Influence of the HLA-DR β shared epitope on susceptibility to and clinical expression of rheumatoid arthritis in Chilean patients

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Abstract

Objective—To analyse the influence of shared epitope positive HLA-DRB1 alleles (QKRAA or QRRAA) on rheumatoid arthritis (RA) susceptibility and severity in Chileans, a population that exhibits a weak association with HLA-DR4.

Methods—Prevalence of alleles DRB1*01 and DRB1*04 alleles was determined by polymerase chain reaction amplification and sequence specific oligonucleotide hybridisation in 129 RA patients with defined clinical features and in 97 healthy controls.

Results—The shared epitope was found in 70 (54%) of the RA patients and in 29 (30%) of controls (odds ratio (OR) = 3; 95% confidence intervals (CI) = 1.5, 5.1; $p = 0.0004$), and was present in a double dose in 20% of patients versus 4% of controls (OR = 6; 95% CI = 2, 21; $p = 0.0009$). HLA-DRB1*0403 was the most prevalent DR4 subtype in controls (19%). HLA-DRB1*0404 or *0408 were the alleles most prominently associated with RA, 19% versus 6% in controls (OR=3; 95% CI = 1.3, 10; $p = 0.01$). The risk of RA in those carrying a double dose of the shared epitope was 7.5 times that seen in patients lacking the epitope. Disease severity was moderate: 33% had extra-articular manifestations. The double dose was associated with an increased risk of vasculitis or extra-articular manifestations. However, 59 patients (46%) did not carry the shared epitope and 18 of them (31%) had extra-articular manifestations.

Conclusions—The weak association of RA with DR4 in Chileans seems to relate to a relatively high frequency of the DRB1*0403 allele among DR4 subtypes. As in other populations, the shared epitope in double dose is associated with RA development, especially in its more severe forms. However, both development and expression of severe forms of the disease were independent of the shared epitope in a high proportion of patients, thus emphasising the genetic heterogeneity of the disease and the possible involvement of other genetic elements.

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A small sequence within the third hypervariable region of HLA DRB1 alleles, which has

been postulated to represent a shared epitope, has been found to be associated with rheumatoid arthritis (RA) in most populations and ethnic groups.¹ In populations in which DR4 is common among patients and the *0401 allele is the most frequent DR4 subtype, the shared epitope has also been found to be associated with disease severity, especially when present in a double dose.^{2,3} RA is a heterogeneous disease, however, and the distribution of DR4 subtypes varies among different populations.¹ There is at least one case, that of African-American patients in which RA is independent of the shared epitope.⁴ Thus, it is important to assess the role of the shared epitope with respect to RA susceptibility and severity in diverse populations. We have previously observed in Chilean patients that the disease is only weakly associated with HLA-DR4. HLA-DR9, which lacks the shared epitope, represented a novel disease marker. However, it was in only 20% of patients^{5,6} and apparently makes no contribution to severity (unpublished observations). This study analyses the effect of positivity for the shared epitope, in either single or double dose, upon susceptibility and disease severity in Chilean patients.

Methods

PATIENTS AND CONTROLS

We examined 129 adult patients with RA, who fulfilled the American Rheumatism Association 1987 revised criteria for RA.⁷ Clinical characteristics were: (mean (SD) (range): age at study 52 (13) (24-79) years; age at disease onset 41 (13) (5-74) years; disease duration 11 (9) (0.4-45) years; 112 women and 17 men; seropositivity (Latex Test > 1/40) 93%; rheumatoid nodules 26%; rheumatoid vasculitis 8%; total extra-articular manifestations 33%. Clinical records were reviewed and patients interviewed by three of the authors and evaluated according to a written protocol, which included: number of disease modifying anti-rheumatic drugs, presence of rheumatoid nodules, rheumatoid vasculitis (diagnosed by histomorphology, non-healing ulcers in absence of atherosclerotic disease or venous disease and mononeuritis multiplex), pulmonary fibrosis and pleuropericarditis, diagnosed by chest x ray and echocardiography. Functional capacity was established with the American College of Rheumatology revised criteria.⁷ Hand radiographs were analysed in 92 patients using the Steinbrocker score.⁷ The

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97 healthy controls had no evidence of arthritis. Patients and controls had similar age and sex distribution and were from the same metropolitan area of Santiago.

