Noninherited Maternal Antigens Do Not Increase the Susceptibility for Familial Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** It has been proposed that noninherited maternal HLA-DR antigens (NIMA) might play a role in the susceptibility for rheumatoid arthritis (RA). This hypothesis has not been thoroughly tested in patients with familial RA, in whom genetic factors, either inherited or not, might have stronger influence than in patients with sporadic RA. We investigated the NIMA hypothesis in a large cohort of European patients with familial RA.

Methods. The distribution of NIMA, noninherited paternal antigens (NIPA), and inherited HLA-DR antigens was assessed in patients with familial RA from all family sets collected from 1996 onwards by the ECRAF. HLA-DRB1 oligotyping from patients and all available nonaffected siblings and parents was carried out. Familial RA was defined by the presence of at least 2 affected first-degree relatives in the same family. The frequencies of HLA-DR NIMA and NIPA were compared using odds ratios after stratification for HLA-DR*04, *0401, and/or *0404 and shared epitope (SE) status. NIMA/NIPA that coincided with inherited parental HLA-DR antigens were considered redundant and were excluded from analysis.

Results. NIMA and NIPA could be analyzed in 165 RA patients with familial RA and 84 nonaffected siblings. Patients were predominantly female, rheumatoid factor positive, and had erosive disease (81, 75, and 84%, respectively). Possession of HLA-DR*04 and *0401/*0404 alleles tended be more frequent in patients than in nonaffected siblings but this did not reach statistical significance. SE possession was similar in patients and healthy siblings, although the former had a double dose SE more often (37.6 vs 17.8%; p = 0.002). Transmission of SE encoding alleles from parents to offspring was skewed only in patients [OR (95% CI) 3.56 (2.55–4.95) vs 1.16 (0.75–1.79) in nonaffected siblings]. Using the NIPA as control, the frequencies of HLA-DRB1*04, *0401/*0404, and SE positive NIMA were not increased in patients lacking these susceptibility alleles. The frequencies of NIMA encoding susceptibility alleles in DR*04 and *0401/*0404 negative patients were lower than in nonaffected siblings.

Conclusion. Our results corroborate the association between RA and inherited SE alleles and do not support a role for noninherited HLA-DR maternal antigens in the susceptibility for familial RA. (J Rheumatol 2001;28:968–74)

Key Indexing Terms:
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Susceptibility for rheumatoid arthritis (RA) involves genetic factors such as those encoded by HLA genes, and is also modulated by environmental and noninherited factors[1]. Noninherited maternal antigens (NIMA) could be considered among the latter since bidirectional maternofetal traffic of cells and antigens does occur during pregnancy, birth, and breast feeding[2]. The so-called NIMA hypothesis states that NIMA might either predispose to, or protect from autoimmune reactions by modulating the immune repertoire in the offspring. A predisposing effect of NIMA in RA was initially suggested in nongenetically predisposed (DR4 negative) Dutch patients with sporadic RA[3]. Similar results were found using HLA-DR oligotyping in a later study by the same authors, although significance was only reached after pooling patient samples in both studies[4]. A more recent analysis in a large cohort of European single-case RA families from the European Consortium on RA Families (ECRAF) could not corroborate these results[5].

RA is a heterogeneous disease, and these results cannot be directly extrapolated to familial RA. The latter is associated with larger sibships[6,7] and, in some studies, with a stronger HLA-DR4 component[8-10], although this has not always been confirmed[7,11]. Individuals from the same sibship are exposed to the same genetic background of inherited and noninherited parental antigens. It seems likely therefore that genetic factors, either inherited or not, might play a more pronounced role in familial RA than in sporadic RA.

With the exception of a small study[12], the effect of NIMA has not previously been studied in familial RA. This is not surprising, since familial clustering is relatively rare[13,14]. Moreover the late onset of RA often hampers parental analysis. Collection of large samples with multiple-case RA and available parents often requires collaborative international efforts. In this study, the NIMA hypothesis was tested in a pooled cohort of European patients with familial RA collected from 1996 onwards by the ECRAF[5,15,16].

MATERIALS AND METHODS

The individuals studied belong to consecutive European family sets recruited by the ECRAF to perform linkage studies in the first European genome scan (set 1 and 2)[15,16] and association studies (set 3)[5]. Enrollment in set 1 and 2 required the presence of at least 2 affected siblings in the same offspring. Enrollment in set 3 required the presence of a single affected case in a sibship having both parents alive. All affected individuals fulfilled the 1987 American College of Rheumatology criteria for RA[17] and gave informed consent. For the purpose of the study, familial RA was defined by the presence of at least 2 affected first-degree relatives in the same family (affected sibling pair or affected parent-offspring pair). To avoid bias, only one affected sibling per family was analyzed.

