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ABSTRACT
In individuals with HIV-1 infection, depletion of CD4+ T cells is often accompanied by a malfunction of CD8+ T cells that are persistently activated and/or exhausted. While the dynamics and correlates of CD8 counts have been well documented, the same does not apply to CD8 counts. Here, we examined the CD8 counts in a cohort of 497 Africans with primary HIV-1 infection evaluated in monthly to quarterly follow-up visits for up to 3 years in the absence of antiretroviral therapy. Statistical models revealed that (i) CD8 counts were relatively steady in the 3- to 36-month period of infection and similar between men and women; (ii) neither geography nor heterogeneity in the HIV-1 set-point viral load could account for the roughly 10-fold range of CD8 counts in the cohort (P > 0.25 in all tests); and (iii) factors independently associated with relatively high CD8 counts included demographics (age ≤ 40 years, adjusted P = 0.010) and several human leukocyte antigen class I (HLA-I) alleles, including HLA-A*03:01 (P = 0.013), B*15:10 (P = 0.007), and B*58:02 (P < 0.001). Multiple sensitivity analyses provided supporting evidence for these novel relationships. Overall, these findings suggest that factors associated with the CD8 count have little overlap with those previously reported for other HIV-1-related outcome measures, including viral load, CD4 count, and CD4/CD8 ratio.

IMPORTANCE
Longitudinal data from 497 HIV-1 seroconverters allowed us to systematically evaluate the dynamics and correlates of CD8+ T-cell counts during untreated primary HIV-1 infection in eastern and southern Africans. Our findings suggest that individuals with certain HLA-I alleles, including A*03 (exclusively A*03:01), persistently maintain relatively high CD8 counts following HIV-1 infection, a finding which may offer an intriguing explanation for the recently reported, negative association of A*03 with HIV-1-specific, broadly neutralizing antibody responses. In future studies, attention to HLA-I genotyping data may benefit in-depth understanding of both cellular and humoral immunity, as well as the intrinsic balances of these types of immunity, especially in settings where there is emerging evidence of antigenicity between the two arms of adaptive immunity.
and examine the dynamics and correlates of CD8 counts before ART initiation, especially in regions where such data remain sparse.

Our findings, based on evaluations of 497 HIV-1-infected Africans with multiple pre-ART visits, now suggest that the independent correlates of the CD8 count have little overlap with those previously seen with the set-point VL, CD4 count, and CD4/CD8 ratio. The underlying biology deserves further investigation and may have implications beyond cellular immunity.

MATERIALS AND METHODS

Study population, laboratory techniques, and outcome measures. Our work here focused on 497 HIV-1 seroconverters (SCs) from Kenya, Rwanda, Uganda, and Zambia who were enrolled under a uniform study protocol developed and implemented by the International AIDS Vaccine Initiative (IAVI). The study design and research procedures, including written informed consent and laboratory testing (e.g., viral sequencing and HLA genotyping), were approved by the institutional review boards at IAVI, Emory University, and the University of Alabama at Birmingham. Clinical and laboratory tests, including centralized, T-cell immunophenotyping during monthly to quarterly follow-up visits, have been described in detail elsewhere (16, 30–33). ART initiation followed appropriate national guidelines (34), but post-ART data were too sporadic (limited to 56 person visits) to allow meaningful analysis. To facilitate a direct comparison with earlier statistical models for establishing correlates of the set-point VL, CD4 count, and CD4/CD8 ratio in primary HIV-1 infection (16, 32, 33), the SCs included in this study must have had at least three virologic and immunologic outcome measures in the 3- to 24-month period after the estimated date of infection (EDI). In addition, all SCs had fully resolved HLA-1 genotypes, as also reported earlier (16, 32, 33).

