Cellular Immune Responses in Asymptomatic Human Immunodeficiency Virus Type 1 (HIV-1) Infection and Effects of Vaccination with Recombinant Envelope Glycoprotein of HIV-1

Geoffrey J. Gorse,1,2,* Ramona E. Simionescu,2† and Gira B. Patel2

Veterans Affairs Medical Center1 and Saint Louis University,2 St. Louis, Missouri

Received 27 June 2005/Returned for modification 31 August 2005/Accepted 18 October 2005

Effects of human immunodeficiency virus type 1 (HIV-1) recombinant envelope glycoprotein vaccines on cell-mediated immune (CMI) responses were assessed in HIV-1-infected patients. Asymptomatic, antiretroviral-treatment-naive, HIV-1-infected patients with CD4+ T-cell counts greater than 400/µl received multiple intramuscular injections of HIV-1 IIIB recombinant envelope glycoprotein (rgp160) vaccine or HIV-1 MN recombinant envelope glycoprotein (rgp120) vaccine (eight patients, referred to as the HIV-1 vaccinees) or placebo or hepatitis B vaccine (three patients, referred to as the controls). Lymphocyte proliferation in response to HIV-1 envelope glycoproteins, both homologous and heterologous to the HIV-1 immunogens, was absent prior to study treatment in all patients but increased significantly during the vaccination series and after the final vaccination in HIV-1 vaccinees (P < 0.05) and remained absent in control patients. In flow cytometric analyses of intracellular cytokines, T-cell receptor stimulation with an anti-CD3 antibody induced gamma interferon (IFN-γ) expression by activated CD4+ and CD8+ lymphocytes at greater frequencies than did stimulation with recombinant envelope glycoprotein and p24 of HIV-1 (P < 0.05). Mean frequencies of HIV-1 envelope glycoprotein-stimulated, activated intracellular IFN-γ-producing CD4+ and CD8+ lymphocytes and of interleukin-2-producing CD4+ lymphocytes did not increase after vaccination, but cytokine-producing cells were detectable in some patients. Comparing pre- to post-HIV-1 vaccination time points, changes in frequencies of activated, IFN-γ-producing CD4+ cells correlated inversely with changes in lymphocyte proliferation in response to recombinant envelope glycoprotein in HIV-1 vaccinees (P < 0.05). Increased CMI responses to HIV-1 envelope glycoprotein measured by lymphocyte proliferation were associated with HIV-1 recombinant envelope glycoprotein vaccines.

Therapeutic vaccination of human immunodeficiency virus type 1 (HIV-1)-infected patients has been evaluated with the goals of improving cell-mediated immunity through enhancing CD4+ T-cell responses and providing help to maintain CD8+ T-cell responses (4, 7, 14, 23, 25, 26, 27, 28, 30, 31, 33). Poor lymphocyte proliferative responses to stimulation with HIV-1 antigens have been associated with progression of HIV-1 disease, lower CD4+ T-cell counts, and higher viral loads (4, 14, 28, 32). Lymphocyte proliferation in response to HIV-1 envelope glycoprotein has been enhanced by vaccination with HIV-1 recombinant envelope glycoprotein vaccines in some, but not all, clinical trials; however, no clear clinical benefit from vaccination has been demonstrated (4, 7, 23, 25, 26, 28, 30, 31, 33).

Quantitative CD4+ and CD8+ T-cell responses following vaccination with HIV-1 recombinant envelope glycoprotein vaccines have received little attention. Ascertainment of frequencies of CD4+ and CD8+ cells that are responsive to antigenic and non-antigenic stimuli before and after vaccination may be an important adjunct to assessment of CD4+ helper cell responses by the lymphoproliferative assay. Our goal was to put the lymphocyte proliferative responses to vaccination in better perspective by also measuring frequencies of interleukin-2 (IL-2)- and gamma interferon (IFN-γ)-producing CD4+ and CD8+ cells in a pilot evaluation. Elaboration of these Th1 cytokines by CD4+ cells may provide some degree of assurance that enhanced lymphocyte proliferation following vaccination reflects a salutary immune benefit, since Th1 responses might be expected to sustain effector CD8+ cytotoxic T lymphocytes, which in turn produce IFN-γ and play a role in chronic control of viremia (15, 27). No assessment of possible clinical benefit of vaccination was done in our study.

MATERIALS AND METHODS

Subjects and study treatments. Eleven HIV-1-infected subjects, who participated at Saint Louis University after giving informed consent in one of two multicenter, institutional review board-approved clinical trials sponsored by the AIDS Vaccine Evaluation Group (AVEG protocols 101 and 104), were assessed for cell-mediated immune responses before and after vaccination. Selection for these laboratory studies was based on availability of cryopreserved peripheral blood mononuclear cells (PBMC) for analysis.

