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Major histocompatibility complex class II *DR* alleles *DRB1*1501* and those encoding HLA-DR13 are preferentially associated with a diminution in maternally transmitted human immunodeficiency virus 1 infection in different ethnic groups: Determination by an automated sequence-based typing method

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ABSTRACT **Transmission of human immunodeficiency virus 1 (HIV-1) from an infected women to her offspring during gestation and delivery was found to be influenced by the infant's major histocompatibility complex class II *DRB1* alleles. Forty-six HIV-infected infants and 63 seroreverting infants, born with passively acquired anti-HIV antibodies but not becoming detectably infected, were typed by an automated nucleotide-sequence-based technique that uses low-resolution PCR to select either the simpler *Taq* or the more demanding T7 sequencing chemistry. One or more *DR13* alleles, including *DRB1*1301, 1302*, and *1303*, were found in 31.7% of seroreverting infants and 15.2% of those becoming HIV-infected [OR (odds ratio) = 2.6 (95% confidence interval 1.0–6.8); $P = 0.048$]. This association was influenced by ethnicity, being seen more strongly among the 80 Black and Hispanic children [OR = 4.3 (1.2–16.4); $P = 0.023$], with the most pronounced effect among Black infants where 7 of 24 seroreverters inherited these alleles with none among 12 HIV-infected infants (Haldane OR = 12.3; $P = 0.037$). The previously recognized association of *DR13* alleles with some situations of long-term nonprogression of HIV suggests that similar mechanisms may regulate both the occurrence of infection and disease progression after infection. Upon examining for residual associations, only the *DR2* allele *DRB1*1501* was associated with seroreversion in Caucasoid infants (OR = 24; $P = 0.004$). Among Caucasoids the *DRB1*03011* allele was positively associated with the occurrence of HIV infection ($P = 0.03$).**

There is increasing interest in the extent to which genetically controlled host factors either influence the occurrence of human immunodeficiency virus 1 (HIV-1) infection or affect the rate at which the infection progresses. The vertical transmission of HIV-1 during gestation and delivery offers a special opportunity to examine the influence of host factors on the acquisition of infection, since fewer than one in four infants born of infected mothers become persistently infected (1). Efforts to describe why some children become infected and others do not have identified important maternal and delivery variables (2–5).

However, in addition, several observations suggest the possibility that nonmaternal factors are also involved. They include the sometimes random pattern of vertical HIV transmission to the children born of a multiparous HIV-infected woman and the presence of cell-mediated reactivity to HIV-1-encoded peptides in uninfected infants borne of HIV-1-infected mothers. This suggests that these infants may have been exposed to viral products, although whether this exposure has been to infectious virus with consequent elimination of the infection is unknown (6–12). Further to this point, a well-documented instance of the apparent clearing of neonatal HIV infection has been reported (13). Thus these observations suggest that the exposure of infant tissues to infectious virus may be a more prevalent occurrence than would be suggested by the current transmission rates but that some of these exposed infants, especially in situations of challenge with minimal amounts of virus, may effectively clear the agent. Were such a response to exist, one might expect to find genetic differences in the regulation of this trait contributing to the variability in vertical transmission rate.

The genetic polymorphisms of the major histocompatibility complex (MHC) genes that regulate the immune response are a logical site to examine for relevant genetic host factors. Accordingly, the association of particular HLA alleles is used as an index to gauge whether a particular immune response or disease trait may depend on a specific immune recognition event involving the trimolecular interaction of an MHC molecule, peptide, and T-cell receptor or alternatively to other linked genes in the MHC. The methods used in identification of the alleles of the MHC have progressively increased in precision and now reveal more than 100 alleles at the *DRB1* locus alone. Recently, the introduction of various methods to determine MHC class II alleles by direct nucleotide sequencing have added considerable additional precision to the process of HLA typing, compared to earlier and much more indirect techniques (14–18) that involved serologic or restriction fragment length polymorphism (RFLP) typing.

