Human Leukocyte Antigen Variants B*44 and B*57 Are Consistently Favorable during Two Distinct Phases of Primary HIV-1 Infection in Sub-Saharan Africans with Several Viral Subtypes[▽][†]

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As part of an ongoing study of early human immunodeficiency virus type 1 (HIV-1) infection in sub-Saharan African countries, we have identified 134 seroconverters (SCs) with distinct acute-phase (peak) and early chronic-phase (set-point) viremias. SCs with class I human leukocyte antigen (HLA) variants B*44 and B*57 had much lower peak viral loads (VLs) than SCs without these variants (adjusted linear regression beta values of -1.08 ± 0.26 $\log_{10}$ [mean $\pm$ standard error] and -0.83 ± 0.27 $\log_{10}$, respectively; $P < 0.005$ for both), after accounting for several nongenetic factors, including gender, age at estimated date of infection, duration of infection, and country of origin. These findings were confirmed by alternative models in which major viral subtypes (A1, C, and others) in the same SCs replaced country of origin as a covariate ($P \le 0.03$). Both B*44 and B*57 were also highly favorable ($P \le 0.03$) in analyses of set-point VLs. Moreover, B*44 was associated with relatively high CD4$^+$ T-cell counts during early chronic infection ($P = 0.02$). Thus, at least two common *HLA-B* variants showed strong influences on acute-phase as well as early chronic-phase VL, regardless of the infecting viral subtype. If confirmed, the identification of B*44 as another favorable marker in primary HIV-1 infection should help dissect mechanisms of early immune protection against HIV-1 infection.

HIV-1 viral load (VL or viremia), in the typical form of viral RNA concentration in plasma, has both epidemiological and clinical implications because of its dual impact on transmission (18, 30, 71) and on the rate of disease progression (58, 59). In most studies, a quantitative measure of VL is used without reference to estimated date of infection (EDI), under the assumption that patients are seldom observed during acute-phase (peak) infection and that the early chronic-phase (set-point) VL is usually stable for years in patients with no apparent manifestations of immunode-ficiency. Factors known or suspected to influence VL range from viral mutations (changes in replication fitness or switches in coreceptor tropism) (15, 28, 39, 72) to host genes that govern innate and adaptive immune responses (54, 75, 81, 83, 84, 86).

Within the human nuclear genome, human leukocyte antigen (HLA) class I genes are the most convincing (and universally applicable) quantitative trait loci for HIV-1 viremia (14, 16, 17, 66). However, the individual HLA alleles, haplotypes, and supertypes with reported impacts on HIV-1 VL are not always clear because their distribution and patterns of linkage disequilibrium often differ from one population to another (7, 53, 63). Consensus findings have been limited to a few variants like B*27 and B*57 (9, 80), although more recent work based on African cohorts has yielded consensus results for additional variants, including A*74, B*13, and B*81, that are often favorable (49, 81). The HLA class I heavy chains encoded by these alleles differentially present highly conserved viral epitopes for cytotoxic T-lymphocyte (CTL) responses (5, 29, 39). Such HLA-restricted immune mechanisms provide clues for designing better vaccine antigens that can drive immune responses

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a

Subjects included → **134 seroconverters (SCs) with acute infection**
— 106 SCs with peak viral load (VL) within 10 wk after EDI
— 28 SCs with peak VL between 10 wk and 3 mth
— All had early set-point VL (3-12 mth after EDI)

Subjects excluded → **241 SCs not suitable for analyses here**
— 129 SCs without frequent or adequate follow-up for VL
— 95 SCs without visits corresponding to acute infection
— 12 SCs with uncertainty about EDI
— 5 SCs with liver and kidney problems

b

Screening step ⋯▸ HIV-1 VL and CD4+ T-cell counts as repeated measures
— Mixed models adjusted for non-genetic factors
  • First 12 mth after EDI
  • 3-12 mth after EDI (early chronic phase)

Primary analyses → Acute phase and set-point VL (log₁₀) as separate outcomes
— Tested for linear regression (beta, SE, and p value)