Molecular HLA-DR genotyping was performed by polymerase chain reaction (PCR) using biotinylated generic primers amplifying exon 2 of DRB1 and DRB3 to DRB5. The amplification product was hybridized using a commercial assay (Inno-lipa, Innogenetics) that allows high resolution HLA-DRB1 typing. Subtyping for DRB1*04 (*0401 to *0411) was performed using the same principle. HLA-DRB1*01, DRB1*11, DRB1*13, and DRB1*14 subtyping was performed using PCR sequence-specific primers (PCR-SSP). DRB1*06 alleles were subtyped in *1301/1302/*1305, *1303, *1304, and *1401/*1404. These typing methods allow discerning between homo- and heterozygosity. The frequencies of shared epitope (SE) encoding alleles from patients with RA were compared with those found in a group of 265 healthy Caucasian French individuals oligotyped by an ECRAF member (DC). This seemed warranted in view of the predominant French nationality of the families studied.

NIMA and noninherited paternal antigens (NIPA) are defined as those HLA-DRB1 alleles from mother or father not inherited by the patient. Allocation of NIMA/NIPA therefore requires HLA typing of at least one parent. If one of the parents was missing, NIMA/NIPA were not included in the study unless unequivocal assessment was possible based on the typing of available siblings. As in previous studies[4,5], NIMA/NIPA that coincided with another inherited parental antigen were considered "redundant" and excluded from analysis.

Statistical analysis. Patients and nonaffected siblings were stratified according to the presence or absence of (1) DRB1*04, (2) DRB1*0401 and/or *0404, and (3) alleles containing the SE motif (i.e., *0101, *0102, *0401, *0405, *0408, *0410, *1001). Moreover the number of SE–containing HLA-DR alleles (0, 1, 2) was taken into account in the analysis. The frequencies of NIMA and NIPA were compared using odds ratios (OR) and 95% confidence intervals. Between-group comparisons among different patient sets were performed using chi-square tests with Yates' correction, one-way ANOVA, and Kruskal-Wallis tests. Patients and nonaffected siblings were compared using chi-square, Student t, and Mann-Whitney rank-sum tests as appropriate.

RESULTS

All family sets recruited from 1996 onwards by the ECRAF were examined for the presence of confirmed familial RA in first-degree relatives. These sets (numbered 1 to 3) consisted of 90, 271, and 170 families, respectively, as described[5,15,16]. From these families, the distribution of NIMA and NIPA could be analyzed in 84 nonaffected individuals and in 165 patients with familial RA. The latter comprised 82 and 62 patients from set 1 and 2 and 21 patients from set 3. All patients from set 1 and 2 had at least one more affected sibling in their offspring and in 6 cases parental RA as well. Patients from set 3 were single-case in their offspring, with both parents alive and at least one parent affected. The distribution of patients with familial RA per country was as follows: Belgium 12, Spain 18, the Netherlands 11, Italy 4, Portugal one, and France 119.

The clinical and demographic characteristics of patients and nonaffected individuals are shown in Table 1. Patients were predominantly female, rheumatoid factor (RF) positive, and had erosive disease (81, 75, and 84%, respectively). Comparison within the 3 different patient sets showed no significant differences apart from the expected younger age ($p < 0.0001$) and shorter disease duration ($p = 0.002$) in probands from set 3 (Table 1). Thus for further analysis patients from all sets were pooled.

Nonaffected siblings were less often female (49%) and slightly older than patients. Possession of HLA-DR*04 and *0401/*0404 alleles (69.1 vs 55.9% and 60 vs 46.4%, respectively) was only slightly higher in patients than in nonaffected siblings (NS). Shared epitope (SE) possession was also similar in patients and nonaffected siblings (Table

Table 1. Clinical and demographic characteristics of patients in this study.

