Absence of Immunodominant Anti-Gag p17 (SL9) Responses among Gag CTL-Positive, HIV-Uninfected Vaccine Recipients Expressing the HLA-A*0201 Allele


*J Immunol* 2004; 173:2126-2133; doi: 10.4049/jimmunol.173.3.2126

http://www.jimmunol.org/content/173/3/2126

References

This article cites 65 articles, 37 of which you can access for free at: http://www.jimmunol.org/content/173/3/2126.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Absence of Immunodominant Anti-Gag p17 (SL9) Responses among Gag CTL-Positive, HIV-Uninfected Vaccine Recipients Expressing the HLA-A*0201 Allele


According to a number of previous reports, control of HIV replication in humans appears to be linked to the presence of anti-HIV-1 Gag-specific CD8 responses. During the chronic phase of HIV-1 infection, up to 75% of the HIV-infected individuals who express the histocompatibility leukocyte Ag (HLA)-A*0201 recognize the Gag p17 SLYNTVATL (aa residues 77–85) epitope (SL9). However, the role of the anti-SL9 CD8 CTL in controlling HIV-1 infection remains controversial. In this study we determined whether the pattern of SL9 immunodominance in uninfected, HLA-A*0201 HIV vaccine recipients is similar to that seen in chronically HIV-infected subjects. The presence of anti-SL9 responses was determined using a panel of highly sensitive cellular immunoassays, including peptide:MHc tetramer binding, IFN-γ ELISPOT, and cytokine flow cytometry. Thirteen HLA-A*0201 vaccinees with documented anti-Gag CD8 CTL reactivities were tested, and none had a detectable anti-SL9 response. These findings strongly suggest that the pattern of SL9 epitope immunodominance previously reported among chronically infected, HLA-A*0201-positive patients is not recapitulated in noninfected recipients of Gag-containing canarypox-based candidate vaccines and may be influenced by the relative immunogenicity of these constructs. The Journal of Immunology, 2004, 173: 2126–2133.

Numerous reports highlight the critical role of MHC class I-restricted CD8+ CTL in the control of several different human viral infections (1–4). Although no clear correlates of immunoprotection against HIV-1 infection have yet been conclusively identified, several studies have pointed to the importance of anti-HIV-1 CD8+ CTL responses in both humans and animal models over the past years. First, CTL activity has been correlated with the initial decrease in viremia during the acute phase of infection in human HIV infection as well as in SIV-infected macaques (5–12). In both HIV-infected patients as well as SIV models, the occurrence of escape mutants is detectable within the first few months of infection, suggesting that CTLs exert considerable selective pressure on the replicating virus population (13, 14). Second, the decline of CTL responses has been temporally related with the onset of overt disease in humans as well as in macaques (15–24). Lastly, CTL activities have been detected in a subset of highly exposed uninfected individuals, and possibly correlated with transient immunoprotection (25–28).

The qualitative and quantitative characteristics of the early anti-HIV cell-mediated immune response may strongly influence the course of infection. In fact, a number of studies have linked virologic control to the breadth of CD8+ CTL as well as the presence of CD4+ Th responses (19, 29). Among the multitude of epitopes recognized by CD8+ cells from infected patients, a number appear at a significantly high frequency (30). One such immunodominant HIV CTL epitope that appears to fit this pattern is the p17 Gag SLYNTVATL epitope (residues 77–85), referred to as SL9. This HLA-A*0201-restricted epitope has been reported to be immunodominant among chronically HIV-infected individuals expressing this HLA allele, because 60–75% of these patients develop a strong CD8+ anti-SL9 response (31–36).

The most recent strategies aimed at developing a protective vaccine against HIV infection are focused on the capacity of a vaccine candidate to elicit MHC class I-restricted CD8+ CTL (reviewed in Ref. 37). Since the clinical testing of these vaccines began, a better understanding has been gained regarding the specificity of the elicited CTL reactivities in humans and animal models (38–43). We report on a study investigating the placement of the immunodominant SL9 epitope response in the ontogeny of cellular responses elicited in noninfected HIV vaccine recipients.

