insight into this phenomenon, we described new *in vitro* infection model utilizing human neural stem cells (hNSCs) and two strains of Western European TBEV subtype varying in the pathogenicity - mild Neudoerfl and severe Hypr. In detail, neurons and astrocytes were artificially differentiated from hNSCs and presence of CNS markers was checked. TBEV infection in both cell types was characterised afterwards. As expected, both cell types proved to be susceptible to TBEV infection. Viability was negatively affected only in infected neurons. In order to identify possible effectors responsible for different susceptibility of neurons and astrocytes, the analyses of changes in poly-(A) and small RNA transcriptome upon TBEV infection were performed. Preliminary results from poly-(A) RNA transcriptome revealed that in both cell types mainly interferon-stimulated genes (ISGs) were up-regulated. However, the expression kinetics of particular ISGs varied. In addition, the vast spectrum of long non-coding RNAs was described to be differentially expressed upon infection. Surprisingly, U1 snRNA was found to be the most down-regulated RNA species among almost all infected samples.

Further analyses are in progress in order to get a complete description of virus-induced changes on the transcriptomic level.

Transcriptional and translation shut-off in TBEV infected neural cells and involvement of viral C protein

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Abstract

Tick-borne encephalitis virus (TBEV, *Flaviviridae*) infection causes severe neurological disease and incapacitates more than 10 000 patients annually in the Eurasian region. Despite extensive studies, some areas of interaction of TBEV with the host cells remain undescribed. Here we investigated the interaction of TBEV and human neural DAOY HTB-186 cells on the transcriptional and translational level.

By labelling of nascent RNA and protein molecules in TBEV-infected DAOY cells, we showed that the virus-induced host translational shut-off. Moreover, TBEV interfered also with the expression of host ribosomal RNAs, in particular with the rRNA species transcribed by RNA polymerase I (18S rRNA, 28S rRNA, and their precursor 45-47S pre-rRNA). Synthesis of host rRNAs is an essential host cell process that is localized in the nucleus, namely nucleoli. By searching for virus factor that could be linked with these effects, we described so far unknown nucleolar localization of TBEV capsid protein C. More importantly, preliminary data from transfection of recombinant C protein led to the reduction in nascent protein synthesis indicating the link between TBEV capsid protein and shut-off phenomena which were described. Furthermore, we identified a potential nuclear localization signal, which seems not to be essential for the shut-down effect.

Taken together we described a brand new type of interaction between TBEV and host neural cells on the transcriptional and translational level and identified viral factor potentially responsible for the observed phenomena. However, further analyses are needed, and the particular mechanism of action remains still elusive.

AurKB ACTIVITY IS NECESSARY FOR DENGUE VIRUS RELEASE

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Abstract

Flaviviruses, such as Dengue (DENV), Zika and Yellow Fever are pathogens with high morbidity and mortality. Around 390 million people per year are infected with DENV, and almost 90 million develop the clinical forms of the infection. In the present work, we analyzed the role of Aurora Kinase B (AurKB) in the replicative cycle of DENV. This Kinase regulates the activation of ESCRT-III complex, which has an essential role in the viral morphogenesis and/or budding from RE to Golgi apparatus. The compound ZM 447439 (ZM) was used to inhibit specifically AurKB, and the viral progeny, viral RNA/protein synthesis efficiency and NS1 secretion were evaluated. The kinase inhibition did not alter the viral protein production/secretion or genome replication but impaired the viral yield without altering the percentage of infected cells.

Moreover, confocal microscopy analysis of DENV-infected ZM447439-treated cells shows a delocalization of viral components from the replicative complexes. In summary, these observations indicate that AurKB participates in DENV viral morphogenesis or release. Together, our results suggest possible participation of AurKB in the viral release of budding through activation of the ESCRT-III complex and suggest a new role for AurKB on flavivirus viral cycle.

The effect of co-infecting *Anaplasma phagocytophilum* on replication of Langat Virus, a model for Louping ill virus, in *Ixodes* spp. tick cells.

