Influence of HLA Supertypes on Susceptibility and Resistance to Human Immunodeficiency Virus Type 1 Infection

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Certain human leukocyte antigens, by presenting conserved immunogenic epitopes for T cell recognition, may, in part, account for the observed differences in human immunodeficiency virus type 1 (HIV-1) susceptibility. To determine whether HLA polymorphism influences HIV-1 susceptibility, a longitudinal cohort of highly HIV-1–exposed female sex workers based in Nairobi, Kenya, was prospectively analyzed. Decreased HIV-1 infection risk was strongly associated with possession of a cluster of closely related HLA alleles (A2/6802 supertype; incidence rate ratio [IRR], 0.45; 95% confidence interval [CI], 0.27–0.72; P = .0003). The alleles in this supertype are known in some cases to present the same peptide epitopes for T cell recognition. In addition, resistance to HIV-1 infection was independently associated with HLA DRB1*01 (IRR, 0.22; 95% CI, 0.06–0.60; P = .0003), which suggests that anti–HIV-1 class II restricted CD4 effector mechanisms may play an important role in protecting against viral challenge. These data provide further evidence that resistance to HIV-1 infection in this cohort of sex workers is immunologically mediated.

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Study participants provided informed consent for their participation in this study. This study was approved by the ethical review boards of the Universities of Nairobi and Manitoba.

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Differential susceptibility to infection and disease expression is observed with almost all infections, although often the reasons for the heterogeneity are unknown. In the case of human immunodeficiency virus type 1 (HIV-1), it has been recognized that not all exposed individuals develop infection and that the course of infection is extremely variable. Viral characteristics, infectious cofactors, individual behaviors, environmental fac-

tors, and host genetics have been shown or are presumed to affect susceptibility to infection and HIV-1 disease progression [1]. Host factors elsewhere associated with susceptibility or resistance to HIV-1 infection and disease progression include chemokine receptor polymorphisms, such as CCR-2 64I [2], SDF-1 α [3], and CCR-5 Δ32 [2, 4–7]; T helper–cell responses [8, 9]; cytotoxic T cell (CTL) responses [10, 11]; and HIV-specific mucosal IgA [12, 13].

One important determinant of resistance and susceptibility to infections is the major histocompatibility complex (MHC). MHC alleles determine the molecular targets of the cellular immune response in a given host. Genetic polymorphism of MHC results from concentrated amino-acid substitutions in the peptide-binding groove of HLA molecules that produce variability in peptide epitope binding and presentation to T cells. At the population level, MHC diversity provides protection against epidemic infection, with the differing precise molecular targets of individuals’ immune responses ensuring that immune-escape–adaptive mechanisms of an infectious organism are countered. This is particularly demonstrable for human infections, such as malaria [14], that affect mortality and is likely true for all infections that have an impact on reproductive fitness.

With respect to HIV, several associations between HLA alleles and differential disease progression [15–17] or disease
manifestations (e.g., Kaposi’s sarcoma) [15, 18] have been reported. However, the role of MHC in resistance and susceptibility to de novo HIV-1 infection has not been well studied [15]. In a prospective study of HIV-1 susceptibility in a cohort of heavily exposed female sex workers in Nairobi, Kenya, over a 13-year period, we identified a subgroup with epidemiologic evidence of resistance to HIV-1 [19]. In this population, HIV-1 resistance is not related to known polymorphisms in HIV-1 coreceptors or chemokine receptors, nor is resistance associated with altered cellular susceptibility to HIV-1 infection in vitro [20, 21]. However, in this population, resistance to HIV-1 infection is correlated with T helper-cell responses to HIV-1 envelope peptides, cytotoxic T cell responses to HIV-1 gene products (K. R. Fowke, personal communication) and peptide epitopes [22], and HIV-1-specific IgA in genital tract secretions [12]. If these immunologic responses are what protecting these individuals, a major unanswered question is why some individuals develop them and exhibit HIV-1 resistance, whereas most others do not. Because MHC alleles are central for determining the specificity of an individual’s immune response, we examined the influence of MHC alleles on susceptibility and resistance to incident HIV-1 infection in the Pumwani Sex Worker Cohort.

