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K Hameed
Aga Khan University

S Bowman

E Kondeatis

R Vaughan

T Gibson

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THE ASSOCIATION OF HLA-DRB GENES AND THE SHARED EPITOPE WITH RHEUMATOID ARTHRITIS IN PAKISTAN

K. HAMEED,* S. BOWMAN,§¶ E. KONDEATIS,‡ R. VAUGHAN‡ and T. GIBSON†

**Department of Medicine, Aga Khan University Hospital, Karachi, Pakistan, †Clinical Rheumatology Unit, ‡Tissue Typing Laboratory and §Molecular Immunogenetics Unit, UMDS, Guy's Hospital, London*

SUMMARY

The association of particular HLA-DR alleles and the shared epitope with rheumatoid arthritis (RA) is now well established. The strength of these links varies between races. Furthermore, the proposition that the presence of the shared epitope is indicative of severe disease has been more difficult to sustain in non-Europeans. This study examines the frequency of HLA-DR and HLA-DRB1 amongst Pakistanis for the first time. Using the polymerase chain reaction (PCR) and sequence-specific oligonucleotide probes (PCR-SSOP) and primers (PCR-SSP), HLA-DR phenotype and genotype frequencies were ascertained in 86 RA hospital out-patients and 79 healthy controls matched for age, gender and ethnicity. HLA-DR1 and HLA-DR4 frequency was similar in patients and controls. HLA-DR10 occurred in 26 instances (15%) in RA and in eight (5%) controls (Pcorr = 0.048). HLA-DR2 was also increased in patients (P = 0.053) and its major subtype DR15 was significantly increased (Pcorr = 0.03). HLA-DR5 frequency was 5% in patients and 19% in controls (Pcorr = 0.002). The HLA- DR4 alleles possessing the shared epitope were more common in RA (Pcorr = 0.03) and this difference was enhanced by inclusion of other alleles possessing the shared epitope (Pcorr = 0.002). Shared epitope alleles were observed in 43 (50%) patients and 17 (22%) controls (Pcorr = 0.003). The shared epitope did not distinguish patients with more severe disease, as reflected by pain, joint deformities, disability, rheumatoid factor or X-ray damage. The distribution of HLA-DR alleles in Pakistanis with RA supports the shared epitope hypothesis. In common with other non-European racial groups, HLA-DR4 was not associated with RA. Unlike other groups, there was a weak link of RA with HLA-DR2. A protective effect of HLA-DR5 was apparent. In accord with some other studies, the shared epitope in this hospital out-patient population was not a marker for more severe disease.

KEY WORDS: Rheumatoid arthritis, Pakistan, Shared epitope, Disease severity.

THE association of rheumatoid arthritis (RA) with specific HLA-DR alleles in different racial groups has contributed to the shared epitope hypothesis [1, 2]. The distinctive linking of RA with HLA-DRB1 alleles carrying the amino acid sequence QKRAA/QRRAA provides unifying evidence for the genetic contribution to RA. However, within some populations, possession of the shared epitope contributes only slightly, if at all, toward disease susceptibility [3]. Furthermore, there remains doubt about whether the shared epitope is a disease susceptibility or severity marker [4, 5]. There is some evidence that the shared epitope on HLA-DRB1*0401 and *0404 is associated with more serious disease in Caucasians [6], but amongst other races this relationship is less evident [7, 8]. In people of African and Hispanic derivation, the association of the shared epitope with RA is tenuous [8, 9]. Thus, although the shared epitope hypothesis is supported by a growing body of evidence from different communities, the strength of the association with disease susceptibility or severity is inconsistent. Understanding the relevance of particular HLA alleles and the shared epitope to RA disease pathogenesis might be advanced by wider exploration of the world's populations. We sought to determine the association of HLA-DR specificities and their alleles with RA and its severity amongst Pakistanis, a national group not previously investigated in this way.

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¶Present address: Department of Rheumatology, University of Birmingham, Birmingham.

Correspondence to: T. Gibson, Clinical Rheumatology Unit, Guy's Hospital, London SE1 9RT.

PATIENTS AND METHODS

Venous blood samples were taken from 86 RA patients attending a rheumatology clinic at the Aga Khan University Hospital, Karachi. They all satisfied the standard criteria for RA [10]. Clinical evaluation was performed by a single physician using the Stanford Health Assessment Questionnaire [11], the Ritchie joint tenderness score [12], and a count of large and small joint deformities. The erythrocyte sedimentation rate (ESR) was measured by the Westergren method and rheumatoid factor by a latex test. X-rays of hands and feet were scored blind by a single investigator using standard films for comparison [13].

