Immunoglobulin lambda light chain genes in rheumatoid arthritis

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SUMMARY Restriction fragment length polymorphisms (RFLPs) obtained by hybridisation of an immunoglobulin λ constant region probe to Eco RI digests of genomic deoxyribonucleic acid (DNA) obtained from rheumatoid arthritis (RA) and control subjects have been compared. Polymorphic bands of 8, 13, 18, and 23 kb (kilobases) were shown. The 8/8 genotype and 8 kb allele were increased and the 8/18 genotype and 18 kb allele decreased in the RA group. This effect was independent of HLA and Gm. These findings suggest that genes linked to the loci for the immunoglobulin λ constant region may influence susceptibility to RA.

Key words: immunogenetics, RFLPs, genetic predisposition.

Rheumatoid arthritis (RA) results from an interaction between an oligogenic susceptibility and unknown environmental factors.¹ Part of this genetic susceptibility is accounted for by genes within the major histocompatibility complex (MHC). An estimate of the proportion of the total genetic predisposition to RA contributed by genes within the MHC can be calculated from knowledge of the concordance rates in monozygotic and dizygotic twins and the relative frequencies with which affected sibling pairs share both or no haplotypes.² When concordance¹ and haplotype sharing figures³ currently available are used the genes linked to HLA can be estimated to contribute less than 50% of the total genetic contribution to RA and possibly as little as 20–30%. Other potential candidates as susceptibility genes for RA include genes coding for immunoglobulin heavy chains and κ and λ light chains on chromosomes 14, 2, and 22 respectively. Previous studies have shown a population association between RA and particular allotypes of the immunoglobulin heavy chain marker, Gm.^{4–5} This effect appears relatively weak, however, probably dependent on an interaction with genes within the MHC, and still leaves a considerable proportion of the total genetic predisposition to RA unaccounted for. Clinical investigation of the

immunoglobulin light chain loci has been difficult in the past as there are no allotypic λ markers and polymorphism of Km, an allotype on the constant region of the κ light chain, is limited. Even with the limited κ polymorphism, involvement of genes linked to the κ light chain loci has been suggested by population associations found between Km₁ and immunological response to bacterial antigens⁶ as well as associations between Km₁ and SSB auto-antibody formation in patients with connective tissue disease.⁷ Possible involvement of immunoglobulin light chain genes in susceptibility to disease may now be investigated by the use of appropriate immunoglobulin light chain DNA probes. In the present study, by comparing restriction fragment length polymorphisms (RFLPs) obtained by hybridisation of an immunoglobulin λ constant region probe⁸ to restriction endonuclease digests of genomic DNA obtained from RA and control subjects, we have looked for evidence that genes linked to the immunoglobulin λ constant or junctional (J) region loci on chromosome 22 may influence susceptibility to RA.

Patients and methods

PATIENTS

One hundred and eight unrelated patients with classical or definite RA⁹ and 104 unrelated controls

Accepted for publication 17 March 1987.

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Fig. 1 Autoradiograph showing 8 kb, 13 kb, 18 kb, and 23 kb bands obtained with the immunoglobulin C λ probe. Thus lanes 1, 3, 4, 7, 8, 9, 13, and 14 are 8/8 homozygotes, lane 11 an 8/13 heterozygote, lanes 2, 6, 10, and 12 are 8/18 heterozygotes, and lane 5 an 8/23 heterozygote. In some of the strongly hybridising lanes, weak non-polymorphic bands at 14 kb and 16 kb are seen which may be disregarded. These represent partial homology between the probe used and adjoining Eco RI fragments flanking the central polymorphic sequence within the C λ region.

were studied. Both patients and controls were Caucasians living in north west England.

