Influence of non-inherited maternal HLA-DR antigens on susceptibility to rheumatoid arthritis

I E van der Horst-Bruinsma, J M W Hazes, G M Th Schreuder, T R D J Radstake, P Barrera, L B A van de Putte, D Mustamu, D van Schaardenburg, F C Breedveld, R R P de Vries

Abstract

Objective—It has recently been observed that non-inherited maternal DR4 antigens (NIMAs) of DR4 negative rheumatoid arthritis (RA) patients were increased compared with non-inherited paternal DR4 antigens (NIPAs). The aim of this study was to determine the prevalence of non-inherited DR4 antigens and DRB1 alleles in parents of RA patients.

Methods—HLA-DR serology and DRB1 typing was performed in 97 RA patients and their parents. NIMA and NIPA frequencies were compared, stratified according to the presence of DR4 and/or the shared epitope (SE).

Results—In DR4 negative patients, NIMA DR4 was increased compared with NIPA DR4 (OR 3.10, 95% CI 0.76, 12.70). When combined with results from a previous study this increase was significant (OR 3.65, 95% CI 1.29, 10.31). The NIMA effect of SE positive DR4 subtypes in this study (OR 4.73, 95% CI 0.94, 23.8) was stronger than the NIMA effect of combined SE positive DRB1 alleles (OR 2.19 95% CI 0.36, 13.22).

Conclusions—The association between non-inherited maternal HLA-DR4 alleles and the susceptibility to RA was observed in two independent populations.

(*Ann Rheum Dis* 1998;57:672–675)

Departments of Rheumatology
I E van der
  Horst-Bruinsma
J M W Hazes
D Mustamu
F C Breedveld

and Immunohaematology and Bloodbank
G M Th Schreuder
R R P de Vries

Leiden University Medical Centre, the Netherlands

Jan van Breemen Institute Amsterdam, the Netherlands
D van Schaardenburg

University Hospital Nijmegen, the Netherlands
T R D J Radstake
P Barrera
L B A van de Putte

Correspondence to:
Dr I E van der
Horst-Bruinsma, Leiden
University Medical Centre,
Department of
Rheumatology, C4-R, PO
Box 9600, 2300 RC Leiden,
the Netherlands.

Accepted for publication
9 September 1998

The susceptibility to rheumatoid arthritis (RA) is associated with the presence of certain HLA DRB1 alleles sharing a common sequence of aminoacids at position 70–74 of the DRbeta chain, which is known as the shared epitope (SE).[1–3] Particularly the DRB1*0401 and *0404 alleles are strongly associated with RA.[2–7] We have previously proposed that not only inherited factors but also non-inherited HLA-antigens, especially those of the mother (NIMA), can influence the susceptibility to RA. Evidence for this hypothesis was obtained by the finding of an increased frequency of a DR4 NIMA of DR4 negative RA patients (RR 3.5) compared with DR4 non-inherited paternal antigens (NIPAs).[8] In the DR4 positive patients the NIMA DR6 was increased (RR 3.2) and the NIMA DR3 was decreased. No evidence for such a NIMA effect was found in another study that mainly analysed DR4 positive patients from multicase families.[9]

The aim of this study was: (1) to test the NIMA hypothesis for the serologically defined DR4, DR3, and DR6 antigens in another population of RA patients. (2) To test the NIMA hypothesis for DRB1 alleles, taking into account the SE.

Methods

Ninety seven consecutive patients with RA fulfilling the 1987 ARA criteria[10] were recruited in three outpatient clinics: 26 from Leiden (Leiden University Medical Centre), 48 from Amsterdam (Jan van Breemen Institute), and 23 from Nijmegen (University Hospital). The patients in Leiden were not included if they had participated in the former NIMA study.[8] Both parents of all included patients had to be alive. All patients and their parents were interviewed to gain information about first degree relatives with RA. Blood samples were drawn from all patients and their parents to perform HLA class II typing.

