Immunoglobulin heavy chain constant region variants in rheumatoid arthritis

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SUMMARY Polymorphisms within the immunoglobulin heavy chain constant region at switch ($S\mu$) and $S\alpha 1$ loci were studied in patients with rheumatoid arthritis (RA) and controls in parallel with Gm and A2m allotyping. $S\mu$ and $S\alpha 1$ restriction fragment length polymorphisms were defined using a $S\mu$ probe which was hybridised to SstI digests of DNA extracted from circulating white cells. There were no differences in $S\mu$ and $S\alpha 1$ gene or phenotype frequencies between RA and control groups nor within the RA population between DR4 positive and negative subjects. The Gm allotype G1m(x), coded for by genes at the gamma-1 locus, was found in 29/92 (32%) of DR4 positive and 5/52 (10%) of DR4 negative subjects with RA, as compared with 25/115 (22%) of controls. The A2m allotype A2m(2), coded for by the $\alpha 2$ locus, was found in 11/92 (12%) of DR4 positive subjects with RA, zero of 52 DR4 negative subjects, and 5/115 (4%) of controls. These results exclude a major effect of genes within the heavy chain constant region linked to μ or $\alpha 1$ loci on susceptibility to RA, but suggest that further study of variants closely linked to the μ locus is indicated.

There is a genetic predisposition to rheumatoid arthritis (RA), which from family studies appears only partly accounted for by genes within the HLA region.¹⁻² The immunoglobulin heavy chain region locus on chromosome 14 is one of several other 'candidates' which have been proposed to contribute to this susceptibility, but the results of studies of associations between RA and the immunoglobulin heavy chain allotype Gm have been conflicting. Most but not all studies have failed to find any independent effect of Gm on susceptibility to RA,³⁻⁴ whereas three studies have reported an association between a G1m(x) bearing phenotype and

DR4 positive but not DR4 negative RA in white European populations,⁵⁻⁷ but a fourth has not.⁸ Two further studies have reported an association with a different Gm phenotype in a subset of RA characterised by circulating antibodies to native type II collagen, and this effect also appeared to be dependent on DR status.⁹⁻¹⁰ As shown in Fig. 1 the immunoglobulin heavy chain constant region includes μ , δ , gamma-3, gamma-1, pseudo-epsilon-1, alpha-1, pseudo-gamma, gamma-2, gamma-4, epsilon, and alpha-2 loci, and there is linkage disequilibrium between variants coded for by genes within the two main blocks of loci from gamma-3 to gamma-2. Each constant region locus apart from delta is preceded by a switch locus responsible for switching class of immunoglobulin during antibody synthesis. Gm allotypes are coded

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Fig. 1 Arrangement of loci in the immunoglobulin heavy chain constant region. Arrows indicate loci coding for Gm allotypes (G1m, G2m, G3m), A2m allotype, and for the $S\mu$ and $S\alpha 1$ loci. The switch loci are immediately adjacent to the particular constant regions. The variable region is telomeric to the constant region.

for by gamma-1, gamma-2, and gamma-3 loci and the A2m allotype carried on IgA2 molecules is coded for by the $\alpha 2$ locus. The possible effects on disease susceptibility of genes linked to other loci within the immunoglobulin heavy chain constant region may now be sought by studying restriction fragment length polymorphisms obtained with a range of DNA probes.¹¹ In this study we have compared polymorphisms at $\Sigma\mu$ and $\Sigma\alpha 1$ loci in patients with RA and controls in parallel with the results of Gm and A2m allotyping.

Patients and methods

PATIENTS

Serum, DNA, or whole blood samples for subsequent DNA extraction were stored from a group of 177 unrelated subjects with classical or definite RA.¹² All were white and were living in the north-west of England. DNA samples were available from 126, serum samples for Gm allotyping from 144, and both DNA and serum samples were available from 93. The results of Gm allotyping have previously been reported in 98 of the subjects with RA.⁶ The local control group included 135 subjects, with 115 providing samples for Gm allotyping, 84 providing DNA samples, and 64 providing both DNA and serum samples.

