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Impact of HLA Allele-KIR Pairs on HIV Clinical Outcome in South Africa

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Background. HLA class I contributes to HIV immune control through antigen presentation to cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. In contrast to investigations of CTL, studies of NK cells in HIV control through HLA-killer immunoglobulin-like receptor (KIR) interactions remain sparse in African cohorts.

Methods. Treatment-naive, chronically HIV-infected adults (N = 312) were recruited from South Africa, and the effects of HLA-KIR pairs on clinical outcome were analyzed.

Results. There was no significant difference in viral load among all subjects with HLA alleles from the HLA-C1 group ($P = .1$). However, differences in HLA-C type significantly influenced viremia among 247 *KIR2DL3* positives ($P = .04$), suggesting that specific HLA-KIR interactions contribute to immune control. Higher viral load ($P = .02$) and lower $CD4^+$ T-cell counts ($P = .008$) were observed in subjects with *HLA-C*16:01*$^+$*KIR2DL3*$^+$. Longitudinal analysis showed more rapid progression to AIDS among *HLA-C*16:01*$^+$*KIR2DL3*$^+$ subjects (adjusted hazard ratio 1.9, $P = .03$) than those without this genotype, independent of $CD4^+$ T-cell count and viral load.

Conclusions. These results highlight the existence of unique anti-HIV innate immunity within distinct populations and the contribution of KIR on NK cells and some CTLs to the well-described HLA-mediated impact on HIV disease progression.

Keywords. HIV; HLA; KIR; South Africa; disease outcome.

class I human leukocyte antigen (HLA) molecules interact with both cytotoxic T lymphocytes (CTLs) through their T-cell receptors, and natural killer (NK) cells through their killer immunoglobulin-like receptors (KIRs). Associations between certain HLA-KIR pairs and clinical outcomes were previously reported in several diseases such as hematopoietic stem cell transplantation and its outcome in leukemia [1], fetal growth, preeclampsia, and miscarriage in reproductive diseases [2], and autoimmune diseases [3, 4].

In HIV infection, however, the contribution of NK cells to disease control in vivo is less clear compared with that of CTLs. NK cells are regulated by the combinatorial effect of many activating and inhibitory receptors, including KIRs [5]. Recently, HLA-KIR–associated viral sequence polymorphisms were demonstrated in chronically HIV-infected individuals, suggesting ongoing viral adaptation against NK cell-mediated immune pressure [6, 7]. KIRs contain 2 or 3 external immunoglobulin-like domains (2D, 3D) and have either long or short cytoplasmic tails, which determine whether they are inhibitory or activating receptors, respectively [8, 9]. Some KIRs recognize HLA class I molecules as their ligands: for example, the ligands for the KIR3DL1 subtypes are HLA-B molecules containing the Bw4 motif at residues 77–83 [10, 11]. The high level of expression of the KIR allotype KIR3DL1 and its ligands HLA-Bw4 alleles with isoleucine at residue 80 (Bw4-80I) has been associated with improved clinical outcome via modulation of antiviral NK activity [12, 13]. Interestingly, some of the most protective class I HLA alleles in HIV infection, such as HLA-B*57 in different ethnic groups [14–17] and HLA-B*58:01 in African populations [15], are included in the Bw4-80I group.

HLA-C alleles, which are ligands of KIR2D, are classified into either an HLA-C group 1 (HLA-C1) or group 2 (HLA-C2), depending on sequences at residue 77 and 80 [18, 19], such that HLA-C molecules expressing serine at residue 77 and asparagine at 80 belong to the HLA-C1 group, and HLA-C molecules expressing asparagine at residue 77 and lysine at 80 belong to the HLA-C2 group. The former has a binding affinity for KIR2DL2, KIR2DL3, and KIR2DS2, and the latter for KIR2DL1 and KIR2DS1 [5, 20]. However, whether there is an association between HLA-C–KIR2D interactions and HIV control, as has been demonstrated for HLA-Bw4–KIR3D, has not been fully investigated.

