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Mismatched Human Leukocyte Antigen Alleles Protect Against Heterosexual HIV Transmission

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Summary: Genetic variation at the human leukocyte antigen (HLA) loci has been shown to be an important risk factor for progression to HIV disease, but its significance in infection is less well understood. We have investigated its role in HIV transmission in a cohort of individuals at risk for heterosexual infection. Analysis of over 80 individuals revealed that that the degree of concordance at HLA A, B, and DR loci differs significantly between transmitting and nontransmitting couples at risk for heterosexual HIV transmission ($p < .02$), suggesting that allogeneic immune responses may confer a degree of protection against HIV infection. Analysis of the frequencies of specific alleles at the A, B, and DR loci revealed a significantly higher frequency of HLA DR5 among exposed uninfected individuals, relative to population controls.
Key Words: Major histocompatibility complex—HIV—Heterozygosity—Sexual transmission.

Individuals who appear to be resistant to HIV-1 infection despite multiple exposure (EUs) have been identified among homosexual men (1), prostitutes in Africa (2), and long-term sexual partners of HIV-seropositive individuals (3–6). The discovery that homozygotes for a 32-base pair (bp) deletion in the CCR5 receptor gene are highly resistant to infection (1) was significant for our understanding of virus–cell interactions but explains only a small proportion of EUs due to the absence of this allele from nonwhite populations, and its low frequency even among whites (7). In some studies, major histocompatibility complex (MHC)-restricted, HIV-specific, cellular immune responses have been detected in the absence of any evidence of HIV infection, suggesting that exposure to virus has induced an immune response that may be protective (8). Nevertheless, what determines which individuals are at lower risk of infection remains unclear (2).

Associations with progression to HIV disease have been observed for several HLA haplotypes and it has been demonstrated that heterozygosity at HLA loci is also protective against disease progression (9). Less is known about the role of HLA in protection against infection; in a study of perinatal transmission in Kenya, concordance at HLA-A, B, and C loci was studied and found to increase risk for vertical transmission (10). We earlier analyzed the effect of genotype at chemokine receptor loci on HIV transmission in the Edinburgh Heterosexual Partner Study (EHPS), a cohort that includes couples both concordant and discordant for HIV serostatus. Despite the strong protection against infection conferred by the CCR5 Δ32 mutation, in the homozygous state and the delayed disease progression observed in heterozygotes (11), we found that heterozygosity at CCR5 did not reduce risk of infection significantly, and

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although the allele frequency was high in this population, homozygotes were too infrequent to explain the number of EUs (12). We have now analyzed the same individuals for HLA alleles at three loci and present evidence that the number of alleles shared between partners differs significantly between HIV-concordant and HIV-discordant couples and that possession of HLA-DR11 is also associated with protection against HIV infection from heterosexual contact.

METHODS

The patient population has been described previously (3,12). Briefly, the partner study was initiated following an epidemic of HIV associated with injection drug use and most of the index patients were men who had been infected by that route. Length of relationship and frequency and nature of sexual contacts were determined by interview when an HIV test and counseling was offered. Couples discordant for HIV status received a follow-up interview and HIV test at approximately 6-month intervals. Informed consent was obtained from each study subject, all of whom are white and 95% of whom reside in central Scotland.

Complete HLA typing for A, B, and DR has been carried out on both partners on a total of 41 couples recruited to this study, 11 concordant and 30 discordant for HIV serostatus.

HLA typing was performed using serologic and sequence-specific oligonucleotide hybridization methods, with sera provided by the UK National Transplant Service.

RESULTS

From the subjects recruited by the EHPS (4), couples were selected for whom the only risk factor for HIV infection in the contact reported at interview was sexual contact with the index. We tested for differences in frequencies of specific alleles in exposed uninfected (EU) and HIV-infected (HIV-positive) contacts. For the three loci, A, B, and DR, a total of 39, 51, and 54 EUs and 17, 17 and 16 HIV-positives were typed, respectively. The frequency of HLA alleles differed in the two groups, strikingly so with respect to DR11 (14 of 54 EUs; none of 16 HIV-positives). However, because of the low numbers of HIV-positive individuals this comparison ($p < .02$, Fisher exact test, two-tailed) loses significance when corrected for the number of alleles tested. To allow a more powerful test for alleles that might be associated with protection, a second comparison was performed between the frequencies observed in EHPS EUs and those published for the general Scottish population (13). No significant differences in allele frequencies were observed for HLA-A or -B, but frequencies at the DR locus differed significantly between the two samples (Table 1; heterogeneity $\chi^2 = 18.4$, degrees of freedom [df], 6). In the population survey, the DR5 allele class was not split to DR11 and DR12 as in the current study. The groups therefore have to be compared in terms of frequency of DR5 (= [DR11 + DR12]), which was significantly different between the two samples (16.5% in EUs, 6.5% in the general population; $\chi^2_1 = 10.5$; $p < .02$, corrected for number of tests). Because 14 of the DR5-bearing individuals among the EUs carried DR11, and only 1 was DR12, we therefore conclude that HLA DR11 probably confers some protection against heterosexual HIV infection.

TABLE 1. *Frequency of alleles at the human leukocyte antigen (HLA) DR locus*

	Allele frequency (%)						
	DR2	DR3	DR4	DR5	DR6	DR7	Other
EU	12.5	18.3	19.2	16.3[b]	9.6	15.4	8.7
Population[a]	15.8	17.2	13.4	6.5	19.5	17.9	9.7

Frequencies observed in exposed uninfected individuals ($n = 54$) and in a sample from the Scottish general population ($n = 264$) are shown for alleles present in either population at a frequency >10%.

χ^2 test for heterogeneity = 18.44 (6 df); $p < .01$.

