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Potentiation of Simian Immunodeficiency Virus (SIV)-Specific CD4⁺ and CD8⁺ T Cell Responses by a DNA-SIV and NYVAC-SIV Prime/Boost Regimen

Zdeněk Hel,* Wen-Po Tsai,* Arthur Thornton,* Janos Nacska,* Laura Giuliani,* Elżbieta Tryniszewska,* Monita Poudyal,* David Venzon,† Xiaochi Wang,‡ John Altman,‡ David I. Watkins,§ Wenhong Lu,§ Agneta von Gegerfelt,¶ Barbara K. Felber,‖ James Tartaglia,‖ George N. Pavlakis,‖ and Genoveffa Franchini†*

T cell-mediated immune responses play an important role in the containment of HIV-1 replication. Therefore, an effective vaccine against HIV-1 should be able to elicit high frequencies of virus-specific CD8⁺ and CD4⁺ T cells. The highly attenuated poxvirus-based vaccine candidate, NYVAC-SIV-gag-pol-env (NYVAC-SIV-gpe), has been shown to induce and/or expand SIV-specific CD4⁺ and CD8⁺ T cell responses in both naive and infected macaques. In this study, the immunogenicity of NYVAC-SIV-gpe alone was compared with a combination regimen where priming with an optimized DNA-SIV-gag-env vaccine candidate was followed by a NYVAC-SIV-gpe boost. In macaques immunized with the prime-boost regimen, the extent and durability of CD8⁺ T cell response to an immunodominant SIV gag epitope was increased and these animals recognized a broader array of subdominant SIV epitopes in the cytolytic assay. In addition, the prime-boost regimen significantly enhanced the proliferative responses to both SIV gag and env proteins. Thus, the combination of these vaccine modalities may represent a valuable strategy in the development of a vaccine for HIV. The Journal of Immunology, 2001, 167: 7180–7191.

An effective vaccine able to halt or limit the HIV epidemic is urgently needed. The development of an HIV vaccine has encountered numerous difficulties. Although the presence of a sufficient amount of neutralizing Abs specific to the challenge virus can prevent infection or modify disease progression in animal models (1, 2), the induction of Abs with a neutralizing spectrum broad enough to neutralize various primary HIV-1 isolates remains a challenge. Several lines of evidence indicate the importance of virus-specific CD4⁺ and CD8⁺ T cell responses in the containment of HIV/SIV infection (3–6). In infected individuals, immunological pressure exerted by virus-specific CTLs has been demonstrated by the selection of immune escape variants (7–12). CD4⁺ and CD8⁺ T cell responses have been shown to be associated with viremia containment in long-term nonprogressors (5, 13–15) and may be important in prevention of infection in high-risk individuals, such as commercial sex workers or discordant couples (16, 17). The role of CD8⁺ T cells in suppressing HIV-1 replication is also supported by the finding that autologous HIV gag-specific CD8⁺ CTL clones transferred to infected individuals temporarily decrease viral load (18).

Effective cytolytic activity of CD8⁺ T cells depends on help provided by CD4⁺ T lymphocytes. Indeed, virus-specific CD4⁺ lymphoproliferative responses (LPR) are associated with CD8⁺ T cell responses in HIV-1 infection (5, 19, 20). Control of viral rebound following the suspension of antiretroviral therapy in primary HIV-1 or SIV infection of humans or monkeys is associated with both CD4⁺ and CD8⁺ T cell responses to HIV or SIV Ags (21–24). Altogether, these results indicate an important role for cell-mediated immunity in control of HIV/SIV replication and suggest that an important feature of effective HIV vaccine must be its ability to induce high frequency of virus-specific CD4⁺ and CD8⁺ T cells.