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An additional aspect of HLA-mediated NK cell immunity involves the inhibitory HLA-E–NKG2A interaction [21]. The HLA-E–NKG2A interaction is mediated by HLA-E binding of an epitope within the signal peptide present at the N-terminus of classic class I HLA molecules, coded at residues −22 to −14 [22, 23]. Within this signal peptide epitope, methionine is a predominant amino acid at residue −21 (−21M), and all of HLA-A and C alleles share this −21M variant. However, some HLA-B alleles encode threonine at this position (−21T) [22, 23]. Compared to the signal peptide with −21T, the peptide with −21M binds with stronger affinity to HLA-E, consequently leading to higher expression of HLA-E, increased interaction with inhibitory NKG2A, and possibly reduced target cell lysis by NK cells [23]. HLA-E expression and its interaction with NKG2A are increased in diseases such as cytomegalovirus [24], HIV [25], and cancers [26–28], escaping from cell lysis by NK cells.

Our objective here was to identify the effect of KIRs and their interaction with their HLA ligands on HIV viral control in a cohort of 312 chronically infected, treatment-naive adults from South Africa.

METHODS

Subjects and Data Collection

Chronically HIV-infected adults were recruited from outpatient clinics in Durban, South Africa, from March 2003 to March 2007 [29]. All the 312 study participants were antiretroviral treatment (ART) naive with absolute $CD4^+$ T-cell counts of ≥200/mm^3 at enrollment, measured by flow cytometry. Viral load was measured using the Roche Amplicor version 1.5 assay. Data regarding timing of ART introduction were also collected. For class I HLA genotyping, high-resolution genotyping for HLA class I loci was performed by polymerase chain reaction (PCR)–sequence-based typing, as recommended by the 13th International Histocompatibility Workshop (available at: http://www.ihwg.org/tmanual/TMcontents.htm). HLA sequences were analyzed using the ASSIGN software (Conexio Genomics, Fremantle, Western Australia, Australia). KIR genotyping for the presence or absence of each KIR gene was conducted by PCR with sequence-specific priming as described previously [30]. The presence and absence of specific PCR products was detected by agarose gel electrophoresis containing ethidium bromide and products were visualized under ultraviolet light. This research was approved by the ethical review boards at the University of KwaZulu-Natal, South Africa, and the University of Oxford, United Kingdom. All patients provided written informed consent for the collection of samples and subsequent analysis.

Statistical Analysis

Statistical analysis was performed using SPSS 21.0 (IBM, Armonk, New York). Kruskal-Wallis test was used to assess viral load differences among HLA-C1–comprising alleles, HLA-C2–comprising alleles, HLA-Bw4–comprising alleles, and HLA-Bw4 80I–comprising alleles. The effect of HLA-KIR pairs on viral load was tested with Mann–Whitney U test for univariate analysis, and linear regression model for multivariate analysis. To avoid overestimation of the CTL-related effect of an HLA allele on clinical outcome, in univariate analysis we analyzed viral load differences between subjects with a particular HLA allele-KIR pair versus subjects with the same HLA but lacking that KIR [31] (for example, difference in viral load between *HLA-B*57:01*$^+$*KIR3DL1*$^+$ subjects vs *HLA-B*57:01*$^+$*KIR3DL1*$^-$ subjects). A Log-rank test was performed for the longitudinal analysis of ART introduction (time to ART initiation or CD4 count <200 cells/mm^3 before ART initiation), and Cox hazard model was applied for multivariate analysis.

RESULTS

Characteristics of the Cohort

Of the 312 HIV-infected individuals, 253 (81%) were women and 59 (19%) were men (Table 1). HLA allele frequency and its linkage disequilibrium (Supplementary Table 1) and KIR frequency (Table 1) are shown in tables. Median absolute $CD4^+$ T-cell count at enrollment was 394 cells/mm^3 (interquartile range [IQR], 306–516), CD4/CD8 ratio was 0.4 (IQR, 0.3–0.6), and viral load was 4.6 log copies/mL (IQR, 3.8–5.1).

