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MAJOR ARTICLE

Therapeutic Vaccination With Dendritic Cells Loaded With Autologous HIV Type 1–Infected Apoptotic Cells

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Background. We report the results of a phase I/II, open-label, single-arm clinical trial to evaluate the safety and anti–human immunodeficiency virus type 1 (HIV-1) efficacy of an autologous dendritic cell (DC)–based HIV-1 vaccine loaded with autologous HIV-1–infected apoptotic cells.

Methods. Antiretroviral therapy (ART)–naive individuals were enrolled, and viremia was suppressed by ART prior to delivery of 4 doses of DC-based vaccine. Participants underwent treatment interruption 6 weeks after the third vaccine dose. The plasma HIV-1 RNA level 12 weeks after treatment interruption was compared to the pre-ART (ie, baseline) level.

Results. The vaccine was safe and well tolerated but did not prevent viral rebound during treatment interruption. Vaccination resulted in a modest but significant decrease in plasma viremia from the baseline level (from 4.53 $\log_{10}$ copies/mL to 4.27 $\log_{10}$ copies/mL; $P = .05$). Four of 10 participants had a >0.70 $\log_{10}$ increase in the HIV-1 RNA load in plasma following vaccination, despite continuous ART. Single-molecule sequencing of HIV-1 RNA in plasma before and after vaccination revealed increases in G>A hypermutants in *gag* and *pol* after vaccination, which suggests cytolysis of infected cells.

Conclusions. A therapeutic HIV-1 vaccine based on DCs loaded with apoptotic bodies was safe and induced T-cell activation and cytolysis, including HIV-1–infected cells, in a subset of study participants.

Clinical Trials Registration. NCT00510497.

Keywords. HIV-1; therapeutic vaccine; dendritic cell; apoptotic cell; residual viremia.

Despite receipt of suppressive antiretroviral therapy (ART) for years, elimination of human immunodeficiency virus type 1 (HIV-1) reservoirs or immune control is not achieved. Residual plasma viremia below the level of detection of commercial assays can be detected in most individuals even after receipt of effective ART for many years [1, 2]. Strategies that can activate latent proviruses and induce killing of infected cells (the so-called kick-and-kill approach) could decrease the size of viral reservoirs and improve HIV-1-specific immune responses to achieve durable ART-free virus control (ie, functional cure). Shan et al [3] reported that after proviral reactivation, antigen-specific cytotoxic T lymphocytes (CTLs) are needed to kill infected cells, which underscores the need to enhance HIV-1–specific immune function.

Dendritic cells (DCs) have been used to make therapeutic vaccines because they are potent antigen-presenting cells (APC) that can link innate and adaptive immune responses [4]. DC-based therapeutic HIV-1 vaccines have been shown in vitro to enhance HIV-1–specific T-cell responses [5–8], leading to a number of clinical trials [9]. Although the vaccines have been safe and well tolerated, immunologic and virologic responses have been variable [10]. In a recent study [11], vaccination with DCs expressing autologous HIV-1 antigens (ie, HIV-1 antigens derived from the participants' contemporary virus pool) led to a decrease of at least 1 $\log_{10}$ in the plasma HIV-1 set point after analytic treatment interruption (ATI) in 55% of vaccine recipients, compared with 9% of controls. This decrease correlated with increased HIV-1–specific T-cell responses.

We previously showed a modest and transient immunologic response to a vaccine consisting of autologous DCs loaded with major histocompatibility complex class I HIV-1 consensus epitope peptides [12, 13]. Our additional work showing that loading DCs with HIV-1–infected, apoptotic $CD4^+$ T cells enhanced interferon γ (IFN-γ) production by autologous $CD8^+$ T cells in vitro [14] led us to develop a new vaccine composed of autologous DCs loaded with autologous HIV-1–infected apoptotic cells (ApB DC vaccine) [15]. We now report the results of a phase I/II, single-arm, single-site clinical trial to evaluate the

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safety and antiviral efficacy of an ApB DC therapeutic HIV-1 vaccine.

MATERIALS AND METHODS

Study Design

The study was a phase I/II evaluation of therapeutic immunization with autologous DCs loaded with autologous, inactivated, HIV-1–infected apoptotic cells (NCT00510497). Eligible participants were HIV-1–infected, ART-naive adults with a $CD4^+$ T-cell count of ≥300 cells/mm^3 and a plasma HIV-1 RNA load of 3000–100 000 copies/mL. The study was approved by the University of Pittsburgh Institutional Review Board. Written informed consent was obtained from all participants.

The study design is summarized in Figure 1. After enrollment, autologous virus was isolated as previously described [15]. The average of the HIV-1 RNA load at 2 visits immediately preceding ART initiation served as the pre-ART baseline. Participants initiated ART once an adequate amount of autologous HIV-1 was available (at least 50 mL of supernatant with >24 ng/mL of p24) to produce the vaccine. Adherence to ART was assessed through participant self-report. After at least 8 weeks of virologic suppression (defined as a plasma HIV-1 RNA load of <50 copies/mL), participants underwent leukapheresis to obtain monocytes and lymphocytes for vaccine production. Four weeks after leukapheresis, ApB DC vaccine (10^7 ApB DCs per dose, divided into 2 syringes) was administered subcutaneously into the upper medial area of the arm bilaterally every 2 weeks for a total of 3 doses (V1–V3). Six weeks after the third vaccine dose, the participants discontinued ART for at least 12 weeks. A fourth vaccine dose (V4) was administered 2 weeks after the start of ATI. Levels of plasma HIV-1 RNA, $CD4^+$ T-cell count, and immunologic parameters in peripheral blood specimens were measured at the end of the 12-week ATI period, which was the end point for the study. Participants were followed for an additional 36 weeks. Adverse events, including local and systemic reactions, were graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, version 1.0, December 2004.