Blood was also obtained from 79 healthy controls. These were either hospital employees or visitors who were unrelated to the patients. All subjects were of South Asian origin, but because of the ethnic heterogeneity of Pakistan, controls were matched closely for age, sex and the religious or regional affiliation by which subjects defined their ethnicity, e.g. Pathan, Memon, Punjabi, Ismaili, etc.

All blood samples were taken into anticoagulant and stored at −40°C, and later transported to London in dry ice. The extraction of genomic DNA, HLR-DR genotyping and HLA-DR2 and DR4 subtyping were performed using previously described methods involving polymerase chain reaction (PCR) and sequence-specific oligonucleotide probes (PCR-SSOP) [14, 15]. Confirmatory testing of some samples was conducted using sequence-specific primers (PCR-SSP) [16, 17]. The results were evaluated using χ^2 tests with a correction factor of 10 for the number of HLA-DR types examined. Clinical comparisons between patients with and without the shared epitope were made using χ^2 and Mann–Whitney *U*-tests. There were insufficient subjects carrying HLA-DR4 to allow a similar comparison of this specificity and its associated alleles.

RESULTS

Demographic details of the patients and controls are shown in Table I. The distribution of HLA-DR alleles is shown in Table II. There was no significant difference in the frequency of HLA-DR1 and -DR4 between patients and controls. There was a significant reduction of HLA-DR5 amongst patients. All positive DR5 samples were HLA-DR5 [11], except in one control where DR5 [12] was identified. Amongst RA subjects, HLA-DR2 was found in 45 (52%) and amongst controls in 27 (34%) (Pcorr = 0.3). The total frequency of HLA-DR2 in RA subjects almost reached the conventional level of significance ($P = 0.053$) when the frequency was compared with that of controls (Table II). There was a statistically significant increase of HLA-DR10 frequency in RA (Table II). The HLA-DR2 and -DR4 subtypes, and the shared epitope frequencies, are shown in Table III. The major subtype of HLA-DR2 was DR15 and this was significantly more frequent in RA ($P = 0.03$), but no difference was apparent for the separate DR2 alleles, DR2*1501, *1502, *1601/2. Similarly, the HLA-DR4 alleles were not significantly different in frequency, although it is noteworthy that DR4 alleles possessing the shared epitope (*0401, *0404, *0405/8) were more common in RA and the sum of their frequencies was significantly greater (Pcorr = 0.03). This significance was enhanced by the inclusion of the other shared epitope alleles *0101/2, *1402 and *1001 (Pcorr = 0.002). Shared epitope alleles were observed in 43 (50%) RA patients and 17 (22%) controls (Pcorr = 0.003). Homozygosity for the shared epitope was observed in 10 RA patients and in three controls (NS).

Comparison of RA patients with and without the shared epitope did not reveal any difference in disease activity or severity, the latter being measured by the number of joint deformities, a disability score and X-ray damage (Table IV). The two groups were similar in sex distribution, age and disease duration. Disease-modifying anti-rheumatic drug (DMARD) use was increased in those with the shared epitope, but not significantly.

The RA population in Pakistan has a higher frequency of rheumatoid factor positivity than white Caucasians [18]. Those with the shared epitope had a

TABLE I

The gender, age and ethnic distribution of RA patients and their controls. Ethnicity was determined by subjects with reference to their language, branch of the Muslim faith or the places of origin of their families in the South Asian subcontinent

	RA patients (n = 86)	Healthy controls (n = 79)
Gender	28 M 58 F	25 M 54 F
Median age (range)	44 (18–81)	45 (24–80)
Ethnicity		
	No. (%)	No. (%)
North Indian	33 (38)	33 (42)
South Indian	3.0 (3.5)	–
Central Indian	3.0 (3.5)	–
Punjabi	17 (20)	16 (20)
Sindhi	4.0 (5.0)	2.0 (2.0)
Pathan	7.0 (8.0)	6.0 (8.0)
Bengali	1.0 (1.0)	–
Baluch	1.0 (1.0)	1.0 (1.0)
Memon	10 (12)	11 (14)
Ismaili	7.0 (8.0)	10 (13)
Total	86	79

