



EMORY
LIBRARIES &
INFORMATION
TECHNOLOGY

OpenEmory

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

Heather A. Prentice, *University of Alabama at Birmingham*
Hailin Lu, *University of Alabama at Birmingham*
Matthew A. Price, *University of California at San Francisco*
Anatoli Kamali, *MRC/UVRI Uganda Virus Research Unit on AIDS*
Etienne Karita, *Project San Francisco*
[Shabir A. Lakhi](#), *Emory University*
Eduard J. Sanders, *Kenya Medical Research Institute*
Omu Anzala, *Kenya AIDS Vaccine Initiative*
[Susan Allen](#), *Emory University*
Paul A. Goepfert, *University of Alabama at Birmingham*

Only first 10 authors above; see publication for full author list.

Journal Title: Journal of Virology

Volume: Volume 90, Number 22

Publisher: American Society for Microbiology | 2016-09-14, Pages 10423-10430

Type of Work: Article | Final Publisher PDF

Publisher DOI: 10.1128/JVI.01467-16

Permanent URL: <https://pid.emory.edu/ark:/25593/s2jpb>

Final published version: <http://dx.doi.org/10.1128/JVI.01467-16>

Copyright information:

© 2016, American Society for Microbiology. All Rights Reserved.

Accessed January 26, 2020 1:12 PM EST

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

Heather A. Prentice,^{a,*} Hailin Lu,^a Matthew A. Price,^{b,c} Anatoli Kamali,^d Etienne Karita,^e Shabir Lakhi,^f Eduard J. Sanders,^{g,h} Omu Anzala,ⁱ Susan Allen,^{e,f,j} Paul A. Goepfert,^a Eric Hunter,^k Jill Gilmour,^l Jianming Tang,^a for the International AIDS Vaccine Initiative African HIV Research Network

Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA^a; International AIDS Vaccine Initiative, New York, New York, USA^b; Department of Epidemiology and Biostatistics, University of California at San Francisco, San Francisco, California, USA^c; MRC/UVRI Uganda Virus Research Unit on AIDS, Masaka Site, Masaka, Uganda^d; Project San Francisco, Kigali, Rwanda^e; Zambia-Emory HIV Research Project, Lusaka, Zambia^f; Centre for Geographic Medicine Research, Kenya Medical Research Institute, Kilifi, Kenya^g; Centre for Clinical Vaccinology and Tropical Medicine, University of Oxford, Headington, United Kingdom^h; Kenya AIDS Vaccine Initiative, Nairobi, Kenyaⁱ; Department of Pathology and Laboratory Medicine, Emory University, Atlanta, Georgia, USA^j; Vaccine Research Center, Emory University, Atlanta, Georgia, USA^k; International AIDS Vaccine Initiative, Human Immunology Laboratory, Chelsea and Westminster Hospital, London, United Kingdom^l

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 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 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, the 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 the 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 the 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 antiretro-

viral therapy (ART) is likely to change this paradigm for three reasons. First, the 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

Received 25 July 2016 Accepted 7 September 2016

Accepted manuscript posted online 14 September 2016

Citation Prentice HA, Lu H, Price MA, Kamali A, Karita E, Lakhi S, Sanders EJ, Anzala O, Allen S, Goepfert PA, Hunter E, Gilmour J, Tang J, for the International AIDS Vaccine Initiative African HIV Research Network. 2016. Dynamics and correlates of CD8 T-cell counts in Africans with primary human immunodeficiency virus type 1 infection. *J Virol* 90:10423–10430. doi:10.1128/JVI.01467-16.

Editor: F. Kirchhoff, Ulm University Medical Center

Address correspondence to Jianming Tang, jtang@uab.edu.

* Present address: Heather A. Prentice, Kaiser Permanente, San Diego, California, USA.

Copyright © 2016, American Society for Microbiology. All Rights Reserved.

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-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 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 \log_{10} -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 $\geq 5\%$ 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 (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 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 \log_{10} 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 supporting 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