Table 1. Characteristics of Subjects (N = 312)

Characteristics	Value, No. (%)
$CD4^+$ T cell, median (IQR), counts/μL	394 (306–516)
CD4/CD8 ratio, median (IQR)	0.4 (0.3–0.6)
Viral load, median (IQR) log copies/mL	4.6 (3.8–5.1)
Sex, female	253 (81)
HLA-Bw4$^+$	175 (56)
HLA-Bw4 80I$^+$	134 (43)
HLA-Bw6$^+$	273 (88)
HLA-C1$^+$	201 (64)
HLA-C2$^+$	254 (81)
HLA-C1C1$^+$	58 (19)
HLA-C2C2$^+$	111 (36)
KIR2DL1$^+$	308 (99)
KIR2DL2$^+$	220 (71)
KIR2DL3$^+$	247 (79)
KIR2DL4$^+$	310 (99)
KIR2DL5$^+$	215 (69)
KIR2DS1$^+$	49 (16)
KIR2DS2$^+$	191 (61)
KIR2DS3$^+$	92 (29)
*KIR2DS4*219*$^+$	257 (82)
KIR2DS5$^+$	160 (51)
KIR3DL1$^+$	308 (99)
KIR3DL2$^+$	312 (100)
KIR3DL3$^+$	312 (100)
KIR3DS1$^+$	31 (10)

Abbreviations: HLA, human leukocyte antigen; IQR, interquartile range; KIR, killer immunoglobulin-like receptor.

Contribution of NK Cells to HLA-Viral Load Association Through HLA-KIR Interaction

Previous population-specific studies conducted in various geographical regions have reported associations between HLA class I gene carriage and identified disease-protective and susceptible HLA alleles [14–17]. To investigate whether HLA-KIR interactions contribute to such associations, we first investigated whether viral load differed significantly among subjects with the combination of particular KIRs together their corresponding HLA ligands (Figure 1 and Supplementary Figure 1). Whilst viral load did not differ among subjects who were positive for 1 of the 8 HLA-C1 group alleles (P = .1) (Figure 1A) among *KIR2DL3*-positive subjects, but not *KIR2DL2*-positive or *KIR2DS2*-positive subjects, viral load differed significantly according to HLA-C1 allele (P = .04, P = .3, and P = .2, respectively) (Figure 1B–D). However, in post hoc analysis, there was no significant difference in viral load between HLA-C1 group alleles among *KIR2DL3*-positive subjects (Figure 1B), even including the subjects without HLA-C1 comprising alleles as control group (Supplementary Figure 2). This suggests that within HLA-viral load associations previously reported, NK cells might contribute to such an effect through HLA-KIR interactions, particularly through the HLA-C1–KIR2DL3 interaction in this population.

