inexpensive and provides a new technique for the diagnosis of non-immunologically mediated bronchial hyperreactivity.

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Requests for reprints should be sent to SDA.

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DR antigens and rheumatoid arthritis: a study of two populations

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Abstract

The prevalence of HLA-DR antigens was determined in a group of white patients with rheumatoid arthritis, similar patients originating from the Indian subcontinent, and corresponding controls. Rheumatoid arthritis was found to be highly associated with DR4 in the white patients but with DR1 in the Indian patients.

These results raise the possibility that the DR antigens themselves do not play a part in increasing susceptibility to rheumatoid arthritis, but the locus for increased susceptibility is probably closely linked to the DR locus.

Introduction

An association has been shown between HLA-DR4 and rheumatoid arthritis.¹⁻³ The populations studied have been largely European or of European origin. When an HLA antigen is shown to be positively associated with a disease two main possibilities arise. Firstly, the HLA antigen itself may play a direct part in producing increased susceptibility. Secondly, the association may arise because the susceptibility gene is not that determining the HLA antigen itself but is present at a locus within the HLA region and is in linkage disequilibrium with the gene for the HLA antigen. Supportive evidence for this second concept is afforded when what appears to be the same disease is associated with different HLA antigens in different ethnic populations. To elucidate this further with regard to rheumatoid arthritis we tested for their DR phenotypes two ethnically different groups of patients and corresponding healthy controls.

Patients and methods

Two separate studies were carried out and the results analysed together. In the Liverpool study DR typing was carried out in 100 consecutive white patients with seropositive erosive rheumatoid arthritis attending a rheumatology clinic. Typing was also carried out on blood samples from 100 healthy subjects (medical, nursing, and laboratory staff) living in the same geographical area.

In the Midlands study DR typing was similarly carried out in 35 patients originating from the Indian subcontinent and having seropositive rheumatoid arthritis. Typing was also performed in 42 healthy controls of the same ethnic origin living in the same geographical area.

DR typing was done in one laboratory (department of medicine, Liverpool University) by means of a cytotoxicity technique using an adaptation of the two-colour fluorescence method.⁴ The panel of sera used permitted typing for seven DR specificities. The relative risks were calculated using Haldane's modification of the Woolf method.⁵ The derived probability values (p) are twice the p values obtained from the normal probability integral, giving values almost identical with those obtained with Fisher's exact test. Taking into account that seven antigens were tested for, a significant association was associated with a p value of 0.007 or less.

Results

Table I shows the numbers of patients and controls positive for each DR antigen in the two series. In the Liverpool study there was a highly significant positive association with DR4, the relative risk of a person positive for DR4 getting rheumatoid arthritis being 4.56 ($p=0.61 \times 10^{-6}$). There was a non-significant increase in the relative risks associated with DR1 and DR3. The relative risks for DR6 and DR7 were 0.22 and 0.37 respectively, and the corresponding p values of 0.005 and 0.0067 suggest that the negative associations were real. In the Midlands study a significant positive association was found with DR1, with a relative risk of 7.0 ($p=0.0022$). There was a small non-significant increase in the prevalence of DR4. No significant negative associations were found.

Table II gives the outcome of statistical analysis of the results in the two series. There was significant heterogeneity between the two populations in respect of DR1 ($p=0.013$) and DR4 ($p=0.049$).

Discussion

The main finding in this study was the significantly different patterns of association of DR antigens with rheumatoid

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arthritis between the two ethnically different populations. In the indigenous white population rheumatoid arthritis was significantly associated with the presence of DR4 and there was evidence of a negative association with DR6 and DR7. In contrast, in the Indian immigrant population the disease was significantly associated with DR1 and not with DR4.

These findings support the concept that HLA-linked susceptibility genes for rheumatoid arthritis may not be the

patterns of association between DR phenotypes and rheumatoid arthritis argues for the susceptibility genes not being those that determine the DR antigens themselves.

