Evidence That Intermittent Structured Treatment Interruption, but Not Immunization with ALVAC-HIV vCP1452, Promotes Host Control of HIV Replication: The Results of AIDS Clinical Trials Group 5068

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Background. The ability to control human immunodeficiency virus (HIV) replication in vivo in the absence of antiretroviral therapy (ART) is a measure of the efficiency of antiviral immunity. In a study of patients with chronic, ART-suppressed HIV infection, AIDS Clinical Trials Group 5068 investigated the effects of immunization with an exogenous HIV vaccine and pulse exposure to the subject’s unique viral epitopes, by means of structured treatment interruptions (STIs), on the dynamics of viral rebound during a subsequent analytical treatment interruption (ATI).

Methods. Ninety-seven subjects receiving stable ART with an HIV-1 RNA load <50 copies/mL and CD4+ T lymphocyte count >400 cells/mm³ were randomized to undergo continued ART, STIs, ALVAC-HIV vCP1452 immunization, or STIs and ALVAC-HIV vCP1452 immunization.

Results. Subjects in the 2 STI arms had a significantly longer median doubling time in the period of the initial rise of viral load, a significantly lower median peak viral load, a significantly lower median end-of-ATI viral load set point, and a greater proportion of subjects with an end-of-ATI viral load set point <1000 copies/mL, compared with the subjects in the 2 arms without STIs. With an immunization schedule of 3 sets of 3 weekly injections, ALVAC-HIV vCP1452 did not affect viral load measures.

Conclusions. In this randomized, controlled study of intermittent STI as a therapeutic autoimmunization strategy, evidence of enhanced immunologic control of HIV replication was demonstrated.

Although antiretroviral chemotherapy raises CD4+ T lymphocyte counts and improves general immunity against other potential pathogens, it does not substantially augment immune responses against HIV-1 itself [1]. Inducing the immune system of an infected person to control HIV-1 replication better on its own would reduce the need for long-term antiretroviral chemotherapy, with its attendant cost and risks of toxicity.
HIV demonstrates wide genetic diversity, even within each infected individual [2, 3]. To be effective, a therapeutic immunization strategy needs to improve immune responses against an HIV-infected person’s own “quasi species” of viral strains. Cycles of structured treatment interruptions (STIs) could serve as a means of providing pulses of autologous viral antigens to the host immune system. However, the efficiency of the induction of new immune responses may be decreased by the immunosuppressive effects of viral products [4–7] and the preferential infection of proliferating HIV-specific CD4+ T lymphocytes [8]. Immunization with an exogenous HIV vaccine expressing consensus HIV-1 antigens in the absence of viremia might be more effective than immunization with endogenous virus but may lack patient-specific viral epitopes and is dependent on the potency of the vaccine product.

In AIDS Clinical Trials Group (ACTG) 5068, we examined these 2 approaches to immunization with HIV antigens to determine whether either approach was effective and whether the combination of the 2 would provide a synergistic enhancement of HIV immune responses. The HIV vaccine used in this study was ALVAC-HIV vCP1452, a recombinant, attenuated canarypox virus that expresses the products of the HIV-1 env and gag genes and a synthetic polypeptide encompassing the known cytotoxic T lymphocytes epitopes from the nef and pol gene products. This vaccine elicits cellular immune responses against HIV in a subset of persons not infected with HIV [9–12].

**METHODS**

**Study design.** ACTG 5068 was a prospective, randomized, partially double-blind study (figure 1). Subjects receiving their first potent antiretroviral therapy (ART) regimen who had plasma HIV-1 RNA levels <400 copies/mL and CD4+ T lymphocyte counts >400 cells/μL for >6 months and who had HIV-1 RNA levels <50 copies/mL at study entry were randomized to 1 of 4 arms. Subjects randomized to arms A and C received continuous ART for 44 weeks. Subjects randomized to arms B and D underwent 2 cycles of treatment interruption (the first lasting 4–6 weeks, depending on the time to viral load rebound, and the second lasting 4 weeks) followed by 16-week periods of treatment resumption. Immunization with ALVAC-HIV vCP1452 (arms C and D) or placebo (arms A and B) was performed in 3 sets of 3 weekly injections, beginning 4 weeks before treatment interruption for arms B and D and at comparable time points for arms A and C.

