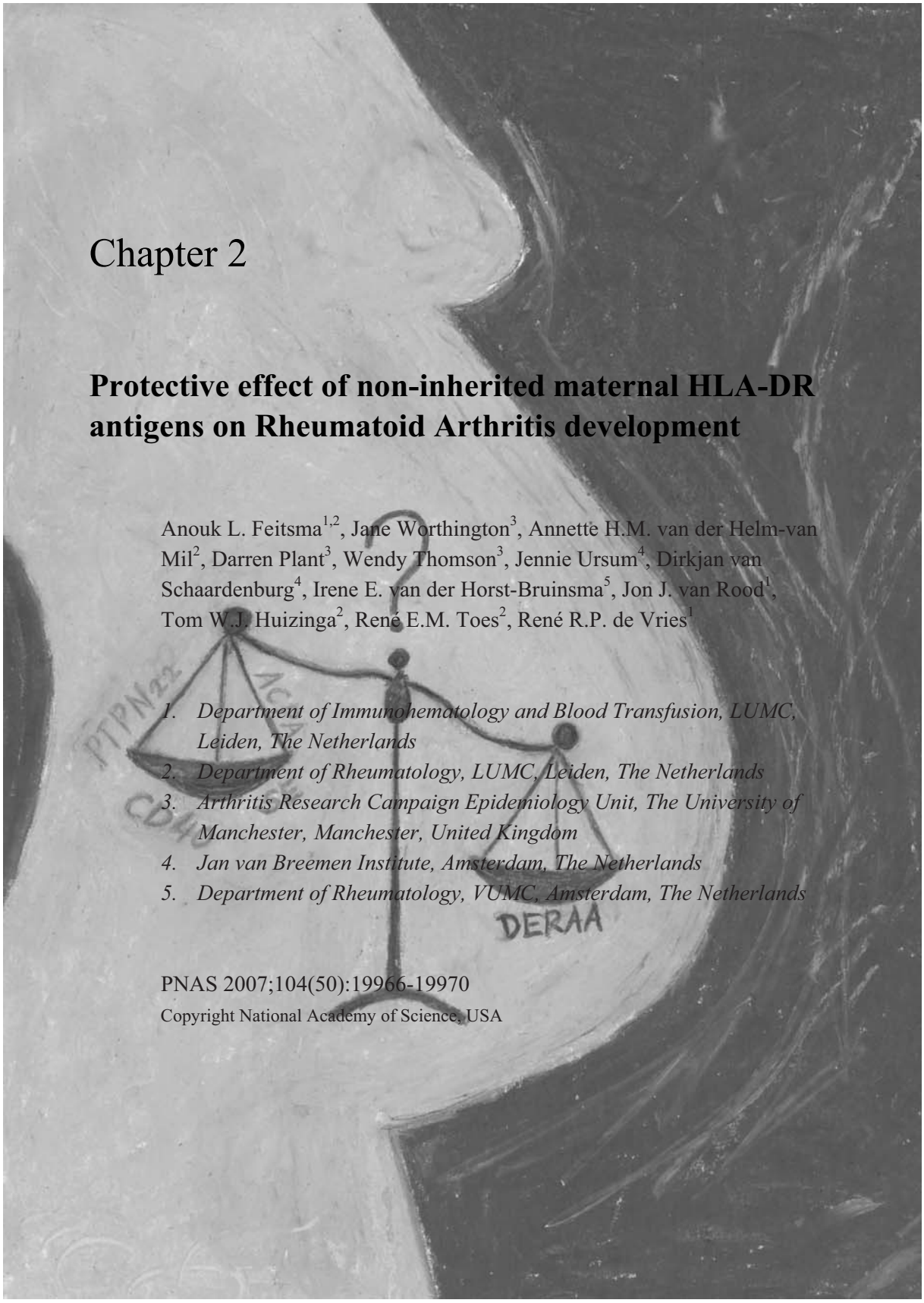
Immunogenetic and immunological aspects of rheumatoid arthritis : DERAA and anti-citrulline reactivity can make the difference

Feitsma, A.L.



Chapter 2

Protective effect of non-inherited maternal HLA-DR antigens on Rheumatoid Arthritis development

Anouk L. Feitsma[1,2], Jane Worthington[3], Annette H.M. van der Helm-van Mil[2], Darren Plant[3], Wendy Thomson[3], Jennie Ursum[4], Dirkjan van Schaardenburg[4], Irene E. van der Horst-Bruinsma[5], Jon J. van Rood[1], Tom W.J. Huizinga[2], René E.M. Toes[2], René R.P. de Vries[1]

1. *Department of Immunohematology and Blood Transfusion, LUMC, Leiden, The Netherlands*
2. *Department of Rheumatology, LUMC, Leiden, The Netherlands*
3. *Arthritis Research Campaign Epidemiology Unit, The University of Manchester, Manchester, United Kingdom*
4. *Jan van Breemen Institute, Amsterdam, The Netherlands*
5. *Department of Rheumatology, VUMC, Amsterdam, The Netherlands*

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Abstract

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 non-inherited 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.

