A Canarypox Vaccine Expressing Multiple Human Immunodeficiency Virus Type 1 Genes Given Alone or with Rgp120 Elicits Broad and Durable CD8+ Cytotoxic T Lymphocyte Responses in Seronegative Volunteers

Thomas G. Evans,1 Michael C. Keefer,1 Kent J. Weinhold,2 Mark Wolff,4 David Montefiori,3 Geoffrey J. Gorse,7 Barney S. Graham,8 M. Juliana McElrath,9 Mary Lou Clements-Mann,4a Mark J. Mulligan,10 Patricia Fast,6b Mary Clare Walker,6 Jean-Louis Excler,11,b Ann-Marie Duliege,12 James Tartaglia,2 and the NIAID AIDS Vaccine Evaluation Group

Induction of CD8+ cytotoxic T cells is considered one of the important correlates for the protective efficacy of candidate human immunodeficiency virus type 1 (HIV-1) vaccines. To induce CD8+ cytotoxic T lymphocytes (CTLs) along with neutralizing antibody and CD4+ T cell help, a live canarypox virus construct expressing gp120, transmembrane gp41, the gag and protease genes, and sequences containing CTL epitopes in nef and pol was given simultaneously or, followed by, rgp120 SF2. CD8+ CTLs were detected in 61% of volunteers at some time during the trial. Three to 6 months after the last immunization, the gene-specific responses were gag, 26/81; env, 17/77; nef, 12/77; and pol, 3/16. Simultaneous immunization with the canarypox vector and the subunit, beginning with the initial immunization, resulted in earlier antibody responses. In summary, a strategy of immunization with a canarypox vector expressing multiple genes of HIV-1 given with gp120 results in durable CD8+ CTL responses to a broad range of epitopes.

The development of an effective prophylactic vaccine for human immunodeficiency virus type 1 (HIV-1) is hindered by the lack of a known immunologic correlate of protection. However, until an efficacy trial is undertaken, those evaluating candidate vaccines must rely on criteria chosen from other clinical settings, such as the immunologic responses found in HIV-1-infected long-term nonprogressors and in HIV-1–exposed but uninfected children and adults, and primate vaccine studies. It is widely believed that two of the more important responses for preventing or controlling HIV-1 infection are a vigorous CD8+ cytotoxic T cell lymphocyte (CTL) response (CD8+ CTL) and the development of antibody that would neutralize primary, transmitted viruses [1, 2].

Previously, live vector recombinants have successfully induced CD8+ CTL in a number of HIV-1 vaccine trials [3–12]. A critical factor may be the degree and breadth of CTLs induced by these vector-based vaccine candidates. Each volunteer is able to respond to a finite number of MHC class I–restricted epitopes presented by a vaccine, and evidence of either mutational escape or variant peptide antagonism is well documented in HIV-1–infected individuals [13–17]. This limited recognition can potentially be overcome by increasing the number of CTL epitopes in the immunogen, either by including the target genes of more HIV-1 strains or by including genes for several molecular components of HIV-1.

The poxvirus vector constructs that have induced CTLs in humans have not effectively elicited high-titer neutralizing antibodies [3, 11, 18]. However, the use of an envelope protein immunization after the live vector recombinant has resulted in
higher levels of binding and homologous isolate neutralizing antibodies, antibody-dependent cytotoxicity, and T cell help as measured by lymphoproliferation [3, 11, 19, 20]. In addition, murine models have revealed that CD4 help, although not required for CD8+ CTL generation, is needed for persistence of CD8 memory [21–25]. On the other hand, there has been concern that attempts to elicit antibody and CTLs simultaneously may result in down-regulation of the antibody or CTL response [26–28].

To address some of these questions, a Phase I vaccine trial was undertaken in which an ALVAC (canarypox virus) vector (vCP300) encoding multiple genes (MN gp120, the transmembrane portion of IIIB gp41, IIIB gag and protease, 3 CTL-dense regions of LAI pol, and 2 CTL-dense regions of LAI nef) was given in conjunction with a recombinant SF-2 gp120 in MF59 adjuvant (rgp120). The study design allowed comparison of the vector given alone, simultaneously with, or followed by rgp120.

