Mother-Child Class I HLA Concordance Increases Perinatal Human Immunodeficiency Virus Type 1 Transmission

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Major histocompatibility complex (MHC) gene products are expressed on human immunodeficiency virus (HIV)-infected cells and incorporated into the lipid envelope of HIV virions. Macaques immunized with human MHC gene products are protected from simian immunodeficiency virus challenge when the virus is grown in cells expressing the same MHC alleles. To relate these findings to mother-to-child transmission of HIV-1, investigations of whether sharing HLA between mother and infant influenced the risk of transmission of HIV-1 to the child were carried out. Class I HLA concordance was independently associated with a stepwise increase in the risk of perinatal HIV-1 transmission for each additional concordant allele (odds ratio, 2.63; 95% confidence interval, 1.36–5.07; P = .003). Thus, discordant HLA may provide infants with a means of protection against HIV-1 as a result of allogeneic infant anti-maternal MHC immune responses.

Human immunodeficiency virus type 1 (HIV-1)–infected mothers may transmit the virus to their infants during gestation or during birth. Infection may occur through maternal blood or mucosal secretions, which contain either free HIV-1 virions or HIV-1–infected cells. Postnatal transmission through breastfeeding may occur via free virions or infected cells as well. While the relative contribution of each of these routes and modes of transmission to the burden of vertical HIV-1 transmission remains unclear, there is increasing evidence that the fetus is exposed to maternal blood, and thus to free HIV-1 and HIV-1–infected cells, during both gestation and delivery. Sensitive studies using polymerase chain reaction (PCR) have shown that maternal cells are ubiquitous in cord blood samples, at an estimated frequency of 1/10^4–10^5 nucleated cells [1, 2]. In addition, extended survival of maternal cells in the newborn can occur, particularly when mother and child are HLA–identical or when fetal immune surveillance is compromised [3]. Since HIV-1 is known to infect cells within the placenta [4–6], the increased frequency of chorioamnionitis in mothers with AIDS could potentially result in an increased exchange of maternal and fetal cells and thus of HIV-1 [7, 8]. Thus, the chance of a child born to an HIV-infected mother having been exposed to free virus or to HIV-infected cells would seem to be very high. Yet, only a minority of children are ultimately infected by their mother. 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 at least in some circumstances, fetuses or newborns become infected but are able to contain or eliminate infection through virus-specific cellular effector mechanisms [9–11].

Fetal or newborn alloimmune responses directed at maternal HIV-1–infected cells or directed at free virus bearing maternal major histocompatibility complex (MHC) determinants could also account for some children remaining uninfected. Evidence suggesting that anti-MHC immune responses can protect against HIV-1 infection comes from a macaque model in which immunization with a human lymphoblastoid cell line protected macaques against subsequent simian immunodeficiency virus (SIV) challenge, when the virus was grown in the same cell line [12, 13]. Protection correlated best with antibodies against class I MHC [14]. Studies by Arthur et al. [15] showed that class I and II MHC is present on the envelope of HIV-1 and that antisera to these proteins precipitated intact virions. Chan et al. [16] also showed that immunization with purified class I HLA molecules can protect macaques from challenge with cell-free virus expressing the same class I HLA. It is not known whether alloimmune anti-MHC responses would afford the same level of protection, but it is known that alloimmune responses can be even more vigorous than xenoimmune responses [17]. Stott et al. [18] have reported that immunization with allogeneic cynomolgus lymphocytes can protect macaques against SIV grown in simian cells. It has been suggested that
exploration of this mechanism of protection from HIV-1 challenge may provide important clues for vaccine development [8, 19]. In the situation of mother-to-child transmission of HIV-1, both free HIV-1 virions and HIV-1--infected cells of maternal origin display maternal MHC, and either fetal or newborn anti-MHC antibodies or alloreactive T cell responses could potentially protect against infection from the mother. Such mechanisms would only be operative in the case of some degree of HLA discordance between mother and child, and it could be reasonably expected that any protection provided might increase with the extent of HLA discordance between mother and child. We designed a study to explore this hypothesis, by examining the relationship between HLA mother-child concordance and the risk of mother-to-child transmission of HIV-1.

