HLA haplotypes in non-familial rheumatoid arthritis

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SUMMARY The frequencies of HLA-A, B, C, DR, and BF haplotypes in 44 unrelated Caucasian patients with definite seropositive rheumatoid arthritis (RA) were compared with haplotype frequencies in controls. Overall, the patients had an increased risk for HLA-DR4, DR3, and DR2 antigens, but frequencies of certain DR4 or DR3 haplotypes were not increased, suggesting the importance of other HLA loci for the evaluation of risk. The presence of DR4 alone was not found to produce an increased risk for RA since the frequencies of certain DR4 haplotypes were similar in patients and controls. Increased frequencies of HLA-B18,DR4, HLA-B15,DR4, and HLA-A1,B8,Cw7,DR3 haplotypes were found in patients. RA susceptibility has been found to be associated with the last two haplotypes in some studies of multiple case families, suggesting that similar genetic mechanisms may underlie the disease in familial and sporadic forms.

Genetic factors have an important role in the pathogenesis of seropositive rheumatoid arthritis (RA) as shown by the strong association with HLA-DR4¹⁻⁴ and non-random sharing of haplotypes in affected relatives in multiple case families.⁵⁻⁶ The mechanism of genetic predisposition to RA, however, is still poorly understood.

Previously, we have reported positive associations between RA and HLA-DR1, DR2, and DR3 antigens (with smaller relative risks than HLA-DR4),⁷ and several studies have shown increased risks associated with other loci of the HLA region, alone or in combination with HLA-DR4.²⁻⁸⁻¹¹ An investigation of haplotype frequencies in multiple case families suggested that relative risks vary for different haplotypes carrying the same HLA-DR antigen,¹¹ but no studies of haplotype distributions in non-familial RA have been reported. To gain further insight into the association between HLA loci and RA we have undertaken a study of the distribution of HLA haplotypes in 44 unrelated seropositive patients with RA and 184 controls.

Patients and methods

Forty four unrelated patients with definite rheuma-

toid arthritis according to the criteria of the American Rheumatism Association¹² were studied. All patients were Caucasians living in the region of Paris. All had positive titres for rheumatoid factor by the Waaler-Rose ($\geq 1/32$) and latex ($\geq 1/80$) tests. Sjögren's syndrome, defined as the conjunction of an abnormal Schirmer's test and keratoconjunctivitis demonstrated by rose bengal staining, was found in 16 of the 42 patients who underwent these tests. HLA-A, B, and C antigens were identified with the conventional microcytotoxicity test. Enriched suspensions of B cells were prepared as described by Pellegrino *et al* for HLA-DR determinations.¹³ BF typing was obtained by electrophoresis followed by immunodiffusion.¹⁴ Family studies allowed haplotype determinations for all patients. The control series consisted of 184 unrelated healthy Caucasians living in the Paris area. Family studies also allowed determinations of haplotypes in controls.

The total heterogeneity χ^2 statistic¹⁵ was used to assess HLA-DR antigen and haplotype frequencies in patients and controls. The number of comparisons made is reflected in the degrees of liberty assigned to the χ^2 statistic, and therefore the significance levels are not adjusted for the number of antigens at each locus.¹⁶ The results were confirmed using a χ^2 test with Yates's correction.¹⁷ Two-locus haplotype analysis was performed for HLA-DR antigens that were significantly associated

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with RA, and alleles at the HLA-A, B, C, or BF loci when they were present for 15 or more haplotypes. Haplotype association was deemed to be present when third order terms were significant in the log-linear model.¹⁵ Association with specific haplotype combinations was evaluated using deviations from expected and observed values¹⁵ and confirmed with Fisher's exact test or a χ^2 test with Yates's correction.

Results

Table 1 shows the HLA-DR frequencies in patients with RA and in controls. The distributions differed significantly in the two groups ($\chi^2_8=45.97$, $p<0.001$). The control group had gene frequencies similar to those reported for other European populations.¹⁸ The strong association between RA and HLA-DR4 may mask associations with other antigens,⁷ and thus the distributions were also compared when HLA-DR4 had been removed. The test for heterogeneity was again significant ($\chi^2_8=22.31$, $p<0.005$). In addition to HLA-DR4, both HLA-DR3 and HLA-DR2 were significantly increased in patients. Risks for these antigens relative to others were 6.81 ($p<0.0001$), 3.95 ($p<0.001$), and 2.84 ($p<0.005$) respectively. There was no heterogeneity of risk for the other HLA-DR antigens once the associations with HLA-DR4, DR3, and DR2 had been taken into account ($\chi^2_7=2.42$, $p=0.92$).