Methods

Vaccine and placebo constructs. ALVAC-HIV vCP300 (Pasteur Merieux, Lyon, France) is a recombinant canarypox virus capable of entering mammalian cells, expressing its genes, and producing noninfectious, virus-like particles. These viruses are nonreplicative in humans, pose no risk of productive poxvirus infection such as that seen with recombinant vaccinia viruses, and are equally immunogenic in persons with prior vaccinia immunization [29–31]. vCP300 expresses the following HIV-1 gene products: LAI gag p55; LAI protease p15; part of env expressing MN gp120 and the anchoring transmembrane region of LAI; 2 nef genes encoding 9.1- and 3.3-kDa peptides (amino acids [aa] 66–147 and 182–206); and 3 LAI pol regions encoding 5.5-, 7.2-, and 7.0-kDa peptides (aa 172–219, 325–383, and 461–519). The pol and nef sequences were chosen for their high density of known MHC class I-restricted epitopes. The canarypox is grown on chick embryo cells and purified to a final titer of 10^6.3 TCID50. During this trial, it was noted that the vaccine was not completely stable, resulting in a fall in titer from 10^6.3 to 10^5.8 TCID50 over an 8-month period. The control vaccine was an ALVAC recombinant expressing Rabies virus glycoprotein 65 (rgp120). The study design allowed comparison of the vector given alone, simultaneously with, or followed by rgp120.

HIV-1 SF-2 rgp120 (Chiron, Emeryville, CA) was produced from Chinese hamster ovary tissue culture cells and formulated in a citrate buffer with MF59 adjuvant as an emulsion, containing 0.5% polysorbate 80, 0.5% sorbitan trioleate, and 5% squalene, as described elsewhere [34]. The rgp120 (50 μg) or MF59 placebo was given intramuscularly (im) in 1-mL volumes.

Study design and monitoring. This multicenter, randomized, double-blind, Phase I safety and immunogenicity study was carried out by the National Institute of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group (AVEG). Randomization, data, and study management were performed by The EMMES Corporation. An AIDS Vaccine Data Safety and Monitoring Board met regularly to review safety. Only volunteers at lower risk for HIV-1 infection as defined by AVEG criteria were enrolled at the 6 clinical sites [35, 36]. All volunteers, coordinators, and laboratory personnel were blinded to the immunizations received. Safety considerations included close monitoring for local toxicity, systemic symptoms, and hematologic, immunologic, hepatic, renal, and other reactions after each immunization and throughout the trial. To obtain data on the shorter schedule and simultaneous immunizations more quickly, enrollment was staggered such that arms D–G were fully enrolled prior to the enrollment of arms A–C (table 1). The first volunteer was enrolled in February 1996, and the last was enrolled in January 1997.

CTL assay ([AVEG standard protocol]) [10, 11]. Initial studies were undertaken in 31 selected subjects, to define an optimal in vitro stimulation method that compared the use of canarypox immunogen with a cocktail of vaccinia vectors (vP1291-containing gp120, gp41, gag, and protease regions of the vaccinia immunogen, vP1288-IIIB pol, vP1218-MN nef) for infecting the stimulator peripheral blood mononuclear cells (PBMC). A higher rate of bulk false-positive assays was found by use of the canarypox stimulation than the vaccinia stimulation vectors, and canarypox vector stimulation was discontinued. Also, to compare the env- and gag-specific CD8+ CTL activity with other AVEG phase 1 trials in which the pol and nef epitopes were not included (by use of the canarypox immunogen vCP205), most of the 12-month assays were conducted with the vP1291 plus vP1218 stimulation strategy.

Table 1. Study design of vCP300 phase 1 trial.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Immunization schedule (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>D</td>
<td>A</td>
</tr>
<tr>
<td>E</td>
<td>A</td>
</tr>
<tr>
<td>F</td>
<td>A</td>
</tr>
<tr>
<td>G</td>
<td>A+SF</td>
</tr>
</tbody>
</table>

NOTE. Each group contains 17 vaccine recipients and 3 volunteers who received either MF59 alone, ALVAC-rabies glycoprotein 65, or both. A, ALVAC-HIV (vCP300) dose of 10^6 TCID₅₀; SF, SF-2 rgp120 (50 μg) in MF59 adjuvant.
<30% maximal detergent lysis. A positive assay was scored when the HIV-1–specific target had lysis 10% greater than the vaccinia vP1170 control. The assay is considered to be CD8 positive when at least 50% of the activity is lost, when the PBMC are depleted by use of anti-CD8 monoclonal antibodies linked to magnetic beads (Dynalbeads; Dynal, Great Neck, NY). Specimens assayed at E:T ratios of both 50:1 and 25:1 were reduced to a single outcome by the following algorithm: The CTL assay is negative if the 50:1 E:T ratio results do not support the findings of elevated cytolytic activity at the 25:1 E:T ratio. Percent-specific lysis results at 25:1 are used when available, unless 50:1 E:T indicates cytolytic activity and the 25:1 E:T results are either negative or not performed. In a rare number of cases in which multiple tests were conducted for the same gene target at a given time point (gag), the maximum lysis result was used.

