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Maternal HLA Class II Compatibility in Men with Systemic Lupus Erythematosus

Anne M. Stevens[1], **Betty P. Tsao**[2], **Bevra H. Hahn**[2], **Katherine Guthrie**[3], **Nathalie C. Lambert**[3], **Allison J. Porter**[3], **Tracy S. Tylee**[3], **J. Lee Nelson**[1]

[1]Fred Hutchinson Cancer Research Center and University of Washington, Seattle

[2]David Geffen School of Medicine, University of California, Los Angeles

[3]Fred Hutchinson Cancer Research Center, Seattle, Washington.

Abstract

Objective—Maternal–fetal cell transfer during pregnancy can lead to long-lasting microchimerism, which raises the question of whether microchimerism sometimes contributes to autoimmune disease later in life. In an experimental model, transfusion of parental lymphocytes homozygous for major histocompatibility complex alleles results in systemic lupus erythematosus (SLE). We identified male patients with SLE and healthy male subjects and their mothers in order to investigate the mother–son HLA relationship in SLE risk. Male subjects were selected in order to avoid confounding due to fetal microchimerism, which may occur in women.

Methods—HLA genotyping for DRB1, DQA1, and DQB1 was conducted for sons and their mothers. Thirty men with SLE and their mothers were compared with 76 healthy men and their mothers.

Results—Sons with SLE were HLA-identical with their mothers (bidirectionally compatible) for the basic HLA–DRB1 groups encoded by DRB1*01 through DRB1*14 more often than were healthy sons (odds ratio [OR] 5.0, $P = 0.006$). Each DRB1 group contains multiple allelic variants; male patients with SLE and their mothers often were identical for both DRB1 allelic variants (OR 3.2, $P = 0.08$). For DQA1 and DQB1, the ORs were 2.3 ($P = 0.08$) and 2.0 ($P = 0.21$), respectively. When analysis was limited to male subjects with SLE-associated HLA genes (encoding HLA–DR2 or HLA–DR3), the differences further increased for DRB1 basic groups (OR 7.2, $P = 0.01$), DRB1 alleles (OR 15.0, $P = 0.018$), DQA1 6.4 ($P = 0.006$), and DQB1 (OR 5.7, $P = 0.027$). No increase in (unidirectional) compatibility of the mother from the son's perspective was observed at any locus.

Conclusion—We observed increased bidirectional HLA class II compatibility of male SLE patients and their mothers compared with healthy men and their mothers. This observation implies that maternal microchimerism could sometimes be involved in SLE and therefore merits further investigation.

Address correspondence and reprint requests to Anne M. Stevens, MD, PhD, Children's Hospital and Regional Medical Center, 307 Westlake Avenue N, Suite 300, Seattle, WA 98109; anne.stevens@seattlechildrens.org.

Naturally acquired microchimerism from pregnancy has been implicated in some autoimmune diseases (1–3). In an experimental mouse model, the transfusion of homozygous parental lymphocytes into heterozygous progeny resulted in disease that resembled human systemic lupus erythematosus (SLE) (4). Thus, cells from the mother are potentially capable of inducing experimental SLE in her progeny in vivo. To our knowledge, no studies have investigated maternal microchimerism in human SLE. In limited studies of fetal microchimerism, male DNA (presumed to be from prior pregnancies with male fetuses) was observed in women with SLE but also in controls, with a trend toward increased microchimerism in SLE patients, especially those with nephritis (for review, see ref. 3). According to one case report, male cells were identified in organs obtained from a female SLE patient at autopsy (5).

