Effects of BEI inactivation and sucrose purification on Rift Valley fever virus

MaRyka R. Smith, A. Sally Davis

Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS

Rift Valley fever virus (RVFV) is a zoonotic arbovirus endemic to Sub-Saharan Africa and the Arabian Peninsula. It is a significant public health and agricultural threat due to its ability to cause fatal disease in ruminants and humans. To facilitate the elucidation of RVFV's host cell receptor(s), we sought to develop inactivation and purification methods that minimally disrupted the virion. Using the Select Agent (SA) excluded RVFV vaccine strain, MP-12, we tested RNA alkylating agent binary ethylenimine (BEI), for inactivation, and discontinuous sucrose gradients and cushions, for purification. Virus integrity was analyzed by plaque assay, SDS-PAGE, western blot, and protein quantification. BEI consistently inactivated RVFV MP-12. In Vero cell cultures, the typical RVFV cytopathic effect was absent and plaque assays were negative. Purification by sucrose cushion was easier to conduct and yielded sufficient virus for further analysis. All purified virus was replication competent and anti-RVFV glycoprotein antibodies still bound their epitopes but total protein and virus titers decreased. Verifiable BEI inactivation of virulent RVFV strains in high containment will enable work with these viruses at biosafety level 2. Next steps include confirming purification reproducibility, more extensive blind passaging of inactivated virus for further verification, testing these methods on SA excluded RVFV ZH501-ΔNSs-ΔNSm, development of an RVFV *in vitro* binding assay, and fluorophore bioconjugation of purified virus for glycan binding experiments.

Research Grant: Department of Diagnostic Medicine and Pathobiology, College of Veterinary Medicine (CVM), Kansas State University (KSU); Kansas State University Small Research Grant

Student Support: American Society of Investigative Pathology, KSU CVM Office of Research