Materials and Methods

Subjects and study protocols

PBMC were selected from HLA-A*0201-positive volunteers enrolled in the National Institutes of Health-sponsored AIDS Vaccine Evaluation Group protocols 022, 022A, 032, 033, and 020. For protocols 022, 022A, 032, and 020, a recombinant canarypox (ALVAC) vector vCP205 (Pasteur-Mérieux Connaught Laboratories, North York, Canada) comprised of the following HIV-1 genes was used: gp120M, the transmembrane portion of gp41L(M), the transmembrane portion of gp41L(A), the cytoplasmic domain of gp41, and protease. The immunization schedule consisted of either three or four vector injections, followed by two HIV-1 envelope subunit boosts with either HIV-1 Env, gp120 (Chiron, Emeryville, CA) alone or in combination with vCP205. For protocol 033, volunteers...
received vCP205, either alone or in combination with GM-CSF, and also received two booster immunizations with a Gag-Pol DNA vaccine (Wyeth-Lederle Vaccine and Pediatrics, Pearl River, NY).

**Human HLA typing**

Molecular HLA typing was performed by Dr. Kostyu (Duke University, Durham, NC) using generic and family- or subtype-specific primers and sequence-specific oligonucleotide probes, as previously described (44, 45).

**Vaccinia constructs**

Autologous stimulator cells for in vitro stimulation (IVS) were infected with the recombinant vaccinia virus vP1291 expressing the following epitopes: HIV-1MN env gp120 and gp41 transmembrane protein and HIV-1 LA gag/protease. Autologous B lymphocyte cell line (BLCL) targets for the CTL assays were infected with the following recombinant vaccinia viruses: vP1170 (Western Reserve parent control), vP1174 (HIV-1MN env gp160), and vDK1 (HIV-1_M1 gag clade B). All the recombinant vaccinia constructs were used at a multiplicity of infection of 5:1. These viruses were provided by Dr. J. Tartaglia (Aventis-Pasteur, Lyon, France) and the National Institutes of Health AIDS Research and Reference Reagent Programs.

**Peptides**

The 15- and 20-mer overlapping peptides of the HIV-1MN subtype B gag and peptides selected from previously defined B clades epitopes (as described in the HIV Molecular Immunology Database (46)) were synthesized by stepwise solid phase Fmoc chemistry using a Rainin Symphony automated peptide synthesizer (SynPep, Dublin, CA), and purity was established by HPLC. For the purpose of epitope mapping, a peptide matrix was used consisting of five pools of consecutive 10 (P1–10 through P40–49) and 10 pools of five (M1 through M10) overlapping 20-mer peptides representing the entire gag (HXB2) protein. The peptides were arranged such that any particular peptide could be found in only two pools, as previously described (47). The peptides were used at a final concentration of 2 μg/ml for the ELISPOT analysis and at 10 μg/ml to generate CTL lines from frozen PBMC as well as to coat the B cell lines used as targets in the CTL assay.

**CTL studies in HIV seronegative vaccine recipient volunteers**

PBMC were separated from 50 ml of acid citrate dextrose anticoagulated blood, and CD8 CTL responses were evaluated after IVS with recombinant poxvirus-infected autologous PBMC stimulators according to the method described by Ferrari et al. (48). These effectors were termed Ag+CTL. To generate SL-9-specific cell lines, frozen PBMC were stimulated on day 0 with 10 μg/ml peptide in the presence of 330 U/ml IL-7 (R&D Systems, Minneapolis, MN). The cell lines (SL-9 IVS) were subsequently expanded with peptide-pulsed autologous PBMC and maintained with recombinant vaccinia virus vP1291 expressing the following extrinsic gene inserts: HIV-1MN env gp120 and gp41 transmembrane protein and the National Institutes of Health AIDS Research and Reference Reagent Programs.

