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Differences in immune control of HIV-1 infection are often attributable to the highly variable HLA class I molecules that present viral epitopes to CTL. In our immunogenetic analyses of 429 HIV-1 discordant Zambian couples (infected index partners paired with cohabiting seronegative partners), several HLA class I variants in index partners were associated with contrasting rates and incidence of HIV-1 transmission within a 12-year study period. In particular, A*3601 on the A*36-Cw*04-B*53 haplotype was the most unfavorable marker of HIV-1 transmission by index partners, while Cw*1801 (primarily on the A*30-Cw*18-B*57 haplotype) was the most favorable, irrespective of the direction of transmission (male to female or female to male) and other commonly recognized cofactors of infection, including age and GUL. The same HLA markers were further associated with contrasting viral load levels in index partners, but they had no clear impact on HIV-1 acquisition by the seronegative partners. Thus, HLA class I gene products not only mediate HIV-1 pathogenesis and evolution but also influence heterosexual HIV-1 transmission. The Journal of Immunology, 2008, 181: 2626–2635.

Materials and Methods

Study population and HIV-1 transmission as the primary outcome

From 1995 to 2006, HIV-1 discordant Zambian couples were identified and enrolled continuously by investigators who are now part of the Rwandan/Zambia HIV-1 Research Group in Lusaka, Zambia. Procedures for initial screening, voluntary counseling and testing, as well as prospective (quarterly) medical examination have been described elsewhere (19, 20). As the

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a

Transmission pairs with linked viruses
-205 transmission pair index (TP1) partners
-205 seroconverters (SCs)

Non-transmission pairs with 29 months follow-up
-224 non-transmission pair index (NTI) partners
-224 exposed seroconverters (ESNs)

Excluded couples
-28 pairs had unlinked HIV-1
-27 pairs received treatment
-18 pairs remained discordant, follow-up <9 months
-64 pairs without adequate samples (lost or partners died)

b

Time to HIV-1 transmission (TP1 + NTI)
-Kaplan-Meier curves & Cox proportional hazard models

Transmission (TP1) versus non-transmission (NTI)
-Logistic regression (univariate & multivariable models)

Tertiary analyses
-Index partner (TP1 + NTI) viral load (low, medium, high)
-Logistic regression (univariate & multivariable models)

Secondary analyses

Primary analyses

FIGURE 1. Classification of 566 Zambian couples (1132 individuals) enrolled for longitudinal study from 1995 to 2006 (a), along with the statistical approach to analyzing correlates of HIV-1 transmission and acquisition (b). Characteristics of 429 (205 + 224) couples included in the final analyses are detailed in Table I. For all association analyses, HLA class I factors (common haplotypes or their component alleles) serve as the independent variables, while nongenetic factors (age, direction of HIV-1 transmission, and GUI) are treated as covariates. Of note, tertiary analyses of HIV-1 acquisition are restricted to HLA factors identified by primary analyses of HIV-1 transmission.

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-205 seroconverters (SCs)

Non-transmission pairs with 29 months follow-up
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Index partner (TP1 + NTI) viral load (low, medium, high)
-Logistic regression (univariate & multivariable models)

Kaplan-Meier curves & logistic regression models

HIV-1 acquisition (SCs + ESNs)

HIV-1 viral load (RNA copies/ml of plasma) in index partners as secondary outcome

HIV-1 RNA copies in patient plasma were measured universally by the Roche Amplicor 1.0 assay (Roche Diagnostics Systems) in laboratories certified by the Virology Quality Assurance Program of the AIDS Clinical Trials Group. The assay was highly comparable with other commercial viral assays (Chiron Quantiplex HIV-1 branched DNA, Roche Amplicor version 1.5, and nucleic acid sequence-based amplification — NASBA HIV-1 RNA QT) (22), with a lower limit of detection at 400 RNA copies/ml of plasma. In earlier analyses (19), index partners with medium (10^2–10^3 copies/ml) and high (>10^3 copies/ml) concentrations of HIV-1 RNA transmitted viruses more readily than those with low-level viremia (<10^3 copies/ml). Thus, these three categories of viral load were treated as a separate outcome for secondary analyses (Fig. 1b) and were further assessed as predictive covariates in analyses of primary outcome.