Vaccine Preparation

ApB DC vaccine production was performed under certified good manufacturing practice regulations, as previously reported [15]. Briefly, monocytes and lymphocytes were recovered by centrifugal elutriation. The lymphocyte fraction was used for separation of $CD4^+$ T cells, which were superinfected with previously isolated autologous virus. Psoralen (7H-Furo[3,2-g][1]benzopyran-7-one; 20 μg/mL) and ultraviolet B light (3 mW/m^2) were used to inactivate 99.99% of infectious virus and form the ApB. Monocytes were cocultured with interleukin 4 and granulocyte-macrophage colony-stimulating factor for 6 days to generate immature DCs. These were then cocultured with the ApB (1:1 ratio) for 24 hours at 37°C in a medium containing tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), IFN-α, IFN-γ, and polyinosinic:polycytidylic acid to induce DC maturation. The ApB DC vaccine was considered ready for release testing once there was uptake of ApB into >80% of DCs and the DC maturation profile was confirmed by flow cytometry. Prior to cryopreservation, the final ApB DC vaccine product was sampled for analysis of microbial sterility, evaluated by a mycoplasma assay, and determination of endotoxin levels, cell viability (>80%), cell purity, and potency (based on interleukin 12p70 [IL-12p70] levels). The IL-12p70 production assay involved incubation of 2×10^4 mature DCs alone or with a CD40 ligand–transfected J588 cell line for 24 hours at 37°C. Soluble IL-12p70 and interleukin 10 (IL-10) levels were obtained using multiplex beads (Biosource, East Spring Valley, New York) and a multibead fluorescent platform (Luminex,

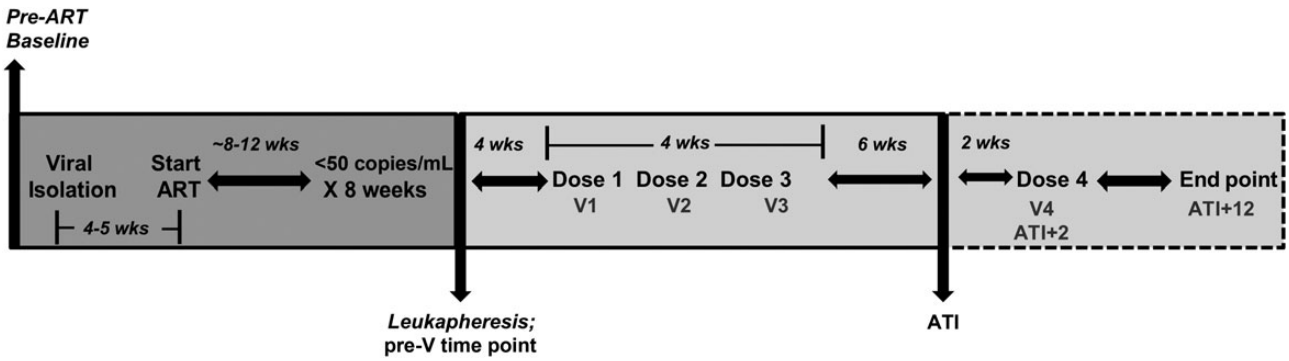

Figure 1. Study design. Virus was obtained from antiretroviral (ART)–naive study participants prior to initiating ART consisting of a protease inhibitor–based regimen. Human immunodeficiency virus type 1 (HIV-1) RNA obtained prior to the viral isolation phase served as the pre-ART specimen (pre-ART). After viral suppression for at least 8 weeks, participants underwent leukapheresis to obtain monocytes and lymphocytes that were used for the ApB DC vaccine. Blood samples obtained at the date of leukapheresis served as the time point before vaccine receipt (pre-V). Four weeks after leukapheresis, participants received 3 vaccine doses (V1–V3) 2 weeks apart. Six weeks after the V3, blood samples were again obtained, after which participants underwent antiretroviral treatment interruption (ATI). A fourth vaccine dose was given 2 weeks after ATI (V4; ATI+2). The primary end point was 12 weeks from the start of ATI.

Austin, Texas). The vaccine product was aliquoted into sterile vials and cryopreserved until the time of administration. Similar tests were done after the ApB DC vaccine was thawed for vaccine delivery.

Analysis of Polyfunctional Response and T-Cell Activation

Peripheral blood mononuclear cells (PBMCs) were obtained and cryopreserved at 3 time points (before vaccine receipt, immediately before ATI, and at the end point), thawed, stimulated with a Gag p55 peptide pool (15mers overlapped by 11 amino acids spanning the consensus B HIV-1 proteome; 5 μg/mL; AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases [NIAID], National Institutes of Health [NIH]), and evaluated for intracellular staining as previously published [13], using the following monoclonal antibodies (mAb): anti-CD3 APC-H7 (5 μL), anti-CD4 V450 (5 μL), anti-CD8 PE-Cy7 (5 μL), anti-CD14/19 PE-CF594 (5 μL), Live/Dead Aqua (1 μL), anti-IFN-γ Alexa 488 (5 μL), anti-TNF-α Alexa Fluor 700 (5 μL), anti-macrophage inflammatory protein 1β (MIP-1β) APC (5 μL), anti-interleukin 2 (IL-2) PerCP-Cy5-5 (5 μL), and anti-CD107α PE-Cy5 (20 μL); all were from BD Pharmingen, except for Live/Dead (Invitrogen). Specimens were analyzed within 24 hours of staining, using an LSR Fortessa flow cytometer (BD Biosciences). $CD8^+$ T-cell polyfunctional responses were assessed using the SPICE Program (version 5.3033; Mario Roederer, Vaccine Research Center, NIAID, NIH). To evaluate changes in T-cell activation before and after vaccine administration, PBMCs were obtained and cryopreserved at 3 time points (before vaccine receipt, at V1+1, and immediately before ATI); thawed; stained with anti-CD4 FITC mAb (20 μL), anti-CD8 mAb PE (20 μL), anti-CD3 ECD (10 μL), anti-HLA-DR PC5 (10 μL), and anti-CD38 PC7 (5 μL; Beckman Coulter); and analyzed within 24 hours of staining, using a Beckman Coulter FC-500 flow cytometer.

Extraction of Nucleic Acid

Cell-associated HIV-1 DNA and RNA were isolated from cryopreserved PBMCs, as previously published [16, 17]. All samples were quantified by NanoDrop 1000 (Thermo Scientific, Massachusetts). DNA samples were diluted to a final concentration of <170 ng/μL, whereas RNA samples were diluted to a final concentration of <30 ng/μL, to prevent inhibition of quantitative polymerase chain reaction (qPCR).

Quantification of Plasma HIV-1 RNA Levels and Total HIV-1 DNA and RNA Levels

Residual plasma HIV-1 RNA from samples before vaccine receipt, 1 week after V2 (V2+1), and immediately before ATI was quantified by a modified version of the single-copy assay (SCA) with a 2-step reverse-transcription (RT)–qPCR assay targeting the integrase region of *pol* as described previously [17]. No RT controls were included to detect DNA contamination. The limit of detection for HIV-1 RNA was 0.8 copies/mL. Total HIV-1 DNA and unspliced HIV-1 RNA levels were quantified from PBMC specimens before vaccine receipt and immediately before ATI, as previously reported [16, 18]. The limit of detection for both total HIV-1 DNA and unspliced HIV-1 RNA assays were 2–5 copies/10^6 PBMCs.

Single-Genome Sequencing (SGS) of Virus and CTL Escape Analysis

SGS of the p6-Pro-Pol region of HIV-1 RNA in plasma or of proviral DNA in PBMCs was performed as previously described [19]. No RT controls were included in SGS analysis of plasma HIV-1 RNA. Sequences were aligned using ClustalW. Population genetic diversity was calculated as the average pairwise difference, using MEGA5 (available at: http://www.megasoftware.net). Neighbor-joining phylogenetic analyses were done using MEGA5. Trees were rooted on the subtype B consensus sequence (available at: http://www.HIV-1.lanl.gov) shown as the lowest (unmarked) branch of each tree. CTL escape mutations were identified by mapping changes in epitopes matching the participants' HLA, as defined in the Los Alamos Database.

