

DEVELOPMENT OF A VACCINE BASED ON RECOMBINANT SUBUNIT PROTEINS TO PROTECT HUMANS AND ANIMALS AGAINST FILOVIRUS DISEASE

Axel T. Lehrer, University of Hawaii
lehrer@hawaii.edu
Teri-Ann S. Wong, University of Hawaii
Michael M. Lieberman, University of Hawaii

Key Words: Ebola, filovirus, recombinant, subunit, preclinical

Ebola Virus Disease (EVD) is the most prominent example of filovirus disease and as a zoonotic virus fits the characteristics of a neglected tropical disease. Despite being characterized as a Category A Priority Pathogen by NIH/NIAID over a decade ago, EVD lacked public and private research resources due to the absence of a commercial market. Previously, outbreaks of limited scale linked to transmission from livestock or wild animals into the human population occurred in the 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 recently in 2013-2015 when an Ebola virus (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. As typical in an epidemic with a significant number of infectious patients traveling within and from the endemic area, the disease was also exported outside the outbreak region as has been shown with introductions into Nigeria, Mali, and the United States (amongst other countries averting in-country transmission from imported cases). This demonstrated the threat posed to the global public health systems if spread of Ebola or a related filovirus cannot be contained at its source.

We have produced three soluble recombinant filovirus glycoproteins (GP) and the matrix proteins of EBOV (VP24 and VP40) using the *Drosophila* S2 cell expression system. For each antigen, a specific immunoaffinity chromatography method was developed to allow purification to purity levels >90%. The immunogenicity of recombinant subunits and admixtures formulated with or without clinically relevant adjuvants was subsequently 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 in mice for protection against challenge with mouse-adapted EBOV. All vaccine formulations containing EBOV GP generated protective responses and serum transfer from such animals into naïve mice demonstrated that humoral immunity alone can be fully protective. Furthermore, the transfer of immune splenocytes into naïve mice showed that recombinant GP and VP24 subunits elicit functional T cell responses that lead to protection against live virus challenge.

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 suggest that vaccination with GP+VP40+VP24 and an emulsion-based adjuvant consistently produces high anti-EBOV IgG and virus neutralizing titers. This 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.

Current efforts in our laboratory are focused on defining correlates of protection to allow clinical development of a monovalent vaccine candidate for protection against EVD and further formulation optimization towards a trivalent, recombinant subunit vaccine with protective efficacy against EBOV, *Sudan ebolavirus* (SUDV) and *Marburgvirus* (MARV) infection.