Is rheumatoid arthritis in Indians associated with HLA antigens sharing a DR β ₁ epitope?

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Abstract

HLA class II antigens were identified in a group of 44 patients with rheumatoid arthritis (RA) originating largely from the north or northeast of the Indian subcontinent and resident now in east London. Compared with 67 locally typed east London Asian controls, the prevalence of three HLA-DR antigens was raised in the patients: DR1 18.2% *v* 6.0% $\chi^2=3.99$, DR4 20.5% *v* 11.9% $\chi^2=1.48$, and DRw10 27.3% *v* 8.9% $\chi^2=6.56$. These differences were also found when the patients with RA were compared with a larger control group of 110 northern Indians: DR1 18.2% *v* 7.2% $\chi^2=4.02$, DR4 20.5% *v* 7.2% $\chi^2=5.56$, and DRw10 27.3% *v* 8.1% $\chi^2=9.7$. Twenty five (57%) of the patients expressed at least one of these antigens. All patients were also characterised for HLA-Dw types by mixed lymphocyte culture typing. The prevalence of the HLA-DR4 associated Dw types in the patients was: Dw4 2.3%, Dw10 0%, Dw14 11.4%, and Dw15 6.8%.

The DR β ₁ chains of DR1 and DRw10 together with the Dw types of DR4 other than Dw10 share amino acid residues in a region of the third hypervariable region considered to be critical in antigen presentation. It is concluded that RA in Indians is associated with these HLA antigens, and data from this study support the hypothesis of a cross reactive epitope common to HLA specificities associated with RA.

Many studies have confirmed the original observation of an association between HLA-DR4 and rheumatoid arthritis (RA).¹ Many of these previous analyses, however, have largely concentrated on white patients from Europe and North America.

An interesting exception was described by Nichol and Woodrow, who reported an association between DR1 and RA in Asian Indians resident in the United Kingdom.² This association was not noted in other studies of Indian patients with RA, in whom the prevalence of DR4 was found to be significantly raised.³⁻⁵ Further studies have also implicated DR1 in RA susceptibility, including those of Israeli Jewish,⁶ Spanish,⁷ and British⁹ populations. The possibility existed that the involvement of DR4 and DR1 in RA might be through some degree of shared molecular structure. Preliminary evidence for this came with demonstration of the antigenic determinant MC1 common to both DR1 and DR4, which could be defined by alloantisera.¹⁰ Further evidence for a

shared epitope came by using monoclonal antibodies¹¹⁻¹² which broadly react with DR1 and DR4, together with some additional specificities. HLA-DR9 and DRw10 also seem to share this antigenic site with DR1 and DR4¹³⁻¹⁴ and to be associated with RA.¹⁰⁻¹⁵

HLA-DR4 can be subdivided into Dw4, Dw10, Dw13, Dw14, and Dw15 subtypes by mixed lymphocyte culture.¹⁶⁻¹⁷ HLA-Dw15 is associated with RA in Japanese¹⁸ and Dw4, Dw14 with RA in some white populations.¹⁹⁻²¹ Dw10 has not been associated with RA.⁶ The relation between these subtypes associated with RA, and DR1, DR9, and DRw10 has been made clearer by analysis of amino acid residues and by DNA sequencing.

HLA-DR alleles—namely, Dw4, Dw14, Dw15, DR1, and DR10—found with increased prevalence in patients with RA have in common a sequence of basic amino acids at residues 69 to 71 in the third hypervariable region of the DR β ₁ gene.²² This has led to the hypothesis that DR specificities associated with RA share a cross reactive epitope, which may operate at a level of antigen presentation.²³ With this in mind we analysed a group of Indian patients with RA in an attempt to clarify the previous contradictory findings.