IMMUNOGLOBULIN ALLOTYPING

Gm and A2m allotypes were detected by haemagglutination inhibition assay of serum samples.¹³ The following allotypes were defined: G1m(z, a, x, f), G2m(n), G3m(b0, b1, b3, b5, g, c3, c5, s, t), and A2m(1,2).¹³ The A2m(2) allotype is uncommon in European populations and is found mainly in linkage disequilibrium with Gm haplotypes zag and zaxg.

HLA TYPING

HLA typing results were available in 108 of the subjects with RA and were used to subdivide the patients into DR4 positive and DR4 negative groups. Methods were as previously described.⁶

DNA STUDIES

DNA was extracted from circulating white cells and stored at -20°C or -70°C . After digestion with *Sst*I fragments were separated by agarose gel electrophoresis (0.7% agarose gels, 1 volt/cm for 20 hours) and transferred to nylon filters (Amersham Hybond N) by the Southern blotting technique.¹⁴ A 2.2 kb $\Sigma\mu$ probe was used which was derived from the phage clone H24.¹⁵ This probe cross hybridises to other switch loci and when hybridised to *Sst*I DNA digests shows polymorphisms at both $\Sigma\mu$ and $\Sigma\alpha 1$

loci.¹¹ The probe was radiolabelled by oligonucleotide primed synthesis.¹⁶ Filters were incubated for two hours at 65°C in prehybridisation solution (6 $\times$ SSC, 5 $\times$ Denhardtts, 200 $\mu\text{g/ml}$ denatured salmon sperm DNA, 0.5% sodium dodecyl sulphate (SDS), followed by hybridisation solution (similar to prehybridisation solution with the addition of 10% dextran sulphate and 100 ng of denatured probe DNA). Filters were hybridised for 16–20 hours at 65°C . Filters were washed at 65°C in 2 $\times$ SSC/0.1% SDS followed by 0.2 $\times$ SSC/0.1% SDS.

Polymorphic bands at 7.4, 6.9, 6.8, 7.1, and 7.0 kilobases were detected at the $\Sigma\alpha 1$ locus and polymorphic bands of 2.7, 2.2, 2.6, and 2.75 kilobases at the $\Sigma\mu$ locus (Fig. 2). Accurate assignment of bands at the $\Sigma\alpha 1$ locus proved to be difficult initially because of the 100 base pairs difference between each of four variants. This problem was overcome where appropriate by running samples at 2 V/cm for 40 hours in 0.7% agarose with human marker DNAs of known sizes. Separation of $\Sigma\mu$ polymorphisms proved adequate with the standard electrophoretic conditions.

STATISTICAL ANALYSIS

The significance of differences in phenotype and allotype frequencies was analysed by χ^2 or Fisher's

Fig. 2 Autoradiograph after hybridisation of $\Sigma\mu$ probe to *Sst*I digested DNA from normal subjects. Polymorphic bands at $\Sigma\mu$ and $\Sigma\alpha 1$ loci are indicated. Most other bands shown represent cross hybridisation of the probe to other switch regions.

exact test where appropriate. The significance of linkage disequilibrium between variants at different loci was also determined by χ^2 analysis.¹⁷

Results

Tables 1 and 2 show the gene and phenotype frequencies at the *S_u* locus. There were no statistically significant differences between RA and control groups. There was no linkage disequilibrium between *S_u* and Gm variants.