Statistical analysis. Using software packages in SAS, version 9.4 (SAS Institute, Cary, NC), data analyses focused on pre-ART CD8 counts, with further consideration being given to earlier work that analyzed the pre-ART VL, CD4 count, and CD4/CD8 ratio (16, 32, 33). We began with a full assessment of log10-transformed CD8 counts in the 3- to 36-month period after EDI, using Pearson’s correlation coefficients (r), local regression (LOESS) curves, mixed models for repeated measurements, analysis of variance (ANOVA) of cross-sectional data (i.e., visit-specific data or mean CD8 counts over a given time period), and logistic regression models for cross-sectional data. Association analyses targeted HLA variants that were adequately prevalent (present in ≥5% of the study population), with a focus being on individual alleles that met two thresholds of statistical significance, i.e., a P value of <0.05 and a q value of <0.10. Summary statistics included (i) P values and associated false discovery rates (FDR; q values) when multiple testing was applied and (ii) the effect size of individual factors on the CD8 count, as measured by mean regression beta estimates (Δ), the standard error (SE) of Δ, and the degree of variance explained by each factor (R²). In multivariable models, statistical adjustments were made for demographics (sex and age), geography (eastern versus southern Africa), and three categories (low, medium, and high) of the set-point VL that have clinical and epidemiological implications (32). The final statistical models were also subjected to sensitivity analyses that were restricted to data from the 3- to 24-month period after EDI. For individual correlates of the CD8 count (with log10 transformation), the statistical significance was accepted at the level of a P value of <0.05 and a q value of <0.10 in the initial screening models, followed by an adjusted P value of <0.05 in multivariable tests.

Bioinformatics. Several public databases were surveyed for following evidence of genomics data pertinent to HLA/major histocompatibility complex (MHC) gene expression and effective tagging of individual HLA alleles by single nucleotide polymorphisms (SNPs). Specifically, MHC SNPs that tag HLA class I alleles in Africans (35) were first queried in HaploReg, version 4.0 (http://www.broadinstitute.org/mammals/haploreg/haploreg_v4.php, last accessed on 2 September 2016) (36) for patterns of linkage disequilibrium (LD) uncovered by The 1000 Genomes Project and for functional properties annotated by the ENCODE project (37, 38). SNPs already associated with immune disorders and/or gene expression quantitative trait loci (eQTLs) (39) were checked in the NCBI Global Cross database (http://www.ncbi.nlm.nih.gov/) and the SCAN database (http://www.scandb.org/newinterface/index.html, last accessed on 11 March 2016). Findings on HLA-1 variants were interpreted in light of these bioinformatics data, with further reference being made to a panel of fine-mapped, causal SNPs linked to various genome-wide association studies (40).

RESULTS

Steady CD8 counts in 497 SCs. In the 3 to 36 months after EDI, CD8 counts were available for a total of 4,131 person visits. Overall, CD8 counts ranged from 2.40 to 3.30 log10 (a roughly 10-fold range), being relatively stable within individuals and similar between 185 women and 312 men (P > 0.60) (Fig. 1). For example, the linear correlation between the first CD8 count after 3 months of infection and the last count before 36 months was quite strong for both men (Pearson r = 0.77, P < 0.0001) and women (r = 0.78, P < 0.0001). Evaluation of other demographic features revealed that longitudinal CD8 counts differed between individuals.
in two age groups ($P = 0.027$ for individuals $>40$ years old versus individuals $\leq 40$ years old) but were similar between individuals from eastern and southern Africa ($P = 0.47$). Differences between age groups were confirmed by analysis of the mean CD8 counts (with logarithmic transformation) during the 3- to 36-month intervals ($P = 0.018$) (Fig. 2).

**HLA variants as genetic correlates of CD8 counts.** In the study cohort, 12 HLA-A, 16 HLA-B, and 9 HLA-C variants had to be observed in at least 25 (5%) individuals to facilitate statistical screening for potential associations with repeated measures of CD8 counts (Table 1). After statistical adjustments for potential confounding by age, sex, geography, and duration of HIV-1 infection, individual alleles that met the thresholds of statistical significance included A*03 (exclusively A*03:01 ($\Delta = 0.08 \pm 0.03$, $P = 0.003$, $q = 0.048$), B*15:10 ($\Delta = 0.06 \pm 0.02$, $P = 0.005$, $q = 0.059$), and B*58:02 ($\Delta = 0.07 \pm 0.02$, $P < 0.001$, $q = 0.015$). The only variant that appeared to have a negative impact on CD8 counts was B*58:01, but the borderline statistical significance ($P = 0.049$) had a high probability of false discovery ($q = 0.394$) (Table 1).

Visualization using LOESS curves indicated steady differences between subjects with and without these HLA variants (e.g., for the A*03-positive [A*03 $^+$] versus A*03-negative [A*03 $^-$] groups in Fig. 3). In multivariable models, all three genetic correlates were independent of other potential confounders (adjusted $P$-value range, $<0.001$ to 0.013) (Table 2). An alternative model for mean CD8 counts led to almost identical results for the HLA variants of interest (adjusted $P$-value range, $<0.001$ to 0.013) (Table 2). In contrast, both statistical models failed to detect differences in CD8 counts that could be attributed to the three HIV-1 VL groups (adjusted $P$-value range, 0.251 to 0.795).