Methods

Study subjects. The study was conducted among women enrolled in the Pumwani Sex Worker Cohort, an open observational cohort study of risk factors for HIV-1 infection and disease, which was established in 1985. The cohort design and follow-up have been described elsewhere [19]. In brief, informed consent was obtained, and women were interviewed at enrollment for demographic information and sexual, reproductive, and prostitution history. They were examined and tested for sexually transmitted pathogens, including Neisseria gonorrhoea, Haemophilus ducreyi, and Chlamydia trachomatis infection. Blood was collected for HIV-1, HIV-2, and syphilis serologic analysis; serologic HLA typing; and the preparation ofuffy coats or peripheral blood mononuclear cells (PBMC) as a source of DNA for molecular HLA typing. Women were scheduled for follow-up at 6-month intervals but were free to attend the clinic for intervening health problems. At each visit, interval history on sexual behavior, prostitution, and general health was obtained; physical examinations were repeated; and specimens for sexually transmitted disease diagnosis and HIV-1 serology were obtained.

Laboratory methods. All individuals were tested for HIV-1 and HIV-2 antibodies with commercial EIAs, as described elsewhere [19]. All seroconversions were confirmed by immunoblot from 1985 to 1991 (Novapath Immunoblot; BioRad Laboratories, Richmond, CA) and with Recombigen HIV-1/2 EIA (Cambridge Biotech, Rockville, MD) after 1991. Women with persistently negative HIV serologic findings were confirmed to be seronegative by immunoblot on ≥1 occasion. Starting in 1991, PBMC from persistent HIV-1–seronegative individuals were tested on ≥1 occasion. In persistent HIV-1–seronegative patients who remained in follow-up after 1992, ≥2 tests were done at least 6 months apart. Primers and probes for conserved sequences of env, vif, and nef were used. These primers have been validated elsewhere on African samples [23]. The limit of detection of these primer pairs is 3.8 copies per 150,000 cells, as described elsewhere [19]. Methods for the detection of other sexually transmitted pathogens have been described elsewhere [24].

Serologic MHC typing. Standard methods for serologic class I MHC (A and B locus) typing were used [25]. Immunomagnetic beads (BioRad, Hercules, CA) were used to purify CD8+ T cells for class I MHC determination. Cells were placed in 72-well Terasaki typing trays (typing trays specific for black populations [BL-72, One Lambda Inc., Conoga Park, CA] and/or trays specifically designed for black populations by the Canadian Red Cross). Complement-mediated cell lysis was detected by reading trays with an inverted fluorescent microscope. Supplemental typing trays were used for ambiguous or incompletely typed samples.

Molecular MHC typing. Molecular methods were used for all class II (DRB1 locus) typing and for class I in the following circumstances: (1) to confirm alleles that were found to be associated with increased or decreased risk of HIV-1 seroconversion; (2) to clarify alleles that were ambiguous or untypable by serologic methods; or (3) to subtype selected serologic determinants (e.g., HLA-A2). DNA from frozen buffy coats was extracted [26]. For class I, locus-specific primers were used for an initial amplification, and allele-specific primers were used for a second amplification [27–29]. Polymerase chain reaction products were detected by gel electrophoresis. A 400-bp fragment of the β2-microglobulin gene was included in every well as an internal positive control. All study subjects with serologically defined HLA-A2 or A28 were subtyped for both HLA-A2 (HLA A*0201-0214) and HLA A28 (HLA A*6801-6802, A*6901). Other alleles were similarly confirmed. Complete molecular typing [27–29] was also used to define serologically uninterpretable typings. Class II typing was performed molecularly by use of sequence-specific primers and oligonucleotide probes, as described elsewhere [30–34].