Hundred-seventy-nine families were studied, 88 from the Netherlands and 91 from the UK. The frequency of "DERAA"-containing HLA-DRB1-alleles of the Dutch mothers (16.1%), but not of the fathers (26.2%), was lower compared to 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 as compared to 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.

Introduction

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 to 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 to a HLA-DR4 or SE-positive father (10, 11). This observation was confirmed in one study (8) while in two other studies there was a non-significant trend in the same direction (12, 13). 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 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 as compared to fathers of patients negative for one or both of these antigens (7).
As there is so far no evidence for a protective effect in human autoimmune disease for NIMA we were interested to study whether "DERAA"-containing HLA-DRB1 alleles 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.

Patients and Methods

Dutch RA families: 88 consecutive patients with RA fulfilling the 1987 ACR criteria were recruited in 1996 in two outpatient clinics: 37 from the Leiden University Medical Centre, Leiden, and 51 from the Jan van Breemen institute, Amsterdam. At time of inclusion, both parents of the patient 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 mothers and 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 Centre.

English families: Potential multi-case 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 ARA 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 previously (11). In England HLA-DRB1 typing was performed by polymerase chain reaction, using specific primers and hybridization with sequence-specific biotin labeled oligonucleotides (Dynal kit, Dynal Biotech, Wirral, UK). In four of the 88 fathers and one of the 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 Chi-square (dichotomous variables) or independent T-test (continuous variables). For the patient groups of table 3 (<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 Chi-square 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 using odds ratios with 95% confidence intervals combined with a Chi-square test. The observed frequency of "DERAA"-positive mothers was compared to 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 Chi-square, independent T-tests and the Binomial test were performed using SPSS_12.0 Software (Chicago, IL, USA). The odds ratios and 95% confidence intervals were calculated using Statcalc Software (EpiInfo version 5, Statcalc, December 1990).

Results

Two different data sets were studied: Dutch RA patients with their parents and English RA patients with their brothers, sisters and parents. The characteristics of both data sets at the time of taking the blood sample for HLA-DRB1 typing are listed in Table 1.

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

	Dutch (n=88)	English (n=223*)
Age at onset (years)	30	32
Disease duration (years)	7.8	11.5
female sex (%)	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. *out of 91 (multi-case) families.*

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 HLA-DRB1 typing was taken. These differences are most probably the consequence of including multi-case families in the English data set and mainly single case families (only 1 multi-case family) in the Dutch data set. Patients of

multi-case families more often are carriers of predisposing HLA-DRB1 alleles (the SE-alleles), often have more severe disease and therefore have a higher frequency of rheumatoid factor antibodies (15). Since these differences were as expected and were not considered to interfere with our research question, the patients from both data sets 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) as 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 non-inherited "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 to the general population. Therefore, we determined whether the frequency of "DERAA"-containing HLA-DRB1 alleles of mothers and fathers of RA patients was different as compared to 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 in only 14 mothers (16.1%) a "DERAA"-containing HLA-allele was present. When these frequencies were compared to the frequency of "DERAA"-containing HLA-DRB1 alleles in a Dutch healthy control population (29.3 %), the mothers showed a significantly lower frequency (p=0.02) compared to the control

population. In contrast, the frequencies of the fathers of the RA patients and the individuals of the healthy control group were comparable.

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

	"DERAA"+ n =	"DERAA"- n =	frequency (%)	OR	(95% CI)	p-value
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 Centre. OR= odds ratio compared to healthy controls. 95% CI= 95% confidence interval.

These findings were replicated in the English multi-case families from Manchester. In these English RA families 9 mothers out of a total of 91 (9.9%) carried a "DERAA"-containing HLA-DRB1 allele, compared to 14 fathers (15.7%). When these frequencies were compared to 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 fact that the "DERAA" frequency of the fathers was also (non-significantly) lower that that of the controls is probably due to the fact that the English families were multi-case families which are expected to have a lower frequency of the protective "DERAA"-containing 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 out of 186 women carried one or two "DERAA"-containing HLA-DRB1 alleles (26.8%) compared to 67 out of 232 men (29.5%). These frequencies were not significantly different (OR= 0.91; 95%CI 0.58-1.42; p=0.73), indicating that the lower frequency of "DERAA"-containing HLA-DRB1 alleles in the mothers as compared to the fathers of RA patients points 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 non-inherited 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 to the data shown in Table 1 except for a borderline significant difference in sex in the English patient group (p=0.04). Since 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).

