

Markedly improved Ab responses to DNA vaccination for HIV Env using CD8+ T cells and BAFF

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ABSTRACT

Objective: DNA vaccines for transmembrane proteins often fail to elicit high titer antibodies (Abs), and this problem has plagued DNA vaccines for HIV Env (pEnv). A report by Dyer et al (1) indicated that CD8+ T cells could promote Ab production by lysing DNA transfected muscle cells, thereby liberating antigen (Ag) for transit to B cells in the draining lymph node (DLN) and initiating Ab production. This was tested by immunizing mice with pGag to generate anti-Gag CD8+ T cell responses, followed by vaccination with pEnv ± pGag and measuring anti-Env Abs by ELISA. Additionally, a plasmid for BAFF (pBAFF), a powerful B cell stimulant, was also studied.

Methods: BALB/c mice were vaccinated with pGag plus a plasmid for 4-trimer soluble CD40L as previously reported (2). Two weeks later, mice were vaccinated with a codon-optimized plasmid for subtype C Env (pEnv) either alone or mixed with a pGag ± pBAFF (constructed as a 4-trimer soluble protein).

Results: When pGag was included with pEnv, there was a 7-fold GMT increase in anti-Env IgG, but if and only if Gag contained the immunodominant MHC-I epitope for BALB/c mice (AMQMLKETI). When combined with pEnv + pGag, pBAFF enhanced Ab production by another 2.6-fold GMT only 1 week after a single vaccination.

Conclusions: These data are consistent with a need for CD8+ T cells to lyse transfected muscle cells, thereby liberating cell debris containing membrane-bound Env which in turn moves to the draining lymph node to interact with B cells. We call this vaccine strategy '**CD8+ T cell-mediated Antibody-Eliciting Vaccine**' (CAEVac). By incorporating BAFF into the vaccine, further improvements in Ab responses can occur. In addition, the use of pEnv to produce correctly folded Env in vivo may circumvent the need to provide an artificially stabilized soluble Env immunogen.

BACKGROUND

DNA vaccines for eliciting antibodies (Ab) are often effective in mice, sometimes effective in macaques, but rarely effective in humans. In spite of this, a VLP-encoding DNA vaccine for West Nile Virus is approved for horses because it elicits protective Ab. To explain these inconsistencies, we hypothesize that the i.m. injection of DNA vaccines encoding non-secreted antigens results in protein expression only in muscle cells and that the antigen fails to migrate to the draining lymph node (DLN). However, if CD8+ cytolytic T cells lyse the transfected muscle cells, then B cells in the DLN can become exposed to antigen and Ab production can result.

This hypothesis is based in part upon a report from Dyer et al showing that CD8+ T cells can enhance the Ab response to a DNA vaccine for ovalbumin where the ovalbumin plasmid used encodes a membrane form of this antigen (1).

In this study, we show how the strategy of CD8+ T cell-mediated lysis can be used to enhance anti-Env Ab responses. In addition, we found that two molecules in the TNF superfamily (TNFSF), BAFF and GITRL, can adjuvant Ab responses using this vaccine format.

DNA VACCINE METHODS

Prevaccinations to elicit anti-Gag CTLs: As we previously described (2), the combination of plasmids for secreted, codon-optimized HIV-1LAI Gag (pScGag, 80 µg) plus secreted 4-trimer CD40L (pSP-D-CD40L, 20 µg) is an effective way to elicit strong anti-Gag CD8 T cell responses. BALB/c mice were vaccinated this way every 2 weeks X 3.

Secondary vaccination: Once anti-Gag CTLs were formed, mice were vaccinated i.m. with plasmids for membrane pEnv (p96ZM651-gp160-opt, 40 µg) ± pGag (p96ZM651-Gag-opt, 40 µg) ± pTNFSF (20 µg) in a total of 100 µl PBS, 50 µl per quadriceps muscle. Wild-type pGag lacked the H-2K^d immunodominant AMQMLKETI MHC-I epitope, whereas pGag-D193E was mutated to contain this epitope.

Immunoassays: 7, 13, or 28 days after the last vaccination, sera were collected for IgG Ab measurement by ELISA using lectin-purified 96ZM651 gp120 Env as the coating antigen.

CAEVac DNA VACCINE STRATEGY

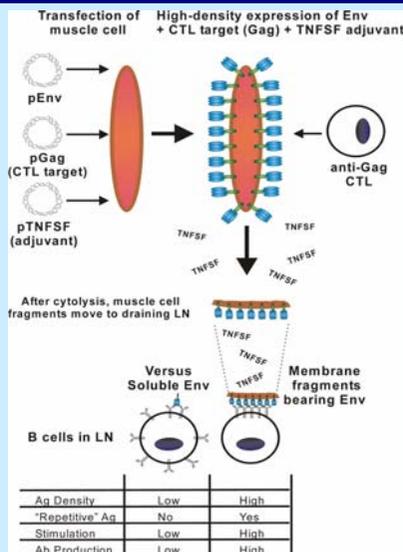


Fig. 1. CAEVac schematic.

