CONCISE COMMUNICATION

The HLA A2/6802 Supertype Is Associated with Reduced Risk of Perinatal Human Immunodeficiency Virus Type 1 Transmission

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Certain HLAs may, in part, account for differences in human immunodeficiency virus type 1 (HIV-1) susceptibility by presenting conserved immunogenic epitopes for T cell recognition. The HLA supertype A2/6802 is associated with decreased susceptibility to HIV-1 among sex workers. The alleles in this supertype present the same HIV-1 peptide epitopes for T cell recognition in some cases. This study sought to determine whether the HLA A2/6802 supertype influenced HIV-1 transmission in a prospective cohort of HIV-1–infected mothers and children in Kenya. Decreased perinatal HIV-1 infection risk was strongly associated with possession of a functional cluster of related HLA alleles, called the A2/6802 supertype (odds ratio, 0.12; 95% confidence interval, 0.03–0.54; $P = .006$). This effect was independent of the protective effect of maternal-child HLA discordance. These data provide further evidence that HLA supertypes are associated with differential susceptibility to HIV-1 transmission.

Mother-to-child transmission of human immunodeficiency virus type 1 (HIV-1) may occur during gestation, during delivery, or after birth through breast-feeding. Even when antiretroviral agents are not given to the mother or infant, transmission occurs in a minority of cases [1]. Reports of HIV-1 clearance in perinatally exposed infants and the presence of cytotoxic T cell responses against HIV-1 in exposed uninfected infants suggests that, in some circumstances, fetuses or newborns can abort or eliminate infection through virus-specific cellular effector mechanisms [2–5].

Why do some infants develop what appear to be protective cytotoxic T cell responses and not others? One important determinant of resistance and susceptibility to infection is the major histocompatibility complex (MHC). Class I MHC alleles determine the molecular targets of the cytotoxic T lymphocytes (CTL) in a given host. We showed elsewhere that certain clusters of functionally related class I MHC alleles, called HLA supertypes (e.g., HLA A2/6802), are associated with reduced risk of sexual transmission of HIV-1 among sex workers in Kenya [6]. We sought to determine whether this same HLA supertype is associated with protection from HIV-1 in an independent population with another mode of transmission.

Methods

Study population. HIV-1–infected mothers and their children enrolled in the University of Nairobi HIV-1 Perinatal Transmission Study were recruited for this study. The cohort and study design have been described elsewhere [1, 7, 8]. HIV-1 testing by serology and proviral polymerase chain reaction (PCR) was done at birth, at ages 6 and 14 weeks, and at 3-month intervals thereafter, as described elsewhere [8]. This study was based partly on a group of mothers and children enrolled from May 1993 until December 1996, whom we reported elsewhere as having an association between HLA discordance and reduced perinatal HIV-1 transmission [8].

HLA typing. Class I HLA typing by serology was performed on all mothers and infants by standard microlymphocytotoxicity methods described elsewhere [9]. Molecular HLA typing was done by using nested sequence-specific PCR on frozen buffy coats and cell pellets [6]. We performed class I molecular HLA typing to clarify alleles that were ambiguous by serologic methods and to subtype serologic determinants (e.g., HLA-A2 and HLA-A28) that form the A2/6802 supertype. Alleles screened included A*0201, A*0202, A*0205, A*0214, A*6801, A*6802, and A*6901. Of note, although A*6801 serologically is part of the HLA-A28 determinant

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

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Table 1. Comparison of demographic, obstetric, and other characteristics of perinatally human immunodeficiency virus (HIV)–1–infected and –uninfected children and their mothers.

Characteristic	HIV-1 uninfected at birth (n = 152)	HIV-1 perinatal infection (n = 19)	P (2-tailed)
Child			
Birth weight, g	3079 (530)	3150 (436)	.61
No. female	72	8	.85[a]
Gestational age at delivery	39.1 (2.4)	38.6 (3.2)	.21
CD4 cell count at <6 months old, cells/mm^3	1968 (597)	1626 (523)	.04
Duration of follow up, months	48.8 (25.0)	41.2 (30.8)	.25
Mother			
No. married	114	12	.26[a]
Age, years	22.6 (3.3)	21.9 (2.8)	.36
No. of sex partners in past 5 years	2.9 (8.5)	2.4 (1.9)	.42
Duration of labor, h	10.6 (5.2)	12.2 (6.3)	.30
Gravida	2.3 (1.5)	1.7 (1.1)	.09
Duration of membrane rupture, h	4.32 (6.9)	4.1 (5.4)	.88
CD4 cell count, 1–6 months postpartum, cells/mm^3	590 (393)	741 (647)	.2

NOTE. Data are mean (SD).
[a] By the χ^2 test; all other comparisons were by Student's t test.

and it cross-reacts with HLA-A2, functionally it forms part of the HLA-A3 supertype on the basis of motif and epitope specificity [10]. Class II typing was not possible because of the very small amounts of DNA available. A maternal-child HLA concordancy score was calculated by the addition of the number of matches at all 3 class I loci; therefore, scores ranged from 3 to 6 [8].