To investigate the effect of NK cells on viral control, we next analyzed the differences in viral load and $CD4^+$ T-cell count associated with individual HLA-KIR pairs (Supplementary Tables 2 and 3). We found that there was a significantly higher viral load in the individuals with *KIR2DL3* compared to the individuals without *KIR2DL3* (median 4.7 vs 4.4 log copies/mL; P = .01 by Mann–Whitney U test; Supplementary Table 2). The absolute CD4 count was a median of 45 cells/mm^3 lower among subjects with *KIR2DL3*, although this difference did not reach statistical significance (median 432 versus 387 cells/mm^3; P = .1; Supplementary Table 3). However, there was no significant viral load and $CD4^+$ T-cell count difference between KIR-positive and KIR-negative subjects when the subjects were grouped by their ligands of HLA-C1, HLA-C2, or HLA-Bw4 groups (Supplementary Table 2 and 3). Two HLA-KIR combinations were significantly associated with changes in viral load: (1) there was lower viral load in subjects with *HLA-C*02:02-KIR2DS1* (viral load median 4.2 log copies/mL in *HLA-C*02:02*$^+$*KIR2DS1*$^+$ vs 4.9 log copies/mL in *HLA-C*02:02*$^+$*KIR2DS1*$^-$; P = .04; Figure 2A); and (2) although there was a small number of subjects with *HLA-C*16:01*$^+$*KIR2DL3*$^-$ (n = 2), there was higher viral load in *HLA-C*16:01-KIR2DL3* (viral load median 5.0 log copies/mL in *HLA-C*16:01*$^+$*KIR2DL3*$^+$ vs 3.4 log copies/mL in *HLA-C*16:01*$^+$*KIR2DL3*$^-$; P = .04; Figure 2B and Supplementary Table 4). The *HLA-C*16:01*$^+$*KIR2DL3*$^+$ combination was also significantly associated with lower absolute CD4 count (median 357 cells/mm^3 in *HLA-C*16:01*$^+$*KIR2DL3*$^+$ vs 674 cells/mm^3 in *HLA-C*16:01*$^+$*KIR2DL3*$^-$; P = .03; Figure 2C

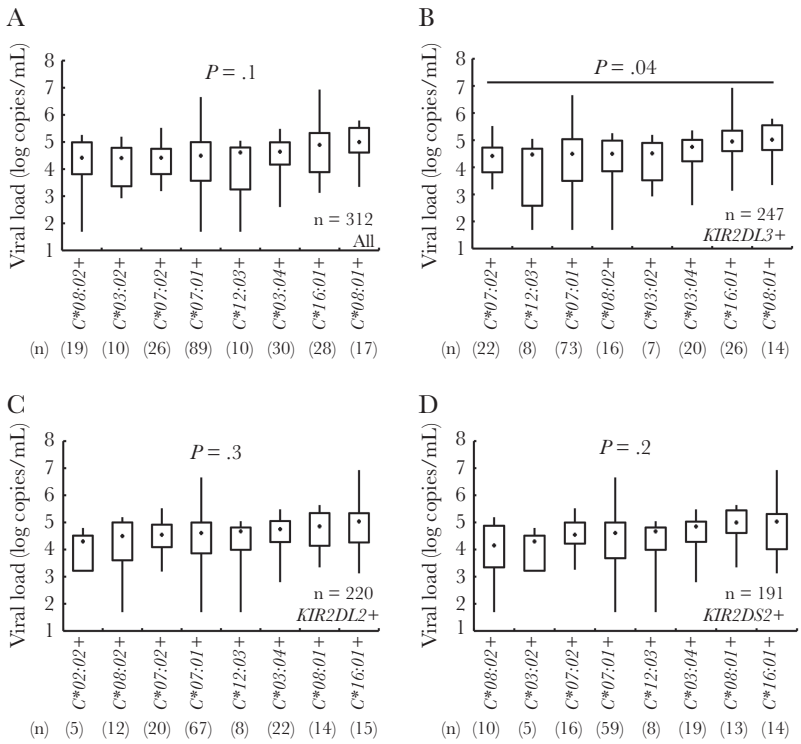

Figure 1. Viral load differences among human leukocyte antigen-C1 (HLA-C1) group comprising alleles under the existence of their receptor killer immunoglobulin-like receptors (KIRs). Kruskal–Wallis tests are shown in all 312 subjects (*A*), 247 *KIR2DL3* positives (*B*), 220 *KIR2DL2* positives (*C*), and 191 *KIR2DS2* positives (*D*). In a post hoc analysis, there were no significant differences between HLA alleles in *KIR2DL3* positives, by the Steel-Dwass test (*B*). The boxplot indicates minimum and maximum (line), 25th centile and 75th centile range (box), and median (dot in a box).