Because maternal and fetal microchimerism occurs in healthy individuals as well as in patients with autoimmune disease (1–3,6), additional environmental and genetic factors are likely important if naturally acquired microchimerism contributes to autoimmune diseases such as SLE. Major histocompatibility complex (MHC) molecules are important in chronic graft-versus-host disease (GVHD), autoimmune diseases, and in the mouse parental-to-progeny transfusion GVHD/SLE model. MHC genes could contribute to disease risk in more than one way. First, specific MHC alleles are known to increase susceptibility to autoimmune diseases. For example, HLA–DRB1*1501, DRB1*0301, and DRB1*0801 and the DQ genes that are in linkage disequilibrium (DQA1*0102-DQB1*0602, DQA1*0501–DQB1*0201, and DQA1*0401-DQB1*0402) are associated with an increased risk of SLE (7,8). In most studies, however, fewer than 50% of patients have HLA alleles associated with SLE, suggesting that additional factors predispose to SLE. The HLA relationship of naturally acquired microchimerism from pregnancy to the host may represent another risk factor. In the mouse GVHD/SLE model, development of immune complex-mediated nephritis is dependent on class II MHC genes carried by the parent and the parent-F_1 MHC relationship (4). The aim of the present study was to test the hypothesis that maternal HLA class II compatibility is a risk factor for subsequent SLE in her progeny.

PATIENTS AND METHODS

Study population

The study protocols were approved by the Human Subjects Committees at the Fred Hutchinson Cancer Research Center (University of Washington Consortium) and the University of California, Los Angeles (UCLA). Informed consent was obtained from all study subjects. Male probands and their mothers were studied. The study was limited to men, because females are subject to fetal microchimerism that could be confounding. Subjects who were twins were also excluded, because microchimerism can derive from a twin. Healthy control male subjects and their mothers were recruited as part of other ongoing studies based in King County, Washington, by response to posters, flyers, advertisements, and referral from friends. The majority of male SLE patients were identified as part of previously described studies of the genetics of SLE in multiplex families conducted at UCLA (9). All SLE patients satisfied the American College of Rheumatology criteria for a diagnosis of SLE (10). In healthy families with more than 1 male child, 1 male

child per family was randomly chosen for analysis. One SLE family had 2 affected males, and only 1 male child from this family was included in the analysis. For male SLE patients, the age range was 5–50 years (median 20 years), and the age range for healthy male subjects was 5–53 years (median 17 years) (*P* not significant). Ethnicity was determined by 2 methods. For SLE patients, each subject reported the ethnicity of all 4 of his grandparents. For control subjects, each subject reported his own ethnicity. Differences in ethnicity between groups were not statistically significant. The large majority of male SLE patients and healthy male subjects were Caucasian American (25 [83%] and 71 [93%], respectively); 1 patient (3%) and 2 healthy subjects (3%) were African American, and 4 patients (13%) and 3 healthy subjects (4%) were Asian American.

DNA extraction

DNA was extracted from whole blood or peripheral blood cells, using the Wizard DNA extraction kit according to the manufacturer's instructions (Promega, Madison, WI). For some study subjects, DNA was extracted from hair roots or from mouthwash specimens, using the High Pure PCR Template Preparation Kit standard protocol (Roche Diagnostics, Indianapolis, IN).

HLA genotyping

DNA-based HLA typing was conducted to determine DRB1, DQA1, and DQB1 alleles, as previously described (6). For DRB1, the initial assay detects the basic DRB1 groups (also called HLA–DR families), DRB1*01 through DRB1*14. This was followed by 1 or more high-resolution assays or direct sequencing for specific DRB1 alleles within the basic DRB1 groups. Alternatively, some initial typing of DRB1 and DQB1 was done using Dynal RELI SSO HLA–DRB and DQB typing kits (Dynal, Oslo, Norway). HLA typing methods did not discriminate DQB1*0401 from DQB1*0402 or DQB1*0201 from DQB1*0202; therefore, these alleles were designated as DQB1*04 and DQB1*02, respectively. DQA1 alleles were determined by sequence-specific oliognucleotide probe typing, using methods similar to those previously described, to which other probes were added to detect newly identified alleles of DQA1. For some SLE patients and controls, DQA1 alleles were inferred based on allele-level typing results for both DRB1 and DQB1.

Statistical analysis

The hypothesis tested was that HLA class II compatibility of the mother is associated with increased risk of SLE in her progeny. The possible mother–son HLA relationships are shown in Table 1. HLA relationships that result in compatibility include compatibility from the mother's perspective only, compatibility from the proband's perspective only, and bidirectional compatibility attributable to heterozygous or homozygous HLA identity.