Of relevance is the growing body of information suggesting that the course of HIV-1 infection evolves differently in the presence of particular MHC alleles. This laboratory has been interested in the possible regulatory role of several structurally similar alleles of *DR11* (*DR5*) and *DR13* (*DR6*) because they have been associated with a clinically distinctive group of long-term slow progressors who develop an infiltrative CD8 T-cell lymphocytosis syndrome with features suggesting an antigen-driven response to HIV-1 (19–21). The immunologic environment created by these allelic products appears to influence the evolution of the viral infection (22). Interestingly, the presence of some of these same *DR5* and *DR13* alleles has also been correlated with an enhanced immune response to certain HIV-encoded peptides (23, 24). Reciprocally, either a more rapid decline in the levels of CD4 T cells or an earlier

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Abbreviations: MHC, major histocompatibility complex; HIV, human immunodeficiency virus; OR, odds ratio; RFLP, restriction fragment length polymorphism.

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appearance of frank immune deficiency or other AIDS-defining events have been associated with other HLA alleles including *HLA-DR3* (25–31). Thus, these observations suggest that variations in the transport of virally encoded peptides to (32) or presentation by (33) MHC molecules may importantly influence the host response to HIV infection. Accordingly, the present study was undertaken to specifically evaluate the hypothesis of whether the presence of specificities such as DR11 and DR13, which have been associated with differences in viral progression, might also act in infants exposed to maternal HIV-1 to decrease the occurrence of vertically transmitted infection.

METHODOLOGY

Patient Population. Children born to HIV-infected mothers were recruited from Bellevue Hospital as a case control study. The "seroreverter" children from a cohort (7) who have become HIV-antibody-negative and have no other evidence of HIV infection by repetitive viral culture and at least two PCR determinations were the source of all seroreverter samples in this study. Because the study was initiated in 1989, the seroreverter and infected children have been followed to the present, affirming their diagnostic classification.

HLA Typing. DNA was isolated from 2×10^6 lymphoblastoid cells that were initiated from the subjects by digesting the cells with proteinase K. The following primers were used in PCR amplification, with primer specificity and codon positions indicated in parentheses. All were designed according to the approach of Spurkland *et al.* (16) and they were prepared on a model 392 oligonucleotide synthesizer (Applied Biosystems). DRBAMP-1 (*DRB1*01*), 5′-TTCTTGTGGCAGCTTAAGTT-3′ (codons 7–13); DRBAMP-2 (*DRB1*15*, **16*), 5′-TTCCTGTGGCAGCCTAAGAGG-3′ (codons 7–13); DRBAMP-3 (*DRB1*03*, **08*, **11*, **12*, **13*, **14* (except **1410*), 5′-CACGTTTCTTGGAGTACTCTAC-3′ (codons 5–12); DRBAMP-4 (*DRB1*04*, **1410*), 5′-GTTTCTTGGAGCAGGTTAAAC-3′ (codons 6–13); DRBAMP-7 (*DRB1*07*), 5′-TTCCTGTGGCAGGGTAAGTATA-3′ (codons 7–14); DRBAMP-9 (*DRB1*09*), 5′-GTTTCTTGAAGCAGGATAAGTTT-3′ (codons 6–13); DRBAMP-10 (*DRB1*10*), 5′-GGAGGAGGTTAAGTTTGAGTGTC-3′ (codons 8–16); DRBAMP-3, 5′-CACGTTTCTTGGAGTACTCTACG-3′ (codons 5–12); DRBAMP-B (*DRB1* generic), Bio-5′-CTCGCCGCTGCACTGTGAAG-3′ (codons 8–15). All primers contain the M13 sequence TGTAAAACGACGGCCAGT 5′ to the DRBAMP sequence. This design is for subsequent use of the M13 dye-primer to sequence the PCR products by using T7 chemistry. DRBAMP-B is a 3′-biotinylated primer to isolate single-strand DNA with avidin-magnetic beads.