Highly attenuated live recombinant poxviruses, such as the modified virus Ankara (MVA) (26–29), the genetically attenuated vaccinia-based vector NYVAC (21, 25, 30, 31), or the canarypox-based vector designated ALVAC (32, 70) have proven to be safe and immunogenic vaccine vehicles in monkeys. However, immunizations with various strains of recombinant poxvirus-based vaccines have demonstrated limited protective efficacy against highly pathogenic SIVmac251 challenge (25, 26, 27, 33, 70). Potential limitation of the use of poxvirus-based vectors as immunization vehicles is related to the expression of vector-specific gene products present in the inoculum or derived de novo in the recipient, which could lead to immunogen competition with the transgene product and/or vector-specific immune responses. The latter is known to impair the reuse of vectors, such as adenovirus or poxvirus-based vaccine candidates (34–39). One potential strategy to overcome these hurdles has been suggested by studies using the murine model of...
malaria whereby the use of DNA-based immunogen before administration of the poxvirus-based vaccine appeared to focus the immune response to the desired Ag expressed by the DNA (40–42). A combination of DNA priming followed by MVA or NYVAC boost induced higher CD8+ T cell responses than any of the individual vaccines alone and resulted in protection against malaria. DNA-based HIV and SIV vaccines were shown to be immunogenic in chimpanzees (43, 44) and rhesus macaques (45–47), but failed to protect the latter against a vigorous SIVmac251 challenge (46, 47) or SIV Ab670 (45). DNA priming followed by MVA boost induced cell-mediated immune responses in nonhuman primates (26, 45, 48) and decreased viremia following exposure to a nonpathogenic SHIV-IIIB virus (44), pathogenic SHIV-89.6P (49), or HIV-1 (50).

In a previous study we demonstrated that immunization with the highly attenuated NYVAC-SIV-gag-pol-env (NYVAC-SIV-gpe) resulted in a significant reduction of viremia in about one-third of macaques exposed i.v. to the highly pathogenic SIVmac251 (25). In the same study, 5 of 11 immunized macaques exposed intrarectally to SIVmac251 experienced transient low-level viremia at 1 wk postinfection and contained the virus below the limit of detection for several years thereafter (Ref. 25 and our unpublished observations). Importantly, i.v. or intrarectal challenge exposure was performed 6 or 9 mo following the last immunization, respectively, suggesting that the NYVAC-SIV-gpe vaccine candidate induced long-lasting virus-specific memory responses. In this study, we investigated whether the immunogenicity of NYVAC-SIV-gpe vaccine might be further enhanced by prior immunization with DNA-based immunogens expressing both the gag and env proteins of SIV239 in a Rev-independent manner. We demonstrate that DNA priming and subsequent boosting with NYVAC-SIV-gpe resulted in an enhancement of SIV-specific LPR and in an increase in the frequency and durability of CD8+ T cell responses to the immunodominant gag181 epitope. In addition, several of the immunized animals recognized a number of subdominant gag-, env-, and pol-specific epitopes in a cytolytic assay. Thus, the combination of these vaccine modalities deserves further evaluation in human trials of preventive and therapeutic HIV vaccine.

Materials and Methods

Construction of Rev-independent SIV gag and env expression vectors

The backbone of both plasmid vectors was derived from the kanamycin-expressing pVR1332 (provided by Vical, San Diego, CA) (51). The SIV gag expression vector contained the CMV promoter (without introns), the RNA-optimized SIV p57 gag coding region, and the bovine growth hormone polyadenylation site. To optimize for RNA expression, the previously identified gag inhibitory sequences (INS) were mutated by introducing multiple silent point mutations not affecting the encoded protein precursor, as previously described for HIV-1 gag (52–54). The SIV-op timized SIV gag160 env gene contains 29 point mutations eliminating the Rev-responsive elements (55) and is conjugated to the 3' untranslated region to the constitutive transport element of simian retrovirus type 1, which further promotes mRNA export (56, 57). DNA plasmid preparations of a clinical-grade quality were produced by Qiagen (Hilden, Germany).