Internal consistency
— Based on univariate & multivariable models

Secondary analyses ⋯▸ CD4+ T-cell counts at viral load set-point (incomplete data)
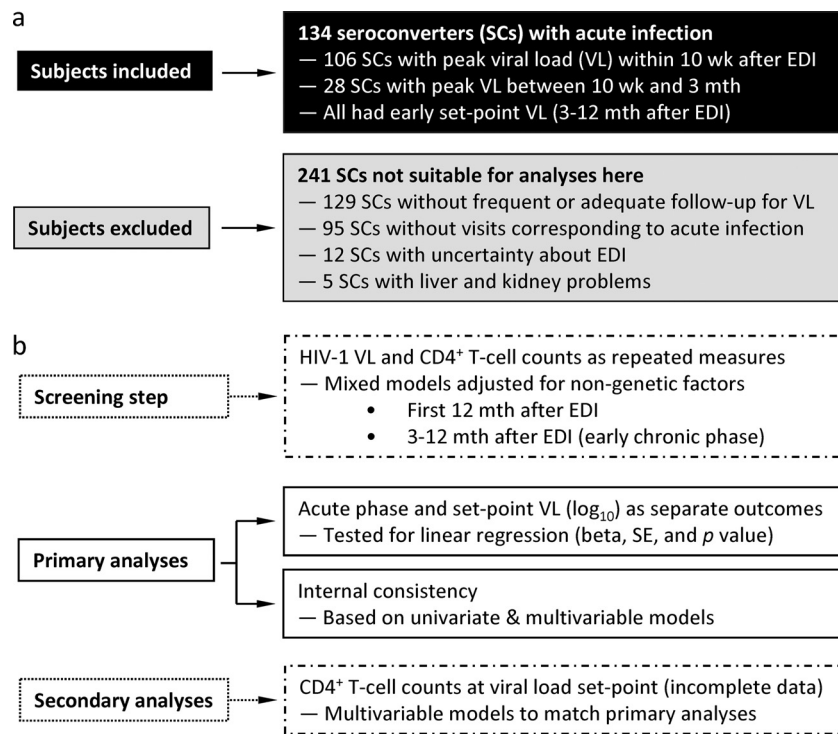— Multivariable models to match primary analyses

FIG. 1. Flow chart for selecting and analyzing informative HIV-1 seroconverters (SCs), with a focus on two distinct phases of primary HIV-1 infection. (a) Selection was based on availability and adequacy of clinical data, including acute-phase viral load (VL), early set-point VL, and set-point CD4+ T-cell counts (missing in some subjects). (b) Streamlined association analyses rely on univariate and multivariable models.

toward desirable epitopes, i.e., those are highly relevant to viral fitness (13, 37, 38, 65, 91).

We and others have reported previously that both host and viral factors can independently impact HIV-1 VL during the early and chronic phases of infection (54, 81, 84, 85). Host factors ranged from coreceptor gene (e.g., *CCR5*) polymorphisms to HLA variants (24, 50, 83, 84). The importance of viral characteristics is reflected by a correlation between donor and recipient VL after controlling for the effects of age and gender, as well as HLA (85). Supporting evidence from several recent studies suggests that the magnitude of the influence of the transmitted virus on the recipient's VL can be highly variable (26, 27). While heterogeneity in viral subtypes and other characteristics might raise some doubts about the relative contribution of host factors, our analyses of recent African seroconverters (SCs) with acute- and early chronic-phase VL measurements indicate that HLA class I factors can influence VL during primary HIV-1 infection with at least two major viral subtypes (A1 and C).

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MATERIALS AND METHODS

Study population. Between 2006 and 2010, over 370 HIV-1 seroconverters (SCs) were enrolled from Kenya, Rwanda, Uganda, South Africa, and Zambia in studies sponsored by the International AIDS Vaccine Initiative (IAVI). The procedures for written informed consent and all other research protocols were approved by institutional review boards at all sponsoring organizations, with further compliance to human experimentation guidelines set forth by the U.S. Department of Health and Human Services. Clinical and laboratory tests have

been described in detail elsewhere (35). For this study, we focused on a subset of 134 SCs observed in two distinct phases of primary HIV-1 infection (PHI). They were selected (Fig. 1) based on (i) the availability of more than 20 SCs from a single study site (grouped by country) with biological specimens for HLA class I genotyping, (ii) a narrow interval (<4 months) between the last seronegative and first seropositive visits to allow reliable calculation of the estimated date of infection (EDI), i.e., either the midpoint between the last seronegative and first seropositive visit or 2 weeks prior to the detection of HIV-1 p24 antigen in plasma, (iii) adequate follow-up for measuring VL during acute phase (<3 months) and early chronic phase (3 to 12 months) without antiretroviral therapy, (iv) the availability of CD4+ T-cell (CD4) counts for at least one of the two targeted infection intervals, (v) no apparent liver malfunction, i.e., serum alanine transferase concentration of <183 IU/liter (3 times the upper range in healthy adult Africans) (34), and (vi) no apparent kidney malfunction, i.e., serum creatinine concentration of <327 μM (3 times the upper range in healthy adult Africans) (34). The remaining SCs (n = 241), excluded from this work, all had insufficient data or uncertain EDI (Fig. 1; also see Table S1 in the supplemental material).

HIV-1 VL as primary and CD4 count as secondary outcome. Plasma VL (RNA copies/ml) was measured using the Amplicor monitor assay, version 1.5 (Roche Applied Science, Indianapolis, IN) (70). For log₁₀ transformation, a VL below the lower limit of detection (50 RNA copies/ml) was assigned a value of 0.849 (half of log₁₀50). CD4 counts were based on T-cell immunophenotyping, with assays done at individual clinics using the FACScount System (Beckman Coulter Ltd., London, United Kingdom). For this study, CD4 counts during the early chronic phase (corresponding to earliest VL set-point) were not collected for 5 of 134 SCs available for analyses.