Subjects and methods

Forty four patients originating from the Indian subcontinent fulfilling American Rheumatism Association criteria for definite or classical RA²⁴ were typed for class II HLA antigens (class I data and clinical details were available for 45 patients). These patients attended rheumatology clinics at either Newham, St Andrews, or The London Hospital in the east end of London. Clinical features of this group were collected (Stevens C, Jawad A, Perry D, unpublished data). Briefly, 42/45 (93%) patients were rheumatoid factor positive, 11/45 (24%) had nodules, and 17/45 (38%) had features of extra-articular disease. Patients originated largely from either northern areas of the Indian subcontinent (Punjab 28%, Kashmir 11%, Gujarat 17%) or from the north east (Sylhet, Bangladesh 20%). A control group of healthy unrelated Indians from comparable areas were contacted locally in east London and HLA typed (class II *n*=67, class I *n*=71). Published control HLA prevalences for a north Indian group were also available for comparison.²⁵ HLA-DR typing was performed by a two colour fluorescence technique²⁶ and a panel of well characterised antisera. HLA-Dw typing was by mixed lymphocyte culture with irradiated

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homozygous typing cells.²⁷ The homozygous typing cells had been accredited through previous International Histocompatibility Workshop studies and were either of local origin or exchanged with other laboratories. A minimum of two homozygous typing cells per specificity were used in replicate experiments. Typing assignments were based on procedures described elsewhere.^{28, 29} Statistical comparison was by the χ^2 test and relative risks calculated as cross product ratios.

Results

Table 1 summarises the prevalence of HLA-DR and DQ antigens in patients and controls. When compared with a local Indian control panel a significantly higher prevalence of two antigens was found in the patients with RA: DR1 (18.2% *v* 6.0%, $\chi^2=3.99$, RR=3.4) and DRw10 (27.3% *v* 8.9%, $\chi^2=6.56$, RR=3.8). the prevalence of DR4 was also substantially increased (20.5% *v* 11.9%), though it did not reach statistical significance. Similar differences were seen when the patients were compared with published north Indian controls²⁵: DR1 (18.2% *v* 7.2%, $\chi^2=4.02$, RR=2.8), DR4 (20.5% *v* 7.2%, $\chi^2=5.56$, RR=3.3), and DRw10 (27.3% *v* 8.1%, $\chi^2=9.7$, RR=4.2). Twenty five (57%) of the patients with RA were either DR1, DR4, or DRw10 compared with 16 (24%) of the local controls. All of the patients identified as DRw8 were present as a previously described distinct subtype (DR8I).³⁰ Table 2 summarises the HLA-Dw typing performed on the patients with RA. The DR4 positive patients were typed as Dw4, Dw13, Dw14, or Dw15, with Dw14 being the most common. HLA-Dw10 was the only DR4 subtype not represented. The subtype Dw15, previously reported only in Japanese and Chinese patients,³¹ was also identified in this group.

Table 1 Prevalence of class II HLA antigens in Indian patients with rheumatoid arthritis (RA) and controls

HLA	Indian patients with RA (n=44)	London Hospital Indian controls (n=67)	North Indian* controls (n=110)
DR1	18.2	6.0†	7.2
DR2	40.9	40.3	47.2
DR3	20.5	14.9	27.2
DR4	20.5	11.9‡	7.2¶
DRw11 (5)	11.4	16.4	23.6
DRw12 (5)	0.0	5.9	
DRw13 (6)	11.4	19.4	22.7
DRw14 (6)	2.3	8.9	
DR7	22.7	40.3	24.5
DRw8	0.0	2.9	13.6
DR8I	9.0	7.5	NT
DR9	0.0	1.5	1.8
DRw10	27.3	8.9§	8.1**
DRw52	54.5	71.6	70.9
DRw53	40.9	50.7	38.1
DQw1	83.7	73.1	78.1
DQw2	37.2	38.8	29.1
DQw3	27.9	46.2	46.3

*Ref 26.

† $\chi^2=3.99$, *p*<0.05, RR=3.4.

‡ $\chi^2=1.48$, *p* NS.

§ $\chi^2=6.56$, *p*<0.01, RR=3.8.

|| $\chi^2=4.02$, *p*<0.05, RR=2.8.

