Conserved HIV-1 Epitopes Continuously Elicit Subdominant Cytotoxic T-Lymphocyte Responses

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Background. The epitope specificities and antiviral activities of class I HLA-restricted CD8+ T cells, especially those induced during human immunodeficiency virus type 1 (HIV-1) primary infection, are important considerations in designing HIV-1 vaccines. Conserved epitopes may be more commonly and persistently recognized than variable epitopes, as they may be more likely to be present in infecting viruses. However, some studies have shown preferential or similar targeting of variable versus conserved epitopes during primary infection.

Methods. We analyzed cytotoxic T-lymphocyte (CTL) responses toward predefined conserved and variable epitopes in 45 subjects during primary (n = 34) and/or chronic infection (n = 16).

Results. Conserved and variable CTL epitopes were recognized with similar probabilities, whereas conserved epitopes generally elicited subdominant responses during both primary and chronic infection. During primary infection, CTL responses against Gag versus responses against Env and variable epitopes tended to be associated with lower and higher viral loads, respectively. During chronic infection, Env-specific responses tended to be associated with lower CD4+ cell counts.

Conclusions. Subdominant CTL recognition of conserved HIV-1 epitopes commonly occurs from the primary through chronic stages of HIV-1 infection. These findings underscore the challenge in designing T cell–based vaccines that can induce immunodominant CTL responses to conserved HIV-1 regions.

Extensive viral genetic variability found among and within individuals is a major obstacle to the development of effective vaccines against human immunodeficiency virus type 1 (HIV-1). Although cytotoxic T-lymphocyte (CTL) responses [1–3] are critically involved in immune defense against HIV-1 infection, they are unable to eradicate the virus. Selective escape from CTL responses has been shown to be a major driving force of HIV-1 evolution [4–6]. However, escape mutations could be associated with replication fitness costs that could therefore limit evolution in conserved CTL epitopes [7–13].

Indeed, HIV-1 control appears to be associated in some instances with CTL escape mutations in highly conserved regions of the virus [14]. This has led to HIV vaccine development efforts aimed at eliciting CTL responses exclusively toward highly conserved regions [15, 16].

Because epitopes in conserved regions of the HIV-1 proteome are more likely to be present in infecting viruses and could thus be less likely to undergo escape mutations, one might expect these epitopes to be more commonly and persistently recognized by CTL responses. Whole-proteome mapping of CD8+ CTL responses demonstrated that conserved 15- to 20-mer peptides were targeted more frequently than were variable peptides [17]. Frequent targeting of antigens at conserved regions of Gag and Nef was also found in subtype C–infected Indian subjects [18]. These observations are consistent with a study of CTL responses to viral protease and integrase [19] and an in silico analysis of experimentally defined HIV CTL epitopes listed in the Los Alamos HIV Sequence and Immunology Database [20]; both studies found CTL epitopes concentrated in relatively conserved regions. In con-
Table 1. Study Subjects and Cytotoxic T-Lymphocyte (CTL) Responses to Conserved and Variable Epitopes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Infection</th>
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<tbody>
<tr>
<td></td>
<td>Primary</td>
<td>Chronic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 34)</td>
<td>(n = 16)</td>
<td></td>
</tr>
<tr>
<td>Median (range) days post onset of acute symptomsa</td>
<td>27 (3–91)</td>
<td>357 (109–1182)</td>
<td></td>
</tr>
<tr>
<td>Viral load, median log_{10} copies/mL (range)</td>
<td>4.98 (2.88–6.92)</td>
<td>4.36 (&lt;1.7 to 5.57)</td>
<td></td>
</tr>
<tr>
<td>CD4 T cell count, median cells/μL (range)</td>
<td>684 (241–1090)</td>
<td>574 (130–1325)</td>
<td></td>
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<tr>
<td>Epitopesb</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fraction of subjects with CTL responses</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conserved</td>
<td>0.5</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Variable</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Median number of epitopes recognized (range)</td>
<td>1 (0–3)</td>
<td>1 (0–2)</td>
<td></td>
</tr>
<tr>
<td>Variable</td>
<td>2 (1–8)</td>
<td>3 (1–9)</td>
<td></td>
</tr>
<tr>
<td>Probability of recognition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conserved</td>
<td>0.145</td>
<td>0.143</td>
<td></td>
</tr>
<tr>
<td>Variable</td>
<td>0.173</td>
<td>0.239</td>
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**NOTE.** Primary and chronic infection were defined as occurring before or after 91 days after onset of acute symptoms of viral infection, respectively. Symptoms usually occurred within ~2 weeks after exposure to human immunodeficiency virus (HIV) in this cohort (J.I. Mullins, data not shown).

