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Induction of Strong HIV-1-specific CD4⁺ T Cell Responses using an HIV-1 gp120/NefTat Vaccine adjuvanted with AS02_A in ARV Treated HIV-1-Infected Individuals

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Abstract

Background—Induction of HIV-1-specific CD4⁺ T cell responses by therapeutic vaccination represents an attractive intervention to potentially increase immune control of HIV-1.

Methods—We performed a double-blinded, randomized, placebo-controlled clinical trial to determine the safety and immunogenicity of GSK Biologicals' HIV-1 gp120/NefTat subunit protein vaccine formulated with the AS02_A adjuvant in subjects with well controlled chronic HIV-1 infection on HAART. Ten individuals received the vaccine; while adjuvant alone or placebo was given to five subjects each. Immunogenicity was monitored by intracellular cytokine flow cytometry and CFSE-based proliferation assays.

Results—The vaccine was well tolerated with no related SAEs. Vaccine recipients had significantly stronger gp120-specific CD4⁺ T cell responses which persisted until week 48 and greater gp120-specific CD4⁺ T cell proliferation activity as compared to controls. In the vaccine group, the number of participants that demonstrated positive responses for both gp120-specific CD4⁺ T cell IL-2 production and gp120-specific CD8⁺ T cell proliferation was significantly higher at week 6.

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Conclusions—The gp120/NefTat/AS02_A vaccine induced strong gp120-specific CD4⁺ T cell responses, and a higher number of vaccinees developed both HIV-1-specific CD4⁺ T cell responses and CD8⁺ T cell proliferation. The induction of these responses may be important in enhancing immune-mediated viral control.

Keywords

HIV-1; Vaccination; Therapeutic Vaccination; HIV-1-specific CD4⁺ cells

Introduction

Infection with HIV-1 causes progressive immune dysfunction, and the vast majority of infected individuals will ultimately require indefinite treatment with combination antiretroviral therapy (ART)^{1–2}. However, despite suppression of viral load to the limit of detection, the immune system remains impaired in many patients, especially if treatment is started at lower CD4⁺ T cell counts^{3–5}. The failure of the immune system to control HIV-1, and the postulated role of immune activation in rate of HIV-1 disease progression^{6–7} and in higher rates of non-HIV-1 related diseases, including cardiovascular disease^{8–11} and cancer^{1, 8, 11–14}, has generated interest in treatments aimed at modulating the immune system, including therapeutic HIV-1 vaccines. A therapeutic HIV-1 vaccine could serve to harness the immune system to allow for better control of the virus potentially delaying the start of ART.

Multiple strategies have been used to design HIV therapeutic vaccines including vaccination with a DNA encoding Gag-Pol-Nef fusion protein and envelope¹⁵, DNA encoding cytotoxic T lymphocyte epitopes¹⁶, Tat protein¹⁷, pox-virus with HIV genes encoding multiple HIV proteins^{18–24}, HIV lipopeptides^{19, 23, 25–26}, recombinant envelope protein²⁷, inactivated envelope-depleted whole virus²⁸, or a combination of the above. Thus far, these vaccination approaches have not yielded durable improvements in immune responses directed against HIV-1. Models of viral infections in mice and non-human primates have suggested that CD4⁺ T cell help is crucial for the development and maintenance of effective virus-specific CD8⁺ T cell responses^{29–31}. In addition, a recent study indicated strong HIV-2-specific CD4⁺ T cell responses are maintained in chronic HIV-2 infection, which may in part account for the significantly lower disease progression and mortality rates as compared to HIV-1³². Therefore, a therapeutic HIV-1 vaccine that induces HIV-1-specific CD4⁺ T cell responses could improve antiviral immunity resulting in delayed disease progression.