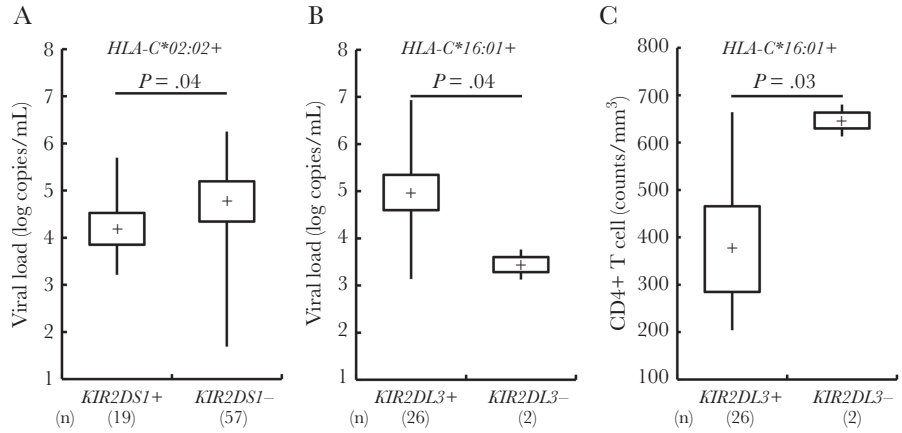

Figure 2. *A–C,* Viral load and CD4$^+$ T-cell count differences between killer immunoglobulin-like receptor (KIR) positives versus negatives under the existence of their ligand human leukocyte antigen (HLA) alleles. *P* values by Mann–Whitney U test are shown. The boxplot indicates minimum and maximum (line), 25th centile and 75th centile range (box), and median (dot in a box).

and Supplementary Table 5). However, *HLA-C*02:02-KIR2DS1* was not associated with a higher absolute CD4 count (median 363 cells/mm^3 in *HLA-C*02:02*$^+$*KIR2DS1*$^+$ vs 376 cells/mm^3 in *HLA-C*02:02*$^+$*KIR2DS1*$^-$; *P* = .6; Figure 2C and Supplementary Table 5). The HLA-C*16:01-KIR2DL3 associations with viral load and CD4 count remained significant after adjusting for the impact of *KIR2DL2* and *KIR2DS2*, which are the other receptors for HLA-C*16:01 (*P* = .02 and *P* = .008, respectively; Table 2). These data suggest that significant clinical outcome differences are associated with certain HLA allele-KIR pairs identified in this South African cohort.

Linkage Disequilibrium Between HLA-C*16:01 and HLA-B*45:01, but Less Impact of HLA-E–NKG2A Interaction on Disease Outcome Among HLA-C*16:01 Positives

As another aspect of HLA-mediated NK cell immunity, HLA-E–NKG2A interaction was considered [21]. In this study population, HLA-C*16:01 is in linkage disequilibrium with HLA-B*45:01 (Supplementary Table 1). However, HLA-B*45:01 expresses the −21T HLA-E variant within the signal peptide, suggesting less impact of HLA-E–NKG2A interaction on disease outcome among HLA-C*16:01 positives.

Table 2. Viral Load and CD4$^+$ T-Cell Count Differences Between KIR Positives versus Negatives Under the Existence of Their Ligand HLA Alleles

	R square	B (95% CI)	β	*P*
Viral load, log copies/mL				
*HLA-C*02:02*$^+$	0.07			
KIR2DL1$^+$		−1.7 (−3.7 to 0.3)	−0.2	.1
KIR2DS1$^+$		−0.6 (−1.3 to 0.08)	−0.2	.08
*HLA-C*16:01*$^+$	0.2			
KIR2DL2$^+$		0.7 (−1.2 to 2.6)	0.4	.5
KIR2DL3$^+$		1.6 (0.2 to 3.1)	0.5	.02
KIR2DS2$^+$		−0.3 (−2.2 to 1.6)	−0.1	.8
CD4$^+$ T cell, counts/mm^3				
*HLA-C*16:01*$^+$	0.3			
KIR2DL2$^+$		−149 (−417 to 119)	−0.5	.3
KIR2DL3$^+$		−276 (−473 to −78)	−0.5	.008
KIR2DS2$^+$		119 (−150 to 388)	0.4	.4

P values by multivariate linear regression model are shown.