Eight subjects were enrolled in AVEG protocol 101 in 1992; they were asymptomatic HIV-1-infected patients who had mean CD4+ T-cell counts of at least 600/µl at entry, had no history of a condition that met the definition for AIDS, and had received no antiretroviral chemotherapy in the previous 6 months. Subjects enrolled in AVEG protocol 101 received study injections in the deltoid muscle monthly between study days 0 and 140. The HIV-1 envelope glycoprotein vaccine was recombinant HIV-1 IIIB gp160 (rgp160 IIIB) that had been produced in Vero tissue culture cells using recombinant vaccinia virus as described elsewhere (1, 2), formulated with aluminum hydroxide and deoxycholate adjuvant (IMMUNO-AG, Vienna, Austria), and given at 50 µg per injection dose. Of five recipients of the HIV-1 vaccine, three received six injections of rgp160 IIIB vaccine at monthly intervals and two received three injections of rgp160 IIIB...
TABLE 1. Lymphocyte proliferation measured prevaccination, during the vaccination series, and postvaccination

| Proliferation measure | HIV-1 vaccines (n = 8) | Mean result ± SEM (no. with SI of ≥3.0) |
|-----------------------|------------------------|----------------------------------------|
|                       | Prevaccination          | During vaccination series               | Postvaccination |
|                       | Control subjects (n = 3)| During vaccination series               | Postvaccination |

- **Tetanus toxoid**
  - Δcpm: 6.221 ± 3.635 (50.1 ± 31.9) (7)
  - SI: 7.682 ± 3.355 (94.0 ± 83.9) (5)
  - Prevaccination: 5.149 ± 3.235 (39.1 ± 31.6) (4)
  - During vaccination series: 4.365 ± 2.131 (23.5 ± 16.0) (3)
  - Postvaccination: 436 ± 361 (5.8 ± 4.6) (1)
  - Control subjects: 12,004 ± 7,439 (36.9 ± 14.3) (1)

- **HIV-1 immunogen**
  - Δcpm: 124 ± 101 (1.2 ± 0.2) (0)
  - SI: 4,726 ± 2,261 (12.3 ± 6.2) (4)
  - Prevaccination: 9,729 ± 6,252 (18.9 ± 7.2) (6)
  - During vaccination series: 26 ± 68 (1.1 ± 0.3) (0)
  - Postvaccination: 17 ± 60 (0.5 ± 0.2) (0)

- **Baculovirus-expressed rpg160 MN**
  - Δcpm: 2 ± 58 (1.3 ± 0.2) (0)
  - SI: 1,033 ± 725 (11.8 ± 5.9) (5)
  - Prevaccination: 6,7 ± 3.3 (0.3)
  - During vaccination series: 69 ± 110 (1.0 ± 0.3) (1)
  - Postvaccination: 33 ± 54 (1.1 ± 0.2) (1)

- **PHA**
  - Δcpm: 76,147 ± 22,189 (432.2 ± 19,011 (3)
  - SI: 7,628 ± 338,08 (537.4 ± 237.4) (3)
  - Prevaccination: 94,315 ± 18,899 (933.1 ± 326.9) (3)

- **Notes:**
  - *The time point during the vaccination series was 84 days after the first study injection for all subjects; the postvaccination time point was 168 (n = 2) or 336 (n = 6) days after the first study injection for AVEG protocol 101-enrolled subjects (n = 8) and 4 to 6 months after the first study injection for AVEG protocol 104-enrolled subjects (n = 3). See Materials and Methods.
  - *Δcpm, counts per minute of [3H]thymidine incorporated in unstimulated cells incubated in medium.**
  - *[3H]thymidine incorporated in stimulated cells. SI is calculated as counts per minute of stimulated cells divided by counts per minute of unstimulated cells.
  - *The recombinant envelope glycoprotein of HIV-1 antigen used as a vaccine in the protocols—either HIV-1 IIB rgp160 (AVEG protocol 101, with five vaccine recipients and three control subjects) or HIV-1 MN rgp120 (AVEG protocol 104, with three vaccine recipients)—was used as the in vitro antigen. See Materials and Methods.*
  - *P < 0.05; mean during vaccination series and postvaccination were each greater than prevaccination values.*
  - *<i>P < 0.01</i>; proportion of HIV-1 vaccines with an SI of ≥3.0 to HIV-1 immunogen was higher postvaccination than prevaccination.*
  - *<i>P < 0.05</i>; proportion of HIV-1 vaccines with an SI of ≥3.0 to baculovirus-derived rpg160 MN was higher during the vaccination series and postvaccination than prevaccination.*

Results were expressed as Δcpm (calculated as mean counts per minute of stimulated cell cultures minus mean counts per minute of medium control cell cultures) and as the stimulation index (SI), calculated as the ratio of the mean counts per minute of stimulated cell cultures to the mean counts per minute of medium control cell cultures.