Methods

Study population. HIV-1--infected mothers and their children, enrolled in the University of Nairobi HIV-1 Perinatal Transmission and Pediatric AIDS Study, were recruited for this study. This observational cohort study was initiated in 1986, and its enrollment protocol and study design have been previously described [20, 21]. Breast-feeding was advocated according to the World Health Organization breast-feeding guidelines. Blood for HIV-1 serologic assays was obtained at birth, at 6, 14, and 24 weeks, and every 3 months thereafter. Blood for HIV-1 provirus PCR was collected on the same visits after 1990. From May 1993 until December 1996, mothers and children seen during follow-up were invited to participate in this nested study. Because of the volume of blood required for HLA typing, only children who were ≥1 year of age were studied. Only class I serologic typing was done because of the difficulties of interpretation of class II serologic typing and also because of the volume of blood available.

HLA typing. Class I HLA typing was done by use of microlymphocytotoxicity according to standard methods [22]. Class I 72-well typing trays, selected for the presence of sera reactive against determinants seen in blacks (as observed in previous studies by our group in Nairobi), were provided for use by the Canadian Red Cross (Ottawa). Extended trays were used or typings were repeated in the case of ambiguous results. Serologic HLA subtypes were assigned when detected (e.g., HLA-A74[19]); otherwise, a broad determinant was assigned (e.g., HLA-A19). Blank alleles (e.g., only 1 determinant detected at a locus) were retyped to detect missed determinants. Persistent blank alleles were present in <3% of A and B locus typings and in 19% of C locus typings.

Classification of mother-child HLA matching. The number of matches between mother and child at each of the A, B, and C loci were counted. Since mother and child should share at least one match at all HLA loci (this was indeed the case), all children had at least a 3 of 6 match. The occurrence of additional matches between mother and child at the class I A, B, and C loci was scored. Persistent blank typings in mothers and children were considered homozygotes at that locus. If mothers were homozygous at a locus, their child was considered to have two matches at that locus (i.e., the mother’s cells would be recognized as foreign at that locus). If a child was homozygous and the mother was not, the child was considered to have one match with the mother at that locus (the mother’s cells would be recognized as foreign at that locus). An HLA concordance score was calculated as the sum of the number of matches at all three loci. Therefore scores ranged from 3 to 6.

HIV-1 serologic testing and HIV-1 PCR. HIV-1 screening serology was done by ELA (Vironostika; Organon Teknika-Cappel, Durham, NC). Confirmation tests were done by immunoblot (Du Pont, Geneva). All tests were done according to manufacturers’ instructions. Immunoblots were considered positive if antibody to one core protein and one envelope protein were present. HIV-1 provirus PCR was available to assist in the diagnosis of HIV-1 infection in children after 1990. One hundred thirty children had HIV-1 provirus PCR done, including 16 of 19 perinatally infected infants and 17 of 20 infants infected through breast-feeding. The mean number of PCR tests on those children was 3.27 (range, 1–9). PCR was done with novel primers and probes developed to the regulatory genes vif and nef. For comparison, primers SK68’ (AGCAGCGAGGAAGCAATGG) and SK69’ (CCGACACGTGAGTTGCAACAG), based on published sequences, were used to amplify part of the env gene [23, 24].

The limit of detection of these primer pairs is 3.8 viral copies/150,000 cells. Cord and peripheral blood mononuclear cells from HIV-1--exposed children were used as a source of DNA for HIV-1 provirus detection, with use of the above primers. Children were considered positive if two or more primers were positive.

Classification of HIV-1 infection status of children. All children were initially HIV-1--seropositive because of passive transfer of maternal IgG. Children who became seronegative and remained seronegative were considered uninfected. Children who became seronegative and remained seronegative on two or more visits but who later seroconverted, as well as children who were HIV-1--negative in the first 6 months but later became HIV-1--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--positive were considered to be perinatally infected. Children who were seronegative on only one occasion were also classified as perinatally infected. The perinatally infected group of children thus comprise, as intended, both intruterine and intrapartum infections. It is recognized that breast milk transmission occurring soon after birth would be included in this group.