PCR-based genotyping of HLA class I variants

Genomic DNA was extracted from whole blood or buffy coats using QIAamp blood kits (Qiagen). HLA class I genotyping was performed using a combination of PCR-based techniques, including PCR with sequence-specific primers (Dynal/Invitrogen), automated reference-strand conforma-
acquisition were confined to HLA factors identified by primary analyses of HIV-1 transmission.

Results
Classification and characteristics of Zambian couples at the end of follow-up

Using data from 10,278 person-visits for the initially seronegative (nonindex) partners, 429 couples eligible for primary analysis here were classified into 205 virologically linked transmission pairs and 224 nontransmission pairs (involving 108 female and 116 male index partners) (Table I). For the 205 TPI partners and 224 NTI partners, enrollment dates were highly comparable, as were within-couple age differences (ageΔ, 6.8 ± 4.2 years in the TPI group and 7.5 ± 5.2 years in the NTI group, p > 0.10). In contrast, mean and median follow-up time (FUT) of NTI was nearly double that of TPI (p < 0.001).

Consistent with earlier observations in the Zambian study population (19), MTF transmission (124 pairs) was more common than FTM transmission (81 pairs) (Table I). Among index partners of the transmission couples, 48.3% had GUI within the 6 mo prior to transmission for the transmission pairs or at end of follow-up for nontransmission pairs.

Table II. Major HLA class I haplotypes in 429 PIPs with or without HIV-1 transmission events

<table>
<thead>
<tr>
<th>Major Local (B-C) Haplotypes and Observed Freqa</th>
<th>Major Extended (A-B-C) Haplotypes and Estimated Freqb</th>
</tr>
</thead>
<tbody>
<tr>
<td>23 most frequentd</td>
<td>Freq_PIP</td>
</tr>
<tr>
<td>B<em>42-Cw</em>17</td>
<td>0.129</td>
</tr>
<tr>
<td>B<em>53-Cw</em>04</td>
<td>0.077</td>
</tr>
<tr>
<td>B<em>14-Cw</em>08</td>
<td>0.072</td>
</tr>
<tr>
<td>B<em>15-Cw</em>02</td>
<td>0.070</td>
</tr>
<tr>
<td>B<em>07-Cw</em>07</td>
<td>0.059</td>
</tr>
<tr>
<td>B<em>15-Cw</em>03</td>
<td>0.057</td>
</tr>
<tr>
<td>B<em>57-Cw</em>18</td>
<td>0.085</td>
</tr>
<tr>
<td>B<em>58-Cw</em>06</td>
<td>0.040</td>
</tr>
<tr>
<td>B<em>45-Cw</em>16</td>
<td>0.036</td>
</tr>
<tr>
<td>B<em>58-Cw</em>07</td>
<td>0.034</td>
</tr>
<tr>
<td>B<em>81-Cw</em>18</td>
<td>0.031</td>
</tr>
<tr>
<td>B<em>35-Cw</em>04</td>
<td>0.029</td>
</tr>
<tr>
<td>B<em>44-Cw</em>04</td>
<td>0.028</td>
</tr>
<tr>
<td>B<em>08-Cw</em>07</td>
<td>0.027</td>
</tr>
<tr>
<td>B<em>45-Cw</em>06</td>
<td>0.024</td>
</tr>
<tr>
<td>B<em>13-Cw</em>06</td>
<td>0.016</td>
</tr>
<tr>
<td>B<em>39-Cw</em>12</td>
<td>0.014</td>
</tr>
<tr>
<td>B<em>44-Cw</em>07</td>
<td>0.014</td>
</tr>
<tr>
<td>B<em>51-Cw</em>16</td>
<td>0.013</td>
</tr>
<tr>
<td>B<em>18-Cw</em>07</td>
<td>0.012</td>
</tr>
<tr>
<td>B<em>15-Cw</em>04</td>
<td>0.010</td>
</tr>
<tr>
<td>B<em>40-Cw</em>07</td>
<td>0.010</td>
</tr>
<tr>
<td>B<em>53-Cw</em>06</td>
<td>0.010</td>
</tr>
</tbody>
</table>