The main predictor of interest was disease status (SLE versus no SLE), and the potential confounders considered were age and ethnicity. Each compatibility outcome was treated as a binary variable, in a logistic regression model. The models were used to estimate unadjusted and adjusted odds ratios (ORs) to describe the association of each compatibility measure with disease. *P* values from regression models were two-sided and were calculated using the

Wald test. No adjustments were made for multiple comparisons. SAS software (Cary, NC) was used for all analyses.

RESULTS

The bidirectional HLA compatibility of mothers and sons was greater in pairs of male SLE patients and their mothers than in pairs of healthy men and their mothers. The increase was most evident for bidirectional compatibility at the DRB1 locus (Table 2). HLA compatibility for the basic HLA–DR groups encoded by DRB1*01 through DRB1*14 was significantly greater among male SLE patients and their mothers than among healthy men and their mothers (OR 5.0 [$P = 0.006$]). The basic DRB1 groups have shared sequences that define groups of HLA–DR molecules, previously detected by using antibodies as reagents and corresponding to DR1 through DR14. However, each HLA–DR group (often referred to as HLA–DR families and designated as $DRB1_1$ in Tables 2 and 3) contains members that differ elsewhere on the DRβ1 chain, and >300 allelic variants of DRB1 are currently recognized (designated as $DRB1_2$ in Tables 2 and 3).

Male SLE patients and their mothers were more often identical for both DRB1 allelic variants, although the difference was not significant (OR 3.2 [$P = 0.08$]). Increases in DQA1 and DQB1 compatibility were also observed but did not reach statistical significance (OR 2.3 [$P = 0.08$] and OR 2.0 [$P = 0.21$], respectively). Adjusting for age and ethnicity did not significantly change the associations of HLA compatibility with SLE. Homozygosity (resulting in unidirectional compatibility from the son's perspective) was not more common in mothers of SLE patients than in mothers of healthy male subjects (Table 2, mother from son's perspective). We also examined compatibility of the son from the mother's perspective (unidirectional compatibility from the mother's perspective) and observed that the rates of compatibility were similar in SLE patients and controls (Table 2, son from mother's perspective).

Because specific HLA class II genes are known to be increased among SLE patients, we next addressed the potential explanation that increased mother–son HLA compatibility occurs because the HLA genes associated with SLE are common in the population, thus resulting in a greater likelihood of the mother's nontransmitted HLA gene being shared. The most consistent HLA class II associations with SLE are for genes encoding HLA–DR3 (DRB1*03) and HLA–DR2 (DRB1*15/16) (7,8). We therefore conducted analyses restricted to mother–son pairs in which the son carried an HLA gene encoding DR3 or DR2.

Rather than diminishing, the differences in compatibility at DRB1 between male patients with SLE and their mothers versus healthy sons and their mothers increased (Table 3). Moreover, compatibility at DQA1 and DQB1 was significantly increased. The OR for bidirectional compatibility for the basic HLA–DR groups was 7.2 ($P = 0.011$), and for DRB1 alleles the OR was 15.0 ($P = 0.018$). For DQA1 and DQB1, the ORs were 6.4 ($P = 0.006$) and 5.7 ($P = 0.027$), respectively. Results were also significant when analyses were conducted after adjusting for age (for DRB1 basic groups, OR 7.2 [$P = 0.01$]), DRB1 alleles (OR 14.6 [$P = 0.02$]), DQA1 (OR 5.8 [$P = 0.01$]), and DQB1 (OR 5.8 [$P = 0.03$]). Analyses restricted to white subjects only remained significant for DRB1 basic groups (OR 5.5 [$P =$

0.04]), DRB1 alleles (OR 12.7 [$P = 0.03$]), and DQA1 (OR 5.4 [$P = 0.02$]) but not for DQB1 (OR 4.0 [$P = 0.10$]). In 4 families, the mother also had a diagnosis of SLE, and in a fifth family the mother had a possible diagnosis of SLE; the HLA compatibility rates in these families were similar to the overall rates (data not shown).

DISCUSSION

The fact that naturally acquired microchimerism from pregnancy persists decades after birth raises the question of whether microchimerism sometimes contributes to autoimmune disease later in life (1–3,6). Because MHC molecules play a central role in complications of iatrogenic chimerism such as that observed in the setting of GVHD and transplant rejection, we hypothesized that HLA class II compatibility of the mother affects the risk of SLE in her progeny. We observed a significant increase in bidirectional HLA class II compatibility (identical HLA alleles) in mother–son pairs in which the son had SLE compared with pairs in which the son was healthy.