HLA TYPING
Serological HLA-DR typing as well as generic DRB1 typing was performed with a polymerase chain reaction and biotin labelled sequence specific oligonucleotide (PCR-SSO) method as described previously.[11] This method allows medium resolution DRB1 typing. DRB1*04 subtyping (DRB1*0401–0411) was performed by group specific amplification of DNA from all DRB1*04 positive subjects and hybridisation with relevant SSOs.[12] In this way it was possible to distinguish homozygosity and heterozygosity for all DRB1*04 alleles. With the same technique the alleles known to express the shared epitope—that is, DRB1*0101, *0102, *0401, *0404, *0405, *0408, *0410, and *1001 were identified. DRB1*06 alleles were subtyped in *1301/*1302, *1303, and *1401. Differentiation between *1301 and *1302 was not possible.

The HLA DRB1 typings of 421 healthy organ donors served as controls for the HLA profile of the RA patients.

NIMA or NIPA were defined as serological DR antigens or DRB1 alleles present in respectively mother or father and not inherited by the patient.

Table 1 *Comparison of frequencies of rheumatoid arthritis (RA) associated DRB1 alleles between RA patients and controls*

HLA	Patients n=97(%)	Controls n=421(%)	OR (95% CI)
DR4	58 (60)	95 (23)	5.10 (3.20, 8.13)
SE	72 (74)	178 (42)	3.93 (2.40, 6.45)
*0401/*0404	56 (58)	94 (22)	4.75 (2.99, 7.55)

SE: shared epitope, that is, DRB1*0101, *0102, *0401, *0404, *0405, *0408, and *1001 alleles. *0401/*0404: DRB1*0401 or DRB1*0404 allele present.

Table 2   Frequency of HLA-DR non-inherited maternal(NIMA) and paternal antigens (NIPA) in RA patients

HLA	NIMA	NIPA	OR (95% CI)
DR4 negative patients (n=39)			
DR3	6	7	0.83 (0.25, 2.74)
DR4	8	3	3.10 (0.76, 12.70)
DR6	6	11	0.46 (0.15, 1.41)
DR4 positive patients (n=58)			
DR3	6	11	0.49 (0.17, 1.44)
DR4	10	9	1.13 (0.42, 3.04)
DR6	8	11	0.68 (0.25, 1.85)

To compare the NIMA and NIPA frequencies in non-RA families HLA-DR typing data from the healthy Dutch families of the previous study were used.[8]

STATISTICS

The association between the presence and absence of HLA-DR3, DR4, and DR6 as a NIMA or a NIPA were calculated using odds ratios with 95% confidence intervals. This analysis was done in patients stratified according to the presence or absence of DR4. The frequencies of DRB1*0401 and/or *0404 or all SE positive alleles as NIMAs or NIPAs were calculated separately in the same way after stratification for the presence or absence of these DRB1 alleles or the SE. The data of the previous study[8] were reanalysed calculating the odds ratio and confidence intervals instead of the relative risk. The odds ratio of the DR4 positive NIMA compared with DR4 positive NIPA in DR4 negative patients was calculated after combining the results of the previous study with this study.

Results

Most patients were women (85%) and, because of the selection for both parents alive, were young with a median age of RA onset at 29 years (range 16–53). The median age of disease onset did not differ among DR4 negative compared with DR4 positive patients (29 v 28 years, Kruskall-Wallis (KW) test p=0.51) or in DRB1*0401/*0404 negative versus DRB1*0401/*0404 positive patients (29 v 27 years, KW p=0.21) or in SE negative versus SE positive patients (29 v 26 years, KW p=0.29).

The frequencies of the DR4, SE, and the SE positive DRB1*04 alleles *0401/*0404 alleles were increased in patients compared with healthy blood donors (table 1). The association of DR4 and DRB1*04 subtypes with RA did not differ much because most DRB1*04 subtypes were DRB1*0401 and *0404 alleles.