Similarly, Tables 3 and 4 show allele and phenotype frequencies at the *Sal* locus. Once again there were no statistically significant differences between RA and control groups. Allele frequencies in controls were similar to those previously reported.¹¹ Also as previously reported¹¹ the 6.9 kb and 6.8 kb fragments were in linkage disequilibrium with Gm haplotypes zag and zaxg, while the 7.4 kb fragment was in linkage disequilibrium with Gm haplotypes fb

and fnb as calculated from the formula of Mattiuz *et al.*¹⁷

There were no significant differences in Gm or A2m allotype frequencies between patients with RA overall and controls as reported previously.⁶ Thirty-eight of the controls were DR4 positive and 77 DR4 negative. There were no significant differences in G1m(x) or A2m(2) allotype frequencies between DR4 positive and negative control groups (G1m(x) 0.16 v 0.25 and A2m(2) 0.08 v 0.03 in DR4 positive and DR4 negative subjects respectively). Controls were therefore pooled in the subsequent analysis. The frequency of the allotype G1m(x) in DR4 positive RA was greater than in both DR4 negative RA and controls, though the differences compared with the control group studied here were not statistically significant (Table 5). The frequency of the A2m(2) allotype was also increased in DR4 positive RA, but here the differences were statistically significant compared with both DR4 negative RA and overall control groups.

Table 1 Allelic designations and frequencies for *S_u* in patients with rheumatoid arthritis and in controls (frequencies in parentheses)

	Molecular length (kb) of <i>S_u</i> alleles			
	2.7	2.2	2.6	2.75
Controls (n=176)	78 (0.44)	91 (0.52)	1 (0.005)	6 (0.03)
URAs* (n=252)	123 (0.48)	118 (0.47)	2 (0.008)	9 (0.04)
DR4+ RA* (n=138)	59 (0.43)	70 (0.50)	1 (0.007)	8 (0.06)
DR4- RA (n=82)	45 (0.55)	36 (0.44)	—	1 (0.01)

*URAs represent pooled results for DR4+, DR4-, and non-tissue-typed patients with rheumatoid arthritis; RA=rheumatoid arthritis.
Differences were not statistically significant.

Table 3 Allelic designations and frequencies for *Sal* in patients with rheumatoid arthritis and in controls (frequencies in parentheses)

	Molecular lengths (kb) of <i>Sal</i> alleles				
	7.4	6.9	6.8	7.1	7.0
Controls (n=168)	105 (0.63)	33 (0.20)	26 (0.16)	2 (0.01)	2 (0.01)
URAs* (n=252)	165 (0.66)	47 (0.19)	37 (0.15)	1 (0.004)	2 (0.008)
DR4+ RA* (n=136)	85 (0.63)	28 (0.20)	21 (0.15)	—	2 (0.015)
DR4- RA (n=80)	55 (0.69)	10 (0.13)	14 (0.18)	1 (0.01)	—

*URAs represent pooled results for DR4+, DR4-, and non-tissue-typed patients with rheumatoid arthritis; RA=rheumatoid arthritis.
Differences were not statistically significant.

Table 2 Phenotypic designations and frequencies for *S_u* in patients with rheumatoid arthritis and in controls (frequencies in parentheses)

	Molecular length (kb) of <i>S_u</i> phenotypes						
	2.7	2.7/2.2	2.2	2.7/2.6	2.75/2.7	2.75/2.2	2.6/2.2
Control (n=88)	23 (0.26)	29 (0.33)	29 (0.33)	1 (0.01)	2 (0.02)	4 (0.045)	—
URAs* (n=126)	35 (0.28)	48 (0.38)	32 (0.25)	1 (0.008)	4 (0.03)	5 (0.04)	1 (0.008)
DR4+ RA* (n=69)	14 (0.20)	27 (0.40)	19 (0.28)	—	4 (0.06)	4 (0.06)	1 (0.014)
DR4- RA (n=41)	14 (0.34)	17 (0.41)	9 (0.22)	—	—	1 (0.02)	—

*URAs represent pooled results for DR4+, DR4-, and non-tissue-typed patients with rheumatoid arthritis; RA=rheumatoid arthritis.
Differences were not statistically significant.