Evidence is accumulating of the existence of more than one locus at which Ia molecules are determined within the D/DR region, homologous to the loci in the I region of the mouse H-2. It has recently been suggested that the DR molecules may correspond to the I-E molecules of the mouse.^{9,10} There is

TABLE I—Prevalence of DR antigens in white subjects (Liverpool study) and subjects from Indian subcontinent (Midlands study)

Antigen	Liverpool study				Midlands study			
	No of patients positive (n = 100)	No of controls positive (n = 100)	Relative risk	p	No (%) of patients positive (n = 35)	No (%) of controls positive (n = 42)	Relative risk	p
DR1	28	20	1.54	0.25	21 (60.0)	7 (16.7)	7.0	0.0022
DR2	21	25	0.80	0.61	15 (42.9)	15 (35.7)	1.3	0.69
DR3	29	20	2.19	0.19	3 (8.6)	12 (28.6)	0.3	0.05
DR4	74	38	4.56	0.61 × 10 ⁻⁶	6 (17.1)	6 (14.3)	1.2	0.98
DR5	3	11	0.28	0.05	6 (17.1)	12 (28.6)	0.5	0.36
DR6	4	17	0.22	0.005	7 (20.0)	14 (33.3)	0.5	0.29
DR7	14	31	0.37	0.0067	7 (20.0)	8 (19.0)	1.1	1.00

TABLE II—Results of statistical analysis comparing findings in the two series

	Combined χ^2	p	χ^2 for heterogeneity	p
DR1	9.93	0.016	6.15	0.013
DR2	0.03	0.86	0.84	0.36
DR3	0.1	0.76	6.66	0.01
DR4	21.32	0.39 × 10 ⁻⁵	3.89	0.049
DR5	5.26	0.022	0.67	0.4
DR6	8.48	0.004	1.32	0.25
DR7	5.35	0.02	2.66	0.10

genes determining the DR antigens themselves. Such a gene or genes may well be the same in the two different populations but have a differing pattern of linkage disequilibrium with the various alleles of the DR locus.

A positive association with DR4 has now been found in populations of wide ethnic diversity—namely, Caucasians, Japanese, and American blacks.^{3,6} Most of the studies carried out in these populations also give some evidence of a small positive association with DR1, and a combination of 14 reports^{2,3,6-8} gives a combined relative risk for DR1 of 1.46 ($\chi^2=13.4$, $p=2.5 \times 10^{-10}$; χ^2 for heterogeneity=19.5, $p=0.1$). In contrast, a small study³ of patients of Ashkenazim origin with rheumatoid arthritis gave the relative risk associated with DR1 of 8.3, a figure similar to that for the Indian Asians, with no evidence of a significant association with DR4.

When one HLA antigen is positively associated with a disease it is to be expected that the antigens determined by the other alleles of the same locus will show, to a greater or lesser degree, a negative association when compared with all other antigens. In the present study there was evidence of an appreciable negative association with DR6 and DR7 in the white patients and controls, though the pattern in the Indian population was somewhat different, with DR3 showing a relative risk of 0.3 ($p=0.005$). Analysis of various reported studies suggests that DR2 is negatively associated with rheumatoid arthritis in white Caucasian populations (though not significantly in the present study) but not in Asian, Indian, or Jewish populations. It is too early to be sure what exactly is the true pattern of negative associations in different ethnic groups.

A strong indication that the gene for B27 is the major HLA-linked gene providing increased susceptibility to ankylosing spondylitis is the fact that the association with spondylitis is present in many populations throughout the world, differing ethnically and in particular in their prevalences of the various HLA antigens. The evidence for significant differences in the

strong evidence that the products of the MCH-linked immune response genes in the mouse are the Ia glucoprotein molecules themselves,¹¹ being expressed on macrophages, B cells, and activated T cells. If, as seems likely, this also applies in man the gene product of loci in the D/DR region must have a key role in the development of the ability to discriminate self and non-self and hence in the development of an individual's immune responses. Knowledge of the key immunological events in the development of rheumatoid arthritis is at present fragmentary, and there is no evidence to point to any particular aspect of the immunological features characteristic of rheumatoid arthritis as being the most likely to be influenced by genes in the D/DR region. Better definition of other loci in the D/DR region may lead to identification of the actual genes whose product leads directly to increased susceptibility to the development of the disorder.

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