Table 3   Frequency of HLA-DR non-inherited maternal (NIMA) and paternal antigens (NIPA) in DR4 negative RA patients from this study and the study of Ten Wolde[8] and in DR4 negative non-RA controls

		NIMA	NIPA	OR (95% CI)
This study	DR4+	8	3	3.10 (0.76, 12.70)
	DR4−	31	36	
Ten Wolde et al	DR4+	12	2	4.26 (0.88, 20.62)
	DR4−	38	27	
Both studies	DR4+	20	5	3.65 (1.29, 10.31)
	DR4−	69	63	
Controls	DR4+	8	9	0.87 (0.31, 2.42)
	DR4−	58	57	

Table 4   Frequencies of RA associated DR4 subtypes (*0401 and/or *0404) as NIMAs and NIPAs of *0401 and/or *0404 negative RA patients and *0401 and/or *0404 positive RA patients

	*0401/*0404− patients (n=41)		*0401/*0404+ patients (n=56)	
	NIMA	NIPA	NIMA	NIPA
*0401	4	1	7	6
*0404	4	1	2	1
Total	8	2	9	7
OR (95% CI)	4.73 (0.94, 23.82)		1.34 (0.46, 3.89)	

*0401/*0404: DRB1*0401 or DRB1*0404 allele present (+) or both absent (−).

Firstly, we analysed the NIMA and NIPA frequencies at serological level using the same methodology as in the previous study.[8] In the 39 DR4 negative patients, the DR4 positive NIMAs were increased compared with the DR4 positive NIPAs, but this increase failed to reach significance (OR 3.10, 95% CI 0.76, 12.70, table 2). Neither an increase of DR6-NIMA nor a strong decrease of DR3-NIMA was found in DR4 positive patients (table 2).

The results of the former study[8] were reanalysed in the same way as in this study calculating the odds ratio instead of the relative risk. Thus it appeared that the increase of DR4 NIMAs compared with DR4 NIPAs of DR4 negative RA patients was also non-significant (OR 4.26, 95% CI 0.88, 20.62) (table 3). When the results of this study are combined with those of the previous study the increase of DR4 NIMAs of DR4 negative RA patients was significant (OR 3.65, 95% CI 1.29, 10.31, table 3).

In non-RA control families[8] the frequency of DR4 positive NIMAs was equal to that of DR4 positive NIPAs in DR4 negative controls (table 3).

Next we focused on the DRB1*04 alleles as defined by PCR-SSO typing. In the DR4 negative patients the eight DR4 NIMAs were: DRB1*0401 alleles in four cases and the *0404 also in four cases; the three DR4 NIPAs were: DRB1*0401, *0402, and *0404. In the DR4 positive patients the 10 DR4 NIMAs were: DRB1*0401 in seven cases, *0403 in one case, and *0404 in two cases, whereas the nine DR4 NIPAs were: *0401 in six cases, the *0404 in one, and the *0407 in two cases. The DRB1*0405 and *0408 alleles were not found in this population. The other DRB1*04 alleles carrying the SE motif (*0401 and *0404) were increased as NIMAs compared with the DRB1*0401 and/or *0404 positive NIPAs in patients lacking these alleles (OR 4.73, 95% CI 0.94, 23.82, table 4).

The effect of all SE positive alleles as a NIMA compared with the SE positive NIPA (OR 2.19, 95% CI 0.36, 13.22, table 5) was smaller than the effect of the SE positive DR4 subtypes (table 4).

To exclude a bias caused by other genetic factors that might play a part in multicase families, the family history of the patients was obtained. Thirteen patients belonged to multicase families: five (19%) from Leiden, four (8%) from Amsterdam, and four (17%) from Nijmegen respectively. A separate analysis of

Table 5 Frequency of shared epitope (SE) alleles as non-inherited maternal (NIMA) and paternal antigens (NIPA) in SE positive and SE negative RA patients

	SE−patients (n=25)		SE+patients (n=72)	
SE	NIMA	NIPA	NIMA	NIPA
*0101		1	5	12
*0102			1	
*0401	1		10	7
*0404	2		4	2
*1001	1	1	2	
Total	4	2	22	21
OR (95% CI)	2.19 (0.36, 13.22)		OR 1.07 (0.52, 2.18)	

the NIMA frequencies in these patients was not possible because of the small number of DR4 negative patients (3 of 13).