a Local haplotypes are assigned manually and confirmed by the EM algorithm.
b Tabulation of extended haplotypes based on the EM algorithm.
c Restricted to those with predicted Freq > 0.010 and sorted in order of descending Freq (overall p = 0.197 between TPI and NTI, by log likelihood χ² test).
d Also restricted to those with Freq ≥ 0.010 and sorted in order of descending Freq (overall p = 4.4 × 10⁻⁸ between TPI and NTI, by log likelihood χ² test).
e All enriched in the TPI group (p ≤ 0.010).
f Enriched in the NTI group (p ≤ 0.010).
partners (21.9%, in the 6 mo before end of follow-up) \( (p < 0.001) \).

The proportion of GU was also statistically different between recipient partners who became infected (SC group, 45.9%) and those who remained uninfected (ESN group, 10.7%) \( (p < 0.001) \). These differences persisted in stratified analyses of FTM and MTF transmission (Table I). Accordingly, GU in all partners was retained as a covariate in subsequent analyses of HLA genotypes.

**Global tests of HLA class I variants at the two-digit resolution (allele group)**

At two-digit resolution, which is largely equivalent to serologic specificity, 12 HLA-A alleles, 18 HLA-B alleles, 11 HLA-C alleles, 21 local (HLA-B-HLA-C = B-C) haplotypes, and 25 extended (3-locus = A-B-C) haplotypes were found at Freq \( \geq 0.10 \) in the 429 Zambian couples (1716 chromosomes). Within the 429 paired index partners (PIP) (858 chromosomes), the respective numbers (12, 17, 11, 23, and 22) were similar. The major B-C haplotypes assigned manually correlated extremely well with haplotypes inferred using the EM algorithm, with correlation coefficients (Pearson and Spearman) all exceeding 0.99 in analyses of overall data from the 429 Zambian couples and in analyses of either index (TPi plus NTi) or initially seronegative (ESN plus SC) Zambians.

Three global tests generated evidence for a high level of heterogeneity in the Freq of HLA class I genotypes between TPI and NTI subjects as a result of HIV-1 transmission. First, overall distribution of extended haplotypes differed between TPI and NTI subjects \( (p = 7.1 \times 10^{-12}) \) by log likelihood \( \chi^2 \) tests, mostly due to the major haplotypes with predicted Freq \( \geq 0.010 \) (4.4 \( \times 10^{-3} \); Table II). Second, in tests of 22 extended (A-B-C) haplotypes with Freq \( \geq 0.010 \), four differed in their distribution between the two patient groups \( (p < 0.010) \) (Table II). Third, distribution of HLA-A diplotypes in the TPI (rather than NTI) patients deviated from HWE \( (p < 0.001) \), whereas the distribution of HLA-B and HLA-C diplotypes all conformed to HWE in the two patient groups (data not shown, available upon request).

Global tests based on all members of 429 couples also indicated that LD between HLA-B and HLA-C variants \( (p < 1.0 \times 10^{-14}) \) was much stronger than LD between HLA-A and HLA-B variants \( (p = 1.1 \times 10^{-69}) \) or HLA-A and HLA-C variants \( (p = 1.1 \times 10^{-53}) \), as reflected by pairwise tests of individual two-digit allele groups from each locus (Table III). For the index partners alone, LD patterns were the same, with \( p \) values ranging from 3.4 \( \times 10^{-116} \) (B-C LD) to 0.005 (A-C LD). A total of 30 pairs of HLA variants had \( p < 0.0001 \) in tests of \( r \) and \( D' \) (Table III); the strongest LD was between B*42 and Cw*17 and between B*14 and Cw*08 in the entire cohort \( (r = 0.94, D' = 1.00 \) and \( r = 0.85, D' = 0.95 \), respectively), as well as in the subset of index partners \( (r = 0.93, D' = 1.00 \) and \( r = 0.85, D' = 0.91 \), respectively). The strength of LD provided strong rationale for analysis of major haplotypes (local and extended) as separate entities in association analyses.

