 Thermostable Lyophilized Ebola Vaccine Formulations

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Purpose

- No licensed vaccines against Ebola virus infections are currently available
- Ebola virus vaccine candidates in development as well as most current licensed vaccines require transport and storage under a continuous cold chain in order to prevent potential decreases in product efficacy
- Cold chain requirements are particularly difficult to maintain in countries with a history of Ebola virus
- To reduce costly cold chain requirements, a thermostable subunit protein vaccine against Ebola was developed using lyophilization and ThermoVax™ technology

Vaccine Formulation

- Formulations of the key antigen, Ebola glycoprotein (EBOV-GP), adjuvanted with microparticulate aluminum hydroxide (alum) were prepared in liquid and lyophilized forms

Vaccine Formulation

- 0.1 mg/mL EBOV GP
- 10 mM ammonium acetate pH 7
- 9.5% (w/v) trehalose
- ±0.5 mg/mL aluminum hydroxide

Characterization

- No changes were detected between non-incubated and incubated lyophilized vaccine formulations by SDS-PAGE and Coomassie staining or western blotting

SE-HPLC

- Monomeric and high and low molecular weight protein species were monitored using size-exclusion HPLC and a TSKgel G3000SWXL column
- Liquid EBOV GP formulations incubated for 12 weeks at 40°C had loss of monomer and increased levels of HMW species
- Lyophilized EBOV GP formulations incubated for 12 weeks at 40°C had small increases in HMW species compared to the incubated liquid formulation

Intrinsic Fluorescence Quenching

- Tertiary structure changes in EBOV GP were monitored using intrinsic fluorescence quenching of protein tryptophan residues using acrylamide
- Stern-Volmer constants (Ksv) were obtained from the slope of the Stern-Volmer plot and indicate the relative accessibility of solvent to tryptophan residues

Immunogenicity Testing

- Intramuscular injections of vaccine formulations were administered to adult female BALB/c mice
- Antibody responses against EBOV GP in mice were monitored using a bead-based multiplex immunoassay
- Followed University of Colorado IACUC approved protocol 2318-4DEC2018 for animal experiments

Conclusions and Future Work

- Degradation of protein assembly state was observed in incubated liquid formulations, whereas changes in protein assembly state were minimal in incubated lyophilized formulations
- Lyophilization of EBOV-GP formulations did not decrease vaccine potency in mice and immunogenicity of lyophilized formulations was not diminished after high-temperature storage
- In order to better differentiate immune responses between vaccine formulations, passive protection studies in mice are being conducted

Non-Incubated vs. Incubated Vaccines

- Antibody responses in mice injected with reconstituted lyophilized vaccine formulations that had been incubated at 40°C for 12 weeks prior to injection were similar to responses in mice injected with non-incubated reconstituted lyophilized vaccines

Liquid vs. Lyophilized Vaccines

- Corresponding liquid and lyophilized vaccine formulations produced similar anti-EBOV-GP IgG antibody responses in mice
- Mice administered with alum-containing vaccine formulations exhibited stronger antibody responses than mice administered with non-alum containing vaccine formulations
- Lyophilization of EBOV-GP formulations did not decrease immunogenicity of the vaccine

No soluble protein was detected in chromatograms of formulations containing alum, indicating that 100% of EBOV GP was adsorbed to alum (data not shown)