Discussion

The increase of DR4 NIMAs compared with DR4 NIPAs of DR4 negative RA patients found in this study was in accordance with the previous study[8] and does reach significance if the results of both studies are combined (OR 3.65, 95% CI 1.29, 10.31).

Focusing on the DR4 subtypes that were significantly associated with RA in the patients studied, the frequency of DRB1*0401 and/or *0404 NIMAs of *0401 and/or *0404 negative RA patients was increased compared with the NIPAs carrying these alleles. This increase (OR 4.73, 95% CI 0.94, 23.82) was stronger than that observed for DR4 (OR 3.10, 95% CI 0.76, 12.70). The effect of the SE positive NIMA of SE negative patients was less impressive (OR 2.19, 95% CI 0.36, 13.22).

Therefore we conclude that the risk of RA seems to be increased in patients who are genetically not predisposed (that is, DR4 and shared epitope negative) but have contact during their fetal period with DRB1*0401 and/or *0404 positive HLA antigens derived from the mother. Because we ascertained RA patients with a young age of onset (median age 29 years) as both parents had to be alive for patients to be included in the study, this conclusion may only be valid for RA with early onset.

To our knowledge there has been only one other study on the subject of the NIMA hypothesis and the onset of RA.[9] This study showed no increase of either maternal DR4 or the HLA-DRB1 SE compared with the same non-inherited paternal antigens. These RA patients were all derived from multicase families, who might have a different genetic background than sporadic RA patients, the latter group constituting the majority of the RA patients. Of more importance is that there were too few DR4 negative patients in that study to allow an analysis of NIMAs of DR4 negative patients. As the DR4 NIMA effect was observed only in DR4 negative patients, the results of that study are not in contrast with our findings.

In two other HLA associated autoimmune diseases, insulin dependent diabetes mellitus and myasthenia gravis, no increase of non-inherited maternal disease predisposing HLA antigens was found.[13 14]

The mechanism of the influence of the non-inherited maternal HLA antigens might be that these HLA antigens are associated with the acquisition or course of a viral infection in the mother, which then could be transmitted to the fetus. However, we favour the hypothesis that these non-inherited maternal HLA antigens themselves are transmitted to the fetus and exert their effect through the shaping of the T cell repertoire of the child in utero.[15] The observation that 50% of the patients that receive multiple blood transfusions do not form antibodies against the non-inherited maternal HLA antigens supports this hypothesis.[16] Moreover several studies show that maternal cells do enter the fetal circulation[17–20] and thus might change the immune system of the child.

The finding that DR4-subtypes that carry the SE motif (DRB1*0401 and *0404) seem to have a stronger NIMA effect than DR4, is compatible with a pathogenetic influence of these alleles on RA. During the fetal period *0401 and/or *0404 positive T cells, molecules or peptides of the mother might change the T cell repertoire of the child, which might make it prone to develop RA later in life.

In conclusion, in DRB1*0401 and *0404 negative RA patients the frequency of DRB1*0401 or DRB1*0404 alleles is increased in the mothers compared with the fathers. This suggests that maternal *0401 and/or *0404 positive cells, molecules or peptides may exert a predisposing effect on the development of RA in fetuses who are negative for these alleles.