Statistical Analyses

Averaged data are expressed as medians. HIV-1 RNA values were $\log_{10}$ transformed. For SCA values below the limit of detection of 0.8 copies/mL, an imputed value of 0.4 copies/mL was used in the analyses. Two-tailed Wilcoxon matched pairs signed rank tests were used to compare plasma HIV-1 RNA loads, cell-associated HIV-1 RNA/DNA loads, $CD4^+$ T-cell counts, and T-cell activation levels between time points. Comparison between subgroups of participants was done using the Mann–Whitney *U* test. Analyses were done using GraphPad Prism, version 9.05. Statistical analysis of Boolean-gated data used in polyfunctional responses was done using the SPICE software.

RESULTS

Demographic Characteristics and Vaccine Safety

Eighteen participants were screened for the study; 11 were enrolled, but 1 did not receive any study vaccinations and was excluded from analysis. Ten subjects received all planned study vaccinations and completed follow-up at least through the primary end point visit. Participants had a median pre-ART baseline plasma HIV-1 RNA load of 4.53 $\log_{10}$ copies/mL (range, 3.65–4.81 $\log_{10}$ copies/mL) and a median $CD4^+$ T-cell count of 486 cells/mm^3 (range, 377–881 cells/mm^3). All participants initiated an ART regimen consisting of ritonavir-boosted atazanavir and coformulated tenofovir/emtricitabine and reported adherence to the daily doses.

ApB DC vaccinations were safe and well tolerated. Vaccine-related adverse events included mild-to-moderate inflammation at the injection site (grade 1–2). Two study participants experienced grade 3 events (severe pruritus and injection site pain). Symptoms resolved within 24 hours. No grade 4 events occurred. Plasma HIV-1 RNA remained below the limit of detection (<50 copies/mL) during ART.

ApB DC Vaccine Did Not Prevent Virologic Rebound After Treatment Interruption

Six weeks after the third ApB DC vaccine dose (V3), participants discontinued ART and then received a fourth vaccine dose (V4) 2 weeks later (Figure 1). Viral rebound was detected in 2 of 10 participants by the second week of ATI, prior to receiving V4. By 6 weeks from the start of ATI, viremia was observed in all participants, with a median HIV-1 RNA load of 4.29 $\log_{10}$ copies/mL. The median $CD4^+$ T-cell count at this time point was 534 cells/mm^3 (Supplementary Table 1). At the end point, 3 of 10 participants had a >0.4 $\log_{10}$ decrease in HIV-1 RNA load, compared with their pre-ART baseline (Figure 2*A*). The median HIV-1 RNA load for all participants at the end point was 4.27 $\log_{10}$ copies/mL, which was modestly lower than the pre-ART baseline load of 4.53 $\log_{10}$ copies/mL ($P = .049$; Figure 2*B*). $CD4^+$ T-cell counts at the pre-ART time point and at the end point were not significantly different ($P = .77$; data not shown).

$CD8^+$ T-Cell Polyfunctional Responses and T-Cell Activation

We investigated $CD8^+$ T-cell polyfunctional *gag*-specific responses at 3 time points (before vaccine receipt, immediately before ATI, and at the end point) since response to *gag* has been associated with anti-HIV-1 response [20, 21]. No significant postvaccine increase in polyfunctional response to HIV-1 *gag* was observed. Four of the 10 participants appeared to have more polyfunctional responses, while 2 of 10 appeared to have decreased responses (Figure 3). These did not correlate with viral control at end point. $CD8^+$ T-cell activation increased significantly 1 week after the first vaccine (V1+1), compared with values before vaccine receipt ($P = .004$) and immediately before ATI ($P = .031$; Figure 4*A*). Levels of $CD4^+$ T-cell activation were also elevated at V1+1 as compared to before vaccine receipt ($P = .002$) and immediately before ATI ($P = .016$; Figure 4*B*).

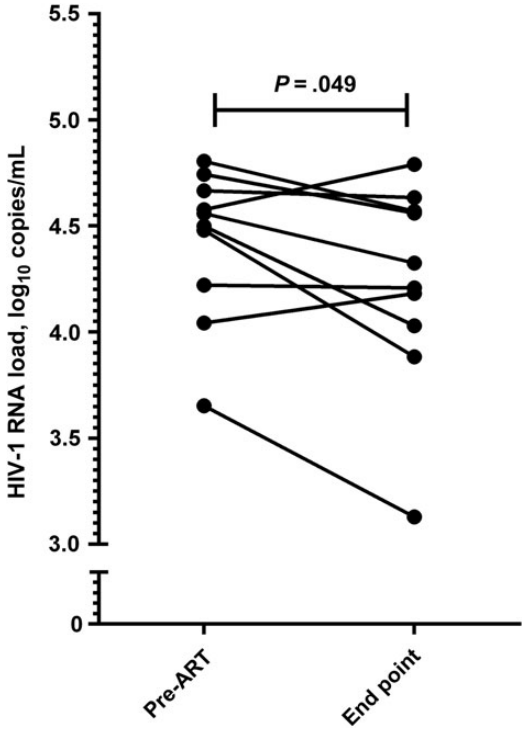

Figure 2. Human immunodeficiency virus type 1 (HIV-1) RNA levels in the vaccinated subjects. The figure shows the changes in levels of HIV-1 RNA before antiretroviral therapy (Pre-ART) and at the primary end point, which was 12 weeks from the start of treatment interruption. We observed a modest decrease in the HIV-1 RNA load, from 4.53 $\log_{10}$ copies/mL to 4.27 $\log_{10}$ copies/mL ($P = .049$, by the Wilcoxon signed rank test).