**Tetramer analysis**

For tetramer analyses, 1–2 × 10^6 cells (either PBMC or Ag-IVS cells) were incubated for 30 min at 4°C with 5 μg of PE-labeled HLA A*0201 tetramer complexes with epitopes (HIV-1 Gag p17 aa 77–85: SLYNTVATL) (32) or control peptide (HTLV-I Tax aa 11–19: LLFGYPVYYV) (51). HLA-B*0701 tetramers loaded with the epitopic CMV pp65 peptide (aa 417–426: TPRVTGGGAM) were also used as a control (52). All tetramers were obtained from Beckman Coulter (San Diego, CA). After initial incubation, cells were washed in 2% FCS-PBS, followed by a 15-min incubation at 4°C with anti-CD8αβ-PE/FITC (Caltag Laboratories, Burlingame, CA) and either anti-CD45RO-allophycocyanin (BD Biosciences, Mountain View, CA) or anti-CD8 BV421 (BD Biosciences, San Diego, CA). Finally, cells were washed, fixed in 1% formaldehyde-PBS, and analyzed on a FACS-Calibur using CellQuest software (BD Biosciences) and FlowJo software (Tree Star, San Carlos, CA). Tetramer binding cells are expressed as a percentage of CD8^+^ lymphocytes. The intensity of tetramer staining of CTL lines over a 4-decade logarithmic plot was measured.

**Ag-specific cytokine flow cytometry assay**

Functional populations of IFN-γ-producing memory CD4^+^ and CD8^+^ lymphocytes that respond to soluble Ag were detected using a short term restimulation assay. Flow cytometry-based analysis of intracellular cytokine production of IFN-γ by CD4^+^ and CD8^+^ lymphocyte subsets in response to Ag-specific stimulation was performed using intracellular cytokine staining (ICS) to detect IFN-γ secretion. A panel of markers for analysis of PBMC. Staphylococcal enterotoxin B, HIV-1 Gag pool of 122 15-mer peptides overlapping by 11 aa, and optimal epitope peptides were used as recall Ags. Briefly, 1 × 10^6 PBMC were stimulated with 2 μg/ml of specific Ag in the presence of 1 μg/ml CD28 and CD49d costimulatory mAbs for 2 h at 37°C. The protein transport inhibitor, brefeldin A (10 μg/ml), was added, and the cells were incubated for another 4 h at 37°C. After specific Ag stimulation, PBMCs were placed at 4°C overnight. The following day cells were treated with EDTA for 15 min, followed by FACS Lysing Solution (BD Biosciences) to lyse RBCs. Simultaneous permeabilization and fixation were completed using FACS Permeabilizing Solution 2 (BD Biosciences). PBMC were aliquoted into two tubes and simultaneously stained for the following surface and intracellular markers made against fixed Ags: CD3^+^ CD62P-FITC/CD69-PE/CD4-PerCP-Cy5.5/IFN-γ-allophycocyanin, and CD3^+^ CD62P-FITC/CD4-PerCP-Cy5.5/IFN-γ-allophycocyanin. Fluorochrome and Ab combinations had been optimized for this technique and were purchased from BD Biosciences (San Jose, CA) as a customized kit. After a 30-min incubation with Ab mixtures, cells were washed once and reconstituted in a solution of 1% formaldehyde in PBS for acquisition and analysis. During the final analysis, CD3^+^ CD62P-FITC expression was used as an exclusion channel for monocytes and activated platelets. Final analysis plots of IFN-γ-allophycocyanin vs CD69-PE were gated on CD8^+^ lymphocytes. Positive responses were defined as those that were 2-fold above the background and >0.05% IFN-γ^+^CD69^+^ CD8^+^ lymphocytes.
Results

HIV-recombinant pox virus vectors can expand memory anti-SL9-specific CD8 responses