	Set 1	Set 2	Set 3	p*	Total Patients	Nonaffected Siblings	p**
No. of patients	82	62	21		165	84	
Female sex, %	84	73	90	NS	81	49	< 0.0001
Age, median (range), yrs							
Present	47 (23–74)	47 (29–72)	36 (22–47)	< 0.0001	45 (22–74)	48 (30–70)	0.05
At onset	33 (12–61)	32 (14–60)	27 (15–42)	0.09	33 (12–61)		
Disease duration, median (range), yrs	9.5 (0–38)	12 (2–37)	5 (0–12)	0.002	9.5 (0–38)		
RF positive, %	73	74	81	NS	75		
Erosive disease, %	83	87	76	NS	84		
Rheumatoid nodules, %	24	24	10	NS	22		
Subjective Sjögren, %	12	11	5	NS	11		
Extraarticular manifestations, %	4	8	0	NS	5		
HLA-DRB1*04 positive, %	72	66	67	NS	69	56	0.056
*0401/*0404 positive, %	67	56	43	NS	60	46	0.057
Shared epitope positive, %	83	79	81	NS	81	82	NS

*Comparison within patient sets and **between patients and unaffected siblings, respectively.
NS: not significant.

1), although the former carried the SE double dose more often than the latter (37.6 vs 17.8%; p = 0.002).

The distribution of inherited and noninherited HLA-DRB1 alleles, including "redundant" NIMA and/or NIPA in patients and controls, is shown in Table 2. HLA-DRB1*0410 was absent in this population.

Considering all SE encoding alleles, possession of DR*04 and *1001 was more frequent in patients than in healthy siblings, but this was not the case for DR*0101 and *0102 (Table 2). After excluding possession of DR4 SE positive alleles, 17 patients and 26 unaffected siblings were DR*0101 or *0102 positive and 6 patients and one nonaffected sibling were DR*1001 positive.

The frequencies of SE-encoding specificities among patients, nonaffected siblings, and healthy Caucasian controls are shown in Table 3. As shown, all *04 specificities and *1001 were more frequent in patients compared to healthy controls and to a lesser extent also compared to nonaffected siblings from the same family. Nonaffected siblings were more often positive for *0401, *0101, and *0404 than the controls.

Analysis of transmitted (IMA + IPA) and noninherited

Table 2. Distribution and odds ratios of transmitted (IMA, IPA) versus noninherited (NIMA, NIPA) HLA-DR alleles in patients and healthy siblings.

	RA Patients, n = 165					Nonaffected Siblings, n = 84				
	Number of Alleles				OR (95% CI)	Number of Alleles				OR (95% CI)
Serological	IMA	IPA	NIMA	NIPA		IMA	IPA	NIMA	NIPA	
DRB1 allele										
*0101 DR1	17	20	11	22	1.09 (0.66–1.79)	15	11	18	6	1.07 (0.58–1.95)
*0102 DR1	2	2	6	1	0.54 (0.16–1.88)	3	4	2	1	2.33 (0.59–9.19)
*0401 DR4	45	36	17	16	2.82 (1.82–4.38)	15	12	8	11	1.46 (0.78–2.75)
*0404 DR4	22	7	5	3	3.74 (1.68–8.31)	11	3	11	5	0.84 (0.40–1.78)
*0405 DR4	6	5	2	3	2.16 (0.74–6.28)	3	1	2	1	1.31 (0.29–5.94)
*0408 DR4	5	7	0	0		0	2	1	5	0.32 (0.06–1.59)
*1001 DR10	10	7	4	2	2.82 (1.10–7.26)	1	1	2	1	0.65 (0.11–3.92)
Total SE alleles	109	84	45	47	3.56 (2.55–4.95)	48	34	44	30	1.16 (0.75–1.79)
*04 non-SE DR4	2	4	1	6	0.82 (0.27–2.47)	2	2	2	2	0.98 (0.24–3.97)
*15 and *16 DR15(2),DR16(2)	12	12	19	21	0.54 (0.32–0.93)	9	7	7	10	0.91 (0.44–1.87)
*03 DR17(3),DR18(3)	8	12	21	16	0.49 (0.28–0.86)	4	8	9	14	0.47 (0.23–0.98)
*11 DR11(5)	4	10	16	22	0.33 (0.17–0.61)	4	17	3	7	2.20 (1.00–4.83)
*12 DR12(5)	2	2	2	0	1.94 (0.35–10.65)	1	0	0	4	0.24 (0.03–2.17)
*13 DR13(6)	11	7	19	12	0.53 (0.29–0.97)	11	3	11	1	1.15 (0.52–2.57)
*14 DR14(6)	3	5	8	3	0.69 (0.27–1.75)	1	5	0	3	1.99 (0.49–8.09)
*07 DR7	7	15	23	23	0.42 (0.25–0.72)	3	4	5	6	0.60 (0.23–1.60)
*08 DR8	1	5	1	2	1.94 (0.48–7.84)	0	0	1	0	
*09 DR9	2	3	0	0		0	2	0	0	
*0103 DR103	2	1	0	2	1.45 (0.24–8.73)	0	1	0	4	0.24 (0.03–2.17)
Total non-SE alleles	52	76	110	107	0.28 (0.20–0.39)	35	49	37	51	0.86 (0.56–1.33)

Table 3. Distribution of SE-encoding specificities among patients with RA, nonaffected siblings and healthy Caucasian controls.