ApB DC Vaccination Led to an Increase in Plasma HIV-1 RNA Despite Ongoing ART in a Subset of Participants

Median levels of residual plasma HIV-1 RNA for all participants did not change significantly from before vaccine receipt to the 2 postvaccine time points (data not shown). However, in 4 of 10 participants, the residual plasma HIV-1 RNA load consistently increased from before vaccine receipt (or V2+1 in participant 2, due to a lack of samples obtained before vaccine receipt) to V2+1 and to immediately before ATI (Figure 5 and Supplementary Table 2); all 4 having a ≥0.7 $\log_{10}$ increase from before vaccine receipt (V2+1 in participant 2) to immediately before the ATI time point (median increase, 0.75 $\log_{10}$). However, between before vaccine receipt and immediately before the ATI time points, there was no decrease in cell-associated HIV-1 DNA in these 4 participants. Analysis of DC phenotype in these 4 participants showed no differences in the expression of the costimulatory molecules CD80 and CD86 and the DC maturation markers CD40, HLA-DR, and CD11c, compared with the 6 of 10 who had no increase in plasma HIV-1 RNA load (Supplementary Figure 1*A*). However, DC functional potency, evaluated by the production of IL-12 and IL-10 (Supplementary Figure 1*B*), revealed that, although levels of IL-12 for the 10 participants were lower than what we obtained during preliminary in vitro studies, the median ratio of IL-12 to IL-10 levels in these 4 participants were significantly higher and thereby more proinflammatory (median, 1.28 vs 0.04; $P = .038$; Figure 6). It is important to note though that, when participant 2 was removed from the analysis (owing to a lack SCA data from before vaccine receipt), the median ratio of IL-12 to IL-10 levels was not significantly different for the remaining 3 participants ($P = .095$). The addition of participant 8 (who had a ≥0.7 $\log_{10}$ increase from V2+1 to immediately before ATI but an initial decrease from before vaccine receipt to immediately before ATI) to the group with increasing HIV-1 RNA loads resulted in a trend of higher ratios of IL-12 to IL-10 levels ($P = .056$).

Source of Vaccine-Induced Increases in Plasma HIV-1 RNA During ART

To evaluate the source of the vaccine-induced increases in plasma HIV-1 RNA level, we characterized the sequences of the HIV-1 RNA that increased after ApB DC vaccination and also those that rebounded after ATI, by performing p6-Pro-Pol SGS of plasma HIV-1 RNA and PBMC HIV-1 DNA in those

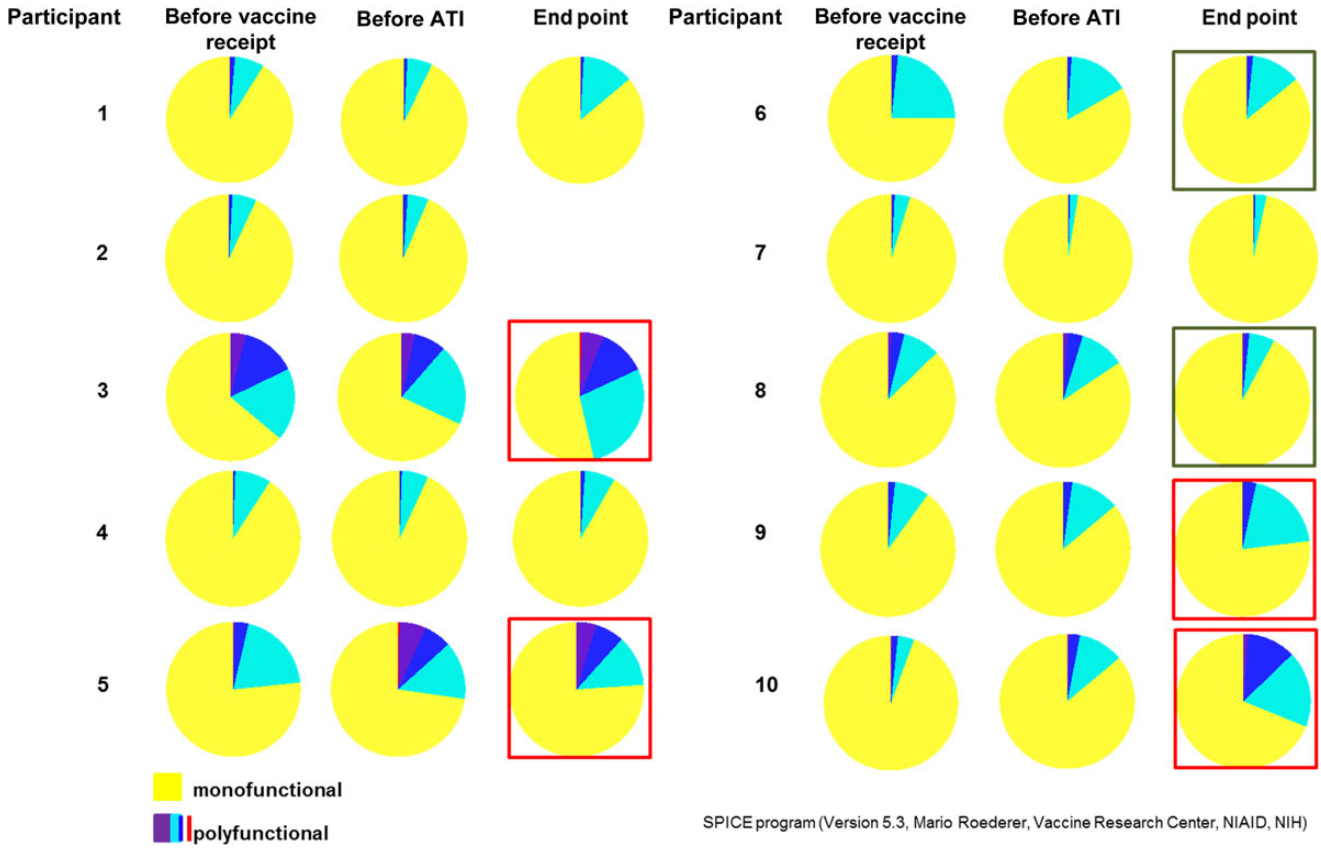

Figure 3. Changes of $CD8^+$ T-cell polyfunctional response to Gag p55 peptide pool before vaccine receipt and after vaccine receipt (immediately before antiretroviral treatment interruption [ATI] and at the study end point). The pie charts show the proportion of $CD8^+$ T cells that secreted >1 immune mediator (interleukin 2 [IL-2], interferon γ [IFN-γ], tumor necrosis factor α [TNF-α], macrophage inflammatory protein 1β [MIP-1β], and CD107α) in response to stimulation with Gag peptide. The portion of the pie chart in yellow indicates the proportion of $CD8^+$ T cells that secreted only one immune mediator. CD107α was the most common immune mediator expressed by $CD8^+$ T cells that secreted only 1 immune mediator following stimulation with Gag. Among those that secreted 2 immune mediators, the combination of CD107α and IL-2 was the most common. There were no significant differences in polyfunctional response following vaccinations. There appeared to be a modest increase in polyfunctional response in 4 of 10 participants (red squares) but a decrease in 2 of 10 participants (green squares). Polyfunctional responses did not correlate with viral control at end point.