With the aim of inducing helper T-cell responses and neutralizing antibodies, GlaxoSmithKline developed a subunit vaccine composed of a NefTat fusion protein and envelope glycoprotein gp120 formulated with AS02_A adjuvant. Primate studies of this vaccine approach showed lower viral load and protection against progression to AIDS following SHIV challenge in vaccinees³³. This subunit vaccine was found to be safe and well-tolerated in previous trials in non-HIV-1-infected adults^{34–35} and induced durable HIV-1-specific CD4⁺ T cell responses but not detectable CD8⁺ T cell responses. Here, we report the results of a double blinded, placebo-controlled study of this vaccine composed of three recombinant HIV-1 clade B viral antigens (envelope glycoprotein gp120, Nef and Tat) formulated with AS02_A adjuvant in participants with chronic HIV-1 infection well-controlled on combination ART.

Methods

Study Participants

Men and women participants aged 18 to 60 with chronic HIV-1 infection were recruited at Massachusetts General Hospital (MGH), in Boston, Massachusetts. To be eligible for the study, volunteers had to be taking at least three antiviral drugs for at least 12 consecutive months prior to screening, have had an undetectable HIV-1 RNA in ultra-sensitive assays on at least two occasions in the 6 months prior to screening, and have had a CD4+ T cell count >400 cells/mm³ within 28 days of study entry. This study was approved by the Institutional Review Board at MGH and was conducted in accordance with Helsinki Declaration of 1975, as revised in 2000. Written informed consent was obtained from each individual. The study was overseen by an Internal Safety Review Committee and a Safety Monitoring Committee.

Vaccine and Adjuvant

The HIV-1 vaccine (gp120/NefTat/AS02A) administered consisted of three recombinant HIV-1 clade B viral antigens: envelope glycoprotein gp120 and two regulatory proteins, Nef (derived from HIV_{LAI}) and Tat (derived from HIV_{BH10}). The latter are expressed as one recombinant fusion protein, NefTat. The recombinant gp120 is a truncated form of the gp120 envelope protein of HIV-1 isolate W6.1D. The antigens were formulated in a proprietary adjuvant, AS02_A, comprised of two immunostimulants (MPL and QS-21) in an oil-in-water emulsion. The placebo used was NaCl.

Study Design

In this randomized, double blind clinical trial of the gp120/NefTat/AS02_A vaccine, participants were randomized in a 2:1:1 distribution to vaccine plus adjuvant (10 individuals), AS02_A adjuvant alone (5 individuals), or placebo (5 individuals). Injections with vaccine, adjuvant alone or placebo were administered intramuscularly at weeks 0, 4, and 12 (Supplemental Figure 1). The volunteers were monitored for 60 minutes following vaccination for adverse events. Participants recorded reactogenicity events on a diary card for 7 days following vaccination. Local reactogenicity events were defined as pain, tenderness, erythema, or induration at the site of vaccination, and systemic reactogenicity events included body temperature, malaise, fatigue, chills, headache, myalgias, arthralgias, nausea, vomiting, and diarrhea. Participants underwent a clinical evaluation on the day of each immunization and 2 weeks after each immunization. Blood samples were obtained for safety monitoring, HIV-1 RNA (Roche Amplicor HIV-1 Monitor, ultrasensitive version 1.5), T cell subsets, and immunological assays at study baseline and weeks 2, 4, 6, 12, 14, 24, 48.

The primary safety endpoint was the occurrence, intensity and relationship of any local and general signs and symptoms during a 7-day follow-up period after each vaccination. The primary immunogenicity endpoint was the change in the frequency of IL-2+ CD4+ T cells in response to at least one vaccine antigen, assessed two weeks after the third vaccination (week 14). The secondary immunogenicity endpoints were the changes in cell-mediated immune (CMI) responses following vaccination. CMI responses were assessed by measuring lymphoproliferative HIV-1-specific CD4+ and CD8+ T cell responses, HIV-1-specific cytokine production by CD4+ and CD8+ T cells, and CD8+ T cell degranulation following stimulation with HIV-1 peptides (see details below).

Lymphocyte Separation

Fresh blood was collected in ACD tubes. Peripheral blood mononuclear cells (PBMCs) were extracted from whole blood by Ficoll-Hypaque as described previously³⁶ and frozen for subsequent CMI analyses.

