Cumulative Impact of Host and Viral Factors on HIV-1 Viral-Load Control during Early Infection

Ling Yue, Emory University
Heather A. Prentice, University of Alabama at Birmingham
Paul Farmer, Emory University
Wei Song, University of Alabama at Birmingham
Dongning He, University of Alabama at Birmingham
Shabir Lakhi, Emory University
Paul Goepfert, University of Alabama at Birmingham
Jill Gilmour, International AIDS Vaccine Initiative
Susan A Allen, Emory University
Jianming Tang, University of Alabama at Birmingham

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

Journal Title: Journal of Virology
Volume: Volume 87, Number 2
Publisher: American Society for Microbiology | 2013-01, Pages 708-715
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1128/JVI.02118-12
Permanent URL: http://pid.emory.edu/ark:/25593/f4xqd

Final published version: http://jvi.asm.org/content/87/2/708

Copyright information:
© 2013, American Society for Microbiology. All Rights Reserved.

Accessed January 11, 2020 7:50 AM EST
Cumulative Impact of Host and Viral Factors on HIV-1 Viral-Load Control during Early Infection

Ling Yue, Heather A. Prentice, Paul Farmer, Wei Song, Dongning He, Shabir Lakhi, Paul Goepfert, Jill Gilmour, Susan Allen, Jianming Tang, Richard A. Kaslow, Eric Hunter

Emory Vaccine Center and Yerkes National Primate Research Center, Emory University, Atlanta, Georgia, USA; Department of Epidemiology, University of Alabama at Birmingham, Birmingham, Alabama, USA; Zambia-Emory HIV Research Project, Lusaka, Zambia; Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA; International AIDS Vaccine Initiative, London, England; Department of Pathology, Emory University, Atlanta, Georgia, USA

In HIV-1 infection, the early set-point viral load strongly predicts both viral transmission and disease progression. The factors responsible for the wide spectrum of set-point viral loads are complex and likely reflect an interplay between the transmitted virus and genetically defined factors in both the transmitting source partner and the seroconverter. Indeed, analysis of 195 transmission pairs from Lusaka, Zambia, revealed that the viral loads in transmitting source partners contributed only ~2% of the variance in early set-point viral loads of seroconverters (P = 0.046 by univariable analysis). In multivariable models, early set-point viral loads in seroconverting partners were a complex function of (i) the viral load in the source partner, (ii) the gender of the seroconverter, (iii) specific HLA class I alleles in the newly infected partner, and (iv) sharing of HLA-I alleles between partners in a transmission pair. Each of these factors significantly and independently contributed to the set-point viral load in the newly infected partner, accounting for up to 37% of the variance observed and suggesting that many factors operate in concert to define the early virological phenotype in HIV-1 infection.

The HIV-1 set-point viral load (VL) in infected individuals directly affects the transmission rate of the virus (1–3) and early disease progression (4–6). Recent studies have shown a trend of increasing set-point VLs over the last 30 years of the HIV-1 epidemic (7, 8), raising the possibility of both increasing transmission rates and virulence in treatment of naive HIV-1 infection. A plausible explanation for this observation is viral adaptation to the host at the population level over time (9, 10), providing a further challenge for HIV-1 vaccine design. The determinants of set-point VL in newly infected individuals include viral genetic factors (11–13) and host genetic factors (14–16). Thus, to comprehensively define the role of underlying factors in determining early set-point VL, it is necessary to understand the complexity of the interaction between the transmitted founder virus, with its embedded footprints of the immune response of the transmitting source partner (TSP), and the de novo immune defense of the seroconverting partner (SC).