DRB1 Allele	Serological	Familial RA: RA Patients N (%)	Familial RA: Nonaffected Siblings N (%)	p*	Healthy Controls N (%)	p**	p†
*0101	DR1	37 (22)	26 (31)		51 (19)		0.04
*0102	DR1	4 (2)	7 (8)		8 (3)		
*0401	DR4	77 (47)	26 (31)	0.02	37 (14)	< 0.0001	< 0.001
*0404	DR4	29 (18)	12 (14)		17 (6)	< 0.0005	0.04
*0405	DR4	11 (7)	4 (5)		7 (3)	0.04	
*0408	DR4	12 (7)	2 (2)		2 (1)	< 0.0005	
*1001	DR10	17 (10)	2 (2)	0.03	3 (1)	< 0.0001	

*Comparison between patients with RA and healthy siblings, **patients with RA and healthy controls, and †nonaffected siblings and healthy controls.

(NIMA + NIPA) HLA-DR alleles showed significant skewing in the transmission of SE-encoding alleles to affected offspring (IMA+IPA vs NIMA+NIPA: OR 3.56, 95% CI 2.55–4.95), but not to unaffected siblings (OR 1.16, 95% CI 0.75–1.79). This was true for all SE-encoding specificities except for *0101 and *0102 (Table 2).

We next studied the distribution of NIMA and NIPA after stratification according to the presence or absence of HLA-DRB1*04, HLA-DRB1*0401, and/or *0404 and alleles carrying the SE motif in patients and nonaffected siblings (Tables 4A, 4B, respectively). NIMA/NIPA that coincided with an inherited allele were excluded from this analysis. Moreover, the dose of alleles (0, 1, or 2) was taken into account, because in the presence of a double dose of transmitted HLA-DRB1 susceptibility alleles an additional effect of NIMA seems unlikely[12].

As shown, among HLA-DRB1*04 negative individuals, DR*04 NIMA were overrepresented in nonaffected siblings (OR 5.33, 95% CI 1.73–16.4), but neither DR*04 nor SE positive NIMA were more frequent than NIPA in patients. In HLA-DRB1*04 positive individuals, HLA-DR6 NIMA were in excess of NIPA, but this was more marked in nonaffected siblings than in patients (OR 4.26, 95% CI 1.09–16.7 vs OR 2.05, 95% CI 0.90–4.69, respectively). HLA-DRB1*03 alleles were evenly distributed in NIMA and NIPA in DRB1*04 positive and negative individuals (Table 4A).

No effect of HLA-DRB1*0401 and/or *0404 or SE positive NIMA was observed in the patients who were negative for *0401/*0404, although this was the case in nonaffected siblings (OR 2.63, 95% CI 0.96–7.21 and OR 5.85, 95% CI 2.25–15.2 for *0401/*0404 and SE, respectively). SE-encoding alleles were equally distributed among NIMA and NIPA in SE negative individuals, but were overrepresented

Table 4A. Odds ratio (95% CI) of NIMA versus NIPA in patients with familial RA after stratification for HLA-DR*04, 0401/0404, and SE status. The numbers of patients negative, single dose, and double dose positive were 51, 83, and 31, respectively for DRB1*04; 66, 86, and 13 for *0401/0404; and 31, 72, and 62 for the shared epitope.