¶ $\chi^2=5.56$, *p*<0.05, RR=3.3.

** $\chi^2=9.7$, *p*<0.01, RR=4.2.

Table 2 Prevalence of HLA-Dw types in 44 Indian patients with rheumatoid arthritis

HLA	Prevalence (%)
Dw1	20.4
Dw2	18.2
Dw2+12	22.7
Dw3	18.2
Dw4 (DR4)	2.3
Dw10 (DR4)	0
Dw13 (DR4)	6.8
Dw14 (DR4)	11.4
Dw15 (DR4)	6.8
Dw5	11.4
Dw18 (DRw6)	9.0
Dw19 (DRw6)	0
Dw7	20.4
Dw8	0

Data were also available on class I HLA antigens in both patients and controls. No major differences in the prevalence of antigens were detected. HLA-B37, however, was significantly more prevalent in patients with RA (8.8%) than in local Indian controls (1.4%). This may be explained by the known linkage disequilibrium between this antigen and DRw10. Interestingly, the antigen Bw62, which is in strong linkage disequilibrium with DR4 in white Europeans, was absent in the patients and present in 9.8% of controls. This may reflect an absence of DR4 haplotype associations noted in other groups.

Discussion

Previous studies of HLA associations with RA in Indians have led to some apparently conflicting findings. Notably, studies by Nichol and Woodrow showed an association with DR1,² whereas others found a prevalence of DR4 in Indian patients with RA (60–70%) similar to that described in white British patients.^{3, 4}

Findings from our study show a significantly raised prevalence of DR1, DR4, and DRw10 in a series of Indian patients with RA resident in the United Kingdom. All these antigens have been implicated in RA susceptibility in a variety of populations and, recently, the molecular basis for a shared epitope in the hypervariable region of the DR β chain of these specificities was described.²³ This indicates that the component of DR4 critical for RA development may reside in only a small region of the molecule and that this epitope can also be found in a range of other DR specificities. This is supported by the observation that 57% of our patients have at least one of the DR1, 4, or 10 types. Furthermore, an analysis of the DR4, Dw subtypes showed that an appreciable number were Dw14, and although Dw4, Dw13, and Dw15 were also present, no DR4 patient was Dw10. The prevalences of HLA-Dw4, Dw14, and Dw15 have been shown to be specifically raised in RA,^{19–21, 32} whereas Dw10 does not seem to be associated with RA, even in Jewish populations where it accounts for the major proportion of DR4 subjects.³³ This is in keeping with a shared epitope hypothesis as Dw10 differs from other DR4/Dw types within this region.

A shared DR epitope might therefore provide

a basis for explaining the range of DR specificities associated with RA, but it cannot account for the preferential association of one specificity with RA, above another, in different panels of Indian patients.

The Indian patients studied by Nichol and Woodrow² were Ugandan, largely originating from Gujarat State. Other studies have probably included a better cross sectional sample of the population in north India. This has been suggested as one possible explanation for the differences in HLA associations found. This might apply if one antigen was more prevalent in one population than in another. For example, in Japan the Dw15 variant almost completely accounts for DR4 subtypes and is associated with RA rather than others which are rare or absent. Both DR1 and DR4 were present in all of the Indian groups previously studied, however, and it is difficult to appreciate why different RA associations were found.

One possible explanation may be that populations from different geographical locations are exposed to varying environmental factors and loads. For example, if this region of the DR β_1 chain is important for the ability to present a particular antigen to T cells, subtle differences elsewhere in the β molecule of different DR types may be more or less efficient in presenting this antigen. For different strains of organisms, such as mycobacteria species, where the degree and type of exposure may vary from one area to another, such differences in DR may be important.

The lack of DRw10 association in previous studies of Indian patients with RA may be due to antigen assignment. Although this specificity was originally identified in 1980, adequate well defined antisera were not widely available until after 1984. Until this time, many DRw10 subjects were assigned as DR1 or DR blank.

It will be of interest to investigate further well defined patients from other areas of the Indian subcontinent.

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