We previously observed that cytotoxic T-lymphocyte (CTL) epitope escape mutations selected by immune pressure in the TSP can modulate the early set-point VL in the SC (11, 17), suggesting that mutations that would be expected to positively impact VL in the TSP can negatively impact VL in the seroconverter. This observation is consistent with our previous studies of the ZEHRP cohort and with other studies of heterosexual cohorts of limited size. These studies demonstrated a relatively weak correlation between VLs in TSPs and SCs in linked heterosexual transmission partners (18–20). In contrast, a more significant VL correlation was shown for a transmission pair cohort of men who have sex with men (MSM) in San Francisco (21), and a phylogenetic analysis of MSM in the Swiss HIV Cohort Study concluded that up to half of the variance in set-point VL can be heritable (22). These observations reinforced the hypothesis that the genetic characteristics of the transmitted founder virus could be an important determinant for defining the early set-point VL in the linked SC (18, 19, 21, 22). The contribution of the virus may, however, be modulated by human genetic factors in both the SC and the TSP. In the newly infected SC, HIV-specific CD8+ T-cell responses, facilitated by specific HLA class I (HLA-I) molecules, clearly have a substantial immediate impact on VL, as documented for recently and chronically infected individuals (16, 23–25). The effects of favorable and unfavorable HLA-I alleles (23, 26, 27) and their additive effect (28) on VL control have been reported. Gender has also been observed to modulate VL in both early and chronic infections in antiretroviral-naive and -treated populations (29–31), although the exact mechanism for this is not known (32). Finally, sharing of HLA-I alleles between the TSP and the SC can lead to reduced control of virus following transmission because of the existence of CTL escape mutations selected in the TSP that reduce the efficacy of the de novo immune response (11, 19, 33). In our current study of 195 phylogenetically linked heterosexual transmission couples, we had a sufficiently large population to document for the first time that while the phenotype of the transmitted founder virus had a significant impact on early set-point VL, the gender of the SC as well as the immunogenetic characteristics of both the TSP and the SC significantly and independently modulated this effect. Taken together, we show that the early set-point VL established by a particular transmitted founder virus in a newly infected individual will be influenced by a complex interplay of virus and host factors.
TABLE 1 Characteristics of HIV SC partners in entire study population and in phylogenetically linked couples only

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All SCs</th>
<th>Linked SCs</th>
<th>P value</th>
<th>Male-to-female transmission</th>
<th>Female-to-male transmission</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of SCs</td>
<td>195</td>
<td>23</td>
<td></td>
<td>117</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>No. (%) of individuals aged &gt;40 yr</td>
<td>13 (6.7)</td>
<td>2 (8.7)</td>
<td>0.663</td>
<td>3 (2.6)</td>
<td>10 (12.8)</td>
<td>0.005</td>
</tr>
<tr>
<td>Sex ratio (no. of males/no. of females)</td>
<td>0.7 (78/117)</td>
<td>1.6 (14/9)</td>
<td>0.055</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Index partner log_{10} VL* (mean ± SD)</td>
<td>5.0 ± 0.7</td>
<td>4.3 ± 1.2</td>
<td>0.009</td>
<td>5.1 ± 0.7</td>
<td>4.8 ± 0.6</td>
<td>0.004</td>
</tr>
<tr>
<td>DOI (days) until set-point VL (median [IQR])</td>
<td>231 (134–339)</td>
<td>222 (130–251)</td>
<td>0.218</td>
<td>210 (131–322)</td>
<td>268 (140–378)</td>
<td>0.058</td>
</tr>
<tr>
<td>DOF (days) for index partner until SC EDI (median [IQR])</td>
<td>408 (157–972)</td>
<td>688 (292–1596)</td>
<td>0.059</td>
<td>421 (224–963)</td>
<td>379 (149–972)</td>
<td>0.037</td>
</tr>
<tr>
<td>No. (%) of individuals with Zambian HLA-I marker set score&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.6</td>
<td>0.002</td>
<td>0.176</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥2</td>
<td>5 (2.6)</td>
<td>4 (17.4)</td>
<td>1 (0.8)</td>
<td>4 (5.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>35 (17.9)</td>
<td>0 (0.0)</td>
<td>20 (17.1)</td>
<td>15 (19.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>155 (79.5)</td>
<td>19 (82.6)</td>
<td>96 (82.0)</td>
<td>59 (75.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. (%) of individuals with HLA-B sharing with TSP&lt;sup&gt;b&lt;/sup&gt;</td>
<td>47 (24.1)</td>
<td>21 (18.0)</td>
<td>0.014</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> VL measurement taken on the visit date closest to the estimated date of infection of the SC.