Abbreviations: CI, confidence interval; HLA, human leukocyte antigen; KIR, killer immunoglobulin-like receptor.

More Rapid Progression to ART Initiation Among Subjects With Certain HLA Allele-KIR Pairs

Next, we assessed the impact of HLA allele-KIR pairs on time to meeting CD4 criteria for ART initiation prevailing at this time (absolute CD4 count of <200 cells/mm^3) among 289 out of 312 patients with follow-up data. In detail, 134 out of 289 patients (46%) initiated ART during the period of follow up, of whom 118 had absolute CD4 counts of <200 cells/mm^3, and 16 initiated ART before drop of CD4 <200 cells/mm^3 (at a median 232 cells/mm^3). First we found that the subjects with *HLA-C*06:02*$^+$*KIR2DL1*$^+$ (*P* = .02 by log rank test; Figure 3A), *HLA-C*16:01*$^+$*KIR2DL3*$^+$ (*P* = .004; Figure 3B), or *HLA-B*58:02*$^+$*KIR3DL1*$^+$ (*P* = .001; Figure 3C) met CD4 criteria to start ART earlier than subjects without those HLA-KIR pairs. On the other hand, the subjects with *HLA-B*44:03*$^+$*KIR3DL1*$^+$ met CD4 criteria to start ART later than subjects without this HLA-KIR pair (*P* = .04; Figure 3D). These results remained significant when the analysis was undertaken using an endpoint of ART initiation at an absolute CD4 count of <200 cells/mm^3, therefore excluding these 16 individuals (Supplementary Figure 3). In order to control for the effect of other factors on rate of progression to meet CD4 criteria for ART initiation, a multivariate analysis was performed. Included in the model were sex, CD4 counts, CD4/8 ratio, viral load, *HLA-C*06:02*$^+$*KIR2DL1*$^+$, *HLA-C*16:01*$^+$*KIR2DL3*$^+$, *HLA-B*44:03*$^+$*KIR3DL1*$^+$, and *HLA-B*58:02*$^+$*KIR3DL1*$^+$ (Table 3). In the multivariate analysis, carriage of the *HLA-C*16:01*$^+$*KIR2DL3*$^+$ (adjusted hazard ratio [aHR], 1.9; 95% confidence interval [CI], 1.1–3.5; *P* = .02) pair remained a significant factor associated with higher rates