1 Nepom GT, Nepom BS. Prediction of susceptibility to rheumatoid arthritis by human leukocyte antigen genotyping. Rheum Dis Clin North Am 1992;18:785–92.
2 Nelson JL, Dugowson CE, Koepsell TD, Voigt LF, Branchaud AM, Barrington RA, et al. Rheumatoid factor, HLA-DR4, and allelic variants of DRB1 in women with recent-onset rheumatoid arthritis. Arthritis Rheum 1994;37: 673–80.
3 Gough A, Faint J, Salmon M, Hassell A, Wordsworth P, Pilling D, et al. Genetic typing of patients with inflammatory arthritis at presentation can be used to predict outcome. Arthritis Rheum 1994;37:1166–70.
4 Deighton CM, Cavanagh G, Rigby AS, Lloyd HL, Walker DJ. Both inherited HLA-haplotypes are important in the predisposition to rheumatoid arthritis. Br J Rheumatol 1993;32:893–8.
5 Salmon M, Wordsworth P, Emery P, Tunn E, Bacon PA, Bell JI. The association of HLA DR B alleles with self-limiting and persistent forms of early symmetrical polyarthritis. Br J Rheumatol 1993;32:628–30.
6 Wordsworth BP, Stedeford J, Rosenberg WM, Bell JI. Limited heterogeneity of the HLA class II contribution to susceptibility to rheumatoid arthritis is suggested by positive associations with HLA-DR4, DR1 and DRw10. Br J Rheumatol 1991;30:178–80.
7 MacGregor A, Ollier W, Thomson W, Jawaheer D, Silman A. HLA-DRB1*0401/0404 genotype and rheumatoid arthritis: increased association in men, young age at onset, and disease severity. J Rheumatol 1995;22:1032–6.
8 Wolde ten S, Breedveld FC, Vries de RRP, D'Amaro J, Rubenstein P, Schreuder GMT, et al. Influence of non-inherited maternal HLA antigens on occurence of rheumatoid arthritis. Lancet 1993;341:200–2.
9 Silman AJ, Hay EM, Worthington J, Thomson W, Pepper L, Davidson J, et al. Lack of influence of non-inherited maternal HLA-DR alleles on susceptibility to rheumatoid arthritis. Ann Rheum Dis 1995;54:311–3.
10 Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
11 Verduyn W, Doxiadis IIN, Anholts J, Drabbels JJM, Naipal A, D'Amaro J, et al. Biotinylated DRB sequence-specific

oligonucleotides; comparison to serologic HLA-DR typing of organ donors in Eurotransplant. Hum Immunol 1993;37:59–67.

12 Gao X, Olsen NJ, Pincus T, Stastny P. HLA-DR alleles with naturally occurring amino acid substitutions and risk for development of rheumatoid arthritis. Arthritis Rheum 1990;33:939–46.

13 Kockum I, Wassmuth R, Holmberg E, Michelsen B, Lenmark A. Inheritance of MHC class II genes in IDDM studied in population-based affected and control families. Diabetologia 1994;37:1105–12.

14 Matsuki K, Maeda H, Nomura Y, Segawa M. Influence of non-inherited maternal HLA on disease development. Lancet 1993;341:639–40.

15 Zhang L, Rood JJ van, Claas FHJ. The T-cell repertoire is not dictated by self antigens alone. Res Immunol 1991;142:441–5.

16 Claas FHJ, Gijbels Y, Velden-de Munck J van der, Rood JJ van. Induction of B cell unresponsiveness to inherited maternal HLA antigens during fetal life. Science 1988;241: 1815–17.

17 Lo YMD, LO ESF, Watson N, Noakes L, Sargent IL, Thilaganathan B, *et al*. Two-way cell traffic between mother and fetus: biologic and clinical implications. Blood 1996;88:4390–5.

18 Hall JM, Lingenfelter P, Adams SL, Lasser D, Hansen JA, Bean MA. Detection of maternal cells in human umbilical cord blood using fluorescence in situ hybridization. Blood 1995;86:2829–32.

19 Artlett CM, Smith B, Jimenez SA. Identification of fetal DNA and cells in skin lesions from women with systemic sclerosis. N Engl J Med 1998;338:1186–91.

20 Nelson JL. Microchimerism and autoimmune disease. N Engl J Med 1998;338:1224–5.