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Molecular analysis of HLA-DRβ and DQβ polymorphism in Chinese with rheumatoid arthritis

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Abstract

***Objectives*—Several studies have suggested that genetic predisposition to rheumatoid arthritis may be related to the presence of specific polymorphic HLA sequences that are often associated with HLA-DR4 haplotypes. This study was performed to determine if an association exists between Chinese with rheumatoid arthritis and a particular HLA-DRβ or DQβ subtype.**

***Methods*—This study used the polymerase chain reaction to amplify HLA-DRβ and DQβ genes, and oligonucleotide probe hybridisation to examine the association of certain polymorphic sequences with rheumatoid arthritis in 23 Chinese patients from Shanghai.**

***Results*—An HLA-DR4 associated sequence was significantly increased in the Chinese patients (43%) compared with healthy controls (14%) from the same location (relative risk=4·6, 95% confidence limits 1·1 to 19·3). Analysis of the third hyperpolymorphic region of DR4 positive samples was performed to detect polymorphic sequences associated with Dw4, Dw10, Dw13, Dw14, Dw15, and KT2 cellular specificities. Examination of this region showed that 91% of patients had sequences encoding amino acids QRRAA (associated with Dw14 and Dw15) or QKRAA (associated with Dw4) compared with 64% of the DR4 positive controls.**

***Conclusions*—Rheumatoid arthritis in the Chinese is associated with HLA-DR4. There is a possible relationship between sequences within the third hyperpolymorphic region of the DRB allele and rheumatoid arthritis in the Chinese.**

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The association between HLA-DR4 and genetic susceptibility to rheumatoid arthritis (RA) is well established. This observation was originally described by Stastny[1] in a white, North American population, and has since been reported in many other ethnic groups, including black Americans, Mexicans, and Japanese.[2] Additional studies were performed to determine the involvement of the HLA-DQ loci. Several of these studies observed an association between RA and the DQw7 specificity,[3] but this relation was not confirmed by another investigation.[4]

The apparent complexity of the relation between HLA specificities and RA was partially attributed to the molecular heterogeneity of DR4 specificities. Cellular typing methods define at least six different DR4 associated specificities which are designated Dw4, Dw10, Dw13, Dw14, Dw15, and KT2.[5 6] Stastny first suggested that the DR4 Dw4 specificity was associated with RA, and several additional studies have suggested that the Dw4, Dw14, and Dw15 specificities are also associated with RA.[7 8] In contrast, Dw10 and Dw13 do not appear to confer risk for RA.

Each cellular specificity has been associated with one or more HLA-DR alleles, and examination of the sequences of RA associated cellular specificities has demonstrated an increased risk when the sequences QKRAA or QRRAA are present at positions 70–74 of the HLA-DRβ1 chain.

We investigated whether this pattern of association is seen in Chinese from the Shanghai area, an ethnic population that has not previously been studied. We used the polymerase chain reaction to amplify HLA genes specifically and sequence specific probe hybridisation to detect specific polymorphic sequences, a technique collectively known as oligotyping. The data provide the first assessment of the frequency of DR and DQ alleles in the Shanghai Chinese population using this technique, and confirm the association of RA and DR4 in this ethnic group.

Materials and methods

SUBJECTS

Twenty three patients with RA in the Shanghai area were selected by the department of clinical immunology, Ren-Ji Hospital. They all had definite or classic rheumatoid arthritis as defined by the criteria of the American Rheumatism Association.[9] Of the 23 patients, 15 were women and eight were men. An initial group of 21 randomly selected healthy controls was obtained: of these only three were subsequently found to carry DR4. In addition to these controls, another group of 14 DR4 positive controls was collected. Thus a total of 17 DR4 positive, normal subjects were available for analysis of DR4 subtype frequencies in the normal population. All DR4 positive and negative controls were obtained from a population of normal subjects, native to the city of Shanghai and are not known to differ

from the patient group in their ethnic or geographical origins.

STATISTICS

A statistically significant association of RA with the DRβ1*04 oligotype was established by calculating an odds ratio (estimated relative risk) using a standard 2×2 table and χ^2 analysis. A two tailed Fischer's exact test was used for the DR4 subtype analysis.

OLIGOTYPING

DNA isolation was performed by a procedure described by Wyman and White.[10] DNA polymerase chain reaction amplification of DRβ and DQβ alleles, dot blot hybridisation, probe labelling, and non-radioactive detection were achieved as described previously.[11] Data representing the overall procedure of oligotyping have been given previously.[11] DR4 associated alleles were selectively amplified and hybridised according to procedures established in this laboratory and described elsewhere.[12 13]

NOMENCLATURE

Probes were designed to detect polymorphic sequences that can be correlated with routinely recognised serological specificities, DRw1-w12, DRw52, DRw53, DQw1-w4, and DQw7-w9 (DQw3). Samples were assigned oligotypes according to the polymorphic sequences identified by this procedure and named according to the World Health Organisation allele designation. Tables 1–3 give the oligotypes together with their associated serological or cellular specificity.

Table 1 HLA-DRβ oligotypes in patients and healthy controls

Oligotype	*Associated serological specificity*	*Number of alleles (% of patients)*	
		Patients (n=23)	*Controls (n=21)*
DRB1*01	DR1	0	0
DRB1*15, *16 DRB5*01, *02	DR2	7 (30)	6 (29)
DRB1*03	DR3	1 (4)	2 (10)
DRB1*04*	DR4	11 (48)	3 (14)
DRB1*11	DR5	5 (22)	2 (10)
DRB1*13, *14	DR6	5 (22)	3 (14)
DRB1*07	DR7	1 (4)	2 (10)
DRB1*08	DR8	1 (4)	4 (19)
DRB1*09	DR9	8 (35)	10 (48)
DRB1*10	DR10	1 (4)	1 (5)
DRB1*12	DR12	3 (13)	6 (29)
DRB3*52	w52	14 (61)	12 (57)
DRB4*0101	w53	16 (70)	13 (62)

*This represents 11 haplotypes from 10 patients, one sample was a DRB1*0401, *0405.

