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Association between Virus-Specific T-Cell Responses and Plasma Viral Load in Human Immunodeficiency Virus Type 1 Subtype C Infection

V. Novitsky, P. Gilbert, T. Peter, M. F. McLane, S. Gaolekwe, N. Rybak, I. Thior, T. Ndung’u, R. Marlink, T. H. Lee, and M. Essex

Harvard School of Public Health, Boston, Massachusetts; University of Washington, Seattle, Washington; and Botswana-Harvard Partnership for HIV Research and Education and National Health Laboratory/National Blood Transfusion Center, Gaborone, Botswana

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Virus-specific T-cell immune responses are important in restraint of human immunodeficiency virus type 1 (HIV-1) replication and control of disease. Plasma viral load is a key determinant of disease progression and infectiousness in HIV infection. Although HIV-1 subtype C (HIV-1C) is the predominant virus in the AIDS epidemic worldwide, the relationship between HIV-1C-specific T-cell immune responses and plasma viral load has not been elucidated. In the present study we address (i) the association between the level of plasma viral load and virus-specific immune responses to different HIV-1C proteins and their subregions and (ii) the specifics of correlation between plasma viral load and T-cell responses within the major histocompatibility complex (MHC) class I HLA supertypes. Virus-specific immune responses in the natural course of HIV-1C infection were analyzed in the gamma interferon (IFN-γ)-enzyme-linked immunospot assay by using synthetic overlapping peptides corresponding to the HIV-1C consensus sequence. For Gag p24, a correlation was seen between better T-cell responses and lower plasma viral load. For Nef, an opposite trend was observed where a higher T-cell response was more likely to be associated with a higher viral load. At the level of the HLA supertypes, a lower viral load was associated with higher T-cell responses to Gag p24 within the HLA A2, A44, B27, and B58 supertypes, in contrast to the absence of such a correlation within the HLA B44 supertype. The present study demonstrated differential correlations (or trends to correlation) in various HIV-1C proteins, suggesting (i) an important role of the HIV-1C Gag p24-specific immune responses in control of viremia and (ii) more rapid viral escape from immune responses to Nef with no restraint of plasma viral load. Correlations between the level of IFN-γ-secreting T cells and viral load within the MHC class I HLA supertypes should be considered in HIV vaccine design and efficacy trials.

An essential role for virus-specific CD8+ T-cell responses in the control of simian immunodeficiency virus (SIV) and human immunodeficiency virus (HIV) infection has been documented (29, 34, 61, 70). Potent cytotoxic-T-lymphocyte (CTL) responses can apparently cause a dramatic drop of plasma viral load in acute HIV type 1 (HIV-1) infection (7, 38) and contain viral replication (54). The emergence of HIV-1C-specific CTLs in acute infection coincides with a plasma viral load decline (7, 38). The presence of CD8+ T cells was directly correlated with the control of SIV infection in the macaque model (29, 61). Moreover, vaccine-induced CTL responses protected macaques against the development of AIDS (1, 3, 4, 64). Taken together, these data provide evidence for a protective role of virus-specific CD8+ T-cell responses. However, taken alone, CTL responses are unable in most cases to prevent HIV-1 infection or thwart viral escape from immune recognition (8, 25, 58), which necessitates a cooperation with CD4+ T-helper responses and neutralizing antibodies. An additional challenge for efficient CTL responses is the accumulating genetic diversity of HIV-1 in the global AIDS epidemic driven by a relatively high evolution rate of the virus (18, 36). HIV-1 subtype C (HIV-1C) is the most prevalent HIV-1 subtype worldwide (15, 55) and is responsible for the majority of new HIV-1 infections (16, 55). The emergent ascendancy of HIV-1C indicates a need for a comprehensive analysis of HIV-1C-specific immune responses, both CD4+ and CD8+, in the context of vaccine design.

The association between CTL responses and plasma viral load has been addressed in previous studies of HIV-1B infection (10, 27, 34, 59). An inverse correlation between virus-specific CTL responses and plasma viral load was observed for Gag (14, 53), Pol (6, 53), and Env (47). In contrast, an association with high viral load was reported for Env- and Nef-specific CTL responses (39) or for the total frequency of CD8+ T cells (5). No significant correlation between CTL responses and plasma viral load was found in a number of other recent studies (13, 14, 19, 42). A correlation between CTL responses and higher HIV production was observed during highly active antiretroviral therapy, but an inverse correlation was seen at rapid virus rebound (44) or in patients with relatively high CD4+ counts (37). The discrepancies in correlation between plasma viral load and virus-specific T-cell immune responses might be explained, at least in part, by differences in assay methodologies, the targeting of different epitopes across the viral genome, different disease stages, and differences between diverse population groups. Discrepancies have also been reported between CD8+ T-cell responses as assayed by tetramer staining and immune func-
tion indicators such as gamma interferon (IFN-γ) release (21, 26, 37).