<sup>b</sup> Favorable and unfavorable HLA markers.

Virologic assessment. Plasma VL was measured as the number of HIV RNA copies/ml, using the Roche Amplicor 1.0 assay (Roche Diagnostics Systems Inc., Branchburg, NJ), with a lower limit of detection of 400 copies/ml. Phylogenetic analysis and criteria for establishing identity between viruses from the two partners have been documented previously (34). In all 195 SCs available for analysis, set-point VL was the first VL measured ≥9 weeks after the estimated date of infection (EDI), which was defined as the midpoint between the last seronegative and first seropositive visits, resulting in a median interval of 231 days for linked couples and 222 days for unlinked couples. In a subset of SCs (n = 143) with multiple VL measurements, set-point VL was calculated as the geometric mean of all VLs collected between 3 and 12 months after the EDI. In TSPs, the VL measured at the visit date closest to the SC’s EDI was analyzed (median interval, 89 days for linked couples and 113 days for unlinked couples).

Genotyping. HLA-1 genotyping resolved alleles to the first four digits by use of a combination of PCR-based methods, including PCR with sequence-specific primers (Dynal/Invitrogen, Brown Deer, WI), automated sequence-specific oligonucleotide probe hybridization (Innogenetics, Alpharetta, GA), and sequencing-based typing (Abbott Molecular, Inc., Des Plaines, IL) tailored to capillary electrophoresis and an ABI model 3130xl DNA analyzer (Applied Biosystems, Foster City, CA).

Favorable and unfavorable HLA markers. The Zambian-specific HLA-1 marker set included three population-specific markers previously identified (27, 35) as favorable soon after seroconversion (A*74, B*13, and B*57); the A*30-C*03 combination was excluded because of a lack of statistical significance in this subset of SCs, and HLA-C*18 was excluded due to its strong linkage disequilibrium (LD) with B*57. No HLA-1 marker has been confirmed unequivocally as unfavorable in Zambian SCs with early infection. In order to summarize the impact of the Zambian SCs on set-point VL for each SC, each favorable marker was given a value of +1 and then summed, for a total set score ranging from 0 to ≥2.

**HLA class I allele sharing.** HLA-1 allele sharing and SC set-point VLs were assessed for the phylogenetically linked population only. Sharing was defined as carriage by both the TSP and the SC of one or both alleles at HLA-A, HLA-B, or HLA-C. Because two-digit alleles at HLA-B tend to be rather homogeneous in our Zambian population, we considered sharing at the two-digit level of genotyping closely equivalent to sharing at the four-digit level, with two exceptions: the more heterogeneous B*15 and B*58 alleles, whose effects on VL have shown differences at the four-digit level (26). Although tight linkage disequilibrium between HLA-B and HLA-C alleles produces very similar associations for B and B-C haplotype sharing, analyses were performed with B allele sharing, since the evidence for involvement of HLA-B is stronger.

Statistical analysis. Differences in characteristics between partners of epidemiologically linked and unlinked couples were assessed using Student’s t test (for continuous variables) and the χ² test or Fisher exact test (for categorical variables). All VL data were log_{10} transformed. Generalized linear models (GLMs) were applied separately to linked and unlinked couples. Multivariable models included adjustments for other known factors associated with set-point VL, including SC age, sex, CD4 cell count, and SCVL. Non-HLA factors included in the multivariable model were age, sex, CD4 cell count, SCVL, and smoking status.

RESULTS

Study population and virus phylogeny. The study population consisted of 195 heterosexual transmission pairs in which the HIV-1 subtype C viruses in the TSPs and SCs were phylogenetically linked using methods described previously (34). An additional 23 couples in which the SC was infected with viruses clearly unrelated to those of the chronically infected index partner were available as controls. Among the linked pairs, male-to-female (MTF) transmissions outnumbered female-to-male (FTM) transmissions by about 50%, and male SCs were significantly older than female SCs (Table 1). The TSPs of linked pairs had a significantly higher mean VL than that of the index partners of the 23 unlinked couples.