To determine whether HIV-recombinant vaccinia and canarypox vectors can stimulate anti-SL9 HLA-A*0201-restricted memory T cell responses, we tested two recombinant constructs for their capacity to expand anti-SL9 CD8+ cells obtained from HIV-infected individuals. PBMC were obtained from six long term nonprogressor HLA-A*0201+ HIV-infected subjects and were tested for the presence of circulating anti-SL9 specific CD8+ cells by tetramer analysis. Three subjects had no detectable anti-SL9 circulating CD8+ lymphocytes. In the three other subjects, the frequency of anti-SL9-specific CD8+ cells ranged between 0.13 and 1.23%. PBMCs obtained from five subjects were subsequently stimulated with the two HIV-recombinant vectors that were used either as the vaccine construct in the clinical trials (canarypox virus vCP205) or to derive stimulator cells in our previously described Ag-IVS stimulation strategy to expand CD8+ CTL in vaccinees and infected individuals (vaccinia virus vP1291) (48). PBMC from patient 3 were stimulated with the vaccinia virus vP1291, but not with the vCP205 construct. PBMCs from all six subjects were also stimulated in parallel with 10 μg/ml SL9 peptide as a positive control. After 14 days the cultures were tested for the presence and frequency of anti-SL9 reactivities by tetramer analysis. The results obtained in these experiments are reported in Fig. 1. In three of six patients, we could expand SL9-specific CD8+ cells with vP1291 (vaccinia) construct, and in two of five with vCP205 (canarypox) construct. The frequencies of the Ag-specific cells after IVS were 4- to 10-fold higher than those observed in freshly isolated PBMC. The results suggest that the vaccinia construct may be more efficient than the canarypox vector in expanding this Ag-specific effector cell population. Using the SL9 peptide, we observed concordant expansion of the effector population in three of six patients. These data confirm that SL9 determinants are functionally expressed in the context of Ag-specific stimulation/expansion of CD8+ by APCs infected with either recombinant poxvirus construct. It should be noted that during in vitro expansion of the anti-SL9-specific CD8+ effector populations, these cells become activated, and therefore, they appear larger and more granular than the original population detected in the PBMC. As shown in the bottom panel of Fig. 1A, using the back-gating procedure, these cells migrate toward the left in the side-vs-forward scatter analysis.

Detection of anti-Gag reactivity in vaccinees

Based on the results obtained using our standard Ag-specific IVS (Ag-IVS) to detect the presence of vaccine-induced, HIV-specific CD8 CTL responses, we selected 13 vaccine recipients who were HLA-A*0201 positive and had a detectable CTL response against Gag. The results obtained by stimulating fresh PBMC from these individuals with the vP1291 vaccinia recombinant construct for 14 days are reported in Table I. The cytolytic activity observed at the E:T cell ratio of 25:1 ranged between 11 and 27% of specific lysis (%SL). These %SL values were similar to those observed in HIV-infected individuals after IVS with vP1291 (mean, 22 ± 17%SL; data not shown). All these reactivities were mediated by CD8+ lymphocytes, as demonstrated by the lack of detectable activity after removal of the CD8+ population (data not shown). Subsequently, we used frozen PBMC samples obtained from eight of these subjects on the same visit date and performed an Ag-IVS using the optimal SL9 peptide. After 14 days, the cultures were tested for lytic activity against autologous BLCL coated with the peptide as target cells in the standard 51Cr release assay. The results are also presented in Table I as SL9-IVS. It is striking that despite the capacity of this IVS strategy to expand the Ag-specific effector population in infected individuals, as demonstrated in the tetramer analysis reported above, we could not detect any anti-SL9-specific CTL reactivity among the vaccinee samples.

Evaluation of anti-SL9 reactivities in vaccinees using tetramer analysis

The presence of SL9-specific CD8 responses in vaccinees was also studied using HLA A*0201 tetramers loaded with the SL9 epitopic peptide in both cryopreserved PBMC and cell cultures after Ag-IVS. Fresh PBMC samples were not available for this analysis. Based on the results obtained in HIV-infected individuals, we analyzed the cultures from eight of the vaccinees that had detectable anti-Gag CTL reactivities. The cells that were not consumed in the 51Cr release assay after Ag-IVS with vP1291 were frozen on the same day of the assay and subsequently tested in parallel with unstimulated PBMC obtained from the same visit. For comparison Ag-IVS as well as unstimulated PBMC from patient 3 were analyzed in this assay. As shown in Fig. 2 and summarized in Table I (SL9 tetramer), we could not detect anti-SL9 binding of CD8+ lymphocytes from vaccinees in either PBMC or cultures after Ag stimulation. Once again, these Ag-stimulated cultures were the same ones that had demonstrable anti-Gag CTL reactivities. The data obtained from patient 3 show that the frequency of the original SL9-specific CD8 population expanded in vitro from an initial frequency of 0.52% CD8+ cells to 5.02% after Ag-IVS.