After the intervention period, all subjects had an analytical treatment interruption (ATI) that lasted 12–20 weeks, with subjects continuing to not receive therapy for at least 8 weeks after the first quantifiable plasma HIV-1 RNA level >50 copies/mL. Subjects whose viral load reached a new steady state, defined as a change in HIV-1 RNA level of <0.2 log10 copies/mL per week over 3 consecutive weeks before week 12 could end the ATI at that point but were encouraged to complete the 12–20-week ATI. Subjects with steady-state plasma viral loads <10,000 copies/mL whose absolute CD4+ T lymphocyte counts or percentages remained >50% of baseline levels were encouraged to continue the treatment interruption for 48 weeks.

The protocol was approved by local institutional review boards. Written informed consent was obtained from potential subjects. Human experimentation guidelines of the US Department of Health and Human Services and those of the authors’ institutions were followed in the conduct of the clinical research.

**Evaluations.** Subjects were evaluated by history, physical examination, and laboratory safety monitoring at protocol-defined intervals. During treatment interruptions, CD4+ T lymphocyte count and viral load were obtained weekly.

Plasma HIV-1 RNA levels were measured in real time at a single ACTG laboratory (Johns Hopkins University) by use of the AmpliCap HIV-1 Monitor Test (version 1.5; Roche Molecular Systems). Lymphocyte proliferation assays were performed on fresh blood specimens in accordance with the standard ACTG protocol [13]. Stimulants included pokeweed mitogen (0.1 μg/mL; Sigma), Casta antigen (10 μg/mL; Greer), tetanus toxoid (1U/mL; Wyeth-Lederle), IRC HIV antigen (5 μg/mL p24; Immune Response), recombinant p24, and a control baculovirus preparation (5 μg/mL; Protein Sciences). The stimulation index (the geometric mean counts per minute for each stimulant divided by the geometric mean counts per minute for medium alone) was used for analysis of the response. Cryopreserved lymphocytes were obtained for future additional measurements of CD4+ and CD8+ T cell responses to HIV antigens.

**Subject safety.** Subjects in arms B and D (STI arms) did...
Table 1. Baseline characteristics of the 97 subjects in AIDS Clinical Trials Group Protocol 568, by treatment arm.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (n = 97)</th>
<th>A (n = 24)</th>
<th>B (n = 24)</th>
<th>C (n = 25)</th>
<th>D (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median, years</td>
<td>43</td>
<td>44</td>
<td>44</td>
<td>45</td>
<td>41</td>
</tr>
<tr>
<td>Sex, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>80 (82)</td>
<td>22 (92)</td>
<td>18 (75)</td>
<td>21 (84)</td>
<td>19 (79)</td>
</tr>
<tr>
<td>Female</td>
<td>17 (18)</td>
<td>2 (8)</td>
<td>6 (25)</td>
<td>4 (16)</td>
<td>5 (21)</td>
</tr>
<tr>
<td>Race/ethnicity, no. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>55 (57)</td>
<td>20 (83)</td>
<td>11 (46)</td>
<td>13 (52)</td>
<td>11 (46)</td>
</tr>
<tr>
<td>Black</td>
<td>25 (26)</td>
<td>2 (8)</td>
<td>8 (33)</td>
<td>7 (28)</td>
<td>8 (33)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>14 (14)</td>
<td>1 (4)</td>
<td>5 (21)</td>
<td>5 (20)</td>
<td>3 (13)</td>
</tr>
<tr>
<td>Asian</td>
<td>2 (2)</td>
<td>1 (4)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>American Indian</td>
<td>1 (1)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
<tr>
<td>CD4+ T lymphocyte count, median (IQR), cells/mm³</td>
<td>730 (605–866)</td>
<td>779 (595–865)</td>
<td>754 (666–1011)</td>
<td>689 (648–796)</td>
<td>684 (559–849)</td>
</tr>
</tbody>
</table>

NOTE. Treatment arms are as follows: A, continuous therapy and placebo vaccination; B, structured treatment interruption (STI) and placebo vaccination; C, continuous therapy and vCP1452 vaccination; D, STI and vCP1452 vaccination. IQR, interquartile range.