For *DRB1* typing, seven standard PCRs were set up for each sample with each of the allele-group-specific primers, and the 3′-biotinylated primer. The standard PCR was performed by using 20 pmol of the 3′-biotin-tagged primer and 20 pmol of the 5′ primer in 1.5 mM $MgCl_2$ with 100 ng of DNA template. Thermal cycling consisted of 8 cycles at 98°C for 5 sec, 62°C for 20 sec, and 72°C at 60 sec, followed by 32 cycles at 96°C for 10 sec, 57°C for 20 sec, and 72°C for 1 min, in a GeneAmp PCR system 9600 thermal cycler (Perkin–Elmer). Five microliters of the PCR product of each reaction was electrophoresed on an agarose gel to determine whether the reaction produced a product for sequencing. In the event that this low-resolution typing yielded a positive result with two primer combinations, the sequencing component of the typing was performed by using the simpler and less expensive *Taq* chemistry. In the alternative event that no product was encountered in the preliminary low-resolution typing, suggesting the possible presence of two alleles in a group, Sequenase-based T7 sequencing chemistry was used. The PCR with DRBAMP-3 uses the higher-stringency thermal cycling parameters of six cycles at 98°C for 5 sec, 68°C for 20 sec, and 72°C for 60 sec, followed by 32 cycles at 96°C for 20 sec, 67°C for 30 sec, and 72°C for 40 sec. The DRBAMP-4 reaction is similar to DRBAMP-3 with the exception that the second stage annealing temperature is 65°C instead of 72°C. The single-stranded PCR product of all of these reactions served as the template and was purified on streptavidin-coupled Dynal M280 magnetic beads. In the cases where a product is obtained, single-strand DNA is isolated by using purification of the biotin-tagged sense strand with 200 μg of streptavidin beads and the Applied Biosystems protocol for magnetic bead purification of PCR products. The supernatant containing the antisense strand is discarded.

For automated fluorescent sequencing of the *DRB1* exon 2 gene, the 5′ primers specific for different *DRB1* allele families, and one generic 3′-biotinylated primer are used with dideoxynucleotide-terminator (DyeDeoxy; Perkin–Elmer) *Taq* cycle sequence or dye-primer Sequenase (T7) sequence methods to directly sequence PCR products purified by Dynal magnetic beads. In the case of *Taq* dye terminator chemistry, 9.5 μl of prepared sequencing reagents containing *Taq* polymerase is added and the single-strand template attached to the beads is sequenced according to the Applied Biosystems DyeDeoxy product literature for dye termination methods with 3.2 pM of the locally prepared appropriate 5′ sequencing primer. The sequencing primer DRB1-19 (5′-GAGTGTCATTTCTTCAA-3′) is used to sequence *DRB1*04*, *DRB1*07*, *DRB1*09*, and *DRB1*10* templates, and DRB1-seq (5′-ATGGGACGGAGCGGGTGCGGT-3′) is used for all other alleles. In the case of the Sequenase-based reactions, the four sequencing reactions are set up according to the product literature for the Prism Sequenase sequencing kit with the −21 M13 dye primer, but using twice the recommended amounts of enzyme and primer (Perkin–Elmer). The beads are applied to a sequencing gel of a model 373A fluorescent sequencer (Perkin–Elmer). In instances of possible ambiguity in the reactions the sequencing reactions were performed in the reverse direction. *DPB1* locus typing was performed as described (17, 18) by using the Sequenase chemistry and reagents as the previously described method with primers specific for *DPB1* locus alleles provided in a kit from the Applied Biosystems Division of Perkin–Elmer.

The sequences obtained with these procedures were edited and the bases were recalled by using IUB nomenclature in the SEQUENCE NAVIGATOR program and a specialized program based on the FACTURA batch analysis program, provided by the Applied Biosystems Division of Perkin–Elmer. The HLA allele-calling software module of this program was used to assign alleles. Statistical analyses were performed by using SPSS for Windows (SPSS, Chicago). The tables contain uncorrected *P* values. A Bonferroni-type correction for multiple comparisons based on the appropriate test situation such as the 10 principal DR specificities was used when not testing the *a priori* hypothesis concerning the role of *DR5* (*DR11* and *DR12*) and *DR6* (*DR13* and *DR14*) or that of the *DR2* and *DR3* alleles.

