Protective effect of noninherited maternal HLA-DR antigens on rheumatoid arthritis development

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Rheumatoid arthritis (RA) is a complex genetic disorder in which the HLA-region contributes most to the genetic risk. HLA-DRB1-molecules containing the amino acid sequence DERAA (i.e., HLA-DRB1*0103, *0402, *1102, *1103, *1301, *1302, and *1304) are associated with protection from RA. It has been proposed that not only inherited but also noninherited HLA-antigens from the mother (NIMA) can influence RA-susceptibility. Up to now, no protective NIMAs were described. Here, we studied whether DERAA-containing HLA-DRB1-alleles as NIMA are associated with a protective effect. One hundred seventy-nine families were studied, 88 from the Netherlands and 91 from the United Kingdom. The frequency of DERAA-containing HLA-DRB1-alleles of the Dutch mothers (16.1%), but not of the fathers (26.2%), was lower compared with the general Dutch population (29.3%; $P = 0.02$). This was replicated in the English set of patients and controls ($P = 0.01$). Further, of all families, 45 contained at least one DERAA-negative child with RA and at least one DERAA-positive parent. The odds for the DERAA-negative RA patients of having a DERAA-positive mother was significantly lower compared with having a DERAA-positive father (OR 0.25; $P = 0.003$). These data show a protective NIMA-effect in a human autoimmune disease and indicate that a DERAA-positive mother can transfer protection against RA to her DERAA-negative child.

autoimmunity | DERAA | NIMA

Rheumatoid arthritis (RA) is a complex genetic disorder in which the HLA-region contributes most to the genetic risk. Especially HLA-DRB1 molecules sharing a common epitope, R(Q)K(R)RAA, [i.e., the amino acids arginine, (glutamine), lysine, (arginine), arginine, alanine, alanine] at position 70–74, the so-called shared epitope (SE), are associated with both susceptibility to and severity of RA (1–4). At the same position of the HLA-DRB1 molecules as the SE, the amino acids DERAA (i.e., the amino acids aspartic acid, glutamic acid, arginine, alanine, alanine) can be present. Individuals carrying HLA-DRB1 alleles that express this DERAA-sequence (DERAA-positive individuals) (DERAA is present in HLA-DRB1*0103, *0402, *1102, *1103, *1301, *1302, and *1304) have a lower susceptibility to develop RA and less severe disease compared with individuals with 'neutral' (SE- and DERAA-negative) HLA-DRB1 alleles. DERAA-containing HLA-DRB1 alleles protect in both SE-negative and SE-positive individuals, and therefore this effect is independent of the effect of SE-alleles (5).

It has been proposed that not only inherited but also non-inherited HLA-antigens from the mother (NIMA) as opposed to those from the father (NIPA) can influence the immune reactivity of an individual with implications for tissue transplant survival and susceptibility to autoimmune disease (6–8). During pregnancy, the immune systems of mother and child are in close contact, and trafficking of cells, antibodies, and/or antigens can occur. Confrontation of the fetal/newborn immune system with the NIMA may have a lifelong influence on the immune response of the child. It has been shown in transplantation studies, that haplo-identical NIMA-mismatched sibling transplants had a graft survival similar to that of HLA-identical siblings, whereas NIPA-mismatched sibling transplants did as poorly as did recipients of maternal and paternal grafts (9).

We have described that HLA-DR4 or SE NIMA but not HLA-DR4 or SE NIPA are associated with susceptibility to RA, because HLA-DR4 or SE-negative RA patients have more often a HLA-DR4 or SE-positive mother compared with a HLA-DR4 or SE-positive father (10, 11). This observation was confirmed in one study (8), whereas, in two other studies (12, 13), there was a nonsignificant trend in the same direction. When the studies were combined, a significant HLA-DR4 and SE NIMA effect in DR4 or SE-negative patients was observed (8). This is not or is less clearly the case for HLA-DR4 or SE-positive RA patients (10, 11, 14). The strongest genetic risk factors for type I diabetes, HLA-DR3-DQ2 and HLA-DR4-DQ8, are also more frequent in mothers compared with fathers of patients negative for one or both of these antigens (7).

Because there is so far no evidence for a protective effect in human autoimmune disease for NIMA, we were interested in studying whether DERAA-containing HLA-DRB1 alleles, such as NIMA, are associated with a protection against RA.