not undergo their second STI if, by the time the second STI was to begin, they had not experienced resuppression of plasma HIV-1 RNA level to <200 copies/mL while receiving therapy. Any subject with a plasma HIV-1 RNA level not resuppressed to <1000 copies/mL or with a CD4+ T lymphocyte count and percentage <70% of baseline values at the scheduled start of the ATI continued to receive therapy. HIV drug-resistance monitoring was performed on the last available plasma sample from these subjects that had an HIV-1 RNA level >500 copies/mL. Sequencing of HIV-1 pol (codons 41–237 of the reverse transcriptase) was performed by standard bulk viral population resistance testing by use of the US Food and Drug Administration–approved TruGene HIV-1 Sequencing Kit and OpenGene DNA Sequence Analysis System (GeneObjects, version 3.2; Bayer Nucleic Acid Diagnostics) [14, 15] at a single ACTG laboratory (University of Alabama, Birmingham) (sequences are available from GenBank [accession numbers DQ497400–DQ497408]). Phylogenetic trees of the aligned, manually edited sequences were constructed using PHYLIP and Treeview, to verify that each patient’s viral sequence was unique [16, 17].

Statistical analysis. Virological end points included the end-of-ATI viral load (geometric mean of the values at the last 2 weeks of the ATI), steady-state viral load (geometric mean of 3 consecutive weekly viral load values in which the slope of the RNA versus time curve for all 3 values and the slope of the last 2 values were both between −0.2 and 0.2 log₁₀ copies/mL per week), time from the start of ATI to viral load rebound of >50 copies/mL, the rate of initial rise of viral load during the ATI (calculated from the series of consecutive viral load measurements in which the slope for each consecutive pair of values was >0.2 log₁₀ copies/mL per week), the peak viral load in the ATI, and the postpeak low viral load. Immunologic end points included HIV-1–specific and antigen/mitogen lymphoproliferative responses.

The primary end point of the study was the end-of-ATI viral load. The primary prespecified analysis was a comparison of the primary end point between the control arm (no vaccine and no STIs) and each of the 3 other arms (STIs only, vaccine only, and vaccine and STIs). The sample size was determined on the basis of providing the study 80% power to detect—with 2-sided, 5% significance level, exploratory rank-based tests—a shift in the distribution of the primary end point of 0.62 log₁₀ copies/mL in a single arm versus another single arm. A secondary prespecified analysis was to compare the combined 2 STI arms with the combined 2 non-STI arms and also to compare the combined 2 vaccine arms with the combined 2 non-vaccine arms.

Analyses were exploratory at the nominal, 2-sided, 5% level of significance, with no adjustment for multiple testing. Time-to-event end point distributions were estimated with the Kaplan-Meier method and were compared using the log-rank test. Other continuous end points were compared using the 2-sided Wilcoxon rank sum test. Proportions were compared using Fisher’s exact test. Correlations were estimated and tested with Spearman’s rank correlation. In intent-to-treat analyses, the worst rank in the rank-based tests was assigned to subjects without an observed end point; as-treated analyses were based only on subjects with an observed end point.
results

study population. Between May 2001 and October 2003, 97 patients were enrolled; 83 (86%) had an end-of-ATI plasma HIV-1 RNA set point determined. Twenty-four patients were randomized to arm A, 24 to arm B, 25 to arm C, and 24 to arm D. The patients in the 4 groups had similar baseline characteristics, except for race/ethnicity (table 1). Fewer than 20% of the subjects enrolled in arm A were nonwhite, whereas 50% of the subjects in arms B, C, and D were nonwhite. The median CD4+ T lymphocyte count was 730 cells/mm³. At study entry, 56 subjects were receiving a nonnucleoside reverse-transcriptase inhibitor (NNRTI), and 45 subjects were receiving a protease inhibitor. Eighty-five subjects were receiving lamivudine.

viral kinetics during the ATI. After treatment interruption, a rise in plasma HIV-1 RNA level occurred, usually within 2–4 weeks (figure 2). The initial rate of rise in viremia fit a first-order exponential growth curve. Viremia peaked several weeks after initial detection, then fell to a lower level, and tended to stabilize at an equilibrium (set point) viral load.