* Peripheral blood mononuclear cell specimens from these time points were derived and examined for CTL responses.

Conserved epitopes were defined as belonging to the upper quartile of the tested epitopes in terms of database frequency (conserved in >78.68% of the sequences of HIV-1 subtype B in Las Alamos HIV Sequence Database for 2005 [30]), whereas epitopes in the lower 3 quartiles were considered to be variable epitopes.

To compare the prevalence and immunodominance of CTL responses to conserved and variable epitopes, we analyzed CTL responses in 45 HIV-1 subtype B–infected male subjects during primary and/or chronic infection. We found that conserved epitopes were recognized by CTL with probability similar to variable epitopes and generally elicited subdominant responses during both primary and chronic infection.

**MATERIALS AND METHODS**

**Study subjects.** We measured CTL responses in 50 HIV-1–infected male subjects living in Washington state. These subjects were enrolled in a longitudinal study of HIV infection in the University of Washington Primary Infection Clinic. At the time of enrollment, all subjects were either HIV antibody–negative or HIV antibody–positive, which was determined with a negative or indeterminate Western blot test result, a negative “detuned” antibody test result, or a negative HIV test result within 365 days prior to screening [25]. Clinical information about these subjects is summarized in Table 1. The cutoff for assignment of primary infection in this study was set at 91 days after onset of acute symptoms of HIV-1 infection (such symptoms occurred, on average, within 2 weeks following HIV-1 exposure in this cohort; J.I.M., unpublished data), or ~105 days after exposure (corresponding to Fiebig stages V or VI of primary infection [26]), and samples obtained before or at this time were classified as being obtained during primary infection. Samples from 39 subjects were obtained during primary infection. Samples from 16 subjects were obtained during chronic infection. In 5 subjects, samples were obtained during both primary and chronic infection. In 9 subjects, samples were obtained at 2 time points during chronic infection. All subjects provided written consent, and the University of Washington Human Subjects Committees approved this study.

CTL responses were measured by interferon γ (IFN-γ) ELISpot assays using predefined optimal epitopes [27, 28] (Table 2). All subjects’ HLA class I alleles were determined by sequence-specific primer molecular typing [29]. IFN-γ ELISpot assays were performed as previously described [27]. CTL re-
sponses in 21 subjects were examined during primary infection in a previous study by Cao et al [27], with a set of class I HLA-restricted HIV-1 epitopic peptides that were used in a study by Goulder et al [28] that matched the subjects’ HLA alleles. In addition, 460 overlapping 15- or 20-mer peptides spanning the entire HIV-1 proteome, derived from HXB2, MN, or subtype B consensus sequences, were examined for CTL responses in these 21 subjects, and 15 previously unreported epitopes were defined and HLA restriction determined [27]. CTL responses to these 15 newly defined epitopes were also assessed in all 21 subjects. CTL responses were measured in an additional 29 subjects, with defined epitopic peptides used by Goulder et al [28] and Cao et al [27] that matched the subjects’ HLA alleles. CTL responses were measured before combination antiretroviral therapy (ART) with 2 exceptions: responses in 1 subject were measured during failed ART (71 and 148 days after the start of ART) with viral loads consistently >50,000 copies/mL and in another subject 149 days after the termination of 11 months of ART.

**Determination of epitope conservation and probability of recognition.** For each epitope, the frequency of occurrence in HIV-1 subtype B sequences in the Los Alamos HIV Sequence Database for 2005 [30] was calculated. The database frequency of an epitope represents the conservation of the entire epitope in the known HIV-1 subtype B population. Conserved epitopes were arbitrarily defined as those belonging to the upper quartile of tested epitopes in terms of database frequency (conserved in ≥78.68% of the subtype B sequences), whereas epitopes in the lower 3 quartiles were considered variable epitopes. The probability of recognition of an epitope was taken as the number of subjects that recognized the epitope (x) divided by the number of subjects in whom the epitope was tested (x). Therefore, the probability of recognition of conserved or variable epitopes is \( \Sigma x / \Sigma x_r \), where \( n \) is the number of conserved or variable epitopes tested.