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Table 4 Phenotypic designations and frequencies with *Sa1* in patients with rheumatoid arthritis and in controls (frequencies in parentheses)

	Molecular length (kb) of <i>Sa1</i> phenotypes								
	7.4	7.4/6.9	7.4/6.8	7.4/7.0	7.4/7.1	6.9	6.8	6.9/6.8	7.1/6.9
Controls (n=84)	31 (0.37)	20 (0.24)	19 (0.23)	2 (0.02)	2 (0.02)	6 (0.007)	3 (0.035)	1 (0.01)	—
URAs* (n=126)	54 (0.43)	27 (0.21)	28 (0.22)	2 (0.015)	—	9 (0.07)	4 (0.03)	1 (0.007)	1 (0.007)
DR4+ RA* (n=68)	29 (0.43)	13 (0.19)	12 (0.18)	2 (0.03)	—	7 (0.10)	4 (0.06)	1 (0.015)	—
DR4- RA (n=40)	18 (0.45)	5 (0.13)	14 (0.35)	—	—	2 (0.05)	—	—	1 (0.025)

*URAs represent pooled results for DR4+, DR4-, and non-tissue-typed patients with rheumatoid arthritis; RA=rheumatoid arthritis. Differences were not statistically significant.

Table 5 Immunoglobulin Gm and A2m allotype frequencies in rheumatoid arthritis (RA) and control groups (frequencies in parentheses)

	DR4 positive RA (n=92)	DR4 negative RA (n=52)	Controls (n=115)
G1m(x)	29 (0.32)*	5 (0.1)	25 (0.22)
A2m(2)	11 (0.12)†	0 (0)	5 (0.04)

*p=0.003 v DR4 negative RA (Fisher's exact test).
†p=0.006 v DR4 negative RA; p=0.038 v controls (Fisher's).

Discussion

The results of some previous studies based mainly on Gm allotyping⁵⁻⁷ and more recently on the study of DNA variants¹⁷ have suggested that genes linked to the immunoglobulin heavy chain constant region loci may have an effect on susceptibility to RA. We have attempted to examine this possibility further by studying polymorphisms at *Su* and *Sa1* loci in parallel with a study of Gm and A2m allotypes. There is linkage disequilibrium between the 6.9 kb *Sa1* variant and Gm haplotypes zag and zaxg and between the 7.4 kb *Sa1* fragment and Gm haplotypes fnb and fb.¹¹ In our study there are no statistically significant differences in *Su* and *Sa1* frequencies between RA, either DR4 positive or DR4 negative, and control groups. These results contrast with those of Sakkas and coworkers,¹⁸ who did not distinguish between 6.9 kb and 6.8 kb *Sa1* fragments, but reported a decreased frequency of their '6.9 kb' homozygotes in a group of London Euroid subjects with RA. The main differences between the two studies lie in the control frequencies of '6.9 kb' homozygotes with the London group reporting 19.1% in controls, whereas in our study the equivalent frequency is between 10% and 11% for both RA and control subjects (pooling 6.9 kb, 6.8 kb, and

6.8 kb/6.9 kb phenotypes). It is possible in this study that with larger numbers some of the minor differences seen between patients with RA and controls may become statistically significant, but our results make it unlikely that genes closely linked to μ or $\alpha 1$ loci have any major effect on susceptibility to RA.

As previously reported the frequency of the G1m(x) allotype (gamma-1 locus) is greater in DR4 positive RA than in DR4 negative RA and controls, though with the numbers reported here the differences compared with controls fail to reach statistical significance. In this study, however, we report a stronger association between RA and the IgA2 allotype, A2m(2), which is found in linkage disequilibrium with Gm haplotypes zaxg and zag. Both G1m(x) and A2m(2) are found in only a minority of subjects with RA, and these associations are likely to represent linkage disequilibrium with susceptibility gene(s) elsewhere in this region of chromosome 14 rather than any direct effect. These associations could represent linkage disequilibrium with variable region genes. There is evidence, however, for a recombination hotspot between variable and constant regions,¹⁹ so that the possibility of a direct contribution of genes within the heavy chain constant region on disease susceptibility cannot be discounted. In view of the association now described between RA and A2m(2) study of further variants closely linked to the $\alpha 2$ locus would be of interest.

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