Synthetic Peptides

We used a panel of overlapping peptides (15 amino acids overlapping by 11 amino acids) representing unique sequences (gp120, Nef, Tat) present in the vaccine construct³⁴. In addition, we used a panel of overlapping peptides (15–20 amino acids overlapping by 10–11 amino acids) spanning Gag based on the HIV-1 clade B 2001 consensus sequence.

Analysis of Antigen-specific T cell Function by Multiparameter Flow Cytometry

Intracellular cytokine staining assays were performed on cryopreserved PBMCs. One million PBMCs were incubated with peptide pools of Gag as well as the Tat, Nef and gp120 sequences contained in the vaccine (final concentration per peptide 2 µg/ml). Along with each peptide pool, the cells were incubated with co-stimulatory antibodies (anti-CD28 and anti-CD49d at 1 µg/mL each; Becton, Dickinson and Company - BD) and fluorophore-labeled CD107a/b and CD40L antibodies at 37°C, 5% CO₂ as described previously^{37–38}. After one hour, monensin (GolgiStop) (10 µg/mL, BD) was added. Following a 16-hour incubation at 37°C, 5% CO₂, the cells were washed, stained with fluorophore-labeled CD8, CD4, and CD3 (BD), washed again, and cells were fixed and permeabilized with Caltag Fixation/permeabilization Kit (Invitrogen). Next, the PBMCs were intracellularly stained with fluorophore-labeled anti-IL-2, IFN-γ, and TNF-α antibodies (Becton, Dickinson, and Company). Cells were analyzed on a FACS Sort Flowcytometer (Becton, Dickinson and Company). Control samples were unstimulated autologous PBMCs otherwise treated identically. Results were analyzed with FlowJo software version 7.6.1 (Tree Star) and are reported as percentages of CD4+ T cells or CD8+ T cells that produce cytokines following stimulation with the corresponding peptide pool, following subtraction of the negative control values. A vaccine responder was defined as an individual with ≥ 0.03% antigen-specific cells above the background level following peptide stimulation and at least an increase of 0.05% antigen-specific cells (after week 0). This cutoff was based on the 95th percentile for peptide-specific cytokine producing cells observed prior to vaccination.

Proliferation Assay

Ex vivo proliferation was measured using carboxyfluorescein succinimidyl ester (CFSE; Invitrogen) as described previously³⁶. Briefly, one million PBMCs were stained with 0.25 µM CFSE at 37°C for 7 minutes. Following the addition of serum, the cells were washed with PBS and suspended at 1×10^6 /mL in medium (RPMI 1640 supplemented with glutamine, 10% fetal calf serum, penicillin, and streptomycin). Pools of overlapping HIV-1-specific peptides described above were added at a concentration of 20 ng/mL. On day 6, cells were washed with PBS and stained with fluorophore-labeled anti-CD8, CD3, and CD4 antibodies. Cells were then washed and fixed in 1% paraformaldehyde (Becton, Dickinson and Company). Flow cytometric data were acquired on a FACS Sort Flowcytometer. Negative control samples were unstimulated autologous PBMCs, and positive control samples were autologous PBMCs that were incubated with phytohemagglutinin (PHA). Data were analyzed with FlowJo software version 7.6.1 (Tree Star). The percentage of proliferating cells in response to a specific antigen was calculated by subtracting the background proliferation (proliferating fraction in media alone) from the proliferating fraction in response to antigen. A vaccine responder was defined as an individual with ≥ 0.5% antigen-specific proliferating cells above the background level following peptide stimulation and at least an increase of 0.1% (after week 0) antigen-specific proliferating cells. This cutoff was based on the 95th percentile for peptide-specific proliferating cells observed prior to vaccination.