Statistical analysis. Statistical methods are reported in tables for univariate analysis. For analysis of associations of transmission risk with individual HLA alleles, only those class I alleles with a population frequency of >10% were examined. Only those reaching statistical significance are reported, uncorrected for multiple comparisons. Logistic regression was used to analyze the effect of several variables simultaneously on infection at birth. Variables included as independent variables, in addition to the HLA-A, -B, and -C locus concordance score, were individual alleles that accounted for at least 10% of the allele frequency at their loci, parity, marital status of the mother, age, and the average maternal CD4 cell count during the period 1–6 months after delivery. Reported Ps are two-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 concordance score. We also used survival
Table 1. Comparison of demographic, obstetric, and other characteristics of perinatally HIV-1–infected and uninfected children and their mothers.

| Child | HIV-1–uninfected at birth (n = 141) | HIV-1 perinatal infection (n = 19) | P  
|-------|------------------------------------|-----------------------------------|-----
| Birth weight (g) | 3074 (542) | 3150 (436) | 0.59
| No. female | 68 | 8 | 0.80
| Gestational age at delivery | 38.0 (2.4) | 38.6 (3.2) | 0.52
| CD4 cell count, <6 months of age | 1978 (599) | 1626 (523) | 0.04
| Duration of follow-up (months) | 48.6 (25.8) | 31.2 (30.8) | 0.25

| Mother | HIV-1–uninfected at birth (n = 141) | HIV-1 perinatal infection (n = 19) | P  
|-------|------------------------------------|-----------------------------------|-----
| Married | 110 | 13 | 0.52
| Age | 22.6 (3.4) | 21.9 (2.8) | 0.13
| Lifetime no. of sex partners | 2.9 (8.5) | 2.4 (1.9) | 0.80
| Duration of labor (h) | 10.6 (5.2) | 12.2 (6.3) | 0.28
| Gravida | 2.3 (1.5) | 1.7 (1.1) | 0.09
| Duration of membrane rupture (h) | 4.2 (6.2) | 4.1 (5.4) | 0.94
| CD4 cell count, 1–6 months postpartum (mm³) | 581 (402) | 741 (647) | 0.19

NOTE. Data are mean (SD). * x²; all other comparisons were by Student’s t test.

Results

**Demographics.** One hundred sixty children born to 125 mothers were evaluated. These included 29 mothers with 2 or more children enrolled, including two sets of dizygotic twins. The children were born between July 1986 and January 1996. Of the 160 children, 19 were classified as perinatally infected, while 141 were uninfected at birth. Of these, 20 subsequently HIV-1–seroconverted during follow-up, acquiring HIV from prolonged breast-feeding. The mean time of seroconversion of this group was 15.6 months (median, 13.6). Table 1 shows demographic, obstetric, and other characteristics of uninfected and perinatally infected children and their mothers. With the exception of children’s CD4 cell counts during the first 6 months of life, there were no statistically significant differences in the characteristics between uninfected and perinatally infected children.

**HLA concordance and HIV-1 transmission.** Table 2 shows class I HLA concordance between mother and children with respect to the three class I loci, HLA-A, -B, and -C. The data clearly indicate that increasing class I HLA concordance represents a risk factor for intrauterine, intrapartum, or early breast milk HIV-1 transmission (P < .008). While persistent blank alleles occurred in <3% of persons at the A and B loci, the HLA-C locus, as in all serologic surveys of HLA, showed a large proportion (30/160) of undefined typings, or “blanks,” due to the poor reactivity of sera against it. The poor seroreactivity of the C locus gene products may be in part a reflection of its lower baseline expression on the surface of most cells compared with that of the A and B locus products [25, 26]. Consequently, we also examined the concordance score at only the A and B locus in relation to the risk of children being infected perinatally. The results are shown in table 3. These data also show a significant (P < .02) association between concordance and risk of HIV-1 infection. The increased risk of perinatal infection associated with concordance occurs across the loci and is still significant even if the B locus is considered alone (A locus, P = .16; B locus, P = .03; C locus, P = .23).

Table 4 shows the relationship between HIV-1 seroconversion (associated with late breast milk transmission) and HLA concordance. No correlation was observed. Survival analysis using Cox regression (results not shown) also did not show any relationship between concordance and time of seroconversion. Inclusion of some early breast milk transmission in the HIV-1 perinatally infected group thus might dilute the association between concordance and intrapartum and intrapartum transmission and could thereby slightly bias the findings toward the null hypothesis.