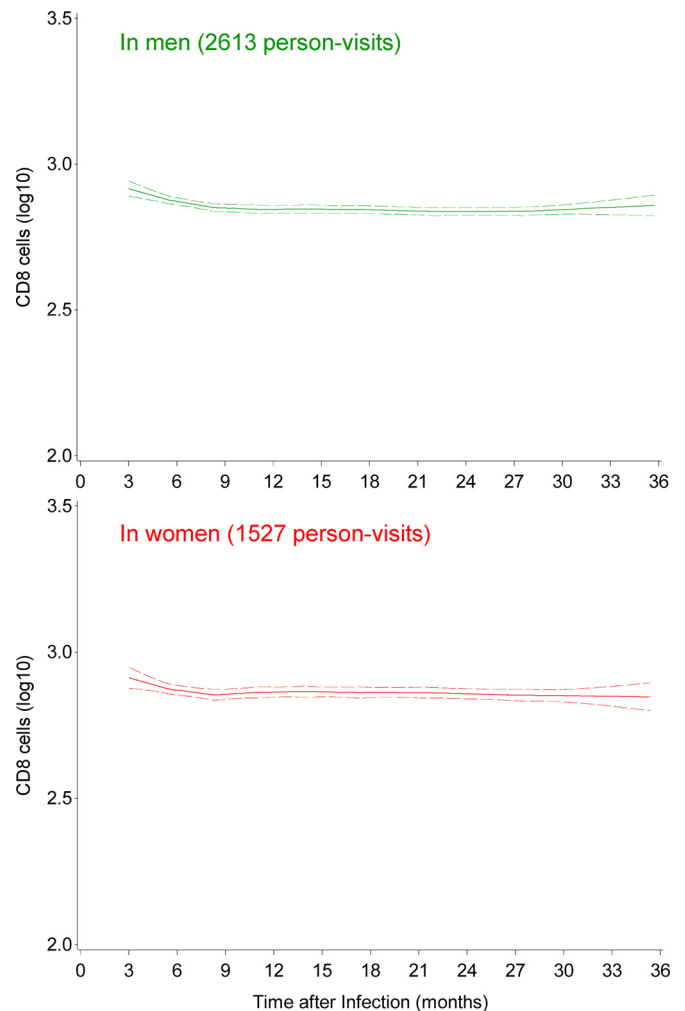


FIG 1 Local regression curves for CD8 counts in seroconverted men and women (3 to 36 months after the estimated date of HIV-1 infection). Solid and dotted lines, mean values and 95% confidence intervals, respectively ($P > 0.60$ between the two patient groups).

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-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 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 \log_{10} (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

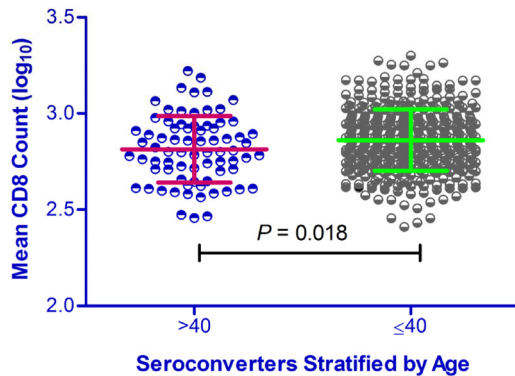


FIG 2 Mean CD8 counts in HIV-1 seroconverters defined by two age groups.

in two age groups ($P = 0.027$ for individuals >40 years old versus individuals ≤ 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 \log_{10} 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 VL 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 1 Univariable analyses of major HLA-I variants for potential association with \log_{10} -transformed CD8 counts^b

HLA variant	No. of individuals	Frequency	Impact ($\Delta \pm$ SE)	Adjusted P^a	FDR (q)
<i>A*01</i>	62	0.12	0.02 ± 0.02	0.305	0.727
<i>A*02</i>	173	0.35	0.01 ± 0.02	0.678	0.784
<i>A*03</i> (*03:01)	45	0.09	0.08 ± 0.03	0.003	0.048
<i>A*23</i>	75	0.15	-0.01 ± 0.01	0.605	0.747
<i>A*29</i>	47	0.09	-0.02 ± 0.02	0.432	0.727
<i>A*30</i>	177	0.36	0.01 ± 0.02	0.427	0.727
<i>A*33</i>	25	0.05	0.03 ± 0.03	0.357	0.727
<i>A*34</i>	36	0.07	-0.04 ± 0.03	0.145	0.619
<i>A*36</i>	41	0.08	-0.04 ± 0.03	0.179	0.653
<i>A*66</i>	32	0.06	-0.02 ± 0.03	0.517	0.747
<i>A*68</i> (mostly *68:02)	121	0.24	0.00 ± 0.02	0.945	0.945
<i>A*74</i>	63	0.13	-0.01 ± 0.02	0.609	0.747
<i>B*07</i>	66	0.13	-0.01 ± 0.02	0.761	0.853
<i>B*14</i>	46	0.09	0.03 ± 0.03	0.262	0.727
<i>B*15:03</i>	87	0.17	0.02 ± 0.02	0.393	0.727
<i>B*15:10</i>	55	0.11	0.06 ± 0.02	0.005	0.059
<i>B*15:xx</i> (other <i>B*15s</i>)	24	0.05	0.00 ± 0.03	0.932	0.945
<i>B*18</i>	34	0.07	-0.01 ± 0.03	0.626	0.747
<i>B*35</i> (*35:01)	28	0.06	0.01 ± 0.03	0.838	0.912
<i>B*42</i>	66	0.13	-0.02 ± 0.02	0.432	0.727
<i>B*44</i>	51	0.1	-0.02 ± 0.02	0.343	0.727
<i>B*45</i>	81	0.16	0.00 ± 0.02	0.866	0.916
<i>B*49</i>	38	0.08	0.04 ± 0.03	0.143	0.619
<i>B*53</i>	94	0.19	-0.01 ± 0.02	0.543	0.747
<i>B*57</i>	46	0.09	-0.04 ± 0.02	0.074	0.458
<i>B*58:01</i>	55	0.11	-0.05 ± 0.02	0.049	0.394
<i>B*58:02</i>	72	0.14	0.07 ± 0.02	<0.001	0.015
<i>B*81</i>	25	0.05	-0.02 ± 0.03	0.581	0.747
<i>C*02</i>	91	0.18	-0.01 ± 0.02	0.549	0.747
<i>C*03</i>	71	0.14	0.04 ± 0.02	0.053	0.394
<i>C*04</i>	158	0.32	-0.01 ± 0.02	0.589	0.747
<i>C*06</i>	146	0.29	0.02 ± 0.02	0.151	0.619
<i>C*07</i>	179	0.36	-0.02 ± 0.02	0.279	0.727
<i>C*08</i>	63	0.13	0.02 ± 0.02	0.402	0.727
<i>C*16</i>	67	0.13	-0.02 ± 0.02	0.282	0.727
<i>C*17</i>	80	0.16	-0.01 ± 0.01	0.589	0.747
<i>C*18</i>	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.

