HLA and Discordance in Manifestations of HIV-1 Infection

HLA-B*57 versus HLA-B*81 in HIV-1 Infection: Slow and Steady Wins the Race?

Running title: HLA and Discordance in Manifestations of HIV-1 Infection

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Abstract
Two human leukocyte antigen (HLA) variants, HLA-B*57 and -B*81, are consistently known as favorable host factors in HIV-1-infected Africans and African-Americans. In our analyses of prospective data from 538 recent HIV-1 seroconverters and cross-sectional data from 292 subjects with unknown duration of infection, HLA-B*57 (mostly B*57:03) and -B*81 (exclusively B*81:01) had mostly discordant associations with virologic and immunologic manifestations before antiretroviral therapy. Specifically, relatively low viral load (VL) in B*57-positive subjects ($P \leq 0.03$ in various models) did not translate to early advantage in CD4$^+$ T-cell (CD4) counts ($P \geq 0.37$). In contrast, individuals with HLA-B*81 showed little deviation from the norm of set-point VL ($P > 0.18$) while maintaining high CD4 count during early and chronic infection ($P = 0.01$). These observations suggest that discordance between VL and CD4 count can occur in the presence of certain HLA alleles and that effective control of HIV-1 viremia is not always a prerequisite for favorable prognosis (delayed immunodeficiency). Of note, steady CD4 count associated with HLA-B*81 in HIV-1-infected Africans may depend on country of origin, as observations differed slightly between subgroups enrolled in southern Africa (Zambia) and eastern Africa (Kenya, Rwanda, and Uganda).
In Africans infected with diverse HIV-1 subtypes (clades), the virologic and immunologic outcomes can be highly variable, often depending on complex viral and host factors, especially human leukocyte antigen (HLA) variants that facilitate innate and adaptive immune responses (1-7). Population- and methodology-specific issues aside (8), strong and consistent evidence suggests that two relatively common HLA-B variants, B*57 and B*81, may be universally favorable in the context of viral load (VL) control (4-6, 9). Confirmatory findings based on analyses of African-Americans are also convincing (10, 11), so are efforts to define the underlying functional mechanisms (12-16). Further translational research may require close attention to the timing of HLA-I-mediated immune pathways (17-20).

The effects of favorable HLA class I (HLA-I) alleles like B*57 are known to be more readily detectable in primary (early) than chronic infection (4), presumably because viruses acquire immune escape and compensatory mutations over time to gain new phenotypes (14, 20-22). The long-term benefit of HLA-B*57 and -B*81 is still uncertain as immunologic outcomes and disease progression are rarely examined for resource-poor African populations (3, 5). Relying on new prospective data from 538 African seroconverters with early chronic infection and cross-sectional data from 292 AIDS-free subjects with unknown duration of infection, our work here suggest that virologic and immunologic manifestations of HIV-1 infection are often discordant in subgroups of Africans defined by HLA-B*57 and -B*81.

Methods

Study population and follow-up intervals. HIV-1-infected Africans, including 538 recent seroconverters (SCs) and 359 subjects already seropositive (seroprevalent, SPs) at first testing, were enrolled from Kenya, Rwanda, Uganda, and Zambia under a uniform study protocol developed and implemented by the International AIDS Vaccine Initiative (IAVI). The procedures for written informed consent and all other research protocols were approved by institutional review boards at all sponsoring organizations, with further compliance to human experimentation guidelines set forth by Department of Health and Human Services in the United States. Clinical and laboratory tests during regular follow-up...
visits have been described in detail elsewhere (6, 23, 24). Access to health care and HIV-1 prevention is similar across all clinical sites in terms of HIV risk reduction, management of sexually transmitted infections, CD4+ T-cell (CD4) counts, general medical care, as well as family planning counseling (25). Initiation of antiretroviral therapy (ART) followed appropriate national guidelines (25), and all visits after ART were excluded. Analyses of the remaining longitudinal data targeted three time intervals in SCs, i.e., (i) acute-phase within the first three months (91 days) after the estimated date of infection (EDI) (6), (ii) set-point in the 3-12 months period after EDI (4), (iii) early chronic phase corresponding to 12-24 months after EDI. The EDI was established in three ways (whichever came first): (i) 10 days prior to the first detection of viral RNA in plasma accompanied by negative serology, (ii) two weeks prior to the first detection of HIV-1 p24 antigen in plasma with negative serology, and (iii) the midpoint between last seronegative and first seropositive test. The chronically infected SPs were enrolled as the suspected transmission source partners of SCs. They attended a single study visit to provide cross-sectional data for analysis here.