RESULTS

Thirty-eight alleles of the *DRB1* locus were identified with the direct sequence-based typing method in 45 HIV-infected infants and 63 seroreverting infants. Grouping of all of the alleles encoding the DR13 serologic specificity revealed that the frequency of this virtual allele, or allele group, was significantly increased in the HIV-negative or seroreverter infants, being 15.2% in those who became HIV-infected and 31.7% in the seroreverters [$P = 0.048$; odds ratio (OR) = 2.6 (95% confidence interval 1.0–6.8); Table 1]. The intensity of this association varied with ethnicity. Among Black or Hispanic infants, 10.3% of infected infants and 33.3% of uninfected infants expressed these alleles ($P = 0.022$) with the

Table 1. Frequency of the group of alleles encoding DR13 is significantly increased among Black or Hispanic HIV-negative seroreverting infants born of HIV-infected mothers

	HIV infection of infant						
	Present		Absent				95% confidence
Ethnicity	% +	*n*	% +	*n*	*P*	OR	limit
Black or Hispanic	10.3	29	33.3	51	0.023	4.3	1.2–16.4
Black	0.0	12	30.4	24	0.037	12.1*	—
Hispanic	17.6	17	37.0	27	0.170	2.8	0.6–12
Caucasian	23.5	17	25.0	12	0.927	1.1	0.2–6.1
All	15.2	46	31.7	63	0.048	2.6	1.0–6.8

All OR values are Woolf ORs except that marked with an asterisk, which is a Haldane OR.

effect greatest among Black infants where 7 of 24 seroreverters inherited these alleles and none of 12 HIV-1-infected Black infants had any of the *DRB1*13* alleles (Haldane OR = 12.3; $P = 0.037$). Conversely, among Caucasoids the association with the *DRB1*13* alleles was not evident. Among the Black and Hispanic subjects 5 of the 6 *DRB1*13* alleles identified in this study were increased in frequency among the seroreverters and contributed to the association, with the *DRB1*1301* allele the most heavily represented (Table 2).

A search for secondary associations was made by eliminating all *DRB1*13* individuals from HIV-positive and seroreverter categories and repeating the analysis on this sample subset. Table 3 illustrates that there is a trend for overrepresentation of the group of *DR2* alleles that was significant among Caucasoids [OR = 11.0 (1.4–85.2); $P = 0.014$]. The association with the DR2 virtual allele was entirely driven by the *DRB1*1501* allele in the entire sample and the Caucasoid subset. The *DRB1*1501* allele was present in 6 of 9 seroreverters and 1 of 13 HIV-positive children [OR = 24 (2.0–287.7); $P = 0.0035$]. *DRB1*1503* and *DRB1*1601* had no trend toward a similar association. Interestingly, within the *HLA-DR3* group of alleles, only *DRB1*13011* exhibited a sharply different association in the Black and Caucasoid ethnic groups, being significantly associated with progression to HIV positivity in the latter ($P = 0.034$) and with seroreversion in the former ($P = 0.021$).

Testing of the study population for *DPB1* locus alleles was less complete, due to limited availability of specialized sequencing reagents. No statistically significant associations were identified in this incomplete study. While the action of other genes in linkage disequilibrium with these *DR* alleles remains a possible explanation, none of the *HLA-DR13* alleles could be uniformly associated with any alleles of the *DPB1* locus determined by the same method, suggesting that the correlation of *DR13* alleles with seroreversion was not the property of an extended haplotype that included alleles of the *DPB1* locus.

