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Comparison of multiple adjuvants on the stability and immunogenicity of a clade C HIV-1 gp140 trimer

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ABSTRACT

Immunogens based on the human immunodeficiency virus type-1 (HIV-1) Envelope (Env) glycoprotein have to date failed to elicit potent and broadly neutralizing antibodies against diverse HIV-1 strains. An understudied area in the development of HIV-1 Env-based vaccines is the impact of various adjuvants on the stability of the Env immunogen and the magnitude of the induced humoral immune response. We hypothesize that optimal adjuvants for HIV-1 gp140 Env trimers will be those with high potency but also those that preserve structural integrity of the immunogen and those that have a straightforward path to clinical testing. In this report, we systematically evaluate the impact of 12 adjuvants on the stability and immunogenicity of a clade C (CZA97.012) HIV-1 gp140 trimer in guinea pigs and a subset in non-human primates. Oil-in-water emulsions (GLA-emulsion, Ribi, Emulsigen) resulted in partial aggregation and loss of structural integrity of the gp140 trimer. In contrast, alum (GLA-alum, Adju-Phos, Alhydrogel), TLR (GLA-aqueous, CpG, MPLA), ISCOM (Matrix M) and liposomal (GLA-liposomes, virosomes) adjuvants appeared to preserve trimer integrity as measured by size exclusion chromatography. However, multiple classes of adjuvants similarly augmented Env-specific binding and neutralizing antibody responses in guinea pigs and non-human primates.

1. Introduction

The development and evaluation of novel HIV-1 Env glycoprotein immunogens that can induce potent and broad neutralizing antibodies (nAbs) against diverse HIV-1 strains is a critical priority of the HIV-1 vaccine field [1–3]. HIV-1 Env is the sole target of nAbs and consists of two non-covalently associated fragments: the receptor-binding fragment gp120 and the fusion fragment gp41. Three copies of each heterodimer constitute the mature, trimeric viral spike $(gp120/gp41)_3$ which facilitates viral entry into target CD4 T-cells [4].

With the failure of monomeric gp120 immunogens to elicit broadly reactive nAbs in animal models [5,6] and humans [7,8], trimeric gp140 immunogens have been developed [9–12] and have shown improved nAb responses in several studies [9,11,13]. However, HIV-1 Env trimers typically require adjuvants to activate innate immunity and to optimize immunogenicity. Adjuvants can be classified into two general categories: improved delivery systems and immune potentiators [14–16]. Delivery-system adjuvants, whose mode of action have traditionally been thought to involve controlled release or a depot effect, although newer evidence suggests they may enhance immunogenicity by triggering inflammasome processes [17], include aluminum compounds, emulsions, liposomes, virosomes and immune stimulating complexes (ISCOMs). Immune potentiating adjuvants, on the other hand, rely on directly stimulating the innate immune system and include TLR ligands, saponins, cytokines, nucleic acids, bacterial products and lipids. Several adjuvants have been formulated

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Table 1
Summary of adjuvants used in study. Descriptions of the adjuvants tested with HIV-1 clade C gp140 Env trimer and their mechanisms of action.

Adjuvant format	Adjuvant	Description	Mechanism of action
Aluminum based	Adju-Phos[a]	Aluminum phosphate wet gel suspension	Depot effect, induction of inflammation, conversion of soluble antigen to particulate form for efficient APC phagocytosis [27,28]
	Alhydrogel[a]	Aluminum hydroxide wet gel suspension	Depot effect, induction of inflammation, conversion of soluble antigen to particulate form for efficient APC phagocytosis [27,28]
	GLA-alum	Aluminum formulation of synthetic glucopyranosyl lipid adjuvant (GLA)	TLR4 agonist [29,30]
TLR based	GLA-aqueous[c]	Nanosuspension of GLA in an aqueous base	TLR4 agonist [30]
	CpG[b]	Synthetic immunostimulatory di-nucleotide	TLR9 agonist [31]
	MPLA[b]	Low-toxicity derivative of the lipid A region of lipopolysaccharide (LPS)	TLR4 agonist [32]
ISCOM based	Matrix M[b]	Saponin-based cage-like nano-particles formulated with cholesterol and phospholipids	Mechanism undefined but induces strong T-helper 1 and 2 responses [33]
Emulsion based	GLA-emulsion[c]	Oil-in-water formulation of GLA	TLR4 agonist [29,30]
	Ribi	Monophosphoryl lipid, synthetic trehalose dicorynomycolate in 2% oil (squalene)–Tween 80–water	TLR2/TLR4 agonist [34]
	Emulsigen	Stable mineral oil-in-water emulsion	Depot effect
Liposomal based	Virosome[a]	Spherical vesicles with mixture of synthetic and natural phospholipids holding influenza hemagglutinin (HA) and neuraminidase (NA) proteins	HA and NA proteins provide properties which facilitate efficient vesicle uptake by and subsequent activation of cells of the immune system [26]
	GLA liposome	Liposomal formulation of GLA	TLR4 agonist [30]