**Virologic and immunologic phenotypes.** Plasma viral load (VL = RNA copies/mL) was measured using the Amplicor Monitor v1.5 assay (Roche Applied Science, Indianapolis, IN) (24). For log_{10} transformation, VL below the lower limit of detection (400 RNA copies/mL) was assigned a value of 1.300 (half of log_{10}400). CD4 counts were based on T-cell immunophenotyping, with assays done at individual clinics using the FACScount System (Beckman Coulter Ltd., London, UK). Duration of infection for each VL and CD4 measurement was calculated from the blood sample date and the EDI.

**Viral sequencing and HLA genotyping.** Methods for HIV-1 pol gene sequencing and HLA class I genotyping have been described elsewhere (4, 6, 24). Viruses were grouped into subtypes (mostly A1, C and D) and recombinant forms (rare). Allelic variants at three HLA class I loci (HLA-A, HLA-B and HLA-C) were fully resolved to their 4-digit specificities.
Statistical analysis. Using software packages in SAS, version 9.2 (SAS Institute, Cary, NC), SCs and SPs were evaluated primarily for virologic and immunologic outcomes. All SCs with adequate data in the first 24 months after EDI were analyzed, while 67 SPs with end-stage infection (CD4 count <200 cells/μL) were excluded, leading to an effective sample size of 292 chronically infected SPs suitable for analysis of VL (Table 1). Comparative analyses followed strategies established in similar studies (4, 9), with an emphasis on (i) local regression (LOESS) and mixed models for repeated measures, (ii) analysis of variance (ANOVA), (iii) t-test for quantitative variables with a normal distribution (CD4 count and log10 VL), (iv) Cochran-Armitage tests for trend across three ordinal VL categories (high, medium and low) with differential impact on HIV-1 transmission (4, 9, 26). In addition, Kaplan-Meier curves were used to compare time from EDI to severe immunodeficiency (CD4 count <350 cells/μL) in SCs, with individual plots generated using GraphPad Prism (GraphPad Software, Inc.). Collectively, these analyses aimed at defining the durability and concordance of HLA-I associations with virologic and immunologic manifestations of primary and chronic HIV-1 infection. Separate analyses of two subgroups with subjects from Zambia (southern Africa) and subjects from eastern Africa allowed a partial evaluation of region-specific relationships. The statistical significance was accepted at the level of \( P < 0.05 \) in multivariable models, i.e., with full adjustment for cofactors and potential confounders.

Results

Overall characteristics of the study population. HIV-1 seroconverters (SCs) available for analysis here included 106 Kenyans, 85 Rwandans, 128 Ugandans, and 219 Zambians, with an overall male to female ratio of 1.7 (Table 1). The vast majority (74.4%) of SCs were less than 40 years old at enrollment, and their estimated dates of infection (EDI) ranged from May 2005 to August 2011. HIV-1 subtypes C and A1 were dominant among these SCs, accounting for 39.2% and 33.3% of the total, respectively. Other subtypes, i.e., D, B, and recombinant forms, were present at low frequencies. There were a median of 9 follow-up visits (interquartile range, 7-10) per patient in the first two years after EDI. HIV-1 viral load
(VL) and CD4+ T-cell (CD4) count measured at these visits provided longitudinal data for testing. AIDS-free subjects (SPs) who were seropositive at first test mostly came from three countries (Rwanda, Uganda, and Zambia), with an overall male to female ratio of 0.8. Their ages at enrollment were similar to SCs, but HIV-1 subtypes remained largely unknown (viral sequencing not done). HIV-1 VL and CD4 count measured at the first available visit were treated as cross-sectional data for analysis.

Two HLA-B variants of interest, B*57 (mostly B*57:03) and B*81 (exclusively B*81:01), were found in 49 (9.1%) and 25 (4.6%) SCs, respectively, with two (0.4%) SCs having both (no different from the co-existence frequency expected from a random distribution). In the SP group, the frequency was slightly higher for both, as 38 (13.0%) of them had B*57, 21 (7.2%) had B*81, and two (0.7%) had both.

Relationships between timing of data collection and manifestations of HIV-1 infection. In the SC group, virologic and immunologic outcomes showed weak and sporadic correlation with duration of infection (DOI) at three major intervals (peak, set-point, and nadir) within the first 24 months of infection (Table 2). Correlation with the greatest statistical significance ($P < 0.0001$) was for peak VL and DOI (time elapsed after EDI) (Spearman rho = -0.37). Timing of data collection was not an issue for the SP group, as there was little variation in the duration of follow-up (DOF) until the first VL and CD4 count measurements were taken (Table 2). For consistency, however, DOI and DOF were treated as covariates in all subsequent analyses, regardless of their statistical significance.