^b By repeated measures for 497 HIV-1 seroconverters during the 3- to 36-month period after EDI.

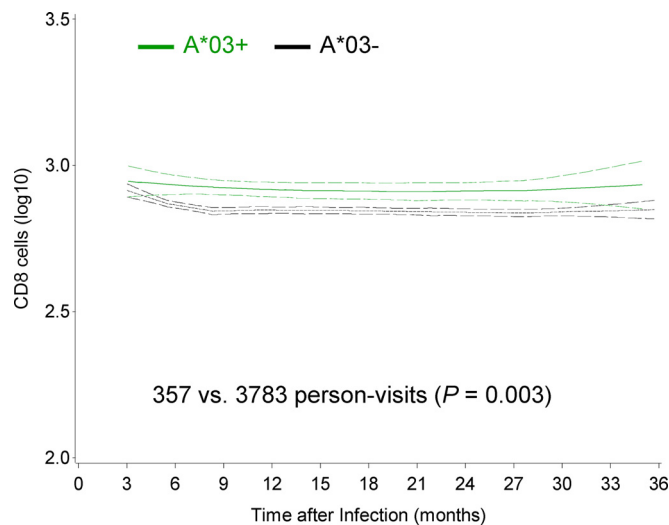


FIG 3 Local regression curves for CD8 counts in seroconverters with and without HLA-A*03 (3 to 36 months after the estimated date of HIV-1 infection). For each stratum (presence and absence of A*03), solid and dotted lines correspond to the mean values and 95% confidence intervals, respectively.

cans, but none of them have been associated with outcomes related to HIV-1 infection (41–43). On the other hand, B*58:02 is a somewhat unfavorable allele in HIV-1 infection (44) and 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

TABLE 2 Correlates of CD8 counts in 497 HIV-1 SCs revealed by two multivariable models

Individual factors in each model	For repeated measurements ^a		For mean CD8 counts ^a	
	$\Delta \pm SE$	<i>P</i>	$\Delta \pm SE$	<i>P</i>
Age >40 (<i>n</i> = 75) vs ≤ 40 yr (<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

^a CD8 counts in the 3- to 36-month period after the estimated date of infection with \log_{10} transformation. NA, not applicable.

^b 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.

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

^d 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, 57), with the medium VL (*n* = 265) being the reference group for comparison.

TABLE 3 Sensitivity analyses of two multivariable models for CD8 counts in the 3- to 24-month period after EDI

Individual factors in each model	For repeated measurements ^a		For mean CD8 counts ^a	
	$\Delta \pm SE$	<i>P</i>	$\Delta \pm SE$	<i>P</i>
Age >40 (<i>n</i> = 75) vs ≤ 40 yr (<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) ^b	0.01 ± 0.02	0.721	0.01 ± 0.02	0.725
Zambia (southern Africa, <i>n</i> = 195) ^{b,c}	-0.01 ± 0.02	0.552	-0.01 ± 0.02	0.541
Low VL (<i>n</i> = 140) ^d	-0.01 ± 0.02	0.527	-0.01 ± 0.02	0.497
High VL (<i>n</i> = 92) ^d	-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

^a See footnote a of Table 2.

^b See footnote b of Table 2.

^c See footnote c of Table 2.

^d See footnote d of Table 2.

populations (45, 46). In study cohorts 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

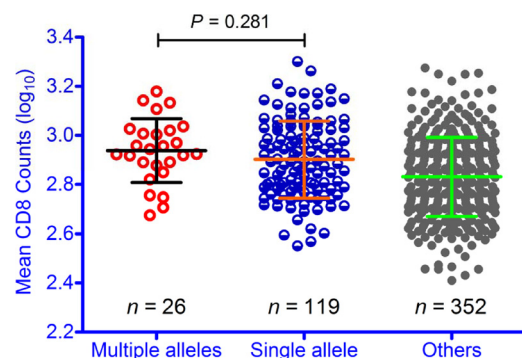


FIG 4 Lack of additive effect of HLA variants on CD8 counts. The mean CD8 counts (3 to 36 months after the estimated date of HIV-1 infection) in three subgroups of HIV-1 seroconverters were compared on the basis of the presence and absence of three HLA variants of interest (A*03, B*15:10, and B*58:02).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

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 intervals (Tables 1 and 2), mostly within a magnitude of a 15 to 17% (0.06- to 0.07- \log_{10}) difference. 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 the 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 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) to be a major correlate of CD8 counts in healthy adolescent twins from Australia (effect size = $-0.31 \pm 0.03 \log_{10}$). 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 metabolism is expected to ex-

pedite future research on immunogenetic control of the 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 to be unfavorable (associated with a 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 impacts on CD8 counts, but the statistical power in our study favors the analysis of B*58:02 rather than B*58:01 (which were found in 72 versus 55 subjects, respectively, 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 count data before HIV-1 infection and after ART initiation. As our study cohort was designed for the evaluation of primary HIV-1 infection, preinfection 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 data set 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 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 (56).

ACKNOWLEDGMENTS

We thank all members of the IAVI African HIV Research Network for their valuable contributions to cohort assembly and data collection. We are also grateful to several associates, especially Travis Porter and Xuelin Li, for technical assistance.