Table 2. Frequencies of alleles of *DR6* (*DR13* and *DR14*) in Black and Hispanic infants

Allele	HIV-positive, % ($n = 29$)	Seroreverter, % ($n = 51$)
*DRB1*13011*	6.9	17.6
*DRB1*13012*	0.0	2.0
*DRB1*1302*	0.0	5.9
*DRB1*1304*	0.0	3.9
*DRB1*1305*	3.4	2.0
*DRB1*1401*	3.4	0.0
*DRB1*1412*	0.0	2.0

DISCUSSION

The central observation in this study suggests that vertical transmission of HIV-1 to infants during pregnancy is significantly influenced by the infant's particular *HLA-DRB* alleles, emphasizing the need for additional studies of this phenomenon. Ethnicity appeared to be a very important variable for interpreting the effect of *HLA* alleles. Among Black and Hispanic infants, a protective influence of certain *HLA* alleles is expressed as relative resistance to infection associated with the presence of the *DRB1*13* alleles as a group. Among Caucasoids a similar protective effect is observed with the *DRB1*1501* allele, but not with other alleles encoding the DR2 serologic specificity. Presumably, the contrasting protective effect of these alleles occurs either through the action of what could be a common linked gene present on haplotypes that exhibit a differential ethnic distribution or by epistatic interaction between the particular *DRB1* allelic product and one or more non-MHC polymorphic genes differentially distributed in the two ethnic groups. The study design does not permit distinguishing between these two alternatives. The association with *HLA-DR13* alleles is of special interest because the presence of these alleles correlates with slower progression to opportunistic infection (20) and with a heightened response to HIV-encoded peptides (23), suggesting the possibility that there may be parallels between certain mechanisms of immunologic resistance to infection and those involved in a beneficial response to infection.

A point emerging from these studies is the utility of direct sequence-based typing of *DRB1* alleles, particularly in situations where the subjects are drawn from less completely

Table 3. Frequencies of selected alleles and allelic groups in HIV-positive and seroreverter outcomes of various ethnicities after exclusion of all children with *DR13* alleles

	HIV-positive		Seroreverter					
Allele	%	*n*	%	*n*	*P*	OR	CI	Eth
DR2	38.5	39	44.2	43	0.60	1.3	0.7–53.7	All
DR2	50.0	26	38.2	34	0.36	0.6	0.2–1.7	B/H
DR2	15.4	13	66.7	9	0.014	11.0	1.4–85.2	C
*DRB1*1501*	15.4	39	23.4	43	0.37	1.7	0.5–3.1	All
*DRB1*1501*	19.2	26	11.8	34	0.42	0.56	0.1–2.3	B/H
*DRB1*1501*	7.7	13	66.7	9	0.004	24.0	2.0–282.7	C
*DRB1*11011*	2.6	39	11.6	43	0.12	5.0	0.6–44.8	All
*DRB1*1102*	15.4	39	11.6	43	0.62	0.7	0.2–2.6	All
*DRB1*03011*	17.9	39	18.6	43	0.94	1.1	0.3–3.2	All
*DRB1*03011 1*	7.7	26	23.5	34	0.10	3.7	0.7–19.1	B/H
*DRB1*03011*	38.5	13	0	9	0.034	12.3*	—	C
*DRB1*03011*	0	12	35.3	17	0.021	0.07*	—	B

Eth, ethnicity; B, Black; H, Hispanic; C, Caucasoid; CI, 95% confidence interval. The Woolf OR is shown except for samples marked with an asterisk for which the Haldane OR is shown. All children with *DRB1*13* alleles were excluded.

studied population groups. In the present approach to sequence-based typing, the use of *Taq* is a less expensive alternative, from the technical aspect. But, in situations in which the amplified product came from two alleles, *Taq* chemistry proved incapable of discriminating two different templates in the same reaction, necessitating the use of Sequenase. After the completion of these studies, we were provided a preprint of a manuscript by McGinnis *et al.* (34) describing a similar method for identification of *DRB1* alleles, also based on the approach of Spurkland *et al.* (16), but using T7 (Sequenase)-based sequencing in all situations. The consistent use of T7-based chemistry offers a definite advantage in precision of results and simplicity of design but involves somewhat more technical effort. The work of McGinnis *et al.* (34) and that of the present paper clearly emphasize the feasibility and utility of automated-sequence-based typing.