**HLA class I variants in index partners in relation to heterosexual HIV-1 transmission**

In univariate analyses of all major HLA variants, only A*36 showed a positive association with HIV transmission at \( p < 0.01 \) (for proportional hazard analysis). At \( p < 0.05 \), haplotype A*36-B*53-Cw*04 also showed positive association. Two other variants, B*57 and Cw*18, and their haplotype, B*57-Cw*18, were negatively associated with HIV-1 transmission. Multivariable models demonstrated that A*36 and Cw*18 were the two major
factors independently associated with HIV-1 transmission (adjusted relative hazard (RH) = 1.79 and 0.64, \( p = 0.002 \) and \( p = 0.043 \), respectively) (Fig. 2). Within the study period, 69% of index partners with A*36 transmitted HIV-1 to their seronegative partners (median transmission-free time = 899 days, 95% CI = 454–1235 days), while 35% of index partners with Cw*18 and without A*36 did so (median transmission-free time = 1823 days, 95% CI = 1460 to 2400 days). These estimates of infection-free time differed between the two patient groups in both log-rank (\( p = 0.001 \)) and Wilcoxon (\( p = 0.001 \)) tests; they further differed from those (median = 1560 days, 95% CI = 1327–2458 days) for the remainder of index partners without A*36 or Cw*18 (\( p = 0.041 \) and \( p = 0.019 \) by log-rank and Wilcoxon tests, respectively). After further statistical adjustment for age within couples, direction of viral transmission (MTF and FTM), and GUI, these contrasting HLA relationships remained intact (Table IV). Replacement of Cw*18 by B*57 led to very similar findings (Table IV and Fig. 3b). In addition, all but one individual who had both A*36 and Cw*18 (\( n = 4 \)) or A*36 and B*57 (\( n = 5 \)) behaved just like those with A*36 alone (data not shown).

A reduced logistic regression model confirmed the relationships of A*36 and Cw*18 to HIV-1 transmission (Table IV). For A*36, the adjusted odds ratio (OR) for HIV-1 transmission status was 2.62 (95% CI = 1.30–5.27) and for Cw*18, the OR was 0.57 (95% CI = 0.31–1.03). Age, direction of transmission, and GUI also showed independent associations based on multivariable analyses (adjusted \( p = 0.034 \) for all). In an alternative logistic regression model, the relationship of B*57 to HIV-1 transmission status (Fig. 3a) was somewhat weaker (adjusted OR = 0.66, 95% CI = 0.33–1.32) in the presence of other more prominent factors (adjusted \( p = 0.035 \) for all).

HLA class I variants in relation to HIV-1 viral load in index partners
In 202 TPIs and 218 NTIs, HIV-1 viral load was measured at a median interval of 546 days after enrollment. Consistent with

<table>
<thead>
<tr>
<th>Factors Tested in Models</th>
<th>Cox Proportional Hazard Model(^a)</th>
<th>Logistic Regression Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RH</td>
<td>Adjusted ( p )</td>
</tr>
<tr>
<td>Best reduced model(^a)</td>
<td>1.77</td>
<td>1.21–2.59</td>
</tr>
<tr>
<td>A*36 (( n = 52 ))</td>
<td>0.59</td>
<td>0.37–0.92</td>
</tr>
<tr>
<td>Cw*18 (( n = 65 ))</td>
<td>0.96</td>
<td>0.93–0.99</td>
</tr>
<tr>
<td>Age( \Delta ) (per year)</td>
<td>1.47</td>
<td>1.10–1.96</td>
</tr>
<tr>
<td>GUI</td>
<td>2.45</td>
<td>1.84–3.25</td>
</tr>
<tr>
<td>Alternative model(^b)</td>
<td>1.82</td>
<td>1.25–2.66</td>
</tr>
<tr>
<td>A*36 (( n = 52 ))</td>
<td>0.62</td>
<td>0.37–1.04</td>
</tr>
<tr>
<td>B*57 (( n = 45 ))</td>
<td>0.96</td>
<td>0.93–0.99</td>
</tr>
<tr>
<td>Age( \Delta ) (per year)</td>
<td>1.45</td>
<td>1.08–1.93</td>
</tr>
<tr>
<td>GUI</td>
<td>2.42</td>
<td>1.82–3.20</td>
</tr>
</tbody>
</table>

\(^a\) Best model has factors with adjusted (multivariable) \( p \leq 0.05 \); four patients with both A*36 and Cw*18 are treated as part of the A*36 group.

