

Dynamics and Correlates of CD8 T-cell Counts in Africans with Primary Human Immunodeficiency Virus Type 1 Infection

Running title: CD8 T-cell counts in primary HIV-1 infection

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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 CD4 counts have been well-documented, the same does not apply to CD8 counts. Here, we examined CD8 counts in a cohort of 497 Africans with primary HIV-1 infection and monthly to quarterly follow-up visits for up to three years in the absence of antiretroviral therapy. Statistical models revealed that i) CD8 counts were relatively steady in the 3-36 months period of infection and similar between men and women; ii) neither geography nor heterogeneity in 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 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 have 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 a relatively high CD8 counts following HIV-1 infection, 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 their intrinsic balances, especially in settings where there is emerging evidence of antagonism between the two arms of adaptive immunity.

CD8⁺ cytotoxic T-lymphocytes (CTLs) are critical to early immune control of HIV-1 infection, and many studies have documented the dynamics and evolution of HIV-1-specific CTLs that target viral epitopes in the context of differential presentation (restriction) by the highly variable human leukocyte antigen class I (HLA-I) molecules (1-5). More often than not, immune protection provided by CTLs is transient, as CTL escape mutations are abundant in the circulating viruses, even in the presence of favorable HLA-I variants like B*57 and B*81 (6-8). Concomitantly, depletion of CD4⁺ helper T-cells can exacerbate the losing battle for CTLs, leading to the accumulation of activated and exhausted CD8 cells (9-13), as well as a persistent reversion of CD4:CD8 T-lymphocyte ratio (14-16). Moreover, the orchestration of cellular and humoral immunity can be problematic when CTL impairment occurs early, as broadly neutralizing antibodies usually take years to develop (17-19).

In the clinical realm, attention to the dynamics and functions of CD8 cells *per se* has been rather limited, as much of the decision-making process relies almost exclusively on HIV-1 viral load (VL) and CD4⁺ T-cell (CD4) counts following diagnosis of HIV-1 infection. However, the new era of early and intensified antiretroviral therapy (ART) is likely to change this paradigm for three reasons. First, CD4 count alone is unable to fully gauge immunologic health after ART (20-22). Second, CD8 cells are essential to the eradication of residual HIV-1 reservoirs after ART initiation (23-26). Third, CD8 cells can be induced to enhance the efficacy of vaccination (27), as reported recently in nonhuman primate models (28, 29). To this end, it is worthwhile to take a step back and examine the dynamics and correlates of CD8 counts before ART initiation, especially in regions where such data remain sparse.

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

Methods

Study population, laboratory techniques, and outcome measures. Our work here focused on 497 HIV-1 seroconverters (SCs) who were enrolled from Kenya, Rwanda, Uganda and Zambia 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 institutional review boards at IAVI, Emory University and 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 set-point VL, CD4 count, and CD4:CD8 ratio in primary HIV-1 infection (16, 32, 33), SCs included in this study must have at least three virologic and immunologic outcome measures in the 3-24 months period after the estimated date of infection (EDI). In addition, all SCs had fully resolved HLA-I 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 of earlier work that analyzed 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 3-36 months period after EDI, using Pearson's correlation coefficients (r), local regression (LOESS) curves, mixed models for repeated measurements, analysis of variance (ANOVA) in 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 $\geq 5\%$ of study population), with a focus on individual alleles that met two thresholds of statistical significance, i.e., $P < 0.05$, $q < 0.10$. Summary statistics included: i) P values and associated false discovery rates (FDR, q) when multiple testing was applied, ii) effect size of

individual factors on CD8, as measured by mean regression beta estimates (Δ), the standard error (SE) of Δ , and the degree of variance explained by each factor (R^2). 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 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 3-24 months period after EDI. For individual correlates of CD8 count (with \log_{10} -transformation), the statistical significance was accepted at the level of $P < 0.05$ and $q < 0.10$ in the initial screening models, followed by an adjusted $P < 0.05$ in multivariable tests.

Bioinformatics. Several public databases were surveyed for supporting evidence of genomics data pertinent to HLA/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 September 2, 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 March 11, 2016). Findings on HLA-I 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 3-36 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 \log_{10} (a roughly 10-fold range),

being relatively stable within individuals and similar between 185 women and 312 men ($P > 0.60$) (Figure 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 two age groups ($P = 0.027$ for >40 versus ≤ 40 years old) but were similar between eastern and southern Africa ($P = 0.47$). Differences between age groups were confirmed by analysis of mean CD8 counts (with \log_{10} -transformation) during the 3-36 months intervals ($P = 0.018$) (Figure 2).