The analytic approach of grouping alleles was used in the present study because power is lost if there are parallel associations with related alleles when each allele is tested separately. The problem of fractionation of specificities into multiple single alleles was addressed by formation of virtual analytic alleles such as the *DR13* group used in the present study, the occurrence of which was inferred from the presence of any *DR13* allele. The biologic rationale for construction of this virtual allele is based on the presence of considerable shared molecular structure encoded by each of these alleles and the fact that other methods such as serologic determination should optimally define the same virtual allele. Grouping alleles into virtual alleles does not decrease power of the analysis unless it is used without *a priori* structural rationale. Alternatively, all individuals with the allele of the primary association can be removed to examine the residue or "tail" of the data set for secondary associations, reducing power because the sample size is decreased.

Although considerable caution is required in the interpretation of studies with relatively small numbers of subjects such as this, because of the possibility of sampling errors, the present findings extend the reports of three laboratories that have studied the influence of HLA on maternal–infant transmission (35–37). Kilpatrick *et al.* (35), in a study of Scottish Caucasoids with serologic typing, observed that *HLA-DR2* was significantly increased among seroreverter infants, providing the hypothesis for this study. The present observations suggest that the *DR2* allele *DRB1*1501* or a gene on its haplotype is responsible for the *DR2* association. Kilpatrick *et al.* (35) also found that *HLA-DR3* was increased in frequency among HIV-infected children. In the present study only the *DR3* allele *DRB1*13011* occurred significantly more frequently among the subset of Caucasoid infants who became infected. The association of susceptibility with a *DR3* allele is consistent with other reports concerning other circumstances of HIV-1 transmission (26, 28, 29, 32, 38, 39). In uninfected subjects, *DR3* has been associated with abnormalities in *in vitro* cytokine production, possibly affecting TH1 T-cell responses, and natural killer activity (40) and exhibit deficient responses to HIV-encoded peptides (23).

The study of Greggio *et al.* (36) from Italy on the role of HLA on maternal–infant transmission in infants of undefined ethnicity was performed with the RFLP method, which indirectly identifies certain *DR* alleles (41–44). These workers reported a DR polymorphism, DRB1-13a4, associated with the *DRB1*1301* allele, to be significantly increased among seroreverters. The present data are in agreement with their data, if it is postulated that the Mediterranean subset of the Caucasoid population exhibits the well-recognized presence of some genes of African origin (45) or that African children were included. Determination of DR polymorphisms with *Taq* results in a great many bands and the results of this enzyme–probe combination are considered difficult to interpret even using homozygous cell lines (43).

The report by Just *et al.* (37) studied vertical transmission in Black and Hispanic infants with the same RFLP methods used by Greggio *et al.* (36). These investigators did not observe any association between infant susceptibility and resistance to infection and DR polymorphisms. In support of the possibility that inherent limitations of RFLP analysis account for the apparent difference in results are the other observations by these authors that *DQA1*0102* and *DQA1*0103* are associated with seroreversión status since these alleles are reported to be in linkage with the *DRB1*1301* and **1302* alleles (46, 47).

The present research and these previous reports (35–37) underscore the importance of performing an independent larger study of the role of host MHC alleles on susceptibility to infection. Because maternal immunogenetics may likely influence the evolution of HIV infection and, correspondingly, influence the likelihood of its vertical transmission, this will also be an important variable to explore. The results of the present study are not likely attributable to proxy typing of the mother for a maternal genetic effect, since examination of nine mothers of seroreverting infants with *DRB1*13* alleles revealed them to be paternally derived in four instances. The mechanism of action of candidate MHC genes in the infant can only be speculated upon. If classic MHC molecules were to be identified as the responsible structures (20), it would suggest the operation of immune recognition events in the fetus or neonate to HIV-1, possibly similar to those immune responses evident at birth that are initiated by infection of the fetus with herpes viruses and the agents responsible for toxoplasmosis, rubella, and syphilis. Alternatively, the effect might map to other genes within the MHC that encode molecules operating in entirely different ways, perhaps even within the placenta rather than the infant, to influence the outcome of an encounter with HIV-1.

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