Thermostable Lyophilized Ebola Vaccine Formulations

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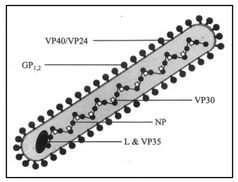
Purpose

- 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



The structure of Ebola virus.
Feldmann et al. 1999

- Suspensions of alum were added to vaccine formulations and samples were rotated end-over-end for 1 hour before lyophilization
- Vaccine formulations were lyophilized under the following conditions:
  - Lyophilizer shelves were pre-cooled to -10°C; shelf temperature was decreased at a rate of 0.5°C/min to −40°C and then held at−40°C for 1 hour
  - Primary drying at 60 mTorr and shelf temperature was increased at a rate of 1°C/min to − 20°C and then held at − 20°C for 20 hours
  - Secondary drying at 60 mTorr and shelf temperature was increased at a rate of 0.2°C/min to 0°C, followed by an increase to 30°C at 0.5°C/min, and then held at 30°C for 5 hours
- Lyophilized vaccine formulations were reconstituted immediately after lyophilization or after 12 weeks of incubation at 40°C and were characterized

Characterization

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

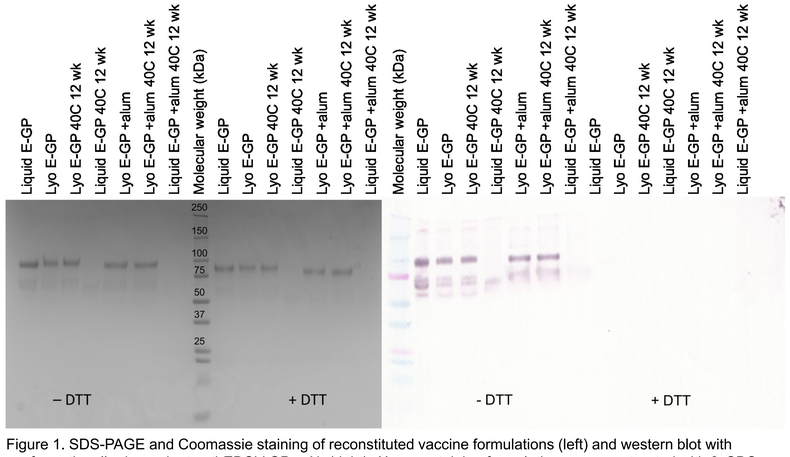


Figure 1. SDS-PAGE and Coomassie staining of reconstituted vaccine formulations (left) and western blot with conformationally-dependent anti-EBOV GP mAb (right). Alum-containing formulations were pretreated with 2xSDS 10xPBS solution to desorb protein from alum.

SE-HPLC

- Monomeric and high and low molecular weight protein species were monitored using size-exclusion HPLC and a TSKgel G3000SW$_{XL}$ column
- Liquid EBOV GP formulations incubated for 12 weeks at 40°C had loss of monomer and increased levels of HMW species and LMW species
- Lyophilized EBOV GP formulations incubated for 12 weeks at 40°C had small increases in HMW species compared to the incubated liquid formulation

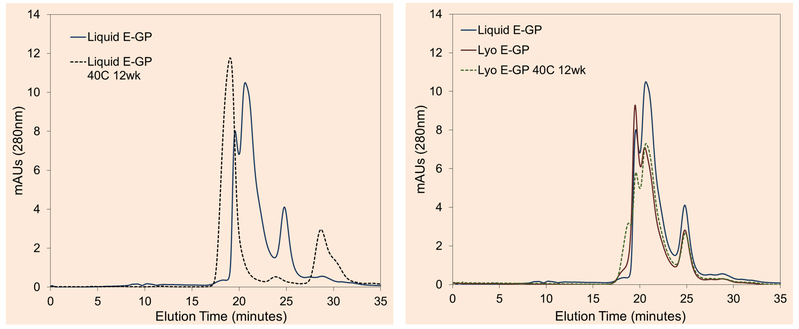


Figure 2. Size-exclusion chromatograms of liquid vaccine formulations (left) and lyophilized and reconstituted vaccine formulations (right). The absorbance of the eluent was monitored at 280 nm.