[a] Licensed adjuvants.
[b] Has undergone early phase clinical testing.
[c] Soon entering early phase clinical testing.

to provide both delivery and immune potentiating components simultaneously [14–16]. We hypothesize that it will likely be important to maintain HIV-1 Env trimer integrity in any given adjuvant. We therefore sought to address the understudied question of the impact of various adjuvants on HIV-1 Env trimer immunogen stability, as well as their ability to augment the magnitude of binding and neutralizing antibodies. We observed that emulsion-based adjuvants led to Env trimer aggregation and dissociation, but that multiple classes of adjuvants augmented antibody responses to the Env trimer to a similar extent in guinea pigs and non-human primates.

2. Materials and methods

2.1. Production of C97ZA.012 Clade C gp140 Env trimer

For protein production, a stable 293T cell line expressing biochemically stable, His-tagged CZA97.012 (clade C) gp140 trimer was generated by Codex Biosolutions as previously described [11]. The stable line was grown in Dulbecco's Modified Eagle Medium (DMEM) (supplemented with 10% FBS, penicillin/streptomycin and puromycin) to confluence and then changed to serum-free Freestyle 293 expression medium (Invitrogen) supplemented with the same antibiotics. The cell supernatant was harvested at 96–108 h after medium change. His-tagged gp140 protein was purified by Ni-NTA (Qiagen) followed by gel-filtration chromatography as previously described [11,12].

2.2. Adjuvants and size-exclusion chromatography

Clade C gp140 trimer was evaluated for stability in aluminum-based [Adju-Phos, Alhydrogel, Glucopyranosyl Lipid Adjuvant (GLA)-alum], TLR-based (GLA-aqueous, CpG, MPLA), ISCOM-based (Matrix M), emulsion-based (GLA-emulsion, Ribi, Emulsigen) or liposome-based (virosome, GLA-liposome) adjuvants (Table 1). GLA adjuvants were kindly provided by the Infectious Disease Research Institute (IDRI) (Seattle, WA, USA), and virosomes were provided by Crucell (Leiden, the Netherlands). All other adjuvants were purchased commercially from Sigma (Ribi, MPLA), Isconova (Matrix M), Brenntag (AdjuPhos, Alhydorgel), MVP Laboratories (Emulsigen), and Midland Certified Reagent Company (CpG). Clade C gp140 trimer (100 μg) was mixed with the various adjuvants according to each supplier's instructions and incubated for 1-h at 37 °C. Protein was re-purified from the adjuvants by mini Ni-NTA columns (Pierce) and assessed by size exclusion chromatography on a Superose 6 column (GE Healthcare) in 25 mM Tris (pH 7.5) and 150 mM NaCl.

2.3. Animals and immunizations

Outbred female Hartley guinea pigs (Elm Hill) (n = 5/group) were housed at the Animal Research Facility of Beth Israel Deaconess Medical Center under approved Institutional Animal Care and Use Committee (IACUC) protocols. Guinea pigs were immunized intramuscularly (i.m.) with clade C gp140 trimer (100 μg/animal) in the presence or absence of the various adjuvants at weeks 0, 4, 8 in 500 μl injection volumes divided between the right and left quadriceps. Serum samples were obtained 4 weeks after each immunization by vena cava blood draws. For non-human primate studies, specific-pathogen-free rhesus monkeys (*Macaca mulatta*) were housed at the New England Primate Research Center (Southborough, MA) and Bioqual Incorporated (Rockville, MD) under approved IACUC protocols. Monkeys were immunized i.m with clade C gp140 trimer (250 μg/animal) in GLA aqueous, Matrix M or virosome adjuvants at weeks 0, 4, 8 (500 μl injection volume) divided equally between the two quadriceps muscles. Env trimers used in all animal immunizations were mixed immediately before immunizations and not subjected to the 1-h, 37 °C incubation