with available specimens from time points before ART initiation, before vaccine receipt (only HIV-1 DNA), at V2+1, immediately before ATI, and at 2 points after ATI (ATI+2, ATI+4, only plasma RNA). HIV-1 RNA from the virus isolated for vaccine production (vaccine prep) was also sequenced by SGS. Figure 7 shows the results of SGS of plasma and PBMCs for participants 9 and 10, both of whom had increases in plasma HIV-1 RNA load after vaccination and during receipt of ART. The viral populations that were used to generate the vaccines were highly diverse and were not different from the plasma populations found before ART. Analysis of HIV-1 RNA in plasma during the postvaccination time points showed diverse viral variants, including G>A hypermutants. In participants 9 and 10, these hypermutants occurred at a frequency of <1% in the pre-ART virus population and in the vaccine prep but at a frequency of 29% (Figure 7*A*) and 56% (Figure 7*B*), respectively, after vaccination, immediately before ATI ($P < .002$). The hypermutants contained stop codons in *gag* and *pol*, indicating that these genomes were not packaged in virions but were likely released from cells undergoing cytolysis after vaccination. HIV-1 DNA was not detected in plasma, indicating that the sequences obtained, including the hypermutants, were HIV-1 RNA. The hypermutated HIV-1 RNA detected in the plasma that was not associated with virion may have been protected from degradation, at least temporarily, by encapsulation in membrane vesicles or by binding of host proteins. Detection of hypermutant RNA in plasma does not necessarily indicate that HIV-1–infected cells carrying hypermutant sequences were more susceptible to cell killing as a result of the ApB DC vaccine; rather, the hypermutant sequences could just reflect nonspecific killing of T-cells following vaccination. Indeed, analysis of apoptosis markers in participant 10, who had available specimens in the time points evaluated, showed an increase in $CD4^+$ T-cell annexin V expression from 13% to 34.4% after vaccination (data not shown). The finding of both nonmutant and hypermutant HIV-1 RNA in plasma after vaccination suggests

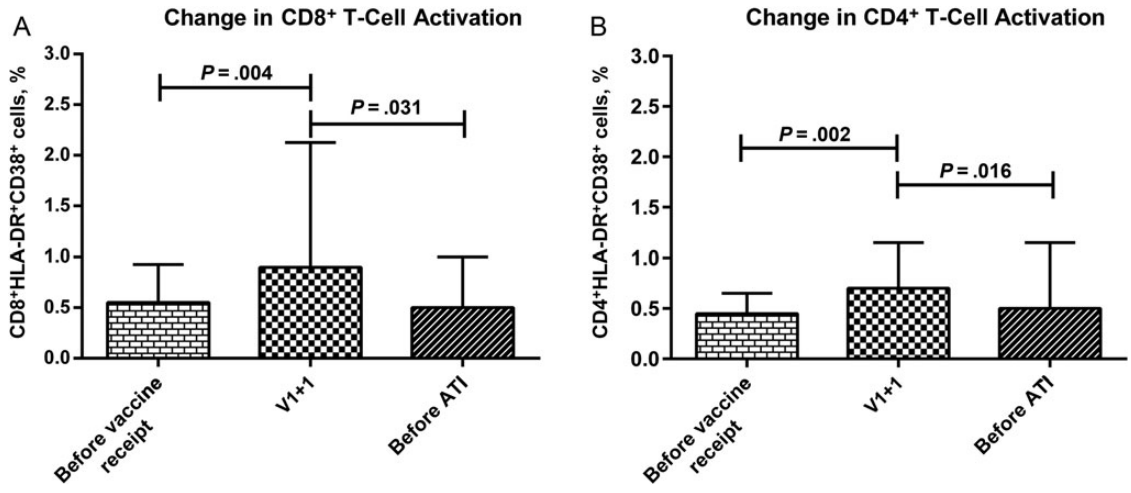

Figure 4. Effect of dendritic cell (DC)–based vaccination on T-cell activation. *A*, Changes in $CD8^+$ T-cell activation before and after vaccine receipt. The frequency of $CD8^+$ T cells coexpressing HLA-DR and CD38 significantly increased after the first vaccine dose (V1). This decreased to frequencies before vaccine receipt by the time immediately before antiretroviral treatment interruption (ATI; ie, 6 weeks after V3). Bar graphs show median values and interquartile ranges. *P* values were determined by the Wilcoxon signed rank test. *B*, Changes in $CD4^+$ T-cell activation before and after vaccine receipt. Similar to $CD8^+$ T-cell activation, the frequency of $CD4^+$ T cells coexpressing HLA-DR and CD38 increased after V1 but returned to levels before vaccine receipt 6 weeks after V3. Bar graphs show median values and interquartile ranges. *P* values were determined by the Wilcoxon signed rank test.