Table 2 HLA-DQβ oligotypes in patients and healthy controls

Oligotype	*Associated serological specificity*	*Number of alleles (% of patients)*	
		Patients (n=23)	*Controls (n=21)*
DQB1*01	DQ1	10 (44)	10 (48)
DQB1*02	DQ2	2 (9)	4 (19)
DQB1*0301	DQ7	9 (39)	9 (43)
DQB1*0302	DQ8	3 (13)	0
DQB1*0303	DQ9	9 (39)	11 (52)
DQB1*04	DQ4	7 (30)	3 (14)

Table 3 Analysis of HLA-DR4 oligotypes

Oligotype	*Associated serological specificity*	*Number of alleles (% of patients)*	
		Patients (n=11)*	*Controls (n=17)*
DRB1*0401	Dw4	2 (18)	0
DRB1*0402	Dw10	0	1 (6)
DRB1*0403	Dw13	1 (9)	0
DRB1*0404	Dw14	1 (9)	2 (12)
DRB1*0405	Dw15	7 (64)	9 (53)
DRB1*0406	KT2	0	5 (29)

*This represents 11 haplotypes from 10 patients, one sample was a DRB1*0401 *0405.

Results

Twenty three patients with rheumatoid arthritis from Shanghai, China and 21 healthy, random controls from the same geographical area were characterised by oligotyping. This method detects specific HLA-DRβ and DQβ polymorphic sequences associated with routinely detected serological specificities. The results show that 43% of the patients with RA have the DRβ1*04 oligotype (DR4), compared with 14% of non-selected control individuals (table 1). Based on the odds ratio calculation, the estimated relative risk of developing RA is 4·6 for subjects who carry the DR4 oligotype (95% confidence limits 1·1 to 19·3, p=0·03 by χ^2 analysis). The relative risk obtained in this analysis is also similar to that which has been reported for DR4 and RA in many other populations. No significant association of RA with any particular DQβ oligotype was observed (table 2).

HLA polymorphism in the third hyperpolymorphic region of DR4 alleles was investigated by specifically amplifying DR4 alleles and hybridising the amplified DNA with a panel of seven probes that define oligotypes DRβ1*0401–*0406 (table 3). A total of 17 DRβ1*04 positive, local, healthy controls were used for comparison with the 10 DRβ1*04 positive patients with RA (11 alleles). Our analysis indicates a trend for an increase in the polymorphic sequences encoding QKRAA or QRRAA from the third hyperpolymorphic region of DR4 in the patients with RA (91% of DRB1*04 positive patients *v* 65% in DRB1*04 positive controls, p=0·19 by two tailed Fischer's exact test). Thirteen of the patients with RA (57%) in this study lacked the DR4 allele, and also lacked sequences encoding either QKRAA or QRRAA.

Discussion

Oligotyping provides many advantages for the analysis of HLA polymorphism, among which is the ability to characterise specifically individual stretches of coding sequence. It has been previously proposed that a shared, disease predisposing epitope might explain both DR4 positive and non-DR4 RA (reviewed in Gregersen, Silver, and Winchester[14]). The sequence QKRAA or QRRAA lies in the third hyperpolymorphic region of the DRβ1 chain of alleles and is associated with a variety of serological and cellular specificities, including DR4 Dw4, DR4 Dw14, DR4 Dw15, DR1 Dw1, DR1 Dw20, and DR6 Dw16. Oligotyping was performed to allow the specific analysis of sequences encoding these disease predisposing epitopes.

Thirteen of the Chinese patients in our study did not have a DRβ1*04 oligotype. We therefore investigated the possibility that these patients might have an RA associated sequence in the third hyperpolymorphic region. None of the 13 samples contained the reported sequences encoding QKRAA or QRRAA. Thus while a trend was evident within the DR4 positive patients with RA for a disease predisposing epitope, patients without DR4 lacked this association in the Chinese. Several explanations could account for this. Rheumatoid arthritis is a disease with a heterogeneous aetiology. It is unknown how the HLA associations seen in this disease relate to its pathogenesis. DR4 may regulate immune responsiveness to some, but not all, the environmental triggers that may initiate the disease. In all populations studied, some subjects with what appears to be classic RA, lack the HLA determinants that have been associated with disease. Disease in these patients may be initiated by a distinct environmental trigger. Such heterogeneity in disease pathogenesis could account for the lack of an observed association in 13 non-DR4 samples.

After this work was submitted, Seglias *et al*[15] reported HLA associations in Chinese patients with RA from the Hong Kong area. Their results are strikingly similar to those reported here. DR4 was present in 42·4% of the RA group *v* 17·8% of the controls, giving a relative risk of 3·4. The DR4 subtype distribution also revealed a relative enrichment of the DRβ1*0404 and *0405 subtypes in these patients. Because the Hong Kong study had only seven DR4 positive controls, however, a striking contrast was not evident, whereas our study contained 17 DR4 positive controls for subtype comparison. Taken together, the results firmly establish a linkage between DR4 and RA in the Chinese ethnic population. The data also establish a linkage between sequences within the third hyperpolymorphic region of DR4 positive patients and RA.

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