**Antibody studies.** Serum specimens collected before and 2 weeks after immunizations and at other defined time points were analyzed at a single laboratory (at Duke University) by ELISA for binding antibodies to recombinant SF-2 and MN gp120 (VaxGen, South San Francisco, CA) and to 24-residue V3 loop peptides [19]. Fusion inhibition assays were performed by blocking the binding of soluble CD4 to MN gp120 immobilized on 96-well plates [37]. Neutralization antibody assays were performed with the SF-2 and MN HIV-1 strains in CEMx174 and MT-2 cells, respectively, by using neutral red to quantitate viable cells that survived viral-induced cytopathic effects. In short, 500 TCID$_{50}$ of HIV-1 were incubated with multiple dilutions of test sera in triplicate at 37°C for 1 h, after which time cells were added. Viable cells are quantified 4–5 days later, when >70% cell killing had occurred in the virus control wells (cells plus virus but no serum sample). Neutralization titers are defined as the reciprocal of the serum dilution needed to produce 50% protection [38].

**Statistical tests.** Data from the 6 participating sites and the Central Laboratory for this study were collected in a distributed data system developed specifically for AEG trials. Statistical analysis was done with SAS Version 6.12. χ² tests were used to compare frequency distributions; Fisher’s exact test was used in 2 × 2 tables. Logistic regression and generalized estimating equations were used to explore the relationship of CD8$^+$ CTLs with vaccine group, schedule, and regimen.

**Results**

A total of 140 individuals were enrolled in the 7-arm trial (table 1). Dosing and immunization schedules were based on previous studies [3, 11, 19]. Volunteers in arms A–F received vCP300 im at months 0 and 1. These volunteers were then reimmunized im with either vCP300, rgp120, or vCP300 plus rgp120 on either a 3- and 6-month or a 6- and 9-month schedule. Volunteers in arm G received vCP300 and rgp120 simultaneously at 0, 1, 6, and 9 months. Each arm contained 17 vaccine and 3 control (ALVAC-rabies glycoprotein vCP65 and/or MF59) recipients. For the simultaneous inoculations, volunteers received the vCP300 or control rabies glycoprotein in one deltoid and the gp120 or control MF-59 in the opposite deltoid. Three volunteers discontinued immunization and another was lost to follow-up because of relocation.

CTL activity was measured in fresh PBMC as both bulk CD4$^+$ and CD8$^+$-specific activity in all participants 2 weeks after the third and fourth vaccinations. Persistence or late development of CTLs was measured by assays at month 12 (3–6 months after the last vaccination). The results of all CD8$^+$ CTL assays after stimulation with vaccinia vectors against all targets are shown in figure 1 and table 2. Each symbol represents CD8$^+$ CTLs (>10% specific chromium release) against specific vectors at an E:T ratio of 25:1 and 50:1. By the end of the study, the rates of vCP300 immunized volunteers with positive CD8$^+$ CTL activity specific for a particular HIV component at any assay time point ranged from 40% (6/15) in arm F to 81% (13/16) in arm C (table 2). Of these 68 individuals with CD8$^+$ CTLs, 15 (22%) had repeatedly positive assays. Remarkably, the highest frequencies of positive assays were not seen shortly after immunizations but rather later in time. At the 12-month time point, 32/83 (39%) of all vCP300 recipients tested had CD8$^+$CTLs against at least 1 target (table 2). This compared with 2/18 (11%) in the control group. A trend was observed that more vaccinated volunteers had positive CTLs at 12 months after receiving vCP300 plus rgp120 than volunteers receiving ALVAC vCP300 alone (27/60 in arms B, C, E, F, and G vs. 5/23 in arms A and D, P = .08 by Fisher’s exact test). In addition, the rates were significantly higher (P = .002) in those immunized on the 0-, 1-, 6-, and 9-month schedule (73%, arms A–C and G) than in those immunized on the 0-, 1-, 3-, and 6-month schedule (44%, arms D–F). Of note, the former group had received their last vaccines 3 months before the measurement at 12 months.