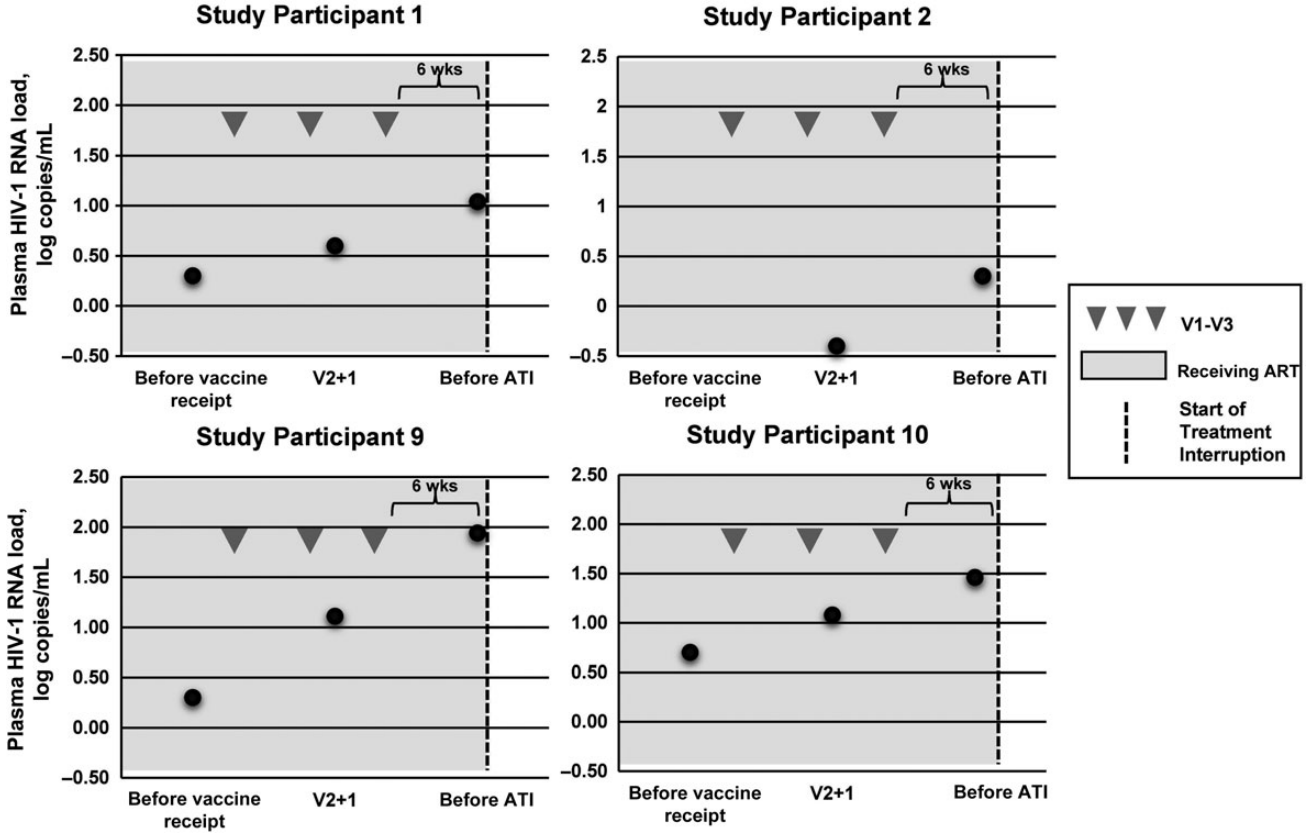

Figure 5. Changes in residual viremia after ApB dendritic cell (DC) vaccination during ART. Single-copy assay was performed on plasma samples obtained at 3 time points: before vaccine receipt, after vaccine receipt (V2+1) and immediately before antiretroviral treatment [ART] interruption [before ATI]). Despite receiving ART, there was a ≥0.7 log increase (median, 0.75 log) in residual plasma human immunodeficiency virus type 1 (HIV-1) RNA between before vaccine receipt/V2+1 and immediately before ATI time points in 4 of 10 participants.

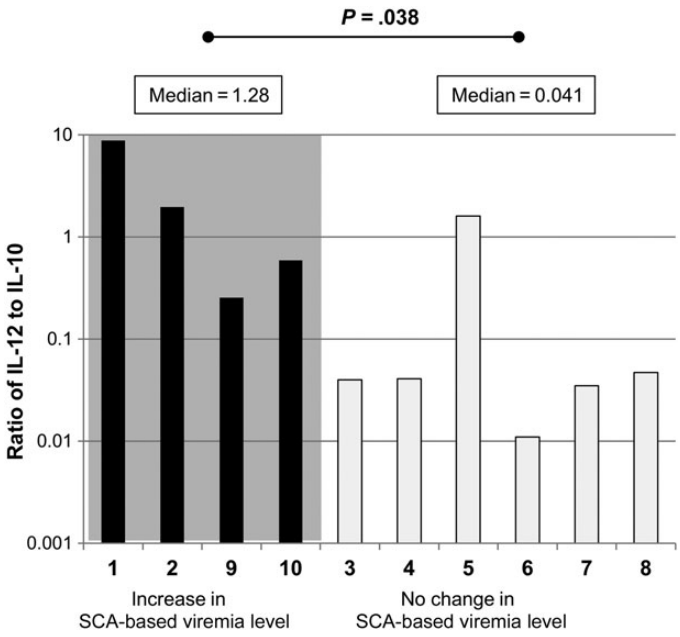

Figure 6. The ratio of interleukin 12 (IL-12) to interleukin 10 (IL-10) levels was evaluated to see whether the vaccine was more proinflammatory. The black bars show the individual ratios of IL-12 to IL-10 levels for the 4 of 10 participants who had an increase in residual viremia level (measured by a single-copy assay [SCA]), while the white bars show the ratios for the other 6 participants. The 4 participants with increasing residual viremia after vaccine receipt had significantly higher ratios than the other 6 (1.28 vs 0.041; $P = .038$).

that the increases in viral RNA levels in the plasma after vaccination could have come from 2 sources: vaccine-induced increases in virus production and/or vaccine-induced killing of infected $CD4^+$ T cells.

Because SGS data provided evidence of vaccine-induced killing of infected cells, we hypothesized that the virus that rebounded after ATI from surviving $CD4^+$ T cells contained CTL escape mutations that allowed these variants to evade an immune response induced by the ApB DC vaccine. We therefore looked for CTL escape variants in the rebound virus in comparison to virus obtained before ART, before vaccine receipt, at V2+1, and in the vaccine prep in participants 9 and 10, who had samples available. CTL escape variants were present in the prevaccine and postvaccine populations, but both the wild-type and CTL variants rebounded after ART was stopped. These data indicate that the vaccine did not induce immune responses that favored rebound of CTL escape mutants over wild-type virus, which is consistent with the marginal effect of the vaccine on the HIV-1 RNA load set point.

DISCUSSION

Our preclinical studies showed increased immunogenicity of DCs loaded with autologous virus-infected apoptotic cells and that production of such a DC-based vaccine was feasible [14, 15]. Although the vaccine was well tolerated, only a modest decrease in HIV-1 RNA load set point was observed at the primary end point. Because the study was uncontrolled, we cannot be certain this modest decrease was due to vaccine effect. Furthermore, despite an increase in both $CD8^+$ and $CD4^+$ T-cell activation, improved polyfunctional response to Gag peptide was observed in less than half of the participants, and polyfunctional responses did not correlate with HIV-1 RNA load set point at the primary end point. In retrospect, we believe that the limited vaccine effect may be due, in part, to suboptimal DC function, as evidenced by low IL-12 production, which has been shown to correlate with the efficacy of DC-based immunotherapy in patients with cancer [22, 23]. As such, this suggests that DC maturation and functional potency are critical factors in the efficacy of DC-based vaccines [24]. Another factor that may have affected vaccine efficacy is the freeze-thaw cycle that occurred before vaccine administration. Furthermore, it is also possible that the immune responses generated by the vaccine were to epitopes that already have CTL escape mutations, especially since the participants initiated ART during chronic HIV-1 infection. The highly diverse viral population present during chronic HIV-1 infection indeed poses a significant challenge in choosing the most appropriate antigen with which to load DCs to generate potent immune responses to epitopes where escape mutations have not been selected.