Dynamics of viremia and CD4 count during acute and early chronic phases of HIV-1 infection. LOESS curves based on 4,615 person-visits (Figure 1A) revealed that three groups of SCs stratified by the presence and absence of HLA-B*57 and HLA-B*81 had distinct VL profiles that were steady beyond 3 months (13 weeks) of infection. The reference group without these two alleles consistently had the highest VL after infection when compared with SCs who were B*57+ or B*81+. In mixed models for testing VL measures in the 3-24 months interval, VLs in B*57+ SCs were about 0.5 log10 lower than in B*57- SCs (adjusted $P < 0.001$) (Table 2). VLs differed by about 0.3 log10 between B*81+ and B*81- SCs, but without reaching statistical significance after adjusting for age, sex, and other potential cofactors (DOI and country of origin) ($P = 0.18$) (Table 2).
When CD4 counts corresponding to 4,676 person-visits was plotted for LOESS curves (Figure 1B), HLA-B*81+ SCs showed relatively steady measures across the first 24 months period of infection. On average, B*81+ SCs had 92 ± 40 more CD4 cells per µl of blood than did B*81- SCs in the 3-24 months after infection (P = 0.02 in analyses of repeated measures) (Table 2). B*57+ SCs, on the other hand, did not demonstrate clear advantage in CD4 count when compared with B*57- SCs (beta estimate = 39 ± 30 cells/µl, adjusted P = 0.19) (Table 2). The slight trend for higher CD4 count in B*57+ SCs diminished over time, especially after 72 weeks of infection (Figure 1B).

**Analysis of cross-sectional data from SPs.** Cross-sectional data from 292 SPs confirmed the discordance between virologic and immunologic phenotypes associated with HLA-B*57 and -B*81 (Table 3). The adjusted effect size for CD4 count was 183 ± 48 cells per µL of blood in differential between B*81+ and B*81- SPs (P = 0.001), but HIV-1 VL was similar between B*81+ and B*81- SPs (P = 0.25). For comparisons between B*57+ and B*57- SPs, the differential in CD4 count was 80 ± 38 cells/µL, in favor of B*57 (P = 0.03), while the differential in HIV-1 VL (0.39 ± 22 log_{10}) did not reach statistical significance (P = 0.09).

**Viremia as a categorical phenotype in SCs and SPs.** Test for trend was also revealing, as HLA-B*57 was clearly less frequent in the subgroups of SCs and SPs with high (>100,000 copies/ml) and medium (10,000-100,000 copies/ml) than low (<10,000 copies/ml) VL (Table 3). The proportional odds ratio (pOR) across the three ordinal subgroups ranged from 0.24 to 0.54 in analysis of peak, set-point, and nadir VL in SCs (adjusted P <0.04 for all tests). Estimates for the SP group was similar (pOR = 0.47, P = 0.02). In contrast, HLA-B*81 did not show much differential distribution across the three VL groups (adjusted P = 0.20-0.89).

**Observations from other analyses.** In further analyses where subjects without HLA-B*57 and HLA-B*81 served as the reference group, an advantage with CD4 count was persistent for HLA-B*81 beyond the acute phase of infection, with the most dramatic difference seen in the SP group (P <0.001) (Figure 2). For HLA-B*57+ subjects, the CD4 profile was always highly comparable to that of the
reference group (Figure 2).

Kaplan-Meier curve further revealed that time from estimated date of infection to severe immunodeficiency (CD4 count <350 cells/μL) differed among SCs with or without HLA-B*57 and HLA-B*81 (P = 0.02) (Figure 3). The overall difference was driven by HLA-B*81 (relative hazards = 0.40; 95% confidence interval = 0.19-0.86) and not B*57 (RH = 0.82, 95% CI = 0.55-1.23). Statistical adjustments for age, gender, and HIV-1 subtype (or country of origin) made no difference to the effects of B*81 or B*57. In sensitivity analysis, the two SCs with both B*57 and B*81 were treated in three ways: (i) left out of the model, (ii) kept in the B*57+ subgroup, and (iii) kept in the B*81+ subgroup. In each case, statistical significance remained with B*81+ SCs (adjusted P = 0.02, <0.01, and = 0.02, respectively). For B*57+ SCs, the corresponding P values associated with RH estimates were 0.34, 0.34, and 0.17, respectively (data available from J.T.).

In our study populations, HLA-B*57 and -B*81 were in strong linkage disequilibrium (LD) with HLA-C*18:01, which is also known to be favorable in Africans (4, 5, 9). Virtually all SCs and SPs with C*18:01 had either B*57 or B*81. The distinct phenotypes in B*57+ and B*81+ subjects, however, could not be explained by C*18:01 or other companion HLA-C variants. Weak LD between HLA-B and HLA-A variants further ruled out the potential confounding by HLA-A alleles (9).