Absence of detectable anti-SL9 reactivities by ELISPOT assay

Although we could not detect anti-SL9 CD8 responses by either standard 51Cr release assay or tetramer analysis in both unstimulated and Ag-stimulated PBMC samples from vaccinees, we used the ELISPOT assay to further test for the presence of the SL9-specific effector cells in cryopreserved PBMC from these vaccine recipients. To increase the sensitivity of this assay we enriched the samples for CD8+ lymphocytes by removing the CD4+ population using magnetic beads. We also used SL9-coated and irradiated C1RA2 cells as stimulator cells to replace the APCs lost during the freezing/thawing procedures. Using this technique in infected individuals, we observed a 3- to 5-fold increase in the frequency of detectable anti-SL9 responses (data not shown). In this set of experiments, PBMC obtained from patient 3 were run as controls in each assay. The data obtained from the experiments using the vaccinee samples are reported in Table I, the frequencies are expressed as spot-forming cells per 10⁶ CD4+ cells. None of the 13 samples from vaccinees had any detectable anti-SL9 response, whereas an average of 4560 spot-forming cells/10⁶ CD4+ cells were detected in patient 3. The results of these experiments are in agreement with what was previously detected by standard CTL assay and tetramer analysis.

Recognition of non-SL9 HLA-A*0201 determinants in vaccinees

To determine whether the anti-Gag responses in these vaccinees were directed against previously described epitopes, we analyzed cryopreserved PBMC using the cytokine flow cytometry assay. In only two volunteers, FS5 and OT7, were we able to detect responses that were significantly above the negative control (anti-CD28/CD49d). Due to limited sample availability, we did not have an opportunity to attempt fine epitope mapping from Ag-IVS cultures. For volunteer FS5, the anti-Gag response was detected using the pool of peptides based on optimal CTL epitopes previously characterized in infected individuals, but not using the pool of 122 15-mer peptides representing the full Gag sequence. These data combined with the results of other assays for this individual
suggest that the immune response was directed against one or more of the previously described epitopes, but not against the SL9 epitope. Limitations in the availability of cell samples from this visit precluded formal epitope mapping of the responses for this individual. In volunteer 0T7, the response was directed against the previously described Gag p24263–272 (KK10) epitope that is recognized in the context of the HLA-B*2705 allele (Fig. 3). The frequency observed using the optimal peptide KK10 was of the

FIGURE 1. Frequency of anti-SL9-specific CD8 lymphocytes before and after Ag-IVS stimulations. For patients 1 (A) and 2 (B) the presence of anti-SL9-specific CD8 lymphocytes was analyzed in PBMC as well as in cultured cells after IVS with vCP205 (construct used in the vaccine trial), vP1291 (construct used to test anti-HIV responses in vaccinees with our standard Ag-IVS), and the p17 (aa 77–85) SLYNTVATL peptide (SL9). For patient 3 (C) PBMC, v1291-IVS, and SL9-IVS were analyzed. Results are reported as the percentage of CD8+ A*0201-SL9 tetramer-binding cells. Results are considered positive when they are 2-fold above the percentage of CD8+ A*0201-Tax tetramer-binding cells used as the control. The percentages of double-positive cells are also indicated. The bottom panels of A show the distribution of CD8+ A*0201-SL9 tetramer-binding cells within the total lymphocyte population using back-gating analysis. These data are representative of the findings observed in all three individuals.
same magnitude as that obtained using the pool of 122 15-mer Gag peptides. These data confirm our earlier observations using ELISPOT analysis, in that the predominant anti-Gag response in this vaccinee was directed against the HLA-B*2705-restricted KK10 peptide (41).

Discussion

In this manuscript we report that the p17 epitope SL9, immunodominant in HLA-A*0201-positive, HIV-1-infected patients, is not recognized by A*0201-positive, uninfected recipients of recombinant canarypox/HIV vaccines who have demonstrable anti-Gag CTL reactivities. This is, to our knowledge, the first such report of differences in the pattern of Ag recognition by infected patients vs uninfected vaccine recipients. As such, these findings could impact on future vaccine strategies based on using epitopes immunodominant in the context of chronic HIV-1 infection.

The SL9 epitope is functionally expressed in cells infected with HIV-recombinant vaccinia and canarypox constructs, as demonstrated by their capacity to expand SL9-specific CD8 precursors during 14-day Ag-IVS. The potency of the vaccinia constructs appears to be higher than that observed with canarypox constructs, in agreement with previous studies in which the stimulatory capacities of these two constructs were compared (53, 54).