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Abstract

Tick borne fever (TBF) caused by *Anaplasma phagocytophilum* (Ap), and louping ill caused by the flavivirus louping ill virus (LIV) are the two most economically important vector-borne diseases in UK sheep populations. Both pathogens are transmitted by the tick *Ixodes ricinus*, which also harbours protozoan parasites and *Borrelia* spp. spirochaetes. *I. ricinus* ticks may be co-infected with multiple microorganisms and potentially transmit more than one pathogen to hosts during blood feeding.

Ap infection is not limited to sheep, causing pasture fever in cattle and granulocytic anaplasmosis in horses, dogs and humans. There is no vaccine available for TBF, and disease control relies on tick control and antibiotic treatment. LIV mainly infects ruminants, but can also infect other livestock including horses, pigs, alpacas and llamas. Since 2016, the LIV vaccine has been unavailable and there is no alternative prophylactic treatment for livestock. Both Ap and LIV are zoonotic diseases with occasional human cases reported in the UK.

Importantly, Ap infection leads to host immunosuppression and consequently increases vulnerability to secondary infections. Both Ap and LIV have been studied as single infections in tick and mammalian cells. However the dynamics and implications of co-infections within the arthropod vector or mammalian host have, to date, not been fully explored.

Using embryo-derived *Ixodes* spp. cell lines infected with Langat virus (a BSL-2 model for LIV) and Ap, we examined the effect of co-infection on viral RNA replication by qRT-PCR and bacterial growth by qPCR. The results of this study will be presented and discussed.

Newly discovered antiviral non-self RNA sensing forms a barrier for flavivirus transmission

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Abstract

In the DNA of most organisms TpA (UpA in RNA) dinucleotides are under-represented. Vertebrate genomes additionally show strong suppression of CpG dinucleotides. In contrast, invertebrates do not suppress CpG dinucleotides. Most RNA viruses mimic host mRNA composition and many mammalian viruses become attenuated when CpG/UpA dinucleotide frequencies are artificially increased. The genus *Flavivirus* contains both vertebrate-infecting viruses (VIF) with suppressed genomic CpG dinucleotide frequencies and insect-specific flaviviruses (ISF) that contain higher frequencies of genomic CpG dinucleotides. We recently showed that the vertebrate Zinc finger antiviral protein (ZAP) can detect and bind RNA that is high in CpG/UpA dinucleotide frequencies and that this non-self RNA recognition pathway effectively attenuates virus replication in vertebrate cells. Here we investigated how this affects the transmission and evolution of mosquito(-borne) viruses that need to cope with two compositionally distinct environments.

We show that knockout of ZAP makes human cells susceptible for infection by ISF, which suggests that ZAP is a barrier that protects vertebrate cells from infection by (CpG-high) insect viruses. Infection of mice with synonymous ZIKV mutants provides the first evidence that arbovirus replication is attenuated by increased CpG/UpA dinucleotide frequencies. Interestingly, infection of live *Aedes aegypti* with these ZIKV mutants suggests that also invertebrates can sense non-self RNA based on the RNA's dinucleotide composition. However, in the mosquito this seems tuned to a different threshold. This suggests that both vertebrate and invertebrate animals may possess distinct non-self RNA sensing pathways that need to be negotiated by arboviruses.

Correlative Light and Soft X-ray Cryo-Tomography Reveals Details of Wolbachia-Mediated Modulations to Cellular Ultrastructure.

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Abstract

Wolbachia is an intracellular symbiont found in an abundance of insects. Upon transinfection into *Aedes aegypti* - the mosquito vector responsible for Dengue, Zika and Chikungunya virus transmission - *Wolbachia* is able to block viral transmission from the vector to new hosts. Thus, *Wolbachia* is emerging as an important biological modulator of viral transmission.

In order to visualise how *Wolbachia* modulates cellular architecture, we employed a newly emerging imaging method - correlative light and soft X-ray cryo-tomography (CLXT) - on frozen, hydrated *Ae. aegypti* cells. With transinfected *Wolbachia* being intracellular endosymbionts of *Ae. aegypti*, SXCT is an excellent imaging technique to visualise and understand the cellular architecture of *Ae. aegypti* cells in a near-native state, and also *Wolbachia*-mediated effects on ultrastructure.