The major histocompatibility complex (MHC) class I HLA alleles are involved in presenting foreign antigens to immune cells and are believed to be essential in host immune responses to viral infections. Some HLA alleles or haplotypes have been associated with altered HIV-1 susceptibility and/or progression to AIDS (9, 11, 12, 28, 31, 40, 60, 65–67). The B*35-Cw*04 haplotype was linked with rapid progression of HIV infection. In contrast, B*27 and B*57 were shown to have a protective effect on progression to AIDS (25, 43, 56). HLA-A29 and HLA-B22 were associated with rapid progression, whereas B14 and C8 were associated with slow progression. (28) Different levels of plasma viral load in HIV-1C infection were associated with several HLA class I alleles and haplotypes (66), suggesting favorable (B*57) and unfavorable (B*39, A*30-Cw*03, *02-Cw*16, A*23-B*14, and A*23-Cw*07) HLA alleles. Differences in frequencies of MHC class I HLA alleles within populations might be one of the reasons for disagreements between different studies that addressed associations between CTL responses and plasma viral load. An introduction of major HLA class I supertypes (62, 63) that grouped the MHC class I alleles on the basis of their functional properties allowed us to address associations between CTL responses and plasma viral load within the HLA supertypes.

In the present study, we addressed associations between virus-specific T-cell immune responses and plasma viral load in the course of natural HIV-1C infection in Botswana, an African country with a severe burden of HIV-1C infection. The virus-specific immune responses were measured in asymptomatic HIV-1-infected blood donors by using overlapping peptides that spanned HIV-1C proteins in the IFN-γ–enzyme-linked immunospot (ELISPOT) assay. Correlations between T-cell immune responses and plasma viral loads were estimated for each HIV-1C protein and subregions of the structural proteins Gag (p17, p24, and p2/p7/p1/p6), Pol (protease, reverse transcriptase, RNase H, and integrase), and Env (gp120 and gp41) by analysis of unadjusted and adjusted (for the CD4 and CD8 counts) sets of data. Correlation was also assessed within five major HLA supertypes that were most common among the study population.

MATERIALS AND METHODS

Study subjects. The sample set used in the present study was the same collection from asymptomatic donors who tested HIV seropositive at the National Blood Transfusion Centre in Gaborone, Botswana, as described previously (48, 50). Details on the HIV testing, sample collection, peripheral blood mononuclear cell (PBMC) isolation, and relevant information of the cohort were described previously (48, 50). The total number of study subjects was 105. Plasma viral load data was available for 103 subjects (median, 37, 769 copies/ml; range, <400 to >750,000 copies/ml). CD4 (median count, 434; mean count, 451) and CD8 (median count, 984; mean count, 1,006) data were available for 98 study subjects. Plasma viral load determinations, CD4 and CD8 counts, and HLA type were performed as described previously (48, 50).

Synthetic peptides. PBMCs were screened for T-cell immune responses in the IFN-γ–ELISPOT assay within HIV-1C Gag, Pol, Vif, Vpr, Tat, Rev, Vpu, Env, and Nef proteins was analyzed (Fig. 1). The opposite trends were revealed in the correlation between T-cell immune responses and viral load for the Gag and Nef proteins (Table 1). Increasing values for HIV-1C Gag-specific T-cell responses were correlated with decreasing levels of plasma viral load (P = 0.020, slope = −0.31, and 95% confidence interval [CI] = −0.52 to −0.05 in the unadjusted analysis [unadjusted], and P = 0.025, slope = −0.27, and 95% CI = −0.51 to −0.04 in the adjusted analysis [adjusted]). No statistically significant correlation was found for other HIV-1C proteins or for total T-cell responses in either the unadjusted analysis or the adjusted analysis (Fig. 1 and Table 1).

Association between T-cell responses and viral load within the Gag, Pol, and Env. To address potential trends within the structural HIV-1C proteins, a similar analysis of association between the HIV-1C-specific T-cell responses and plasma viral load was performed for the p17, p24, and p2/p7/p1/p6 cleavage products of Gag; the protease, reverse transcriptase, RNase H, and integrase of Pol; and the gp120 and gp41 of Env (Fig. 2, accompanied by Table 2). Only Gag p24 demonstrated a strong inverse correlation between T-cell response and viral load (P = 0.0016, slope = −0.40, and 95% CI = −0.60 to −0.16 [unad-
justed], and $P = 0.005$, slope $= -0.43$, and 95% CI $= -0.72$ to $-0.14$ (adjusted).