This work was funded in part by IAVI and made possible by the generous support from many donors, including the Bill & Melinda Gates Foundation, the Ministry of Foreign Affairs of Denmark, Irish Aid, the Ministry of Finance of Japan, the Ministry of Foreign Affairs of the Netherlands, the Norwegian Agency for Development Cooperation, the United Kingdom Department for International Development, and the United States Agency for International Development (USAID). The full list of IAVI donors is available at www.iavi.org. Additional funding for this work came from (i) the United States National Institute of Allergy and Infectious Diseases (NIAID) through an R01 grant (AI064060 to E.H., P.A.G., and J.T.), (ii) the Fogarty AIDS International Training and Research Program (AITRP) (grant FIC 2D43 TW001042 to S.L.), and (iii) the KEMRI-Wellcome Trust Research Programme at the Centre for Geographical Medicine Research-Kilifi (Wellcome Trust award 077092).

Submission of this work for publication required approval by the director of the Kenya Medical Research Institute (KEMRI) and by KEMRI and IAVI representatives, but the contents are the responsibility of the

individual authors and do not necessarily reflect the views of IAVI, KEMRI, NIAID, USAID, or the United States government.

FUNDING INFORMATION

This work, including the efforts of Eric Hunter, Paul A. Goepfert, and Jianming Tang, was funded by NIAID (R01 AI064060). This work, including the efforts of Shabir Lakhani, was also funded in part by NITRP (FIC 2D43 TW001042).