Through using CLXT we have discovered what *Ae. aegypti* cells look like at the ultrastructural level. Mosquito cells are filled with vesicles and vacuoles and, curiously, contain intranuclear vesicles. In the presence of *Wolbachia*, these intranuclear vesicles disappear, providing a potential cellular basis of *Wolbachia*-mediated virus block. In the presence of *Wolbachia* we also see an increase in lipid droplets and dense, intracellular compartments that correlate with our fluorescent *Wolbachia* markers. This means that, for the first time, we have performed *in situ* imaging of *Wolbachia* in live cells.

We are now using our experience in CLXM to image alphavirus and flavivirus replication factories *in situ*. Additionally, we are now carrying out molecular biology experiments to better understand the biochemical basis of the cellular architecture we have visualised.

Pan-viral protection against arboviruses by targeting inoculation site-based skin macrophages

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Abstract

Arthropod-borne viruses (arboviruses) are important human and animal pathogens for which there are no specific antiviral medicines. Using an in vivo model of arbovirus infection; which incorporates the bite of *aedes aegypti* mosquitoes and injection with Semliki forest virus and Bunyamwera virus and an *ex vivo* human skin explant model of Zika virus and chikungunya virus infection, we have investigated key aspects of the innate immune response to these viruses at cutaneous bite sites. We suggest that therapeutic intervention at this site represents a novel and plausible strategy for targeting arbovirus infection. We investigated whether augmenting innate immune responses at the inoculation site could affect the local and systemic course of infection and improve disease outcome. *In vivo*, topical or subcutaneous delivery of innate immune agonists significantly decreased viral replication at the inoculation site, reduced dissemination of the virus to remote tissues and limited the development of clinical disease. These effects were dependent on a functioning type I Interferon (IFN) response in skin resident dermal macrophages. *In vitro*, protection against infection of keratinocytes and fibroblasts required a coordinated response by leukocytes in response to innate immune agonists. This work suggests that early events at the mosquito bite site, a common aspect of all mosquito-borne virus infections, are critically important for defining subsequent disease severity and clinical outcome. We show that targeting this site through post exposure prophylactic intervention provides a novel treatment modality for limiting the development of these diseases.

Zika virus utilises the ubiquitin-proteasome pathway during infection of both mosquito and human cells

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Abstract

Arthropod-borne viruses are able to infect vertebrate and non-vertebrate hosts. One such arbovirus is Zika virus (family Flaviviridae) that is mainly transmitted to humans by *Aedes* mosquitoes causing febrile illness and congenital Zika syndrome in infants. An interplay between host and virus proteins enables ZIKV to manipulate its host's cellular machineries in order to facilitate infection and evade antiviral responses. A possible mechanism it utilises is the ubiquitin-proteasome pathway (UPP) where target proteins are ubiquitinated and subsequently degraded by the proteasome. Results of proteomics analysis of *Ae. aegypti* cell lines (AF5) stably expressing V5-tagged ZIKV capsid (C), anchored capsid (AC) or non-structural 3 (NS3) proteins revealed that these viral proteins interact with effector proteins of the UPP. One of these proteins is TER94 an AAA-ATPase that acts as a chaperone segregating ubiquitinated proteins to the proteasome complex. Knockdown experiments of TER94 or its human ortholog VCP using dsRNA or siRNAs showed reduced virus replication in AF5 or A549 cells. Using small molecule inhibitors of UPP proteins also diminished ZIKV replication. Inhibiting different stages of the pathway have led to the identification of critical steps during early stages of infection. The ubiqutination of lysine-rich ZIKV C and its interaction with TER9/VCP could be one of the many universal strategies that the virus employs when it switches between mosquito and human hosts. Understanding how ZIKV is able to infect both human and insect species could provide novel strategies for prevention and therapeutics.

St Abbs Head phlebovirus – a separate virus species or a strain of Uukuniemi phlebovirus?

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Abstract

St Abbs Head virus (SAHV), member of Phlebovirus genus (family *Phenuiviridae*, order Bunyavirales), belongs to the largest group of negative strand RNA viruses. All phleboviruses share a genome structure that comprises three segments of negative-sense or ambi-sense RNA. The viral genome is composed of the small (S), medium (M) and large (L) RNA segments. The S segment encodes the nucleocapsid (N) protein, the M segment encodes the precursor for the viral glycoproteins (Gn and Gc) and the L segment encodes the viral RNA-dependent RNA polymerase (RdRp). Some viruses within the genus also encode non-structural proteins within their S or M segments.