Data analysis. HIV-1 prevalence increased substantially during the 12 years of the study period; thus, each year was not equivalent in terms of HIV-1 exposure risk. We therefore calculated a weighted survival time, as described elsewhere, exponentially weighting each calendar year since 1984 by $\pi = 0.12$ [19]. Incidence rate ratios (IRRs) with exact 95% confidence intervals (CIs) and exact $P$ values were calculated for univariate analysis of MHC associations. In addition to examining the effect of individual MHC alleles on resistance to HIV-1 infection, we also examined the effect of 3 established allelic supertypes (A2/6802, A3, and B7), which consisted of groups of HLA molecules that share peptide epitopes [35–37]. Cox proportional hazards modeling was used for multivariate analysis. MHC associations that were significant at the $P < .05$ level were used in multivariate analysis, with both crude and weighted time to seroconversion or censoring as the dependent variable. $P$ values are reported as both uncorrected and corrected for multiple comparisons.

Results

In total, 498 women initially negative for HIV-1 have been followed up in the Pumwani Sex Worker Cohort, of whom 274 seroconverted to HIV-1 during follow-up. An unselected sample
of 232 women were HLA typed in this study, of whom 122 seroconverted to HIV-1. Almost all study subjects were from East African tribes of Bantu origin. There were no significant differences in MHC allele frequencies among these tribes (data not shown). Potential risk factors for HIV-1 seroconversion [19] are compared among persistently seronegative and seroconverting women involved in the current study in table 1.

Twenty-one serological HLA-A locus determinants and 26 HLA-B locus determinants were detected. Those present at a frequency of \( \geq 5\% \) were examined for associations with risk of HIV-1 seroconversion (A locus, \( n = 11 \); B locus, \( n = 7 \)). HLA alleles associated with increased or decreased risk of seroconversion (HLA-A2, HLA-A28, HLA-A23, HLA-A24, and HLA-B18) were typed by molecular methods.

Molecular subtyping was used to examine alleles comprising both HLA-A2 and HLA-A28 because of their close serological and genetic relationship. HLA-A2 allelic subtypes detected included A*0201, A*0202, A*0205, and A*0214, with non-A*0201 subtypes accounting for 40\% of HLA-A2. HLA-A28 was composed predominantly of A*6802 but also of A*6801 and, very rarely, of A*6901. Both HLA-A*0202 and HLA A*6802 were associated with a significantly decreased rate of HIV-1 seroconversion (table 2). Because these alleles have also been shown to belong to a functional supertype [37], we analyzed the effect of the functionally similar HLA-A2 and HLA-A28 subtypes as an allelic supertype (table 2). The A2/6802 supertype comprising A*0202, A*0205, A*0214, and A*6802 was associated with a significantly decreased rate of HIV-1 seroconversion (IRR, 0.45; 95\% CI, 0.27–0.72; \( P = .0003 \); figure 1). Each individual allele within the supertype was associated with a somewhat decreased risk of HIV-1 seroconversion. However, because of the rarity of some of these individual alleles, individually, only A*6802 and A*0202 were statistically significant (table 2) at uncorrected \( P < .05 \). There was no apparent added effect of homozygosity for multiple A2/6802 supertype alleles, but only 8 such individuals were found in the cohort. The allelic supertype A3 was marginally associated with an increased risk of seroconversion (IRR, 1.55; 95\% CI, 1.05–2.27; \( P = .02 \)), but this appeared to be due to the absence of the A2/6802 supertype, because it was not independent of A2/6802 on multivariate analysis. The B7 supertype did not influence the risk of HIV-1 seroconversion (IRR, 1.06; 95\% CI, 0.72–1.56; \( P = .73 \)).

