Evidence to Show MHC-linked Factors Other Than HLA-B27 Governing Susceptibility to Spondylo-arthropathies in Asian Indians*

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SUMMARY The HLA antigen profile of 129 North Indian patients with ankylosing spondylitis, 66 patients with Reiter's syndrome and 57 patients with 'unclassifiable' arthritis was compared with 380 normal, healthy controls. Besides B27 which appeared with a significantly increased frequency in the three patient groups, other HLA antigens, *viz.* A2 and B35, showed deviated frequencies. The HLA supratype A2, B27 was found to be at an elevated frequency in patients with ankylosing spondylitis and unclassifiable arthritis whereas the B35, B27 combination showed a decreased frequency in our Reiter's syndrome sample. These data suggest that besides B27, other HLA-linked factors influence susceptibility to spondylitic disorders and might act as 'modifier' genes for the type and severity of spondylo-arthropathy in a B27-positive individual.

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During the past few years, various investigations have revealed a strong association of HLA-B27 with ankylosing spondylitis (AS) and Reiter's syndrome (RS) among almost all ethnic groups.[1] B27 has also been reported to be increased in other related spondylo-arthropathies, e.g. ulcerative colitis, psoriatic arthritis, Behcet's disease, acute anterior uveitis (AAU), reactive arthropathies etc.[2,3] as well as "unclassifiable" arthritis,[4] thus supporting the genetic basis for susceptibility to these disorders. Recent studies have shown that besides B27, other genes, possibly HLA-linked, are also likely to have a role in increasing susceptibility to AS. Thus, HLA-A2 has been reported to occur with an appreciably increased frequency both in AS as well as RS[5,6] patients of Caucasoid origin. By contrast, antigen B35 confers protection against certain features of Reiter's syndrome.[6]

Earlier studies on the native Asian Indian population have reported an association of B27 with the spondylo-arthropathies.[7-10] In the present investigation, we studied a larger sample of Indian patients to determine whether (a) any HLA-A or HLA-B antigen other than B27 or any supratypes tend to occur more frequently and define a particular group of patients and (b) to see if any negative association could be found in North Indian Caucasian patients of non-European descent.

MATERIALS AND METHODS

Patients

Two hundred and fifty-two unrelated North Indian Hindu patients with spondylitic disorders, who had been attending the Immunology Clinic of the All-India Institute of Medical Sciences Hospital over a period of four years, were selected for the study. A detailed history on joint symptoms, low back pain and stiffness, heel pain, symptoms of uveitis, conjunctivitis, urethritis, inflammatory bowel disease, skin lesions, mucosal ulcerations and thrombophlebitis was obtained on each patient. A thorough physical examination was done with special attention to joints, spine, skin, eyes, buccal mucosa and genitalia. Tests for rheumatoid factor in sera by the standard latex agglutination test using the Rheuma-Welcotest Kit (Burroughs-Wellcome, U.K.) and the postero-anterior and oblique view radiographs of the sacroiliac joints were done in each case. The

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HLA test results and the radiographs were read independently of the clinical diagnosis. The 252 spondylitic patients were categorised into the following three groups:

(i) 129 patients with ankylosing spondylitis with or without extra-axial involvement and diagnosed according to Romes' criteria,[11]

(ii) 66 patients with Reiter's disease diagnosed according to criteria published earlier,[12]

(iii) 57 patients with unclassifiable arthritis, i.e. patients with seronegative peripheral inflammatory arthritis with other features of spondylo-arthropathies including asymmetrical, predominantly lower extremity arthritis, heel pain, low back pain, mouth ulcers and balanitis.[4]

Controls

The data on HLA antigen frequencies in the patients were compared with those of 380 unrelated individuals belonging to the same ethnic background. The controls consisted essentially of healthy individuals and a random selection of potential kidney transplant donors.

Methods

All individuals were tissue typed for 20 antigens in the HLA-A and HLA-B loci using (with slight modifications) the standard NIH microlymphocytotoxicity test.[13] The phenotype frequencies of antigens in patients and controls expressed as population percentages were compared on a 2 x 2 table and their "P" values were calculated accordingly (correction of those values was made by multiplying by 20).