Classification of HIV-1 infection status of children. All children were HIV-1 seropositive at birth because of passive transfer of maternal IgG. Children who became seronegative and remained so were considered to be uninfected. Children who became seronegative and remained so on ⩾2 visits, but who later seroconverted, and children who were HIV-1 PCR negative in the first 6 months but later became HIV-1 PCR positive and/or HIV-1 seropositive were considered to represent late infections due to breast-feeding and were classified as uninfected at birth. Children who were HIV-1 seropositive at ⩾12 months of age and were always HIV-1 seropositive and HIV-1 PCR positive were considered to be perinatally infected. Children who were seronegative only once were also classified as perinatally infected. The perinatally infected group of children thus comprise, as intended, both intrauterine and intrapartum infections. It is recognized that breast milk transmission soon after birth would be included in this group, thus biasing the study toward the null hypothesis, in terms of discriminating between differences in susceptibility on the basis of route of infection.

Statistical analysis. Statistical methods are reported in table 1 and in the text for univariate analysis. Logistic regression was used to analyze the effect of several variables simultaneously on infection at birth. Variables included as independent variables were HLA-A, -B, and -C locus concordancy score, A2/6802 supertype, parity, marital status of the mother, age, and the average maternal CD4 cell count 1–6 months after delivery. P values given are 2-tailed. To explore the possible effect of the inclusion of early breast milk transmission in the group of children who were perinatally infected, we cross-tabulated subsequent seroconversion among those classified as uninfected at birth against the A, B, and C locus concordancy score and against the A2/6802 supertype. We also used survival analysis (Cox regression) to study a potential effect of concordancy and the A2/6802 supertype on the risk of late seroconversion.

Results

In total, we evaluated 171 children born to 135 mothers. These included 29 mothers with ⩾2 children enrolled, including 2 sets of dizygotic twins. The children were born between July 1986 and June 1996. Of the 171 children, 19 were classified as perinatally infected and 152 were uninfected at birth. Of these, 21 subsequently HIV-1 seroconverted during follow-up, acquiring HIV-1 from prolonged breast-feeding. Table 1 shows demographic, obstetric, and other characteristics of uninfected and perinatally infected children and their mothers. With the exception of CD4 cell counts of the children during the first 6 months of life, there were no statistically significant differences in the characteristics between uninfected and perinatally infected children.

Figure 1 shows the rate of perinatal HIV-1 transmission in children who possessed the HLA alleles that form part of the A2/6802 supertype versus those who did not. Perinatal transmission occurred in 2 (2.8%) of 72 children with the A2/6802 supertype versus 17 (17.2%) of 99 children without the A2/6802 supertype (odds ratio [OR], 0.17; 95% confidence interval [CI], 0.03–0.53; P = .005). Consistent with our previous report [8], maternal-child class I HLA matching from 3 of 6 to 6 of 6 was associated with an increased risk of perinatal HIV-1 transmission, with transmission increasing 3.0%, 9.3%, 15.7%, and 30.7% in each group, respectively (P = .001, χ^2 for trend).

Of interest, possession of alleles within the A2/6802 supertype was not associated with protection from late breast milk transmission. Of 72 children with the A2/6802 supertype, 10 (13.9%) became infected from late breast milk transmission versus 11 (11.1%) of 99 children without the A2/6802 supertype (OR, 1.1;

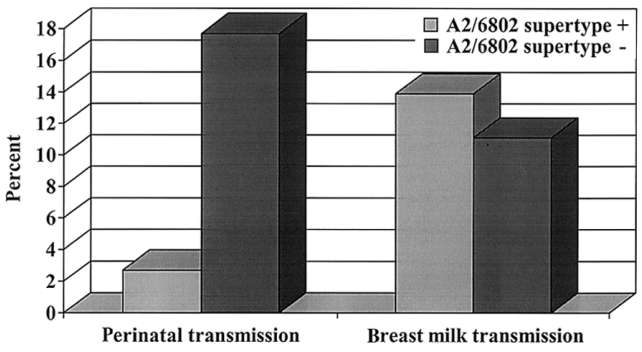

Figure 1. Comparison of infants with (n = 72) and without (n = 99) HLA A2/6802 supertype, with respect to perinatal human immunodeficiency virus (HIV)–1 transmission (P = .005) and late HIV-1 transmission from breast-feeding (P = .99).