- **Intracellular cytokine assay:** Flow cytometry was performed to detect intracellular cytokines expressed by activated CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes in PBMC that had been collected and cryopreserved before the first study injection and 19 to 23 months after the final study injection for subjects enrolled in AVEG protocol 101 and 0 to 3 months after delivery for subjects enrolled in AVEG protocol 104. Cells were not available for flow cytometric analysis at other study time points. PBMC from both time points for each subject were assayed simultaneously. Cells were thawed, washed, suspended in RPMI medium supplemented with 40% autologous plasma, and either left unstimulated in medium with 40% autologous plasma or stimulated with either baculovirus-expressed rgp160 MN (Protein Sciences) at 1 μg/ml, baculovirus-expressed HIV-1 LAI p24 Gag (p24; Protein Sciences) at 1 μg/ml, or a mouse monoclonal antibody to CD3 (Immunotech/Coulter Co., Marseilles, France) at 1 ng/ml. The manufacturer’s procedures (FastImmune immunostaining system; Becton Dickinson Immunocytometry Systems, San Jose, CA) for cell activation, staining, and flow cytometric analysis were used as described elsewhere (29). One recombinant envelope glycoprotein antigen was chosen due to the limitation in the number of cells for testing by flow cytometry. The antigen chosen was able to induce lymphocyte proliferation in recipients of either HIV-1 vaccine. Both stimulated and unstimulated control cells were incubated with mouse monoclonal antibodies to CD25 and CD49d (Immunotech/Coulter Co.) at 1 μg/ml for 6 h at 37°C under 5% CO<sub>2</sub> and brefeldin A (Sigma) was added at 10 μg/ml after the second hour to inhibit cytokine secretion. EDTA was then added at 2 mM, and the cells were fixed in formaldehyde. Cells were washed and incubated in a permeabilizing solution (BD FACS; Becton Dickinson Immunocytometry Systems). After an additional wash, about 2 × 10<sup>5</sup> cells were distributed per tube for staining. Cell aliquots were stained for intracellular and surface molecules with anti-IFN-γ or anti-IL-2 fluorescein isothiocyanate (FITC), anti-CD69 phycoerythrin (PE), and anti-CD4 or anti-CD8 PerCP-Cy5.5 mouse monoclonal antibodies (BD FastImmune; Becton Dickinson Immunocytometry Systems). After incubation for 30 min at room temperature, the cells were washed and fixed in 1% (vol/vol) paraformaldehyde for flow cytometric analysis.

Three-color flow cytometric analysis was done by acquiring data using...
FIG. 1. Intracellular IFN-γ expression with and without stimulation in peripheral blood mononuclear cells from blood drawn at the prevaccination time point from one of the HIV-1-infected patients studied. In the three-color flow cytometric analysis, gating was done to
TABLE 2. CD8+ cell activation and IFN-γ induction measured pre- and postvaccination

| In vitro stimulation | CD8+ cell population | Mean % positive cells ± SEM* (no. of subjects higher than unstimulated) |
|----------------------|----------------------|--------------------------------------------------|
|                      |                      | HIV-1 vaccines (n = 8)                           | Control subjects (n = 3) |
|                      |                      | Pre     | Post     | Pre     | Post     |
| None                 | CD69+                | 6.4 ± 1.5 (NA) | 1.8 ± 0.76 (NA) | 3.0 ± 1.9 (NA) | 2.8 ± 0.23 (NA) |
|                      | CD69+ IFN-γ          | 0.37 ± 0.21 (NA) | 0.22 ± 0.21 (NA) | 0.17 ± 0.17 (NA) | 0.39 ± 0.39 (NA) |
| Anti-CD3             | CD69+                | 20.9 ± 4.0 (8) | 10.9 ± 2.6 (8) | 14.0 ± 6.1 (3) | 32.4 ± 8.5 (3) |
|                      | CD69+ IFN-γ          | 2.6 ± 0.75 (7) | 2.1 ± 0.9 (8) | 1.6 ± 0.60 (3) | 7.4 ± 1.5 (3) |
| rgp160 MNb           | CD69+                | 9.6 ± 2.1 (5) | 8.8 ± 5.8 (6) | 5.2 ± 0.44 (2) | 7.8 ± 1.9 (3) |
|                      | CD69+ IFN-γ          | 0.80 ± 0.42 (5) | 1.1 ± 0.67 (3) | 0.62 ± 0.58 (2) | 0.29 ± 0.15 (1) |
| rp24b                | CD69+                | 8.0 ± 0.83 (5) | 2.8 ± 1.0 (3) | 3.4 ± 1.7 (1) | 4.7 ± 1.7 (2) |
|                      | CD69+ IFN-γ          | 1.0 ± 0.89 (2) | 0.04 ± 0.04 (0) | 0.09 ± 0.09 (0) | 0.01 ± 0.01 (1) |

* Net percentage after subtraction of staining by isotype controls. Pre, prevaccination; Post, postvaccination; NA, not applicable.

** P < 0.05; mean percentage higher than respective postvaccination value.

The anti-CD4 antibody was used to set an acquisition gate for analysis of CD4+ cells. The percentage of CD4+ T cells that stained CD69+ under the following in vitro conditions: unstimulated (b and f) or stimulated with anti-CD3 antibody (c and g), and recombinant gp160 of HIV-1 (d and h).

** Statistical methods.** Arithmetical means of continuous variables with standard errors of the means (SEM) are reported. A two-sided Fisher exact test was used for 2-by-2 comparisons of proportions. Means of paired data were compared using the Wilcoxon matched-pairs test. Correlations between lymphocyte proliferation and percentages of cells that were positive for CD69 and cytokines were assessed with the Spearman rank order correlation test.