RESULTS

The HLA antigen profile in the patient group was comparable to that of the normal controls except for antigens A2, B27 and B35 which showed significant deviations from the expected frequencies (Table 1). An analysis of the simultaneous presence of these antigens in combination in the same patient revealed that whereas the supratype A2, B27 occurred with an increased frequency in the patient group (Table 2), the B35, B27 combination was found with a significantly reduced frequency in the patients particularly those with Reiter's syndrome (Table 3).

Ankylosing spondylitis (AS)

In the 129 patients (119 males and 10 females) with AS, the mean age of onset was 21 years. B27 occurred in 119 patients (χ^2 = 359.45, $p < 0.001$) with a relative risk of 194. In addition, HLA-A2 was present in excess among AS patients as compared with the

Table 1 Percentage of phenotype frequencies of HLA-A2, B27 and B35 in ankylosing spondylitis, Reiter's syndrome and 'unclassifiable" arthritis of North Indian origin.

HLA	Healthy controls (n = 380)	Ankylosing spondylitis (n = 129)	χ^2	Reiter's syndrome (n = 66)	χ^2	Unclassifiable arthritis (n = 57)	χ^2
A2	21.32	31.36	5.02 $p < .025$	27.11	NS	33.96	4.2 $p < .05$
B27	5.78	92.25	359.45 $p < .0001$	80.30	223.2 $p < .0001$	78.94	204.35 $p < .0001$
B35	25.52	18.33	$p < .001$*	3.03	16.5 $p < .001$*	10.52	6.2 $p < .025$

NS = not significant. *p corrected < 0.05.

Table 1 Occurrence of HLA-A2 in ankylosing spondylitis, Reiter's syndrome and "unclassifiable" arthritis in patients with and without B27.

	B27-positive				B27-negative			
	AS (n = 119)	RS (n = 53)	Unclassifiable (n = 45)	Controls (n = 22)	AS (n = 10)	RS (n = 13)	Unclassifiable (n = 12)	Controls (n = 358)
$A2^+$	35 (29.4%)	12 (22.6%)	14 (31.1%)	2 (9%)	2 (20%)	4 (30.7%)	4 (33.3%)	79 (22%)
$A2^-$	84 (70.5%)	41 (77.3%)	31 (68.8%)	20 (90.9%)	8 (80%)	9 (69.2%)	8 (66.6%)	279 (77.9%)
χ^2	3.96	NS	3.94		NS	NS	NS	

NS = not significant.

Table 3 Occurrence of HLA-B35 in ankylosing spondylitis, Reiter's syndrome and "unclassifiable" arthritis in patients with and without B27

	B27-positive				B27-negative			
	AS (n = 119)	RS (n = 53)	Unclassifiable (n = 45)	Controls (n = 22)	AS (n = 10)	RS (n = 13)	Unclassifiable (n = 12)	Controls (n = 358)
$B35^+$	8	2	4	3	2	0	2	94
	(6.7%)	(3.7%)	(8.8%)	(13.6%)	(20%)	(0%)	(16.6%)	(26.2%)
$B35^-$	111	51	41	19	8	13	10	264
	(93.2%)	(96.2%)	(91.1%)	(86.4%)	(80%)	(100%)	(83.3%)	(73.7%)
χ^2	NS	NS	NS		NS	4.5	NS	

NS = not significant.

controls ($\chi^2 = 5.02$, $p < 0.025$), whereas antigen B35 showed a significantly reduced frequency ($\chi^2 = 18.33$, p corrected < 0.05). The 129 patients were categorised into B27 positive (119) and B27 negative (10) group. Of the B27 positive patients, 29 per cent carried the allele HLA-A2 compared with 9 per cent of the controls ($\chi^2 = 3.96$) (Table 2). In the B27 negative group, however, the frequency of A2 in patients did not differ significantly from that of the controls. Conversely, B35 occurred in 6.7 per cent of the B27-positive AS patients compared with 13.6 per cent of the controls (this figure was not statistically significant). Similarly, in the B27-negative group, the frequency of B35 did not differ much from that of the controls (Table 3).