[a] Data from Jazwinska and Kilpatrick (13).

[b] For EUs split typing to HLA DR11 and DR12 was performed (DR11, 14.8%; DR12, 0.92%).

EU, multiply exposed uninfected study subjects.

Complete HLA typing for the A, B, and DR loci on both partners was achieved for a total of 41 couples, 11 concordant and 30 discordant. Alleles at all three loci were compared between index and contact and a simple mismatch score devised, recording how many (potentially between 0–6) were common to both. The range observed in both concordant and discordant patients was from 1 to 6. However, the distribution of mismatch scores was different in the two groups (Fig. 1): the median number of mismatches for concordant couples was 3 of 6 whereas that for discordant couples was 4.5 of 6. The means of the two groups were significantly different ($t = 2.51$; $p < .02$, on square-root transformed means). Despite the small numbers available for analysis, the difference was also significant under a nonparametric test (Wilcoxon rank sum $z = 2.34$; $p < .02$). We therefore further conclude that concordance between sexual partners at these HLA loci is associated with increased risk of heterosexual HIV transmission. This effect was independent of DR11 because the results remained significant when DR11 individuals were removed from the analysis.

In previous work (12), we did not find evidence for significant association between heterozygosity for CCR5 Δ32 and EU status, but, surprisingly, CCR2 64I heterozygosity was associated with HIV infection among women. We have therefore tested a logistic model for HIV infection in women incorporating both CCR2

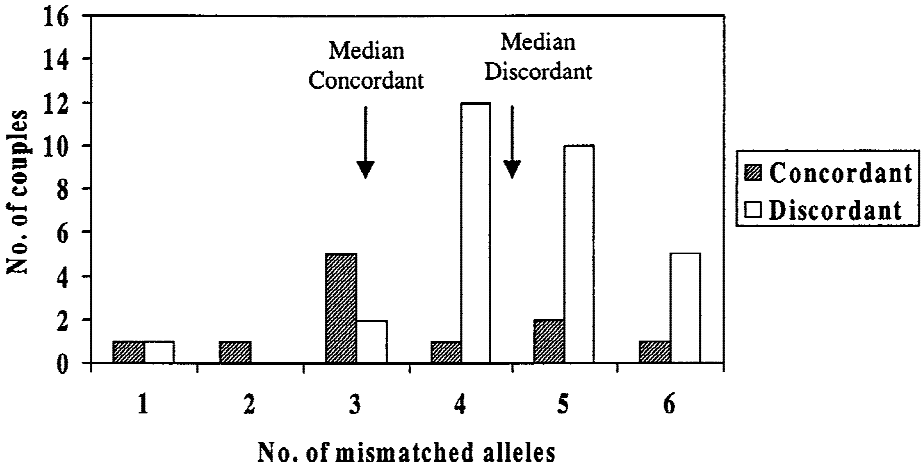

FIG. 1. Human leukocyte antigen (HLA) mismatch in HIV-concordant and -discordant heterosexual couples. The distribution of numbers of mismatched HLA alleles at the A, B, and DR loci, together with the median value, is shown for HIV-concordant (n = 11) and HIV-discordant (n = 30) couples from the Edinburgh Heterosexual Partner Study. The two groups differed significantly under the Wilcoxon rank sum test ($p < .02$).

genotype and HLA mismatch score. This model was highly significant ($p < .01$, likelihood ratio test) and no significant interaction was detected between the two variables. CCR2 64I was associated with an odds ratio of 6.7 for infection, whereas each unit change in mismatch score was associated with an odds ratio of 1.5.

DISCUSSION

An earlier analysis of data from a European study of heterosexual HIV transmission suggested the relationship between number of accrued sexual contacts between discordant couples and probability of transmission was not simple (14). A model assuming a constant risk of infection per contact underestimated the risk for small numbers of sexual contacts between discordant partners and overestimated it for larger numbers. One interpretation of this observation is that a degree of protection against HIV infection can be acquired following repeated exposure. A further suggestion that exposure is itself protective has come from the more recent observation that among long-term exposed uninfected prostitutes in Nairobi, protection could be lost as a result of a decrease in exposure level (15). One possible component of the protective response is alloreactivity, an immune response to a mismatched HLA antigen (16). It has been shown in a macaque monkey model that vaccination with purified Class 1 molecules can be protective against virus grown in cells expressing the same Class 1 protein, by virtue of its acquisition on the viral envelope during budding (17). As the average duration of a partnership in the EHPS was about 4 years and the median number of sexual contacts accrued was over 100 (3,12), there were substantial opportunities for activation of alloresponses in this group.

One analysis of vertical HIV transmission also revealed evidence of HLA concordance acting as a risk factor for infection (10), but another study of heterosexual HIV transmission failed to identify significant frequencies of mismatch between concordant and discordant couples (6). However, that study differed from this in that HLA concordance was scored as presence or absence of a single mismatch for any of the scored loci; thus, the cumulative effect described here was not tested. Notwithstanding, individual effects of specific alleles were also tested in that study and a twofold elevation of frequency of DR5/DR11 in EUs was observed, as described here, although it was not significant due to low numbers (6). HLA DR has been found to be protective in a vaccine challenge study in the macaque (18), it is possible that Class II molecules are particularly significant in HIV protection.

We have obtained direct evidence that degree of mismatch at HLA loci between partners can be associated with probability of heterosexual HIV transmission in a cohort of long-term partnerships independent of genotype at chemokine receptor loci. It has recently been shown that alloimmunization in humans can elicit cellular immune responses that inhibit HIV replication in vitro (19), taken together these observations suggest that the role of alloreactivity should be explored in larger studies in view of its potential as a contributor to an HIV vaccine (16).

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