**RESULTS**

**Subjects and CD4+ T-cell counts.** PBMC from eight HIV-1 vaccine recipients (four male and four female) with a mean age ± SEM of 28.8 ± 2.3 years and from three control subjects (male) with a mean age ± SEM of 32.5 ± 5.6 years were available for assay. Mean CD4+ T-cell counts ± SEM at postvaccination time points for the eight HIV-1 vaccine recipients were 952 ± 170 cells/μl and 915 ± 135 cells/μl, and for the three control subjects these values were 802 ± 39 cells/μl and 666 ± 109 cells/μl.

**Lymphocyte proliferation.** None of the HIV-1 vaccine recipients or control subjects exhibited lymphocyte proliferation in response to recombinant envelope glycoprotein antigens (respective immunogen and baculovirus-expressed rgp160 MN) prior to receiving study treatment injections (Table 1). The HIV-1 vaccine recipients had significant increases in mean lymphocyte proliferative responses to recombinant envelope glycoprotein antigens (respective immunogen and baculovirus-expressed rgp160 MN) during the vaccination series and at the postvaccination time point, but none of the control subjects had an SI of 3.0 or greater in response to recombinant envelope glycoprotein antigens (Table 1). The sole recipient of zidovudine therapy had an SI below 3.0 at all three time points to recombinant envelope glycoprotein antigens despite receiving rgp120 MN vaccine. Lymphocyte proliferative responses to tetanus toxoid antigen and to PHA did not change between study time points (Table 1).

**Flow cytometric analysis of cell activation and intracellular cytokine staining.** Figure 1 consists of representative two-color dot plots of gated CD4+ and CD8+ cells showing intracellular IFN-γ and CD69 surface staining of cells obtained from one subject at the prevaccination time point. CD69+ staining of unstimulated CD4+ cells and, to a lesser extent, CD8+ cells indicated T-cell activation either in vivo or as a result of in vitro cell manipulations, or both (Tables 2 and 3). In vitro stimulation with an anti-CD3 antibody increased the mean percentages of CD4+ and CD8+ cells that stained CD69+ and both CD69+ and IFN-γ+ at the two study time points (Tables 2 and 3) (P < 0.05). The means of the percentages of CD4+ and CD8+ cells that stained CD69+ IFN-γ+ and of CD4+ cells that were CD69+ IL-2+ did not differ statistically between pre- and postvaccination time points.
TABLE 3. CD4+ cell activation and IFN-γ induction measured pre- and postvaccination

| In vitro stimulation | CD4+ cell population | Mean % positive cells ± SEMa (no. of subjects higher than unstimulated) | HIV-1 vaccinees (n = 8)b | Control subjects (n = 3)c |
|----------------------|----------------------|-------------------------------------------------|--------------------------|----------------------------|
|                      |                      |                                                | Pre | Post | Pre | Post | Pre | Post |
| None                 | CD69+                | 29.4 ± 5.8 (NA) | 26.1 ± 6.2 (NA) | 41.5 ± 6.4 (NA) | 27.5 ± 12.3 (NA) |                                |
|                      | CD69+ IFN-γ          | 0.79 ± 0.43 (NA) | 0.64 ± 0.33 (NA) | 0.16 ± 0.11 (NA) | 0.07 ± 0.05 (NA) |                                |
| Anti-CD3             | CD69+                | 51.8 ± 7.1 (8)  | 45.7 ± 8.2 (7)  | 67.2 ± 1.5 (3)  | 62.3 ± 11.4 (3) |                                |
|                      | CD69+ IFN-γ          | 3.2 ± 1.2 (8)  | 5.9 ± 3.8 (6)  | 2.6 ± 1.3 (2)  | 4.8 ± 3.4 (3) |                                |
| rgp160 MNd           | CD69+                | 36.6 ± 5.1 (6)  | 27.0 ± 7.1 (4)  | 44.8 ± 2.5 (2)  | 25.6 ± 8.9 (2) |                                |
|                      | CD69+ IFN-γ          | 0.62 ± 0.27 (3)  | 1.0 ± 0.92 (4) | 0.89 ± 0.45 (2) | 0.23 ± 0.17 (3) |                                |
| rp24f                | CD69+                | 34.9 ± 6.6 (6)  | 29.8 ± 8.6 (4) | 44.8 ± 2.4 (2)  | 29.7 ± 13.7 (2) |                                |
|                      | CD69+ IFN-γ          | 0.80 ± 0.44 (5)  | 0.55 ± 0.52 (1) | 1.2 ± 1.0 (3)   | 0.02 ± 0.01 (2) |                                |

a Net percentage after subtraction of staining by isotype controls. Pre, prevaccination; Post, postvaccination; NA, not applicable.

b There was an inadequate number of cells to test one of the eight vaccinees postvaccination.

c rgp160 MN, baculovirus-expressed recombinant gp160 of HIV-1 MN; rp24, baculovirus-expressed recombinant p24 Gag of HIV-1 LAI.

d P < 0.05; mean value for anti-CD3-stimulated CD4+ CD69+ cells higher than means for unstimulated, rgp160 MN-stimulated, and rp24-stimulated CD4+ CD69+ cells in comparison of postvaccination versus unstimulated and higher than unstimulated and rgp160 MN-stimulated CD4+ CD69+ cells in comparison of prevaccination values.

e P < 0.05; mean value for anti-CD3-stimulated CD4+ CD69+ IFN-γ+ cells higher than means for unstimulated and rgp160 MN-stimulated CD4+ CD69+ IFN-γ+ cells in comparison of prevaccination values and higher than rp24-stimulated CD4+ CD69+ IFN-γ+ cells in comparison of postvaccination values.

poststudy treatment injection time points for each stimulation condition (Tables 2, 3, and 4). Cytokine responses declined from pre- to postvaccination time points for the recipient of rgp120 MN vaccine who was also treated with zidovudine.

