

SCIENTIFIC PAPERS

HLA-DR antigens and HLA-DQ β chain polymorphism in susceptibility to rheumatoid arthritis

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Abstract

Forty four patients with rheumatoid arthritis (RA) were studied for HLA-DR antigens and for HLA-DQ β chain gene restriction fragment length polymorphism using DNA hybridisation. A significant increase in the prevalence of the DR4 antigen and a tendency towards an increase of DR1 was found in patients with RA. No allelic form of HLA-DQ restriction fragment length polymorphism patterns was increased, but the prevalence of an allele characterised by a combination of 7.5 and 3 kb fragments was decreased among patients with RA. The DQw8 subtype represented by a 12 kb fragment was the most common DR4 associated allele, and a 3.7 kb fragment related to DQw7 was found in only 5/25 (20%) DR4 positive patients and 2/12 (17%) controls. The results support the hypothesis that RA susceptibility factors are primarily located within HLA-DR genes but HLA-DQ genes may have a role in protection from the disease.

Susceptibility to rheumatoid arthritis (RA) is associated with the presence of the HLA-DR4 antigen.^{1,2} This antigen is closely associated with HLA-DQw3 specificity, which can be divided into 3.1 (DQw7) and 3.2 (DQw8) alleles.³ This dichotomy may be used to define further disease susceptibility genes located in the HLA area. The strong association of, for example, insulin dependent diabetes with the DQw8 allele^{4,5} suggests that determinants in the HLA-DQ β chain are of major importance in disease susceptibility.

The association of RA with different DQw3 subtypes seems less clear. Strong association with the DQw7 allele has been reported, especially in severe disease,^{6,7} but there are also studies in which an equivocal or even a controversial association with the DQw8 allele has been detected.⁸⁻¹⁰ In our study a group of 44 Finnish patients with RA was analysed for HLA-DR and DQ alleles in an attempt to clarify the location of the HLA area susceptibility genes. In addition to standard serological HLA typing defining DR specificities, DNA was isolated from blood samples and studied using a short DQ- β intervening sequence I probe.⁵

Patients and methods

PATIENTS AND CONTROLS

Forty four consecutive patients with recently diagnosed definite or classical RA were included in the study.¹¹ One hundred and ninety six healthy laboratory staff members and blood donors from Oulu served as controls.

HLA-DR TYPING

Mononuclear cells were isolated from heparinised peripheral blood by Lymphoprep (Nygard, Oslo, Norway) gradient centrifugation and B lymphocytes further enriched by depletion of T cells with 2-aminoethylisothiuronium bromide treated sheep red blood cells and monocytes by plastic adherence. HLA-DR antigens were determined from B cells by a standard two stage microlymphocytotoxicity method and commercial tissue typing trays (Biotest AG, Frankfurt/M, FRG).

RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS BY DNA HYBRIDISATION

The HLA-DQ β gene intervening sequence I specific probe was constructed from a BamHI 3.7 kb fragment corresponding to the 166 base pairs of the first intron sequence of the HLA-DQ β chain gene.⁵

For DNA analysis leucocytes were separated from EDTA-blood samples by red cell haemolysis (155 mM NH₄Cl, 10 mM KHCO₃, 0.1 mM EDTA, pH 7.4). The white cell pellet was suspended in TRIS-EDTA (TE) buffer and stored at -80°C. For analysis the cells were lysed at 37°C overnight (2 mM TRIS, 0.4 mM EDTA, 2 mM NaCl, 1% sodium dodecyl sulphate (SDS), 0.4% proteinase K), then extracted by phenol and chloroform. DNA was precipitated from the aqueous phase with ethanol. The air dried pellet was dissolved in an appropriate volume of TE buffer. DNA (10 μ g) was digested with BamHI according to the manufacturer's specifications (Boehringer Mannheim) and electrophoresed in 0.6% agarose gel at 40 V overnight. After alkaline denaturation the DNA was transferred to the nylon filter (Hybond-N, Amersham) according to the method of Southern, dried, and fixed with ultraviolet light for three minutes.

The purified intervening sequence I probe was labelled by α -³²P labelled dCTP by nick

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translation to a specific activity of $3-5 \times 10^8$ cpm/ μ g. Hybridisations were performed at 42°C overnight in the presence of 50% formamide and 10% dextran sulphate. After subsequent washings (2×10 minutes, at room temperature in $2 \times$ SSC, 0.1% SDS and 3×20 minutes at 55°C in $0.1 \times$ SSC, 0.1% SDS) the filters were exposed to x rays for three days at -70°C with intensifying screens.

Results

HLA-DR typing results showed a significantly increased prevalence of the DR4 antigen in patients with RA ($p < 0.001$, χ^2 test) (table 1). An increase of the DR1 antigen was also detected, but this did not reach significance ($p < 0.1$).

None of the alleles defined by restriction fragment length polymorphism analysis of the HLA-DQ β chain gene was significantly increased in patients with RA (table 2). The prevalence of the 7.5/3.0 kb fragment combination was in fact lower in the group with RA than in the controls ($p < 0.05$). This DQ β allele is associated with both DR2 and DRw6 antigens. The difference was no more significant after multiplication by the number of alleles compared.