To answer this question, 88 Dutch and 91 English families were typed for HLA-DRB1. Families in which the RA patient did not carry a HLA-DRB1 allele containing DERAA and either the father, the mother, or both carried DERAA-containing HLA-DRB1 alleles were analyzed for the presence of a NIMA effect mediated by DERAA-containing HLA-DRB1 loci.

Results

Two different datasets were studied: Dutch RA patients with their parents and English RA patients with their brothers, sisters, and parents. The characteristics of both datasets at the time of taking the blood sample for HLA-DRB1 typing are listed in Table 1. Both patient populations had a comparable age of onset, sex distribution, and a similar percentage of patients with joint erosions. The young age at onset is probably due to the selection of patients with living parents. The English patients were more often rheumatoid factor (RF)- and SE-positive and had a longer disease duration at the time the blood sample for

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Table 1. Clinical and laboratory characteristics of the Dutch and English patients used for this study

Characteristic	Dutch (n = 88)	English (n = 223*)
Age at onset, years	30	32
Disease duration, years	7.8	11.5
female, %	86.5	79.8
Rheumatoid factor-positive, %	57	84
SE-positive, %	74	86
Erosive disease, %	87	84

The age at onset and disease duration show the mean values in years. Disease duration is the duration of rheumatoid arthritis at the time of taking the sample for HLA-DRB1 typing. The positivity of rheumatoid factor was also determined at the time of taking the blood sample for HLA-DRB1 typing.
*Of 91 (multicase) families.

HLA-DRB1 typing was taken. These differences are most probably the consequence of including multicase families in the English dataset and mainly single-case families (only 1 multicase family) in the Dutch dataset. Patients of multicase families more often are carriers of predisposing HLA-DRB1 alleles (the SE alleles) and often have more severe disease and therefore have a higher frequency of rheumatoid factor antibodies (15). Because these differences were as expected and were not considered to interfere with our research question, the patients from both datasets were pooled for some analyses.

The frequency of DERAA-containing HLA-DRB1 alleles present in the Dutch RA patients (DERAA-positive RA patients) (14.6%) was significantly lower than that of the Dutch healthy control population (29.3%; $P = 0.007$). A similar observation was made in the English patients (only the oldest RA child of every family was included), because the frequency of DERAA-containing HLA-DRB1 alleles (8.6%) was significantly lower than that of the English control population (23.8%; $P = 0.002$). These data confirm the protective effect associated with DERAA-containing HLA-DRB1 alleles. Before studying a possible effect of DERAA-containing HLA-DRB1 alleles as NIMA, we studied whether there was no difference in inheritance of DERAA-containing HLA-DRB1 alleles from fathers or mothers to their children. Therefore, we analyzed the frequency of fathers and mothers that have passed on a DERAA-containing HLA-DRB1 allele to DERAA-positive RA patients. As expected, DERAA-containing HLA-DRB1 alleles were equally inherited from fathers or mothers in both the Dutch and English families (data not shown). These data indicate that there is no gender difference in inheritance of DERAA-containing HLA-DRB1 alleles.

If noninherited DERAA-containing HLA-DRB1 alleles of the mother protect the child to RA development, it is expected that the frequency of mothers of RA patients bearing a DERAA-containing HLA-DRB1 allele is lower compared with the general population. Therefore, we determined whether the frequency of DERAA-containing HLA-DRB1 alleles of mothers and fathers of RA patients was different compared with controls. The frequencies of DERAA-containing HLA-DRB1 alleles of the mothers and fathers of the 88 Dutch RA families were therefore compared with the frequency of DERAA-containing HLA-DRB1 alleles of a Dutch healthy control population (Table 2). Twenty-two Dutch fathers (26.2%) carried a DERAA-containing HLA-DRB1 allele, whereas, a DERAA-containing HLA-allele was present in only 14 mothers (16.1%). When these frequencies were compared with the frequency of DERAA-containing HLA-DRB1 alleles in a Dutch healthy control population (29.3%), the mothers showed a frequency significantly lower ($P = 0.02$) compared with the control population. In contrast, the frequencies of the fathers of the RA patients and the individuals of the healthy control group were comparable.