95% CI, 0.39–2.9; $P = .99$). Class I HLA matching was not associated with increased risk of late breast milk transmission; in fact, there was a trend toward the opposite ($P = .06$). This is consistent with our previous observation [8]. Survival analysis with Cox regression (results not shown) also found no relationship between concordancy and time of seroconversion.

Multivariate analysis using logistic regression as described in Methods showed that the A2/6802 supertype was independently associated with protection from perinatal HIV-1 transmission (OR, 0.12; 95% CI, 0.03–0.54; $P = .006$). Each additional matching class I HLA allele was independently associated with an OR of 2.2 (95% CI, 1.2–4.0; $P = .009$), thus confirming that HLA alleles within the A2/6802 supertype and a lack of HLA matching were independently protective against perinatal HIV-1 transmission. After we controlled for class I MHC discordance, there was no significant difference whether the child inherited the A2/6802 supertype from the mother or the father (data not shown).

Discussion

Our findings show that, among infants, the A2/6802 supertype is associated with an estimated 7-fold protective effect from perinatal HIV-1 transmission. Furthermore, this effect is independent of the potent protection associated with maternal-child class I HLA discordance that we previously reported. These 2 observations are important because both point to potential immunologic approaches to preventing HIV-1 infection.

We reported elsewhere that the A2/6802 supertype is associated with protection against HIV-1 infection among female sex workers in Nairobi [6]. We sought to confirm this association in an independent population of the same ethnic group. We focused molecular HLA typing on the A2/6802 supertype, to avoid the problem of multiple comparisons. There have been no prospective molecular studies of class I HLA and mother-to-child HIV-1 transmission, although there was a small molecular study of class II HLA alleles [11]. The A2/6802 supertype among East Africans is composed of the following alleles, in decreasing order of frequency: A*0201, A*6802, A*0202, A*0205, A*0214, and rarely A*6901 (not found in this study). This contrasts with findings in white persons, in whom A*0201 is predominant. Worldwide, the subtypes that make up the A2/6802 supertype vary, but there is a remarkable consistency across populations in the frequency of the overall supertype [12]. This has prompted the hypothesis of convergent evolution in response to infectious disease pressure [13]. In our previous study of this phenomenon in sex workers, each subtype was associated with ORs that were protective, although A*0201 was relatively less protective than the others. In the current study, the A*0201 subtype, even when considered individually, approaches significance, with an OR of 0.16 ($P = .08$), thus supporting the composition of the supertype. Consistent with the rationale for grouping alleles by functional supertypes, the serologically related but functionally different allele A*6801 is not associated with protection (OR, 2.0; $P = .72$).

The consistent lack of protection from late breast milk transmission provided by either HLA discordance or the A2/6802 supertype deserves comment. Late HIV-1 transmission from breast milk differs from intrauterine and intrapartum transmission by several factors, notably the route and the immune maturation and cytokine milieu of the infant [14]. We previously hypothesized that, as an infant becomes older, antimaternal alloimmune responses may wane and provide less protection [15]. The difference may also be explained by the differing mucosal site of viral shedding for the mother (hence potential differences in viral quasi species) and the differing challenge site of the child. Finally, the form of viral challenge (cell-associated or cell-free virus) may differ. These factors could all influence the relative protection afforded by CTLs that may have been primed at a different site. This study therefore underscores the importance of understanding the role of the maturation of the infant's immune system in regard to vulnerability to HIV-1 and the site specificity of potential mechanisms of protection.

One intriguing additional possibility is that a reciprocal response or an immune hierarchy occurs with protection from alloimmune antimaternal HLA immune responses and virus-specific effectors (restricted by the A2/6802 supertype). In this model, infants with maximal HLA discordance would be protected prenatally and at birth through this mechanism and hence may not receive enough "viral challenge" to prime A2/6802-restricted anti-HIV effectors. As antimaternal alloimmune responses wane, these infants might be paradoxically at increased risk of infection, because they do not have the potential benefit of antiviral effectors generated earlier. This is consistent with our observation that HLA discordance is associated with a trend toward increased risk from late breast-feeding. In this scenario, one would expect that the effects of HLA matching and the A2/6802 supertype might oppose each other at this later stage in this subset of infants. This small study does not have the statistical power within the subset of HLA-discordant infants ($n = 32$) to address this intriguing question. In fact a more definitive answer would come from serial measurements over time of anti–HIV-1 CTL and anti-maternal HLA effectors in HIV-1–exposed infants.

The association of the HLA A2/6802 supertype with decreased susceptibility to HIV-1 has particular significance with respect to vaccine design since the frequency of the supertype worldwide approaches 40% [12]. HIV-1 epitopes that can be presented by multiple members of the supertype, especially if they are identified in HIV-1–exposed uninfected persons, could form important constituents of an epitope-based HIV-1 vaccine.

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