HLA variants as genetic correlates of CD8 counts. In the study cohort, 12 *HLA-A*, 16 *HLA-B*, and 9 *HLA-C* variants were 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) (adjusted $\Delta = 0.08 \pm 0.03$, $P = 0.003$, $q = 0.048$), B*15:10 (adjusted $\Delta = 0.06 \pm 0.02$, $P = 0.005$, $q = 0.059$), and B*58:02 (adjusted $\Delta = 0.07 \pm 0.02$, $P < 0.001$, $q = 0.015$). 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., Figure 3 for A*03+ versus A*03- groups). In multivariable models, the three genetic correlates were all independent of other potential confounders (adjusted P values ranging from <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 values ranging from <0.001 to 0.013) (Table 2). In contrast, both statistical models failed to detect CD8 differences that could be attributed to three HIV-1 VL groups (adjusted $P = 0.251$ -0.795).

Supporting evidence from sensitivity analyses. When analyses were restricted to the 3-24 months 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 ($\Delta = 0.07 \pm 0.02$, $P = 0.003$), and B*58:02 ($\Delta = 0.07 \pm 0.02$, $P < 0.001$) (Table 3), so did the alternative model for mean CD8 counts (adjusted P values ranging from <0.001 to 0.019 as well) (Table 3). Again, variance in mean CD8 counts was not attributable to distinct VL groups (adjusted $P = 0.340-0.527$).

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 3-36 months intervals were the highest in this small subgroup when compared with SCs with a single HLA factor and the reference group (all others) without any HLA variants of interest (Figure 4) ($P < 0.0001$ in ANOVA), but the difference between the first two subgroups was modest ($P = 0.281$ by t -test). The mean CD8 counts over the 3-24 months intervals yielded similar results ($P = 0.272$ for multiple versus 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 Africans, but none of them have been associated with outcomes related to HIV-1 infection (41-43). On the other hand, B*58:02 as a somewhat unfavorable allele in HIV-1 infection (44) has no strong LD with any neighboring SNP variants. Thus, high-throughput SNP genotyping platforms are not expected to provide sufficient coverage of all three HLA alleles being highlighted here.

Genetic evidence from other studies. At least two studies have examined the genetic impact on CD8 T-cell counts in human populations (45, 46). In study cohorts from Australia and 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 HLA-A*03-related MHC haplotype and CD8 T-cell counts was inconclusive for highly selected hereditary hemochromatosis (iron overload) patients 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 two other commonly studied outcomes, i.e., HIV-1 VL and CD4 T-cell counts. First, unlike VL and CD4 counts that 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, log₁₀-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 statistically significant in the overall analyses and robust in sensitivity models, the effects of three HLA variants on CD8 counts are all relatively modest during the study intervals (Tables 1-2), mostly within a magnitude of 15-17% difference (0.06-0.07 log₁₀). The biological consequences may depend on the longevity of these seemingly minor differences and the subsets of CD8 T-cells that are mostly affected. Earlier research has suggested that steady CD8 T-cell counts during chronic HIV-1 infection may reflect a prolonged differentiation rather than elevated activation (9). This long-lasting phenomenon may indirectly impair other arms of immune responses, at least in individuals with HLA-A*03 (exclusively A*03:01 in the study cohort) because this allele is enriched in subjects who did not develop HIV-1-specific, broadly neutralizing antibody responses (48). Assuming that antagonism and competition do exist between cellular and humoral arms of adaptive immunity, especially in lymphoid tissues where both space and resources are limited (49, 50), one can also envision that HLA alleles B*15:10 and B*58:02 may operate in a similar fashion. Meta-analyses of data from different studies should offer new insights into this new hypothesis. Indeed, a recently reported association between HLA-A*02 and enhanced humoral (IgG) responses to HIV-1 vaccination (the RV144 trial in Thailand) (51) may be viewed as an anecdotal evidence for this hypothesis, although it is still not clear if such conclusions can apply to various populations that differ in HLA-I allelic profiles and/or allele frequencies.