An interesting and novel finding in our study was the observed increase in the plasma HIV-1 RNA load following vaccine administration, despite continuation of suppressive ART and medication adherence, in a subset of 4 participants. Such increases in HIV-1 RNA load are not attributable to assay

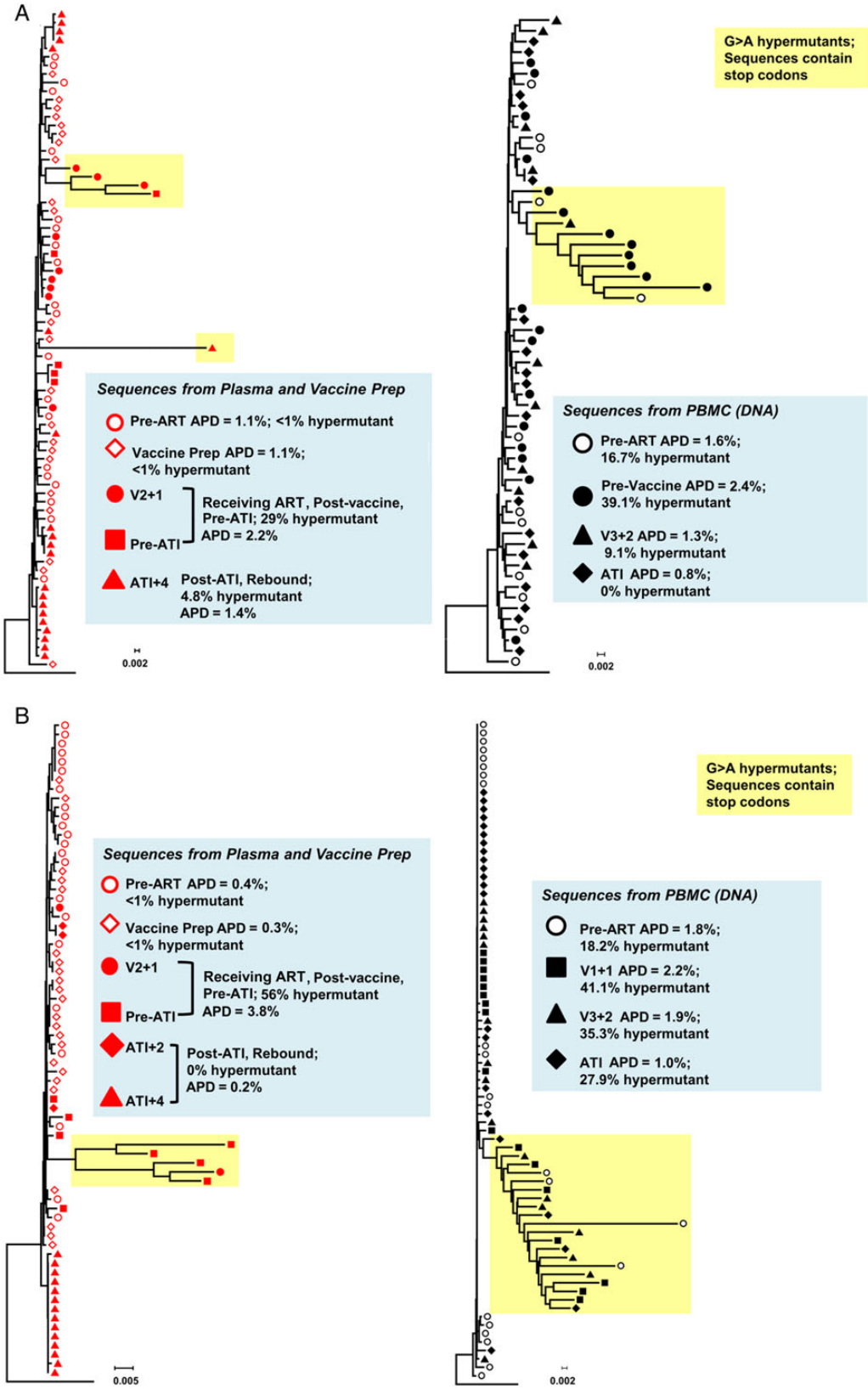

Figure 7. Neighbor-joining phylogenetic analyses of single-genome p6-Pro-Pol RNA or DNA sequences obtained from the virus isolated for vaccine production (vaccine prep), before antiretroviral therapy (pre-ART), after vaccine receipt (post-vaccine), and after antiretroviral treatment interruption (ATI) from study participant 9's plasma and peripheral blood mononuclear cells (PBMCs; *A*) and patient 10's plasma and PBMCs (*B*). Hypermutant RNA was observed in plasma after vaccination, suggesting that their presence resulted from cell death rather than from packaging into virions. Abbreviation: APD, average pairwise distance.

variation [25] or biological variation during stable ART [1, 26, 27] and have only been reported with deintensification of ART [28], which did not occur in the current study. Since in vitro studies have shown that latent HIV-1 provirus can be activated by contact with DCs [29, 30], we hypothesized that the increase in plasma HIV-1 RNA load may be due to reactivation of latent HIV-1. To test this, we first confirmed that the HIV-1 RNA detected in the plasma by SCA was indeed HIV-1 RNA and not contaminating HIV-1 DNA. SGS was performed to assess the diversity and detect mutated HIV-1 RNA as a means of differentiating cellular HIV-1 RNA from HIV-1 RNA packaged into virions. SGS showed that up to 56% of the plasma HIV-1 RNA after vaccination was hypermutated, including multiple stop codons in *gag* and *pol*, and thus was very unlikely to have been packed into virions. The observed increase in annexin V expression after vaccination supports $CD4^+$ T-cell apoptosis as the mechanism for release of cell-associated HIV-1 RNA into the plasma. Whether the nonhypermutated HIV-1 RNA detected in the plasma was within virions released from infected cells or from apoptotic HIV-1–infected $CD4^+$ T cells is unclear and cannot be readily differentiated. It is possible that the postvaccine increase in $CD4^+$ T-cell activation brought about by a more proinflammatory DC function led to an increase in virus production by $CD4^+$ T-cells and/or triggering of activation-induced $CD4^+$ T-cell apoptosis, both of which would increase HIV-1 RNA levels in the plasma. We could not determine whether induction of apoptosis was specific for HIV-1–infected cells or nonspecific for all $CD4^+$ T-cells, and, thus, observed effects must be interpreted with caution.

Although we were unable to fully determine whether HIV-1 RNA detected in the plasma was exclusively from apoptotic HIV-1–infected $CD4^+$ T cells or also from virions released from infected cells, in vitro studies showing activation of latent HIV-1 provirus by DCs [29, 30] support that DC-based vaccines can potentially be used to activate latent proviruses. Thus, apart from evaluating the plasma HIV-1 RNA load set point and the time to rebound following ATI, DC-based therapeutic vaccine studies should include measurements of plasma HIV-1 RNA and cell-associated HIV-1 loads during ART and whether changes in these measures are associated with a lower viral load set point and a prolonged time to viral rebound. Furthermore, the mechanisms behind the ability of DC-based vaccines to activate latent HIV-1 should be further investigated. Last, DC-based vaccines should be optimized for their ability to induce specific killing of HIV-1–infected cells apart from just IFN-γ production or polyfunctional responses. Cytotoxicity should be the main immunologic readout of vaccine efficacy. In conclusion, future studies of DC-based therapeutic vaccines in HIV-1 infection should concentrate on the discovery of preparations that are able to both activate latent HIV-1 and induce potent CTL activity to kill HIV-1–infected cells.