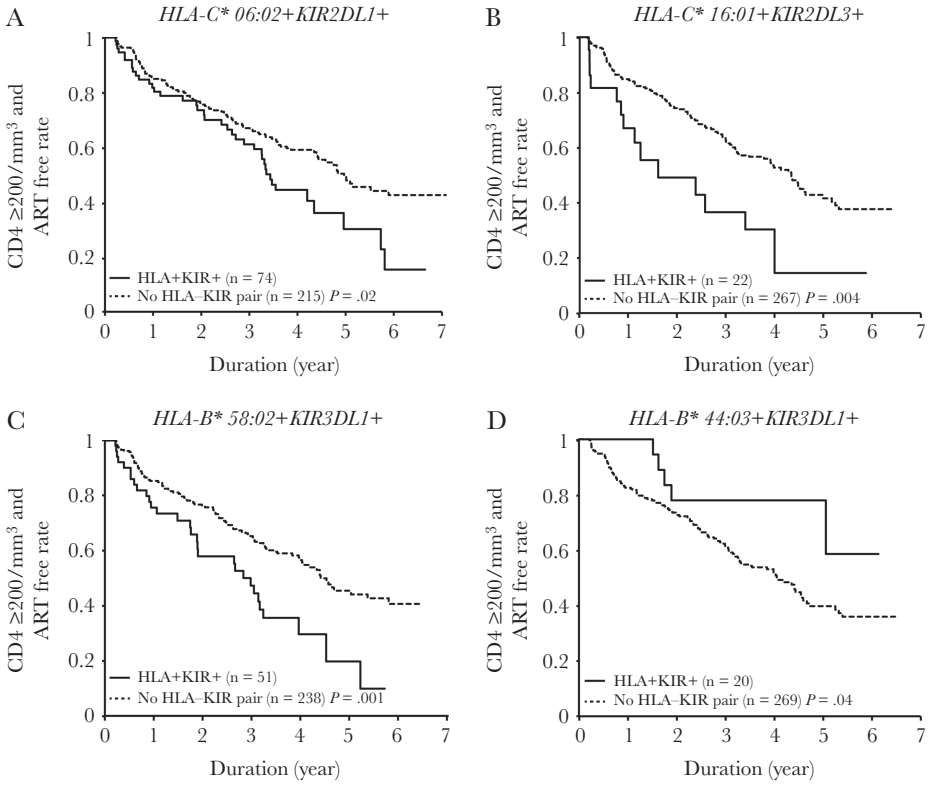

Figure 3. *A–D,* CD4$^+$ T-cell counts ≥200/mm^3 and antiretroviral treatment (ART)-free rate differences between human leukocyte antigen-killer immunoglobulin-like receptor (HLA-KIR) pair positives and negatives. Log-rank tests are shown.

of progression to an absolute CD4 count of <200 cells/mm^3, which was independent of CD4$^+$ T-cell count (aHR, 0.9; 95% CI, 0.9–0.9; $P < .001$) and viral load (aHR, 2.0; 95% CI, 1.5–2.6; $P < .001$) (Table 3). This result strongly suggests the deleterious effect of HLA-C*16:01$^+$KIR2DL3$^+$ on HIV clinical outcome in the studied South African population.

DISCUSSION

This study systematically investigated the effect of particular KIRs and their HLA ligands on clinical outcome in a South African cohort of chronically HIV-infected ART-naive adults. Here, in both cross-sectional and longitudinal analyses, we identified the deleterious effect of HLA-C allele-KIR2D pairs, in particular of HLA-C*16:01$^+$KIR2DL3$^+$, on HIV clinical outcome.

The protective effect of HLA-Bw4 in HIV infection was first described in 2001 [32], and the subsequently advantageous effect of high cellular surface expression allotype of KIR3DL1*h/*y in combination with HLA-Bw4-80I was reported [13, 33]. In contrast, whether the HLA-C–KIR2D pair confers protection against HIV infection has remained unclear. The deleterious effect of the HLA-C1–KIR2DL3 pair on HIV disease outcome observed here is consistent with 2 previous studies, in Cote d'Ivoire [34] and in Thailand [35]. However, the HLA-C1–KIR2DL3 combination has also been reported as protective against mother-to-child transmission in South Africa [36], and against HIV infection among intravenous drug users (IDUs) in Vietnam [37]. A possible explanation for the discrepancy between the reported effects of HLA-C1–KIR2DL3 gene carriage in HIV infection may be found in the study examining the impact of KIRs in hepatitis C virus (HCV) infection [38]. This study showed that the HLA-C1–KIR2DL3 pair was advantageous for HCV resolution in cases of low-dose viral transmission such as needle prick injuries and IDU injections, but not in those with high-dose exposure, such as through blood transfusions. Indeed, in the mother-to-child transmission study [36], the protective impact of the

Table 3. CD4$^+$ T-Cell Counts <200/mm^3 or ART Initiation Rate Difference Among HLA-KIR Pairs

Variables	aHR (95% CI)	*P*
Sex, female	1.2 (0.7–1.9)	.5
CD4$^+$T cell, counts/mm^3	0.9 (0.9–0.9)	<.001
CD4/CD8 ratio	0.4 (0.1–1.0)	.05
Viral load, log copies/mL	2.0 (1.5–2.6)	<.001
*HLA-C*06:02$^+$KIR2DL1$^+$*	0.9 (0.5–1.7)	.7
*HLA-C*16:01$^+$KIR2DL3$^+$*	1.9 (1.1–3.5)	.02
*HLA-B*44:03$^+$KIR3DL1$^+$*	1.0 (0.4–2.6)	.9
*HLA-B*58:02$^+$KIR3DL1$^+$*	1.5 (0.7–3.0)	.3

Multivariate Cox hazard model analysis is shown.