Previously, a genome-wide association study (45) identified a single SNP (rs2524054) as a major correlate of CD8 counts in healthy adolescent twins from Australia (effect size = -0.31 ± 0.03 log₁₀). Located between *HLA-C* and *HLA-B*, rs2524054 has some functional attributes (gene expression patterns), but there is no indication that rs2524054 tags specific HLA-I alleles (35) or SNPs (rs2524024, rs3819294, and rs2523638) that are in strong LD with A*03:01 and B*15:10. Recent fine-mapping data

do suggest that LD between rs2524054 and a functional (causal) SNP variant (rs2247056-T) can account for the association of rs2524054 with serum triglycerides in healthy subjects (40). Although fine-mapping can be influenced by ethnic backgrounds, a focus on gene expression and lipid metabolisms is expected to expedite future research on immunogenetic control of CD8 T-cell function in health and diseases.

On the other hand, the positive impact of B*58:02 on CD8 counts is not complicated by neighboring SNPs (35). In several studies of HIV-1-infected Africans (52-54), B*58:02 has been recognized as unfavorable (associated with high viral load and low CD4 counts), being functionally and epidemiologically distinct from another closely related allele, B*58:01 (52-54). By our analysis, B*58:01 and B*58:02 do seem to have opposing impact on CD8 counts, but statistical power in our study favors the analysis of B*58:02 rather than B*58:01 (72 versus 55 subjects in our cohort). A more definitive conclusion will obviously require a larger sample size to strengthen the analysis of B*58:01.

One major limitation in this study is the lack of CD8 data before HIV-1 infection and after ART initiation. As our study cohort was designed for the evaluation of primary HIV-1 infection, pre-infection and post-ART data from other study populations will help assess the relationships between HLA-I alleles and the dynamics of CD8 counts in Africans. For example, a hematology reference panel has included CD8 counts in 2,105 healthy subjects from eastern and southern Africa (55). Preparation for vaccine trials may justify HLA-I genotyping in this large study population. Meanwhile, assembling a prospective post-ART dataset will likely require years of concerted efforts, as the implementation of new guidelines for early HIV-1 therapy has been a slow process.

The frequencies of HLA-I alleles being highlighted in this study range from 9% to 14% in our study cohort (Table 1). Collectively, they are found in over 29% of subjects (Figure 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 (56).

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TABLE 1 Univariable analyses of major HLA-I variants for potential association with log₁₀-transformed CD8 counts (repeated measures in 497 HIV-1 seroconverters, during the 3-36 months period after EDI).

HLA variants	<i>n</i>	Frequency	Impact ($\Delta \pm \text{SE}$)	Adjusted <i>P</i> ^a	FDR (<i>q</i>)
A*01	62	0.12	0.02 ± 0.02	0.305	0.727
A*02	173	0.35	0.01 ± 0.02	0.678	0.784
A*03 (*03:01)	45	0.09	0.08 ± 0.03	0.003	0.048
A*23	75	0.15	-0.01 ± 0.01	0.605	0.747
A*29	47	0.09	-0.02 ± 0.02	0.432	0.727
A*30	177	0.36	0.01 ± 0.02	0.427	0.727
A*33	25	0.05	0.03 ± 0.03	0.357	0.727
A*34	36	0.07	-0.04 ± 0.03	0.145	0.619
A*36	41	0.08	-0.04 ± 0.03	0.179	0.653
A*66	32	0.06	-0.02 ± 0.03	0.517	0.747
A*68 (mostly *68:02)	121	0.24	0.00 ± 0.02	0.945	0.945
A*74	63	0.13	-0.01 ± 0.02	0.609	0.747
B*07	66	0.13	-0.01 ± 0.02	0.761	0.853
B*14	46	0.09	0.03 ± 0.03	0.262	0.727
B*15:03	87	0.17	0.02 ± 0.02	0.393	0.727
B*15:10	55	0.11	0.06 ± 0.02	0.005	0.059
B*15:xx (other B*15s)	24	0.05	0.00 ± 0.03	0.932	0.945
B*18	34	0.07	-0.01 ± 0.03	0.626	0.747
B*35 (*35:01)	28	0.06	0.01 ± 0.03	0.838	0.912
B*42	66	0.13	-0.02 ± 0.02	0.432	0.727
B*44	51	0.1	-0.02 ± 0.02	0.343	0.727
B*45	81	0.16	0.00 ± 0.02	0.866	0.916
B*49	38	0.08	0.04 ± 0.03	0.143	0.619
B*53	94	0.19	-0.01 ± 0.02	0.543	0.747
B*57	46	0.09	-0.04 ± 0.02	0.074	0.458
B*58:01	55	0.11	-0.05 ± 0.02	0.049	0.394
B*58:02	72	0.14	0.07 ± 0.02	<0.001	0.015
B*81	25	0.05	-0.02 ± 0.03	0.581	0.747
C*02	91	0.18	-0.01 ± 0.02	0.549	0.747
C*03	71	0.14	0.04 ± 0.02	0.053	0.394
C*04	158	0.32	-0.01 ± 0.02	0.589	0.747
C*06	146	0.29	0.02 ± 0.02	0.151	0.619
C*07	179	0.36	-0.02 ± 0.02	0.279	0.727
C*08	63	0.13	0.02 ± 0.02	0.402	0.727
C*16	67	0.13	-0.02 ± 0.02	0.282	0.727
C*17	80	0.16	-0.01 ± 0.01	0.589	0.747
C*18	42	0.08	-0.03 ± 0.03	0.194	0.653