MATERIALS AND METHODS

Study population. We studied 218 HIV-concordant couples from the Zambia-Emory HIV Research Project, among which 195 had phylogenetically linked and 23 had unlinked viruses based on viral sequencing (34). More than 95% of couples were infected with HIV-1 subtype C (34). This study was approved by the institutional review boards at Lusaka, Emory University, and the University of Alabama at Birmingham; all subjects gave written informed consent for participation.

Virologic assessment. Plasma VL was measured as the number of HIV RNA copies/ml, using the Roche Amplicor 1.0 assay (Roche Diagnostics Systems Inc., Branchburg, NJ), with a lower limit of detection of 400 copies/ml. Phylogenetic analysis and criteria for establishing identity between viruses from the two partners have been documented previously (34). In all 195 SCs available for analysis, set-point VL was the first VL measured ≥9 weeks after the estimated date of infection (EDI), which was defined as the midpoint between the last seronegative and first seropositive visits, resulting in a median interval of 231 days for linked couples and 222 days for unlinked couples. In a subset of SCs (n = 143) with multiple VL measurements, set-point VL was calculated as the geometric mean of all VLs collected between 3 and 12 months after the EDI. In TSPs, the VL measured at the visit date closest to the SC’s EDI was analyzed (median interval, 89 days for linked couples and 113 days for unlinked couples).

Genotyping. HLA-1 genotyping resolved alleles to the first four digits by use of a combination of PCR-based methods, including PCR with sequence-specific primers (Dynal/Invitrogen, Brown Deer, WI), automated sequence-specific oligonucleotide probe hybridization (Innogenetics, Alpharetta, GA), and sequencing-based typing (Abbott Molecular, Inc., Des Plaines, IL) tailored to capillary electrophoresis and an ABI model 3130xl DNA analyzer (Applied Biosystems, Foster City, CA).

Favorable and unfavorable HLA markers. The Zambian-specific HLA-1 marker set included three population-specific markers previously identified (27, 35) as favorable soon after seroconversion (A*74, B*13, and B*57); the A*30-C*03 combination was excluded because of a lack of statistical significance in this subset of SCs, and HLA-C*18 was excluded due to its strong linkage disequilibrium (LD) with B*57. No HLA-1 marker has been confirmed unequivocally as unfavorable in Zambian SCs with early infection. In order to summarize the impact of the Zambian SCs on set-point VL for each SC, each favorable marker was given a value of +1 and then summed, for a total set score ranging from 0 to ≥2.

**HLA class I allele sharing.** HLA-1 allele sharing and SC set-point VLs were assessed for the phylogenetically linked population only. Sharing was
SCs, consistent with the finding from prior studies that the likelihood of transmission in discordant couples increases with VL in the chronically infected partner (1, 3). The fraction of individuals with HLA-I markers reported previously to downmodulate VL (23, 27, 36) did not differ significantly between linked and unlinked SCs or between linked male and female SCs, although the distribution was significantly different, since in this small number of unlinked SCs all four individuals carried two protective alleles ($P = 0.002$) (Table 1).

For all 195 SCs, at least one VL determination from more than 9 weeks postinfection was available to establish the early set-point VL (VL1), while for 143 SCs sufficient longitudinal samples were available to determine the geometric mean early set-point VL (VL2). Univariable and multivariable analyses are presented for both early set-point VL calculations. We first analyzed the impact of host genetic factors on early set-point VL in the SC and then, in this context, the impact of TSP VL on this parameter.

Effects of age and gender. To quantify the impact of each suspected host factor on SC VL, we first tested each one individually in univariable models. Both the age and gender of infected expected host factor on SC VL, we first tested each one individually in this context, the impact of TSP VL on this parameter.

Effects of host genetic factors. Evidence for favorable or unfavorable relationships of certain HLA-I markers to virologic and immunologic outcomes of HIV infection is conclusive (19, 27, 28, 33, 35, 37–39). Here we evaluated a set of these markers chosen on the basis of their documented impact and population frequencies in this Zambian HIV-discordant heterosexual transmission cohort. The Zambian-specific markers included three “favorable” HLA alleles (A*74, B*13, and B*57) that were significantly associated with lower VLs within the early weeks of infection (Table 2). The effect on early set-point VL control for each favorable HLA class I marker in the group of 195 SCs is shown in Fig. 2A (left panel) and Table 2. In univariable analyses, the presence of each of these protective alleles had a large negative impact on early set-point VL ($\beta = -0.57$ to $0.87 \log_{10}$) (Table 2) in the SCs, and in the case of A*74 and B*57, this was highly statistically significant. In multivariable models, all three alleles exhibited highly significant and similarly large effects on SC set-point VL ($P \leq 0.003; \beta = -0.53$ to $0.87 \log_{10}$) (Table 2).