	Negative		SE Single Dose		SE Double Dose		Positive	
DRB1*04								
Total NIMA/NIPA	41/45		71/65		23/26		94/91	
DRB1*04	8/5	1.94 (0.58, 6.50)	7/9	0.68 (0.24, 1.95)	0/4	0	7/13	0.48 (0.18, 1.27)
DRB1*03	5/6	0.90 (0.25, 3.22)	9/9	0.90 (0.33, 2.44)	5/1	6.94 (0.75, 64.6)	14.10	1.42 (0.59, 3.38)
DRB1*06	8/5	1.94 (0.58, 6.50)	14/7	2.04 (0.77, 5.41)	5/3	2.13 (0.45, 10.1)	19/10	2.05 (0.90, 4.69)
SE positive	10/8	1.49 (0.52, 4.24)	19/18	0.95 (0.45, 2.03)	3/10	0.24 (0.06, 1.02)	22/28	0.69 (0.36, 1.32)
*0401/*0404								
Total NIMA/NIPA	55/58		70/68		10/10		80/78	
*0401.*0404	8/7	1.24 (0.42, 3.69)	1/1	0.97 (0.06, 15.8)	0/1		1/1	0.97 (0.06, 15.8)
SE positive	15/12	1.44 (0.60, 3.43)	16/18	0.82 (0.38, 1.79)	1/6	0.07 (0.01, 0.84)	17/24	0.61 (0.30, 1.25)
Shared epitope								
Total NIMA/NIPA	23/26		59/58		53/59		112/110	
*0101/*0102	3/3		10/6		1/13		11/19	
*0401	4/2		2/4		2/2		4/6	
*0404	0/0		5/0		0/1		5/1	
*0405	0/0		0/0		1/3		1/3	
*1001	0/1		3/1		1/0		4/0	
All SE positive	7/6	1.46 (0.41, 5.21)	20/11	2.19 (0.94, 5.12)	5/19	0.18 (0.06, 0.53)	25/30	0.77 (0.42, 1.41)

Table 4B. Odds ratio (95% CI) of NIMA versus NIPA in nonaffected siblings after stratification for HLA-DR*04, 0401/0404, and SE status. The numbers of nonaffected siblings negative, single dose, and double dose positive were 37, 41, and 6, respectively, for DRB1*04; 45, 36, and 3 for *0401/0404; and 15, 54, and 15 for the shared epitope.

	Negative		SE Single Dose		SE Double Dose		Positive	
DRB1*04								
Total NIMA/NIPA	31/36		38/36		4/3		42/39	
DRB1*04	16/6	5.33 (1.73, 16.4)	6/13	0.33 (0.11, 1.0)	0/0		6/13	0.33 (0.11, 0.99)
DRB1*03	2/8	0.24 (0.05, 1.24)	5/5	0.94 (0.25, 3.56)	2/0		7/5	1.36 (0.39, 4.70)
DRB1*06	0/1		9/3	3.41 (0.84, 13.8)	2/0		11/3	4.26 (1.09, 16.7)
SE positive	20/10	1.49 (0.52, 4.24)	17/15	0.95 (0.45, 2.03)	0/0	0.24 (0.06, 1.02)	17/15	0.69 (0.36, 1.32)
*0401/*0404								
Total NIMA/NIPA	38/44		33/31		2/0		35/31	
*0401/*0404	14/8	2.63 (0.96, 7.21)	3/3	0.93 (0.17, 5.01)	0/0		3/3	0.88 (0.16, 4.69)
SE positive	27/13	5.85 (2.25, 15.2)	10/12	0.69 (0.24, 1.94)	0/0		10/12	0.63 (0.23, 1.77)
Shared epitope								
Total NIMA/NIPA	14/14		46/52		13/9		59/51	
*0101/*0102	6/3		9/4		0/0		9/4	
*0401	2/3		4/5		0/0		4/5	
*0404	2/0		9/2		0/1		9/3	
*0405/*0408	0/1		3/5		0/0		3/5	
*1001	0/1		2/0		0/0		2/0	
All SE positive	10/8	1.88 (0.39, 9.01)	27/16	3.20 (1.39, 7.34)	0/1		27/17	2.18 (1.02, 4.66)

in NIMA from nonaffected siblings and patients with a single SE dose (OR 3.20, 95% CI 1.39–7.34 and OR 2.19, 95% CI 0.94–5.12, respectively) (Table 3).

DISCUSSION

Our results do not support the hypothesis that noninherited maternal HLA-DR antigens play a role in the susceptibility for RA among patients with familial RA. This conclusion was achieved using HLA oligonucleotide typing and a strict definition of NIMA/NIPA. The number of patients studied was large (n = 165) and was derived from a much larger cohort encompassing a total of 531 European families, which illustrates the scarcity of families with parents alive and/or available for oligotyping.

Using the NIPA as control, there was no significant excess of NIMA-encoding susceptibility alleles in DR*04, DR*0401/0404, or SE negative patients (Table 4).