\(^b\) B*57 replacing Cw*18 (the two variants are in strong LD); five patients with both A*36 and B*57 are treated as part of the A*36 group.
earlier observations (19), TPIs more frequently had high (>10^5) or medium (10^4-10^5 copies/ml) than low (<10^4 copies/ml) concentrations of viral RNA in plasma, while the opposite was seen in NTIs. For example, 106 (52.3%) of TPIs and 65 (29.8%) of NTIs had high viral load (p < 0.0001). Patients with very high viral load (>5.0 × 10^5 copies/ml), which might be indicative of acute-phase or late-stage infection, was also more common in TPIs (13.9%) than NTIs (4.6%) (p = 0.0001).

Index partners with A*36, which was associated with accelerated HIV-1 transmission, were over-represented in the subgroups defined by high and medium viral load when compared with patients defined by low viral load (univariate p = 0.037 in test for trend) (Table V). The association of A*36 with viral load diminished after statistical adjustments for age, sex, and membership of patient groups (TPI and NTI) (p = 0.072). Association with the A*36-B*57-Cw*04 haplotype did not better account for these findings. Likewise, patients with HLA class I alleles and haplotypes showing association or a tendency toward association with delayed HIV-1 transmission were always enriched among index partners with low viral load, as reflected by analyses of Cw*18 alone (adjusted p < 0.0001), Cw*18 without B*57 (p = 0.002), Cw*18 without B*81 (p = 0.007), and B*81 alone (p = 0.011) (Table V).

The association of Cw*18 with low viral load could not be fully captured by B*57 and B*81 despite their tight LD with Cw*18 (r = 0.88, D’ = 0.97 when the two HLA-B alleles were treated as one entity). For example, 16 patients with the two HLA-B variants but without Cw*18 (i.e., Cw*18-, B*57+, or B*81+) were in viral load categories comparable with the categories of patients without
those alleles (adjusted \( p = 0.256 \)) (Table V). No informative test could be done for the two patients who had Cw*18 in the absence of B*57 or B*81 (data not shown), but data from the 24 patients with Cw*18 and without B*57, along with 43 others with Cw*18 and without B*81, all seemed to suggest that the effect of Cw*18 on viral load was almost exclusively due to the two haplotypes, i.e., B*57-Cw*18 and B*81-Cw*18 (adjusted on viral load was almost exclusively due to the two haplotypes, and without B*81, all seemed to suggest that the effect of Cw*18 with Cw*18 and without B*57, along with 43 others with Cw*18 of B*57 or B*81 (data not shown), but data from the 24 patients could be done for the two patients who had Cw*18 in the absence of viral load data.

Joint analyses of host and viral factors in index partners in relation to HIV-1 transmission

A close relationship between viral load in index partners and the likelihood of HIV-1 transmission status during follow-up was recognized earlier in the Zambian cohort (19). When high and medium index partner viral load (relative to the reference low level) were included in regression models as cofactors for HIV-1 transmission, only age, GUI, and HLA-A*36 were retained as independent contributors (adjusted RH for A*36 = 1.71, 95% CI = 1.17–2.49; OR = 2.42, 95% CI = 1.21–4.85) (Table VI, reduced model). In contrast, neither B*57 nor Cw*18 or its haplotype remained as independent contributor (protective factor) when viral load was included in the multivariable model (e.g., adjusted RH for Cw*18 = 0.75, \( p = 0.212 \)) (Table VI, full model).

Other confirmatory analyses

Several alternative analytic strategies confirmed the major HLA associations presented above. For example, the relationships of A*36 and Cw*18 to HIV-1 viral load in index partners were confirmed in linear regression models in which \( \log_{10} \)-transformed viral load was

Table VI. Multivariable models that account for both host and viral factors provisionally associated with differential HIV-1 transmission from 429 index Zambians to their seronegative partners

