

Summer 2022 OVPR Seed Grant Program

Cover Page and Overview/Abstract

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TITLE: Developing Reverse Genetic Systems for Powassan Virus and Hantavirus

Overview: The goal of this proposal is to develop Reverse Genetics systems for Powassan virus (POWV) and Hantavirus (HV) that permit analysis of virulence determinants and attenuating mutations, and the development of recombinant vaccines for these human pathogens.

Reverse genetics is the term coined for the generation of infectious RNA viruses from cloned DNAs. Reverse genetics permits genetically modifying infectious RNA viruses and this permits defining proteins and residues that confer virulence and attenuate recombinant viruses. Without a viable reverse genetics system in hand it is not possible submit proposals analyzing determinants of pathogenesis, defining pathogenic mechanisms or evaluating viral changes that may attenuate POWV or HV and serve as vaccines. With a number of different virulence determinants defined for both of these viruses we remain unable to propose studies that validate their role in pathogenesis or investigate mutations that are likely to attenuate POWV or HVs. The development of reverse genetics for POWV and HV are key limitations to further development of attenuated vaccines for these viruses and for acquiring NIH funding for the rational design of recombinant POWV and HV vaccines.

Reverse genetics has been accomplished for several RNA viruses, but is lacking for key human pathogens including BSL3 viruses, POWV and HV, that we study. While no one has successfully developed reverse genetics for HV or POWV, reverse genetics approaches has been successful for other Bunyaviruses (BVs) and Flaviviruses (FVs) that suggest the potential for developing these systems for POWV and HVs. A Circular Polymerase Extension Reaction (CPER) approach⁽¹⁻⁷⁾ was used to successfully generate recombinant FVs (Dengue virus, West Nile virus). Here we propose systematically developing POWV and HV reverse genetics using approaches successful for other RNA viruses with attention to FV and HV specific hurdles. We propose using a CPER approach to generate recombinant versions of the virulent POWV LI9 and attenuated POWV, LI9-P that we have isolated and developed and which permit analysis of POWV mutations that direct POWV attenuation.

Reverse genetics systems effective for other tripartite negative-stranded bunyaviruses have been unsuccessful for HVs. Reasons include low level cytoplasmic HV replication and an endonuclease (Endo) that prevents detectable polymerase (Pol) expression^(8, 9). We propose systematically developing HV reverse genetics using 1) approaches from other RNA viruses (capping enzymes, fusion proteins, HDVRs); 2) our experience with HV constructs, capped mRNA leaders, cell targets and helper protein expression of HV proteins; and 3) nucleoside stabilized mRNAs. We recently generated CPER products that express N, GnGc and Pol-K44A-HA proteins and suggest the efficacy of the CPER approach for generating recANDVs in proposed studies.

The goal of these studies is to develop initial reverse genetics systems using overlapping complementary approaches to generate infectious POWV and HV that can be manipulated to study their pathogenic determinants. The development of POWV and HV reverse genetics is critical to proposing NIH studies with a high probability of funding and which have the ability to permit development of live attenuated POWV and HV vaccines.