TABLE II

HLA-DR phenotype frequency among 86 RA patients and 79 healthy controls

HLA-DR	RA [no. (%)]	Controls [no. (%)]	Odds ratio (95% CI)	Significance (corrected)
1*	5 (2.9)	8 (5.1)	0.55 (0.18, 1.67)	NS
2	57 (33.1)	30 (19.0)	3.21 (1.70, 6.06)	$P = 0.053$
3	23 (13.4)	28 (17.7)	0.66 (0.34, 1.29)	NS
4	23 (13.4)	12 (7.6)	2.04 (0.95, 4.39)	NS
5†	9 (5.2)	30 (19.0)	0.19 (0.09, 0.43)	$P = 0.002$
6	12 (7.0)	19 (12.0)	0.51 (0.23, 1.13)	NS
7	13 (7.6)	20 (12.7)	0.53 (0.24, 1.13)	NS
8	1 (0.6)	0 (0)	–	NS
9	3 (1.7)	3 (1.9)	0.92 (0.21, 4.09)	NS
10	26 (15.1)	8 (5.1)	3.85 (1.65, 8.95)	$P = 0.048$

*All DRB1*0101, except for one control who was *0102.
†All DR5 [11], except for one control who was DR5 [12].

TABLE III
HLA-DR genotype frequency among 86 RA patients and 79 healthy controls

HLA-DR	RA [no. (%)]	Controls [no. (%)]	Odds ratio (95% CI)	Significance (corrected)
HLA-DR2				
*1501	28 (16)	12 (7.0)	2.7 (1.27, 5.71)	NS
*1502	24 (14)	13 (8.0)	1.97 (0.93, 4.15)	NS
*1601/2	5 (3.0)	5 (3.0)	0.91 (0.27, 3.08)	NS
HLA-DR4				
*0401	5 (3.0)	1 (0.5)	4.81 (0.55, 42.14)	NS
*0404	7 (4.0)	1 (0.5)	6.91 (1.07, 30.8)	NS
*0405	4 (2.0)	2 (1.0)	1.88 (0.33, 10.55)	NS
*0408	3 (2.0)	0	–	NS
*0405/08	1 (1.0)	0	–	NS
*0401/4/5/8	20 (12)	4 (2.0)	5.68 (1.92, 16.65)	$P = 0.03$
*0402	2 (1.0)	2 (1.0)	0.92 (0.16, 5.33)	NS
*0403	1 (0.5)	6 (4.0)	0.14 (0, 0.94)	NS
Shared epitope*	52 (30)	20 (13)	4.51 (2.33, 8.75)	$P = 0.002$
No. with shared epitope homozygosity				
*0401/*0404	1	0		
*0404/*0404	1	0		
*0405/*0405/8	1	0		
*0401/*1001	1	0		
*0405/*1001	1	0		
*1001/*1001	5	0		
*0101/*0101	0	2		
*0101/*1001	0	1		
Total (%)	10 (12)	3 (4.0)	3.33 (0.95, 11.64)	NS

*Shared epitope alleles in this analysis were *0101/2, *0401/4/5/8, *1402, *1001.

higher prevalence of positive RA latex tests, but this did not reach statistical significance ($\chi^2 = 2.71$; $P = 0.09$).

The 10 patients who were homozygous for the shared epitope did not exhibit more severe disease measurements than those who were heterozygous. Similarly, those with HLA-DR2 had similar age, sex, disease duration and DMARD use to those without HLA-DR2, and the indices of disease activity and severity were not significantly different between them (data not shown).

DISCUSSION

RA is a ubiquitous disorder, although in urban Pakistan and amongst Pakistanis living in England the prevalence seems to be less than amongst white Caucasians [19, 20]. We have speculated that this apparent reduced susceptibility may be genetic. The available evidence indicates that the frequency with which the shared epitope occurs is roughly proportional to the estimates of disease prevalence [21]. For example, the striking frequency of the allele HLA-DRB1*1402 amongst Tlingit Indians with RA, and amongst the healthy Tlingit, probably contributes to the high prevalence of RA in this population [7]. By contrast, the prevalence of the shared epitope in Singaporean Chinese with RA and their controls is similar to that reported by us amongst Pakistanis

TABLE IV
Details of RA patients who did or did not possess the shared epitope. Results are expressed as median (range) or no. (%). Statistical significance was sought by χ^2 or Mann–Whitney U-tests