The mean change in the percentage of CD4+ cells that were CD69+ IFN-γ+, subtracting pre- from poststudy treatment injection time points, was higher under all stimulation conditions among HIV-1 vaccine recipients than among control subjects (mean change ± SEM for vaccinees [n = 7] versus control subjects [n = 3], 2.9% ± 2.7% versus 2.2% ± 2.8% for anti-CD3, 0.60% ± 0.80% versus −0.66% ± 0.38% for rgp160 MN, and −0.30% ± 0.54% versus −1.1% ± 1.1% for rp24; P = not significant [NS] for each comparison). The mean change in the percentage of CD4+ cells that were CD69+ IL-2+, subtracting pre- from poststudy treatment time points, was higher under all stimulation conditions among HIV-1 vaccine recipients than among control subjects (mean change ± SEM for vaccinees [n = 7] versus control subjects [n = 3], 0.32% ± 1.5% versus −2.7% ± 5.2% for anti-CD3, 0.87% ± 1.0% versus 0.12% ± 0.63% for rgp160 MN, and 3.9% ± 3.7% versus −2.5% ± 2.1% for rp24; P = NS for each comparison). While the IFN-γ and IL-2 responses of CD4+ CD69+ cells may have appeared to be better preserved among vaccine recipients than among control subjects at the poststudy treatment injection time point, no conclusions can be drawn, because the differences did not achieve statistical significance.

Correlations between lymphocyte proliferation, cell activation and intracellular cytokine staining and between lymphocyte proliferation and cell activation among HIV-1 vaccine recipients. The difference in the SI (ΔSI) in response to stimulation with recombinant envelope glycoprotein, subtracting prevaccination from postvaccination time points, was negatively correlated with the change between time points in the percentage of rgp160 MN-stimulated CD4+ cells that were CD69+ IFN-γ+ (r = −0.85 [P < 0.05] for ΔSI in response to HIV-1 immunogen and change in rgp160 MN-stimulated percentage of CD4+ cells that were CD69+ IFN-γ+; r = −0.76 [P < 0.05] for ΔSI in response to baculovirus-expressed rgp160 MN and change in rgp160 MN-stimulated percentage of CD4+ cells that were CD69+ IFN-γ+) but not with the changes between time points in the percentages of rgp160 MN-stimulated CD4+ cells that were CD69+ or CD69+ IL-2+ and of CD8+ cells that were CD69+ or CD69+ IFN-γ+.

DISCUSSION

Vaccination of asymptomatic HIV-1-infected patients with HIV-1 recombinant envelope glycoprotein vaccines resulted in

TABLE 4. IL-2 production by activated CD4+ cells measured pre- and postvaccination

| In vitro stimulation | Mean % CD4+ CD9+ IL-2+ cells ± SEMa (no. of subjects higher than unstimulated) |
|----------------------|-------------------------------------------------|----------------------------|
|                      | HIV-1 vaccinees (n = 8)b | Control subjects (n = 3)c |
|                      | Pre | Post | Pre | Post | Pre | Post | Pre | Post |
| None                 | 1.6 ± 0.40 (NA) | 1.8 ± 0.88 (NA) | 1.4 ± 1.1 (NA) | 2.7 ± 2.3 (NA) |                                |
| Anti-CD3             | 1.8 ± 0.40 (4)  | 1.9 ± 1.3 (4)  | 4.5 ± 4.0 (3)  | 1.8 ± 1.8 (0) |                                |
| rgp160 MNd           | 2.0 ± 0.95 (3)  | 2.8 ± 2.0 (3)  | 0.64 ± 0.13 (2) | 0.75 ± 0.52 (0) |                                |
| rp24f                | 2.2 ± 0.47 (6)  | 6.2 ± 4.1 (5)  | 3.4 ± 2.3 (2)  | 0.84 ± 0.43 (1) |                                |

a Net percentage after subtraction of isotype controls. Pre, prevaccination; Post, postvaccination; NA, not applicable.

b There was an inadequate number of cells to test one of the eight vaccinees postvaccination.

c rgp160 MN, baculovirus-expressed recombinant gp160 of HIV-1 MN; rp24, baculovirus-expressed recombinant p24 Gag of HIV-1 LAI.
a significant increase in lymphocyte proliferation in response to HIV-1 envelope glycoprotein in our study. None of the patients had demonstrable lymphocyte proliferation in response to HIV-1 envelope glycoprotein prior to the first study treatment injection. Since lymphocyte proliferation in response to tetanus toxoid and mitogen was detectable before and after study treatment injections without any significant change, there was a possible defect in lymphocyte proliferative capacity that was HIV-1 specific prior to vaccination.