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

	DERAA+ n =	DERAA- n =	frequency (%)	OR	(95% CI)	p-value
Mothers	17	28	37.8	0.25	(0.09-0.65)	0.003*
Fathers	32	13	71.1			

The data of the English and Dutch families are combined.
"DERAA"+: carriership of at least one "DERAA"-containing HLA-DRB1 allele.
"DERAA"-: no "DERAA"- containing HLA-DRB1 allele present. The frequency is the percentage DERAA-positive individuals. OR= odds ratio of mothers compared to fathers. 95% CI= 95% confidence interval.

The odds ratio (OR) for "DERAA"-negative RA patients of having a "DERAA"-positive mother compared to a "DERAA"-positive father was 0.25 (95% CI 0.09-0.65; p=0.003). The observed frequency of "DERAA"-positive mothers (37.8%) was also significantly decreased compared to 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 LUMC 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 non-inherited 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 to 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) can be present or the amino acids "DERAA". 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 to 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). Since 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 as 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.

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References

(1) Deighton, C. M., Walker, D. J., Griffiths, I. D. & Roberts, D. F. (1989) *Clin. Genet.* **36**, 178-182.

(2) MacGregor, A., Ollier, W., Thomson, W., Jawaheer, D. & Silman, A. (1995) *J. Rheumatol.* **22**, 1032-1036.

(3) Moreno, I., Valenzuela, A., Garcia, A., Yelamos, J., Sanchez, B. & Hernanz, W. (1996) *J. Rheumatol.* **23**, 6-9.

(4) Kaltenhauser, S., Wagner, U., Schuster, E., Wassmuth, R., Arnold, S., Seidel, W., Troltzsch, M., Loeffler, M. & Hantzschel, H. (2001) *J. Rheumatol.* **28**, 735-744.

(5) van der Helm-van Mil AH, Huizinga, T. W., Schreuder, G. M., Breedveld, F. C., de Vries, R. R. & Toes, R. E. (2005) *Arthritis Rheum.* **52**, 2637-2644.

(6) Claas, F. H., Gijbels, Y., van der Velden-de Munck & van Rood, J. J. (1988) *Science* **241**, 1815-1817.

(7) Pani, M. A., Van Autreve, J., Van der Auwera, B. J., Gorus, F. K. & Badenhoop, K. (2002) *Diabetologia* **45**, 1340-1343.

(8) Harney, S., Newton, J., Milicic, A., Brown, M. A. & Wordsworth, B. P. (2003) *Rheumatology. (Oxford)* **42**, 171-174.

(9) Burlingham, W. J., Grailer, A. P., Heisey, D. M., Claas, F. H., Norman, D., Mohanakumar, T., Brennan, D. C., de Fijter, H., van Gelder, T., Pirsch, J. D. *et al.* (1998) *N. Engl. J. Med.* **339**, 1657-1664.

(10) ten Wolde, S., Breedveld, F. C., de Vries, R. R., D'Amaro, J., Rubenstein, P., Schreuder, G. M., Claas, F. H. & van Rood, J. J. (1993) *Lancet* **341**, 200-202.

(11) van der Horst-Bruinsma IE, Hazes, J. M., Schreuder, G. M., Radstake, T. R., Barrera, P., van de Putte, L. B., Mustamu, D., van Schaardenburg, D., Breedveld, F. C. & de Vries, R. R. (1998) *Ann. Rheum. Dis.* **57**, 672-675.

(12) Barrera, P., Balsa, A., Alves, H., Westhovens, R., Maenaut, K., Cornelis, F., Fritz, P., Bardin, T., Ceu, M. M., Lopes-Vaz, A. *et al.* (2001) *J. Rheumatol.* **28**, 968-974.

(13) Barrera, P., Balsa, A., Alves, H., Westhovens, R., Maenaut, K., Cornelis, F., Fritz, P., Bardin, T., de Almeida, G., Lopes-Vaz, A. *et al.* (2000) *Arthritis Rheum.* **43**, 758-764.

(14) Silman, A. J., Hay, E. M., Worthington, J., Thomson, W., Pepper, L., Davidson, J., Dyer, P. A. & Ollier, W. E. (1995) *Ann. Rheum. Dis.* **54**, 311-313.

(15) Jawaheer, D., Lum, R. F., Amos, C. I., Gregersen, P. K. & Criswell, L. A. (2004) *Arthritis Rheum.* **50**, 736-741.

(16) van Rood, J. J., Loberiza, F. R., Jr., Zhang, M. J., Oudshoorn, M., Claas, F., Cairo, M. S., Champlin, R. E., Gale, R. P., Ringden, O., Hows, J. M. *et al.* (2002) *Blood* **99**, 1572-1577.

(17) el Hashimy, M., Angelico, M. C., Martin, B. C., Krolewski, A. S. & Warram, J. H. (1995) *Diabetes* **44**, 295-299.