HLA-DR1 (*0101), DR4 (*04) AND DR4 SUBTYPING

Genomic DNA was extracted from mononuclear cells isolated by the Ficoll-Hypaque method, starting from 10 ml blood samples. The generic primers GLPDR β and GAMPDR β A⁸ were used in polymerase chain reaction to obtain a template from all known HLA-DRB1 alleles, for hybridisation with DR1 (*0101) and DR4 (*04) specific oligonucleotides. The DR4 alleles were then specifically amplified for subtyping, using described methods.⁹ All the DR4 or DR1 patients and controls were subsequently analysed for the second allele using the Quick-Type HLA Class-II DRB sequence specific oligonucleotide typing (Lifecodes Corp, Stanford, CT). Because we did not find RA patients with DR10 (*1001) or DR* 1402 the study was restricted to DR1 and DR4. For statistical analysis, ANOVA, χ^2 test with Yates's correction and Fisher's exact tests were used where indicated. Results are expressed as the mean (SD) (range). The associations between RA and (a) specific DRB1 subtypes and (b) genotypic combinations either lacking (-/-) or carrying the shared epitope in single (-/+) or double dose (+/+), are expressed as odds ratios (OR) with Cornfield 95% confidence intervals. Unless specified, all odds ratios were calculated relative to the RA risk in subjects without the shared epitope. Subgroup analysis was accomplished with RA patients categorised by severity features: seropositivity, radiological erosions, vasculitis, rheumatoid nodules and total extra-articular manifestations. Sex and age at onset of disease (date of first joint swelling) were also analysed.

Results

DISTRIBUTION OF HLA-DRB1 ALLELES AND DISEASE RISK

HLA-DR4 (*04) and DR1 (*0101) were more frequent in patients (n=58, 45%; and n=31, 24%) than in controls (n=32, 33%; and n=17,

18%, respectively; p=NS). The shared epitope (QKRAA of DRB1 *0401, and QRRRA of DRB1 *0404, *0408, *0405, and *0101), was found in 70 (54%) of the RA patients and in 29 (30%) of controls (OR=3; 95% CI=1.5, 5.1; p = 0.0004). It was present as double dose in 26 (20%) patients versus four (4%) controls (OR = 6; 95% CI = 2, 21; p = 0.0009). Considered together, the related HLA-DRB1 alleles *0404 and *0408 were the only allele group showing a significant association with RA. HLA-DRB1*0404 or *0408 were found in 24 (19%) patients versus six (6%) controls (OR = 3; 95% CI = 1.3, 10; p = 0.01). HLA-DRB1*0403 was the most prevalent DR4 allele in controls (n=18; 19%), being present in 13 (10%) patients (p=NS). The risk of RA was found to be increased twofold in shared epitope heterozygotes (-/+) and 7.5-fold in those bearing double dose (+/+) when patients and controls having these allelic combinations were compared with those lacking the shared epitope (-/-) (table 1).

DRB1 GENOTYPE AND DISEASE MANIFESTATIONS

Of 129 patients, 59 (46%) were negative for the shared epitope and 18 (31%) of these had extra-articular manifestations. Even when analysing a small subgroup of 59 patients selected by erosive hand radiographs (Steinbrocker class III or IV) and similar disease duration it was found that only 31 (52%) had the shared epitope. In this group of 59, 24 patients (40%) had extra-articular manifestations and nine of 24 (38%) did not carry the shared epitope. However, evidence for a positive association between a double dose of the shared epitope and disease severity was found. The frequency of the double dose genotype in patients without extra-articular manifestations was similar to controls whereas it was significantly increased in the more severe group of patients. Patients with two shared epitope alleles had an increased risk of having extra-articular manifestations. The odds ratios for patients with shared epitope +/+ and vasculitis was 17 (95% CI = 2, 174; disease duration = 17 (7) years), and for those with extra-articular manifestations was OR = 11 (95% CI = 3, 47;