Separate analyses of subjects from southern and eastern Africa to assess regional consistency. HLA-related, discordant relationship between VL and immunodeficiency was evident in subgroup analyses (Table 4 and Figure 4). Among 219 southern African (Zambian) SCs, the association of HLA-B*57 with lower VL (adjusted P <0.01) but not higher CD4 count (P = 0.48) was in clear contrast with the concordant association of female sex with lower VL and higher CD4 count (Table 4). SCs from eastern Africa (n = 319) provided similar results, except that neither CD4 count nor time from infection to CD4 count <350 cells/μL reached statistical significance for the subgroup of SCs defined by HLA-B*81 (adjusted P >0.25). In the comparison of cross-sectional data from Zambian SPs (n = 138) and eastern African SPs (n = 154), HLA-B*81 was unequivocally advantageous in analyses of CD4 count
regardless of country of origin (Table 4). Nonetheless, the adjusted effect size attributable to HLA-B*81 was more substantial in Zambian SPs ($\Delta = 219 \pm 72 \text{ cells/\mu L}$, $p < 0.01$) than eastern African SPs ($\Delta = 161 \pm 63 \text{ cells/\mu L}$, $p = 0.01$).

**Discussion**

Spontaneous control of HIV-1 infection is typically manifest by low or undetectable viremia, but our work here provides compelling evidence that virologic control is not always a prerequisite for durable benefit during the first two years of infection (when most subjects were available for analysis), as seroconverters (SCs) with HLA-B*81 had relatively steady CD4 count without much advantage in suppressing viral load (VL). Time to CD4 count <350 cells/µL, a well-recognized threshold for initiation of antiretroviral therapy (27), was delayed so substantially in HLA-B*81+ individuals that this relationship persisted in the cross-sectional analysis of chronically infected subjects (SPs) with CD4 count over 200 cells/µL. Overall, HLA-B*81 may not be the most favorable allele for virologic control, but its steady benefit can become obvious when CD4 counts (immunologic outcomes) are evaluated. HLA-B*57+ subjects showed the inverse to be true, i.e., clear advantage with VL was accompanied by rather limited impact on CD4 count and progression to severe immunodeficiency.

Recognition of HLA-B*57 as a favorable host factor in the course of HIV-1 infection began with cohorts of European ancestry (28-30), with B*57:01 as a single dominating allele highly enriched among elite controllers with undetectable VL or viremic controllers with VL <2,000 copies/mL and steady CD4 count (31). In cohorts of African ancestry, B*57 is primarily represented by B*57:03, while B*57:02 and B*57:01 are present at much lower frequencies. Recent work does suggest that “micropolymorphisms” within B*57-related alleles have functional consequences in the context of antigen presentation and HIV-1 immune escape (22). While rarity of B*57:01 in African populations precludes any meaningful analysis of this particular allele here, evidence from comparison of HIV-1-infected African-Americans controllers and progressors suggests that the effective size (odds ratio) for three B*57 alleles can vary by up to 2-fold.
HLA and Discordance in Manifestations of HIV-1 Infection

Region-specific HIV-1 viruses and HLA alleles (e.g., A*30 and A*74) in strong linkage disequilibrium with HLA-B*57 alleles may offer another explanation for disparity in the relative effects of three alleles in the HLA-B*57 group (9, 32).

Compared with HLA-B*57, B*81 is less common, with population frequencies ranging from 2.7% in Kenyans, 4.8% in Rwandans, 5.9% in Zambians to 7.1% in Ugandans enrolled into this study. Other rare HLA-B alleles occasionally associated with advantageous outcomes (e.g., B*13, B*27, and B*39) (10, 33) did not show much differential impact on VL or CD4 count in our cohort, often as a result of limited statistical power. Given the maturity of the HIV/AIDS epidemic in sub-Saharan Africa (34, 35), rare allele advantage is expected to be less obvious because the circulating viruses have had ample opportunities to encounter and adapt to specific HLA-I profiles, especially since these alleles have no reported advantage in delaying or preventing the acquisition of HIV-1 infection (9, 19).

HLA-B*81-restricted, HIV-1-specific cytotoxic T-lymphocyte (CTL) responses are known to induce several mutations in Gag, a matrix protein important to HIV-1 virion assembly and maturation (15, 36). The single amino acid substitution (S186T) in one Gag epitope (TS9) is of particular interest, as HIV-1 subtype C viruses encoding 186S cannot replicate in vitro unless compensatory mutations are introduced to neighboring sites (e.g., Q182S plus T190A/I) (16). The biology underlying this rather dramatic “fitness cost” may point to gag codon 186 and neighboring sites as a viral Achilles heel.