	Shared epitope		
	Positive	Negative	Significance
Males	15 (35%)	13 (30%)	NS
Females	28 (65%)	30 (70%)	NS
Age (yr)	44 (23–81)	43 (18–72)	NS
Disease duration (yr)	5.0 (1.0–28)	7.0 (1.0–42)	NS
DMARD	32 (74%)	29 (67%)	NS
Ritchie index	9.0 (0–25)	8.0 (0–33)	NS
No. of deformities	5.0 (0–24)	5.0 (0–24)	NS
HAQ (raw scores)	12 (0–52)	10 (0–58)	NS
ESR (mm/h)	56 (6–128)	55 (12–130)	NS
Rheumatoid factor (latex positive)	39 (91%)	34 (81%)	NS
X-ray score	8.2 (0–67)	9.0 (0–55)	NS

[22]. The prevalence of RA amongst neighbouring Hong Kong Chinese is relatively low, as it is in Pakistan [23]. However, amongst the Chinese of urban Taiwan, the shared epitope frequency amongst RA patients and controls is similar to that of the West, and the prevalence of the disease in this population is similar to that of white Caucasians [24, 25]. The importance of the shared epitope to the genetic contribution of RA susceptibility is further emphasized by monozygotic twins in whom concordance for RA is rare in the absence of the epitope [26]. However, concordance for RA is a feature in $< 25\%$ of identical twins, indicating that although inheritance is important to susceptibility, it does not account entirely for disease determination [27]. Furthermore, the shared epitope appears less relevant to disease susceptibility in some non-white racial groups, such as North Americans of Hispanic [7] and African descent [8]. Even amongst white Caucasians in southern Europe, evidence for the hypothesis is less strong [28].

Surveys of RA patients of South Asian derivation indicate marked genetic heterogeneity. Without exception, these studies have been consistent with the shared epitope hypothesis. However, amongst Indians living in the West, RA is associated predominantly with the HLA-DR1 or -DR10 specificities [29, 30], but in North India, it is with HLA-DR4 and the allele HLA-DRB1*0405 more specifically [31]. Amongst South Asian people living in South Africa, haplotype associations are strongest with HLA-DR4 amongst Muslims, and with HLA-DR10 amongst Tamils and Hindus [32]. Interestingly, this study also revealed a weak relationship with HLA-DR2 amongst the Hindu and Tamil groups, but not the Muslims. The association of HLA-DR2 with RA in our Pakistani population accords with this earlier observation. There is no clear ethnic link between Pakistanis and the two populations in South Africa. Furthermore, in common with findings elsewhere, HLA-DR2 frequency was found to be significantly reduced in North Indian RA patients [31]. The relevance of the association with HLA-DR2 in our patients was not furthered by our exploring its possible role as a disease severity marker. The disparate observations relating to this specificity are of interest, but at present cannot be explained. Our finding of a significantly lower distribution of HLA-DR5 in Pakistani RA patients has not been observed in other South Asian studies. It suggests a protective role in this population.

The possibility that the shared epitope is a determinant of more severe disease rather than susceptibility is supported by studies of early cases and the course of their illness [6, 33]. However, in both the community setting and in hospital, there are conflicting data [4, 5]. It is argued that an association of the HLA-DRB1*0401/0404 alleles with severity in men is related to the higher threshold of males for severe, progressive RA [34]. A view has been propagated that amongst Caucasians, at least, there is clinical value in the predictive potential of patient genotyping [35]. Our own study of hospital RA patients failed to demonstrate any difference of severity between those with and without the shared epitope. The established nature of the disease in these cases could have militated against our demonstrating such an effect. This criticism applies equally to other hospital-based studies. It is possible that in non-Caucasians, the relationship is less apparent. It is noteworthy that Pakistanis have less severe erosive disease than white Caucasians and it is X-ray damage which has been most convincingly linked with the shared epitope [6, 33]. The relative paucity of shared epitope frequency in Pakistanis could account for this observation despite the failure to demonstrate any epitope-related characteristics in the RA study population of the current study.

The mode of influence of HLA-DR genes on RA disease evolution is unknown. Evidence that there is a functional association with the T-cell receptor or tumour necrosis factor are amongst the latest proposals [36, 37]. Whatever the mechanism, more information is yet to be gleaned from studies such as ours in other communities. The incomplete and variable association of the shared epitope with RA in different races may indicate that RA has miscellaneous environmental causes dependent on geographical location and the presence of appropriate HLA and non-HLA genotypes [38].

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