As well as comparing the frequencies of NIMA and NIPA in patients, we studied their distribution in all available nonaffected siblings from these families. We hypothesized that if disease related NIMA enhance the susceptibility for RA in nongenetically predisposed individuals, a skewed NIMA to NIPA distribution would only occur, or be more marked, in patients than in healthy siblings. However, all relevant deviations from the expected NIMA/NIPA distribution (odds ratio roughly 1) were more pronounced in nonaffected siblings than in patients. This was the case for DR*04 positive NIMA in DR*04 negative individuals, *0401/*0404 and SE positive NIMA in DR*0401/*0404 negative individuals, SE positive NIMA in individuals carrying a single SE dose, and DR6 NIMA (DR*13 and DR*14) in DR*04 positive individuals (Table 4).

The more pronounced skewing in healthy siblings might be explained by the relatively lower number of families with nonaffected siblings available for analysis. This might have introduced some bias in the NIMA to NIPA ratios among healthy siblings. Nevertheless, the findings in healthy siblings show that "susceptibility-encoding NIMA" do not increase the chance of disease in the offspring.

Taking the results of the analysis of NIMA with NIPA as control and the comparison of odds ratios in patients and nonaffected siblings together, our results confirm the role of inherited susceptibility alleles, and do not favor a role of NIMA in the susceptibility for RA. These results are in agreement with our observations in sporadic RA[5] and with a study in a relatively small sample with familial RA from the Arthritis and Rheumatism Council's National Repository of RA[12]. Our results do not corroborate the findings of 2 Dutch studies in sporadic RA[3,4]. This discrepancy is unlikely to be explained by diversity in SE-encoding alleles in Europe, since the frequencies of DR*04, *0401/*0404, and SE positive patients in our study were similar to those in the Dutch families (69.1 vs 60%, 60 vs 65.4%, and 82.1 vs 74.2%, respectively, for DR*04, *0401/*0404, and SE in subjects in the present study versus the Dutch families)[3,4].

Some brief observations. First, the main differences between patients and nonaffected siblings were the higher prevalence of female sex and double SE dose in the former. As expected, the transmission of SE-encoding epitopes to the offspring was skewed only in patients, but not in unaffected siblings. Nonetheless, the prevalence of SE positivity was similar in patients and unaffected individuals and DRB1*04 and *0401/*0404 positivity was only slightly higher among patients. These results confirm the association

of familial RA with female sex and do not corroborate the idea that male sex is a major risk factor for familial RA[18,19]. Our results suggest that double SE dose, rather than DR4 or SE positivity, increases the risk for RA within a given family.

Of note, in contrast to other SE-encoding alleles, we observed neither association between RA and DRB1*0101 and/or *0102 nor skewed transmission of these alleles. DRB*01 alleles are associated with RA in Mediterranean countries including Spain, Italy, and Greece[16,20,21]. Such association was confirmed in European families collected by the ECRAF. Among the latter, the prevalence of DRB1*0101/*0102 was high (> 30%) in Italy, Portugal and Spain, intermediate (roughly 25%) in France, and low (< 15%) in the Netherlands and Belgium[16]. The lack of association and/or increased transmission of DRB*01 susceptibility alleles in the present study are likely explained by the higher proportion of French and non-Mediterranean patients analyzed. In those countries, DR*04 alleles play a predominant role in the susceptibility for RA.

Alternatively, these results may support the notion that not all SE-encoding alleles have the same effect in RA susceptibility. This theory was put forward in 2 studies investigating the HLA component in RA. The first found no support for direct involvement of HLA-DR alleles considered as either one (*0401, *0404, *01001) or 2 epitopes (QKRAA for *0401 and QRRAA for *0404 and *0101)[22]. A second study was compatible with a recessive mode of inheritance of susceptibility alleles in linkage disequilibrium with HLA-DR, although allelic association was lowest for *0101[23].

Therefore, although both inherited HLA haplotypes have been shown to be important in susceptibility for RA[24], our findings suggest a predominant role of DR*04 alleles in populations where these are more frequent.

NIMA have been proposed to play a tolerizing role in the longterm survival of renal transplants[25,26] and in sensitization by random transfusions[27,28]. With these exceptions, their role in autoimmune reactions either has not been studied or has not been supported[29,30]. Our results together with our previous findings in sporadic RA[5] confirm the association of inherited HLA-DR antigens with RA, but do not show any effect of NIMA in susceptibility for the disease. These findings provide strong evidence against the NIMA hypothesis in RA.

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