Immunization with vCP300 induced CD8$^+$ CTLs to all of the encoded genes in at least some volunteers. In vaccinees, the rates of responses at the 12-month time point to env were 17/77 (22%), gag 26/81 (32%), nef 12/77 (16%), and pol 3/16 (19%; table 3). In the control recipients, the response rates of CD8$^+$ CTLs were 1/17 (6%), 2/17 (12%), 0/16 (0%), and 0/5 (0%), respectively. Positive CTLs in control recipients were spread randomly over epitopes and volunteers (figure 1). In contrast to the 22% of ALVAC recipients with CD8$^+$ CTLs, no control recipient had repeatedly reactive CTLs against the same target, which emphasizes the random nature of the false-positive results. The overall results can also be analyzed as the rates of positive CD8$^+$ CTLs per assay for each target (table 3). The background false-positive average results of 7% per assay (defined as per each gene target) are in accord with rates seen in previous immunization trials: 5.5% for 1151 control assays performed at the AEG central laboratory.

There was no statistically significant increase in those individuals who had received 2 canarypox immunizations followed by rgp120 compared with those who received 4 canarypox immunizations along with rgp120, although a trend toward a better response with more vCP300 immunizations was noted (P = .08). Indeed, 36% of individuals who received only 2 can-
Figure 1. Upper half presents specific lysis of relevant human immunodeficiency virus type 1 (HIV-1) targets for each positive CD8⁺ cytotoxic T lymphocyte (CTL) response from both vaccinees (V) and control (C) recipients at each measurement time point (2 weeks after third and fourth vaccines and at 1 year after study initiation). Definition of positive result is found in Methods. Only CTLs that had at least 50% depletion with CD8⁺ T cell removal by magnetic beads are shown. Bar represents geometric mean for each group. Lower half shows percent positive and number of CD8⁺ CTLs performed in both controls and vaccinees. A, gag; B, env; C, nef; D, pol.
arypox immunizations, at months 0 and 1, had a positive CTL assay at 1 year. Simultaneous administration of both the ALVAC vCP300 and rgp120 induced greater CD8+ CTL response at 12 months than in all of the other treatment arms combined (10/16 vs. 22/67, *P* = .04), as well as the more rapid development of both binding and neutralizing antibodies.

The level of the antibody response was most dependent on the gp120 subunit administration. The recipients of the vCP300 alone had less-frequent and lower titer-binding antibodies than any group that received the vCP300 with rgp120. These results were obtained whether antibodies to MN gp120, MN V3 loop, SF-2 gp120, or SF-2 gp120 V3 loop were assessed by ELISA. A representative example of the differences in mean optical density for the 7 arms of the study is shown in figure 2 for the binding antibodies to the MN gp120 V3 peptide. These same increases in antibody with subunit administration were mimicked by the MN and SF-2 strain-specific neutralization results (table 4). Simultaneous immunization (arm G) led to a more rapid rise in neutralizing antibody and to similar titers at the end of the overall immunization period. These neutralization responses were more frequent and of higher titer (geometric mean titer [GMT] of 173–506) in volunteers receiving ALVAC vCP300 plus rgp120 (regardless of schedule) compared with those receiving ALVAC vCP300 alone (GMT of 32 and 35).

As limited primary isolate neutralization had been seen in previous trials by use of the SF-2 gp120 immunogen, no assays were performed against such strains in this study. Lymphoproliferation was measured from cryopreserved PBMC in a subset of volunteers (*n* = 38) representing each of the study arms. All immunization arms except the controls resulted in proliferation of PBMC in vitro to gp160 antigen (minimum positive cutoff of SI = 3.0). After 4 immunizations, the stimulation indices achieved with 10 μg/mL recombinant gp160 (either IIIB or MN strain) used as the antigen ranged from 3.8 to 21.4 (data not shown). Tetanus toxoid and Candida used as the in vitro recall antigen controls averaged stimulation indices of ~10–30 in these assays using cryopreserved cells. There were no significant differences between study arms.