REFERENCES

- Koup RA. 1994. Virus escape from CTL recognition. *J Exp Med* 180:779–782. <http://dx.doi.org/10.1084/jem.180.3.779>.
- Mollet L, Li TS, Samri A, Tournay C, Tubiana R, Calvez V, Debre P, Katlama C, Autran B. 2000. Dynamics of HIV-specific CD8⁺ T lymphocytes with changes in viral load. The RESTIM and COMET Study Groups. *J Immunol* 165:1692–1704.
- Edwards BH, Bansal A, Sabbaj S, Bakari J, Mulligan MJ, Goepfert PA. 2002. Magnitude of functional CD8⁺ T-cell responses to the Gag protein of human immunodeficiency virus type 1 correlates inversely with viral load in plasma. *J Virol* 76:2298–2305. <http://dx.doi.org/10.1128/JVI.76.5.2298-2305.2002>.
- Crawford H, Lumm W, Leslie A, Schaefer M, Boeras D, Prado JG, Tang J, Farmer P, Ndung'u T, Lakhani S, Gilmour J, Goepfert P, Walker BD, Kaslow R, Mulenga J, Allen S, Goulder PJ, Hunter E. 2009. Evolution of HLA-B*5703 HIV-1 escape mutations in HLA-B*5703-positive individuals and their transmission recipients. *J Exp Med* 206:909–921. <http://dx.doi.org/10.1084/jem.20081984>.
- Wright JK, Naidoo VL, Brumme ZL, Prince JL, Claiborne DT, Goulder PJ, Brockman MA, Hunter E, Ndung'u T. 2012. Impact of HLA-B*81-associated mutations in HIV-1 Gag on viral replication capacity. *J Virol* 86:3193–3199. <http://dx.doi.org/10.1128/JVI.06682-11>.
- Carlson JM, Brumme ZL, Rousseau CM, Brumme CJ, Matthews P, Kadie C, Mullins JJ, Walker BD, Harrigan PR, Goulder PJ, Heckerman D. 2008. Phylogenetic dependency networks: inferring patterns of CTL escape and codon covariation in HIV-1 Gag. *PLoS Comput Biol* 4:e1000225. <http://dx.doi.org/10.1371/journal.pcbi.1000225>.
- Goepfert PA, Lumm W, Farmer P, Matthews P, Prendergast A, Carlson JM, Derdeyn CA, Tang J, Kaslow RA, Bansal A, Yusim K, Heckerman D, Mulenga J, Allen S, Goulder PJ, Hunter E. 2008. Transmission of HIV-1 Gag immune escape mutations is associated with reduced viral load in linked recipients. *J Exp Med* 205:1009–1017. <http://dx.doi.org/10.1084/jem.20072457>.
- Carlson JM, Schaefer M, Monaco DC, Batorsky R, Claiborne DT, Prince J, Deymier MJ, Ende ZS, Klatt NR, DeZiel CE, Lin TH, Peng J, Seese AM, Shapiro R, Frater J, Ndung'u T, Tang J, Goepfert P, Gilmour J, Price MA, Kilembe W, Heckerman D, Goulder PJ, Allen TM, Allen S, Hunter E. 2014. Selection bias at the heterosexual HIV-1 transmission bottleneck. *Science* 345:1254031. <http://dx.doi.org/10.1126/science.1254031>.
- Ribeiro RM, Mohri H, Ho DD, Perelson AS. 2002. *In vivo* dynamics of T cell activation, proliferation, and death in HIV-1 infection: why are CD4⁺ but not CD8⁺ T cells depleted? *Proc Natl Acad Sci U S A* 99:15572–15577. <http://dx.doi.org/10.1073/pnas.242358099>.
- Trautmann L, Janbazian L, Chomont N, Said EA, Wang G, Gimmig S, Bessette B, Boulassel MR, Delwart E, Sepulveda H, Balderas RS, Routy JP, Haddad EK, Sekaly RP. 2006. Upregulation of PD-1 expression on HIV-specific CD8⁺ T cells leads to reversible immune dysfunction. *Nat Med* 12:1198–1202. <http://dx.doi.org/10.1038/nm1482>.
- Day CL, Kaufmann DE, Kiepiela P, Brown JA, Moodley ES, Reddy S, Mackey EW, Miller JD, Leslie AJ, DePierres C, Mncube Z, Duraiswamy J, Zhu B, Eichbaum Q, Altfeld M, Wherry EJ, Coovadia HM, Goulder PJ, Klenerman P, Ahmed R, Freeman GJ, Walker BD. 2006. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature* 443:350–354. <http://dx.doi.org/10.1038/nature05115>.
- Petrovas C, Casazza JP, Brenchley JM, Price DA, Gostick E, Adams WC, Precopio ML, Schacker T, Roederer M, Douek DC, Koup RA. 2006. PD-1 is a regulator of virus-specific CD8⁺ T cell survival in HIV infection. *J Exp Med* 203:2281–2292. <http://dx.doi.org/10.1084/jem.20061496>.
- Eller MA, Goonetilleke N, Tassanetrithep B, Eller LA, Costanzo MC, Johnson S, Betts MR, Krebs SJ, Slike BM, Nitayaphan S, Rono K, Tovanabutra S, Maganga L, Kibuuka H, Jagodzinski L, Peel S, Rolland M, Marovich MA, Kim JH, Michael NL, Robb ML, Streeck H. 2016. Expansion of inefficient HIV-specific CD8 T cells during acute infection. *J Virol* 90:4005–4016. <http://dx.doi.org/10.1128/JVI.02785-15>.
- Zaman MM, Recco RA, Raguthu L, Likki S, Reddy S. 2000. Characteristics of HIV-1-infected patients with CD4:CD8 lymphocyte ratio normalization on antiretroviral therapy. *AIDS Patient Care STDS* 14:647–649. <http://dx.doi.org/10.1089/10872910050206568>.
- Pahwa S, Read JS, Yin W, Matthews Y, Shearer W, Diaz C, Rich K, Mendez H, Thompson B, Women and Infants Transmission Study. 2008. CD4⁺/CD8⁺ T cell ratio for diagnosis of HIV-1 infection in infants: Women and Infants Transmission Study. *Pediatrics* 122:331–339. <http://dx.doi.org/10.1542/peds.2007-2308>.
- Tang J, Li X, Price MA, Sanders EJ, Anzala O, Karita E, Kamali A, Lakhani S, Allen S, Hunter E, Kaslow RA, Gilmour J. 2015. CD4:CD8 lymphocyte ratio as a quantitative measure of immunologic health in HIV-1 infection: findings from an African cohort with prospective data. *Front Microbiol* 6:670. <http://dx.doi.org/10.3389/fmicb.2015.00670>.
- Walker BD, Korber BT. 2001. Immune control of HIV: the obstacles of HLA and viral diversity. *Nat Immunol* 2:473–475. <http://dx.doi.org/10.1038/88656>.
- Kwong PD, Mascola JR, Nabel GJ. 2013. Broadly neutralizing antibodies and the search for an HIV-1 vaccine: the end of the beginning. *Nat Rev Immunol* 13:693–701. <http://dx.doi.org/10.1038/nri3516>.
- Perreau M, Levy Y, Pantaleo G. 2013. Immune response to HIV. *Curr Opin HIV AIDS* 8:333–340. <http://dx.doi.org/10.1097/COH.0b013e328361faf4>.
- Buggert M, Frederiksen J, Noyan K, Svard J, Barqasho B, Sonnerborg A, Lund O, Nowak P, Karlsson AC. 2014. Multiparametric bioinformatics distinguish the CD4/CD8 ratio as a suitable laboratory predictor of combined T cell pathogenesis in HIV infection. *J Immunol* 192:2099–2108. <http://dx.doi.org/10.4049/jimmunol.1302596>.
- Serrano-Villar S, Sainz T, Lee SA, Hunt PW, Sinclair E, Shacklett BL, Ferre AL, Hayes TL, Somsouk M, Hsue PY, Van Natta ML, Meinert CL, Lederman MM, Hatano H, Jain V, Huang Y, Hecht FM, Martin JN, McCune JM, Moreno S, Deeks SG. 2014. HIV-infected individuals with low CD4/CD8 ratio despite effective antiretroviral therapy exhibit altered T cell subsets, heightened CD8⁺ T cell activation, and increased risk of non-AIDS morbidity and mortality. *PLoS Pathog* 10:e1004078. <http://dx.doi.org/10.1371/journal.ppat.1004078>.
- DeMaster LK, Liu X, VanBelzen DJ, Trinite B, Zheng L, Agosto LM, Migueles SA, Connors M, Sambucetti L, Levy DN, Pasternak AO, O'Doherty U. 2016. A subset of CD4/CD8 double-negative T cells expresses HIV proteins in patients on antiretroviral therapy. *J Virol* 90:2165–2179. <http://dx.doi.org/10.1128/JVI.01913-15>.
- Chun TW, Nickle DC, Justement JS, Large D, Semerjian A, Curlin ME, O'Shea MA, Hallahan CW, Daucher M, Ward DJ, Moir S, Mullins JJ, Kovacs C, Fauci AS. 2005. HIV-infected individuals receiving effective antiviral therapy for extended periods of time continually replenish their viral reservoir. *J Clin Invest* 115:3250–3255. <http://dx.doi.org/10.1172/JCI26197>.
- Williams JP, Southern P, Lissina A, Christian HC, Sewell AK, Phillips R, Pankhurst Q, Frater J. 2013. Application of magnetic field hyperthermia and superparamagnetic iron oxide nanoparticles to HIV-1-specific T-cell cytotoxicity. *Int J Nanomedicine* 8:2543–2554. <http://dx.doi.org/10.2147/IJN.S44013>.
- Sung JA, Lam S, Garrido C, Archin N, Rooney CM, Bollard CM, Margolis DM. 2015. Expanded cytotoxic T-cell lymphocytes target the latent HIV reservoir. *J Infect Dis* 212:258–263. <http://dx.doi.org/10.1093/infdis/jiv022>.
- Jones RB, Walker BD. 2016. HIV-specific CD8⁺ T cells and HIV eradication. *J Clin Invest* 126:455–463. <http://dx.doi.org/10.1172/JCI80566>.
- Walker-Sperling VE, Buckheit RW, III, Blankson JN. 2014. Comparative analysis of the capacity of elite suppressor CD4⁺ and CD8⁺ T cells to inhibit HIV-1 replication in monocyte-derived macrophages. *J Virol* 88:9789–9798. <http://dx.doi.org/10.1128/JVI.00860-14>.
- Hansen SG, Ford JC, Lewis MS, Ventura AB, Hughes CM, Coyne-Johnson L, Whizin N, Oswald K, Shoemaker R, Swanson T, Legasse AW, Chiuhiolo MJ, Parks CL, Axthelm MK, Nelson JA, Jarvis MA, Piatak M, Jr, Lifson JD, Picker LJ. 2011. Profound early control of highly pathogenic SIV by an effector memory T-cell vaccine. *Nature* 473:523–527. <http://dx.doi.org/10.1038/nature10003>.
- Hansen SG, Piatak M, Jr, Ventura AB, Hughes CM, Gilbride RM, Ford