These findings were replicated in the English multicase families from Manchester. In these English RA families, 9 mothers of a total of 91 (9.9%) carried a DERAA-containing HLA-DRB1 allele compared with 14 fathers (15.7%). When these frequencies were compared with the frequency of DERAA-containing HLA-DRB1 alleles in the population of English Caucasians (23.8%), the frequency of DERAA-containing HLA-DRB1 alleles of the mothers was significantly reduced ($P = 0.01$) in contrast to the frequency of DERAA-containing HLA-DRB1 alleles of the fathers ($P = 0.18$). The DERAA frequency of the fathers was also (nonsignificantly) lower than that of the controls, probably because the English families were multicase families, which are expected to have a lower frequency of the protective DERAA-containing HLA-DRB1 alleles.

To exclude the possibility that the difference in DERAA-containing HLA-DRB1 allele frequency between the mothers and fathers is due to a general difference in DERAA-containing HLA-DRB1 allele frequency between males and females, the frequencies of DERAA-containing HLA-DRB1 alleles in males and females of the Dutch healthy control cohort were analyzed. Fifty of 186 women carried one or two DERAA-containing HLA-DRB1 alleles (26.8%) compared with 67 of 232 men (29.5%). These frequencies were not significantly different [odds ratio (OR) = 0.91; 95% C.I. 0.58–1.42; $P = 0.73$], indicating that the lower frequency of DERAA-containing HLA-DRB1 alleles in the mothers compared with the fathers of RA patients points

Table 2. DERAA frequency of mothers and fathers of Dutch and English RA patients compared with healthy Dutch and English controls

Patient	DERAA$^+$, n	DERAA$^-$, n	Freq., %	OR	95% C.I.	P
Dutch						
Mothers of RA patients	14	73	16.1	0.46	0.24–0.88	0.02*
Fathers of RA patients	22	62	26.2	0.86	0.49–1.50	0.66
Contr. fam. mothers	67	141	32.2	1.15	0.79–1.67	0.51
Healthy controls	124	299	29.3			
English						
Mothers of RA patients	9	82	9.9	0.35	0.15–0.80	0.01*
Fathers of RA patients	14	75	15.7	0.60	0.29–1.22	0.18
Healthy controls	42	135	23.8			

DERAA$^+$, carriership of one or two DERAA containing HLA-DRB1 alleles; DERAA$^-$, no DERAA-containing HLA-DRB1 allele present; Contr. fam. mothers, mothers of the control population from the Department of Obstetrics of the Leiden University Medical Center; OR, odds ratio compared with healthy controls.
*Values <0.05 are statistically significant.

Table 3. Mothers of DERAA-negative RA patients carry a DERAA-containing HLA-DRB1 allele less often than fathers

Parent	DERAA$^+$, *n*	DERAA$^-$, *n*	Freq., %	OR	95% C.I.	*P*
Mother	17	28	37.8	0.25	0.09–0.65	0.003*
Father	32	13	71.1			

The data of the English and Dutch families are combined. DERAA$^+$ and DERAA$^-$ are as defined in Table 2. The frequency is the percentage DERAA-positive individuals. OR, odds ratio of mothers compared with fathers. *Values <0.05 are statistically significant.

to a mother-specific effect of DERAA-containing HLA-DRB1 alleles on the child.

To further ascertain that the observed difference in frequency of DERAA-containing HLA-DRB1 alleles between mothers and fathers of RA patients could indeed be attributed to an effect of noninherited HLA-antigens, the DERAA-positive families with a DERAA-negative child (the RA patient) were selected for further analysis. The patient characteristics of this group were comparable with the data shown in Table 1 except for a borderline significant difference in sex in the English patient group ($P = 0.04$). Because the patient characteristics between the Dutch and English patients (as shown in Table 1) only differed for the expected characteristics (RF, SE, and disease duration), the patients were pooled for further analysis. From the 45 families fulfilling the selection criterion, 17 DERAA-positive mothers and 32 DERAA-positive fathers were identified (Table 3). The OR for DERAA-negative RA patients of having a DERAA-positive mother compared with a DERAA-positive father was 0.25 (95% C.I. 0.09–0.65; $P = 0.003$). The observed frequency of DERAA-positive mothers (37.8%) was also significantly decreased compared with the expected frequency (53.6%; $P = 0.02$). When the data of the 45 families were stratified for SE status of the patient (i.e., either no SE alleles or heterozygous or homozygous for SE), no significant differences were observed between the OR of the DERAA-NIMA versus -NIPA between the different subgroups (data not shown), indicating that the observed NIMA effect of DERAA-containing HLA-DRB1 alleles is probably independent of SE status. However, the numbers in the different subgroups were small, particularly for the SE-negative patients.