Molecular typing of serologically defined HLA-A23 (A*2301), A24 (A*2402), and HLA-B18 (B*1801) did not reveal molecular heterogeneity in this population. As shown in table 2 and figure 2, the HLA class I allele A*2301 was associated with a substantially increased risk of HIV-1 seroconversion (IRR, 3.62; 95\% CI, 2.11–6.06; \( P = .0001 \)). In contrast, a serologically related determinant, A*2402, was associated with a decreased risk of HIV-1 seroconversion, although the \( P \) value (\( P = .04 \)) was marginal. The B-locus allele, B*1801, was also marginally associated with a reduction in the risk of HIV-1 seroconversion (\( P = .04 \)).

A total of 178 individuals were molecularly typed for HLA class II (DR). Sixteen DRB1 determinants were detected, of which the 10 that occurred in the population at a frequency of \( \geq 5\% \) were examined for associations with HIV-1 infection risk. DRB1*01 was strongly associated with a decreased risk of HIV-1 seroconversion (IRR, 0.22; 95\% CI, 0.06–0.60; \( P = .0003 \); figure 3). Allelic subtyping of HLA DRB1*01 revealed 2 subtypes, DRB1*0101 and DRB1*0102. DRB1*0102 was the predominant allele and accounted for most of the protection associated with the DRB1*01 determinant (IRR, 0.23; 95\% CI, 0.04–0.65; \( P = .0011 \)). The less common subtype, DRB1*0101, showed a trend toward protection, although this was not statistically significant (table 2).

Multivariate analysis was done by use of a Cox proportional hazards model. It initially included the HLA-A2/6802 supertype, A*2301, DRB1*01, B*1801, and A*2402, as well as years of prostitution, scoring for frequency of condom use, numbers of sex partners, and proportion of visits with gonorrhea or genital ulcers. In the final model, the HLA-A2/6802 supertype,
A*2301, and DRB1*01 were independently associated with an increased or decreased risk of HIV-1 seroconversion (table 3), whereas the associations with B*1801 and A*2402 were no longer statistically significant. The association with the DRB1 locus remained statistically significant also when only DRB1*0102, instead of DRB1*01 (which includes both subtypes), was included in the multivariate model. Condor use and adjusted years of prostitution were also independently associated with a decrease in risk, but number of sex partners per day was not. Including potential intermediate risk factors, such as gonococcal infections and genital ulcers, in the model did not alter HLA associations with HIV-1 seroconversion (data not shown), which indicates that the HLA associations with HIV were not acting through altered susceptibility to these other infections.

Discussion

In this study, we show that heterogeneity in susceptibility to HIV-1 infection among highly exposed sex workers in Nairobi is associated with several HLA alleles. The HLA A2/6802 supertype and HLA DRB1*01 were associated with a decreased risk of HIV-1 seroconversion, whereas HLA A*2301 was associated with an increased risk of seroconversion. This is the first relatively large cohort study to report an association of HLA alleles with altered risk of HIV-1 seroconversion. Several cross-sectional or case-control studies have associated specific HLA determinants with either decreased or increased risk of HIV infection [15, 38–41]. No consistent class I or class II associations were found among studies, although in a study with a mixed black and white population, HLA-A28 was associated with marginally increased risk in the white subgroup [39]. In view of the diversity of the study populations, the uncertain or differing extents and routes of HIV-1 exposure, the small study sizes, and the lack of molecular confirmation of HLA determinants, the inconsistency of findings is not surprising. The present study overcomes some of the limitations of previous studies. In addition to prospective measurement of HIV-1 incidence, the study population is of a single racial origin and is uniformly heavily exposed to HIV sexually. There appears to be some consistency between the HLA associations found in this cohort study and a study of mother-to-child transmission of HIV-1 and HLA in Nairobi. In that study, serologically defined HLA-A2 in the infant was associated with a 9-fold reduction in risk of perinatal HIV-1 transmission in 176

Table 2. Univariate survival analysis of class I and II HLA alleles for rate of human immunodeficiency virus type 1 seroconversion.