Animals and immunizations

All animals were colony-bred rhesus macaques (Macaca mulatta) obtained from Covance Research Products (Alice, TX). The animals were housed and handled in accordance with the standards of the Association for the Assessment and Accreditation of Laboratory Animal Care International. All rhesus macaques were seronegative for SIV-1, simian T cell leukemia virus type 1, and herpesvirus B before the study. All macaques were screened for the presence of the Mamu-A*01 allele by PCR (58) and the amplified DNA was sequenced to confirm the Mamu-A*01 status. The animals were immunized i.m. with 106 PFU of mock NYVAC or NYVAC-SIV-gpe vaccine. For DNA immunization, 4 mg of each plasmid (CMV/kan-SIVenv and CMV/kan-SIVgag) were administered. Four doses of 0.75 mg of each plasmid were injected i.m. into two sites on each leg; five doses of 0.2 mg of each plasmid were injected intradermally at five different sites in the abdominal area.

Lymphocyte proliferation assay

Ag-specific proliferation was measured using fresh PBMC isolated by density gradient centrifugation on Ficoll lymphocyte separation medium (ICN Pharmaceuticals, Aurora, Ohio). The cells were resuspended in RPMI 1640 medium (Life Technologies, Gaithersburg, MD) containing 5% inactivated human A/B serum (Sigma-Aldrich, St. Louis, MO), and cultured at 106 cells per well in triplicates for 3 days in the absence or presence of native HPLC-purified SIV p27 gag or gp120 Env proteins (Advanced BioScience Laboratories, Rockville, MD) or Con A as a positive control. The cells were then pulsed overnight with 1 μCi of [3H]thymidine before harvest. The relative rate of lymphoproliferation was calculated as fold of thymidine incorporation into cellular DNA over medium control (stimulation index (SI)).

Detection of epitope-specific CD3+CD8+ T lymphocytes by flow cytometry

Fresh PBMC were stained with anti-human CD3 Ab (PerCP labeled, clone SP34; BD Pharmingen, San Diego, CA), anti-human CD8α Ab (FITC labeled, BD Biosciences, San Jose, CA), and Mamma-A*01 tetramer complex refolded in the presence of a specific peptide as described (59) and conjugated to PE-labeled streptavidin (Molecular Probes, Eugene, OR). Samples were analyzed on a FACS C Calibur (BD Biosciences) and the data are presented as percentage of tetramer-positive cells of all CD3+ CD8+ lymphocytes. To amplify the peptide-specific CD8+ lymphocytes in vitro, 3 × 105 cells in 1 ml of medium were incubated with specific peptide at a final concentration of 10 μg/ml for 3 days. IL-2 (Roche, Indianapolis, IN) was added at 40 IU/ml and the cells were cultured for an additional 4 days and stained as described for fresh PBMCs.

ELISPOT assay

Monkey IFN-γ-specific ELISPOT kits manufactured by U-Cytech (Utrecht, The Netherlands) were used to detect the number of cells producing IFN-γ upon in vitro stimulation. Ninety-six-well flat-bottom plates were coated with anti-IFN-γ mAb MD-1 overnight at 4°C and blocked with 2% BSA in PBS for 1 h at 37°C. Cells (104 per well) were loaded in quadruplicates in RPMI 1640 containing 5% human serum and 10 μg/ml of a specific peptide or 5 μg/ml Con A as a positive control. The plates were incubated overnight at 37°C, 5% CO2, and developed according to the manufacturer’s guidelines (U-Cytech).

CTL assay

PBMC (5 × 106) were cultured with 10 μg/ml specific peptide for 3 days, IL-2 (Roche) was added at 40 IU/ml, and the cells were cultured for another 4 days. Twelve hours before the killing assay a second dose of IL-2 at 100 IU/ml was added. The cells were then incubated for 6 h in various E:T cell ratios with Mamma-A*01-positive 31Cr-labeled transfected B cells pulsed overnight with 10 μg/ml of a specific peptide. The killing of cells pulsed with an unrelated peptide in a control experiment was equal to the killing observed in the absence of any peptide.