Identification of HIV-1 subtypes by viral sequencing. HIV-1 *pol* sequencing was performed as a routine procedure for monitoring rates of drug resistance mutation and for providing an indication of infecting viral subtypes (70). Briefly, a 1.7-kb amplicon encompassing the *pol* region was sequenced using five primers and the ABI BigDye terminator kit (version 3.1, Applied Biosystems, Foster City, CA). Sequence identities were established with the REGA HIV-1 Subtyping tool and the Stanford HIV RT and Protease Sequence database (http://hivdb .stanford.edu/). The *pol* sequences can capture four of five major recombinant forms (87). Samples that could not be assigned a specific subtype or recombinant

TABLE 1. Characteristics of 134 seroconverters[a] enrolled from four African countries and suitable for studying primary HIV-1 infection

Overall characteristics[b]	Kenya	Rwanda	Uganda	Zambia
No. of subjects	27	35	27	45
Sex ratio (M/F)	3.50 (21/6)	1.06 (18/17)	1.25 (15/12)	1.50 (27/18)
Age (mean yr ± SD)	27.4 ± 5.0	37.8 ± 9.7	34.7 ± 10.2	34.6 ± 6.8
Age ≥ 40 [no. (%) of subjects]	1 (3.7)	14 (40.0)	8 (29.6)	12 (26.7)
HIV-1 subtype(s) [no. (%) of subjects]				
A1	17 (63.0)	29 (82.9)	8 (29.6)	0 (0.0)
C	5 (18.5)	2 (5.7)	1 (3.7)	43 (95.6)
Others (D, B, recombinants, or unknown)	5 (18.5)	4 (11.4)	18 (66.7)	2 (4.4)
Estimated date of infection (mo/day/yr)				
Earliest	04/21/2006	02/21/2006	04/04/2006	06/26/2006
Latest	11/26/2008	09/06/2008	03/09/2009	05/26/2008
Duration of infection (mo)				
At acute-phase VL visit [median (IQR)]	1.8 (1.3–2.2)	1.5 (0.7–1.9)	1.7 (1.5–2.5)	1.9 (1.5–2.1)
At set-point VL visit [median (IQR)]	8.6 (5.5–11.0)	8.4 (8.2–11.0)	8.6 (8.1–11.0)	8.2 (5.5–11.0)
VL measures taken within 3 mo [median (IQR)]	2 (2–3)	3 (3–4)	2 (2–3)	2 (2–3)
Acute-phase VL (mean $\log_{10}$ ± SD)	5.02 ± 1.00	5.22 ± 0.79	4.78 ± 1.06	5.01 ± 0.88
Set-point VL (mean $\log_{10}$ ± SD)	3.97 ± 1.06	3.53 ± 1.20	3.76 ± 1.39	4.17 ± 0.93
$CD4^+$ T cell count (mean ± SD)				
At acute-phase VL visit	536 ± 185	566 ± 277	643 ± 298	497 ± 183
At set-point VL visit	555 ± 246	573 ± 293	649 ± 282	472 ± 168

[a] All seroconverters have defined HLA class I genotypes.
[b] M, male; F, female; IQR, interquartile range, from 25th to 75th percentile.

form were subjected to phylogenetic analysis using CLUSTAL X, version 2.0 (46), MEGA, version 4 (79), and reference sequences from the Los Alamos HIV database (http://www.hiv.lanl.gov). Selective sequencing of the *env* region was done occasionally to resolve residual ambiguity with *pol* sequences (70).

HLA genotyping. Allelic variants at three HLA class I loci (*HLA-A*, *HLA-B*, and *HLA-C*) were resolved to 4-digit specificities using a combination of PCR-based techniques (81, 82). Reference to fully resolved alleles followed the revised nomenclature effective in April 2010 (55). Because of the limited sample size, HLA specificities were analyzed at the 2-digit level unless there was prior evidence for different outcomes associated with HLA alleles at high resolution (4 digits).

Descriptive statistics and correlation analyses. Using software packages in SAS, version 9.2 (SAS Institute, Cary, NC), SCs were first stratified by country of origin and then compared for demographic data, laboratory findings (VL, viral subtypes, and CD4 counts) (Table 1), and HLA variants of interest (see the supplemental material). Differences between countries and viral subtypes were assessed primarily by (i) analysis of variance (ANOVA) and the *t* test for quantitative variables with a normal distribution, (ii) nonparametric (e.g., Kruskal-Wallis) test for VL data before $\log_{10}$ transformation, or (iii) χ^2 and Fisher exact tests for categorical variables. Several outcome measures were also tested for linearity and strength of correlation, as reflected by Pearson *r* and Spearman rho (ρ) values, respectively. Individual plots were generated using GraphPad Prism (GraphPad Software, Inc.).