### TABLE 2 Multifactorial contributions to set-point VLs in SCs with confirmed TSPs, as revealed by GLMs

<table>
<thead>
<tr>
<th>Host/viral factors used as independent variables (IVs)</th>
<th>Univariable models for VL1</th>
<th>Multivariable model for VL1$^b$</th>
<th>Univariable models for VL2</th>
<th>Multivariable model for VL2$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV1 (age of &gt;40 yr of SC)</td>
<td>Mean $\beta$ $\pm$ SE</td>
<td>P value</td>
<td>Mean $\beta$ $\pm$ SE</td>
<td>P value</td>
</tr>
<tr>
<td>IV2 (SC is male)</td>
<td>0.30 $\pm$ 0.23</td>
<td>0.189</td>
<td>0.29 $\pm$ 0.20</td>
<td>0.154</td>
</tr>
<tr>
<td>IV3 (HV-1 VL $[\log_{10}$ in TSP$^a$)</td>
<td>0.38 $\pm$ 0.11</td>
<td>0.001</td>
<td>0.47 $\pm$ 0.11</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>IV4 (HLA-A*74 in SC)</td>
<td>0.17 $\pm$ 0.08</td>
<td>0.046</td>
<td>0.28 $\pm$ 0.07</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>IV5 (HLA-B*13 in SC)</td>
<td>$-0.39 \pm 0.17 &lt;0.001$</td>
<td>$-0.53 \pm 0.15 &lt;0.001$</td>
<td>$-0.84 \pm 0.19 &lt;0.001$</td>
<td>$-0.72 \pm 0.17 &lt;0.001$</td>
</tr>
<tr>
<td>IV6 (HLA-B*57 in SC)</td>
<td>$-0.57 \pm 0.33 0.081$</td>
<td>$-0.84 \pm 0.29 0.003$</td>
<td>$-0.54 \pm 0.37 0.142$</td>
<td>$-0.79 \pm 0.31 0.012$</td>
</tr>
<tr>
<td>IV7 (HLA-B allele sharing by SC and TSP$^a$)</td>
<td>$-0.84 \pm 0.20 &lt;0.001$</td>
<td>$-0.87 \pm 0.19 &lt;0.001$</td>
<td>$-0.77 \pm 0.24 0.001$</td>
<td>$-0.75 \pm 0.21 &lt;0.001$</td>
</tr>
<tr>
<td>Individual HLA variants 1 (IV4, IV5, and IV6$^a$)</td>
<td>0.36 $\pm 0.13 0.007$</td>
<td>0.26 $\pm 0.11 0.023$</td>
<td>0.43 $\pm 0.16 0.007$</td>
<td>0.37 $\pm 0.14 0.010$</td>
</tr>
<tr>
<td>Individual HLA variants 2 (IV1, IV4, and IV6$^a$)</td>
<td>$-0.61 \pm 0.11 &lt;0.001$</td>
<td>$-0.26 \pm 0.10 &lt;0.001$</td>
<td>$-0.72 \pm 0.12 &lt;0.001$</td>
<td>$-0.74 \pm 0.11 &lt;0.001$</td>
</tr>
</tbody>
</table>

$^a$ First in models using the 9-week set-point VL in 195 SCs and second in models using the alternative geometric mean set-point VL (geometric mean in the 3 to 12 months after infection) in 143 SCs.

$^b$ Testing of all factors (IV1 through IV7) in one model.

$^c$ VL measurement taken at the visit date closest to the estimated date of infection of the SC.

$^d$ HLA sharing was defined as positive if the TSP and SC partners shared at least one HLA-B allele by 2-digit designations, except for B*15 or B*58, which required sharing by 4-digit designations.