(18) Warram, J. H., Krolewski, A. S., Gottlieb, M. S. & Kahn, C. R. (1984) *N. Engl. J. Med.* **311**, 149-152.

(19) de Vries, N., Tijssen, H., van Riel, P. L. & van de Putte, L. B. (2002) *Arthritis Rheum.* **46**, 921-928.

(20) Shadick, N. A., Heller, J. E., Weinblatt, M. E., Maher, N. E., Cui, J., Ginsburg, G. S., Coblyn, J., Anderson, R., Solomon, D. H., Roubenoff, R. *et al.* (2007) *Ann. Rheum. Dis.*

(21) Mattey, D. L., Dawes, P. T., Gonzalez-Gay, M. A., Garcia-Porrua, C., Thomson, W., Hajeer, A. H. & Ollier, W. E. (2001) *J. Rheumatol.* **28**, 232-239.

(22) Snijders, A., Elferink, D. G., Geluk, A., Der Zanden, A. L., Vos, K., Schreuder, G. M., Breedveld, F. C., de Vries, R. R. & Zanelli, E. H. (2001) *J. Immunol.* **166**, 4987-4993.
(23) Lo, Y. M., Lau, T. K., Chan, L. Y., Leung, T. N. & Chang, A. M. (2000) *Clin. Chem.* **46**, 1301-1309.
(24) Petit, T., Dommergues, M., Socie, G., Dumez, Y., Gluckman, E. & Brison, O. (1997) *Br. J. Haematol.* **98**, 767-771.
(25) Maloney, S., Smith, A., Furst, D. E., Myerson, D., Rupert, K., Evans, P. C. & Nelson, J. L. (1999) *J. Clin. Invest* **104**, 41-47.
(26) Vernochet, C., Caucheteux, S. M., Gendron, M. C., Wantyghem, J. & Kanellopoulos-Langevin, C. (2005) *Biol. Reprod.* **72**, 460-469.
(27) Andrassy, J., Kusaka, S., Jankowska-Gan, E., Torrealba, J. R., Haynes, L. D., Marthaler, B. R., Tam, R. C., Illigens, B. M., Anosova, N., Benichou, G. *et al.* (2003) *J. Immunol.* **171**, 5554-5561.
(28) van Rood, J. J., Roelen, D. L. & Claas, F. H. (2005) *Semin. Hematol.* **42**, 104-111.
(29) Loubiere, L. S., Lambert, N. C., Flinn, L. J., Erickson, T. D., Yan, Z., Guthrie, K. A., Vickers, K. T. & Nelson, J. L. (2006) *Lab Invest* **86**, 1185-1192.
(30) Lambert, N. C., Erickson, T. D., Yan, Z., Pang, J. M., Guthrie, K. A., Furst, D. E. & Nelson, J. L. (2004) *Arthritis Rheum.* **50**, 906-914.
(31) Nelson, J. L., Gillespie, K. M., Lambert, N. C., Stevens, A. M., Loubiere, L. S., Rutledge, J. C., Leisenring, W. M., Erickson, T. D., Yan, Z., Mullarkey, M. E. *et al.* (2007) *Proc. Natl. Acad. Sci. U. S. A* **104**, 1637-1642.
(32) Stevens, A. M., Hermes, H. M., Rutledge, J. C., Buyon, J. P. & Nelson, J. L. (2003) *Lancet* **362**, 1617-1623.
(33) Rinkevich, B. (2001) *Hum. Immunol.* **62**, 651-657.
(34) Mommaas, B., Stegehuis-Kamp, J. A., van Halteren, A. G., Kester, M., Enczmann, J., Wernet, P., Kogler, G., Mutis, T., Brand, A. & Goulmy, E. (2005) *Blood* **105**, 1823-1827.
(35) Rastogi, D., Wang, C., Mao, X., Lendor, C., Rothman, P. B. & Miller, R. L. (2007) *J. Clin. Invest* **117**, 1637-1646.
(36) Dankers, M. K., Roelen, D. L., Korfage, N., de Lange, P., Witvliet, M., Sandkuijl, L., Doxiadis, I. I. & Claas, F. H. (2003) *Hum. Immunol.* **64**, 600-606.
(37) Worthington, J., Ollier, W. E., Leach, M. K., Smith, I., Hay, E. M., Thomson, W., Pepper, L., Carthy, D., Farhan, A., Martin, S. *et al.* (1994) *Br. J. Rheumatol.* **33**, 970-976.
(38) MacGregor, A. J., Bamber, S. & Silman, A. J. (1994) *J. Rheumatol.* **21**, 1420-1426.
(39) Middleton, D., Menchaca, L., Rood, H. & Komerofsky, R. (2003) *Tissue Antigens* **61**, 403-407.