Immunogenicity of a stable clade C HIV-1 gp140 Envelope trimer in different adjuvants in guinea pigs

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BACKGROUND: Immunogens mimicking the trimeric structure of Envelope (Env) on the native HIV-1 virion are actively being pursued as antibody-based vaccines. Generation of potent and durable neutralizing antibodies (NAbs) will probably require co-administration of these immunogens in adjuvants. The effects of adjuvants on conformational structure and integrity of Env immunogens, however, is understudied and may be important in the selection of adjuvants for optimal induction of Env-specific NAbs.

AIM: To assess the stability and immunogenicity of the clade C Env gp140 trimer in guinea pigs formulated in aqueous, liposome, emulsion and alun formulations of Glycopyranosyl Lipid Adjuvant (GLA) as well as CpG, Emulsigen, and R848.

METHODS: Stable Env gp140 trimer derived from primary isolate CZA97.012 was further stabilized with a T4-fibritin "foldon" C-terminal trimerization tag (Fd) and generated in T. ni (High 5) cells using the Bac-to-Bac system (Invitrogen).

RESULTS: Although trimer recovery from the various adjuvants was generally low, monodisperse peaks correlating to clade C gp140-Fd trimer were observed when re-purified from CpG, as well as GLA aqueous and emulsion formulations indicating a level of conformational stability in these adjuvants. No peak was observed for GLA alum & liposomes probably due to irreversible antigen adsorption on particles. Ribi, R848, and Emulsigen adjuvants showed varying degrees of protein aggregation.

To assess stability, 100µg of clade C gp140-Fd trimer was incubated separately in the various adjuvants at 37°C for 1 hour, re-purified on mini N-NTA spin columns (Pierce) and assayed by superose 6 size-exclusion chromatography.

For immunogenicity studies, 5 groups of guinea pigs (n = 4 or n=5 per group) were immunized intra-muscularly 3 times, 4 weeks apart, with 100 µg of clade C gp140-Fd in the four GLA formulations or CpG/Emulsigen adjuvants. Animals receiving Ribi adjuvant were immunized subcutaneously and intra-peritoneally as per vendor recommendations while those receiving CpG/R848 were immunized sub-cutaneously. Serum samples were obtained 4 weeks after each immunization and antibody levels determined by an end-point ELISA assay and neutralization activity by the TZM-bl assay.

In all adjuvants tested, the clade C trimer elicited comparable binding antibody ELISA titers of approximately 6.5 logs after three immunizations.

CONCLUSIONS: These data demonstrate the immunogenicity of our stable, clade C gp140-Fd trimer in different adjuvant systems. The effects of adjuvants on conformational structure and integrity may be important in the selection of adjuvants for optimal induction of Env-specific NAbs.