In experimental studies, SLE occurs after infusion of parental lymphocytes that are homozygous for MHC alleles (4). Donor homozygosity is also implicated in cases of transfusion-associated GVHD involving familial donors (11). In these situations, HLA compatibility is unidirectional. We observed an increased risk of SLE associated with mother–son HLA class II compatibility. However, risk was associated with bidirectional compatibility, with no apparent increase in maternal homozygosity; this finding is in contrast with results of experimental studies. Our additional finding that the risk was further increased in male subjects with an SLE-associated HLA allele is, however, analogous to the experimental model in which both the MHC relationship and specific MHC alleles are required for disease (4).

Our study has several limitations. First, SLE is uncommon in boys and men, and the number of male subject–mother pairs in our study was modest. Second, the majority of our study subjects derived from recruitment of families in which more than 1 family member was affected, so that our observations may be influenced by factors contributing to familial SLE. Third, the large majority of our population was Caucasian American, whereas the prevalence of SLE is greater in African Americans and possibly in Asian Americans. However, considering that the HLA associations with SLE have been strongest in Caucasian American populations (7,8), this is a useful population in which to begin to investigate HLA compatibility in SLE. Fourth, although not significantly different, the ethnic composition of our patients and controls differed somewhat and, even within Caucasian American populations HLA allele frequencies differ, which could affect results. Given the limitations of our investigation, it will be important for larger studies to be conducted to examine simplex families as well as non–Caucasian American ethnic/ racial groups. Studies focused on children and female subjects will also be interest.

How maternal–child HLA compatibility might contribute to SLE is unknown. One possibility is that such compatibility occurs through maternal microchimerism, but an alternative theory is that maternal HLA molecules could affect a child’s immune system in utero by influencing T cell development. Whether HLA compatibility affects persistence or

levels of maternal microchimerism later in life is not known, although results of a small study suggested an increase in maternal microchimerism in the context of HLA class II compatibility (12). As suggested in studies of dermatomyositis (13), one consideration is that maternal microchimerism might affect a mother's progeny through anti-fetal alloreactive T cell responses. Female cells (presumed to be maternal) have been described in the tissues of male neonates (14), and an increase in the number of female cells has been reported in the muscle tissues of children with dermatomyositis (15) or idiopathic myositis (16). We recently identified maternal (female) cardiac myocytes in the heart muscle and atrioventricular node of male infants who died of heart block associated with neonatal lupus syndrome (17), suggesting the additional possibility that maternal cells could potentially be tissue targets of immune response (or, alternatively, could be involved in tissue repair). Whether maternal microchimerism is involved in SLE, contributing to blood or tissues, is unknown and will be an area of additional interest for further investigation.

In conclusion, we examined HLA genes in mother–son pairs in which the son had SLE or was healthy, in order to test the hypothesis that the mother–son HLA relationship affects the son's risk for the development of SLE. We observed a significant increase in bidirectional mother–son HLA class II compatibility associated with SLE. The increase in mother–son HLA compatibility was most marked at the DRB1 locus and in patients with SLE-associated DRB1 alleles. Further studies of larger numbers of male SLE patients and studies in differing ethnic/racial groups are needed. Because this observation potentially implicates maternal cells in SLE, studies examining the specificity, reactivity, and phenotype of maternal microchimeric cells will also be of interest for exploring whether maternal–child immune system interactions might lead to chronic inflammatory disease.

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Table 1.

Possible HLA relationships between a mother and her child

Relationship	Mother	Child	Compatibility
1	A, B	A, C	Incompatible
2A	A, A	A, A	Bidirectionally compatible (homozygous, identical)
2B	A, A	A, B	Bidirectionally compatible (heterozygous, identical)
3	A, A	A, B	Mother compatible from son's perspective only (mother homozygous)
4	A, B	A, A	Son compatible from mother's perspective only (child homozygous)

Table 2.