^a Adjusted for demographics (age, sex, and geography), as well as duration of infection.

527 **TABLE 2** Correlates of CD8 counts in 497 HIV-1 seroconverters, as revealed by two multivariable models.

Individual factors in each model	For repeated measurements ^a		For mean CD8 counts ^a	
	$\Delta \pm \text{SE}$	<i>P</i>	$\Delta \pm \text{SE}$	<i>P</i>
Age >40 (<i>n</i> = 75) vs. ≤40 years (<i>n</i> = 422)	-0.05 ± 0.02	0.010	-0.05 ± 0.02	0.007
Women (<i>n</i> = 185) vs. men (<i>n</i> = 312) ^b	0.00 ± 0.02	0.913	0.00 ± 0.02	0.915
Zambia (southern Africa, <i>n</i> = 195) ^{b,c}	-0.01 ± 0.01	0.660	-0.01 ± 0.02	0.685
Low VL (<i>n</i> = 140) ^d	-0.01 ± 0.02	0.588	-0.00 ± 0.02	0.795
High VL (<i>n</i> = 92) ^d	-0.02 ± 0.02	0.251	-0.01 ± 0.02	0.517
Duration of infection (per quarter)	-0.07 ± 0.01	<0.0001	NA	NA
HLA-A*03:01 (<i>n</i> = 45)	0.06 ± 0.03	0.013	0.06 ± 0.03	0.013
HLA-B*15:10 (<i>n</i> = 55)	0.06 ± 0.02	0.007	0.06 ± 0.02	0.010
HLA-B*58:02 (<i>n</i> = 72)	0.07 ± 0.02	<0.001	0.07 ± 0.02	<0.001

528
529 ^a CD8 counts in the 3-36 months period after estimated date of infection, with log₁₀-transformation. NA, not
530 applicable.

531 ^b For consistency with earlier reports, these factors are retained in the models because they have been
532 associated with HIV-1 viral load and CD4 counts in the same cohort.

533 ^c When compared with subjects from other countries in eastern Africa (*n* = 302).

534 ^d Three HIV-1 viral load (VL) categories are defined as low (<10⁴ RNA copies/mL), medium (10⁴-10⁵
535 copies/mL), and high (>10⁵ copies/mL), according to their differential impact on HIV-1 transmission and
536 disease progression (7, 57), with medium VL (*n* = 265) as the reference group for comparison.

537 **TABLE 3** Sensitivity analyses: two multivariable models for CD8 counts in the 3-24 months period after
 538 estimated date of infection.

Individual factors in each model ^a	For repeated measurements ^a		For mean CD8 counts ^a	
	$\Delta \pm \text{SE}$	<i>P</i>	$\Delta \pm \text{SE}$	<i>P</i>
Age >40 (<i>n</i> = 75) vs. ≤40 years (<i>n</i> = 422)	-0.05 ± 0.02	0.010	-0.05 ± 0.02	0.008
Women (<i>n</i> = 185) vs. men (<i>n</i> = 312)	0.01 ± 0.02	0.721	0.01 ± 0.02	0.725
Zambia (southern Africa, <i>n</i> = 195)	-0.01 ± 0.02	0.552	-0.01 ± 0.02	0.541
Low VL (<i>n</i> = 140)	-0.01 ± 0.02	0.527	-0.01 ± 0.02	0.497
High VL (<i>n</i> = 92)	-0.02 ± 0.02	0.340	-0.02 ± 0.02	0.384
Duration of infection (per quarter)	-0.07 ± 0.01	<0.0001	NA	NA
HLA-A*03:01 (<i>n</i> = 45)	0.06 ± 0.03	0.019	0.06 ± 0.03	0.019
HLA-B*15:10 (<i>n</i> = 55)	0.07 ± 0.02	0.003	0.07 ± 0.02	0.004
HLA-B*58:02 (<i>n</i> = 72)	0.07 ± 0.02	<0.001	0.07 ± 0.02	<0.001