**Statistical analysis.** Statistical analyses were conducted using GraphPad Prism software (version 4; GraphPad Software). Categorical data were compared using the Fisher exact test. Paired continuous variables were compared using the Wilcoxon signed rank test. Correlations were tested using the Spearman rank coefficient. For all tests, \( P < .05 \) was considered significant.

Chronic CTL responses were measured at a single time point in 7 subjects and at 2 time points in the other 9 subjects. Therefore, during chronic infection, 2 parallel analyses were conducted unless specifically noted: analysis 1 used data from the former 7 subjects and the data of the first time points from the latter 9 subjects, whereas analysis 2 used data from the former 7 subjects and the data of the second time points from the latter 9 subjects. If results from both analyses were similar, only the result from analysis 1 is reported. Otherwise, results from both analyses are reported.

**RESULTS**

**One-half of the epitopes tested were recognized by CTL.** ELISpot assays were performed using 120 predefined optimal epitopes with samples obtained during primary infection, and 84 of those peptides were also tested in samples obtained during chronic infection (Figure 1). The 120 peptides corresponded to 90 (75%) variable epitopes restricted by 31 different class I HLA alleles and 30 (25%) conserved epitopes restricted by 20 different class I HLA alleles (Figure 1; Table 2). Of the 120 epitopes, 86 corresponded to the subtype B consensus sequence reported in 2005 [30].

Three of the 21 subjects tested in the Cao et al study [27] and 2 of the 29 subjects tested in our study did not show responses and were excluded from our analyses. CTL responses were measured only during primary infection in these 5 subjects. For the remaining 45 subjects, CTL responses were detected at all time points examined. Therefore, CTL responses were analyzed in 34 subjects during primary infection and 16 subjects during chronic infection. All subjects analyzed had at least 1 HLA allele that restricted at least 1 conserved epitope and 1 HLA allele that restricted at least 1 variable epitope. Epitopes recognized during the primary and chronic stages of infections are summarized in Figure 1. Approximately one-half of the epitopes tested were recognized, 66 (55%) in primary and 40 (48%) in chronic infection. During primary infection, the largest numbers of epitopes recognized were found in Env. During chronic infection, however, more Nef and Gag epitopes were recognized than Env epitopes.

**Similar probability of recognition for conserved and variable epitopes.** Although all subjects analyzed had at least 1 HLA allele that restricted conserved epitopes, only one-half (not including the 2 with ART) recognized at least 1 conserved epitope, whereas all recognized at least 1 variable epitope during both the primary and chronic stages of infection (Table 1). Nonetheless, the proportions of the tested epitopes that were recognized during both primary and chronic infection were not significantly different between conserved and variable epitopes (Figure 1). During primary infection, 43% of the conserved and 59% of the variable epitopes tested were recognized (\( P = .15 \); Fisher exact test), and during chronic infection, 33% of the conserved and 52% of the variable epitopes tested were recognized (\( P = .21 \); for the 9 subjects with 2 time point measurements, epitopes recognized at 1 or both time points were counted). Because epitopes were tested in different number of subjects, we then measured the probabilities of recognition and

<table>
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<th>Table 2. Epitopes Tested in This Study</th>
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<td>This table is available in its entirety in the online version of the Journal of Infectious Diseases.</td>
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found that they were similar for both conserved and variable epitopes during primary (\(P = .46\), Fisher exact test; Table 1) and chronic infection (\(P = .12\)).

A subset of CTL epitopes are consistently reported to be targeted during early HIV-1 infection [24, 31]. We next examined whether a similar subset of epitopes were recognized in our 45 subjects and the level of conservation of these commonly recognized epitopes. Epitopes were considered to be commonly recognized if they were tested in \(\geq 5\) subjects and were recognized in more than half of the tested subjects. Eight epitopes fit this criteria (Figure 2; Table 2), with only the B*3501-restricted VPLRPMTY being conserved. VPLRPMTY and the A3-restricted RLRPGGKKK epitope were also commonly targeted during early infection in previous studies [24, 31]. Another commonly reported early epitope, the B*7-restricted IPRRIRQGL, was commonly detected in only the chronic stage of infection in our study.

