DNK-based vaccines have several theoretical and practical advantages over other vaccine types, including safety, relative ease of construction and manufacture, and an ability to revaccinate not limited by induced antivector immunity. However, DNA-based vaccines have not always elicited robust immune responses in humans. The ADVAX vaccine candidate, which is composed of a DNA construct incorporating a clade B/C HIV-1 recombinant insert isolated from the predominant circulating recombinant form in Yunnan Province, China (10), administered by intramuscular electroporation (EP) to 24 individuals (0.2, 1, and 4 mg), was safe and well tolerated and considered acceptable for a prophylactic vaccine (4). EP delivery of ADVAX increased the magnitude of HIV-1-specific cell-mediated immunity by up to 70-fold over IM injection, as measured by gamma interferon (IFN-γ) ELISpot assays. The number of antigens to which a response was detected improved with EP and increasing dosage (22).

In the first study, we showed that, following stimulation with ADVAX insert-matched peptide pools, seven of eight volunteers in the 4-mg-dose group had demonstrable flow cytometry responses. Four had CD3⁺ CD4⁺ responses alone, two had both CD3⁺ CD4⁺ and CD3⁺ CD8⁺ responses, and one had only a CD3⁺ CD8⁺ T cell response. The majority of cellular responses were induced in CD4⁺ T cells that were of a polyfunctional phenotype and targeted HIV gp120 (22). Figure 1 shows a representative example of a gp120-specific response induced in ADVAX vaccinees; the responses tended to be of a CD27⁺ CD45RO⁺ central memory phenotype, producing predominantly interleukin-2 (IL-2), tumor necrosis factor alpha (TNF-α), and IFN-γ, with low levels of MIP-1β and little or no degranulation (22).

Certain regions of the HIV-1 proteome remain highly conserved due to their structural and/or functional importance. Vaccine-induced priming of T-cell responses to such functionally important and conserved regions may limit early virus replication either through destruction of infected cells or through decreasing virus fitness as a consequence of mutating these sites to escape recognition (12, 15, 21, 23, 26, 27). It is important to determine the specificity of immune responses induced following vaccination, to assess whether conserved, functionally important regions are targeted and, if so, how many (5).

A total of 101 15-mer peptides, sequentially overlapping by 11 amino acids and corresponding to the envelope sequence contained within ADVAX, were manufactured to >90% purity by AnaSpec (Fremont, CA). Peptide matrix pools were designed using Deconvolute this! software v1.0 (Mario Roederer, Vaccine Research Center [VRC], NIH); each individual peptide was present in 3 unique pools (18).

The gamma interferon (IFN-γ) ELISpot assay was performed as previously described (6). To facilitate mapping, vaccinees were selected on the basis of ADVAX envelope-specific IFN-γ ELISpot responses above a threshold of >100 spot-forming cells/million peripheral blood mononuclear cells (PBMC). Stimulation used preprepared matrix pools at a final peptide concentration of 1.5 μg/ml.

16 vaccinees were found to fulfill the ELISpot threshold criteria outlined above, with 7, 6, and 3 vaccinees from the 4-mg, 1-mg, and 0.2-mg DNA EP groups, respectively; no vaccinees receiving DNA alone without EP met the criteria. Responses were deconvoluted in 11 of 16 vaccinees, resulting in a total of 22 individual peptides being recognized across the ENV pool (Table 1). Vaccinees responded to up to a total of 5 epitopes, with the majority focusing responses on three regions of the vaccine insert: 5 of 11 volunteers mounted responses against the V2 region spanning peptides VYALFYRLDIVPLNK and FYRLDIVPLNKKNSS, 5 of 11 focused responses against peptides VENFNMWKNDMVQ and/or FNMWKNDMVQNMHED found within the C1 region,
and 4 of 11 individuals targeted the sequence TGDIIGDIRQAHCNI present within the V3/C3 region (Fig. 2).

Delivery of DNA vaccines by EP has been shown to increase their immunogenicity (4, 20, 22, 25). In this report, administration of the DNA-based ADVAX vaccine candidate through EP is shown to induce a breadth of ENV-specific T-cell responses (mean, 2.8 regions/patient) comparable to that seen with vaccination using DNA prime and adenovirus vector boost (11) and (mean responses, 3.3 and 4.2, respectively). Furthermore, the preferential targeting of certain sequences, in particular, V2 (FYRLDIVPLNK), C1 (FNMWKNDMVNQ), and V3/C4 (TG DIIGDIRQAHCNI) sequences, to one or more of which 10 of 11 individuals mounted responses, supported by data from Harari et al. (8) where equivalent CD4 epitopes were targeted following vaccination with NYVAC containing a clade C insert, suggests the potential for vaccine-induced immune focusing.

A phase III clinical trial involving >16,000 adult volunteers conducted in Thailand (RV144) demonstrated that a prime-boost regimen of administration of the canarypox-based vaccine ALVAC plus gp120 protein was 31.2% efficacious in preventing HIV infection, as judged at 3.5 years of follow-up (17). Initial results of the correlate discovery team revealed that levels of antibodies to the first and second variable loops (V1/V2) of gp120 protein was 31.2% efficacious in preventing HIV infection, as judged at 3.5 years of follow-up (17). Initial results of the correlate discovery team revealed that levels of antibodies to the first and second variable loops (V1/V2) of gp120 correlated inversely with subsequent risk of HIV-1 acquisition in RV144, while levels of plasma IgA binding to a panel of HIV-1 envelope proteins showed a direct correlation with risk of infection (9). While these preliminary results require further verification and analysis, it is encouraging that ADVAX delivered by EP can also elicit immune responses to the V2 loop, a functionally important region of the HIV-1 envelope involved in the formation of the glycoprotein trimer, masking of neutralizing epitopes, and interaction with cellular coreceptors (28).

One of the areas targeted within the V2 loop included the $\alpha 4\beta 7$ integrin-binding domain of gp120 (1). HIV-1 gp120 stably binds $\alpha 4\beta 7$ present on CD4 cells, homing to sites of inflammation through an LDI/LDV motif on the V2 loop present in peptide FYRLDIVPLNK (1, 2, 16); recognition of similar peptides by CD4 cells has been previously described with NYVAC (8) and, more recently, in the RV144 trial (3). The ligation event causes the formation of a synapse facilitating events required for HIV-1 entry and resulting in LFA-1 activation, which induces lymphocyte activation, proliferation, further potentiating the formation of a focus of infection (1). Primary HIV-1 infection results in massive CD4 depletion in the gastrointestinal lymphoid tissues (14); one potential reason for this may be HIV-1 infection and destruction of CD4 T cells stimulated via the gp120/$\alpha 4\beta 7$ interaction. Targeting this region during early infection may restrict HIV-1 activity. In conclusion, administration of the DNA ADVAX vaccine through EP elicits polyfunctional responses, primarily in CD4+ T cells.
T-cell populations, to multiple regions of the HIV-1 envelope glycoprotein (22). Here we show that, following epitope mapping, DNA vaccine-induced ENV-specific CD4 responses are of a breadth comparable to the breadth induced by responses elicited by NYVAC (8) and adenovirus-based vectors (11,13). The immunogenicity of DNA-based vectors may be further improved through incorporation of IL-12 adjuvants and/or boosting with viral vectors or proteins (7,24,25).

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