539
 540 ^a As shown in Table 2.

Figure Legends

FIG 1. Local regression curves for CD8 counts in seroconverted men and women (3-36 months after estimated date of HIV-1 infection). Solid and dotted lines correspond to the mean values and 95% confidence intervals, respectively ($P > 0.60$ between the two patient groups).

FIG 2. Mean CD8 counts in HIV-1 seroconverters defined by two age groups.

FIG 3. Local regression curves for CD8 counts in seroconverters with and without HLA-A*03 (3-36 months after after 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. Mean CD8 counts (3-36 months after estimated date of HIV-1 infection) are compared in three subgroups of HIV-1 seroconverters based on 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).

FIG 1

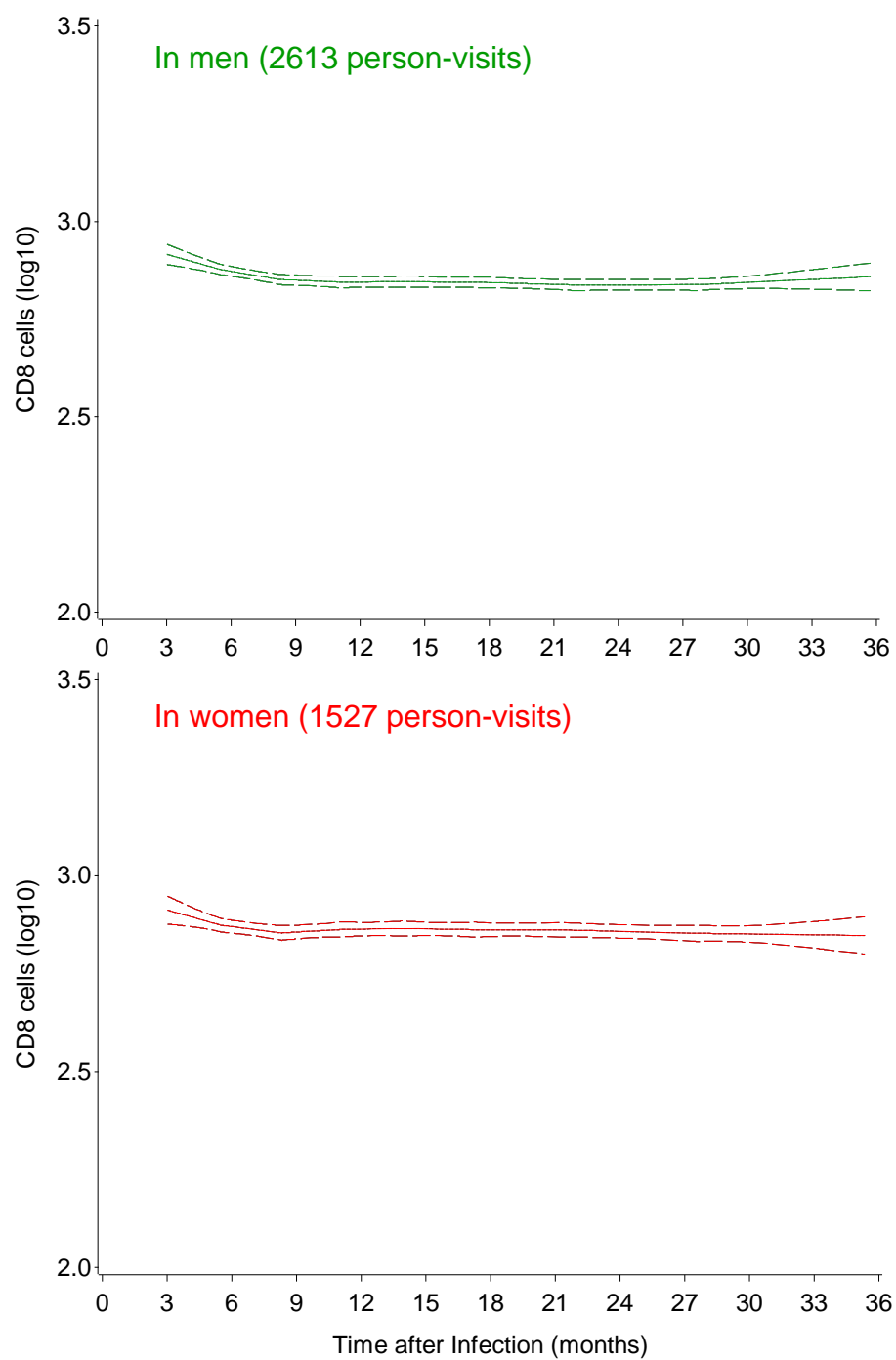


FIG 2

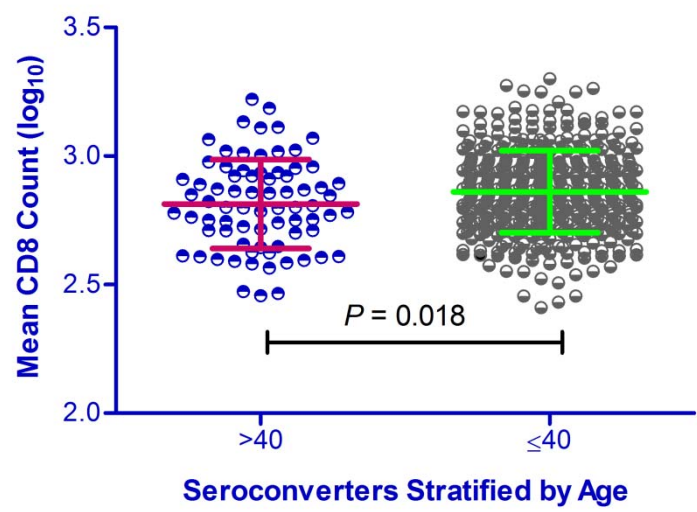


FIG 3

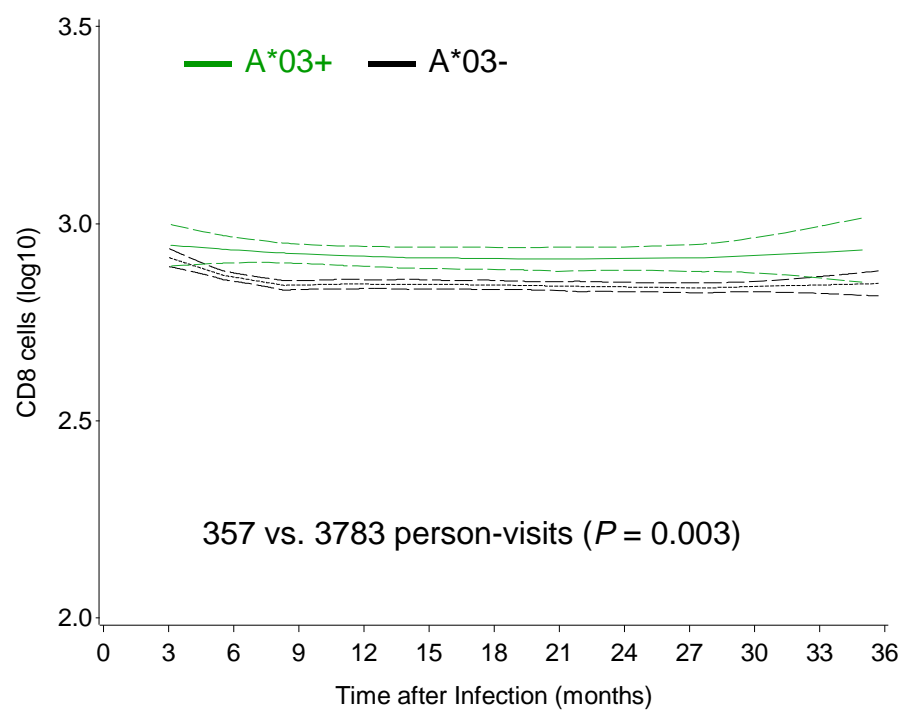


FIG 4