<table>
<thead>
<tr>
<th>HLA allele</th>
<th>n</th>
<th>No. seroconverting</th>
<th>Adjusted follow-up</th>
<th>IRR (95% CI)</th>
<th>P</th>
<th>Corrected P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2/6802 supertype</td>
<td>61</td>
<td>22</td>
<td>431</td>
<td>0.45 (.27–.72)</td>
<td>.0003</td>
<td>.003</td>
</tr>
<tr>
<td>A*0201</td>
<td>47</td>
<td>24</td>
<td>270</td>
<td>0.95 (.58–1.51)</td>
<td>.85</td>
<td>0.33</td>
</tr>
<tr>
<td>A*0202</td>
<td>18</td>
<td>6</td>
<td>140</td>
<td>0.44 (.16–.98)</td>
<td>.03</td>
<td>0.33</td>
</tr>
<tr>
<td>A*0205</td>
<td>8</td>
<td>3</td>
<td>44</td>
<td>0.73 (.15–2.2)</td>
<td>.64</td>
<td></td>
</tr>
<tr>
<td>A*0214</td>
<td>3</td>
<td>1</td>
<td>26</td>
<td>0.41 (.01–2.34)</td>
<td>.39</td>
<td></td>
</tr>
<tr>
<td>A*6801</td>
<td>7</td>
<td>3</td>
<td>36</td>
<td>0.88 (.18–2.63)</td>
<td>.89</td>
<td></td>
</tr>
<tr>
<td>A*6802</td>
<td>40</td>
<td>15</td>
<td>283</td>
<td>0.51 (.28–.88)</td>
<td>.0098</td>
<td>.11</td>
</tr>
<tr>
<td>A*2301</td>
<td>21</td>
<td>18</td>
<td>60</td>
<td>3.62 (2.10–6.06)</td>
<td>.0001</td>
<td>.001</td>
</tr>
<tr>
<td>A*2402</td>
<td>13</td>
<td>4</td>
<td>107</td>
<td>0.38 (.11–1.00)</td>
<td>.035</td>
<td>.39</td>
</tr>
<tr>
<td>B*1801</td>
<td>23</td>
<td>10</td>
<td>189</td>
<td>0.57 (2.6–1.01)</td>
<td>.043</td>
<td>.3</td>
</tr>
<tr>
<td>DRB1*01</td>
<td>21</td>
<td>4</td>
<td>188</td>
<td>0.22 (0.86–6.0)</td>
<td>.0003</td>
<td>.003</td>
</tr>
<tr>
<td>DRB1*0102</td>
<td>4</td>
<td>1</td>
<td>38</td>
<td>0.31 (.007–1.98)</td>
<td>.27</td>
<td></td>
</tr>
<tr>
<td>A*2301</td>
<td>21</td>
<td>18</td>
<td>60</td>
<td>3.62 (2.10–6.06)</td>
<td>.0001</td>
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<td>.003</td>
</tr>
<tr>
<td>DRB1*0102</td>
<td>4</td>
<td>1</td>
<td>38</td>
<td>0.31 (.007–1.98)</td>
<td>.27</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>232</td>
<td>122</td>
<td>1318</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. IRR, incidence rate ratio; CI, confidence interval; HLA, human leukocyte antigen.

* Exposure-weighted, adjusted person-years.

b Corrected for multiple comparisons.

Includes A*0202, A*0205, A*0214, and A*6802 (8 study subjects had 2 alleles within the A*02/6802 supertype; 69 alleles were detected in 61 study subjects).

Figure 1. Kaplan-Meier plot of time to human immunodeficiency virus type 1 (HIV-1) seroconversion with human leukocyte antigen (HLA) A2/A6802 supertype (interrupted line) and in the absence of HLA A2/A6802 (solid line).
mother-baby pairs [42]. Further molecular HLA typing in that cohort reveals that the A2/6802 supertype itself is strongly associated with protection on multivariate analysis (odds ratio, 0.15; 95% CI, 0.03–0.65; \( P = .009 \)) [43]. DRB1 alleles were not determined in that study.