Hypothesis and statistical models. Based on recent evidence from a large African cohort (81), we aimed to test a primary hypothesis that favorable HLA variants generally confer a strong impact on early VL, before the virus acquires mutations to facilitate immune escape. Among all the HLA variants that are potentially favorable in one way or another, 11 are highly relevant to outcomes after HIV-1 infection among native Africans; these include A*29, A*74, B*13, B*44, B*57, B*58:01 (often in contrast to B*58:02), B*81, C*18, B*39-C*10 (haplotype), B*42-C*17 (haplotype), and A*30+C*03 (combination), as reported for three sub-Saharan African cohorts (41, 49, 81). Analytical approaches, including generalized linear models (GLMs) and mixed models ("Proc Mixed" in SAS), followed established strategies for testing independent associations (77, 81, 82). Statistical significance was accepted at the level of a *P* value of ≤0.05, provided that internal consistency was established and that multivariable models could rule out potential confounding by nongenetic factors (Fig. 1b). False

discovery probabilities (*q* values) from screening tests were assessed using the "Proc Multtest" procedure in SAS.

RESULTS

Characteristics of HIV-1 seroconverters available for primary analysis. Our selection process yielded 134 informative SCs from four countries, including 45 from Zambia (Lusaka and Copper Belt), 35 from Rwanda (Kigali), 27 from Kenya (Kilifi and Nairobi), and 27 from Uganda (Entebbe and Masaka) (Table 1). The estimated dates of infection (EDI) ranged from February 2006 to March 2009. The duration of infection was highly comparable across study sites at the visit corresponding to the acute phase (median, 1.5 to 1.9 months) and the visit corresponding to the early set-point (median, 8.2 to 8.6 months). Based on viral sequencing (successful in 95.5% of SCs), HIV-1 subtypes A1 and C were the most common, being found in 54 (40%) and 51 (38%) SCs, respectively. Additional subtypes and recombinant forms were relatively uncommon (2 for subtype B, 18 for subtype D, and 3 for recombinants); these and six SCs with missing information (VL too low to facilitate viral sequencing) were grouped together (Table 1).

Kenyan SCs differed from others in their lower age (27.4 ± 5.0 years), high male-to-female ratio (3.5), and infection with mixed HIV-1 subtypes (A1, C, and others) (18.5 to 63.0%). Rwandan SCs had relatively high acute-phase VLs (5.22 ± 0.79 $\log_{10}$) accompanied by relatively low set-point VLs (3.53 ± 1.20 $\log_{10}$). Zambians were characterized by the predominance of HIV-1 subtype C infection (95.6%) and relatively low CD4 counts in both the acute phase (497 ± 183 cells/μl) and early
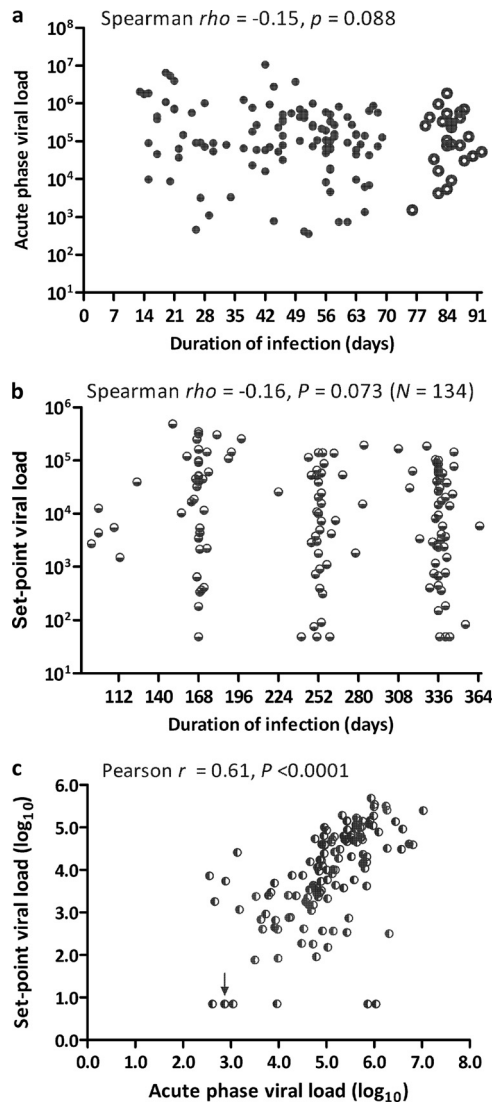
FIG. 2. Distribution of acute-phase and set-point viral load (VL) in 134 seroconverters (SCs). The panels display overall correlation between duration of infection and VL (a and b, drawn to different scales), as well as correlation between acute-phase and set-point VL measurements (c). Open and filled circles (a) correspond to two subgroups (see text). Arrow points to two subjects (from two countries) who share almost identical VL results.
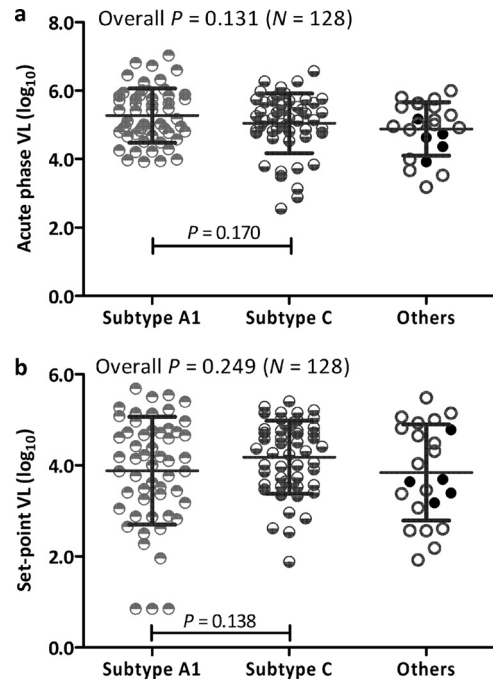