**Duration of CTL recognition of conserved and variable epitopes.** Of the 5 subjects with CTL responses measured during both the primary and chronic stages of infection, 3 recognized a total of 5 epitopes (2 variable and 3 conserved) only during primary infection, whereas in the remaining 2 subjects, all epitopes were recognized during both primary and chronic infection. Again, of these 5 subjects, 3 recognized a total of 6 epitopes (5 variable and 1 conserved) only during chronic infection. Of the 9 subjects with CTL responses measured at 2 time points during chronic infection, 5 recognized a total of 9 epitopes (8 variable and 1 conserved) only at the first time points. In the remaining 4 subjects, all epitopes were recognized at both time points. One variable epitope was recognized only at the second time point in 1 subject. In total, responses to 4 (20%) of 20 conserved epitopes and 10 (24%) of 42 variable epitopes waned over time (\(P > .99\); Fisher exact test). New responses were detected at second time points in a total of 4 subjects and 7 epitopes (11% of all epitopes recognized in these 14 subjects).

**Subdominant CTL responses to conserved epitopes.** We next examined the relationship between epitope conservation and within-subject–dominant CTL responses. We defined the CTL response of the highest magnitude in a subject as the dominant response and all others as subdominant. During primary infection, dominant epitopes were located in Gag, Vpr, Tat, Env, and Nef in 10, 3, 7, 7, and 7 subjects, respectively. Fifty percent of subjects (17 of 34) recognized conserved epitopes during primary infection, whereas in the remaining 2 subjects, all epitopes were recognized during both primary and chronic infection. Again, of these 5 subjects, 3 recognized a total of 6 epitopes (5 variable and 1 conserved) only during chronic infection. Of the 9 subjects with CTL responses measured at 2 time points during chronic infection, 5 recognized a total of 9 epitopes (8 variable and 1 conserved) only at the first time points. In the remaining 4 subjects, all epitopes were recognized at both time points. One variable epitope was recognized only at the second time point in 1 subject. In total, responses to 4 (20%) of 20 conserved epitopes and 10 (24%) of 42 variable epitopes waned over time (\(P > .99\); Fisher exact test). New responses were detected at second time points in a total of 4 subjects and 7 epitopes (11% of all epitopes recognized in these 14 subjects).
Figure 2. Epitopes commonly recognized during primary (A) and chronic (B) infection. Only those epitopes that were tested in >5 subjects and were recognized in more than one-half of the tested subjects were evaluated. Black bars represent the number of subjects tested, and gray bars represent the number of subjects with an immunodominant response to the epitope. White bars represent the number of subjects with an immunodominant response to the epitope. The right panel lists the commonly recognized epitopes and their database frequencies. For HLA B7, the 4 epitopes listed were recognized by the same number of subjects, and the epitope found to elicit immunodominant cytotoxic T lymphocyte responses is labeled with an asterisk. Thus, only single gray and white bars are presented for B7.

topes, yet these corresponded to the dominant responses in only 12% (n = 4; the B27-restricted KRWIILGLNK in 2 subjects and the B*3501-restricted VPLRPMTY in the other 2). During chronic infection, dominant epitopes were located in Gag, Pol, Tat, Env, and Nef in 4, 3, 1, 4, and 4 subjects, respectively (using the data of the first time points if subjects had 2 CTL measurements during chronic infection). Fifty percent of subjects (8 of 16) recognized conserved epitopes; however, they were dominant in only 25% (n = 4; the B27-restricted KRWIILGLNK and the B51-restricted EKEGKISKI in 1 each, and the A26-restricted EVIPMFSAL in 2 others). Therefore, for both primary and chronic infection, dominant responses within a subject were mostly elicited by variable epitopes. Notably, only 2 subjects examined during primary infection and another subject examined during chronic infection had the protective HLA-B27 allele, and all 3 dominantly recognized the conserved B27-restricted epitope KRWIILGLNK. In addition, the 3 epitopes commonly recognized during primary infection in this study were also the dominant epitopes in 40%–88% of the subjects who recognized them (Figure 2).

We next compared the magnitudes of CTL responses with conserved and variable epitopes. During primary infection, the within-subject maximum (Figure 3A) and median (Figure 3C) magnitudes of CTL responses were both significantly lower to conserved epitopes compared with variable epitopes (P < .001, Wilcoxon signed rank test). During chronic infection, the within-subject maximum magnitudes of CTL responses were significantly lower for conserved epitopes than for variable epitopes (P = .015; Figure 3B), whereas median magnitudes were not significantly different (P = .15; Figure 3D). For subjects whose CTL recognized both conserved and variable epitopes, the maximum magnitudes of responses were also significantly lower during primary infection for conserved epitopes than for variable epitopes (P = .01).