Finally, to exclude that also in non-RA families there is a NIMA effect of DERAA-containing HLA-DRB1 alleles, a Dutch control population (mother–child pairs from the Leiden University Medical Center Department of Obstetrics) was analyzed. The frequency of DERAA-containing HLA-DRB1 alleles in both the mothers (32.2%, Table 2) and children (30.3%) were comparable with that of the Dutch healthy control population (29.3%), showing that there is no (NIMA) effect of DERAA-containing HLA-DRB1 alleles in healthy control families. These results together show that there is a protective effect of DERAA-containing HLA-DRB1 alleles as NIMA on development of RA of the child.

Discussion

It has been proposed that not only inherited but also noninherited HLA-antigens from the mother (NIMA) as opposed to those from the father (NIPA) can influence the immune reactivity of an individual. A beneficial NIMA effect has been demonstrated in organ and bone marrow transplantations (6, 9, 16) and a susceptibility-effect of HLA class II molecules as NIMA were shown to be associated with susceptibility to rheumatoid arthritis and diabetes (7, 10, 11). Although it has been shown that diabetes is transmitted less frequently to the offspring of diabetic women than those of diabetic men, no relationship with HLA alleles or other genetic variations was described (17, 18), and, therefore, direct evidence for a protective effect of HLA antigens as NIMA in autoimmune diseases is thus far lacking. In this study, we show that there is a protective effect of HLA-DRB1 molecules that contain the amino acid sequence DERAA as NIMA on the development of RA. The odds ratio (OR) for DERAA-negative RA patients of having a DERAA-positive mother compared with a DERAA-positive father was 0.25. These data show a protective NIMA-effect in a human autoimmune disease and indicate that a DERAA-positive mother can transfer protection against RA to her DERAA-negative child.

HLA-DRB1 molecules play a large role in the genetic risk of developing RA. At position 70–74 of the HLA-DRB1 molecules, either the amino acids of the SE (R(Q)K(R)RAA) or the amino acids DERAA can be present. The odds ratio of individuals carrying HLA-DRB1 alleles that express the DERAA-sequence (HLA-DRB1*0103, *0402, *1102, *1103, *1301, *1302, and *1304) compared with individuals with "neutral" (SE- and DERAA-negative) HLA-DRB1 alleles to develop RA is 0.5–0.7, indicating that DERAA-positive individuals have a lower susceptibility to develop RA (5, 19–21). Because the odds ratio of DERAA was corrected for SE-alleles, it can be concluded that the DERAA-containing HLA-DRB1 alleles are independently associated with a reduced risk to develop RA. The mechanism of protection is unknown, but it has been proposed that it is mediated by T cells recognizing peptides containing the DERAA-sequence presented by HLA-DQ molecules (22). Whether these T cells have a regulatory phenotype or are deleted in the thymus by negative selection is still a subject of research. Our observation of a protective effect of DERAA-containing HLA-DRB1 alleles as NIMA on RA development gives a new dimension to the direction of this research.

During pregnancy, cells of the mother migrate to the fetus and may induce lifelong microchimerism in the child (23–25). Maternal microchimerism has been shown in mice to induce neonatal B cell (26) and probably also T cell (27) tolerance and is therefore one of the possible mechanisms for NIMA effects (28). Although speculative, we postulate therefore that the protective effect of the DERAA-containing HLA-DRB1 alleles as NIMA on the development of RA is most probably mediated by maternal cells entering the bloodstream and tissues of the child, which exert their effect through a change in the immune repertoire and, most likely, the T cell repertoire of the child. These maternal cells might influence thymic selection or act in the peripheral lymphoid organs, for example as a consequence of the sustained presence of cells from the mother in the child. It has been shown that maternal microchimeric cells can be present in many different cell subsets (29) in both healthy and diseased individuals (30, 31) in which they may exert different effects (32, 33). Likewise, immune regulatory mechanisms might directly be induced in the fetus, because it has recently been described that the fetus can already develop cytotoxic T cells directed at a maternal minor H antigen *in utero* (34) or becomes sensitized against foreign antigens to which the mother is exposed during pregnancy (35).