$^e$ A*74, B*13, and B*57 were given a score of $+1$ if present, and the scores were summed into a single score variable. The sums in individual seroconverting partners ranged from 0 to $\pm 2$.

$^f$ Beta result from multivariable model including IV1, IV2, IV3, IV7, and HLA variants.
for the cohort of 143 individuals with geometric mean VLs (Table 2).

Combinations of HLA-I markers associated with more favorable outcomes would be predicted to enhance control of viremia (28, 40). Therefore, in order to summarize the impact of this Zambian-specific favorable HLA-I set on set-point VL for each SC, each of the favorable markers was given a value of +1, and the values were summed to define an overall HLA score for each individual. The additive effect shows a statistically significant inverse relationship between the sum of these values and set-point VL in SCs from the 195 linked pairs (Fig. 2B and Table 2) (univariable analysis $P$ value $= 0.001$; $\beta = 0.61$), with similar results in multivariable analyses and in the subset of individuals with VL2. A broadly comparable relationship was also observed in the small number of SCs from unlinked pairs (Fig. 2A and B, right panels).

A major advantage of a cohort of linked transmission pairs is the capacity to examine the effect of sharing HLA markers between partners, and we were able to document the unequivocal disadvantage for the SCs of sharing HLA-B alleles with their partner. Early set-point VLs were significantly higher in SCs who shared these markers with their respective TSPs (Fig. 2C and Table 2) (univariable analysis $P$ value $= 0.007$; $\beta = 0.36$). Sharing of HLA-A alleles alone showed no effect (Fig. 2C), and strong LD precluded demonstrating a deleterious effect of sharing HLA-C alleles that is independent of the B allele sharing effect (Fig. 2C). Furthermore, neither homozygosity at HLA-A, HLA-B, or HLA-C nor carriage of specific HLA-A or HLA-B supertypes, previously implicated in HIV disease control (41), showed a significant independent association with early set-point VL in the SCs (data not shown).

**Effect of transmitted virus on early set-point VL.** We next determined the impact of the replicative phenotype of the virus, as assessed by VL in the TSP, on the early set-point VL in the SC. In a univariable Pearson correlation analysis of the 195 Zambian pairs, we observed a statistically significant correlation ($P = 0.046$) (Fig. 3, left panel). With an $r^2$ value of 0.02, this indicates that only a small fraction of the effect on SC VL could be related to TSP VL. However, when host factors in both TSPs and SCs were examined jointly with TSP VL in a multivariable analysis, the impact of the latter on early set-point VL in the SC was highly significant (Table 2) ($P < 0.001$; $\beta = 0.28$). For the smaller VL2 cohort,
VL in the TSP lost significance in univariable analyses ($P = 0.180$; $\beta = 0.13$) (Table 2), but it once again became highly significant ($P = 0.003$; $\beta = 0.26$) (Table 2) in multivariable models adjusted for the effects conferred by gender, favorable HLA alleles, and HLA sharing.

Phylogenetically unlinked cohabiting SC partners showed a negative correlation between VL in the nontransmitting index partner and that in the SC (Fig. 3, right panel). Since transmission correlates strongly with VL in the TSP (1, 3), this observation likely reflects the greater probability that SCs will be infected by nonspousal partners with higher VLs than by spousal partners with lower VLs. This explanation is supported by the fact that the mean VL in phylogenetically unlinked Zambian index partners was significantly lower than the mean VL in TSPs (4.3 versus 5.0 log10, $P = 0.009$) (Table 1).

**Complexity of early set-point VL determination.** Finally, the joint impact of host and viral factors in both TSPs and SCs on the variance in early set-point VL was evaluated for the 195 SCs (Table 3). In these analyses, gender and the presence of either the A*74 or B*57 HLA-I allele individually had the greatest impact on early set-point VL (5.5, 6.1, and 8.0% variance in VL, respectively), while HLA-B allele sharing and TSP VL had a more modest impact (3.8 and 2.0% variance, respectively). In multivariable models that take into account all of the host and viral factors, the combined impact on VL was highly significant and explained 29% of the variance observed. Similarly highly significant results were obtained for the 143 SCs with VL2 values, where the overall effect on variance was 36.9%.