We analyzed the combination of the 2 STI arms (B and D) versus the 2 arms that did not have STI (A and C), in a factorial comparison, and found a consistent pattern of statistically significant differences in the kinetics of HIV-1 RNA rebound during the ATI in the combined STI arms compared with the arms that did not have STI (table 2). In analysis of subjects who had the observed evaluations at the end of the ATI period (n = 83), those in the STI arms showed a longer doubling time in viral rebound, a lower peak level of viremia, a lower postpeak low, a lower steady-state level of viremia, and a lower median end-of-ATI level of viremia than did those in the non-STI arms. Although there were multiple differences between these groups in the kinetics of viral rebound once initiated, there was no difference in the time to initial detection of viremia (>50 copies/mL) during the ATI. Finally, and perhaps most importantly, significantly more patients who underwent cycles of STI achieved a stable viral load <1000 copies/mL than did subjects who did not undergo this intervention. As a measure of comparability of the arms, there was no difference between the viral loads at week 4 during the first STI in the STI groups and the viral loads at week 4 during the ATI in the non-STI groups (P = .34). By use of the immunization schedule described above, ALVAC-HIV vCP1452 had no apparent effects on viral load measures during the ATI.

Of the 14 subjects who did not have observed end-of-ATI plasma HIV-1 RNA levels, 3 were in arm A, 3 were in arm B, 3 were in arm C, and 5 were in arm D. Four subjects withdrew from the study prematurely because they could not adhere to the study visit schedule before the ATI, 4 moved or were lost
Table 2. Comparison of virological parameters in the non–structured treatment interruption (STI) arms vs. the STI arms.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Non-STI arms A and C (n = 43)</th>
<th>STI arms B and D (n = 40)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>End of ATI VL, log10 copies/mL</td>
<td>4.40 (3.81 to 4.88)</td>
<td>4.15 (3.00 to 4.58)</td>
<td>.017</td>
</tr>
<tr>
<td>Equilibrium VL, log10 copies/mL</td>
<td>4.37 (4.07 to 4.80)</td>
<td>4.10 (3.29 to 4.43)</td>
<td>.028</td>
</tr>
<tr>
<td>Peak VL, log10 copies/mL</td>
<td>5.36 (4.58 to 5.79)</td>
<td>4.73 (4.07 to 4.91)</td>
<td>.002</td>
</tr>
<tr>
<td>Postpeak VL low, log10 copies/mL</td>
<td>4.10 (3.47 to 4.52)</td>
<td>3.76 (2.51 to 4.28)</td>
<td>.039</td>
</tr>
<tr>
<td>Initial VL rise rate, log10 copies/mL / week</td>
<td>1.08 (0.71 to 1.68)</td>
<td>0.85 (0.58 to 1.14)</td>
<td>.009</td>
</tr>
<tr>
<td>Doubling time, days</td>
<td>1.95 (1.25 to 2.96)</td>
<td>2.48 (1.85 to 3.63)</td>
<td></td>
</tr>
<tr>
<td>Initial decline rate, log10 copies/mL / week</td>
<td>-0.58 (-1.10 to -0.32)</td>
<td>-0.46 (-0.65 to -0.23)</td>
<td>.059</td>
</tr>
<tr>
<td>Time to VL &gt;50 copies/mL, days</td>
<td>21 (13 to 30)</td>
<td>15 (8 to 31)</td>
<td>.94</td>
</tr>
<tr>
<td>Time to peak VL, days</td>
<td>44 (34 to 58)</td>
<td>56 (35 to 70)</td>
<td>.27</td>
</tr>
<tr>
<td>Time to VL equilibrium, days</td>
<td>54 (42 to 75)</td>
<td>47 (23 to 67)</td>
<td>.29</td>
</tr>
<tr>
<td>End-of-ATI VL &lt;1000 copies/mL, ITT, no. (%)</td>
<td>3 (6)d</td>
<td>10 (21)e</td>
<td>.040</td>
</tr>
<tr>
<td>End-of-ATI VL &lt;1000 copies/mL, Obs, no. (%)</td>
<td>3 (7)</td>
<td>10 (25)</td>
<td>.034</td>
</tr>
</tbody>
</table>

NOTE. Data are median (interquartile range) values, unless otherwise indicated. ATI, analytical treatment interruption; ITT, intent-to-treat data; Obs, observed data only; VL, viral load.