Abbreviations: aHR, adjusted hazard ratio; ART, antiretroviral treatment; CI, confidence interval; HLA, human leukocyte antigen; KIR, killer immunoglobulin-like receptor.

HLA-C1–KIR2DL3 pair was significantly elevated in the group of infants with lower maternal viral load, whereas high viral load masked the protective effect of this allele combination. Also, no HLA-KIR pair was reported as a significant factor contributing to HIV infection among highly exposed uninfected hemophilia A patients in the United Kingdom [39]. Such differences in HIV transmission route and inoculum dose may thus explain the discrepancy in the relative contribution of HLA-KIR pairs to viral control in HIV infection.

In terms of the potential mechanisms underlying the association between HLA-KIR pair and susceptibility to HIV infection, Alter et al [7] and Hölzemer et al [6] reported that inhibitory KIR-associated viral mutation sites can enhance binding of NK cells through inhibitory KIRs, causing an inhibition of NK-cell activation and subsequent escape from NK cell-mediated lysis of infected cells. These findings suggest that detection of HLA-KIR pairs that affect clinical outcomes, and definition of the critical viral residues that influence the HLA-KIR interactions would be warranted for further understanding of mechanism of class I HLA-induced antiviral immunity.

HLA-C*16:01 is frequent among African populations (8%–17% population frequency and 9% in this study; Supplementary Table 1) and Caucasians (5%–10%), but rare among Asian populations [40]. HLA-C*16 alleles, including HLA-C*16:01, were also previously reported to be among disease-susceptible HLA-C alleles associated with high viral loads in South Africa [15], and also in a European American population in the United States [41]. Apps et al also reported [41] that HLA-C alleles contribute to HIV disease outcome, with higher cellular surface expression level associated with better CTL activity and improved disease outcome. However, the cellular surface expression level of HLA-C*16:01 was at an intermediate level among HLA-C alleles [41], suggesting that the interaction observed here between HLA-C*16:01 and KIR2DL3 may be one of the mechanisms contributing to a deleterious effect on HIV disease outcome mediated by this allele.

A limitation of this study is the number of participants enrolled, especially the small number with *HLA-C*16:01*$^{+}$*KIR2DL3*$^{-}$ (n = 2), which limited the analyses available. Although we identified statistically significant associations of several HLA allele-KIR pairs and clinical outcome, further analysis with a larger number of participants, greater genetic diversity, and more clinical data, such as ART regimen, its adherence, and viral control rate by multivariate analyses, would be necessary to confirm these findings.

In conclusion, this study investigated the effect of HLA and KIR pairs on viral control in a chronically HIV-infected ART-naive South African population. We have found that certain HLA allele-KIR pairs affects clinical outcome in this population. These data are consistent with the studies demonstrating that the activity of NK cells can make a significant contribution of the impact of HLA on viral load. Our data highlight geographical differences in the frequency and distribution of HLA-KIR pairs, which in turn may affect the NK-cell response and, consequently, the selection pressure exerted on HIV in each endemic area. The contribution of KIR-mediated viral control will most likely be determined by host differences such as HLA class I allele and KIR frequencies in different geographical regions, viral transmission route and inoculum volume, as well as the viral pathogen in question. The identification of uniquely protective and susceptible HLA-KIR pairs in each endemic area will provide the opportunity to better define the nature of HLA-associated immune control of HIV.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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