All the vaccinees were selected for study based on detectable anti-Gag CD8 CTL responses using the Ag-IVS. As such, the lack of an anti-SL9 response in these vaccinees, as measured by a number of assays, including Ag-IVS, was not due to the lack of anti-Gag responses. In this study we were only able to perform epitope mapping on CD8 T cells in two of the original 13 subjects for two reasons. First, the expansion of cells that occurs in the Ag-IVS assay does not occur in the overnight ELISPOT or 6-h ICS

<table>
<thead>
<tr>
<th>Table 1. Anti-SL9 responses in vaccinees</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Anti-Gag CTL&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Vaccinee</td>
</tr>
<tr>
<td>FRC</td>
</tr>
<tr>
<td>FR6</td>
</tr>
<tr>
<td>J1K</td>
</tr>
<tr>
<td>12H</td>
</tr>
<tr>
<td>FRH</td>
</tr>
<tr>
<td>FRN</td>
</tr>
<tr>
<td>FRX</td>
</tr>
<tr>
<td>FS5</td>
</tr>
<tr>
<td>0T7</td>
</tr>
<tr>
<td>EPD</td>
</tr>
<tr>
<td>HY8</td>
</tr>
<tr>
<td>FWN</td>
</tr>
<tr>
<td>ILZ</td>
</tr>
<tr>
<td>Seropositive</td>
</tr>
<tr>
<td>Patient 3</td>
</tr>
</tbody>
</table>

<sup>a</sup> %SL at an E:T cell ratio of 25:1.

<sup>b</sup> Percentage of bright CD8<sup>+</sup> lymphocytes. NEG, %CD8<sup>+</sup>/tetramer<sup>+</sup> <2-fold background.

<sup>c</sup> Not tested.

<sup>d</sup> Mean ± SD.

<sup>e</sup> Spot-forming cells per 10<sup>6</sup> CD4<sup>+</sup> lymphocytes above background.

FIGURE 2. Frequency of SL9-specific CD8 lymphocytes in PBMC. Percentages of CD8<sup>+</sup>/tetramer<sup>+</sup> lymphocytes in PBMC from patient 3 and a noninfected HIV-1 vaccine recipient are shown in A and B, respectively. Left panels, Negative controls; center and right panels, percentages of CD8<sup>+</sup>/A*0201-SL9 tetramer<sup>+</sup> and CD8<sup>+</sup>/B*0701-CMV tetramer<sup>+</sup> lymphocytes, respectively. The percentage of double-positive cells is reported above each panel.
assays. Secondly, limitations in cell numbers prevented us from mapping CD8\(^+\) T cell epitopes using either PBMC or cells derived from the Ag-IVS assay. We recognize that this may be a limitation of the present findings together with the absence of more precise quantification of the anti-Gag reactivities in these vaccines. Based on our ability to detect CD8 responses in PBMC from recombinant HIV-1/canarypox-based vaccine recipients as reported in this study for patient 0T7, we may conclude that this generation of vaccines induces HIV-specific responses that are only 1/10th those observed in infected individuals. This is surprising compared with responses against the CMV pp65 induced by these constructs (55). Even in presence of a high level of virus replication, such as that found during the acute phase of infection, anti-SL9 CD8\(^+\) T cells are not detectable (30, 56), suggesting that levels of Ag expression alone may not be the limiting factor to induce anti-SL9-specific CD8 responses.

Epitope immunodominance is a multifactorial process influenced by a number of parameters (57), including 1) the quantities of peptide-class I complexes expressed on APCs, 2) the differential affinities of the TCR repertoire of the CD8 populations within an individual, and 3) the cross-regulatory events that occur between CD8 cells responding to immunodominant determinants and CD8 cells responding to subdominant determinants. The quantities of peptide-class I complexes are also regulated by the binding affinity of the peptide for the HLA class I molecule. In the case of the SL9 peptide, it has been reported that its binding affinity for the HLA-A*0201 molecule is relatively low compared with that of other HLA-A*0201-restricted epitopes (58). However, in expression models the SL9 peptide is expressed 33-fold more than the subdominant Pol IV9 epitope (aa sequence 476–484) (33). As far as the TCR repertoire of CD8 lymphocytes involved in the anti-SL9 response in infected patients is concerned, it appears that this response may be polyclonal in nature, involving several different TCR \(\gamma\beta\) families, each with a possibly different affinity for the MHC-peptide complex (59). How all these factors contribute to the absence of anti-SL9 immune reactivities among HIV-uninfected vaccine recipients is still under investigation.