**Supporting evidence from sensitivity analyses.** When analyses were restricted to the 3- to 24-month period after EDI, the multivariable model for repeated outcome measurements (3,440 person visits) also supported the independent associations between CD8 counts and A*03 (adjusted $\Delta = 0.06 \pm 0.03$, $P = 0.019$), B*15:10 (adjusted $\Delta = 0.07 \pm 0.02$, $P = 0.003$), and B*58:02 (adjusted $\Delta = 0.07 \pm 0.02$, $P < 0.001$) (Table 3), as did the alternative model for mean CD8 counts (adjusted $P$-value range, $<0.001$ to 0.019 as well) (Table 3). Again, the variance in mean CD8 counts was not attributable to distinct VI groups (adjusted $P$-value range, 0.340 to 0.527).

**No clear additive effects of three HLA factors.** In the study cohort, 26 SCs had a combination of A*03, B*15:10, and B*58:02. The mean CD8 counts over the 3- to 36-month intervals were found to be the highest in this small subgroup when the counts were compared with those in SCs with a single HLA factor and the reference group (all others) without any HLA variants of interest (Fig. 4) ($P < 0.0001$ by ANOVA), but the difference between the first two subgroups was modest ($P = 0.281$ by t test). The mean CD8 counts over the 3- to 24-month intervals yielded similar results ($P = 0.272$ for multiple alleles versus a single allele).

**Findings based on bioinformatics.** In populations of African ancestry (35), HLA-A*03:01 is known to be tagged by rs2524024, a SNP that is distant (30 kb away) from the 5’ end of HLA-A, while B*15:10 is tagged by two SNPs, rs3819294 (an HLA-B intronic SNP) and rs2523638 (a SNP between DHFRP2 and MICA). These SNPs are also in strong LD with multiple neighboring variants, including eQTLs associated with gene expression profiles in Afri-
TABLE 2 Correlates of CD8 counts in 497 HIV-1 SCs revealed by two multivariable models

<table>
<thead>
<tr>
<th>Individual factors in each model</th>
<th>For repeated measurements&lt;sup&gt;a&lt;/sup&gt;</th>
<th>For mean CD8 counts&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ ± SE P</td>
<td>Δ ± SE P</td>
</tr>
<tr>
<td>Age &gt;40 (n = 75) vs ≤40 yr (n = 422)</td>
<td>−0.05 ± 0.02 0.010</td>
<td>−0.05 ± 0.02 0.007</td>
</tr>
<tr>
<td>Women (n = 185) vs men (n = 312)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00 ± 0.02 0.913</td>
<td>0.00 ± 0.02 0.915</td>
</tr>
<tr>
<td>Zambia (southern Africa, n = 195)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>−0.01 ± 0.01 0.660</td>
<td>−0.01 ± 0.02 0.685</td>
</tr>
<tr>
<td>Low VL (n = 140)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>−0.01 ± 0.02 0.588</td>
<td>−0.00 ± 0.02 0.795</td>
</tr>
<tr>
<td>High VL (n = 92)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>−0.02 ± 0.02 0.251</td>
<td>−0.01 ± 0.02 0.517</td>
</tr>
<tr>
<td>Duration of infection</td>
<td>−0.07 ± 0.01 &lt;0.0001</td>
<td>NA NA</td>
</tr>
<tr>
<td>HLA-A*03:01 (n = 45)</td>
<td>0.06 ± 0.03 0.013</td>
<td>0.06 ± 0.03 0.013</td>
</tr>
<tr>
<td>HLA-B*15:10 (n = 55)</td>
<td>0.06 ± 0.02 0.007</td>
<td>0.06 ± 0.02 0.010</td>
</tr>
<tr>
<td>HLA-B*58:02 (n = 72)</td>
<td>0.07 ± 0.02 &lt;0.0001</td>
<td>0.07 ± 0.02 &lt;0.0001</td>
</tr>
</tbody>
</table>

<sup>a</sup> CD8 counts in the 3- to 36-month period after the estimated date of infection with log10 transformation. NA, not applicable.

<sup>b</sup> For consistency with earlier reports, these factors were retained in the models because they were associated with the HIV-1 viral load and CD4 counts in the same cohort.

<sup>c</sup> Compared with subjects from other countries in eastern Africa (n = 302).

<sup>d</sup> The three HIV-1 VL categories are defined as low (<10^4 RNA copies/ml), medium (10^4 to 10^5 RNA copies/ml), and high (>10^5 RNA copies/ml), according to their differential impact on HIV-1 transmission and disease progression (7, 37), with the medium VL (n = 263) being the reference group for comparison.