Further studies on the intriguing interplay between the developing immune system of the child and cells from the mother

are needed both to increase our understanding on how NIMA can influence the immune system of the child and to learn whether and if so how this might be used to combat autoimmune diseases.

Patients and Methods

Dutch RA Families. Eighty-eight consecutive patients with RA fulfilling the 1987 American College of Rheumatology criteria were recruited in 1996 in two outpatient clinics (37 from the Leiden University Medical Center and 51 from the Jan van Breemen Institute). At a patient's time of inclusion, both of their parents had to be alive. Blood samples were drawn from patients and their parents to perform HLA-DRB1 typing.

Dutch Controls. A randomly selected panel of 423 healthy unrelated Dutch individuals served as control population for the Dutch HLA-DRB1 allele frequencies (5).

Dutch Control Families. HLA-DRB1 typings of 208 healthy mother–child pairs were analyzed to control for the specificity of a possible NIMA effect of DERAA-containing HLA-DRB1 alleles in the RA families. These families were collected from a database (36) that includes deliveries that took place in of the Obstetric Department of the Leiden University Medical Center.

English Families. Potential multicase RA families were notified from a number of sources, including consultant rheumatologists, routine questioning of patients in clinics, and direct approaches via the media. Especially families with sibling pairs or extended affected pedigrees were identified (37). The diagnosis was confirmed by a trained rheumatologist. Diagnostic classification was based on the modified 1987 American Rheumatism Association Criteria (38). Blood samples of all individuals were taken for HLA-DRB1 typing. For the NIMA analysis, all DERAA-negative children with RA of each family were taken into account.

English Controls. An English Caucasian study population from the Allele Frequency Database consisting of 177 individuals was used as control population for the English HLA-DRB1 allele frequencies (39).

HLA genotyping. HLA-DRB1 alleles were determined in all RA patients, their parents, brothers, sisters, and controls. In the English families, seven typings of the HLA-DRB1 alleles of either the mother or the father were deduced from the alleles present in the other family members.

HLA-DRB1 typing for the Dutch individuals was performed as described in ref. 11. In England, HLA-DRB1 typing was performed by PCR, using specific primers and hybridization with sequence-specific biotin labeled oligonucleotides (Dynalkit; Dynal). In 4 of 88 fathers and 1 of 88 mothers, no definitive HLA-DRB1 allele could be assigned. Therefore, these individuals were excluded from the analysis.

The following HLA-DRB1 alleles were classified as containing the DERAA epitope: HLA-DRB1*0103, *0402, *1102, *1103, *1301, *1302, and *1304.

Statistics. The patient characteristics of the Dutch and English patients were compared with either a χ^2 (dichotomous variables) or an independent t test (continuous variables). For the patient groups of Table 1 (<30 individuals per group), the patient characteristics were compared with the Fischer exact and Mann–Whitney tests.

The DERAA frequencies of the mothers and the fathers of both the Dutch and English RA patients were compared separately to the DERAA frequency in the Dutch and English healthy control populations, respectively, by using a χ^2 test. In the Dutch healthy control population, the frequency of DERAA-containing HLA-DRB1 alleles in women and men was also compared.

An association between the presence and absence of the DERAA-containing HLA-DRB1 alleles as a NIMA or a NIPA was calculated by using odds ratios with 95% confidence intervals combined with a χ^2 test. The observed frequency of DERAA-positive mothers was compared with the expected frequency, using a binomial test. The expected frequency was calculated with the method of Bayes and a comparable distribution of the English and Dutch families contributing to this analysis was taken into account for the calculation of the expected frequency. These analyses were performed for parents of patients not carrying a DERAA-containing HLA-DRB1 allele.

The χ^2, independent t, and Binomial tests were performed by using SPSS software, Version 12.0 (SPSS). The odds ratios and 95% confidence intervals were calculated by using EpiInfo software, Version 5 (Statcalc).

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