Statistical Analysis

All analyses were based on the intention-to-treat principle, and missing or unevaluable measurements were not replaced. Results are reported as mean or medians, and all immunogenicity results are reported following background subtraction. Differences between vaccine and control groups at a given time point were tested using the two-sided Wilcoxon Rank-Sum Test for non-parametric data. Differences between baseline and later time points in each group were tested using the two-sided non-parametric Wilcoxon matched-pairs signed rank test. Differences among each group over the duration of the study were tested using the two-sided non-parametric Kruskal-Wallis Test. The proportion of individuals with positive responses to gp120 by CD8+ T cell proliferation and by CD4+ T cell IL-2 production (categorical variable) in each group was compared using a two-sided Fischer Exact Test. Tests were considered significant if the p value was greater than or equal to 0.05. For this phase 1, exploratory study, we powered the study to detect safety endpoints. With a sample size of 20 subjects, we had at 87% probability of seeing at least one safety endpoint if the true probability was 0.10 and at 82% probability of seeing at least two safety endpoints if the true probability was 0.15. Reported p-values were two-sided without adjustment for multiple comparisons.

Results

Demographic Data

Twenty participants were enrolled and underwent randomization. Ten subjects were randomly assigned to receive the gp120/NefTat/AS02_A vaccine, 5 subjects to receive AS02_A adjuvant alone, and five subjects to receive placebo (Table 1). At baseline, the participants had a median age of 48 and eighty percent were male. At study entry, the median duration of ART in the 20 volunteers was 11 years (range 3 to 16 years), and the median CD4 count and CD8 count were 633 cells per mm³ (range 405 to 1549 cells per mm³) and 1296 cells per mm³ (range 584 to 1976 cells per mm³), respectively. Interim analysis of immunogenicity data showed no differences between participants receiving placebo or adjuvant; accordingly, the immunogenicity results from subjects in the adjuvant alone and placebo groups for subsequent analyses were combined (control group).

Vaccine Safety

During the study period, 4 serious adverse events (SAE) and 10 adverse events (AE) were observed. All 4 SAE were classified as not related to the study, and none of the 4 SAE occurred in the vaccine arm. Of the 10 AEs, 9 were classified as being not related to the study; 3 of the 9 occurred in the vaccine group. One AE was classified as possibly related to the study, and occurred in a person who received vaccine. Subject #18 reported the worsening of a pre-existing sensory peripheral neuropathy following the 2nd vaccination, and decided to not receive the 3rd vaccination. The participant agreed however to continue to come for the scheduled visits for sample collection and safety/immunogenicity laboratories. Self-reported pain and tenderness at the injection site were common, occurring after almost all injections in the vaccine group (26 of 29) and in the adjuvant only group (14 of 15); these symptoms lasted between 1 to 5 days and were generally mild. Only one vaccination out of 15 in the placebo group was associated with an injection site reaction. Other symptoms that were reported after injections on subject diary cards included malaise, chills, arthralgias, myalgias, fatigue and nausea, but these did not lead to treatment discontinuation and did not differ between the vaccine and the control group.

HIV-1-Specific CD4+ T cell Cytokine Production Following Vaccination

Previous trials of the gp120/NefTat/AS02_A vaccine in healthy, HIV-1-seronegative individuals demonstrated that vaccination induced durable HIV-1-specific CD4+T cell responses without inducing CD8+ T cell responses^{34–35}. We examined CD4+ T cell responses to vaccine-derived peptides before and after vaccination. At the primary immunogenicity endpoint two weeks following the third vaccination (week 14) the median percentage of CD4+ T cells that expressed IL-2 in response to stimulation with the vaccine antigen gp120 (gp120-specific IL-2+ CD4+ T cells) was significantly increased from baseline in vaccine recipients (0 to 0.08%, $p = 0.009$) but not in the control groups (0.005% to 0, $p = 0.62$) (Figure 1A). In addition, the median percentage of gp120-specific IL-2+ CD4+ T cells at week 14 was statistically higher in the vaccine group than in the control group (0.08 versus 0%, $p = 0.0007$) (Figure 1A). In the vaccine group, the percentage of gp120-specific IL-2+ CD4+ T cells peaked at week 6 (median 0.12%) and was maintained throughout the study ($p=0.003$). Vaccination also resulted in greater proportions of gp120-specific CD4+ T cells that produced both IFN- γ and IL-2 (0.015 versus 0%, $p = 0.0062$) or TNF- α (0.052 versus 0.007%, $p = 0.018$) at week 14 (Supplemental Table 1). In response to the vaccine antigen Nef, the frequency of CD4+ T cells producing IL-2 trended up in the vaccine group during the study though this was not statistically significant ($p = 0.19$) (Figure 1A and Supplemental Table 1). Vaccination with gp120/NefTat/AS02_A induced or enhanced production of IL-2 by CD4+T cells in response to the vaccine antigen Tat only at week 14 (0.02 vs 0%, $p = 0.0036$) and did not induce increased IL-2 production over time in the vaccine group ($p = 0.12$). Furthermore, no significant changes in cytokine production by CD4+T cells in response to the non-vaccine antigen Gag were observed ($p = 0.89$ in the vaccine group; Figure 1A and Supplemental Table 1). Taken together, these data show that vaccination with the gp120/NefTat/AS02_A vaccine resulted in the induction of significant levels of vaccine-specific CD4+ T cells, and in particular gp120-specific IL-2+ CD4+ T cells.