There are a number of potential explanations for these findings. Consistent with other epidemiologic and immunologic studies in this population is the explanation that HLA determinants are functionally involved in mediating increased or decreased susceptibility to HIV-1 infection. A systematic correlation of the relative conservation of HIV-1 epitopes presented by certain HLA alleles, compared with their observed protective effect, is not possible because all epitopes are not known for all alleles. Nonetheless, one can reasonably argue that protective alleles may present highly conserved HIV-1 peptide epitopes that elicit strong protective cellular immune responses. In support of this, women in this study have vigorous CTL to highly conserved HIV-1 peptide epitopes that are shared by alleles within the supertype. The protective effect of this family of alleles is important epidemiologically because ~40% of all populations worldwide possess alleles from within the supertype. If these alleles do share HIV-1 epitopes that are highly conserved between viral variants and are the target of protective cellular immune responses, this would greatly inform the rationale for the design of HIV-1 vaccines.

The use of HLA allelic supertypes as a unit for analysis in this study deserves comment. Although HLA alleles are highly polymorphic, small degrees of genetic polymorphism may or may not affect peptide presentation and molecular function. Among genetically related MHC alleles, each HLA molecule preferentially binds peptides with certain anchor amino acids. However, these are not the only peptides that bind with high affinity; within groups of genetically related MHC alleles, there are shared peptide epitopes and a considerable overlap in peptide-binding capacity. Thus, a number of functional HLA supertypes have been identified. These are of obvious significance for vaccine design because the inclusion of “super-epitopes” in vaccines would potentially be immunogenic in large subsets of the population [44]. This concept of an “allelic supermotif” as a restriction element for cellular immune recognition [35–37, 45] has been shown to occur for HLA-A2 and A28. Genetic analysis of HLA-A2 subtypes shows that 2 distinct genetic clusters exist, with diversification from A*0201 at the center of one and diversification from A*0205 at the other (African subtypes A*0202 and A*0214 both represent intermediate variants [46]). The predominant HLA-A28 subtypes found in Africa (A*6801 and A*6802) are genetically related to the A2 group. A functional supertype consisting of A*0201, A*0202, A*0205, A*0214, and A*6802 has been proposed by Sidney et al. and by del Guercio et al. [35, 37] on the basis of the known structure, peptide-binding motif, and epitope presentation of these alleles. Notably, A*6801 is not in the A2 functional grouping and is included in HLA-A3 supertype on the basis of motif and epitope presentation [35, 36]. Other investigators have examined the peptide-binding specificity [37, 47, 48] and CTL recognition [22, 47, 49] of the alleles within the A2/6802 supertype and have identified peptides, including HIV peptides (e.g., HIV Pol 476–484) that promiscuously bind A*0201, A*0202, A*0205, or combinations thereof. In addition, Threlkeld et al. recently...

![Figure 2. Kaplan-Meier plot of time to human immunodeficiency virus type 1 (HIV-1) seroconversion with human leukocyte antigen (HLA) A*2301 (interrupted line) and in the absence of HLA A*2301 (solid line).](image1)

![Figure 3. Kaplan-Meier plot of time to human immunodeficiency virus type 1 (HIV-1) seroconversion with human leukocyte antigen (HLA) DRB1*01 (interrupted line) and in the absence of HLA DRB1*01 (solid line).](image2)
Table 3. Multivariate Cox proportional hazards modeling of risk factors for human immunodeficiency virus type 1 seroconversion.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2/6802 supertype</td>
<td>0.42</td>
<td>0.22–0.81</td>
<td>.009</td>
</tr>
<tr>
<td>A*2301</td>
<td>2.2</td>
<td>1.22–4.00</td>
<td>.009</td>
</tr>
<tr>
<td>DRB1*01c</td>
<td>0.26</td>
<td>0.09–0.71</td>
<td>.009</td>
</tr>
<tr>
<td>Adjusted years of prostitution</td>
<td>0.86</td>
<td>0.8–0.93</td>
<td>.0001</td>
</tr>
<tr>
<td>Condom use frequency score</td>
<td>0.49</td>
<td>0.38–0.63</td>
<td>.0001</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval.