FIGURE 1. Study design. Three groups of eight rhesus macaques were included in the study. The animals were immunized four times at 0, 4, 24, and 52 wk with either mock NYVAC (group A) or NYVAC-SIV-gpe (group B), or at 0, 4, and 12 wk with DNA-gpe followed by two immunizations with NYVAC-SIV-gpe at wk 24 and 52 (group C).
Detection of anti-SIV<sub>mac251</sub> binding Abs by ELISA

Serial dilutions of plasma were incubated with the lysate of SIV<sub>mac251</sub> spiked with native purified gp120 env protein of SIV<sub>mac251</sub> bound to microtiter plates as described elsewhere (60). End-point titers were defined as the reciprocal of the highest sera dilution that gave an optical absorbency at 450 nm, at least two SDs greater than average values obtained with negative control sera.

Statistical analysis

Tests of p27 Gag and gp120 Env lymphoproliferation were performed using repeated measures ANOVA on the log-transformed SI. This procedure combines the t tests at each time point while making the necessary correction for the correlation among the multiple values from each animal. Comparisons between groups at a single time point were done using the nonparametric Mann-Whitney rank sum test. The percentages of tetramer-positive cells of CD3<sup>+</sup>CD8<sup>+</sup> lymphocytes were analyzed using repeated measures ANOVA after the arcsine transformation of the square root of each percentage. This transformation is commonly used with percentage data to bring closer to equality the unequal variances of a range of percentages. All p values reported are two-tailed. The Number Cruncher Statistical System (NCSS, Kaysville, UT) and Sigmastat (version 2.0; SPSS, Chicago, IL) statistical software packages were used for the analyses.
Results

Vaccines and study design

The expression of HIV and SIV genes in infected cells is regulated post-transcriptionally by the Rev protein that binds to the Rev-responsive elements and mediates the export of spliced retroviral mRNA. The expression of these genes in eukaryotic cells in the absence of Rev protein is hampered by the presence of INS that regulate the export of viral RNA from the nucleus. To overcome this problem, the previously identified INS sequences within the gag and env genes were mutagenized without changing the amino
FIGURE 2. (continued)
acid sequence of the p57 Gag and gp160 Env proteins, as previously described for HIV-1 gag (52–54). In the case of the SIV env gene, the constitutive transport element of simian retrovirus type 1, which further promotes mRNA export, was inserted in its 3′ untranslated sequence (55). The introduction of these changes resulted in an efficient expression of both viral proteins in murine and human cells (52). The NYVAC-SIV-gpe vaccine was previously described (25).

Three groups of eight macaques were used in this study (Fig. 1). At wk 0, 4, 24, and 52, animals in group A (Fig. 1, controls) received i.m. inoculation of 10⁸ PFU of mock NYVAC vaccine, while animals in group B were immunized i.m. with the same dose of NYVAC-SIV-gpe. Animals in group C were first immunized at 0, 4, and 12 wk by simultaneous i.m. and intradermal inoculations of DNA-SIV-gag-env (DNA-ge), followed by two boosts with 10⁸ PFU of NYVAC-SIV-gpe given i.m. at wk 24 and 52.

*Primming with DNA-ge significantly enhances the CD4⁺ Th responses following the boost with NYVAC-SIV-gpe vaccine*

The first immunization with either NYVAC-SIV-gpe or DNA-ge did not induce any detectable LPR to p27 or gp120 Ags in the immunized macaques (Fig. 2, A and B). Following the second immunization, positive (SI > 3) proliferative responses to both Ags were detected in only some of the immunized macaques, with no clear difference between groups B and C. However, an additional immunization with DNA-ge of the animals in group C induced a significant expansion of both p27 Gag and gp120 LPR (Fig. 2, C and D). Thus, three inoculations of DNA-ge induced significantly higher Gag-specific LPR than two NYVAC-SIV-gpe inoculations.