Correlations between CTL responses and viral load and CD4+ T cell counts. Finally, we examined the relationship of each of the following factors to viral load and CD4+ T cell counts: the number of recognized epitopes across the viral proteome; the number of recognized conserved and variable epitopes; the fraction of epitopes recognized (total, conserved, and variable), taking the number of tested epitopes into account; and the total and mean magnitude of CTL responses against all tested epitopes, as well as conserved and variable epitopes. As shown in Table 3, during primary infection, an increased
Figure 3. Maximum and median magnitudes of cytotoxic T-lymphocyte (CTL) responses to conserved and variable human immunodeficiency virus type 1 (HIV-1) epitopes within subjects. A, B) Comparison of the maximum magnitudes. C, D) Comparison of the median magnitudes of CTL responses to conserved and variable epitopes during primary and chronic infection. Each line represents results from an individual subject. Red highlighting indicates when the within-subject dominant CTL responses were elicited by conserved epitopes. The horizontal bars represent the mean value for each data set. Only data from the first time point are presented if chronic CTL responses were measured at 2 time points. In 1 subject, the dominant CTL response elicited by a conserved epitope (the A26-restricted EVIPMFSAL) was observed at the second time point measurement, and is thus not presented in the figure. PBMC, peripheral blood mononuclear cell; SFC, spot-forming cells.

DISCUSSION

We analyzed the CTL responses of 45 subjects during the primary and/or chronic stages of HIV-1 infection by using peptides corresponding to known epitopes for each subject as a function of their restricting class I HLA. We found that conserved epitopes were recognized with a probability similar to that for variable epitopes; however, the epitopes that were commonly recognized in subjects that shared the restricting HLA alleles were predominantly variable. Within a subject, CTL responses to conserved epitopes were generally subdominant, especially during primary infection. In addition, we found that an increased number of recognized Gag epitopes, decreased number of recognized Env epitopes, and decreased magnitude of CTL responses to Env and variable epitopes tended to be correlated with lower viral loads during primary infection. Conversely, an increased number of recognized Env epitopes during chronic infection tended to be correlated with lower CD4+ T cell counts. One limitation of our study is that we examined CTL re-
Chronic infection, CD4+ T cell counts and viral load. The latter 9 subjects. Results from both analyses were generally similar. Hence, data from the former 7 subjects and the data of the second time points from the data of the first time points from the latter 9 subjects, and analysis 2 used were conducted. Analysis 1 used data from the former 7 subjects and the time point in 7 subjects and at 2 time points in 9 subjects. Two parallel analyses were conducted. Analysis 1 used data from the former 7 subjects and the data of the first time points from the latter 9 subjects, and analysis 2 used data from the former 7 subjects and the data of the second time points from the latter 9 subjects. Results from both analyses were generally similar. Hence, only the results from analysis 1 are reported, unless specifically noted. VL, viral load.

Table 3. Significant Correlations of Cytotoxic T-lymphocyte (CTL) Responses with Viral Loads and CD4+ T Cell Counts

<table>
<thead>
<tr>
<th>CTL response</th>
<th>Spearman r</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>Primary infection, log10 VL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of epitopes recognized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gag</td>
<td>0.339</td>
<td>.05</td>
</tr>
<tr>
<td>Env</td>
<td>0.3786</td>
<td>.03</td>
</tr>
<tr>
<td>Total magnitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Env</td>
<td>0.438</td>
<td>.01</td>
</tr>
<tr>
<td>Variable</td>
<td>0.401</td>
<td>.02</td>
</tr>
<tr>
<td>Mean magnitude</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variable</td>
<td>0.3412</td>
<td>.048</td>
</tr>
<tr>
<td>Chronic infection, CD4+ T cell counts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of epitopes recognized</td>
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<td></td>
</tr>
<tr>
<td>Env</td>
<td>0.5398</td>
<td>.03</td>
</tr>
<tr>
<td>Gag</td>
<td>a</td>
<td>0.4976</td>
</tr>
<tr>
<td>Env</td>
<td>a</td>
<td>0.513</td>
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</table>

NOTE. The correlations were determined by the Spearman rank coefficient. Chronic stage CTL responses were measured in 16 subjects: at a single time point in 7 subjects and at 2 time points in 9 subjects. Two parallel analyses were conducted. Analysis 1 used data from the former 7 subjects and the data of the first time points from the latter 9 subjects, and analysis 2 used data from the former 7 subjects and the data of the second time points from the latter 9 subjects. Results from both analyses were generally similar. Hence, only the results from analysis 1 are reported, unless specifically noted. VL, viral load.

a Result from analysis 2. Data from the second time points were used for subjects with chronic CTL responses measured twice.