*a* All significant on univariate Cox proportional hazards analysis.


*c* Includes DRB1*0101 and DRB1*0102.

*d* Mean of the self-reported condom use at each study visit on the basis of a semiquantitative scoring system from 1 to 4.

showed promiscuous recognition by CTL of HIV-1 peptides within the A3 supertype [45]. In the current study, we showed that a functional supertype consisting of A*0202, A*0205, A*0214, and A*6802 was strongly associated with a reduced risk of HIV-1 seroconversion (IRR, 0.45; 95% CI, 0.27–0.72; \( P < .0003 \)). We did not include A*0201 in the supertype because it was individually not associated with an altered risk of seroconversion. However, when the supertype including A*0201 is analyzed, the association with protection is similar (IRR, 0.60; 95% CI, 0.40–0.88; \( P = .006 \)) to that observed by use of a supertype that excludes A*0201. The exact constituents of the protective supertype may ultimately prove to include A*0201 when further studies in other populations are done. As noted earlier, in other studies, we have shown that 2 HIV-exposed uninfected women in this population have CTL recognition of an A*0201-restricted HIV peptide Pol 476-484 by A*0202 [22]. In addition, A*6802-restricted cytotoxic T cell responses to conserved protease gene epitopes were shown [22].

Related to the concept of functional supertypes is the possibility that different HLA molecules confer resistance or susceptibility to HIV infection and disease progression through variable tolerance of mutations in their T cell epitopes. In this model, proposed by Hendel et al. [17, 50, 51], HLA alleles that tolerate more epitope variation would be associated with protection and alleles that are sensitive to mutation would be associated with risk. The large A2/6802 supertype appears to tolerate substantial variation in epitopes, as described earlier. We and others have shown that there is cross-clade recognition of mutated epitopes [22, 52].

The strongest protective effect in this study was observed with the DR-locus determinant DRB1*01. A DR-locus association with relative resistance to HIV-1 has not been observed elsewhere with molecularly defined class II MHC gene products. DR molecules participate in immune function by presenting peptides to CD4+ lymphocytes. A highly conserved HIV-1 epitope that is strongly immunogenic and is presented by DRB1*01 could mediate protection by inducing a more vigorous or selective T helper–cell response [53]. In addition, strong T helper–cell responses to an HIV-1 p24 antigen have been correlated with a reduced virus load and risk of HIV-1 disease progression [54] and are likely contribute to a sustained potent cytolytic CD8 response. CD4+ cells can also be involved in the immune response through direct cytolytic activity, as is seen in herpes simplex virus infections [55]. Thus, DRB1*01 may restrict a T helper–cell or cytolytic CD4+ cell response to a conserved HIV-1 epitope. DRB1*0102 differs from DRB1*0101 by only 2 amino acids. Although both changes are in the peptide-binding groove, DRB1*0101 and *0102 have been shown in some cases to present the same peptides for T cell recognition; in other cases, it has been shown that peptide presentation differs between the 2 alleles [56, 57]. Nonetheless, an extended DRB1*01 supermotif for class II molecules may well exist between closely related alleles such as DRB1*0101 and DRB1*0102.