<table>
<thead>
<tr>
<th>Models accounting for host and/or viral factors(^a)</th>
<th>RH (95% CI)(^b)</th>
<th>Adjusted ( p )</th>
<th>OR (95% CI)</th>
<th>Adjusted ( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reduced model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A*36</td>
<td>( 1.71 ) (1.17–2.49)</td>
<td>( 0.005 )</td>
<td>( 2.42 ) (1.21–4.85)</td>
<td>( 0.013 )</td>
</tr>
<tr>
<td>Age (per year)</td>
<td>( 0.96 ) (0.94–0.99)</td>
<td>( 0.008 )</td>
<td>( 0.95 ) (0.91–1.00)</td>
<td>( 0.039 )</td>
</tr>
<tr>
<td>GUI</td>
<td>( 2.23 ) (1.67–2.98)</td>
<td>( &lt;0.001 )</td>
<td>( 3.24 ) (2.10–5.00)</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Viral load: high(^c)</td>
<td>( 2.41 ) (1.47–3.95)</td>
<td>( &lt;0.001 )</td>
<td>( 4.18 ) (2.19–7.98)</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Viral load: medium(^d)</td>
<td>( 1.78 ) (1.07–2.95)</td>
<td>( 0.027 )</td>
<td>( 2.16 ) (1.13–4.11)</td>
<td>( 0.020 )</td>
</tr>
<tr>
<td><strong>Full model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A*36</td>
<td>( 1.74 ) (1.18–2.57)</td>
<td>( 0.005 )</td>
<td>( 2.48 ) (1.19–5.17)</td>
<td>( 0.016 )</td>
</tr>
<tr>
<td>Cw*18</td>
<td>( 0.75 ) (0.47–1.18)</td>
<td>( 0.212 )</td>
<td>( 0.81 ) (0.43–1.53)</td>
<td>( 0.521 )</td>
</tr>
<tr>
<td>Age (per year)</td>
<td>( 0.96 ) (0.94–0.99)</td>
<td>( 0.007 )</td>
<td>( 0.96 ) (0.91–0.99)</td>
<td>( 0.045 )</td>
</tr>
<tr>
<td>GUI</td>
<td>( 2.24 ) (1.68–2.99)</td>
<td>( &lt;0.001 )</td>
<td>( 3.08 ) (1.97–4.83)</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Viral load: high(^c)</td>
<td>( 2.24 ) (1.36–3.72)</td>
<td>( 0.002 )</td>
<td>( 3.98 ) (2.05–7.71)</td>
<td>( &lt;0.001 )</td>
</tr>
<tr>
<td>Viral load: medium(^d)</td>
<td>( 1.73 ) (1.04–2.89)</td>
<td>( 0.036 )</td>
<td>( 2.11 ) (1.10–4.07)</td>
<td>( 0.025 )</td>
</tr>
</tbody>
</table>

\(^a\) Other putative factors tested earlier (Tables I, II, IV, and V) drop out the final models (adjusted \( p > 0.05 \)).

\(^b\) Based on the Wilcoxon proportional hazards models.

\(^c\) In either index (initially seropositive) or nonindex (initially seronegative) partners.

\(^d\) Three categories of HIV-1 viral load in the index partners are defined as high (\( \geq 10^3 \) copies/ml), medium (\( 10^3–10^4 \)), and low (\( <10^3 \) which serves as the reference group).

\(^e\) In tight LD with both B*57 (primarily B*5703) and B*81 (Table III).
treated as a continuous outcome measure (no deviation from normal distribution). The quantifiable viral load differences (i.e., adjusted β estimates) independently attributable to A*36 and Cw*18 were always within 0.50 log10 (data not shown).

**Tertiary analyses of HIV-1 acquisition among the initially seronegative Zambians**

In selective analyses of the 429 initially seronegative Zambians (ESNs plus SCs), GUI in either partner contributed to HIV-1 acquisition ($p < 0.0001$), with adjusted $R^2$ close to 0.37 and OR $= 7.14$ in two multivariable models (data available from J. Tang). In contrast, neither A*36 nor Cw*18 nor B*57 carried by the initially seronegative Zambians was associated with acquisition of infection, although again A*36 tended to be unfavorable, with adjusted $R^2 \leq 1.45$ ($p < 0.071$) in proportional hazard models and OR $\leq 1.85$ ($p \leq 0.066$) in logistic regression models (extra Table available from J. Tang).