FIG. 3. HIV-1 viral load (VL) in 128 HIV-1 seroconverters (SCs) stratified by viral subtype. Acute-phase VL (a) and set-point VL (b) are compared. For each stratum, horizontal bars connected by a vertical line correspond to mean and standard deviation. Five individuals in the third group (filled circles) have subtype B ($n = 2$) or recombinant forms ($n = 3$), while the rest have subtype D (open circles). Six subjects with undetermined viral subtypes are excluded here.

set-point (472 ± 168 cells/μl). Ugandans had the highest frequency (66.7%) of viral subtypes other than A1 and C.

Dynamics of HIV-1 viremia during acute and early chronic phases of infection. Peak (highest) VL during acute-phase infection was readily discernible in 106 SCs because multiple measures within the first 3 months of infection were available. The other 28 SCs each had one single VL measurement taken near the end of the acute phase (Fig. 2a) but still well before set-point VL was established in the early chronic phase (3 to 12 months after EDI) (Fig. 2b).

Overall, the acute-phase VLs ranged from 2.55 $\log_{10}$ to 7.03 $\log_{10}$, showing no correlation with duration of infection (DOI) (Fig. 2a). The early set-point VLs ranged from below detection (in seven SCs) to 5.69 $\log_{10}$ (Fig. 2b). VLs in these two phases showed a strong linear correlation (Pearson $r = 0.61$, $P < 0.0001$) (Fig. 2c); a strong nonlinear correlation was also apparent for VLs before $\log_{10}$ transformation (Spearman $\rho = 0.66$, $P < 0.0001$).

Absence of correlation between HIV-1 subtype and viremia. In 128 of 134 SCs with successful viral sequencing, neither acute-phase nor set-point VL differed by HIV-1 subtype ($P \geq 0.131$ by one-way ANOVA) (Fig. 3). The set-point VLs in three SCs with subtype A1 viruses were below the lower limit of detection. Transformation of VLs to $\log_{10}$ did not alter the results, as nonparametric tests (by VL ranking) led to the same conclusions ($P \geq 0.309$ by Kruskal-Wallis test). Direct comparison between subtype A1 and subtype C alone confirmed similarities between these groups ($P \geq 0.114$ by t test) (Fig. 3).

Distribution of select HLA variants by patient groups and country of origin. Selection of SCs for analysis did not lead to obvious bias in either the national origin (see Table S1 in the supplemental material) or distribution of the 11 HLA variants of interest (see Table S2 in the supplemental material). Stratification by country of origin did not show overall genetic heterogeneity (see Table S3 in the supplemental material), but 3 of 11 candidate variants, i.e., B*13, B*39-C*12, and the A*30+C*03 combination, were too rare to facilitate association analyses (see Table S2 in the supplemental material). For the eight remaining variants, the frequency was highest for A*74 (20 subjects) and lowest for A*29, B*81, and C*18 (10 subjects each).

TABLE 2. Association analyses for eight HLA variants: impact on repeated measures of HIV-1 viral load and
CD4$^+$ T-cell count in 134 seroconverters

HLA variant[a]	Analysis of VL[b]				Analysis of CD4 count[b]			
	No. of subjects	β ± SE	P	q[c]	No. of subjects	β ± SE	P	q[c]
A*29	10	0.18 ± 0.17	0.300	0.384	10	−47 ± 39	0.231	0.582
A*74	20	0.12 ± 0.13	0.336	0.384	20	12 ± 29	0.674	0.839
B*42-C*17	18	0.28 ± 0.14	0.042	0.102	18	108 ± 31	<0.001	0.004
B*44	12	−0.80 ± 0.17	<0.001	0.004	12	213 ± 37	<0.001	0.004
B*57	11	−1.06 ± 0.16	<0.001	0.004	11	30 ± 37	0.415	0.664
B*58:01	14	−0.02 ± 0.15	0.881	0.881	14	−7 ± 34	0.839	0.839
B*81	10	−0.18 ± 0.18	0.325	0.384	10	42 ± 40	0.291	0.582
C*18	10	−0.34 ± 0.17	0.051	0.102	10	−12 ± 38	0.759	0.839

[a] Of 11 candidates, 3 (B*13, B*39-C*12, and A*30+C*03) are too rare (≤5 subjects each) to allow informative analysis.
[b] Mixed models test virological and immunological outcomes in the first 12 months of infection (see text). For consistency, age, sex, country of origin, and duration of infection are retained as covariates in each test.
[c] False discovery rates (q values) are shown for the multiple hypotheses.