HLA A*2301 is associated with a significantly increased risk of HIV-1 infection in this study. There are several potential explanations for this association. HLA A*2301 may present T cell antagonist peptides of HIV. Plebanski et al. [58] have shown in the immune response to malarial antigens that naturally occurring epitope variants can interfere with T cell priming and can narrow the repertoire of the T cell response. Furthermore, McMichael and Phillips have reviewed the mechanisms of T cell antagonism in HIV infection and have argued that even small shifts in repertoire due to T cell antagonism may have a large effect on the efficiency of the CTL response [59]. Another possibility is that HLA A*2301 may present fewer conserved epitopes or may be more intolerant of epitope mutations than are other HLA molecules. If this is the case, homozygosity for HLA A*2301 should be associated with a stepwise increased risk of HIV infection. We were unable to examine this possibility because HLA-A*A*2301 homozygosity was detected in only 2 individuals. Finally, HLA-A*2301 could be linked to another polymorphic gene in the region that acts as a risk factor for HIV-1 acquisition.

Alternative explanations of the associations between HLA molecules and resistance and susceptibility to HIV-1 infection are possible. HLA molecules might operate as intermediate risk factors, altering susceptibility to other infections that enhance susceptibility to HIV-1. This appears unlikely because the associations with HLA alleles are independent of such factors on multivariate analysis, and separate analysis of the effect of the alleles in question for incidence of sexually transmitted infections (including gonococcal infections and genital ulcer disease) shows no associations (F.A.P., unpublished data). It is also possible that the HLA associations were produced through selection bias in the study population. Although the prospective nature of the study helps to minimize this possibility, the cohort was initiated in 1985, and HLA typing was instituted in 1991. Thus, some selection bias could have occurred from enrolled study subjects who died or were lost to follow-up before HLA
typing was done. However, when we compared the overall frequency of class I and class II alleles in this population with the frequency of HLA alleles in other populations from east Africa (data not shown), there were no significant differences, which suggests that a significant selection bias did not occur. In addition, the associations could be a statistical artefact that results from multiple comparisons, which is a problem inherent in any study of HLA and disease associations. This is mitigated to a large extent by the a priori hypothesis that A*02 and A*6802 are involved in resistance, made possible by studies of CTL among these women. In addition, we chose to examine only determinants that are relatively frequent (>5%) and to correlate our findings with the available data on the functional peptide-binding specificity (functional HLA supertypes). Furthermore, the statistical association remained significant at \( P < 0.05 \), after correction for multiple comparisons. Finally, protective HLA associations may also reflect linkage disequilibrium, acting as a marker for another protective polymorphic gene. Given the examination of functional grouping of HLA supertypes that comprise multiple HLA alleles, as well as the correlation with CTL reactivity [22], this possibility seems unlikely but cannot be completely excluded. On balance, it would thus appear that the HLA associations we report here are directly related in some way to HIV-1 resistance and susceptibility.

Evidence is increasing for multiple mechanisms of resistance and susceptibility to HIV-1 infection and disease. Chemokine receptor polymorphisms are now well established as mediating resistance to infection but account for only a small proportion of resistance, even in white populations [60]. An increasing number of studies indicate that immune-mediated resistance occurs [8, 10, 11, 13]. In the Pumwani Sex Worker Cohort, resistance to HIV-1 infection is associated with cellular [22] and mucosal antibody responses to HIV-1 [12] but not with chemokine receptor or receptor-promoter polymorphisms or with altered cellular permissiveness to infection [20]. The associations of HLA alleles with decreased risk of HIV-1 seroconversion also point to immune-mediated mechanisms being involved, perhaps through restriction of T cell responses to conserved HIV-1 antigens. It is also apparent that, although the HLA associations may explain why some individuals are resistant to HIV-1 infection, these alleles are neither completely necessary nor sufficient for resistance. This is highlighted because, in this study, the association between duration of exposure (as measured by the weighted duration of prostitution) was protective against HIV-1 seroconversion, independent of the HLA associations. This result indicates that factors related to exposure, in addition to HLA phenotype, are involved in the development of resistance. Although further studies of genetic and immunologic factors involved in HIV-1 resistance may help to elucidate other mechanisms of protective immunity to HIV-1 and why it develops only in a minority of exposed individuals, these data add substantial information to direct T cell epitope-based HIV vaccine development.

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