Supplementary Data

Supplementary materials are available at http://jid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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References

1. Palmer S, Maldarelli F, Wiegand A, et al. Low-level viremia persists for at least 7 years in patients on suppressive antiretroviral therapy. Proc Natl Acad Sci U S A **2008**; 105:3879–84.
2. Zheng L, Bosch RJ, Chan ES, et al. Predictors of residual viraemia in patients on long-term suppressive antiretroviral therapy. Antivir Ther **2013**; 18:39–43.
3. Shan L, Deng K, Shroff NS, et al. Stimulation of HIV-1-specific cytolytic T lymphocytes facilitates elimination of latent viral reservoir after virus reactivation. Immunity **2012**; 36:491–501.
4. Banchereau J, Briere F, Caux C, et al. Immunobiology of dendritic cells. Annu Rev Immunol **2000**; 18:767–811.
5. Huang XL, Fan Z, Borowski L, et al. Dendritic cells reveal a broad range of MHC class I epitopes for HIV-1 in persons with suppressed viral load on antiretroviral therapy. PLoS One **2010**; 5:e12936.
6. Kurle S, Thakar M, Shete A, Paranjape R. In vitro sensitization of T cells with DC-associated/delivered HIV constructs can induce a polyfunctional CTL response, memory T-cell response, and virus suppression. Viral Immunol **2012**; 25:45–54.
7. Subramanya S, Armant M, Salkowitz JR, et al. Enhanced induction of HIV-specific cytotoxic T lymphocytes by dendritic cell-targeted delivery of SOCS-1 siRNA. Mol Ther **2010**; 18:2028–37.
8. Smith KN, Mailliard RB, Larsen BB, et al. Dendritic cells restore CD8+ T cell reactivity to autologous HIV-1. J Virol **2014**; 88:9976–90.
9. Garcia F, Plana M, Climent N, Leon A, Gatell JM, Gallart T. Dendritic cell based vaccines for HIV infection: The way ahead. Hum Vaccin Immunother **2013**; 9:2445–52.
10. Garcia F, Leon A, Gatell JM, Plana M, Gallart T. Therapeutic vaccines against HIV infection. Hum Vaccin Immunother **2012**; 8:569–81.
11. Garcia F, Climent N, Guardo AC, et al. A dendritic cell-based vaccine elicits T cell responses associated with control of HIV-1 replication. Sci Transl Med **2013**; 5:166ra2.
12. Connolly NC, Whiteside TL, Wilson C, Kondragunta V, Rinaldo CR, Riddler SA. Therapeutic immunization with human immunodeficiency virus type 1 (HIV-1) peptide-loaded dendritic cells is safe and induces immunogenicity in HIV-1-infected individuals. Clin Vaccine Immunol **2008**; 15:284–92.
13. Macatangay BJ, Szajnik ME, Whiteside TL, Riddler SA, Rinaldo CR. Regulatory T cell suppression of Gag-specific CD8T cell polyfunctional response after therapeutic vaccination of HIV-1-infected patients on ART. PLoS One **2010**; 5:e9852.
14. Zhao XQ, Huang XL, Gupta P, et al. Induction of anti-human immunodeficiency virus type 1 (HIV-1) CD8(+) and CD4(+) T-cell reactivity by dendritic cells loaded with HIV-1 X4-infected apoptotic cells. J Virol **2002**; 76:3007–14.

15. Whiteside TL, Piazza P, Reiter A, et al. Production of a dendritic cell-based vaccine containing inactivated autologous virus for therapy of patients with chronic human immunodeficiency virus type 1 infection. Clin Vaccine Immunol **2009**; 16:233–40.
16. Cillo AR, Sobolewski MD, Bosch RJ, et al. Quantification of HIV-1 latency reversal in resting CD4+ T cells from patients on suppressive antiretroviral therapy. Proc Natl Acad Sci U S A **2014**; 111:7078–83.
17. Cillo AR, Vagratian D, Bedison MA, et al. Improved single-copy assays for quantification of persistent HIV-1 viremia in patients on suppressive antiretroviral therapy. J Clin Microbiol **2014**; 52:3944–51.
18. Besson GJ, Lalama CM, Bosch RJ, et al. HIV-1 DNA decay dynamics in blood during more than a decade of suppressive antiretroviral therapy. Clin Infect Dis **2014**; 59:1312–21.
19. Kearney MF, Spindler J, Shao W, et al. Lack of detectable HIV-1 molecular evolution during suppressive antiretroviral therapy. PLoS Pathog **2014**; 10:e1004010.
20. Riou C, Burgers WA, Mlisana K, et al. Differential impact of magnitude, polyfunctional capacity, and specificity of HIV-specific CD8+ T cell responses on HIV set point. J Virol **2014**; 88:1819–24.
21. Turk G, Ghiglione Y, Falivene J, et al. Early Gag immunodominance of the HIV-specific T-cell response during acute/early infection is associated with higher CD8+ T-cell antiviral activity and correlates with preservation of the CD4+ T-cell compartment. J Virol **2013**; 87:7445–62.
22. Okada H, Kalinski P, Ueda R, et al. Induction of CD8+ T-cell responses against novel glioma-associated antigen peptides and clinical activity by vaccinations with {alpha}-type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma. J Clin Oncol **2011**; 29:330–6.
23. Carreno BM, Becker-Hapak M, Huang A, et al. IL-12p70-producing patient DC vaccine elicits Tc1-polarized immunity. J Clin Invest **2013**; 123: 3383–94.
24. Rinaldo CR. Dendritic cell-based human immunodeficiency virus vaccine. J Intern Med **2009**; 265:138–58.
25. Palmer S, Wiegand AP, Maldarelli F, et al. New real-time reverse transcriptase-initiated PCR assay with single-copy sensitivity for human immunodeficiency virus type 1 RNA in plasma. J Clin Microbiol **2003**; 41:4531–6.
26. Maldarelli F, Palmer S, King MS, et al. ART suppresses plasma HIV-1 RNA to a stable set point predicted by pretherapy viremia. PLoS Pathog **2007**; 3:e46.
27. Riddler SA, Aga E, Bosch RJ, et al. Continued slow decay of the residual plasma viremia level in HIV-1-infected adults receiving long-term antiretroviral therapy. J Infect Dis **2016**; 213:556–60.
28. Wilkin TJ, McKinnon JE, DiRienzo AG, et al. Regimen simplification to atazanavir-ritonavir alone as maintenance antiretroviral therapy: final 48-week clinical and virologic outcomes. J Infect Dis **2009**; 199:866–71.
29. van der Sluis RM, van Montfort T, Pollakis G, et al. Dendritic cell-induced activation of latent HIV-1 provirus in actively proliferating primary T lymphocytes. PLoS Pathog **2013**; 9:e1003259.
30. Norton TD, Miller EA, Bhardwaj N, Landau NR. Vpx-containing dendritic cell vaccine induces CTLs and reactivates latent HIV-1 in vitro. Gene Ther **2015**; 22:227–36.