HLA compatibility of SLE son–mother pairs compared with healthy son–mother pairs *

Locus/compatibility	Patients	Controls	OR (95% CI)	*P*
DRB1$_1$ †				
Bidirectional	9 (30)	6 (8)	5.0 (1.6–15.7)	0.006
Mother from son's perspective	4 (13)	11 (14)	0.9 (0.3–3.1)	0.88
Son from mother's perspective	3 (10)	9 (12)	0.8 (0.2–3.3)	0.79
DRB1$_2$ ‡				
Bidirectional	5 (19)	5 (7)	3.2 (0.9–12.2)	0.08
Mother from son's perspective	4 (15)	7 (9)	1.7 (0.5–6.4)	0.42
Son from mother's perspective	2 (7)	4 (5)	1.4 (0.2–8.3)	0.68
DQA1				
Bidirectional	11 (38)	16 (21)	2.3 (0.9–5.8)	0.081
Mother from son's perspective	4 (14)	7 (9)	1.6 (0.4–5.9)	0.50
Son from mother's perspective	3 (10)	13 (17)	0.6 (0.1–2.1)	0.39
DQB1				
Bidirectional	7 (27)	12 (16)	2.0 (0.7–5.7)	0.21
Mother from son's perspective	3 (12)	5 (7)	1.9 (0.4–8.4)	0.42
Son from mother's perspective	2 (8)	10 (13)	0.6 (0.1–2.7)	0.46

* Values are the number (%) of subjects. The analyses were not adjusted for age or ethnicity. For DRB1$_1$, n = 30 patients and 76 controls; for DRB1$_2$, n = 27 patients and 75 controls; for DQA1, n = 29 patients and 76 controls; for DQB1, n = 26 patients and 76 controls. See Table 1 for descriptions of compatibility. SLE = systemic lupus erythematosus; OR = odds ratio; 95% CI = 95% confidence interval.

† Basic DRB1 groups DRB1*01 through DRB1*14 sharing sequence similarities but not necessarily identical throughout the entire DRβ1 chain.

‡ Specific alleles within DRB1 groups.

Table 3.

HLA compatibility of mother–son pairs when the son (SLE or healthy) carries genes encoding HLA molecules associated with SLE risk (HLA–DR2 or DR3)*

Locus/compatibility	Patients	Controls	OR (95% CI)	*P*
$DRB1_1$†				
Bidirectional	7 (39)	3 (8)	7.2 (1.6–32.8)	0.011
Mother from son's perspective	2 (11)	5 (14)	0.8 (0.1–4.6)	0.80
Son from mother's perspective	1 (6)	3 (8)	0.7 (0.1–6.9)	0.73
$DRB1_2$‡				
Bidirectional	5 (29)	1 (3)	15.0 (1.6–142)	0.018
Mother from son's perspective	2 (12)	3 (8)	1.5 (0.2–10)	0.67
Son from mother's perspective	1 (6)	3 (8)	0.7 (0.1–7.4)	0.77
DQA1				
Bidirectional	9 (50)	5 (14)	6.4 (1.7–24)	0.006
Mother from son's perspective	2 (11)	4 (11)	1.0 (0.2–6.2)	0.97
Son from mother's perspective	1 (6)	7 (19)	0.3 (0.1–2.2)	0.22
DQB1				
Bidirectional	6 (33)	3 (8)	5.7 (1.2–26.3)	0.027
Mother from son's perspective	2 (11)	3 (8)	1.4 (0.2–9.3)	0.72
Son from mother's perspective	1 (6)	3 (8)	0.7 (0.1–6.9)	0.73

*Values are the number (%) of subjects. The analyses were not adjusted for age or ethnicity. For $DRB1_1$, n = 18 patients and 37 controls; for $DRB1_2$, n = 17 patients and 37 controls; for DQA1, n = 18 patients and 37 controls; for DQB1, n = 18 patients and 37 controls. See Table 1 for descriptions of compatibility. SLE = systemic lupus erythematosus; OR = odds ratio; 95% CI = 95% confidence interval.

†Basic DRB1 groups DRB1*01 through DRB1*14 sharing sequence similarities but not necessarily identical throughout the entire DRβ1 chain.

‡Specific alleles within DRB1 groups.