**Logistic regression analysis.** The strong association between class I HLA concordance and HIV transmission was confirmed in logistic regression analysis. In logistic regression, the only two variables significantly associated with the risk of early HIV-1 infection were the HLA concordance

Table 2. Class I HLA concordance (A, B, and C loci) and risk of perinatal HIV-1 transmission.

| A, B, C locus match | HIV-1–uninfected at birth (n = 141) | HIV-1 perinatal infection (n = 19) | % transmission P  
|--------------------|------------------------------------|-----------------------------------|-----
| 3 of 6 alleles | 30 | 1 | 3.3
| 4 of 6 alleles | 65 | 7 | 9.7
| 5 of 6 alleles | 37 | 7 | 15.9
| 6 of 6 alleles | 9 | 4 | 30.8

NOTE. P = .008 (Mantel-Haenszel trend test, 2-tailed).

Table 3. Class I HLA concordance (A and B loci only) and risk of perinatal HIV-1 transmission.

| A, B locus match | HIV-1–uninfected at birth (n = 141) | HIV-1 perinatal infection (n = 19) | % transmission P  
|-----------------|------------------------------------|-----------------------------------|-----
| 2 of 4 alleles | 68 | 5 | 6.8
| 3 of 4 alleles | 56 | 8 | 12.5
| 4 of 4 alleles | 17 | 6 | 26.0

NOTE. P = .02 (Mantel-Haenszel trend test, 2-tailed).
Table 4. Class I HLA concordance (A, B, and C loci) and risk of late HIV-1 seroconversion from breast-feeding.

<table>
<thead>
<tr>
<th>A, B, and C loci concordance</th>
<th>Ultimately HIV-1–uninfected (n = 121)</th>
<th>Late HIV-1 seroconversion (n = 20)</th>
<th>% transmission</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 of 6 alleles</td>
<td>24</td>
<td>6</td>
<td>20.0</td>
</tr>
<tr>
<td>4 of 6 alleles</td>
<td>56</td>
<td>9</td>
<td>13.8</td>
</tr>
<tr>
<td>5 of 6 alleles</td>
<td>33</td>
<td>4</td>
<td>10.8</td>
</tr>
<tr>
<td>6 of 6 alleles</td>
<td>8</td>
<td>1</td>
<td>11.1</td>
</tr>
</tbody>
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NOTE. *P* = .31 (Mantel-Haenszel trend test, 2-tailed).

score and the determinant HLA-A2. Using the A, B, and C locus score, the odds ratio (OR) of perinatal HIV infection was 2.63 (95% confidence interval [CI], 1.36–5.07; *P* = .003) for each additional concordant class I allele, and the OR was 0.11 (95% CI, 0.02–0.55; *P* = .006) in children who had the HLA-A2 determinant. Using the A and B locus concordance score, the OR was 2.52 (95% CI, 1.25–5.07; *P* = .009) for each concordant allele and the OR was 0.123 (95% CI, 0.03–0.55; *P* = .008) for HLA-A2. Thus, each extra HLA determinant that a child has in common with its mother more than doubles the estimated risk (measured by OR) of transmission, and this occurs in a dose-effect relationship (figure 1).

Discussion

We examined whether class I HLA discordance was associated with protection from in utero, intrapartum, and early postpartum HIV-1 transmission, which we classified as perinatal infection. It was found that this HIV-1 transmission occurred more frequently in children with a greater degree of class I HLA identity to their mother. The relative protection associated with HLA discordance can best be explained by a protective allogeneic immune response. Exposure to HIV-1 in utero and during birth may involve free or, perhaps more likely, cell-associated virus. When challenged with HIV-1–infected maternal cells, the fetus or infant has the potential to respond to the non-self maternal HLA antigens. Subsequent anti-HLA antibody or cytotoxic T lymphocyte responses could mediate the elimination of HIV-infected maternal cells. Similarly, free HIV virion challenge could be neutralized by antibody responses directed against maternal HLA molecules incorporated into the viral envelope. It is unknown how immunologically reactive neonates are against maternal alloantigens, but fetal cord blood leukocytes have been shown to recognize foreign maternal MHC and mount strong allogeneic immune responses [27]. Although poorly studied overall, at least two instances of fetal anti-maternal HLA antibodies have also been documented [28]. This study was designed to consider the potential for allogeneic immune responses, and therefore it does not directly examine effector mechanisms. Multiple immune effector mechanisms may act synergistically. For example, allogstimulated CD8 lymphocytes have been shown to inhibit the replication of HIV-1 through a noncytolytic mechanism [29].