HIV-1 infection in HLA-B*81 carriers may resemble SIV infection in sooty mangabey, a model of nonprogressive infection despite persistency in high viremia for years (37). In study populations where HLA-B*81 or similar alleles are found at high frequencies, the assumed relationship between set-point VL and disease progression (38, 39) may become obscured (40). When genetic factors associated with VL are distinct from those important to disease progression, as evident from studies of Africans and African Americans (5, 7, 10), systematic evaluation using open-minded approaches is important (8, 41, 42).

Complication by co-morbidity may be relevant (8), as protective alleles like HLA-B*27 (rare in Africans) and -B*57 are known to be unfavorable in the setting of autoimmune diseases, including
HLA and Discordance in Manifestations of HIV-1 Infection

252 ankylosing spondylitis and psoriasis (43, 44). Complication from co-infection is also worth noting as
253 HLA alleles are critical to immunity against all human pathogens.

254 Future research can benefit from HLA-B*81+ individuals in at least two ways. First, B*81-
255 positive subjects and others with steady CD4 count can be ideal for testing therapeutic vaccines, as both
256 CTL and antibody responses depend on regulation by CD4 cells. Second, these individuals may offer an
257 opportunity to examine mechanisms for discordance between VL and immunodeficiency after HIV-1
258 infection. In particular, assessment of T-cell activation status may provide valuable clues about the unique
259 phenotypes associated with HLA-B*81. These efforts may require close attention to country- or region-
260 specific settings, as findings may vary somewhat from one site to another.

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272 authors and do not necessarily reflect the views of KEMRI, USAID or the United States Government.
References


HLA and Discordance in Manifestations of HIV-1 Infection

determinants of heterosexual transmission of human immunodeficiency virus type 1 in Africa.

AIDS Res Hum Retroviruses 17:901-10.


HLA and Discordance in Manifestations of HIV-1 Infection


**TABLE 1** Overall characteristics of 538 HIV-1 seroconverters (SCs) and 292 chronically infected subjects (SPs) enrolled from four African countries.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>SCs (N = 538)</th>
<th>SPs (N = 292)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kenyan: n (%)</td>
<td>106 (19.7)</td>
<td>4 (1.4)</td>
</tr>
<tr>
<td>Rwandan: n (%)</td>
<td>85 (15.8)</td>
<td>81 (27.7)</td>
</tr>
<tr>
<td>Ugandan: n (%)</td>
<td>128 (23.8)</td>
<td>69 (23.6)</td>
</tr>
<tr>
<td>Zambian: n (%)</td>
<td>219 (40.7)</td>
<td>138 (47.3)</td>
</tr>
<tr>
<td>Sex ratio (M/F)</td>
<td>1.7 (336/202)</td>
<td>0.8 (130/162)</td>
</tr>
<tr>
<td>Age at enrollment: mean ± SD (yr)</td>
<td>31.1 ± 8.3</td>
<td>33.1 ± 7.7</td>
</tr>
<tr>
<td>Age ≥40 years: n (%)</td>
<td>84 (15.6)</td>
<td>59 (20.2)</td>
</tr>
<tr>
<td>Estimated dates of infection (EDI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Earliest</td>
<td>May 2005</td>
<td>Unknown</td>
</tr>
<tr>
<td>Latest</td>
<td>Aug 2011</td>
<td>Unknown</td>
</tr>
<tr>
<td>HIV-1 subtype: n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>179 (33.3)</td>
<td>7 (2.4)</td>
</tr>
<tr>
<td>C</td>
<td>211 (39.2)</td>
<td>4 (1.4)</td>
</tr>
<tr>
<td>D</td>
<td>73 (13.6)</td>
<td>2 (0.7)</td>
</tr>
<tr>
<td>Others (B and recombinants)</td>
<td>20 (3.7)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Unknown (no viral sequencing)</td>
<td>55 (10.2)</td>
<td>279 (95.5)</td>
</tr>
<tr>
<td>Viral load measures per patient: median (IQR)</td>
<td>8 (7-10)</td>
<td>1 (1-1)</td>
</tr>
<tr>
<td>Total person-visits with viral load data</td>
<td>4,615</td>
<td>292</td>
</tr>
<tr>
<td>Viral load: mean ± SD</td>
<td>3.3 ± 1.3</td>
<td>4.2 ± 1.3</td>
</tr>
<tr>
<td>CD4 T-cell measures per patient: median (IQR)</td>
<td>9 (7-10)</td>
<td>1 (1-1)</td>
</tr>
<tr>
<td>Total person-visits with CD4 count</td>
<td>4,676</td>
<td>292</td>
</tr>
<tr>
<td>CD4 count: mean ± SD</td>
<td>534 ± 239</td>
<td>475 ± 220</td>
</tr>
</tbody>
</table>

*SPs with <200 CD4+ T-cells/μl were excluded.