* Only 42 subjects were included in the peak VL, and 39 subjects were included in the postpeak VL low and initial decline rates.
  * Only 39 subjects were included in the peak VL, and 38 subjects were included in the postpeak VL and initial decline rates.
  * Wilcoxon rank sum test.
  * A total of 49 subjects were included in the end-of-ATI VL for the ITT analysis of STI arms.
  * A total of 48 subjects were included in the end-of-ATI VL for the ITT analysis of non-STI arms.
  * Fisher’s exact test.

To follow-up before the ATI, 1 underwent interferon-α treatment for hepatitis C before the ATI, 1 restarted ART prematurely during the first STI, 1 decided against undergoing the ATI because their viral load was >1000 copies/mL at the time they were scheduled to start the ATI. Only 1 subject withdrew from the study during the ATI “read-out” period.

Intent-to-treat analyses, which assigned the worst rank to subjects who did not have the required HIV-1 RNA evaluation, gave results similar to those of the analyses of observed data only. The number of subjects who had an end-of-ATI plasma HIV-1 RNA level <1000 copies/mL was greater in the STI arms than in the non-STI arms (10/48 [21%] vs. 3/49 [6%]; P = .04). There was a nonsignificant trend toward a lower median end-of-ATI viral load in the STI arms than in the non-STI arms (HIV-1 RNA level, 4.44 vs. 4.55 log10 copies/mL; P = .12). Although the end-of-ATI viral loads in the control arm were not statistically significantly different from those in any other arm, possibly because of the relatively small sample size, there was a trend toward a lower median value in arm B (STI only) than in arm A (no STI and no vaccine) (HIV-1 RNA level, 4.38 vs. 4.70 log10 copies/mL; P = .14).

There were significant correlations among many of the viral load end points. The steady-state viral load was significantly and positively correlated with the end-of-ATI viral load (P < .0001) (figure 3), the peak viral load (P < .0001), and the rate of the initial rise (P = .025). The time to a viral load >50 HIV-1 RNA copies/mL did not correlate with any other virological measure. The 12-week duration of the ATI period was further validated by comparing the end-of-ATI viral load of the evaluable study population with the steady-state viral load in the 72 subjects who had both end points observed. The median of the absolute difference (end-of-ATI viral load minus steady-state viral load) was only 0.21 log10 HIV-1 RNA copies/mL. Thus, 12 weeks of treatment interruption was probably enough for most subjects to reach new viral load equilibriums.

Those subjects who achieved low end-of-ATI viral loads maintained low viral loads while continuing to not receive ART for extended periods of time after the planned ATI (figure 4). Of the 13 subjects with an end-of-ATI plasma HIV-1 RNA level <1000 copies/mL, 7 maintained this level at 48 weeks of not receiving ART; 2 others maintained this level at their last evaluations at weeks 28 and 42. Four subjects had HIV-1 RNA levels between 1000 and 2000 copies/mL at the week 48 evaluation.

**Immunologic data.** Consistent with the viral kinetics and lower viral load set point we observed during the ATI, there was a smaller decrease in CD4+ T lymphocyte count during
Figure 3. Correlation between viral load (VL) at the end of the analytical treatment interruption (ATI), defined as the geometric mean of the last 2 values, with the new equilibrium ("steady-state") viral load during the ATI, as defined in Methods, for the subjects in the arms with no structured treatment interruption (STI) and in the arms with STIs. Values are expressed exponentially in HIV-1 RNA copies/mL on the X- and Y-axes. The 95% confidence intervals for the correlation coefficient are 0.71–0.92 for the non-STI arms and 0.56–0.87 for the STI arms.