**DISCUSSION**

This study has quantified the relative and cumulative contributions of host and viral factors in each partner of a transmission pair to early set-point VL in the SC partner. Specifically, we have demonstrated that both the VL and HLA-I profile of the TSP, as well as demographic factors and the HLA-I profile of the SC, contribute significantly to early set-point VL, a highly predictive determinant of HIV disease progression.

It is striking that in both the current study and previous analyses of heterosexual transmission pairs (18–20), the significance of the contribution of the TSP VL to SC early set-point VL in univariable analyses (Pearson correlation $P$ value of 0.046; $r = 0.14$) was much lower than that reported for a similar study in MSM from San Francisco (Pearson correlation $P$ value of 0.006; $r = 0.55$) (21). While the fact that HIV-1-infected females present lower VLs than their male counterparts may in part act as a confounder in the VL correlation between TSPs and SCs, it seems unlikely that this completely explains the difference. The other possible difference between these studies is that in the studies of heterosexual transmission pairs, the transmitting partner was already identified as HIV positive prior to inclusion of the couple in discordant couple prevention studies, and in most linked transmission pairs we have studied phylogenetically, the diversity of the TSP virus population is consistent with chronic infection (42–44).

In a study by Hecht et al. (21), the majority of TSPs were identified posttransmission, and 9 of the 24 TSPs represented individuals with recent infection. High frequencies of recently/actively infected source partners were also observed in phylogenic analyses of similar transmission pairs in San Diego, CA (45). In such transmission pairs, it is likely that only limited adaptation to immune pressures was occurring in the TSP, thereby introducing fewer potential confounding components into the analysis. Moreover, in the study of Hecht et al., no correlation was observed between SC VL and SC age, race, or favorable/unfavorable HLA-I alleles, in contrast to the consistent observations from other cohorts (19, 38, 46–50). A second study of MSM, which examined the molecular phylogenetic basis for set-point VL, concluded that up to half of the variance in this parameter could be heritable, even though the stage of infection and host factors were not taken into account (22). By our estimate, host and viral factors in this heterosexual

---

**TABLE 3 Variability in HIV-1 set-point VL attributable to host and viral factors**

<table>
<thead>
<tr>
<th>Host/viral factors used as independent variables (IVs)</th>
<th>Variance in VL1 ($R^2$)</th>
<th>Variance in VL2 ($R^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>$P$ value</td>
</tr>
<tr>
<td>IV1 (age of $&gt;40$ yr of SC)</td>
<td>0.9</td>
<td>0.189</td>
</tr>
<tr>
<td>IV2 (SC is male)</td>
<td>5.5</td>
<td>0.001</td>
</tr>
<tr>
<td>IV3 (HIV-1 VL [log10] in TSP)</td>
<td>2.0</td>
<td>0.046</td>
</tr>
<tr>
<td>IV4 (HLA-A*74 in SC)</td>
<td>6.1</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>IV5 (HLA-B*13 in SC)</td>
<td>1.6</td>
<td>0.081</td>
</tr>
<tr>
<td>IV6 (HLA-B*57 in SC)</td>
<td>8.0</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>IV7 (HLA-B allele sharing by SC and TSP)</td>
<td>3.8</td>
<td>0.007</td>
</tr>
<tr>
<td>Demographic factors only (IV1 + IV2)</td>
<td>5.7</td>
<td>0.003</td>
</tr>
<tr>
<td>Individual HLA-I variants (IV4, IV5, and IV6)</td>
<td>13.8</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Host and viral factors combined$^c$</td>
<td>29.1</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

$^a$ As evaluated in generalized linear models (Table 2).

$^b$ A*74, B*13, and B*57 were given a score of +1 if present, and the scores were summed into a single score variable. The sums in individual seroconverting partners ranged from 0 to $\geq 2$.