*The minor HIV-1 subtypes (D and others) are combined for analysis here. For SPs, viral subtypes are mostly unknown (lack of viral sequencing). M, male; F, female; NA, not applicable; SD, standard deviation of the mean.

*Nadir VL in SCs after 3 months of infection or first available viral load in SPs.

*At visits corresponding to VL measurements.
TABLE 2 Sporadic relationships between timing of follow-up and HIV-1-related outcomes in 538 HIV-1 seroconverters (SCs) and 292 seroprevalent subjects (SPs).

<table>
<thead>
<tr>
<th>Intervals tested</th>
<th>Median (IQR) in weeks</th>
<th>Correlation: Spearman rho (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOI in SCs at three major intervals</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At peak viral load (VL)</td>
<td>17 (8-36)</td>
<td>-0.37 (&lt;0.0001)</td>
</tr>
<tr>
<td>At set-point VL</td>
<td>16 (13-24)</td>
<td>0.02 (&gt;0.600)</td>
</tr>
<tr>
<td>At nadir VL</td>
<td>60 (26-120)</td>
<td>-0.28 (&lt;0.0001)</td>
</tr>
<tr>
<td>At peak CD4+ CD4 cell (CD4) count</td>
<td>16 (8-36)</td>
<td>0.00 (&gt;0.900)</td>
</tr>
<tr>
<td>At set-point CD4 count</td>
<td>16 (13-24)</td>
<td>-0.15 (&lt;0.001)</td>
</tr>
<tr>
<td>At nadir CD4 count</td>
<td>61 (38-84)</td>
<td>-0.11 (0.009)</td>
</tr>
<tr>
<td>DOF in SPs at first test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>For chronic VL</td>
<td>0 (0-0)</td>
<td>0.03 (&gt;0.600)</td>
</tr>
<tr>
<td>For chronic CD4 count</td>
<td>0 (0-0)</td>
<td>-0.07 (&gt;0.200)</td>
</tr>
</tbody>
</table>

* Interquartile range (IQR) is defined by the 25th to 75th percentile.

b Duration of infection (DOI) for HIV-1 seroconverters (SCs) began from the estimated date of infection (see text); duration of follow-up (DOF) for seroprevalent subjects (SPs) began at time of enrollment.
TABLE 3 Discordant relationships of HLA-B*57 and -B*81 to virologic and immunologic phenotypes in early and chronic HIV-1 infection, as seen in 538 seroconverters (SCs) and 292 seroprevalent subjects (SPs).

<table>
<thead>
<tr>
<th>Tests for three series of models</th>
<th>Analysis of HLA-B*57a</th>
<th>Analysis of HLA-B*81b</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Generalized linear models for VL</td>
<td>n, Δ (Mean ± SE), Adjusted P</td>
<td>n, Δ (Mean ± SE), Adjusted P</td>
</tr>
<tr>
<td>SCs: peak (N=538)</td>
<td>49, -0.38 ± 0.12, 0.001</td>
<td>25, -0.05 ± 0.16, 0.760</td>
</tr>
<tr>
<td>SCs: set-point (N=538)</td>
<td>49, -0.31 ± 0.14, 0.032</td>
<td>25, -0.23 ± 0.20, 0.241</td>
</tr>
<tr>
<td>SCs: repeated measures (3-24 months)</td>
<td>49, -0.51 ± 0.12, &lt;0.001</td>
<td>25, -0.23 ± 0.17, 0.178</td>
</tr>
<tr>
<td>SPs: at only available visit (N=292)c</td>
<td>38, -0.39 ± 0.22, 0.087</td>
<td>21, -0.34 ± 0.29, 0.248</td>
</tr>
<tr>
<td>2. Generalized linear models for CD4 count</td>
<td>n, Δ (Mean ± SE), Adjusted P</td>
<td>n, Δ (Mean ± SE), Adjusted P</td>
</tr>
<tr>
<td>SCs: at VL peak (N=538)b</td>
<td>49, 26 ± 34, 0.443</td>
<td>25, 54 ± 46, 0.239</td>
</tr>
<tr>
<td>SCs: at VL set-point (N=538)</td>
<td>49, 28 ± 35, 0.419</td>
<td>25, 36 ± 48, 0.455</td>
</tr>
<tr>
<td>SCs: repeated measures (3-24 months)</td>
<td>49, 39 ± 30, 0.192</td>
<td>25, 92 ± 40, 0.023</td>
</tr>
<tr>
<td>SPs: at only available visit (N=292)c</td>
<td>38, 80 ± 38, 0.034</td>
<td>21, 183 ± 48, &lt;0.001</td>
</tr>
<tr>
<td>3. Ordinal logistic regression for VLd</td>
<td>n, pOR (95% CI), Adjusted P</td>
<td>n, pOR (95% CI), Adjusted P</td>
</tr>
<tr>
<td>SCs: peak (N=538)c</td>
<td>49, 0.38 (0.21-0.66), &lt;0.001</td>
<td>25, 1.06 (0.47-2.38), 0.893</td>
</tr>
<tr>
<td>SCs: set-point (N=538)</td>
<td>49, 0.54 (0.31-0.95), 0.031</td>
<td>25, 0.68 (0.32-1.44), 0.309</td>
</tr>
<tr>
<td>SPs: at only available visit (N=292)c</td>
<td>38, 0.47 (0.25-0.91), 0.024</td>
<td>21, 0.58 (0.25-1.34), 0.201</td>
</tr>
</tbody>
</table>