- JC, Oswald K, Shoemaker R, Li Y, Lewis MS, Gilliam AN, Xu G, Whizin N, Burwitz BJ, Planer SL, Turner JM, Legasse AW, Axthelm MK, Nelson JA, Fruh K, Sacha JB, Estes JD, Keele BF, Edlefsen PT, Lifson JD, Picker LJ. 2013. Immune clearance of highly pathogenic SIV infection. *Nature* 502:100–104. <http://dx.doi.org/10.1038/nature12519>.
30. Karita E, Price M, Hunter E, Chomba E, Allen S, Fei L, Kamali A, Sanders EJ, Anzala O, Katende M, Ketter N. 2007. Investigating the utility of the HIV-1 BED capture enzyme immunoassay using cross-sectional and longitudinal seroconverter specimens from Africa. *AIDS* 21:403–408. <http://dx.doi.org/10.1097/QAD.0b013e32801481b7>.
 31. Price MA, Wallis CL, Lakhi S, Karita E, Kamali A, Anzala O, Sanders EJ, Bekker LG, Twesigye R, Hunter E, Kaleebu P, Kayitenkore K, Allen S, Ruzagira E, Mwangome G, Mutua G, Amornkul PN, Stevens G, Pond SL, Schaefer M, Papathanasopoulos MA, Stevens W, Gilmour J, IAVI Early Infection Cohort Study Group. 2011. Transmitted HIV type 1 drug resistance among individuals with recent HIV infection in east and southern Africa. *AIDS Res Hum Retroviruses* 27:5–12. <http://dx.doi.org/10.1089/aid.2010.0030>.
 32. Prentice HA, Porter TR, Price MA, Cormier E, He D, Farmer PK, Kamali A, Karita E, Lakhi S, Sanders EJ, Anzala O, Amornkul PN, Allen S, Hunter E, Kaslow RA, Gilmour J, Tang J. 2013. HLA-B*57 versus HLA-B*81 in HIV-1 infection: slow and steady wins the race? *J Virol* 87:4043–4051. <http://dx.doi.org/10.1128/JVI.03302-12>.
 33. Prentice HA, Price MA, Porter TR, Cormier E, Mugavero MJ, Kamali A, Karita E, Lakhi S, Sanders EJ, Anzala O, Amornkul PN, Allen S, Hunter E, Kaslow RA, Gilmour J, Tang J, IAVI Africa HIV Prevention Partnership. 2014. Dynamics of viremia in primary HIV-1 infection in Africans: insights from analyses of host and viral correlates. *Virology* 449:254–262. <http://dx.doi.org/10.1016/j.virol.2013.11.024>.
 34. Ngongo PB, Priddy F, Park H, Becker J, Bender B, Fast P, Anzala O, Mutua G, Ruzagira E, Kamali A, Karita E, Mugo P, Chomba E, Bekker LG, Roux S, Nanvubya A, Mbrahtu T. 2012. Developing standards of care for HIV prevention research in developing countries—a case study of 10 research centers in eastern and southern Africa. *AIDS Care* 24:1277–1289. <http://dx.doi.org/10.1080/09540121.2012.656572>.
 35. de Bakker PI, McVean G, Sabeti PC, Miretti MM, Green T, Marchini J, Ke X, Monsuur AJ, Whittaker P, Delgado M, Morrison J, Richardson A, Walsh EC, Gao X, Galver L, Hart J, Hafler DA, Pericak-Vance M, Todd JA, Daly MJ, Trowsdale J, Wijmenga C, Vyse TJ, Beck S, Murray SS, Carrington M, Gregory S, Deloukas P, Rioux JD. 2006. A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. *Nat Genet* 38:1166–1172. <http://dx.doi.org/10.1038/ng1885>.
 36. Ward LD, Kellis M. 2012. HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic Acids Res* 40:D930–D934. <http://dx.doi.org/10.1093/nar/gkr917>.
 37. Rosenbloom KR, Dreszer TR, Pheasant M, Barber GP, Meyer LR, Pohl A, Raney BJ, Wang T, Hinrichs AS, Zweig AS, Fujita PA, Learned K, Rhead B, Smith KE, Kuhn RM, Karolchik D, Haussler D, Kent WJ. 2010. ENCODE whole-genome data in the UCSC Genome Browser. *Nucleic Acids Res* 38:D620–D625. <http://dx.doi.org/10.1093/nar/gkp961>.
 38. ENCODE Project Consortium. 2012. An integrated encyclopedia of DNA elements in the human genome. *Nature* 489:57–74. <http://dx.doi.org/10.1038/nature11247>.
 39. Fairfax BP, Makino S, Radhakrishnan J, Plant K, Leslie S, Dilthey A, Ellis P, Langford C, Vannberg FO, Knight JC. 2012. Genetics of gene expression in primary immune cells identifies cell type-specific master regulators and roles of HLA alleles. *Nat Genet* 44:502–510. <http://dx.doi.org/10.1038/ng.2205>.
 40. Farh KK, Marson A, Zhu J, Kleinewietfeld M, Housley WJ, Beik S, Shosh N, Whitton H, Ryan RJ, Shishkin AA, Hatan M, Carrasco-Alfonso MJ, Mayer D, Luckey CJ, Patsopoulos NA, De Jager PL, Kuchroo VK, Epstein CB, Daly MJ, Hafler DA, Bernstein BE. 2015. Genetic and epigenetic fine mapping of causal autoimmune disease variants. *Nature* 518:337–343. <http://dx.doi.org/10.1038/nature13835>.
 41. Pereyra F, International HIV Controllers Study. 2010. The major genetic determinants of HIV-1 control affect HLA class I peptide presentation. *Science* 330:1551–1557. <http://dx.doi.org/10.1126/science.1195271>.
 42. McLaren PJ, Ripke S, Pelak K, Weintrob AC, Patsopoulos NA, Jia X, Erlich RL, Lennon NJ, Kadie CM, Heckerman D, Gupta N, Haas DW, Deeks SG, Pereyra F, Walker BD, de Bakker PI. 2012. Fine-mapping classical HLA variation associated with durable host control of HIV-1 infection in African Americans. *Hum Mol Genet* 21:4334–4347. <http://dx.doi.org/10.1093/hmg/ddc226>.