HIV-1-Specific CD4+ T cell Proliferation Following Vaccination

Next, we assessed HIV-1-specific CD4+T cell proliferation following vaccination. In agreement with prior studies that indicate that IL-2 is critical for maintaining HIV-1-specific CD4+ T cell proliferation *ex vivo*^{39–40}, the proportion of CD4+ T cells proliferating in response to vaccine-derived gp120 increased significantly ($p = 0.03$) from a median of 0.1 (range 0 to 0.36) at week 0 to 1.16 (range 0 to 6.32) at week 6 (Figure 1B and Supplemental Table 1). This increase in proliferation temporally coincided with the increase in the frequency of gp-120-specific IL-2+ CD4+ T cells. The increase in CD4+ T cell proliferation trended down over time and, by week 48, had returned to baseline. No significant change was seen in CD4+ T cell proliferation in response to vaccine-derived Nef or Tat or non vaccine-derived Gag. Taken together, these data show that the gp120/NefTat/AS02_A vaccine also induced CD4+ T cell proliferation in response to the vaccine peptide gp120 in HIV-1 positive individuals on suppressive ART.

HIV-1-Specific CD8+ T cell IFN- γ Production and Proliferation Following Vaccination

HIV-1-specific CD8+ T cells play a critical role in the control of HIV-1 infection. Therefore, we assessed changes in HIV-1-specific CD8+ T cell responses following vaccination including cytokine production, degranulation, and proliferation. In keeping with the prior study of this vaccine in HIV-1-seronegative adults^{34–35}, vaccination did not result in significant changes from baseline or compared to the control group in the proportion of CD8+ T cells producing IFN- γ , TNF- α , IL-2, or expressing CD107a following incubation with vaccine peptides (Figure 2A and Supplemental Table 2). Because HIV-1-specific CD8+ T cell proliferation has been shown to depend on HIV-1-specific CD4+ T cell help^{36, 41}, we assessed whether the CD4+ T cell responses elicited by the vaccine might be

associated with CD8+ T cell proliferative responses. CD8+ T cell proliferation in response to nef was highest at baseline and declined during the study ($p = 0.02$) (Figure 2B). This significance of this observation is unclear though prior work has demonstrated that CD8+ responses decline over time with suppressed HIV-1 viral load⁴². We found a marginal increase in CD8+ T cell proliferation in response to gp120 at week 6 compared to baseline though it did not reach statistical significance (0.09 to 0.56, $p = 0.08$) and subsequently decreased over time. Nevertheless, at week 6, gp120-specific CD8+ T cell proliferation was significantly higher in the vaccinees compared to controls (0.56 versus 0.06, $p = 0.01$) (Figure 2B and Supplemental Table 2). Furthermore, the proportion of participants that were dual responders for both gp120-specific IL2+ CD4+ T cells and CD8+ T cell proliferation increased significantly following vaccination at week 6 (Figure 3). Thus, these data illustrate that appreciable antigen-specific CD8+ T cell cytokine production or degranulation were not induced by this vaccine, but that vaccination resulted in transiently higher rates of antigen-specific CD8+ T cell proliferation *in vitro* that was associated with an increase in antigen-specific IL-2+ CD4+ T cells.