Improved lymphocyte proliferation in response to HIV-1 envelope glycoprotein and possibly non-HIV-1 antigens has been reported previously after vaccination with HIV-1 recombinant envelope glycoprotein vaccines for HIV-1-infected patients in some, but not all, studies. There has been little evidence of clinical benefit associated with vaccination, and effects of vaccination on CD4+ T-cell trends and plasma HIV-1 viremia have been inferior to those of antiretroviral therapy (4, 7, 23, 25, 26, 28, 30, 31, 33). Induction of new lymphocyte proliferative responses by HIV-1 recombinant envelope glycoprotein vaccines appeared to be associated with CD4+ T-cell counts that were greater than 350 cells/mm³ at baseline and with low plasma HIV-1 loads in one report (28). Positive lymphocyte proliferative responses were associated with lower plasma HIV-1 loads in a longitudinal study (24) and with higher pretreatment CD4+ T-cell counts and longer duration of HIV-1 suppression in patients receiving highly active antiretroviral therapy (16).

The lymphocyte proliferation assay is qualitative but is a functional end point that reflects a complex cellular immune response. It does not necessarily reflect a quantitative per-cell measure of stimulation. The lymphocyte proliferation assay depends on DNA replication and cell division over the course of 7 days, and while it is antigen specific and a reflection of a polyclonal CD4+ T-cell response, it may in part be due to proliferation of CD8+ T cells and non-antigen-specific bystander cells in response to cytokines produced by cells responding to the in vitro stimulus. CD4+ T cells that produce IFN-γ are detectable even in the absence of an in vitro lymphocyte proliferative response, but it is the HIV-1-specific lymphocyte proliferative response that has been linked to reduced HIV-1 load and maintenance of HIV-1-specific CD8+ T-cell responses (3, 11, 13, 21). In particular, high levels of lymphocyte proliferation in response to HIV-1 p24 Gag and HIV-1 envelope glycoprotein have been inversely correlated with HIV-1 loads (18, 27).

An anti-CD3 antibody induced an increased frequency of IFN-γ-producing CD4+ and CD8+ cells compared to no stimulation among subjects in our study, without statistical change between the study time points. Anti-CD3 antibody provides a non-antigen-specific stimulation of the T-cell receptor (TCR). Mean proportions of IFN-γ-producing CD4+ and CD8+ cells after stimulation with HIV-1 antigens were not significantly higher after HIV-1 vaccination. Mean postvaccination changes in frequencies of IFN-γ- and IL-2-producing CD4+ cells compared to prevaccination frequencies appeared higher among HIV-1 vaccine recipients than among control subjects. Higher frequencies of cytokine-producing cells by anti-CD3 stimulation of the TCR than by HIV-1 antigens may reflect anergy of HIV-1 antigen-specific T cells. Suboptimal costimulatory molecule usage is a possible factor that may be more important for antigen-specific stimulation than the stronger TCR signal provided by anti-CD3 (6). The response to anti-CD3 antibody may also be due to polyclonal stimulation of both naive and memory CD4+ and CD8+ cells.

When levels after HIV-1 vaccination were compared to those before vaccination, the change in frequency of IFN-γ-producing CD4+ cells in response to HIV-1 envelope glycoprotein was negatively correlated with the increased lymphocyte proliferative response to envelope glycoprotein among HIV-1 vaccine recipients. This may reflect relative stimulation of HIV-1-specific lymphocyte proliferation by the HIV-1 vaccine over IFN-γ production and a resultant lack of the inhibiting effect of IFN-γ on T-helper cell proliferation. Similarly, lymphocyte proliferation in response to HIV-1 p24 Gag antigen was not necessarily predictive of frequencies of IFN-γ-producing, anti-HIV-1 p24 Gag T cells (20).

Before HIV-1 vaccination and at all time points among the control subjects, there was a lack of demonstrable lymphocyte proliferation in response to HIV-1 envelope glycoprotein, but CD4+ and CD8+ cells capable of responding to HIV-1 antigen by production of IL-2 and IFN-γ were nonetheless present in some patients. Uncoupling between lymphocyte proliferation and Th1 cytokine-producing memory, antigen-specific CD4+ T cells has been noted previously in viremic HIV-1-infected patients, possibly due to CD4+ T-cell dysfunction (10, 17, 19, 22). Although not measured, our patients likely were viremic. In addition to enhanced HIV-1-specific lymphocyte proliferation, low viral load has been associated with HIV-1-specific production of IFN-γ and chemokines such as macrophage inflammatory proteins that are known to inhibit macrophage-tropic strains of HIV-1 (18, 27). Proliferation may also be associated with inflammatory cytokines and non-Th1 responses to antigen (12). Cytokines not measured in our study may have been augmented along with lymphocyte proliferation in response to HIV-1 envelope glycoprotein after vaccination. Hence, some cells that proliferated in response to HIV-1 envelope glycoprotein may have had a cytokine repertoire that did not include IL-2 and IFN-γ.