For both HIV-1C proteins and their subregions, unadjusted and adjusted analyses resulted in similar associations and/or trends (Fig. 1 and 2, Tables 1 and 2).

**T-cell responses and viral load in relation to MHC class I supertypes.** Previously, we identified HLA class I alleles and/or antigen specificities that are most common in the Botswana population (49, 50). This allowed us to determine how HLA alleles common in Botswana are represented within the major HLA class I supertypes (62, 63). The distribution of the Botswana HLA alleles within MHC class I supertypes highlighted specificities of the common HLA alleles in the Botswana population (Table 3). Based on a cumulative allele frequency, the A24 supertype included the HLA alleles that were most common in Botswana (the cumulative frequency of A23 and A30 antigen specificities was 37.2%), followed by the A2, B27, B44, and B58 supertypes that were accountable for a frequency of about 20% each in the Botswana population. However, four HLA class I supertypes—A1, A3, B7, and B62—included HLA alleles that were seen at a relatively low frequency in Botswana (cumulative frequencies of <20%). Based on this analysis, we selected five of the most representative major HLA class I supertypes in Botswana, namely, A2, A24, B27, B44, and B58, and analyzed associations and trends between HIV-1C-specific T-cell responses and plasma viral load within these HLA supertypes. About 97.8% of the study population was covered by at least one HLA allele belonging to the A2, A24, B27, B44, or B58 HLA supertypes.

Distinct patterns of correlation were found within different
MHC class I HLA supertypes (Fig. 3). The correlation patterns also differed across the HIV-1C proteins. Pronounced HLA supertype-based differences were seen in the HIV-1C Gag and Gag p24. Within HIV-1C Gag, a statistically significant correlation between strong T-cell responses and low viral load was found in the A2 supertype (\( P = 0.0058 \), slope = \(-0.53\), and 95% CI = \(-0.76\) to \(-0.17\) [unadjusted], and \( P = 0.0024 \), slope = \(-0.53\), and 95% CI = \(-0.87\) to \(-0.19\) [adjusted]). The A24, B27, and B58 supertypes demonstrated trends toward the same correlation in both unadjusted and adjusted analyses (\( P = 0.065 \), 0.059, and 0.066, respectively, in the adjusted analysis). Interestingly, there was no correlation within the B44 supertype (\( P = 0.95 \)). The observed associations for Gag were more pronounced for HIV-1C Gag p24, revealing a significant correlation between higher T-cell responses and lower plasma viral loads for all but the B44 supertype (\( P = 0.0028 \) for A2, \( P = 0.0086 \) for A24, \( P = 0.0142 \) for B27, and \( P = 0.0144 \) for B58, but \( P = 0.84 \) for B44 [unadjusted]; \( P = 0.0009 \) for A2, \( P = 0.0053 \) for A24, \( P = 0.0073 \) for B27, \( P = 0.0064 \) for B58, but \( P = 0.81 \) for B44 [adjusted]).

![FIG. 2. Association between HIV-1C-specific T-cell responses and plasma viral load in subregions of Gag, Pol, and Env proteins. Regression curves represent results of unadjusted and adjusted analyses. Corresponding statistics are shown in Table 2.](image)

### TABLE 2. Association between HIV-1C-specific T-cell responses and plasma viral load within the structural HIV-1C proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Unadjusted analysis (Spearman rank correlation coefficient)</th>
<th>Adjusted analysis (linear regression)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Slope estimate</td>
<td>95% CI</td>
</tr>
<tr>
<td>Gag p17</td>
<td>0.02</td>
<td>(-0.24, -0.27)</td>
</tr>
<tr>
<td>Gag p24</td>
<td>(-0.40)</td>
<td>(-0.60, -0.16)</td>
</tr>
<tr>
<td>Gag p2/p7/p1/p6</td>
<td>0.12</td>
<td>(-0.14, 0.37)</td>
</tr>
<tr>
<td>Pol protease</td>
<td>(-0.05)</td>
<td>(-0.30, 0.22)</td>
</tr>
<tr>
<td>Pol reverse transcriptase</td>
<td>(-0.01)</td>
<td>(-0.28, 0.26)</td>
</tr>
<tr>
<td>Pol RNase H</td>
<td>0.11</td>
<td>(-0.14, 0.35)</td>
</tr>
<tr>
<td>Pol integrase</td>
<td>0.02</td>
<td>(-0.23, 0.27)</td>
</tr>
<tr>
<td>Env gp120</td>
<td>0.00</td>
<td>(-0.25, 0.26)</td>
</tr>
<tr>
<td>Env gp41</td>
<td>(-0.15)</td>
<td>(-0.41, 0.13)</td>
</tr>
</tbody>
</table>