SAHV was isolated from a pool of Ixodes uriae ticks collected at a seabird colony in Berwickshire, Scotland in 1979. There were quite a few related bunyaviruses found in tick and bird samples on the East Coast of Scotland and England in the 70s and 80s. Recently we have sequenced a sample of SAHV using next generation sequencing technology. The results suggested that this virus is very closely related to the Uukuniemi phlebovirus (UUKV). To determine how similar are SAHV and UUKV, we compared virus growth in various mammalian, bird and tick cell lines.

Alphavirus E1 fusion protein: alternative conformations of the post-fusion trimer depending on the alphavirus

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Abstract

Background

Some of the best characterized alphaviruses are chikungunya virus (CHIKV), Semliki Forest virus (SFV) and Sindbis virus (SINV). E1 is a class II fusion protein, containing 3 domains (DI, DII, DIII) folded essentially as β-sheet, plus a stem region connecting DIII to the transmembrane (TM) segment. The fusion loop (FL) is at the tip of the elongated DII. The X-ray structure of the SFV E1 post-fusion trimer, truncated of the stem region, displayed a tripod-shape with the DII legs open and the FLs away from each other, contrary to the class II viral fusion proteins from other viral genera, in which the FLs interact at the tip of the post-fusion trimer. Here, we set to identify if the stem plays a structural role in zippering together the E1 trimer to bring the fusion loops into contact.

Methods

We produced the recombinant ectodomains of E1 of CHIKV, SINV and SFV containing or not the stem, crystallized them and determined the X-ray structure.

Results

We observed that CHIKV and SINV display E1 in closed conformation, in contrast to SFV, which displays a tripod even with the full stem. We identified a sequence motif in the stem responsible for the conformational difference.

Conclusion

Our results point to potential mechanistic differences between alphavirus E1 in driving fusion. Further functional studies are ongoing to pin-point the significance of these new findings, and the reasons for the alternative post-fusion conformations.

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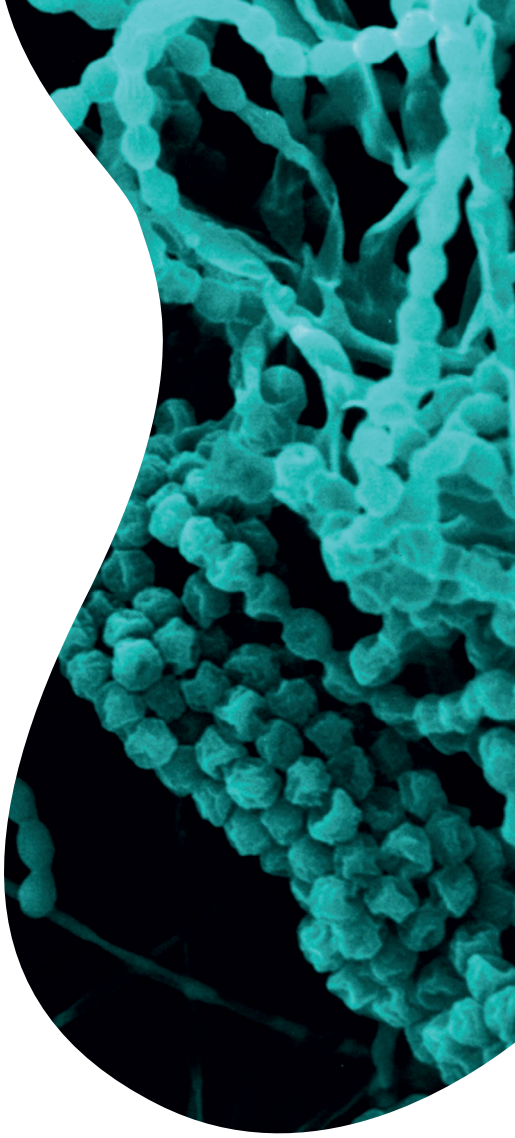

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