In summary, vaccination of asymptomatic HIV-1-infected patients with HIV-1 recombinant envelope glycoprotein vaccines was associated with new lymphocyte proliferative responses to envelope glycoprotein. There was no statistically significant increase in the frequency of in vitro-stimulated IL-2- or IFN-γ-producing cells after study treatment. There was an inverse correlation between pre- versus post–HIV-1 vaccination changes in lymphocyte proliferation and the frequency of CD4+ cells producing IFN-γ in response to HIV-1 recombinant envelope glycoprotein. Future studies should assess other cytokine profiles and chemokines in addition to IL-2 and IFN-γ to help delineate the characteristics of the cells that contribute to enhanced lymphocyte proliferation in response to vaccination. If future vaccination strategies can increase the frequencies of responder Th1 cytokine-producing CD4+ and CD8+ T-cells induced by HIV-1 antigens in addition to increased lymphocyte proliferation, consideration should be given to evaluating their clinical effects as well.

ACKNOWLEDGMENTS

We are grateful to Eric Valdivia and Carolyn Novotny for expert secretarial assistance and to J. Alan Arbuckle for technical assistance.
REFERENCES

1. Barrett, N. A., Mitterer, W. Mundt, J. Eibl, M. Eibl, R. C. Gallo, B. Moss, and F. Dorner. 1989. Large-scale production and purification of a vaccinia recombinant-derived HIV-1 gp160 and analysis of its immunogenicity. AIDS Res. Hum. Retrovir. 5:159–171.

2. Belisle, R. B., M. L. Clements, R. Dolin, B. S. Graham, J. McElrath, G. J. Gorse, D. Schwartz, M. C. Keever, P. Wright, L. Corley, D. P. Bolognesi, T. J. Matthews, D. M. Stabein, F. S. O’Brien, M. Eibl, F. Dornier, W. Koff, and the National Institute of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group, 1993. Safety and immunogenicity of a fully glycosylated recombinant gp160 human immunodeficiency virus type 1 vaccine in normal healthy volunteers. J. Infect. Dis. 168:137–139.

3. Betts, M. R., D. R. Ambrozak, D. C. Douek, S. Bonhoeffer, J. M. Brenchley, J. P. Casazza, R. A. Koup, and L. J. Picker. 2001. Analysis of total human immunodeficiency virus (HIV)-specific CD4+ and CD8+ T-cell responses: relationship to viral load in untreated HIV infection. J. Virol. 75:11987–11991.

4. Birx, D. L., L. L. Loomis-Price, N. Aronson, J. Brundage, C. Davis, L. Dayton, R. Garner, F. Gordin, D. Henry, W. Holloway, T. Kerkering, R. Luskewn, J. M. Neuwell, N. Michael, S. P. Piliers, D. Poretta, S. Rattio, P. Renzullo, N. Ruiz, K. Sitz, G. Smith, C. Tacket, M. Thompson, E. Trotter, R. T. Mitsuyasu, and T. Twaddell. 1996. Randomized trial of MMRng120 HIV-1 vaccine in symptomless HIV-1 infection. Lancet 348:1547–1551.

5. Boyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood. Scand. J. Clin. Lab. Invest. 21(Suppl. 97):77–89.

6. Dubey, C., M. Croft, and S. L. Swain. 1996. Naive and effector CD4 T cells differ in their requirements for T cell receptor versus costimulatory signals. J. Immunol. 157:3220–3229.

7. Eron, J. J., Jr., M. A. Ashley, M. F. Giordano, M. Chernow, W. M. Reiter, S. G. Deeks, J. P. Lavelle, M. A. Conant, B. G. Yangco, P. G. Fata, R. A. Torres, R. T. Mitsuyasu, and T. Twaddell. 1996. Diminished proliferation of human immunodeficiency virus-specific CD4+ T cells is associated with diminished interleukin-2 (IL-2) production and is recovered by exogenous IL-2. J. Virol. 70:10900–10909.

8. Gorse, G. J., and R. B. Belisle. 1991. Enhanced lymphoproliferation to influenza A virus following vaccination of older, chronically ill adults with live-attenuated viruses. Scand. J. Infect. Dis. 23:7–17.

9. Gorse, G. J., R. B. Belisle, F. K. Newman, S. E. Frey, and the NAIDAS Vaccine Clinical Trials Network. 1992. Lymphocyte proliferative responses associated with control of viremia in whole blood by flow cytometry. J. Immunol. 148:1677–1684.

10. Palacios, T. M., R. A. Koup, and C. J. Pilotter. 2000. Discordance between frequency of human immunodeficiency virus type 1 (HIV)-1-specific gamma interferon-producing CD4+ T cells and HIV-1-specific lymphoproliferative responses in HIV-1-infected subjects with active viral replication. J. Virol. 74:5925–5936.

11. Papasavvas, E., J. K. Sandberg, R. Rutstein, E. C. Moore, A. Mackiewicz, B. Thiel, M. Pistilli, R. R. June, K. A. Jordan, R. Gross, V. C. Maino, D. F. Nixon, and L. J. Montaner. 2003. Presence of human immunodeficiency virus-1-specific CD4+ and CD8+ cellular immune responses in children with full or partial virus suppression. J. Infect. Dis. 188:873–882.

12. Piazza, P., Z. Fan, and C. R. Rinaldo, Jr. 2002. CD4+ T-cell immunity in human immunodeficiency virus-infected children. Clin. Infect. Dis. 35:1387–1395.