\( a \) Minimum, maximum.
If T-cell responses and viral load correlate inversely for some HLA supertypes but not for others, then T-cell responses may be HLA supertype specific. To assess this question statistically for HIV-1C Gag and for Gag p24, we used linear regression models to test whether HLA supertype and T-cell responses interacted in their effect on viral load. To test a potential interaction between HLA supertype and T-cell responses, we used linear regression models for the HIV-1C Gag and Gag p24 subregion. Two trends were found: (i) for Gag (HLA supertype A2 versus B44) there was a strong negative correlation for A2 but no correlation for B44 supertype, with an interaction test P value of 0.086; (ii) for Gag p24 (HLA supertype A2 versus B44) there was also a strong negative correlation for A2 but no correlation for B44 supertype, with an interaction test P value of 0.063. The strength of the observed correlation between the MHC class I HLA supertypes and the plasma viral load could be clarified by increasing the sample size in each HLA supertype.

Within the A24 supertype, higher Nef-specific responses were correlated with higher plasma viral load (P = 0.011, slope = 0.45, 95% CI = 0.12 to 0.69 [unadjusted], and P = 0.006, slope = 0.45, and 95% CI = 0.13 to 0.77 [adjusted] [Fig. 4]). Trends for a correlation between higher virus-specific responses and lower viral load within the A24 supertype were seen for gp41 (P = 0.070, slope = −0.35, and 95% CI = −0.65 to 0.03 [unadjusted], and P = 0.091, slope = −0.32, and 95% CI = −0.69 to 0.05 [adjusted]), and the total HIV-1C (P = 0.077, slope = −0.23, and 95% CI = −0.46 to 0.03 [unadjusted], and P = 0.071, slope = −0.23, and 95% CI = −0.49 to 0.02 [adjusted]).

No other significant correlations were found between HIV-1C-specific T-cell responses and viral load.

### Table 1

<table>
<thead>
<tr>
<th>HLA supertype</th>
<th>MHC class I HLA alleles; potential</th>
<th>Frequency (total %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2</td>
<td>A<em>0201-0207, A</em>6802, A*6901</td>
<td>24</td>
</tr>
<tr>
<td>B7</td>
<td>B<em>0702, B</em>3501-03, B<em>51, B</em>5301, B<em>5401, B</em>0703-05, B<em>1508, B</em>5501-02, B<em>5601-02, B</em>6701, B*7801</td>
<td>12</td>
</tr>
<tr>
<td>B44</td>
<td>B<em>3701, B</em>4402-03, B<em>60 (B</em>4001), B<em>61 (B</em>4006), B<em>18, B</em>4101, B<em>4901, B</em>5001</td>
<td>20</td>
</tr>
<tr>
<td>A24</td>
<td>A<em>2301, A</em>2402-04, A*3001-03</td>
<td>37</td>
</tr>
<tr>
<td>B27</td>
<td>B<em>1401-02, B</em>1503, B<em>1509, B</em>1510, B<em>1518, B</em>2701-08, B<em>3801-02, B</em>3902-04, B<em>4801-02, B</em>7301</td>
<td>22</td>
</tr>
<tr>
<td>B58</td>
<td>B<em>1516, B</em>1517, B<em>5701, B</em>5702, B*58</td>
<td>21</td>
</tr>
<tr>
<td>B62</td>
<td>B<em>4601, B</em>52, B<em>1501 (B62), B</em>1502 (B75), B<em>1513 (B77), B</em>1301-02, B<em>1506, B</em>1512, B<em>1514, B</em>1519, B*1521</td>
<td>3</td>
</tr>
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</table>

* Sette and Sidney (62, 63).

### Discussion

In the present study correlation patterns between virus-specific immune responses and plasma viral load were found to vary at two different levels: viral and host. Specifically, differences in correlation were detected (i) between the viral proteins and (ii) between different MHC class I HLA supertypes. Although the results of the present study warrant further research in regards to different stages of disease (i.e., acute versus chronic), these findings have direct relevance for our understanding of HIV pathogenesis, as well as for the design of HIV vaccines and vaccine efficacy trials.