Discussion

Therapeutic vaccinations aimed at the reconstitution of HIV-1-specific immune responses in infected individuals have been proposed as interventions to enhance immune control following interruption of HAART or to reduce the failure rate of HAART. Here we tested the ability of a subunit vaccine composed of a NefTat fusion protein and envelope glycoprotein gp120 formulated with AS02_A adjuvant to reconstitute HIV-1-specific T cell immunity in infected individuals. In HIV-1 positive individuals taking suppressive combination ART, vaccination caused an increase in HIV-1-specific cytokine production and proliferation by CD4+ T cells. While this vaccine did not induce an increase in cytokine production or degranulation of HIV-1-specific CD8+ T cells, and induced only a transient increase in gp120-specific CD8+ T cell proliferation, the proportion of participants that were dual responders for both gp120-specific IL-2+ CD4+ T cells and CD8+ T cell proliferation increased significantly at week 6 following vaccination. The proliferative and immune responses stimulated by the vaccine may represent *de novo* responses or expansion of pre-existing responses that were below the level of detection. Some responses waned over time which can occur in chronic infection with ARV treatment⁴³. These data demonstrate that gp120-specific CD4+ T cell responses can be elicited or reconstituted in infected individuals and may contribute to enhanced proliferation of gp120-specific CD8+ T cells following stimulation *in vitro*.

In addition to significant immunogenicity, the gp120/NefTat/AS02_A vaccine showed an acceptable safety and reactogenicity profile in the study volunteers. Mild to moderate local reactions were common and resolved within a few days, and no SAE that occurred during the trial were attributed to the vaccine. One participant in the vaccine arm experienced a worsening of chronic sensory peripheral neuropathy following the second vaccination and elected not to receive a third vaccination.

The ability of HIV-1-specific CD8+ T cells to respond to antigen by proliferation *in vitro* has been associated with better control of HIV-1 viremia and slower disease progression in a number of studies^{41, 44}. Vaccination with the gp120/NefTat/AS02_A vaccine not only reconstituted HIV-1 specific CD4+ T cell help as shown by increasing IL-2 production in response to vaccine-derived peptides, but also caused a transient increase in the proportion of participants who had a positive response for both antigen-specific IL-2+ CD4+ T cells and CD8+ T cell proliferation. Vaccination could have enhanced CD8+ cell proliferation in the setting of increased CD4+ IL2 production by directly modulating intrinsic CD8+ T cell function or by providing IL-2 in the *in vitro* proliferation assay. In chronic HIV-1 infection,

a prior study demonstrated that loss of HIV-1-specific CD8+ T cell proliferation in progressive infection is in part due to loss of IL-2-secreting HIV-1-specific CD4+ T cells³⁶. In addition, HIV-1 specific CD8+ T cell proliferative responses were restored *in vitro* with the addition of autologous HIV-1-specific CD4+ cells. Therefore, some restoration of CD4+ T cell help with a therapeutic HIV-1 vaccination may help maintain CD8+ T cell function in chronic HIV-1 infection.

Though improvements in both HIV-1-specific CD4+ and CD8+ function were demonstrated following vaccination, no conclusions can be made about the clinical utility of the gp120/NefTat/AS02_A vaccine based on this trial. Several studies indicate that more potent CD8+ T cell functionality is a key difference in long-term non-progressors (LNTP) compared to chronic progressors^{41, 44–47}, which may account for the improved immunologic control of HIV-1 by LNTPs. Based on these findings a therapeutic vaccine that improves CD8+ T cell functionality may lead to improved control of HIV-1. One way to test this hypothesis would be to administer the therapeutic vaccine to HIV-1-infected participants followed by a treatment interruption and concomitant longitudinal assessment of viral load and CD4+ and CD8+ T cell function. However, treatment interruptions in chronic HIV-1 infection should be undertaken with caution given the results of previous trials of CD4 guided antiretroviral therapy versus continuous therapy in which treatment interruption was associated with an increase in opportunistic infections, death from any cause⁴⁸, and morbidity⁴⁹.