13. Pitcher, C. J., Q. Quitter, D. M. Peterson, M. Connors, R. A. Koup, V. C. Maino, and L. J. Picker. 1999. HIV-1-specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. Nat. Med. 5:631–637.

14. Ponteselli, O., E. C. Guerra, A. Ammassari, C. Tomino, M. Carlesimo, A. Antinori, E. Tamburrini, A. Prozzo, A. C. Seeker, S. Vella, L. Ortona, F. Aiuti, and the VaxSyn Protocol Team. 1998. Phase II controlled trial of post-exposure immunization with recombinant HIV-1 Env glycoprotein versus antiretroviral therapy in asymptomatic HIV-1-infected adults. AIDS 12:873–880.

15. Ratko-Kim, S., R. P. Garner, J. H. Kim, L. L. Jagodzinski, N. L. Michael, R. Paris, R. Redfield, and D. L. Birx. 2004. Prospective analysis of HIV-1-specific proliferative responses, recall antigen proliferative responses, and clinical outcomes in an HIV-1-seropositive cohort. J. Infect. Dis. 189:1968–1995.

16. Ratko-Kim, S., K. V. Sitz, R. P. Garner, J. H. Kim, C. Davis, N. Aronson, N. Ruiz, K. Tencer, R. R. Redfield, and D. L. Birx. 1999. Repeated immunization with recombinant gp160 human immunodeficiency virus (HIV) envelope protein in early HIV-1 infection: evaluation of the T cell proliferative response. J. Infect. Dis. 179:337–344.

17. Redfield, R. D., R. L. Birx, N. Ketter, E. Tramont, V. Polonis, C. Davis, J. F. Brundage, G. Smith, S. Johnson, A. Fowler, T. Werthea, A. Fehmeck, T. Volvovitz, C. Oster, D. S. Burke, and the Military Medical Consortium for Applied Retroviral Research. 1991. A phase I evaluation of the safety and immunogenicity of vaccination with recombinant gp160 in patients with early human immunodeficiency virus infection. N. Engl. J. Med. 326:1677–1684.

18. Rosenberg, E. S., J. M. Billingsley, A. M. Caliendo, S. L. Boswell, P. E. Sax, S. A. Kalams, and B. D. Walker. 1997. Viral HIV-specific CD4+ T cell responses associated with control of viremia. Science 278:1447–1450.

19. Schooley, R. T., C. Spinco, D. Kuritzkes, B. D. Walker, F. T. Valentine, M. S. Hirsch, E. Cooney, G. Friedland, S. Kundra, T. C. Merigan, Jr., M. J. McElrath, A. Collifer, S. Peaeger, R. Mitsuyasu, J. Kahn, P. Haslett, P. Uherova, V. deGruttiola, S. Chin, B. Zhang, G. Jones, D. Bell, N. Ketter, T. Twaddell, D. Chernoff, M. Dalban, B. D. Walker, and M. Metcalfe. 2003. Prediction of virologic failure in a phase III randomized clinical trial in HIV-1-infected individuals across a spectrum of disease severity: AIDS Clinical Trials Group Protocol 209 and 214. J. Infect. Dis. 188:1357–1364.

20. Suni, M. A., L. J. Picker, and V. C. Maino. 1998. Detection of antigen-specific T cell cytokine expression in whole blood by flow cytometry. J. Immunol. Methods 212:89–98.

21. Valentine, F. T., S. Kundu, P. J. Haslett, K. Katzenstein, L. Beckett, C. Spinco, M. Borucki, M. Vasquez, G. Smith, J. Korvick, J. Kagan, and T. C. Merigan. 1996. A randomized, placebo-controlled study of the immunogenicity of human immunodeficiency virus (HIV) gp160 vaccine in HIV-1-infected subjects with 3600/mm3 CD4+ T lymphocytes (AIDS Clinical Trials Group Protocol 137). J. Infect. Dis. 172:1336–1346.

22. Wahren, B., G. Bratt, C. Persson, B. Leen, J. Hinkula, G. Gilljam, S. Nordlund, L. Eriksson, F. Volvovitz, P. A. Brodien, and E. Sandstrom. 1994. Improved cell-mediated immune responses in HIV-1-infected asymptomatic individuals after vaccination with envelope glycoprotein gp160. J. Acquir. Immune Defic. Syndr. 7:220–229.

23. Wilson, J. D. K., N. Imami, A. Watkins, J. Gill, P. Hay, B. Gazzard, M. Westby, and F. M. Gotch. 2000. Loss of CD4+ T cell proliferative ability but not loss of human immunodeficiency virus type 1-specificity equates with progression to disease. J. Infect. Dis. 182:792–798.

24. Wright, P. F., J. S. Lambert, G. J. Gorse, R.-H. Hsieh, M. J. McElrath, K. Weinhold, D. W. Wara, E. L. Anderson, M. C. Keever, S. Jackson, L. J. Wagner, D. F. P rancher, and the VaxSyn Protocol Team. 1999. Immunization with envelope MN gp120 vaccine in human immunodeficiency virus-infected pregnant women. J. Infect. Dis. 180:1080–1088.