Responses using only predefined optimal epitopes, most of which are derived from consensus sequences. Our assays likely underestimate HIV-1-specific CTL responses to nonconsensus, or variable epitopes, because we do not know whether the viruses that infected the subjects contained the tested epitopes. Ideally, the testing of CTL responses to highly variable regions should include specific autologous peptides. Without testing autologous peptides, the rate of missed variable epitopes can be as high as one-third of the total epitopes recognized, as we have previously reported [6, 11]. However, we would expect to have observed a greater bias toward recognition of variable epitopes (or more profound dominant responses to variable epitopes) if a more comprehensive epitope panel were assessed.

All commonly recognized epitopes that we found during primary infection were also the immunodominant epitopes in multiple subjects. These results are in agreement with previous studies that showed that the magnitudes of CTL responses positively correlated with their frequencies of recognition in the population [32] and that the CTL responses against the most frequently targeted epitopes tended to be of the highest magnitude [24]. Similarly, these prior studies also examined only predefined epitopes and thus underestimated overall CTL responses.

It is not clear why variable epitopes tend to be dominant. Our results suggest that the conservation level of an epitope plays a minor role in determining the frequency of recognition in the HIV-1–infected population and the immunodominance within a subject. Factors, including the kinetics of viral protein expression [33], antigen processing and presentation [34–36], and interactions between T cell receptors and epitope-HLA complexes [37], might have contributed to the dominant recognition of variable epitopes. Of the subjects examined during primary infection, 41% showed immunodominant CTL responses toward Tat or Nef proteins. As previously hypothesized [38], early expression of Tat and Nef during viral replication may have accelerated presentation of Tat and Nef epitopes to CD8+ T cells and thus might be associated with preferential immunodominant CTL responses toward these variable proteins during primary infection. The highly conserved Pol protein is expressed at a relatively low level in HIV-1 infection [39], which might also contribute to the less frequent immunodominant targeting of conserved epitopes. In our testing epitope panel, 7 of the 8 epitopes that can be presented by >1 HLA alleles are variable. Perhaps variable epitopes are more likely to be presented by multiple HLA alleles and are thus, in sum, more likely to be dominant. It is also possible that variable epitopes might have higher affinity to HLA alleles or higher avidity to CTL. Or perhaps responses to conserved epitopes are partially suppressed by regulatory factors reacting to chronic CTL activity. All these hypotheses should be confirmed by additional detailed studies.

Although early dominant CTL responses tend to exact strong selective pressure [6], our results lead us to hypothesize that the relatively low conservation of epitopes dominantly recognized during early infection may allow the rapid accumulation of escape mutants; therefore, CTL responses to these early epitopes might contribute little to the durable control of HIV-1 infection after initial curtailment of viremia. In addition, our observation that conserved epitopes generally elicited subdominant CTL responses could provide some explanation of previous findings that showed subdominant CD8+ T cell responses to be involved in the control of simian immunodeficiency virus and HIV replication in both monkey [40] and human [41] studies during chronic infection.

Previous studies of chronic HIV-1 infection—including in subtype C–infected heterosexual population from South Africa [42] and subtype B–infected populations from Peru and the United States [43]—have shown that CTL responses to Gag

Table 4. Correlation of Cytotoxic T-lymphocyte (CTL) Responses with Viral Load and CD4+ T Cell Counts

This table is available in its entirety in the online version of the *Journal of Infectious Diseases*.
are associated with viral control, whereas responses to Env are associated with higher levels of viremia. Our study of HIV-1 subtype B–infected homosexual men from the United States further suggests the beneficial effect of CTL responses to Gag and deleterious effect of responses to Env and variable epitopes during primary infection.

Although it has recently been proposed that it could be desirable for an HIV vaccine to have immunodominant CTL responses toward conserved regions of HIV-1 [15, 16], most vaccine strategies have thus far sought to replicate and build on patterns of responses seen in natural infection. However, our results suggest that the immunogenicity of an effective HIV vaccine should not mimic most natural infections, unless it mimics responses induced in those subjects who exhibit exquisite control of viral replication over many years of infection. Overcoming natural immunodominance and direct immunodominant CTL responses toward conserved regions of HIV-1 will be an essential challenge to overcome in the design of an HIV vaccine [15].

Acknowledgments

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References