HLA candidates screened and then dismissed by mixed models. In analyses adjusted for gender, DOI, age at EDI, and country of origin (Table 2), five HLA candidates (A*29, A*74, B*58:01, B*81, and C*18) failed to show appreciable impact on VL or CD4 count ($P \geq 0.051$ and $q \geq 0.102$). Indeed, mean beta estimates for VL were positive (unfavorable) for A*29 and A*74, which were represented by A*29:02 and A*74:01, respectively. On the other hand, B*42-C*17 lacked internal consistency (Table 2), being somewhat unfavorable for VL ($P = 0.042$ and $q = 0.102$) while highly favorable for CD4 count ($P < 0.001$ and $q = 0.004$). As expected, B*42:01 and C*17:01 were the individual alleles accounting for the B*42-C*17 haplotype.

HLA variants persistently associated with acute-phase and set-point viremia. Mixed models consistently identified B*44 and B*57 as markers of relatively low viremia at two intervals of PHI ($P < 0.01$ and $q < 0.01$ for all tests) (Table 2; also see Table S4 in the supplemental material). SCs with HLA-B*44 ($n = 12$) and B*57 ($n = 11$) had significantly lower peak VLs than carriers of other alleles (regression beta values of -1.08 ± 0.26 log$_{10}$ and -0.83 ± 0.27 log$_{10}$, respectively) ($P \leq 0.003$) (Fig. 4a). These relationships were similar when viral subtype replaced country of origin as a nongenetic covariate in the analytic models (data available upon request). The acute-phase mean VL was also somewhat lower in Ugandans than in Rwandans (adjusted beta value of -0.30 ± 0.21 log$_{10}$, $P > 0.15$).

Both B*44 and B*57 were independently associated with lower set-point VLs in the 134 SCs (Fig. 4b), with adjusted VL differences of -0.75 ± 0.33 log$_{10}$ for B*44 ($P = 0.026$) and -1.13 ± 0.34 log$_{10}$ for B*57 ($P = 0.001$). Of note, five of seven (or 71%) SCs with undetectable set-point VLs had either B*44 or B*57. Among nongenetic factors, being female or Rwandan was associated with lower VLs (-0.42 ± 0.20 log$_{10}$, $P = 0.037$, and -0.53 ± 0.25 log$_{10}$, $P = 0.034$, respectively). Age and DOI had no appreciable impact on set-point VL (adjusted P value, >0.16 for all).

Within this study population, B*44 was represented by two 4-digit alleles, namely, B*44:03 ($n = 10$) and B*44:15 ($n = 2$) (Fig. 4). Similarly, B*57 was represented by two 4-digit alleles, B*57:03 ($n = 6$) and B*57:02 ($n = 5$) (Fig. 4). No inference

could be made about possible joint effects of B*44 and B*57 from a single individual who carried both.

Factors associated with CD4 count during early chronic phase of HIV-1 infection. In analyses similar to those performed for set-point VL, B*44 was associated with higher CD4 counts (167 ± 72, $P = 0.022$) (Table 3). The trend toward association with higher CD4 counts in B*57-positive SCs (106 ± 74) was not statistically significant ($P = 0.16$). Higher CD4 counts in females than males (158 ± 44, $P < 0.001$) and
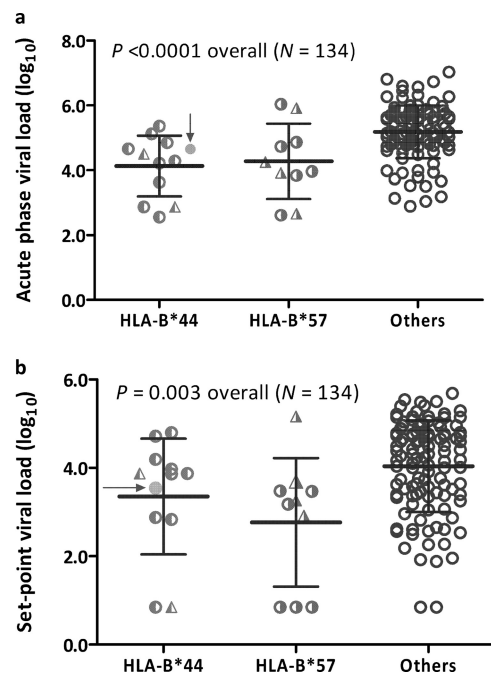

FIG. 4. Acute-phase (a) and set-point (b) viral load in 134 HIV-1 seroconverters after stratification by HLA variant (B*44, B*57, and others). For each stratum, horizontal bars connected by a vertical line correspond to mean and standard deviation. One subject with both B*44:03 and B*57:02 (solid circle indicated by arrow) is grouped with others with B*44 alone. Half-filled circles represent subjects with B*44:03 and B*57:03, while half-filled triangles represent individuals with B*44:15 and B*57:02.