The third NYVAC-SIV-gpe immunization significantly enhanced LPR to both p27 Gag and gp120 Env Ags in group B macaques (p = 0.0009 for p27 and p = 0.0017 for gp120),

![Graph](https://example.com/graph.png)

**FIGURE 3.** Frequency of gag181 epitope-specific CD8⁺ T cell response measured by tetramer staining. The percentage of gag181-specific tetramer-staining cells of total CD3⁺ CD8⁺ T cell population in macaques from control group A (upper panel) and vaccinated macaques from groups B and C (middle and lower panels, respectively). The time of each immunization is indicated by arrows. Animals 21 M (group B) and 26 M (group C) were not tested up to wk 24, because their Mamu-A*01 status was unknown until then.
although the mean value in this group did not exceed an SI of 8 (Fig. 2C). In contrast, boosting of the DNA-primed macaques of group C with a single inoculation of NYVAC-SIV-gpe greatly increased LPR to both p27 Gag and gp120 Env to mean SI values of 62 and 42, respectively (Fig. 2C). The expansion of SIV-specific LPR was significantly greater in macaques primed with DNA than in those primed with NYVAC-SIV-gpe ($p = 0.00006$ for p27 and $p = 0.028$ for gp120) as summarized in Fig. 2D. The last immunization with NYVAC-SIV-gpe at wk 52 significantly increased the level of LPR to both Ags in macaques from both groups. The LPR to p27 Gag was higher and more durable in animals of group C than those of group B, although at wk 64 the response to gp120 Env was equivalent between the two groups (Fig. 2, C and D).

Prior DNA immunization potentiates the CD8+ T cell response to the immunodominant epitope gag181

To assess the CD8+ T cell responses to several Mamu-A*01-restricted SIV CTL epitopes (61), two, four, and five Mamu-A*01-positive macaques were included in groups A, B, and C, respectively. The SIV-specific immune responses to the immunodominant SIV gag epitope gag181 (also referred to as p11C, C→M epitope; Refs. 62 and 63) was enumerated in the blood of the vaccinated animals using Mamu-A*01 tetrameric complex conjugated with the gag181 peptide. Two administrations of NYVAC-SIV-gpe to animals in group B led to gag181-specific CD3+CD8+ T cell response in the blood of Mamu-A*01 macaques that ranged between 0.28 and 3% of total CD3+CD8+ T cells (Fig. 3, group B). Culture of PBMC following stimulation with the gag181 peptide expanded this population up to 27% (data not shown). The overall frequency of the gag181-specific CD8+ T cells was lower in the blood of macaques in group C immunized with DNA-gpe (ranging from 0.3 to 0.9%), and these cells could be expanded following the in vitro peptide stimulation up to 5.1% (Fig. 3, and data not shown).

The third and fourth immunizations of animals in group B with NYVAC-SIV-gpe further expanded gag181-specific CD3+CD8+ T cell population mainly in one macaque (animal 674, peak response 3.7%), whereas this response remained low in the other three Mamu-A*01 macaques (0.2–0.3%; Fig. 3B). In contrast,
boosting with NYVAC-SIV-gpe of DNA-SIV-ge-primed macaques expanded the number of gag181-specific cells in most macaques (peak response 1.5–5.5%; Fig. 3C). The frequency of gag181-staining cells persisted at a higher level in the blood of macaques in group C than in the blood of those in group B (wk 53–64, p = 0.035). In fact, the gag181-specific T cells were still detectable in the fresh blood of vaccinated animals 23 wk following the last immunization (Fig. 4). The levels of gag181-specific T