It has been reported that there is a shift in the recognition pattern of immunodominant CTL epitopes between the acute and the chronic phase in both animal and human viral infection models. In the case of SIV and hepatitis C virus infection, changes in the pattern of immunodominance correlated with the appearance of mutations in the epitopes that determined the emergence of escape mutant virus (3, 14, 42, 60–63). In the case of EBV infection, a switch from the recognition of lytic Ags during acute infection to latent Ags during the chronic phase is dependent upon the differential expression of EBV Ags (64). During the course of HIV infection it has been shown that HLA restriction and the magnitude of anti-HIV CTL responses differed significantly in the acute vs the chronic phase of the disease (30). Anti-SL9 responses do not appear during the acute phase of HIV infection and take up to 5 mo before becoming detectable (56). This absence of detectable SL9-specific CD8 lymphocyte responses during acute infection parallels our findings in vaccine recipients. In the case of our vaccinees, we can exclude factors that could account for these findings in infected individuals, such as competition by Tat, Rev, and Nef Ags not included in the vaccine and CTL escape. Because we did not detect anti-Env CTL responses in these individuals, we can exclude the possibility that recognition of other Env epitopes may have a better binding affinity for HLA-A*0201. However, there is still the possibility that poxvirus epitopes expressed in canarypox vaccine recipients might have a higher affinity for HLA-A*0201 than epitopes derived from the Gag insert. In this possible scenario, vector-related epitopes may cause the SL9 epitope to become a subdominant epitope in uninfected vaccine recipients. Lastly, the pattern of immunodominance may be linked to the relative immunogenicity of the candidate vaccine. We do not know, for example, whether the absence of SL9 cellular reactivity in HIV-1/canarypox vaccine recipients will be recapitulated with a more potent cellular vaccine immunogen.

Another explanation for the delay in the appearance of detectable anti-SL9 responses may reside in the subdominance of HLA-A*0201-restricted responses to other HLA allele-restricted responses, such as HLA-A3- and -B27-restricted responses, during acute HIV infection (30, 65). It is interesting that studies conducted in vaccinees suggest that HLA-B57\(^+\) individuals as well as HLA-B27\(^+\) volunteers can generate anti-HIV CD8-mediated responses more frequently than individuals expressing other HLA alleles (66). In this small cohort of vaccinees, we observed that one vaccinee who was HLA-A*0201 and -B*2705 positive (volunteer 0T7) mounted a dominant response against the HLA-B*2705-restricted Gag p24 epitope KK10 (aa position 263–272), but not against the SL9 epitope. Once again, these data suggest that the HLA-A*0201-restricted response may not be immunodominant in vaccinees in the manner observed in HIV-infected individuals.

Although CD8\(^+\) CTL responses during the acute phase of HIV infection may control early virus replication and determine the set-point of viremia, these responses can also readily induce escape mutant viruses, as observed in human and animal studies (13, 24, 60), and thus fail to control virus replication during chronic infection. If this scenario is true, vaccine strategies should aim to elicit CTL responses that target immunodominant conserved epitopes recognized during both acute and chronic phases of infection that are less likely to mutate. Only large efficacy trials testing vaccine strategies that induce optimal levels of CTL responses will enable...
identification of the relevant immunodominant and potentially protective epitopes.

Acknowledgments
We thank all the HIV-infected patients, the uninfected participants enrolled in the AIDS Vaccine Evaluation Group/HIV Vaccine Trials Network phase 1 vaccine trials, and the HIV Vaccine Trials Network investigators and clinical personnel who made this study possible.

References

ization of macaques with a DNA prime/modified vaccinia virus Ankara boost regimen induces broad simian immunodeficiency virus (SIV)-specific T-cell responses and reduces initial viral replication but does not prevent disease progression following challenge with pathogenic SIVmac239. J. Virol. 76:7167.


