Impact of Dengue Virus Infection on Global Metabolic Alterations in the Aedes aegypti Mosquito Vector

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Aedes aegypti mosquitoes are the primary vectors transmitting dengue virus (DENV), one of the most aggressive re-emerging pathogens worldwide causing more than 390 million infections per year. The spread of the virus is greatly dependent upon successful replication within both the human host and mosquito vector. Much effort has been placed in understanding the dynamics of virus transmission and replication in both organisms, but little is known about the global impact of DENV on metabolic pathways. Previous studies have demonstrated perturbations in human and Aedes albopictus cellular metabolic environments during DENV infection. Some of these perturbations include increasing the production of membranous lipids that had the capability to induce membrane curvature and permeability, as well as visibly altering both human and mosquito intracellular membrane architecture to support DENV replication. In this study, we have explored metabolic changes in Aedes aegypti midgut and salivary glands upon DENV (serotype 2) infection. We have found several significant fluctuations in the lipid and metabolite repertoire from infected tissues compared to uninfected controls, including differential expression of molecules that function as membrane building blocks, bioactive messengers, energy storage and intermediates in lipid biosynthesis and lipolysis pathways. These results and their relevance to dengue virus infection of its mosquito vector will be discussed.

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Alphavirus Infection of the CNS: Entry, Dissemination, and Neurodegeneration

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Alphaviruses most often associated with neuroinvasive disease are limited to the Americas and include strains of EEEV, VEEV, and WEEV. The process of alphavirus entry into the CNS of infected vertebrates following challenge is not well-understood. It is thought that virus entry into the CNS depends on the inoculation route. It is well-established that olfactory sensory neurons provide access to the CNS following challenge with airborne virus. However, less knowledge is available regarding virus entry into the CNS following peripheral, non-olfactory infection, which appears to rely on some form of hematogenous spread. We sought to determine the precise route of CNS entry following footpad inoculation by using a combination of in vivo/ex vivo bioluminescence imaging and traditional histological examination methods. We found a consistent pattern in the spatiotemporal distribution of virus among the imaged brains, none of which involved the olfactory bulb. Extending these studies by performing histological analysis on the imaged tissues, led to the finding that CNS entry by WEEV likely occurs in areas of the CNS where the blood-brain barrier is naturally absent. These areas include the hypothalamus, the subfornical organ, the pineal gland, and the area postrema. Importantly, these results reveal a previously unrecognized method of alphavirus entry into the CNS.

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Sterilization and Disposal of Agricultural Quarantine Waste

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Approximately 150 million people and almost $40 billion worth of agricultural commodities go through U.S. international ports annually. Ports seize animal and plant products potentially contaminated with high risk diseases that then must be decontaminated before entering the waste stream. Currently, there are only 3 methods of decontamination accepted by the Animal Plant and Health Inspection Service at U.S. ports and borders including incineration, high temperature cooking, and discharge of ground waste as sewage. In this study we assess the efficacy of a relatively new decontamination technology, alkaline digestion, to mitigate infectious agents. Transmissible Spongiform Encephalopathies (TSEs), a member of the protein misfolding diseases (ex: Alzheimer’s and Parkinson’s Diseases), were chosen as the infectious agent for this study because they rank as the hardest to kill microbe/pathogen, affect both human and animal species worldwide and are shed by infected hosts into the environment establishing highly infectious biota. Chronic wasting disease (CWD), an emerging TSE of cervid species (deer, elk, moose) in North America, has recently been spotlighted as a potential concern for European countries, and recapitulates human and animal TSE pathogenesis and shedding. For these reasons CWD is ideal for mitigation studies. We processed CWD positive and negative materials by alkaline digestion under standard temperature and pressure at time intervals of 2, 4, and 6 h. Samples were retrieved after digestion, were neutralized and inoculated intracerebrally into transgenic mice expressing the cervid protein to determine remaining prion infectivity. In addition, the samples (pre and post alkaline digestion) were tested for amplification competent prions by Protein Misfolding Cyclic Amplification (PMCA). Preliminary results suggest a lack of amplification competent prions in samples processed by alkaline digestion at 2, 4, and 6 h cycles as compared to nondigested samples. This work will provide a basis for future studies designed to unravel the mechanisms associated with the ability of prions to bind surfaces enhancing prion mitigation strategies for TSEs and by extension, other protein misfolding diseases.