Figure 4. Durability of virological suppression for the 13 subjects who had viral loads <1000 copies/mL at the end of the analytical treatment interruption in the arms with no structured treatment interruption (STI) and in the arms with STIs. Seven of these 13 subjects had HIV-1 RNA levels <1000 copies/mL at the 48-week visit during interruption of antiretroviral treatment (ART); 2 others had such levels at their last evaluations at weeks 28 and 42. Four subjects had HIV-1 RNA levels between 1000 and 2000 copies/mL at the week 48 evaluation. (The values of 3 of the 13 subjects are not shown because their week 48 visit occurred at week 49.)
Figure 5. Lymphocyte proliferation responses to either IRC HIV antigen (Ag) or Protein Sciences recombinant p24 (PS p24) were measured at baseline, after the vaccine and/or structured treatment interruption (STI) interventions but before the analytical treatment interruption (ATI), and at the 12-week end point of the ATI (ATI-EP). The baseline response represents the geometric mean of 2 independent assays at study entry and at 2 weeks before entry, whereas the pre-ATI response represents the geometric mean of the response from assays at weeks 40 and 44. A, Median response of subjects in each study arm. B, Lymphocyte proliferation assay (LPA) response of individual subjects plotted on the X-axis versus the end-of-ATI viral load (VL), the primary study end point. There were no statistically significant differences in the median responses between study arms and no significant correlation between the response at baseline and the viral load at the end of the ATI. However, there was a significant negative correlation between the LPA response measured after the interventions and the end-of-ATI viral load, although the magnitude of the correlation was modest. ALVAC, ALVAC-HIV vCP1452 immunization; HAART, highly active antiretroviral therapy.

Safety and HIV drug-resistance data. There were no deaths, opportunistic infections, or other AIDS-defining events. An acute retroviral syndrome occurred in 1 subject (arm C) during the ATI.

Four subjects never achieved a viral load persistently <50 copies/mL after resuming ART following an STI. Three of the 4 subjects had successful amplification and sequencing of virus from plasma specimens obtained in the post-STI period. All 3 were receiving NNRTI-containing regimens before their STIs and had mutations consistent with NNRTI resistance. Two of the 3 patients were receiving lamivudine and were found to have the M184V mutation. Thus, 3 of the 48 subjects under-
going STI, 26 of whom were receiving NNRTIs, displayed HIV resistance against drugs contained in their pre-STI regimens in bulk plasma viral populations.

One non-STI subject (arm C) did not have a viral load of <1000 copies/mL when the ATI was scheduled to begin. There was no evidence of HIV drug resistance in the plasma of this subject.

**DISCUSSION**

In this partially blind, randomized, controlled study, we found evidence that delivering pulses of exposure to autologous HIV-1 RNA antigens as a result of cycles of brief structured ATIs could enhance host control of viral replication during a subsequent prolonged ATI, whereas immunization with ALVAC-HIV vCP1452 could not. More patients randomized to receive STIs ended the ATI with plasma HIV-1 RNA levels <1000 copies/mL than did those randomized to receive continuous therapy, whether by intent-to-treat analysis or by analysis of observed data only. Other aspects of the viral kinetic curve during the ATI were favorably altered by this treatment strategy, including the initial rate of rise in plasma HIV-RNA level, the peak level achieved, and the new steady-state equilibrium level at which the HIV-1 RNA level settled. In addition, there was a relative preservation of CD4+ T lymphocytes during the ATI in those who underwent STIs.

Although a limitation of the study is that we do not have reliable access to the pre-ART HIV-1 RNA set point values for comparison, the randomized design would make a substantial imbalance in pretherapy viral load across the treatment arms unlikely. In addition, 10 of the 13 subjects with end-of-ATI viral loads <1000 copies/mL achieved viral loads >9000 copies/mL at some point during their treatment interruptions, indicating that these were unlikely to be subjects with chronic low-level viremia when not receiving ART before study entry. Importantly, the low end-of-ATI level of viremia in these subjects was stable for the 48 weeks of follow-up. The durability of the effect suggests that a small fraction of patients could achieve sustained viral suppression with this treatment strategy, potentially sparing them the need to receive antiretroviral chemotherapy for a considerable period of time. The 15%–18% response rate, compared with the control group, is modest, but, hopefully, this response could be improved in future studies with refinements of the STI approach and additional immune manipulations.

The previous negative reports about STIs were based on uncontrolled observations [18, 19] or on study designs that stopped the ATIs before the new viral load equilibrium had been established, either by design or in accordance with safety stopping rules [18–20]. Our study was successful in allowing all but 1 of the subjects who started ATI to complete it, and it demonstrated that premature discontinuation of the ATI would interfere with the ability to interpret the strength of the immune response induced. A common end point in previous studies was the time to the viral load rising above a certain level [18, 20]. Our data show that there is no correlation between the time to initial detection of viremia (HIV-1 RNA level >50 copies/mL) and the ultimate stable viral load steady state established during the ATI. Because antigen drives an immune response, until enough virus is generated, the time to initial detection of viremia in a laboratory assay may depend more on the kinetics of viral replication and the availability of target cells than on the immune response.