<table>
<thead>
<tr>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
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<tr>
<td>671L</td>
<td>674L</td>
<td>676L</td>
</tr>
<tr>
<td>week 53</td>
<td>week 53</td>
<td>week 53</td>
</tr>
<tr>
<td>Gag181 = 0.2%</td>
<td>Gag181 = 11%</td>
<td>Gag181 = 2.5%</td>
</tr>
<tr>
<td>week 56</td>
<td>week 56</td>
<td>week 56</td>
</tr>
<tr>
<td>Gag181 = 11%</td>
<td>Gag181 = 11%</td>
<td>Gag181 = 11%</td>
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FIGURE 6. T cell responses to subdominant SIV epitopes measured using the ELISPOT and ⁵¹Cr-release assay. The bar charts represent the results of an IFN-γ ELISPOT assay with a specific Mamu-A*01-restricted peptide (61) at the time indicated in the legend. The values exceeding the chart scale are indicated by number at the top of the bar. * The values obtained using viably frozen cells. All other assays were done using ex vivo PBMC. Ctrl, Unrelated control peptide; NT, not tested. Line charts represent the percentage of a specific killing of unpulsed control cells or cells pulsed with a specific Mamu-A*01-restricted peptide. All assays were done using the cells from 7-day cultures with a specific peptide at wk 53 or 56, as indicated. Percentage value in top right corner indicates the percentage of gag181 tetramer-staining CD³⁺CD⁸⁺ cells in cultured PBMC.
The gag181-specific CD8+ T cells are functionally competent

Because the IFN-γ ELISPOT assay is generally regarded as a functional assay of specific CD8+ T cell responses, we measured the ability of PBMC to produce IFN-γ following overnight stimulation with the gag181 peptide. Consistent with the higher frequency of gag181 tetramer-staining-positive CD8+ T cells in blood of animals from group C the numbers of IFN-γ spot-forming cells in response to gag181 peptide stimulation were higher in animals of group C than in those of B (Fig. 5). Analysis of the cytolytic activity of PBMC expanded in vitro in the presence of gag181 peptide demonstrated that three of four Banu-A*01 animals in group B vs all Banu-A*01 macaques in group C lysed target cells pulsed with gag181 in a 51Cr-release assay (Fig. 6). Consistent with the results of tetramer staining and ELISPOT assays, the gag181-specific CTL activity was generally higher in DNA-primed animals of group C than in animals of group B.

Response to Banu-A*01-restricted subdominant CTL epitopes in the immunized macaques

To assess the breadth of the CD8+ T cell responses induced by vaccination in Banu-A*01 animals (61), tetrameric complexes conjugated with the subdominant SIV-specific peptides gag254, env235, and env622 were used to stain fresh PBMC of the Banu-A*01-positive animals at several time points. With this assay, no significant responses to env622 or env235 epitopes were detected at any time after immunization. In the case of the gag254 epitope, positive tetramer-staining cells were detected only following in vitro stimulation of cells from macaque 679 at wk 13 (3.3%) and 26 (1.5%) (data not shown).

To further assess CD8+ T cell responses to subdominant epitopes, a panel of 14 different Banu-A*01-restricted peptides was used in an IFN-γ ELISPOT assay. Six of these peptides (gag181, gag254, env133, env235, env622, and pol151) induced IFN-γ production in the blood cells of immunized animals (Fig. 6). No significant difference in the frequency of recognition of subdominant epitopes in ELISPOT assay between the NYVAC-SIV-gpe-primed NYVAC-SIV-gpe-boosted group (p = 0.002), whereas at wk 56 and 76 there was no significant difference between groups B and C (Fig. 7).