In conclusion, we show that therapeutic vaccination with the gp120/NefTat/AS02_A vaccine in chronically HIV-1-infected volunteers on suppressive ART was safe, well-tolerated, and resulted in increases in HIV-1-specific CD4+ T cell help and increases in dual responders for both HIV-1-specific IL-2 production by CD4+ T cells and HIV-1-specific CD8+ T cell proliferation. A vaccine strategy of reconstituting CD4+ T cell help in chronic HIV-1 infection and in turn improving virus-specific CD8+ T cell function may augment virologic control resulting in slower disease progression or decreased immune activation. This vaccine is one potential approach to improve HIV-1-specific CD4+ T cell functionality; to assess the clinical implications of this vaccine would require a larger clinical trial and possible treatment interruption.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A) Longitudinal assessment of CD4+ T cell production of IL-2. Proportion of CD4+ T cells that produce IL-2 quantified by intracellular cytokine staining in response to vaccine-derived HIV-1 peptides (gp120, nef, tat) and a non-vaccine derived HIV-1 peptide (gag) before and after vaccination. B) Longitudinal evaluation of CD4+ T cell proliferation. Proportion of CD4+ T cells proliferating following stimulation with vaccine-derived HIV-1 peptides (gp120, nef, tat) and a non-vaccine derived HIV-1 peptide (gag) before and at four time points following vaccination as measured by an ex-vivo proliferation assay. The top rows represent the vaccine group, and the bottom rows represent the control group. The horizontal lines are the medians. The stated p values were calculated using Kruskal-Wallis test for non-parametric data. Data are reported following background subtraction. & - $P < 0.05$ compared to week 0, and $P < 0.05$ compared to respective timepoint in control group. # - $P < 0.05$ compared to week 0. ‡ - $P < 0.05$ compared to control group.

Figure 2.

A) Longitudinal assessment of CD8+ T cell production of IFN- γ . Proportion of CD8+ T cells that produce IFN- γ quantified by intracellular cytokine staining in response to vaccine-derived HIV-1 peptides (gp120, nef, tat) and a non-vaccine derived HIV-1 peptide (gag) before and after vaccination. B) Longitudinal evaluation of CD8+ T cell proliferation. Proportion of CD8+ T cells proliferating following stimulation with HIV-1 peptides as measured by ex-vivo proliferation assay. The top rows represent the vaccine group, and the bottom rows represent the control group. The horizontal lines are the medians. The stated p values are calculated using Kruskal-Wallis test for non-parametric data. Data are reported following background subtraction. & - $P < 0.05$ compared to week 0, and $P < 0.05$ compared to respective timepoint in control group. # - $P < 0.05$ compared to week 0. ‡ - $P < 0.05$ compared to control group.

Figure 3. Relationship between CD8+ T cell proliferation and CD4+ T cell IL-2 production in response to vaccine-derived gp120 over time

The dashed lines indicate the threshold for a positive test. A responder for cytokine production or CD8+ T cell proliferation following peptide stimulation was defined as an individual with $\geq 0.03\%$ antigen-specific cells and at least an increase of 0.05% (after week 0) or $\geq 0.5\%$ antigen-specific proliferating cells and at least an increase of 0.1% (after week 0), respectively. The p value was calculated by comparing the proportion of dual responders (met criteria for both CD8+ T cell proliferation and for CD4+ T cell IL-2 production) to gp120 in the control group to the proportion of dual responders in the vaccine group using the Fischer Exact test.

Table 1

Baseline characteristics of subjects

Characteristic	Entire Study Population (N=20)	Study Population by Treatment		
		Vaccine (n=10)	Placebo (n=5)	Adjuvant (n=5)
Age (median, yrs)	48	48	45	49
Gender (% male)	80%	60%	100%	100%
Median Duration of Antiretroviral Therapy (yrs) *	11	10	10	11
Nadir CD4 count (median)/mm ³ †	178	218	153	115
Baseline CD4 cell count (median)/mm ³	633	693	629	454
Baseline CD8 cell count (median)/mm ³	1296	1296	820	1545

* Prior to Study Entry

† Data not available for 3 participants