Overall the immunizations were well tolerated. Statistical tests were used to compare vaccinees and controls and to compare vaccinees in arm G (coadministered vaccines) with vaccinees in other treatment arms for the level of systemic symptoms, pain and tenderness, erythema, induration, and temperature. There were no statistically significant (*P* < .05) differences.

**Discussion**

ALVAC canarypox–based vectors have numerous advantages over other vaccine candidates for CD8+ CTL induction in humans. They are nonreplicative in mammalian cells and thus do not pose the risk of progressive infection that accompanies replication-competent vaccinia-based vectors. Cells infected with viral constructs that encode Gag have been shown to produce pseudovirions that bud from the cell surface for up to 3 days after infection in vitro (Spearman P, personal communication), and these particles may stimulate ongoing immune responses. In addition, various canarypox HIV-1 constructs have now been administered to >1600 individuals, of whom 700 received canarypox–HIV-1 vaccine candidates with no serious vaccine-related adverse reactions. In addition, there is no evidence that the humoral or antibody response to ALVAC vectors is influenced by prior vaccinia immunization [39].

An ALVAC-based HIV-1 vaccine candidate encoding gp120, the transmembrane region of gp41, gag, protease, and sequences encoding known human CTL epitopes of pol and nef [40] was able to induce memory CD8+ CTL activity in the majority of recipients in this study. This CTL activity was quite durable. In fact, responses 3–6 months after the final immunization were at least as frequent as those measured 2 weeks after the third and fourth immunizations. This late CTL response is not an artifact of our measurement system, as similar results have been observed in trials of vCP205, which encodes the same sequences as vCP300 except for the pol and nef sequences [39]. In 1 trial of a canarypox recombinant HIV-1 vaccine (AVEG 022), as many as 50% of vaccine recipients had positive Env or Gag CD8+ CTLs when measured 1 year after the last immunization [41].

Other studies using canarypox vectors have noted similar response rates against each gene product included in the immunogen [5, 11]. Of note, the per assay frequency of Env or Gag CD8+ CTLs in this trial (26%) is similar to other AVEG studies, which have used a canarypox vector that does not include the nef and pol epitopes (31% for vCP205 in 1 trial). That is, the inclusion of more sequences in the canarypox vector

### Table 2. CD8+ cytotoxic T lymphocyte responses by treatment group.

<table>
<thead>
<tr>
<th>Group</th>
<th>2 w. after third vaccine</th>
<th>2 w. after fourth vaccine</th>
<th>12 mo. after enrollment</th>
<th>After 3, 4, or 12 mo.</th>
</tr>
</thead>
<tbody>
<tr>
<td>All controls</td>
<td>2/17 (12)</td>
<td>5/18 (28)</td>
<td>2/18 (11)</td>
<td>7/21 (33)</td>
</tr>
<tr>
<td>AA/-/A/A</td>
<td>11/15 (73)</td>
<td>5/14 (36)</td>
<td>3/12 (25)</td>
<td>11/16 (69)</td>
</tr>
<tr>
<td>AA/-/S/S</td>
<td>7/14 (50)</td>
<td>1/12 (8)</td>
<td>4/12 (33)</td>
<td>11/15 (73)</td>
</tr>
<tr>
<td>A/A/-/A/AS/AS</td>
<td>4/9 (44)</td>
<td>8/14 (57)</td>
<td>4/9 (44)</td>
<td>13/16 (81)</td>
</tr>
<tr>
<td>A/A/AA/-</td>
<td>2/14 (14)</td>
<td>4/15 (27)</td>
<td>2/11 (18)</td>
<td>7/16 (44)</td>
</tr>
<tr>
<td>A/A/AS/-</td>
<td>3/16 (19)</td>
<td>1/17 (6)</td>
<td>5/13 (38)</td>
<td>8/17 (47)</td>
</tr>
<tr>
<td>A/A/AS/-</td>
<td>1/14 (7)</td>
<td>4/13 (31)</td>
<td>4/10 (40)</td>
<td>6/15 (40)</td>
</tr>
<tr>
<td>A/AS/AA/-</td>
<td>5/16 (31)</td>
<td>6/16 (38)</td>
<td>10/16 (63)</td>
<td>12/17 (71)</td>
</tr>
</tbody>
</table>