Reiter's syndrome (RS)

Out of the 66 RS patients, 58 were males and 8 females with a mean age of onset 23.8 years (range 10-55 years). B27 occurred with an elevated frequency of 80.3 per cent in these patients. Although antigen HLA-A2 did not reveal any appreciable deviation from that of the control group, B35 occurred with a statistically significant reduced frequency in the patients ($\chi^2 = 16.5$, $p < 0.001$, p corrected < 0.05). The combination of A2, B27 occurred in excess frequency from what had been expected, but was not statistically significant. In the B27-positive group, only two patients carried both B35 as well as B27 (3.7%) i.e. a four-fold decrease compared with the controls. However, none of the 13 B27-negative RS patients carried this allele ($\chi^2 = 4.5$, $p < 0.05$).

Unclassifiable arthritis

This group included patients who could not be categorised as having the symptoms of AS or RS, but had features overlapping AS, RS and rheumatoid arthritis in some combination. The 57 patients clinically classified in this category included 44 males with a mean age of onset of 21.6 years. This group showed the same trend of HLA-antigen distribution as in AS. B27 occurred in 45 patients (78.9%, $x^2 = 204.35$, $p < 0.0001$). HLA–A2 showed increased frequency compared with the controls ($x^2 = 4.2$, $p < 0.05$), whereas B35 was significantly decreased ($x^2 = 6.2$, $p < 0.025$). However, both of these antigens failed to reach statistical significance when correction for the number of antigens studied was made. HLA-A2 was particularly raised in the B27-positive group of patients i.e. a frequency of 31.1 per cent as compared with 9 per cent in the controls, $x^2 = 3.94$. The decrease in B35 was equally distributed in the B27-positive and B27-negative groups compared with that of the controls, although it was not statistically significant.

DISCUSSION

The present study involving a large number of patients of North Indian descent reveals that the presence of HLA-A2 in B27-positive patients with AS and unclassified arthritis enhances the relative risk above that associated with B27 alone. This observation signifies the existence of a "second" spondylitis gene at a different HLA locus, particularly since A2 and B27 have never been reported to be in linkage disequilibrium either in our own series of normal controls or that of others.[14] Also, in a few patients for whom the genotypes were available, the two alleles were found to occur on different HLA haplotypes. Increased frequency of A2 in AS but not in RS is in agreement with the reports on white caucasoids in Europe and North America.[1] Recently, however, Schultz *et al*,[6] have reported an increased occurrence of the A2, B27 haplotype in their series of RS patients, and related this finding to the severity and early onset of symptoms in the course of the disease.

Another interesting finding from this study is the negative association between antigen B35 and the spondylo-arthropathies, particularly the RS series in which only two patients were positive for B35. Incidentally, both of these patients presented milder disease symptoms.

These observations raise the possibility that antigen B35 confers a protective effect against the occurrence of RS or its symptoms. The protection was more pronounced in B27-negative patients, which raises the possibility that a B35 associated protective gene might be responsible for the reduced incidence of sacro-iliitis reported in B27-negative patients with sero-negative peripheral arthritis.[15] So far, only one study has reported a reduced frequency of B35 in RS.[6] Reiter's syndrome in India is predominantly of the post-dysenteric variety, though the frequency of joint involvement is more or less similar to their reported in the West.[12] Also, approximately two thirds of our RS patients did not present any previous history of infection. Considering that the frequency of B35 in the normal Indian population is appreciably high, i.e. 25.5 per cent, it could have some relationship with the reduced incidence of urethritis observed in the Indian RS patients. It is pertinent to note here that the antigen B35 occurs with a high frequency among Blacks, who generally have a less severe RS disease.[16]

A possible model for spondylitis has been hypothesised by Dawkins *et al.*[17] According to this model, HLA-B27 or a linked gene is in itself not sufficient and requires the services of a second and third gene that influence infection and sex related factors, respectively, for the development of classic AS in males. It appears from our study that while B27 might be the primary gene conferring susceptibility in AS and related spondylo-arthropathies, other HLA-linked genetic factors at the same or distant locus might influence the course and severity of the future spondylitic diseases. Thus, whereas the A2, B27 supratype confers greater susceptibility to AS and unclassifiable arthritis, the existence of A9 (A24) in a B27-positive AS patient confers greater susceptibility to AAU.[18] Also, the presence of B35 could confer protection against acquiring a severe variety of Reiter's syndrome. Ultimately, a clinician may be able to design appropriate treatment for a patient based on his or her initial HLA phenotypic expression and early manifestation of symptoms.

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