TABLE 3. Two multivariable models for testing major correlates of CD4$^+$ T-cell counts in 128 HIV-1 seroconverters at the visit corresponding to the earliest set-point viral load

Variable in model	Change in CD4 count[a]			
	Model 1		Model 2	
	β ± SE	P	β ± SE	P
Female	158 ± 44	0.0004	171 ± 43	0.0001
HLA-B*44	167 ± 72	0.022	159 ± 72	0.029
HLA-B*57	106 ± 74	0.158	113 ± 74	0.129
HIV-1 subtype C[b]	NA	NA	−139 ± 46	0.003
Other HIV-1 subtypes[b]	NA	NA	−27 ± 56	0.629

[a] For consistency, age and duration of infection are retained as covariates in all tests although they are not associated with the outcome. Country of origin and HIV-1 subtype are analyzed separately (model 1 versus model 2) because of concerns with colinearity. The beta estimates (β) and standard errors have been adjusted for all factors in each model (NA, not applicable).
[b] HIV-1 subtype A1 (the most frequent subtype) served as the reference group.

lower CD4 counts in Ugandans than Zambians (Δ = 184 ± 60, P = 0.003) mirrored their respective associations with set-point VL. On the other hand, neither age nor DOI had any appreciable effect on CD4 count (adjusted P value, >0.10). In an alternative model where HIV-1 subtype replaced country of origin as a covariate, HIV-1 subtype C was associated with lower CD4 counts than subtype A1 (Δ = −139 ± 46, P = 0.003), despite the lack of difference in set-point VLs between the two major subtypes (Fig. 3). Other subtypes, however, did not differ from subtype A1 in terms of CD4 count. For gender, HLA-B*44, and B*57, there were negligible differences for comparisons of their effects on CD4 count and VL.

DISCUSSION

Despite a modest sample size and limited statistical power, our analysis here demonstrated that HLA-B*44 and B*57 can substantially influence the level of HIV-1 viremia in sub-Saharan Africans with primary HIV-1 infection (PHI). The impact of B*44 and B*57 on viremia well exceeded the threshold value (0.30 log$_{10}$) commonly used to ascribe biological and epidemiological significance (61, 74). In addition, nongenetic host factors, such as age, sex, duration of infection, country of origin and viral subtype, did not obscure the effects attributable to B*44 and B*57—statistical adjustments for those potential confounders did not meaningfully alter the estimates of effect size (i.e., mean regression beta and standard error of the mean). Other HLA candidates had either conflicting (inconsistent) or null associations, so their specific roles in HIV-1 infection might not be generalizable. Larger cohorts may allow further evaluation of these and other variants for country- or virus-specific relationships.

During acute-phase and early chronic infection, a strong association between HLA-B*57 and low viremia was expected, as B*57 variants (mostly B*57:03 and B*57:02 in Africans) have been recognized early and widely as highly favorable (11, 36, 60). The importance of B*57 to clinical and immunological responses during PHI has also been documented (2, 3). Moreover, B*57 in infected partners has been associated with delayed transmission of HIV-1 to their uninfected cohabiting partners in Zambia (82) and more recently in the United States

(21). Three dominant, B*57-restricted CTL epitopes have been mapped to the HIV-1 Gag region. Mutations that alter HIV-1 Gag sequences appear to "cripple" viral replication, as suggested by persistently lower VLs upon transmission to new hosts (1, 23). Viruses from B*57-positive individuals can accumulate immune escape mutations, which ultimately lead to functional compensation and pathogenetic consequences (12). In this study, the SCs with B*57 did have relatively high CD4 counts during early chronic infection compared with those of other SCs, but the small sample size limited statistically meaningful inference. An intact CD4 profile in early infection might further translate to delayed disease progression (58, 59).

Relatively little attention has been paid to HLA-B*44, because it has not been definitively associated with virologic, immunologic, or clinical outcomes before, although one study has identified B*44:03 as a favorable allele in the context of HIV-1 subtype C infection in South Africa (49). In our study population, the association of B*44 with relatively low viremia was accompanied by a corroborative association with high CD4 count. Of the two B*44 alleles (B*44:03 and B*44:15) found in this mixed study population, B*44:03 (previously B*4403) is a common allele that has been studied extensively. According to the HIV Molecular Immunology Database (52), nearly a dozen HIV-1-specific CTL epitopes have been attributed to B*44:03 and its related alleles. The most consistent epitopes targeted by B*44:03 are AW11 derived from Gag/P24 (31, 56) and QW11 from Pol/integrase (39, 73). In South Africans, a single CTL escape mutation (at the QW11 epitope) is associated with low viral load (73). In other populations, B*44:02 is a major allele that is often found on the B*44-C*05 haplotype (53). Understanding the early immune responses facilitated by various B*44 alleles should help with the dissection of protective immunity to HIV-1 infection, especially in the context of subtypes A1 and C in sub-Saharan Africa (22).