**NOTE.** Values are no. positive/volunteers (%). A, ALVAC vCP300; S, SF gp120 (see table 1); w, weeks.

### Table 3. Rate of CD8-positive assays by gene and treatment group.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Vaccinees</th>
<th>Controls</th>
<th>Vaccinees</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD8+ in (%)</td>
<td>CD8+ in (%)</td>
<td>CD8+ in (%)</td>
<td>CD8+ in (%)</td>
</tr>
<tr>
<td>env</td>
<td>17/77 (22)</td>
<td>1/17 (6)</td>
<td>50/269 (19)</td>
<td>4/53 (8)</td>
</tr>
<tr>
<td>gag</td>
<td>26/81 (32)</td>
<td>2/17 (12)</td>
<td>74/383 (19)</td>
<td>7/73 (10)</td>
</tr>
<tr>
<td>nef</td>
<td>12/77 (16)</td>
<td>0/16 (0)</td>
<td>34/170 (20)</td>
<td>2/50 (4)</td>
</tr>
<tr>
<td>pol</td>
<td>3/16 (19)</td>
<td>0/5 (0)</td>
<td>23/177 (13)</td>
<td>2/32 (6)</td>
</tr>
</tbody>
</table>
Figure 2. Antibody response to differing immunization arms and schedules are shown as mean optical density (OD) for each group in binding ELISA assay using human immunodeficiency virus type 1 MN V3 loop as coating antigen. Time points shown represent prevaccination and 2 weeks after first, second, third, and fourth immunization. Early antibody response seen in group receiving simultaneous immunization is significantly different from any of other 6 arms. In addition, the response to volunteers receiving the canarypox immunizations alone (A/A/A/A/– and A/A/–/A/A) are significantly lower after third immunization than in treatment arms that included gp120 subunit.

did not appear to down-regulate the response through competition for antigen processing and presentation. The limit of the number of genes that can be encoded by the pox vectors is large and not exceeded in the vCP300 construct. The nef and pol epitopes were recognized, albeit at a low frequency at the 12-month time point. The induction of a broader CTL repertoire that would encompass a greater number of HLA class I restrictions and allow a wider epitope response upon actual challenge is a desirable vaccine strategy.

The ALVAC HIV-1 vaccine candidate vCP300 alone did not induce high titers of binding or homologous strain neutralizing antibodies. Previous studies of canarypox or vaccinia vectors have also required a recombinant protein booster immunization to induce high titers of antibodies. Although in vitro neutralization of primary isolates has rarely been achieved when T cell line–adapted rgp120 has been used as an immunogen [2, 12, 42, 43], a general strategy of using multivalent primary isolate protein subunit immunogens with viral vector or DNA vaccine(s) could have advantages that warrant investigation. Subunits augment T cell help, leading to an overall increase in antibody production, lymphoproliferative activity and antibody-dependent cellular cytotoxicity [11, 20].

Simultaneous vaccination with a live virus vector and a subunit envelope glycoprotein immunogen was a novel feature of this trial and led to earlier development of an antibody response than when the subunit was administered after the initial pox-virus-based immunization. The responses to each vaccine component in this strategy were also at least equivalent to those seen when either is administered alone. The antibody responses to the rgp120 used in this trial led to similar titers of antibodies after equivalent numbers of immunizations, as is seen in other studies of rgp120 [3, 20, 44]. In addition, the use of the subunit did not interfere with the development of CTLs. Although not statistically different from other treatment arms, the highest number of CTL-positive volunteers was observed in the combination arm G. In settings in which a rapid antibody response is important and in those individuals who may not return for multiple vaccinations, the use of such an accelerated simultaneous live vector plus protein boost strategy may prove useful.

Whether the degree of CD8+ CTL response here will be correlated with protection from natural infection with HIV-1 can be answered only in the context of a human efficacy trial. However, certain aspects of the CTL response induced by canarypox-based immunization should be emphasized. First, the CD8+ CTL response induced in a subset of volunteers who received canarypox-based immunization were able to lyse cells infected with primary HIV-1 isolates from multiple clades [45]. Second, when the precursor frequency of memory CTLs has been measured by use of in vitro restimulation, the response (50–500/10^6) is comparable to that measured in exposed, HIV-1–uninfected individuals and similar to CTLs induced against some other viruses (Weinhold KJ, personal communication).
Table 4. Neutralizing antibody titers to MN in vaccinees.