$^c$ Results from multivariable model including IV1, IV2, IV3, IV7, and HLA variants.
cohort collectively accounted for up to 37% of the variance in early set-point VL. Thus, it is possible that very different factors can determine set-point VL in MSM versus heterosexual transmission pairs, but that hypothesis requires further evaluation in regions where sufficient numbers of MSM and heterosexual transmission pairs can be assembled. As we have pointed out elsewhere (51), the ongoing search for host and viral factors associated with the dynamics of HIV-1 VL should benefit from close attention to the timing of phenotypic measurements as well as careful application of statistical models.

One result of the HLA class I allele-restricted CTL response to HIV infection is the promotion of viral evolution at the population level. CTL epitope escape mutations in the virus that are selected under immune pressure can allow higher levels of virus replication in the selecting host due to reduced immune suppression, despite negatively affecting virus replicative capacity (11, 52). In contrast, upon transmission to an individual with different HLA-I alleles, such mutations can negatively affect virus fitness through decreased replicative capacity (11, 47, 53, 54). Indeed, we have shown previously an inverse correlation between the number of transmitted CTL escape mutations in gag and early set-point VL in the newly infected partner (17), thereby providing an additional source of variation between TSP and SC VLs. This variation may be more pronounced in our cohort, where transmission occurred from a chronically infected partner (with adapted viruses) to a cohabiting seronegative partner. Cohorts with acutely or recently infected source partners might display a different pattern of host-virus interaction.

The strong independent effect of HLA-B allele sharing on early set-point VL observed in the current study (P = 0.007; β = 0.36 to 0.43) implies that escape from HLA-I-mediated CTL recognition results in elevated viremia not only in a chronically infected individual but can also confer a replicative advantage when the virus is transmitted from that individual to a new host displaying a similar HLA-I profile. The reduced immune pressure on the virus in such transmission pairs may also reduce the impact of the CTL escape mutations on overall viral fitness (11). These observations demonstrate that the absence of information about the transmitted virus and its previous host can obscure the pathogenic potential of the virus in a recently infected individual. Such missing information likely accounts for some of the inconsistency in studies of host and viral determinants of control of viremia in recently infected individuals, and it may explain a portion of the observed variability in responses to similar antiretroviral drug regimens at the individual and population levels. Although partners who are the sources of infection have rarely been assessed in populations, our work attests to the potential value of the discordant couple paradigm for investigating host and microbial interactions, not only for HIV but for other infectious agents as well.

ACKNOWLEDGMENTS

We thank all the volunteers who participated in this study and all the staff at the Zambia-Emory HIV Research Project in Lusaka who made this study possible. We also thank Daniel Claiborne, Jessica Prince, Malinda Schaefer, and Jonathan Carlson for thoughtful discussions of the data.

This study was funded by PHS grants R01 AI 64060-08 (E.H.), R37 AI51231-11 (E.H.), and R01 AI071906-03 (R.A.K. and J.T.) from the NI-AID, NIH, grant ULI TR000454 from the Clinical Translational Science Award Program, NIH NCRR, Fogarty International Center grant D43 TW001042, and a grant from the International AIDS Vaccine Initiative (IAVI) to S.A. This work was further supported by the Virology Core at the Emory Center for AIDS Research (P30 AI050409), the IAVI Protocol C research network, and the Yerkes National Primate Research Center base grant (2P51RR000165-51).

REFERENCES

16. HLA-I allele sharing on early set-point VL observed in the current study (P = 0.007; β = 0.36 to 0.43) implies that escape from HLA-I-mediated CTL recognition results in elevated viremia not only in a chronically infected individual but can also confer a replicative advantage when the virus is transmitted from that individual to a new host displaying a similar HLA-I profile. The reduced immune pressure on the virus in such transmission pairs may also reduce the impact of the CTL escape mutations on overall viral fitness (11). These observations demonstrate that the absence of information about the transmitted virus and its previous host can obscure the pathogenic potential of the virus in a recently infected individual. Such missing information likely accounts for some of the inconsistency in studies of host and viral determinants of control of viremia in recently infected individuals, and it may explain a portion of the observed variability in responses to similar antiretroviral drug regimens at the individual and population levels. Although partners who are the sources of infection have rarely been assessed in populations, our work attests to the potential value of the discordant couple paradigm for investigating host and microbial interactions, not only for HIV but for other infectious agents as well.