HIV-1C dominates in the global AIDS epidemic. However, associations between HIV-1C-specific immune responses and viral loads have not been analyzed previously. In addition, differences in geographical distribution of HLA alleles, taken in the context of MHC class I restriction of CTL epitope presentation, may imply another level of divergence between populations and/or ethnic groups. For example, the predominance of HIV-1C in southern Africa (15, 16, 51, 52, 68, 69) and specificities of MHC class I HLA allele frequencies in the Botswana population (49, 50) determined a unique immunodominant profile of cell-mediated immune responses (48, 50).

Patterns of correlation between HIV-1C-specific immune responses and plasma viral load for different viral proteins included an association between strong T-cell responses and lower viral load. A correlation between stronger T-cell responses and lower plasma viral load in the Gag/Gag-p24 proteins coexisted with a trend toward an opposite correlation in the Nef, whereas there was no pattern of correlation between T-cell responses and plasma viral load for the other HIV-1C proteins (analyzed for the entire study group).

The correlation between virus-specific immune responses and plasma viral load in HIV-1C Gag, and particularly in Gag p24, suggests an important role of p24-specific T-cell responses in the control of viremia. The highly conserved p24 appears to have functional constraints and may not be flexible for accumulation of new mutations, which in turn would allow control of the virus by a capable Gag p24-specific T-cell response. It also raises the notion of relative importance of HIV proteins for vaccine design and suggests that the Gag p24 might be the most attractive region to include in vaccine candidates to induce T-cell immune responses that could contain viremia.

In contrast, the correlation (or trend) between increasing Nef-specific T-cell responses and increasing plasma viral load suggests that no benefit may be realized from including Nef or some Nef epitopes in such a vaccine. It also suggests that viral escape from immune recognition is more likely to occur rapidly for CTL epitopes of Nef. The notorious capability of HIV-1 to escape from CTL recognition in Nef was supported by several studies of HIV and SIV (17, 33, 35, 45, 46, 58). HIV-1 Nef is believed to downmodulate the expression of MHC class I molecules, which in turn impairs CTL responses, resulting in an inability to control virus replication (reviewed in references 2,
It is not clear whether Nef-mediated downmodulation of HLA class I molecules plays a role in the observed correlation between increased HIV-1C Nef-specific T-cell responses and elevated levels of plasma viral load.

Diversity in host genetics is likely to be manifested by assorted virus-specific CTL responses for carriers of different MHC class I HLA alleles or HLA supertypes. In the present study the correlation between increasing HIV-1C Gag/Gag p24 immune responses and decreasing viral load within the HLA supertypes A2, A24, B27, and B58 contrasted with the absence of such a correlation for the HLA B44 supertype. This finding demonstrates differences in virus-specific T-cell immune responses in subsets of the population according to expressed HLA class I alleles or HLA supertypes. It is apparent that carriers of the A2 supertype have a strong correlation between T-cell responses and plasma viral load, whereas carriers of B44 supertype do not have such a correlation. In fact, many previous studies that have described a correlation between increasing HIV-1-specific CTL responses and decreasing viral load for HIV-1B have used HLA-A02–Gag epitope tetramer complexes or Gag-specific CTL responses as markers of immune response.

The genetic background of the population might be an important factor for vaccine efficacy, particularly when limited epitope-specific vaccine designs are used. Kaslow et al. described a predictive power for particular HLA class I alleles for the outcome of vaccine trials, showing that individuals vaccinated with HLA-B*27 or HLA-B*57, HLA alleles associated with slower disease progression, had better responses to an ALVAC-HIV recombinant canarypox vaccine (32). The results

FIG. 3. Association between HIV-1C-specific T-cell responses and plasma viral load within the major HLA class I supertypes. Regression curves represent results of unadjusted analysis. (A) Gag associations; (B) Gag p24 associations.
of our study also point, although indirectly, to a potential difference in immune responses among carriers of different HLA supertypes. Assuming that differences in correlation patterns are related to control of viremia, differences between diverse MHC class I HLA supertypes should be taken into account in vaccine design to elicit optimal CTL responses. Divergent MHC class I HLA supertypes are related to control of viremia, differences between HLA supertypes. Assuming that differences in correlation patterns are related to control of viremia, differences between virus-specific T-cell responses and plasma viral load should be considered in vaccine design and development, particularly as they relate to the populations of southern Africa that are severely impacted by the epidemic of HIV-1C, which has the highest rates of infection in the world (15, 16).

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