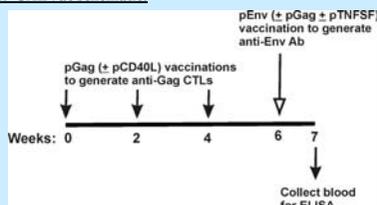


Fig. 2. Schedule of vaccinations. Unlike humans, mice have no known pre-existing CTLs. Consequently, mice were prevaccinated with pGag plus CD40L to generate anti-Gag CD8+ T cells. Two weeks later, the mice were vaccinated with pEnv ± pGag ± pTNFSF. Two TNFSFs were studied – BAFF (a B cell activating factor) and GITRL (a new TNFSF that abrogates CD4-CD25+ regulatory T cells and promotes CD4+ T cell responses).

ADDING A CD8+ T CELL TARGET ANTIGEN TO pEnv VACCINATION ENHANCES ANTI-ENV Ab RESPONSES

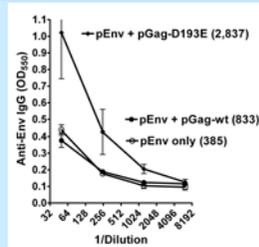


Fig. 3. Addition of a CTL target antigen augmented anti-Env Abs. Mice were prevaccinated to have anti-Gag CTLs as described using a plasmid containing the H-2K^d immunodominant AMQMLKETI MHC-I epitope. Two weeks later, the mice were vaccinated with pEnv ± pGag where the Gag was either wild-type (wt) p96ZM651-Gag (AMQMLKETI) or the same construct mutated to contain this epitope, pGag-D193E. ELISA of sera collected 28 days later demonstrated the need for the MHC-I CTL epitope in order for the pGag CTL target antigen to enhance Ab responses (mean ± SEM, n = 5; geometric mean titer in parentheses).

BAFF AND GITRL ARE ADJUVANTS FOR ANTI-ENV Abs

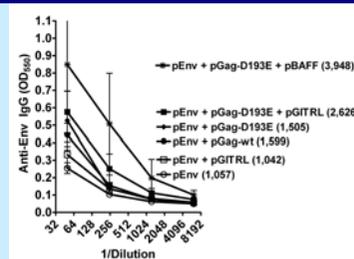


Fig. 4. Plasmid DNA for 4-trimer soluble BAFF and GITRL enhanced antibody responses to an HIV-1 Env vaccine. Mice pre-immunized to have CD8+ T cell responses against HIV-1 Gag were vaccinated with DNA for Env ± Gag ± either BAFF or GITRL (produced in a 4-trimer soluble form as fusion proteins with the body of surfactant protein D (2)). Just 7 days after vaccination, BAFF and GITRL augmented anti-Env IgG responses (mean ± SEM, n = 5; geometric mean titer in parentheses).

CAEVac PROVIDES AN IDEAL MULTI-EPITOPE ANTIGENIC SURFACE FOR Ab RESPONSES

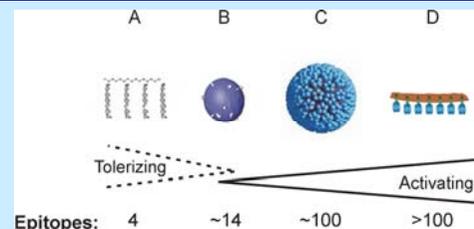


Fig. 5. Immunon concept and CAEVac. The 'Immunon' refers to the minimum 12-16 engaged B cell receptors needed to stimulate a B cell (3). Engaging less than 12-16 receptors is tolerizing (4). Consistent with this concept, a gallery of antigens is shown: (A) LJP 394 (Riquent™) is tolerizing and lowers anti-dsDNA Abs in humans with lupus; (B) HIV is at the edge of tolerizing and activating with 14 ± 7 spikes/virion (5); (C) Gardasil™ has hundreds of epitopes and elicits strong Ab responses; and (D) CAEVac generates membrane fragments of CTL-killed transfected muscle cells that converts HIV-1 Env into a multi-epitope immunogen for maximal B cell stimulation

CONCLUSIONS

- ▶ CAEVac is a useful DNA vaccine strategy for generating Ab against membrane antigens such as HIV Env.
- ▶ CAEVac in humans could utilize epitopes for preexisting CD8+ T cells (against Herpesviruses, for example), thereby obviating the need for prevaccination to generate CTLs.
- ▶ BAFF, and also GITRL, adjuvant the Ab response to CAEVac Env vaccination.
- ▶ CAEVac is a simple way to expose B cells to authentic membrane-bound Env that includes the membrane-proximal external region (MPER). Studies are ongoing to determine if the addition of a furin plasmid is needed to ensure gp160 to gp120/gp41 cleavage.

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