While alloimmune responses seem to be the most likely explanation for our findings, this may not be the sole mechanism. Under maternal class I HLA–restricted CTL pressure, HIV-1 variants may have been selected that are not well contained by HLA-concordant offspring. One would expect this to be observed predominantly in later-stage maternal disease, in which maximal cumulative immune pressure had been exerted. The majority of mothers in this study were early in disease, with a mean CD4 cell count of 603/mm³. Longitudinal studies of HIV-1 mutation in class I HLA–restricted epitopes during the course of maternal infection would be required to examine this. Thus, multiple complementary mechanisms may account for the protective role of mother-child MHC discordance demonstrated here. HLA discordance was not correlated with protection in the subgroup of perinatally uninfected children who subsequently became infected from breast-feeding (late seroconverters). The absence of a protective effect of HLA discordance with breast milk transmission suggests that the type of viral challenge may differ in breast milk transmission. Changes in the infant immune system as it matures or the waning of anti-maternal immune responses could reduce the protective effect of HLA discordance. It also may be that these responses do not afford protection from HIV-1 challenge to the gastrointestinal mucosa.

This study does not take into account the potential concordance or discordance of class II HLA determinants that may be equally important. Studies of the role of class II concordance are problematic because of the extreme molecular diversity at the DR locus, but class II concordance status may well be an important predictor of HIV-1 transmission given the known vigor of class II alloresponses and the high-level expression of DR on both activated lymphocytes and the HIV envelope [15, 30]. Further studies of the role of class II HLA discordance in mediating protection would be of interest.

Figure 1. Mother-child class I HLA concordance and perinatal HIV-1 transmission.
It is known that some HLA alleles in combination elicit a more vigorous alloimmune response. This has been described as the ‘‘taboo concept’’ with respect to organ transplantation [31]. The significance of this phenomenon outside the situation of transplantation is not known. In view of the limited size of our data set, no attempt was made to score specific allelic combinations with respect to immunogenicity. It should be noted that HLA determinants concordant at a serologic level may be discordant when molecularly subtyped, but neither the functional significance nor the likelihood of this happening is yet known (it depends on the number and frequency of molecular subtypes of a given serologic type).

In addition to HLA discordance, the presence of the class I determinant HLA-A2 was associated with a nearly 9-fold reduction in the estimated risk of early HIV-1 infection (as measured by OR). Although the association is significant on univariate analysis and logistic regression ($P = .006$, but not adjusted for multiple comparisons), it requires confirmation in further studies in this population. It is interesting to note that the HLA-A2 phenotype was independently protective only in the perinatally infected group. As HLA-A2 represents a group of distinct molecules at a molecular level (HLA-A*0201, *0202, *0205, *0214 in East Africa) [32], the functional implications of this finding will become clear only when molecular typing has been done.

This study included only children who survived to 1 year of age, so that fetal demise or infant mortality (i.e., death before age 1 year), both potentially related to HIV-1 infection, were not included. With the infant mortality rate of children born to HIV-1–infected mothers in this cohort being ~10%, both can be expected to be common. However, provided increased concordance does not reduce the risk of early mortality (and there is no reason to believe it would), this selection cannot be expected to account for our observations.

In addition to the significance within the field of HIV, these data may have more general significance. Adult women are infected with a wide variety of persistent intra-cellular organisms, and there appears to be significant exposure of the fetus to maternal cells. However, transmission of infection occurs relatively infrequently. It is possible that HLA discordance provides protection of fetuses and infants from a variety of intracellular pathogens. If so, this would contribute to the maintenance of HLA diversity in populations.

To the best of our knowledge, this study provides the first evidence that maternal-child MHC discordance is associated with protection from human HIV-1 infection. These data reinforce the considerable evidence in the SIV-macaque model, that anti-MHC immune responses can provide protection against viral challenge. Collectively, these findings support the potential to use an anti-HLA strategy in the development of an effective HIV-1 vaccine and underscore the need to develop a more complete understanding of the mechanisms mediating this HLA-related protective effect.

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References


10. Bakshi SS, Tetali S, Abrams EJ, Paul MO, Pawha SG. Repeatedly positive human immunodeficiency virus type 1 DNA polymerase chain reaction expected to account for our observations.