Table 1 DRB1 genotype frequencies among RA patients and controls. Association between HLA-DRB1 genotypes and disease

Genotype	RA patients (n=129) Number (%)	Controls (n=97) Number (%)	Odds ratio (compared with controls)	95% confidence intervals
Shared epitope -/-	59 (46)	68 (70)	1	Reference
Shared epitope -/+	44 (34)	25 (26)	2.0	1.1,3.9
*0101/-	22 (17)	14 (14)	1.8	0.8,4.1
*0401/-	5 (4)	3 (4)	1.9	0.4,10.7
*0404/-	11 (9)	6 (6)	2.1	0.7,6.9
*0405/-	6 (5)	2 (2)	3.5	0.6,25.9
Shared epitope +/+	26 (20)	4 (4)	7.5	2.3,27.0
Two identical alleles	15 (12)	3 (4)	5.8	1.5,26.4
*0101/0101	2 (2)	2 (2)	1.2	0.1,11.9
*0401/0401	3 (2)	0 (0)	—	—
*0404/0404	7 (5)	0 (0)	—	—
*0405/0405	3 (2)	1 (1)	3.5	0.3,88.7
Compound heterozygotes	11 (9)	1 (1)	12.7	1.6, 270
*0101/0401	2 (2)	1 (1)	2.3	0.2,66.0
*0101/0404	4 (3)	0 (0)	—	—
*0101/0405	1 (1)	0 (0)	—	—
*0401/0404	2 (2)	0 (0)	—	—
*0401/0405	2 (2)	0 (0)	—	—
*0404/0405	0 (0)	0 (0)	—	—

disease duration = 11 (8) years). In each instance, the OR were higher than in patients with similar disease duration but without these particular clinical features.

Discussion

We have previously found that RA is weakly associated with HLA-DR4^{5,6} and exhibits milder manifestations in Chilean patients.¹⁰ HLA-DR9 seemed to be positively associated with the disease but only accounted for 20% of the patients. The analysis of clinical expression in our previous series showed that severe manifestations were not predominant within haplotypes HLA-DR9, DR4 or DR1 (unpublished observations). As DR9 alleles do not encode the shared epitope and seem irrelevant as severity markers, we focused this study on those genetic features associated with severity in other populations. The Chilean population of the metropolitan area of Santiago has a comparatively genetically homogeneous substrate with no discernible ethnic groups. Based on genetic background variation according to socioeconomic level,¹¹ we estimated that the urban community used in this study would have an aboriginal admixture of about 35-40% with the remainder made up of immigrants mostly derived from Spain and Germany.

Patients had higher prevalence of the shared epitope than controls (54% versus 30%), and those carrying it on both chromosomes had a 7.5-fold higher risk of developing RA than those lacking the epitope. The fact that DRB1*0403 was the most prevalent DR4 subtype in controls could account for the weak association of the disease with DR4 and for the relatively lower RA prevalence (0.1-0.5%) found in Chile¹² (Riedemann P, personal communication). This inference is similar to that made previously in New Zealand Polynesians.¹³ Allelic combinations involving *0401/*0401 or *0401/*0404, which are strongly associated with RA severity,^{2,3,14} were present in seven (5%) of our patients and in none of the controls. This might account for the fact that extra-articular manifestations were seen in only 42 (33%) of our patients, contrasting with other populations.¹⁰

A double allelic dose of the shared epitope may also contribute to the risk of developing severe forms of the disease in Chilean patients, as their odds ratios showed an increased risk of having vasculitis or extra-articular manifestations, or both. The shared epitope was absent, however, in about half of our patients selected for erosions and extra-articular manifestations with similar disease duration, suggesting either

that other genetic factors may also be involved in RA susceptibility and severity in this population or that the entities may be less genetic. The shared epitope has been recently reported as absent in 73% of African-American patients with all forms of the disease.⁴ Furthermore, its prognostic value has been questioned.¹⁵ The results of this study contribute by emphasising the genetic heterogeneity of RA, and illustrate that both its development and the expression of severe forms can be independent of shared epitope positive alleles in a substantial proportion of cases.

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