**Discussion**

Our immunogenetic evaluation of epidemiologic and clinical data collected continuously on HIV-1-discordant Zambian couples produced the first evidence that HLA class I genotypes in the index partners might differentially influence the occurrence and rate of heterosexual HIV-1 transmission during a 12-year study period. In particular, primary analyses clearly pointed to A*36 and Cw*18 in index partners as the major predictors of viral transmission, while secondary analyses revealed contrasting levels of viral load attributable to these HLA genotypes. Overall, these findings extend the well-established role of HLA class I molecules in HIV-1 pathogenesis (13, 14, 17, 30) and viral evolution (31) to include their role (51). As a cautionary note, Zambians with Cw*18 and without A*36 did not show appreciable impact on HIV-1 transmission index partner viral load. Thus, the mechanisms underlying the strong relationship of A*36 in index partners to heterosexual HIV-1 transmission deserve further investigation.

Notably, the association of A*3601 with increased rate as well as incidence of heterosexual HIV-1 transmission remained strong even after statistical adjustment for viral load and other independent cofactors of infection. The weak association between A*3601 and viral load was also seen in our earlier analyses of 168 index partners and 91 SCs (23). Although index partner viral load categories can fluctuate from time to time and for various reasons during the study interval, our incorporation of viral load as a categorical variable should be insensitive to modest (< 5-fold) changes in viral load. Of course, a more critical evaluation of A*3601 or its related haplotype on index partner viral load may require serial and frequent sampling, especially for the few patients who appeared to have low or medium level viral load despite having A*3601 (Table V). Collection of longitudinal viral load data from A*3601-positive SCs should yield that valuable information. In addition, multiple studies (35–43) have demonstrated that viral shedding in the genital tract, rectal mucosa, and semen can be independent of plasma viral load. Those observations raise the alternative possibility that the dynamics of local viral shedding may differ in some of the Zambians with A*3601. For example, local flora, mucosal innate immunity, and coinfection with other pathogens like herpes simplex virus type 2 (44) are key factors in viral shedding that may be influenced by HLA genotypes.

For Cw*18, which is equivalent to Cw*1801 in Zambians (23), the association with reduced rate and incidence of HIV-1 transmission was almost entirely attributable to two haplotypes involving B*57 and B*81, respectively, in the index partners and to the collective impact of these haplotypes on index partner viral load. B*57, especially B*5701 and B*5703, have been widely recognized as favorable factors in HIV-1-infected individuals (reviewed in Refs. 13, 14, 17, whereas B*81 (exclusively B*8101 in Zambians) has been considered favorable in more recent studies of native Africans (25, 34, 45). In Caucasian populations, B*5701 on the B*5701-Cw*0902 haplotype (no Cw*18) is tagged by a single nucleotide polymorphism (rs2395029) in the HCP5 locus (4, 46), but experimental studies have repeatedly demonstrated that B*5701 is responsible for effective and often long-lasting immune control of HIV-1 infection (8, 47, 48). The protein products of B*5703 and B*8101 (B5703 and B8101, respectively) are capable of presenting HIV-1 epitopes (Gag epitopes in particular) known to be restricted by B5701 (15, 49, 50). Therefore, in the same Zambian population where both B*5703 and B*8101 are in tight LD with Cw*1801, Cw*1801 actually tracks the effect of two favorable HLA-B alleles that can target conserved HIV-1 epitopes for protective CTL responses (15, 43, 45, 50) and related function (51). As a cautionary note, Zambians with Cw*18 and without B*57 or B*8101 were too infrequent to allow a clear separation of HLA-B alleles from the two B-C haplotypes (e.g., Table V).

Moreover, two independent research teams have already identified four Cw1801-restricted epitopes: VI9 and FF9 in p24 Gag, VL9 in integrase, and YI9 in GP160 (6, 34, 52), suggesting that the Cw1801 allele itself can also play an important role in mediating CTL responses. Thus, a combination of favorable HLA-B and HLA-C alleles likely underlie the apparent relationships of Cw*1801 to HIV-1 transmission and viral load in more than 60 index partners (Tables IV to VI).