 43. Prentice HA, Pajewski NM, He D, Zhang K, Brown EE, Kilembe W, Allen S, Hunter E, Kaslow RA, Tang J. 2014. Host genetics and immune control of HIV-1 infection: fine mapping for the extended human MHC region in an African cohort. *Genes Immun* 15:275–281. <http://dx.doi.org/10.1038/gene.2014.16>.
 44. Prentice HA, Tang J. 2012. HIV-1 dynamics: a reappraisal of host and viral factors, as well as methodological issues. *Viruses* 4:2080–2096. <http://dx.doi.org/10.3390/v4102080>.
 45. Ferreira MA, Mangino M, Brumme CJ, Zhao ZZ, Medland SE, Wright MJ, Nyholt DR, Gordon S, Campbell M, McEvoy BP, Henders A, Evans DM, Lanchbury JS, Pereyra F, International HIV Controllers Study, Walker BD, Haas DW, Soranzo N, Spector TD, de Bakker PI, Frazier IH, Montgomery GW, Martin NG. 2010. Quantitative trait loci for CD4:CD8 lymphocyte ratio are associated with risk of type 1 diabetes and HIV-1 immune control. *Am J Hum Genet* 86:88–92. <http://dx.doi.org/10.1016/j.ajhg.2009.12.008>.
 46. Costa M, Cruz E, Barton JC, Thorstensen K, Morais S, da Silva BM, Pinto JP, Vieira CP, Vieira J, Acton RT, Porto G. 2013. Effects of highly conserved major histocompatibility complex (MHC) extended haplotypes on iron and low CD8⁺ T lymphocyte phenotypes in HFE C282Y homozygous hemochromatosis patients from three geographically distant areas. *PLoS One* 8:e79990. <http://dx.doi.org/10.1371/journal.pone.0079990>.
 47. Li X, Price MA, He D, Kamali A, Karita E, Lakhi S, Sanders EJ, Anzala O, Amornkul PN, Allen S, Hunter E, Kaslow RA, Gilmour J, Tang J, IAVI Africa HIV Prevention Partnership. 2014. Host genetics and viral load in primary HIV-1 infection: clear evidence for gene by sex interactions. *Hum Genet* 133:1187–1197. <http://dx.doi.org/10.1007/s00439-014-1465-x>.
 48. Landais E, Huang X, Havenar-Daughton C, Murrell B, Price MA, Wickramasinghe L, Ramos A, Bian CB, Simek M, Allen S, Karita E, Kilembe W, Lakhi S, Inambao M, Kamali A, Sanders EJ, Anzala O, Edward V, Bekker LG, Tang J, Gilmour J, Kosakovsky-Pond SL, Phung P, Wrin T, Crotty S, Godzik A, Poignard P. 2016. Broadly neutralizing antibody responses in a large longitudinal sub-Saharan HIV primary infection cohort. *PLoS Pathog* 12:e1005369. <http://dx.doi.org/10.1371/journal.ppat.1005369>.
 49. Mueller SN, Hosiawa-Meagher KA, Konieczny BT, Sullivan BM, Bachmann MF, Locksley RM, Ahmed R, Matloubian M. 2007. Regulation of homeostatic chemokine expression and cell trafficking during immune responses. *Science* 317:670–674. <http://dx.doi.org/10.1126/science.1144830>.
 50. Havenar-Daughton C, Lindqvist M, Heit A, Wu JE, Reiss SM, Kendrick K, Belanger S, Kasturi SP, Landais E, Akondy RS, McGuire HM, Bothwell M, Vagefi PA, Scully E, IAVI Protocol C Principal Investigators, Tomaras GD, Davis MM, Poignard P, Ahmed R, Walker BD, Pulendran B, McElrath MJ, Kaufmann DE, Crotty S. 2016. CXCL13 is a plasma biomarker of germinal center activity. *Proc Natl Acad Sci U S A* 113:2702–2707. <http://dx.doi.org/10.1073/pnas.1520112113>.
 51. Gartland AJ, Li S, McNevin J, Tomaras GD, Gottardo R, Janes H, Fong Y, Morris D, Geraghty DE, Kijak GH, Edlefsen PT, Frahm N, Larsen BB, Tovanabutra S, Sanders-Buell E, deCamp AC, Margaret CA, Ahmed H, Goodridge JP, Chen L, Konopa P, Nariya S, Stoddard JN, Wong K, Zhao H, Deng W, Maust BS, Bose M, Howell S, Bates A, Lazzaro M, O'Sullivan A, Lei E, Bradfield A, Ibitamuno G, Assawadarachai V, O'Connell RJ, deSouza MS, Nitayaphan S, Rerks-Ngarm S, Robb ML, Sidney J, Sette A, Zolla-Pazner S, Montefiori D, McElrath MJ, Mullins JI, Kim JH, Gilbert PB, Hertz T. 2014. Analysis of HLA A*02 association with vaccine efficacy in the RV144 HIV-1 vaccine trial. *J Virol* 88:8242–8255. <http://dx.doi.org/10.1128/JVI.01164-14>.
 52. Leslie AJ, Pfafferoth KJ, Chetty P, Draenert R, Addo MM, Feeney M, Tang Y, Holmes EC, Allen T, Prado JG, Altfield M, Brander C, Dixon C, Ramduth D, Jeena P, Thomas SA, St John A, Roach TA, Kupfer B, Luzzi G, Edwards A, Taylor G, Lyall H, Tudor-Williams G, Novelli V, Martinez-Picado J, Kiepiela P, Walker BD, Goulder PJ. 2004. HIV evolution: CTL escape mutation and reversion after transmission. *Nat Med* 10:282–289. <http://dx.doi.org/10.1038/nm992>.
 53. Lazaryan A, Lobashevsky E, Mulenga J, Karita E, Allen S, Tang J, Kaslow RA. 2006. Human leukocyte antigen B58 supertype and human immunodeficiency virus type 1 infection in native Africans. *J Virol* 80:6056–6060. <http://dx.doi.org/10.1128/JVI.02119-05>.