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Alphavirus E1 Glycoprotein-Liposome-Nucleic Acid Complexes Protect Mice from Lethal Challenge with Multiple Alphaviruses

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Alphaviruses are globally distributed, mosquito borne pathogens that cause death and disease in vertebrates, including humans. Therapeutics to combat alphaviral disease are non-existent and only a handful of IND status vaccines are available. Of the available vaccines most are associated with a poor immunological response and a high rate of Reactivity, and none protects against more than a single alphavirus species. We designed and tested novel alphavirus vaccines comprised of the E1 glycoproteins of western equine encephalitis virus (WEEV) or Venezuelan equine encephalitis virus (VEEV). Immunization with cationic lipid nucleic acid complexes (CLNCs) and alphavirus E1ecto mixture (lipid-antigen-nucleic acid complexes:LANACs) provided significant protection in mice challenged with either WEEV, VEEV or eastern equine encephalitis virus (EEEV) regardless of challenge route. LANAC immunized mice mount a strong humoral immune response lacking neutralizing antibody. Passive transfer of immune sera from LANAC immunized mice to non-immunized mice confers protection to challenge, indicating that non-neutralizing antibody is sufficient for protection. In summary, our LANAC vaccine has both therapeutic and prophylactic potential and is able to offer protection against distinct alphavirus species irrespective of the route of infection.
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Efficient replication and shedding of MERS CoV from the upper respiratory tract of experimentally infected dromedary camels

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The Middle East respiratory syndrome coronavirus (MERS CoV) is a novel coronavirus first recognized in 2012 and is associated with severe respiratory disease in humans. Virus has been isolated from dromedary camels in endemic areas, and many camels also have neutralizing antibodies against the virus, suggesting that they are likely a reservoir host. In order to better understand the role of camels in virus transmission we experimentally infected 3 adult, male dromedary camels with a human isolate of MERS CoV. All animals developed a transient, upper respiratory tract infection associated with very minor clinical disease. Large quantities of infectious virus were isolated from nasal secretions from each animal through 7 days post-inoculation, and viral RNA was detected much longer. Although our study design was limited to 3 animals, these data indicate that MERS CoV readily infects camels, which shed large amounts of virus and likely can efficiently transmit virus to other camels and humans.
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Predicting New Prion Candidates in Yeast

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Prions are infectious proteins capable of self-propagating and transmitting between organisms. Even though there is no homolog to the mammalian prion protein in yeast, several soluble proteins can form heritable aggregates de novo. These proteins provide a model system to investigate the nucleation, aggregation and propagation steps involved in the formation of a prion fibril. Several prion prediction algorithms have been developed to predict yeast proteins that have the propensity to form prions. One of these algorithms was previously developed in our laboratory (Prion Aggregation Prediction Algorithm, PAPA, Toombs et al., 2012). Therefore, we used PAPA to scan the yeast proteome to extract proteins that contain domains predicted to have prion activity (prion-like domains). These prion-like domains will be tested in four prion activity assays to assess their activity in vivo as well as in vitro. Here we provide preliminary evidence that we are successful at predicting yeast proteins that present prion activity in vivo. Following characterization of these prion-like domains, we will test the respective full-length proteins for prion activity using microscopy as well as developing phenotypic assays. Ultimately, we may identify new prion candidates in yeast, which will contribute information about the parameters necessary for prion formation and insight into the functions prions play in yeast. In addition, by confirming PAPA’s ability to predict prion proteins from the yeast proteome, it allows the possibility to apply this methodology to other proteomes.
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Detection of Immunodominant Proteins of Felis catus Gammaherpesvirus 1

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We recently identified and sequenced a novel herpesvirus of domestic cats, Felis catus gammaherpesvirus 1 (FcaGHV1). FcaGHV1 is a member of the gammaherpesvirus subfamily which also includes the human cancer-associated herpesviruses, Epstein-Barr virus (EBV) and Kaposi’s sarcoma-associated herpesvirus (KSHV). As a first step toward developing a serologic assay to detect exposure to FcaGHV1, we are seeking to determine which viral proteins elicit an antibody response in naturally occurring domestic cat infections. We cloned selected FcaGHV1 genes into a mammalian expression vector and performed transfections of a feline cell line for expression of recombinant FcaGHV1 proteins. We fixed cells with paraformaldehyde and methanol-acetone and tested reactivity to serum from cats naturally infected with FcaGHV1 using immunofluorescent antibody staining. An FIV immunofluorescence test was developed as a positive control for transfection and assay function. Serum from specific pathogen-free laboratory cats served as negative controls. Preliminary data from 9 cats with FcaGHV1 infection indicates that capsid protein ORF 65 and tegument protein ORF38 may elicit antibodies during naturally occurring FcaGHV1 infection. Results of this study will suggest which FcaGHV1 proteins are immunodominant during natural infection. With this information we plan to develop a serologic assay and further evaluate FcaGHV1 as a model for EBV and KSHV.
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Further Characterization of Rio Grande Virus and Potential for Serological Cross Reactivity with other Phleboviruses

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Members of the genus Phlebovirus (family Bunyaviridae) are new and emerging disease pathogens of humans and animals. Newly identified viruses include Heartland virus (HRTV), Lone Star virus in the USA, and Severe Fever with Thrombocytopenia Syndrome virus in Asia. Assays to support surveillance, epidemiologic studies, and diagnosis of these viruses may also detect related viruses within the genus, confounding interpretation. Rio Grande virus (RGV) was isolated in 1973 from southern plains