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

Intrinsic Fluorescence Quenching

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

$$\frac{F_0}{F} = 1 + K_{SV}[Q]$$

F_0 = fluorescence intensity in the absence of quencher
F = fluorescence intensity in the presence of quencher
[Q] = quencher concentration
K_{SV} = Stern-Volmer constant

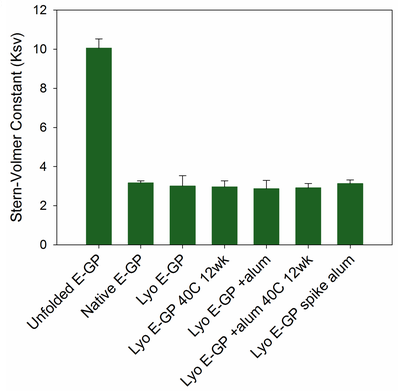


Figure 3. Stern-Volmer constants of reconstituted vaccine formulations

- EBOV GP retained near-native tertiary structure in non-alum and alum containing formulations after lyophilization and reconstitution
- Furthermore, near-native structure was retained in vaccine formulations after 12 weeks of incubation at 40°C

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

Animal Study Design	
Dose	10 µg EBOV GP
Injections	Day 0, 21, 42
Bleedings	Day 0, 14, 35, 56

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

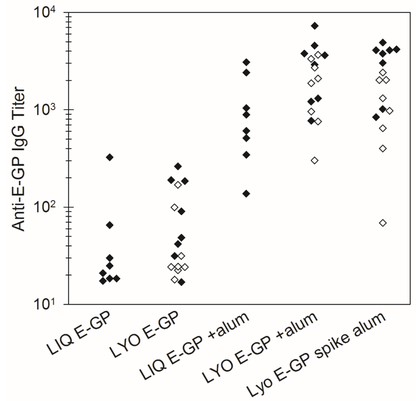


Figure 4. Anti-E-GP IgG antibody titers at Day 35 (open diamonds) and Day 56 (closed diamonds) for mice treated with liquid and lyophilized vaccine formulations with or without alum. Each data point represents the titer value obtained from serum of an individual mouse.

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

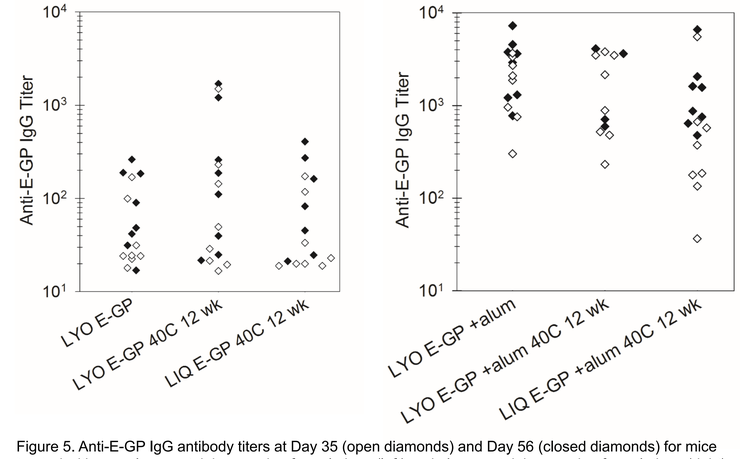


Figure 5. Anti-E-GP IgG antibody titers at Day 35 (open diamonds) and Day 56 (closed diamonds) for mice treated with non-alum containing vaccine formulations (left) and alum containing vaccine formulations (right). Each data point represents the titer value obtained from serum of an individual mouse.

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