Table I. Cumulative frequency of recognition of dominant and subdominant SIV epitopes in ELISPOT and CTL assays

<table>
<thead>
<tr>
<th>Epitope</th>
<th>Group B</th>
<th>Group C</th>
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<tr>
<td></td>
<td>ELISPOT+ positive/tested</td>
<td>CTL+ positive/tested</td>
</tr>
<tr>
<td>gag181</td>
<td>4/4</td>
<td>3/4</td>
</tr>
<tr>
<td>gag254</td>
<td>0/4</td>
<td>0/2</td>
</tr>
<tr>
<td>env235</td>
<td>4/4</td>
<td>1/4</td>
</tr>
<tr>
<td>env622</td>
<td>2/4</td>
<td>0/4</td>
</tr>
<tr>
<td>env133</td>
<td>2/4</td>
<td>0/2</td>
</tr>
<tr>
<td>pol151</td>
<td>1/4</td>
<td>0/2</td>
</tr>
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a ELISPOT assay scored positive at spot-forming cells higher than background established using control peptides in any particular assay (usually >25 spot-forming cells per million).
b CTL, 51Cr-release assay scored positive when specific killing of target peptide-pulsed cells was >10% at E:T ratio 25:1 or lower.

NYVAC-SIV-gpe induces higher titer of Abs binding to SIV

The humoral response induced by the two vaccine regimens was assessed in an ELISA using disrupted SIVmac virions enriched with purified native SIVmac251 env protein. Immunization of group C animals with three doses of DNA-SIV-gpe induced detectable anti-SIV Abs in only one animal. Two weeks following the boost with NYVAC-SIV-gpe (wk 26), the titers of SIV-specific binding Abs were significantly higher in the group that received three immunizations with NYVAC-SIV-gpe than in the DNA-SIV-gpe-primed NYVAC-SIV-gpe-boosted group (p = 0.002), whereas at wk 56 and 76 there was no significant difference between groups B and C (Fig. 7).

Discussion

In this study we demonstrate that DNA priming greatly enhanced the ability of recombinant poxvirus-based NYVAC-SIV-gpe vaccine candidate to induce durable LPR to both the core and envelope proteins of SIVmac251 and significantly increased the frequency of CD8+ T cells specific for the immunodominant epitope gag181 (p11 C-M). The gag181-specific T cells were functionally active and persisted for at least 5.5 mo following the final immunization. We also investigated whether the priming with DNA expanded the breadth of the immune response. Although no clear effect of DNA priming was observed using the tetramer staining or ELISPOT assays, the results of direct cytolytic activity assays following an expansion of cells in vitro suggested that DNA-gag181/NYVAC-SIV-gpe-boosted animals were able to recognize a broader range of epitopes than macaques that received NYVAC-SIV-gpe alone.

A combination of these vaccine modalities has been previously investigated by others (26, 48, 49, 50, 64). However, none of the previous studies in macaques directly compared the immunogenicity of poxvirus-based vaccine to a regimen of DNA priming and poxvirus boost. In this study we demonstrate vigorous SIV-specific LPR elicited by DNA priming and subsequent poxvirus boost in non-human primates. In addition, the prime-boost regimen induced durable CTL responses superior to those induced by immunization with a poxvirus-based recombinant vector alone. The animals in the DNA-primed group recognized a broader array of subdominant
SIV epitopes in the cytolytic, but not the IFN-γ ELISPOT assay, suggesting different levels of sensitivity of these functional assays.

The NYVAC-SIV-gpe-induced proliferative responses were up to 10-fold higher in DNA-SIV-gpe-primed animals than in animals receiving NYVAC-SIV-gpe alone. Interestingly, the level of these responses induced by NYVAC-SIV-gpe immunization in DNA-primed macaques was similar to that induced by NYVAC-SIV-gpe vaccination of SIVmac251-infected macaques treated with antiretroviral therapy (21), suggesting that pharmacologically controlled viral replication may prime similarly to a DNA vaccine.