The two DQw3 subtypes defined by the presence of 3.7 kb (DQw7) and 12 kb (DQw8) fragments were equally common in DR4 positive patients with RA and controls (table 3). HLA-DR phenotypes and restriction fragment length polymorphism patterns suggested in all DR4 positive patients that 12 kb or 3.7 kb fragments detected were associated with DR4. In only two cases was another DR specificity associated with the 12 kb fragment—DR8 is found together with DR4 and both cases were apparently homozygous for the 12 kb fragment. In DR4 positive controls the segregation of 12 kb and

Table 3: HLA-DQw3 subtypes associated with HLA-DR4 antigen in patients with rheumatoid arthritis (RA) and in controls

Fragment size (kb)	DR4 positive patients with RA (n=25)		DR4 positive controls (n=12)	
	No	(%)	No	(%)
12.0 (HLA-DQw8)	19	(76)	10	(83)
3.7 (HLA-DQw7)	5	(20)	2	(17)

3.7 kb fragments with DR4 haplotype was confirmed by family analysis.

Discussion

Results of this study support the hypothesis that the primary association of RA susceptibility within the human HLA class II gene area is with HLA-DR genes. The prevalence of DQ β chain gene alleles associated with DR4 did not significantly differ between patients with RA and controls. The primary association of RA with the HLA-DR gene area is strongly implicated also by the differential association of mixed lymphocyte culture defined subtypes of DR4 with RA susceptibility.¹⁰ RA associated Dw4 and Dw14 molecules share a similar amino acid sequence with the Dw1/DR1 antigen.¹⁰ This antigen has been found in association with RA in some ethnic groups,^{12 13} and marginally also together with DR4 in a white population.¹⁴ A common determinant in the association of HLA-DR1 and DR4 with RA has been recognised also by serology and cellular typing.¹⁵ The DR β chain gene sequence coding this area has a homologous sequence with the Epstein-Barr virus genome, which suggests a possible role for this virus in the cause of RA.

Prevalence of the DR1 antigen was only marginally increased in this study. If DR1, Dw4, and Dw14 associations of the disease are explained by a common shared susceptibility determinant it is difficult to explain why the association with Dw4 and Dw14 antigens dominates in white populations.

The prevalence of the Dw2/DR2 antigen has been found to be decreased in several studies on RA,¹⁶⁻²⁰ including the earlier studies in Finland.^{19 20} Although in our small patient group the prevalence of the DR2 antigen was similar to that in the control population, the DNA hybridisation analysis of the HLA-DQ β chain gene detected that the 7.5/3 kb combination of restriction fragments was significantly decreased in the same patients. This allelic restriction fragment length polymorphism type is strongly associated with DR2 but also found in most DRw6 positive haplotypes and some other haplotypes.²¹ This suggests that there may be some protective factors which are primarily associated with the HLA-DQ gene area. It has to be noted that the disease susceptibility alleles of patients with insulin dependent diabetes mellitus localised to the HLA-DQ β chain gene seem actually to represent protective determinants. The presence of aspartic acid in position 57 of the polypeptide chain seems to protect against the disease.^{22 23}

Table 1: Prevalence of HLA-DR antigens in patients with rheumatoid arthritis (RA) and in controls

Antigen	Patients with RA (n=44)		Controls (n=196)		Significance	RR
	No	(%)	No	(%)		
DR1	20	(45)	60	(31)	$p < 0.1$	1.89
DR2	13	(30)	49	(25)		
DR3	4	(9)	31	(16)	$p < 0.001$	3.90
DR4	26	(59)	53	(27)		
DR5	4	(9)	21	(11)		
DRw6	3	(7)	35	(18)		
DR7	4	(9)	26	(13)		
DRw8	4	(9)	35	(18)		
DR9	2	(5)	12	(6)		

Table 2: Prevalence of HLA-DQ β alleles detected by restriction fragment length polymorphism after BamHI digestion. Hybridisation made with intervening sequence I probe

Fragment size (kb)	Patients with RA (n=44)		Controls (n=118)		Significance	RR
	No	(%)	No	(%)		
12.0	28	(64)	58	(49)	$p < 0.05$	0.45
7.5/3.0	13	(30)	57	(48)		
4.0	8	(18)	30	(25)		
3.7	8	(18)	18	(15)		
3.2	9	(20)	34	(29)		
2.8	1	(2)	0	(0)		
Others	4	(9)	4	(3)		

Protective factors on the HLA-DQ area may affect the expression of DR related susceptibility alleles. This may especially be the case in DR1 associated disease risk, and an analysis of HLA-DR1 positive haplotypes for their HLA-DQ gene area might be informative. This analysis should include comparison of DR1 positive RA and control haplotypes in different ethnic groups. Our own preliminary findings show some heterogeneity within HLA-DR1 positive haplotypes in the control population (Reijonen *et al.*, unpublished).

Our finding of a protective role for HLA-DQ alleles is based on a small number of patients and should be confirmed by larger studies. The results suggest, however, that the DQ area analysis in RA should be directed also towards indicating factors which protect from the disease.

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