a Analysis compares subjects with and without the HLA factor. Potential confounders retained as covariates include age, gender, viral subtype, number of outcome measures, and duration of infection (DOI) for SCs, regardless of statistical significance in each model. For SPs, viral subtype is not available and time after infection is replaced by duration of follow-up (DOF) after enrollment.
b The highest VL within 90 days of infection is analyzed for the acute phase, as reported elsewhere (6).
HLA and Discordance in Manifestations of HIV-1 Infection

CD4+ T-cell counts exceed 200 cells/µL in all SPs. Time interval in all SCs starts from the estimated date of infection (EDI).

Three VL categories, i.e., high (>10^5 copies/mL), medium (10^4-10^5 copies/mL) and low (<10^4 copies/mL), are defined according to their differential impact on HIV-1 transmission (9, 26).
TABLE 4 Multivariable analyses of prospective data from seroconverters (SCs) and cross-sectional data from seroprevalent subjects (SPs) enrolled in southern Africa (Zambia) and eastern Africa (Kenya, Rwanda, and Uganda).

<table>
<thead>
<tr>
<th>Factors in models</th>
<th>SCs from Zambiaa</th>
<th>SCs from eastern Africab</th>
<th>SPs from Zambia</th>
<th>SPs from eastern Africa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ (Mean ± SE)</td>
<td>P</td>
<td>Δ (Mean ± SE)</td>
<td>P</td>
</tr>
<tr>
<td>Female gender</td>
<td>-0.43 ± 0.09</td>
<td>&lt;0.0001</td>
<td>-0.45 ± 0.10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DOI (per week)c</td>
<td>0.002 ± 0.001</td>
<td>0.033</td>
<td>-0.004 ± 0.001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HLA-B*57</td>
<td>-0.41 ± 0.15</td>
<td>0.006</td>
<td>-0.42 ± 0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>-0.47 ± 0.31</td>
<td>0.124</td>
<td>0.04 ± 0.43</td>
<td>&gt;0.500</td>
</tr>
<tr>
<td>HLA-B*81</td>
<td>-0.14 ± 0.20</td>
<td>0.475</td>
<td>-0.46 ± 0.24</td>
<td>0.059</td>
</tr>
<tr>
<td></td>
<td>7 ± 39</td>
<td>&gt;0.500</td>
<td>63 ± 42</td>
<td>0.137</td>
</tr>
<tr>
<td></td>
<td>140 ± 51</td>
<td>0.006</td>
<td>64 ± 59</td>
<td>0.282</td>
</tr>
<tr>
<td></td>
<td>69 ± 23</td>
<td>0.003</td>
<td>89 ± 25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DOI (per week)c</td>
<td>-1 ± 0</td>
<td>&lt;0.0001</td>
<td>-1 ± 0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HLA-B*57</td>
<td>7 ± 39</td>
<td>&gt;0.500</td>
<td>63 ± 42</td>
<td>0.137</td>
</tr>
<tr>
<td></td>
<td>7 ± 39</td>
<td>&lt;0.0001</td>
<td>19 ± 52</td>
<td>&gt;0.500</td>
</tr>
<tr>
<td></td>
<td>140 ± 51</td>
<td>0.006</td>
<td>64 ± 59</td>
<td>0.282</td>
</tr>
</tbody>
</table>

For HIV-1 viral load (VL)

For CD4+ T-cell count

For consistency, age, viral subtype, and time after infection are always treated as covariates in analysis of SCs. For SPs, viral subtype is not available and time after infection is replaced by time after enrollment.

In SCs, analyses test repeated measures of VL and CD4 count in the 3-24 months interval after estimated date of infection; cross-sectional data in SPs are defined in Table 2.

