

PRECLINICAL DEVELOPMENT OF FILOVIRUS AND FLAVIVIRUS VACCINES BASED ON RECOMBINANT INSECT CELL EXPRESSED SUBUNITS

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Ebola Virus Disease (EVD) is the most prominent example of filovirus disease but despite being characterized as a Category A Priority Pathogen by NIH/NIAID over a decade ago, it lacked public and private research resources due to the absence of a commercial market. Transmission from wild animals into the human population typically causes outbreaks of limited scale in endemic areas located in the forested regions of Central Africa and the Philippines (for *Reston ebolavirus*), therefore other public health threats garnered more attention. This changed when a *Zaire Ebolavirus* (EBOV) outbreak of increasing size in several West African countries started to reveal the true epidemic potential that filovirus infections can have when entering an urban setting in a highly mobile society. Despite significant progress with the clinical development of several EBOV vaccine candidates during and after the West African outbreak, no EBOV specific therapeutics and vaccines have yet received regulatory approval. Additional research is needed in particular on understanding the mechanism of protection and defining immune correlates of protection for Ebola and other filovirus vaccines.

For our multivalent filovirus vaccine candidate, we have produced soluble recombinant filovirus glycoproteins (GP) from EBOV, *Marburg marburgvirus* (MARV) and *Sudan ebolavirus* (SUDV) using the *Drosophila* S2 cell expression system. The immunogenicity of highly purified recombinant subunits and admixtures formulated with or without clinically relevant adjuvants was evaluated in mice, guinea pigs and macaques. Strong antigen-specific IgG titers as well as virus neutralizing titers were observed after administering two or three doses of adjuvanted formulations. In mice and non-human primates subunit proteins were also shown to elicit cell mediated immune responses. Analysis of secreted cytokines in batch-cultured, antigen-stimulated splenocytes or PBMC's demonstrated antigen-induced Th1 and Th2 type responses. Recombinant vaccine candidates were tested successfully for protection in the mouse model of EBOV. Further studies allowed us to demonstrate that both humoral and cell-mediated immunity are elicited and can mediate protection. Additional immunogenicity and efficacy studies in guinea pigs were focused on optimized antigen dosing, antigenic balance and adjuvantation. Multiple formulations consistently produced strong antibody responses and demonstrated 100% protective efficacy in the EBOV guinea pig model.

Results from studies in two species of non-human primates demonstrate that vaccination with formulations of recombinant EBOV subunits and an emulsion-based adjuvant consistently produces high anti-EBOV IgG and virus neutralizing titers. Such vaccination prevents viremia subsequent to live virus challenge and protects animals from terminal EBOV disease. These studies suggest that we have defined a viable Ebola virus vaccine candidate based on non-replicating viral subunits. In addition to updates on efficacy testing against EBOV and MARV, we will discuss current formulation optimization efforts in our laboratory including thermostabilization of recombinant subunits as well as defining correlates of protection. These are prerequisites to enable efficient clinical development of a monovalent vaccine candidate for protection against EVD and a multivalent, recombinant subunit vaccine with protective efficacy against EBOV, SUDV and MARV infection. Recently we have also demonstrated the applicability of our vaccine platform for the rapid development of vaccines against emerging diseases with a focus on Zika virus (ZIKV), a flavivirus, where we were able to demonstrate efficacy in mice and cynomolgus macaques within approximately 13 months from designing the synthetic gene for antigen expression. While a completely different disease from EVD, the recent outbreak of ZIKV in the Americas provided a similar challenge as no vaccine development efforts have been conducted prior to 2016 and an increasing body of evidence suggests that rather than causing a typical, mild form of disease as previously reported, ZIKV infections can cause neurological sequelae as well as fetal and infant malformations.

These results demonstrate that the insect cell expression system can be used to rapidly and efficiently produce recombinant viral subunits from a variety of pathogenic viruses that are highly immunogenic in multiple animal species and are capable of providing effective vaccine protection against live virus challenge.