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 pTf against 4-trimer soluble CD40L as previously reported (2). Two weeks later, mice were vaccinated with a codelivered optimized plasmid for subtype C Env (pEnv) either alone or mixed with a pTf plus pBAFF (constructed as a 4-trimer soluble protein).

Results: When pTf was included with pEnv, there was a 7-fold GMT increase in anti-Env IgG, but if and only if Gag contained the immunodominant H-2K b immunodominant AMQMLKETI epitope for BALB/c mice (AMQMLKETI). When combined with pEnv + pTf, 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.

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 occur.

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**

Pre Vaccinations 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 (pScCD40L, 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-gp120ZM651, 40 μg) + pGag (p96ZM651-Gag, 40 μg) + pTf (pTFNSF, 20 μg), in a total of 100 μg PBS, 50 μl per quadriceps muscle. Wild-type pEnv lacked the H-2K b immunodominant AMQMLKETI MHC-I epitope, whereas pGag-D193E was mutated to contain this epitope.

**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 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.

REFERENCES