The frequencies of two locus haplotypes were assessed by direct counting for HLA-A,DR, HLA-B,DR, HLA-C,DR, and BF,HLA-DR. Risks for haplotypes carrying the same HLA-DR antigen were heterogeneous for HLA-A,DR ($\chi^2_{26}=40.20$, $p<0.05$), HLA-B,DR ($\chi^2_{26}=50.78$, $p<0.01$), and HLA-C,DR ($\chi^2_{16}=25.64$, $p<0.01$), suggesting that other HLA loci are important in assessing the risk

Table 2 Distribution of HLA haplotypes in patients (88 haplotypes) and controls (368 haplotypes) typed for HLA-A, B, C, and DR

HLA	Patients with RA* (n=44) No (%)	Controls* (n=184) No (%)	Significance (p value)
B18,DR4	4 (4.5)	0 (0.0)	$p<10^{-5}$
B15,DR4	5 (5.7)	1 (0.3)	$p<10^{-5}$
B27,DR4	0 (0.0)	6 (1.7)	NS
Cw2,DR4	0 (0.0)	6 (1.7)	NS
Other DR4	21 (23.9)	24 (6.4)	$p<10^{-6}$
A1,B8,Cw7,DR3	13 (14.8)	6 (1.7)	$p<10^{-6}$
Other DR3	7 (8.0)	37 (10.2)	NS
DR2	19 (21.6)	57 (15.7)	$p<0.01$
Other DR	19 (21.6)	227 (62.7)	

*Compared with haplotypes not carrying HLA-DR4, DR3, or DR2 (other DR in table).

associated with a given HLA-DR antigen. No significant heterogeneity was found for BF,HLA-DR haplotypes ($\chi^2_7=7.45$, $0.5<p<0.7$).

Only one DR3 haplotype, HLA-A1,B8,Cw7,DR3, was significantly increased in patients. This haplotype had a risk of 17.2 ($p<10^{-6}$) relative to haplotypes not carrying DR4, DR3, or DR2, whereas the relative risk for other HLA-DR3 haplotypes was 1 ($p=0.45$). The HLA-A1,B8,Cw7,DR3 haplotype was not associated with an increased frequency of Sjögren's syndrome, occurring six times in 16 RA patients with this complication, and eight times in the remaining 27 patients that were examined ($p=0.42$).

We found that haplotypes carrying HLA-DR4 and B27 or Cw2 had no increased risk of disease when compared with haplotypes not carrying HLA-DR4, DR3, or DR2 ($p=0.60$ in both cases). In contrast, haplotypes carrying HLA-B18,DR4 or HLA-B15,DR4 may have a higher risk than other HLA-DR4 haplotypes (including frequent haplotypes such as HLA-B40,DR4 and HLA-B44,DR4, even when HLA-B27,DR4 and HLA-Cw2,DR4 are excluded from the comparisons ($p=0.06$ and $p=0.02$ respectively in a one sided test).

A summary of these haplotype associations is given in Table 2.

Discussion

These results show that haplotype combinations involving several loci of the HLA region have a substantial influence on risk in non-familial RA. From our data it appears that the presence of HLA-DR4 alone does not produce an increased risk of disease as the frequency of haplotypes bearing HLA-DR4 and B27 or Cw2 is not increased. On the

Table 1 Distribution of HLA-DR alleles in rheumatoid arthritis patients (88 haplotypes) and controls (368 haplotypes)

	Patients with RA (n=44) No (%)	Controls (n=184) No (%)
DR1	4 (4.2)	29 (7.9)
DR2	19 (19.8)	57 (15.5)
DR3	20 (20.8)	43 (11.7)
DR4	30 (31.3)	37 (10.1)
DR5	8 (8.3)	63 (17.2)
DRw6	2 (2.1)	24 (6.5)
DR7	5 (5.2)	52 (14.2)
DRw8	0 (0.0)	6 (1.6)
DRw9	0 (0.0)	1 (0.3)
'Blank'	8 (8.3)	55 (15.0)

other hand, HLA-B18,DR4 and HLA-B15,DR4 may have greater risks for RA than other HLA-DR4 haplotypes, including common haplotypes such as HLA-B40,DR4 and HLA-B44,DR4. Interestingly, cosegregation of HLA-B15,DR4 with RA susceptibility has also been reported in patients with RA from multiple case families.¹¹

The frequency of the haplotype HLA-A1,B8,Cw7,DR3 is increased in the patients studied here, with a relative risk that is statistically indistinguishable from that for DR4 alone ($p=0.12$). In several multiple case families disease susceptibility has been found to follow this haplotype.^{6,19} In a previous study we have shown that HLA-A1,B8,Cw7,DR3 is strongly associated with intolerance to gold salts in patients with RA.²⁰ Because of the increased frequency of this haplotype in RA it seems that patients with RA are unfortunately at high risk for gold salt intolerance. A similar finding may apply to toxic reactions to D-penicillamine therapy.^{20,21} Our data confirm previous reports that the frequency of the A1,B8,Cw7,DR3 haplotype is the same in RA patients with and without Sjögren's syndrome, whereas this haplotype has an increased frequency in patients with primary Sjögren's syndrome.^{22,23} Other haplotype combinations involving DR3 have no significantly increased risk for RA. We have previously reported the association of HLA-DR2 with an increased risk for RA in a larger series of patients.⁷

Our results support the hypothesis that susceptibility to RA is controlled by a gene, or a group of genes, in the HLA region of chromosome 6, and show that only certain haplotypes carrying HLA-DR4 or DR3 are at increased risk. The latter finding could be explained by linkage disequilibrium between the susceptibility gene(s) and the HLA loci. The variety of associations between HLA and RA in various ethnic groups reported in the literature also supports this hypothesis. Finally, the similarity between haplotypes associated with increased risk in our study, and those frequently described in multiple case families suggests that similar genetic mechanisms underlie familial and non-familial RA.

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