54. Leslie A, Matthews PC, Listgarten J, Carlson JM, Kadie C, Ndung'u T, Brander C, Coovadia H, Walker BD, Heckerman D, Goulder PJ. 2010. Additive contribution of HLA class I alleles in the immune control of HIV-1 infection. *J Virol* 84:9879–9888. <http://dx.doi.org/10.1128/JVI.00320-10>.
55. Karita E, Ketter N, Price MA, Kayitenkore K, Kaleebu P, Nanvubya A, Anzala O, Jaoko W, Mutua G, Ruzagira E, Mulenga J, Sanders EJ, Mwangome M, Allen S, Bwanika A, Bahemuka U, Awuondo K, Omosa G, Farah B, Amornkul P, Birungi J, Yates S, Stoll-Johnson L, Gilmour J, Stevens G, Shutes E, Manigart O, Hughes P, Dally L, Scott J, Stevens W, Fast P, Kamali A. 2009. CLSI-derived hematology and biochemistry reference intervals for healthy adults in eastern and southern Africa. *PLoS One* 4:e4401. <http://dx.doi.org/10.1371/journal.pone.0004401>.
56. Yin L, Rodriguez CA, Hou W, Potter O, Caplan MJ, Goodenow MM, Sleasman JW. 2008. Antiretroviral therapy corrects HIV-1-induced expansion of CD8⁺ CD45RA⁺ CD2⁻ CD11a^{bright} activated T cells. *J Allergy Clin Immunol* 122:166–172.e1-2. <http://dx.doi.org/10.1016/j.jaci.2008.04.029>.
57. Tang J, Shao W, Yoo YJ, Brill I, Mulenga J, Allen S, Hunter E, Kaslow RA. 2008. Human leukocyte antigen class I genotypes in relation to heterosexual HIV type 1 transmission within discordant couples. *J Immunol* 181:2626–2635. <http://dx.doi.org/10.4049/jimmunol.181.4.2626>.