<table>
<thead>
<tr>
<th>Arm</th>
<th>After second immunization</th>
<th>After third immunization</th>
<th>After fourth immunization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GMT</td>
<td>No. positive (%)</td>
<td>GMT</td>
</tr>
<tr>
<td>A/A-/A/A</td>
<td>NA</td>
<td>30/10/16 (63)</td>
<td>35/10/16 (63)</td>
</tr>
<tr>
<td>A/A-/S/S</td>
<td>NA</td>
<td>72/9/16 (64)</td>
<td>287/12/13 (92)</td>
</tr>
<tr>
<td>A/A-/AS/AS</td>
<td>20</td>
<td>1/10 (10)</td>
<td>1/10 (10)</td>
</tr>
<tr>
<td>A/A/A/A/−</td>
<td>NA</td>
<td>21/9/17 (53)</td>
<td>32/10/17 (59)</td>
</tr>
<tr>
<td>A/AS/S−</td>
<td>NA</td>
<td>39/14/17 (82)</td>
<td>193/17/17 (100)</td>
</tr>
<tr>
<td>A/AS/AS/−</td>
<td>15</td>
<td>1/9 (11)</td>
<td>50/10/16 (63)</td>
</tr>
<tr>
<td>AS/AS−/AS/AS</td>
<td>52</td>
<td>106/15/16 (94)</td>
<td>173/16/16 (100)</td>
</tr>
</tbody>
</table>

NOTE. GMT, geometric mean titer; A, ALVAC vCP300; S, SF gp120.

[23, 46, 47]. Lastly, inclusion of nef sequences (expressed in this construct as 2 sequences lacking N-terminal myristylation) in the immunization did not result in any evidence of down-regulation of the CTL response to other components of the vaccine [48], as has been reported in in vitro models in which the nef gene has been replaced by a reporter system and then placed under the influence of a strong promoter [49].

Two new vCP300-like canarypox constructs encoding similar HIV-1 genes that have improved in vitro growth and stability have been developed. These canarypox candidate vaccines will be compared with the vCP205 canarypox construct in a phase I trial to determine which canarypox vector is more immunogenic. The candidate yielding the most frequent and broadest CD8+ CTL response will then be moved forward to larger human studies.

In conclusion, a canarypox vaccine that encodes multiple HIV-1 gene products is able to induce CD8+ CTLs in seronegative, low-risk volunteers against 1 or more HIV-1 antigens. These CTLs were detected as many as 10 months after the last canarypox immunization. The use of a rgp120 immunization given either simultaneously with or after the canarypox immunization resulted in higher levels of antibody responses and a trend toward longer-term memory CD8+ CTL responses. Whether these responses are truly sufficient to induce protection from HIV-1 infection can be determined only in a properly designed clinical trial.

Acknowledgments

We acknowledge the volunteers who made this study possible, as well as the following individuals at each site: University of Alabama at Birmingham: Paul Goepfert, Jiri Mestecky, Susan Duncan, Xiaobing Peng, Steffanie Sabbaj, and Zhong Li; St. Louis University: Robert Belshe (PI), Heidi Israel, Teresa Spitz, Tom Pacatte, Gwen Pendelton, Sharon E. Frey, Donald Kennedy, Mahendra Madava, and Gira B. Patel; Johns Hopkins University: David Schwartz, Clayton Harro, Robert Siliciano, Eric Zimmerman, Jane Reynolds, Lucy Carruth, and Lawrence Chow; University of Washington: Larry Corey (PI), David Berger, Chris Galloway, and Marnie Elizaga; University of Rochester: Raphael Dolin (PI), Lisa Demeter, Shirley Erb, Maryann Pugliese, Carol Gombrone, Theresa Fitzgerald, Esper Kallas, David Gibbons, Curtiss McNair, and Suzan Cole; Vanderbilt University: Peter Wright (PI), Paul Spearman, Mary Braeuner, Denise Owens, Roberto Cornell, Joyce Keltner, Kyle Rybczynk, Katie Crumbo, Frances Robinson, Irina Kuli-zade, Rita Smith, Gwendolyn Rees, and Linda Horton; Duke University: Guido Ferrari, Kelly Coor, and Tom Matthews; The EMES Corporation: Don Stabilein (PI), Carol Smith, Drienna Holman, Sophia Pallas, and Tamara Voss; Pasteur Merieux Connaught: Bernard Meignier, Michel Klein, Olivier Level, and Raphaëlle El-Habib. ALVAC-HIV-1 (vCP300) has been developed with the input of the Agence National de Recherche du SIDA.

References