Duration of infection (DOI) is relevant to analysis of SCs (Table 2).
**Figure Legends**

**FIG 1** Local regression (LOESS) curves showing the dynamics of virologic (A) and immunologic manifestations (B) of HIV-1 infection in 538 seroconverters. Measurements for B*57+ and B*81+ subjects are represented by open circles and dark dots, respectively. Overlap between B*57+ and B*81+ subgroups is minimal (two SCs in the B*81+ subgroup have both B*57 and B*81). Other measurements (grey circles) come from the B*57-B*81- subgroup (treated as the reference group). Thick and thin lines correspond to the expected mean (average) values and 95% confidence intervals for each subgroup. CD4 level at 200 cells/μL is also indicated (dotted line). The numbers of subjects remaining at 14 time intervals are also shown.

**FIG 2** CD4⁺ T-cell (CD4) count observed in 538 HIV-1 seroconverters (SCs) and 292 seroprevalent subjects (SPs) stratified by HLA-B*57 and HLA-B*81. In SCs, the acute-phase (peak) CD4 (A) and nadir CD4 count (B) are based on measurements at <3 months and 3-24 months after estimated date of infection, respectively. In SPs with CD4 count >200 cells/μL, the first available CD4 count (C) is tested. Filled circles denote subjects who have both B*57 and B*81. Subjects without B*57 or B*87 (B*57-/B*81- individuals) serve as the reference group. Horizontal bars connected by a vertical line correspond to the mean and standard deviation within each subgroup. Dotted lines (B and C) indicate CD4 = 200 cells/μL.

**FIG 3** Progression to severe immunodeficiency among 538 HIV-1 seroconverters (SCs) without antiretroviral therapy, as defined by Kaplan-Meier curve. Two SCs with both B*57 and B*81 (B*57+/B*81+) are kept in the B*81+ group (for clarity). The first visit with CD4⁺ T-cell count below 350 cells/μL is counted as the event (outcome). The numbers of subjects remaining at eight time points are all within the 0-70 months interval.
FIG 4 Progression to severe immunodeficiency among HIV-1 seroconverters (SCs) from southern Africa (Zambia) (A, n = 219) and eastern Africa (Kenya, Rwanda, and Uganda) (B, n = 319). The analytical strategies are the same as those shown in Figure 3. The Zambian subgroup has two SC with both B*57 and B*81 (B*57+/B*81+) being kept in the B*81+ group (for clarity). The numbers of subjects remaining at eight time points are all within the 0-70 months interval.
FIG 1

A

HIV-1 Viral Load (log₁₀)

13-104 weeks (3-24 months) interval

B

CD4 Count (cells/µl)

Time after Infection (weeks)

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>HIV-1 seroconverters available at 14 time intervals (0-104 weeks, as shown on the X-axis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B<em>57-/B</em>81-</td>
<td>466 466 432 403 360 321 303 263 232 222 209 190 178 157</td>
</tr>
<tr>
<td>B*57+</td>
<td>25 25 23 23 21 21 21 21 19 19 19 19 15 15 12</td>
</tr>
<tr>
<td>B*81+</td>
<td>47 47 46 42 40 37 36 34 29 27 27 22 20 19</td>
</tr>
</tbody>
</table>
FIG 2

A
HIV-1 seroconverters (overall $P = 0.280, N = 538$)

B
HIV-1 seroconverters (overall $P = 0.055, N = 537$)

C
Seroprevalent subjects (overall $P = 0.011, N = 292$)

$P < 0.001$
FIG 3

All seroconverters (N = 538)

Event-free proportions (%)

Log-rank P = 0.015

Time after Infection (months)

---

<table>
<thead>
<tr>
<th>Subjects available at eight time intervals</th>
<th>Subgroup</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 21 16 11 8 8 7 2</td>
<td>B*81+</td>
</tr>
<tr>
<td>47 37 23 19 9 6 4 3</td>
<td>B<em>57+/B</em>81-</td>
</tr>
<tr>
<td>466 314 191 125 81 50 33 9</td>
<td>B<em>57-/B</em>81-</td>
</tr>
</tbody>
</table>
FIG 4

A

Seroconverters from Zambia (N = 219)

Event-free proportions (%)

Log-rank P = 0.002

Time after infection (months)

B*81+ B*57+ B*81-

B

Seroconverters from other countries (N = 319)

Event-free proportions (%)

Log-rank P = 0.403

Time after infection (months)

B*57+ B*81- B*81+

Subjects available at eight time intervals Subgroup

11 11 10 11 7 5 5 1 B*81+
19 12 7 6 3 2 1 1 B*57+/B*81-
189 118 58 33 23 17 13 7 B*57-/B*81-