The administration of ALVAC-HIV vCP1452, an attenuated canarypox virus loaded with consensus sequences of HIV genes, did not have any effects on measures of viral replication during the ATI. Nor did it demonstrate synergy with exposure to autologous viral antigens, as we had hypothesized. The potency of the vaccine product is critical in testing this hypothesis. Unfortunately, subsequent to the design of this study, the potency of ALVAC-HIV vCP1452 has been disappointing in both prophylactic vaccine studies [9–12] and other therapeutic trials [21–26]. Immunization of HIV-infected subjects with ALVAC-HIV vCP1452 vaccine alone [23, 24] and together with either recombinant gp160 protein [21, 22], Remune vaccine [23], or Lipo-6T vaccine plus interleukin-2 [25] elicited only modest HIV-specific cellular immune responses and, in most studies, demonstrated no clear evidence of control of viremia after ART interruption. It should be noted, however, that, in one study, vaccination was associated with a longer duration of not needing to receive ART [25, 27], and ACTG 5024 did detect a modest level of enhanced viral control after ALVAC-HIV vCP1452 immunization by administration of a single injection every 8 weeks, rather than the 3 cycles of 3 weekly injections administered in this study [26].

We did not find that STIs induced significantly stronger HIV-specific CD4+ T LPA responses, and these responses showed only a modest negative correlation with viremia. Other STI studies have not shown a correlation between laboratory measures of HIV-specific cellular immune activity and levels of viremia [28, 29], whereas some studies of therapeutic immunization have shown such a correlation [24, 27]. A negative correlation between LPA response and chronic viral load when not receiving ART has also been described in subjects without therapeutic immunization [30]. It remains unclear to what extent the variability in these results is due to the technical limitations of the assays in the context of multicenter clinical trials, variability among different patient cohorts, or variability of the interventions examined. It is also conceivable that other immunologic functions not assessed by the assays used play a more important role in controlling virus replication and would give more consistent results.

With regard to safety, of the 48 subjects in the STI arms of
our study, 4 did not experience full resuppression of their virus during the retreatment periods. Mutations associated with resistance to lamivudine and/or NNRTIs were seen in 3 of these 4 subjects. Therefore, STI strategies include a modest risk of resistance, and caution should be exercised. Other trials have shown that the most common drugs to which virus will develop resistance after treatment interruption are lamivudine and NNRTIs, drugs with long half-lives [31–33]. Although detectable viremia does not occur until a median of 2–3 weeks after treatment interruption, the use of agents that have shorter half-lives and higher barriers to resistance than those of lamivudine and NNRTIs may limit the resistance risk in STI studies.

In conclusion, this study showed evidence that STIs as a therapeutic immunization strategy could enhance host control of viral replication. By use of this immunization schedule, ALVAC-HIV vCP1452 did not affect HIV-1 RNA measures during an ATI.

**AIDS CLINICAL TRIALS GROUP 5068 MEMBERS**

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**Acknowledgments**

We are indebted to Gwen Marshall and J. Darren Hazelwood, for technical support; to the clinicians who referred patients to the study; and to the patients who participated. Financial support was provided by the following organizations: the Adult AIDS Clinical Trials Group (AACTG), funded by the National Institute of Allergy and Infectious Diseases (grants AI38858); the Statistical Data and Analysis Center (grant AI38855); the Adult AIDS Clinical Trials Units (grants AI46381, AI217656, AI25879, AI27658, AI25868, AI50410, AI46370, AI25924, AI27664, AI25903, and AI32783); and the General Clinical Research Center Units, funded by the National Center for Research Resources (grants RR00096, RR00044, RR00046, RR00032, and RR00040). V.A.J.’s virology laboratory was supported in part by the AACTG; by virology support funding from the National Institute of Allergy and Infectious Diseases and the AACTG Central Group Grant (grants U01AI38858 and P30 AI27767); by the University of Pennsylvania Center for AIDS Research; and by the Birmingham Veterans Administration Medical Center and University of Alabama, Birmingham, Centers for AIDS Research core clinic and laboratory facilities.

**References**