FIG 3 Local regression curves for CD8 counts in seroconverters with and without HLA-A*03 (3 to 36 months after the estimated date of HIV-1 infection). For each stratum (presence and absence of A*03), solid and dotted lines correspond to the mean values and 95% confidence intervals, respectively.

FIG 4 Lack of additive effect of HLA variants on CD8 counts. The mean CD8 counts (3 to 36 months after the estimated date of HIV-1 infection) in three subgroups of HIV-1 seroconverters were compared on the basis of the presence and absence of three HLA variants of interest (A*03, B*15:10, and B*58:02). Heterogeneity among the three groups is evident (P < 0.001 for the overall comparison).

Traditionally, genetic analyses have been conducted in small patient cohorts and have been limited to the role of HLA alleles in terms of their association with HIV disease progression. However, the recent availability of high-throughput SNP genotyping platforms has provided sufficient coverage of all three HLA alleles being highlighted here.

**GENETIC EVOLUTION FROM OTHER STUDIES.** At least two studies have examined the genetic impact on CD8 T-cell counts in human populations. In one study from Australia and the UK (45), a SNP (rs2524054) located in an intergenic region between HLA-B and HLA-C was associated with absolute CD8 T-cell counts in the general population. However, rs2524054 (close to HLA-B) is not known to tag specific HLA alleles in Africans (35). Instead, it is part of a sequence motif that has potential regulatory function, as reflected by its association with eight quantitative (gene expression) traits. Strong LD between rs2524054 and two downstream SNPs (rs2524143 and rs2853928) precludes a definitive mechanism, but HLA-B gene expression might be a possible connection (45). On the other hand, the relationship between an HLA-A*03-related MHC haplotype and CD8 T-cell counts was inconclusive for highly selected patients with hereditary hemochromatosis (iron overload) from three geographically distant regions (46).

**DISCUSSION.** Our analyses of longitudinal data from HIV-1-infected Africans suggest that CD8 T-cell counts have characteristics that differ...
starkly from those of two other commonly studied outcomes, i.e., the HIV-1 VL and CD4 T-cell counts. First, unlike the VL and CD4 counts, which often differ by sex and geography (a proxy for viral subtypes) (32, 33, 47), CD8 counts and their trajectories during primary HIV-1 infection are similar between men and women and between eastern and southern Africans, which can substantially simplify the search for generalizable and biological correlates using aggregated (instead of stratified) data (47). Second, despite their narrow ranges, log_{10}-transformed CD8 counts are informative quantitative traits for various statistical modeling, as multiple factors associated with CD8 counts can be established. Third, HLA variants (A*03:01, B*15:10, and B*58:02) associated with CD8 counts have little or no overlap with those (e.g., B*18, B*45, B*53, B*57, and B*81) previously reported for VL and CD4 counts in the same study cohort (32, 33, 47), suggesting that the underlying mechanisms should be distinct and may even precede HIV-1 infection (i.e., through intrinsic functions). Analyses of similar data from other cohorts should facilitate a better understanding of CD8 T-cell function in HIV-1 infection and in general populations (45).

Although they were statistically significant in the overall analyses and robust in sensitivity models, the effects of three HLA variants on CD8 counts were all relatively modest during the study analyses and robust in sensitivity models, the effects of three HLA variants (A*03:01, B*15:10, and B*58:02) associated with CD8 counts have little or no overlap with those (e.g., B*18, B*45, B*53, B*57, and B*81) previously reported for VL and CD4 counts in the same study cohort (32, 33, 47), suggesting that the underlying mechanisms should be distinct and may even precede HIV-1 infection (i.e., through intrinsic functions). Analyses of similar data from other cohorts should facilitate a better understanding of CD8 T-cell function in HIV-1 infection and in general populations (45).

The frequencies of HLA-I alleles being highlighted in this study ranged from 9% to 14% in our study cohort (Table 1). Collectively, they were found in over 29% of subjects (Fig. 4). The distribution of these alleles in other ethnic groups can vary, but A*03:01 is a globally common allele and should be readily analyzed in other cohorts, including general populations where CD8 T-cell counts are measured (45, 55). Overall, our findings should broaden the attention to immunogenetic factors, since variability in CD8 counts before antiretroviral therapy may relate to the function of multiple HLA-I variants. This concept can be equally pertinent to studies of CD8 T-cell function after antiretroviral therapy (36).

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