By conventional univariate analyses, HLA-B*57 and the B*57-Cw*18 haplotype were also negatively associated with HIV-1 transmission in Zambians. These relationships were first consistent with the well-recognized role of the B57 product in HIV-1-specific CTL responses and then supported by the association of B*57 with index partners’ HIV-1 viral load (Table V). Although multivariable models dismissed HLA-B*57 and the B*57-Cw*18 as major contributors when the stronger effect (risk) of A*36 and index partner viral load were treated as cofactors, HIV-1 transmission events within the first 6–7 years (i.e., 2192–2557 days) of follow-up did indicate a clear advantage of B*57 and the B*57-Cw*18 haplotype, as can be inferred from Fig. 3. The relative contribution of B*57 and other favorable HLA class I alleles (like B*8101 and Cw*1801) to immune control of HIV-1 infection is expected to diminish with time, due to viral immune escape and accumulation of compensatory mutations during chronic infection (11, 12, 53–55). Accordingly, it may be particularly important to concentrate on HIV-1-related outcomes (i.e., viral load and transmission) during early infection in the effort to identify favorable HLA factors and other correlates of protective immunity.

Viral load during untreated, chronic HIV-1 infection reflects the equilibrium between viral replication and the effect of host adaptive
immunity. Population-based studies have clearly established the predictive value of plasma (cell-free) viral load for heterosexual HIV-1 transmission (18, 19) as well as time to AIDS, especially in Caucasian males (56–58). Assuming that viral load in most of the chronically infected Zambians (i.e., index partners) can serve as a proxy for set-point viral load, patients with low viral load because of Cw*18 or its linked HLA-B alleles may experience a more benign course of disease. However, quantifying the dual impact of viral load on heterosexual transmission and HIV-1 pathogenesis will be difficult in populations where antiretroviral therapy has become increasingly available in the past few years. Confirmatory research will likely depend on analysis of other well-established cohorts of HIV-1 discordant couples with no or limited access to treatment.

Very high levels of viral load (often millions of RNA copies/ml of plasma) during the brief period (usually within the first 9 wk) of acute-phase infection (59) have been associated with highest rates of HIV-1 transmission per coital act (60). Since index partners in the Zambian cohort were identified by serology rather than viral load or p24 Ag tests, our work here might have missed acutely infected patients, although some index partners (4.6% NTIs and 13.9% TPs) indeed had a viral load greater than 500,000 copies/ml of plasma. However, unlike set-point viral loads that differ greatly from one chronically infected patient to another, acute-phase viral loads are so uniformly high that they would likely overwhelm any differential effect of genetic variation. Moreover, our use of viral load as a categorical variable is supported by the recent notion that viral transmission potential in chronically infected Zambians and Ugandans (61) does not increase substantially once set-point viral loads reach 100,000 copies/ml.

In other studies of paired HIV-1 donors and recipients, mother-to-child transmission (MTCT) has provided some unique models for evaluating the varying roles of HLA class I alleles, haplotypes, and diversity (heterozygosity) in HIV-1 infection and/or pathogenesis (62–68). Although consensus findings remain elusive, several maternal HLA class I (mostly HLA-B) alleles, maternal HLA homozygosity, as well as the degree of allele sharing between mothers and infants seem to influence MTCT in one way or another (62, 63, 66, 68). Neither A*36 nor Cw*18 has been reported as critical to vertical HIV-1 transmission, which differs from viral transmission among discordant couples (adults) in two ways: 1) MTCT recipients (infants) and donors always share not only 50% or more of their HLA class I alleles but many other genetic traits throughout the nuclear genome as well and 2) the immature immune system in the infants probably expedites HIV-1 transmission, especially when HLA-adapted viral mutants are being transmitted (67, 68). In any case, maternal HLA alleles previously associated with vertical HIV-1 transmission have invariably differed from infant HLA alleles associated with acquisition of infection. Likewise, the two HLA class I alleles (A*3601 and Cw*1801) strongly implicated in our analyses of viral transmission by the index partners clearly lack association with HIV-1 acquisition by seronegative Zambians. Therefore, within paired donors and recipients, there is more to be learned about the mechanisms of adaptive and innate immunity that control the process of viral transmission as distinct from those that mediate viral acquisition.

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Disclosures

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