Viral subtypes are highly relevant to the design of vaccines (42, 57), and multiple reports have indicated that disease progression may differ somewhat by viral subtype (32, 33, 40, 62, 90). In the cohort under study, the levels of viremia during PHI did not differ by the two major subtypes (A1 and C), but CD4 counts did show clear differences between subtypes A1 and C. Dissociation between virologic and immunologic outcomes has been noted earlier for viral subtypes A and D (4). The distribution of HIV-1 subtypes A1 and C in our cohort correlated closely with the country of origin (Table 1), precluding comparison of different viral subtypes within a homogeneous subpopulation (e.g., Ugandans alone or Zambians alone). The accumulation of enough SCs with different viral subtypes in a subpopulation (e.g., Kenyans) will likely permit such comparison.

The HIV-1 VL presumably reflects the equilibrium between viral replication and immunologically mediated viral clearance. In the context of PHI, the acute phase does not involve intense antibody responses (19, 45, 67). Set-point viremia is typically reached within the first few months after infection (6), well before the debut of high-affinity neutralizing antibodies that require extensive somatic mutation (45, 67). The underlying immune control during acute and early chronic infection appears to depend on cellular immune pathways (8, 43), including CTL and natural killer (NK) cell activities mediated by HLA class I allelic products. Thus, B*44 and B*57 most likely

mediate the level of viremia through these cellular mechanisms to influence immune control of PHI. Our ability to analyze acute-phase viremia in a subset of carefully selected SCs was critical to demonstrating the early impacts conferred independently by B*44 and B*57.

The acute phase of PHI can have multiple long-term implications. First, early events of host-virus interactions are most devastating to mucosal CD4 cells (the "leaky gut" effect) (6, 10), often followed by a multitude of cytokine responses (78). Second, individuals with acute infection are highly contagious (93)—in a study of HIV-1-discordant couples from Uganda, 43.5% of incident cases of heterosexual HIV-1 transmission involved donor/source partners with acute infection (68). Third, acute infection sets the stage for other pathophysiological events, including immune dominance (3, 25), viral genetic drift or recombination (20, 94), and immune escape (39, 64, 69, 76, 89). It is conceivable that relatively effective control of the initial "viral burst," as seen frequently in SCs with B*44 and B*57 during acute infection, can limit viral reservoirs and alter other pathways of HIV-1 pathogenesis. Sequencing of viral and proviral DNA during early infection should facilitate further elucidation of early immune pressure.

Overall, the association of HLA class I variants with different levels of viremia in PHI reiterates the importance of HLA-related immune responses to control of HIV-1 infection. For both B*44 and B*57, the magnitude of the impact on viral load reduction as observed here ($\sim$1.0 $\log_{10}$) is higher than earlier estimates (rarely exceeding 0.3 $\log_{10}$) based on more chronically infected seroprevalent subjects (81). In the latest (and largest) study that focuses on HIV-1 controllers (VL of <2,000 copies/ml for $\geq$12 months), B*57 remains as the most prominent factor in subjects of African and European ancestries (88). However, our results here do not support the attribution of all major favorable effects of the HLA-B molecule to potential peptide-binding properties of three amino acid residues along the peptide-binding groove (88). Indeed, three residues at positions 67, 70, and 97 (around the C and F pockets) all differ between the B*44 and B*57 alleles found in our study population, with Ser, Asn, and Arg for B*44 and Met, Ser, and Val for B*57. One plausible explanation is that other variants in linkage disequilibrium with B*44 might contribute to the observed relationships. Alternatively, at the early phase of HIV-1 infection, the multifaceted HLA-B molecule has deployed its natural killer cell ligand property in addition to its antigen presentation function. In other words, similarity or difference in peptide-binding preferences alone may not fully capture the spectrum of concerted and evolving immune function that is essential to durable containment of HIV-1 infection (44). Either way, ongoing efforts to expand the sub-Saharan African cohorts of seroconverters should allow detailed analysis of HLA sequence motifs and other related properties (e.g., supertypes and frequencies) (47, 48).

Finally, our ability to analyze acute-phase viremia has also produced direct evidence that highly effective viral control, as reflected by undetectable viral load, is not entirely due to defective founder viruses (a plausible alternative hypothesis). For example, seven SCs in our study population had viremia levels of less than 50 RNA copies/ml at the earliest set-point (Fig. 2c), but they all had detectable viremia during acute infection. Indeed, three of them had acute-phase viremia levels

of $\geq$40,000 copies/ml. Since the majority (5/7) of these SCs have B*44 or B*57 (Fig. 4b), frequent testing of HIV-1-sero-negative subjects with these alleles may help identify additional SCs whose course of viremia can offer critical insights about mechanisms of early immune control (25, 51, 92). By our calculation (see Table S3 in the supplemental material), about one in six Africans in Kenya, Rwanda, Uganda, and Zambia carry either B*44 or B*57.

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