Regarding the expansion of virus-specific CD8+ T cells, our findings parallel the observation of others, that in the boost with NYVAC-SIV-gpe of DNA-primed animals resulted in an induction of a high frequency of gag181-specific CD8+ T cells in most Mamu-A*01-positive macaques. In fact, in our study, the DNA priming strategy resulted in an induction in blood of 1.5–5.5% gag181-specific cells, and the response was more durable than in NYVAC-SIV-gpe-only immunized group (Fig. 4). In contrast, only one of four NYVAC-SIV-gpe-immunized macaques mounted a similar response (no. 674). A possible explanation could be that animal 674 is homozygous for the Mamu-A*01 allele, a hypothesis that at present cannot be tested because the PCR-based assay does not discriminate for homozygosity. Hanke et al. (26) have shown that a DNA prime, MVA boost regimen presenting a multiepitope gene construct induced 1–5% of circulating CD8+ lymphocytes specific for epitope gag181. In a study by Allen et al. (48), the use of a single epitope-based vaccine combined with hepatitis B core Ag vector to provide T cell responses resulted in induction of 0.8–20% of epitope-specific cells after the first MVA boost; however, three of six animals displayed levels lower than 1.6%. In our study, all five DNA-primed animals (group C) had from 1.5 to 5.5% of epitope-specific cells at 1 wk after the first NYVAC-SIV-gpe boost. Therefore, our multiepitope vaccine regimen expressing the whole Gag and Env proteins induced CD8+ T cell responses comparable to those induced by vaccine candidates designed for high expression of only a single or a few CTL epitopes. The frequency of gag181 tetramer-staining cells induced by DNA prime, NYVAC-SIV-gpe boost is equal to or higher than those found in the blood of acutely and chronically infected animals (33, 65, 66, 71 and B. R. Mothé, H. Horton, D. K. Carter, M. E. Liebl, S. Fuenger, P. Skinner, T. M. Allen, T. U. Vogel, W. Rehrauer, N. Wilson, G. Franchini, J. D. Altman, A. Haase, L. J. Picker, D. Allison, and D. I. Watkins; unpublished observations).

Likely, the repeated immunizations with recombinant poxvirus vectors may induce sufficient vector immunity to interfere with the presentation of the transgene upon subsequent boosts (35–37, 39). In fact, the CTL response to SIV gag was not further enhanced by the fourth immunization with NYVAC- (25) or MVA- (29, 33) based vaccine candidates. In this study, we show that both LPR and CTL responses were enhanced following the fourth immunization with NYVAC-SIV-gpe alone (group B). However, the level of these responses was not as high as that observed following a single NYVAC-SIV-gpe immunization of DNA-primed macaques (group C). Therefore, three or fewer inoculations with DNA combined with a single recombinant poxvirus-based vaccine boost might be sufficient to reach appropriate immune responses.

DNA priming may augments the overall immune response by focusing the response to the desired Ag; the recombinant poxvirus boost may then amplify the pool of existing Ag-specific cells by presenting the Ag in the context of local induction of cytokines and/or chemokines. Therefore, this strategy could be particularly useful in individuals with a pre-existing immunity to vaccinia virus. Alternatively, DNA priming might induce immune responses qualitatively different from those induced by immunization with recombinant poxviruses or other vaccine delivery vehicles, and the phenotypical difference of the T cell response may be maintained during subsequent booster immunizations or challenge infections (67). The ability of the prime-boost regimen to induce higher proliferative responses than NYVAC-SIV-gpe alone may relate to the ability of vaccinia viruses to interfere with the maturation of infected dendritic cells, decreasing their ability to present Ag to CD4 T cells, while preserving efficient Ag presentation to CD8 T cells (68, 69).

In conclusion, the combination of DNA priming and NYVAC-SIV-gpe boost increases the extent and durability of both the CD4+ and CD8+ T cell responses. In addition, DNA priming broadens the array of subdominant SIV epitopes recognized in the cytolytic, but not the IFN-γ, ELISPOT assay. Because the correlates of protection have not been fully defined, it remains to be ascertained whether these responses may be of sufficient breadth and quantity to afford protection from infection.

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References


POTENTIATION OF IMMUNE RESPONSES BY DNA-PRIME/NYVAC-SIV BOOST


