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 encepha-
ritis 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 chal-
 lenged 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 asso-
ciated 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 \textit{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 Algo-
rithm, 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, \textit{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 immunofluo-
tescence 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 interpreta-
tion. Rio Grande virus (RGV) was isolated in 1973 from southern plains