LABORATORY

DNA was extracted from circulating white cells.¹⁰ After digestion with Eco RI, fragments were separated by agarose gel electrophoresis and transferred to nitrocellulose or nylon (Hybond N) filters.¹¹ An immunoglobulin C λ probe⁸ was kindly donated by Professor Leder and has previously been shown to demonstrate polymorphism in the λ constant region.¹² This polymorphism depends on insertion of additional base sequences between Eco RI restriction sites. A 5th Bam HI fragment from plasmid P1A5 was used which contains flanking J region sequences. The probe was radiolabelled by oligonucleotide-primed synthesis¹³ and hybridised under conditions previously described¹² to the filters to show polymorphic bands of 8, 13, 18, and 23 kilobases (kb) (Fig. 1).

Results

The restriction fragment genotype frequencies in RA and control group are shown in Table 1. The overall distributions of genotype frequencies in RA and control groups were different, and there was a significant increase in the 8/8 genotype frequency and decrease in the 8/18 genotype frequency in the RA group (the latter p values are uncorrected for the number of comparisons made). Genotype frequencies were similar in DR4 positive and negative RA groups, and there was no evidence of an interaction between immunoglobulin λ variants and Gm.

Table 1 Human C λ immunoglobulin locus RFLP genotype frequencies in RA and control groups (non-RA)

Group	Genotype					
	8/8	8/13	8/18	8/23	18/18	13/18
Controls (n=104)	70	4	25	3	1	1
RA (n=108)	90	4	14	0	0	0

* $\chi^2=7.350$; $p<0.01$.

† $\chi^2=4.329$; $p<0.05$.

Overall χ^2 comparing 8/8, 8/13, 8/18, and other genotypes in RA and control groups: $\chi^2=10.531$; $p<0.025$.

Table 2 Allele frequencies in RA and control groups

Group	Allele			
	8 kb	13 kb	18 kb	23 kb
Controls (n=104)	172	5	28	3
RA (n=108)	198	4	14	0

* $\chi^2=7.679$; $p<0.01$.

† $\chi^2=5.785$; $p<0.025$.

Overall χ^2 comparing 8, 13, 18, and 23 kb alleles in RA and control groups: $\chi^2=9.457$; $p<0.025$.

Gene frequencies are shown in Table 2. There was a significant increase of the 8 kb allele (the most striking effect statistically) and a decrease of the 18 kb allele in the RA group (the latter p value loses statistical significance if corrected for the number of alleles). Observed genotype frequencies in both RA and control groups were similar to

Table 3 Comparison of observed genotype frequencies with expected frequencies (calculated from the Hardy-Weinberg law)

Group	Genotype					
	8/8	8/13	8/18	8/23	18/18	13/18
Controls						
Obs.	0.673	0.038	0.240	0.029	0.009	0.009
Exp.	0.684	0.040	0.223	0.023	0.018	0.006
RA						
Obs.	0.833	0.037	0.130	0	0	0
Exp.	0.841	0.035	0.119	0	0.004	0.002

values calculated from gene frequencies using the Hardy-Weinberg formula (Table 3).

Discussion

These studies have shown a significant association between a restriction fragment obtained with an immunoglobulin λ light chain DNA probe and rheumatoid arthritis. The probe contains J flanking region as well as constant region sequences. The association appears weak (the relative risk is only increased twofold) and conceivably could be due to chance. The effect is independent of DR and Gm, and one possible explanation of these findings is that immunoglobulin λ constant or J region genes themselves might influence susceptibility towards rheumatoid arthritis. The polymorphism demonstrated with the C λ probe depends on insertion of additional base sequences between Eco RI restriction sites. Thus the 8 kb fragment contains two C λ genes, the 13 kb fragment contains three, the 18 kb fragment contains four, and the 23 kb fragment five.¹² Hence the association between RA and the 8 kb fragment might conceivably reflect loss of protective or control genes in the immunoglobulin λ constant or J regions, although it is uncertain how many of these duplicated genes are active and in any case no immunological function has been assigned to the λ constant region.

An alternative and more likely explanation for the association is that this is due to linkage disequilibrium between the 8 kb fragment and a gene at a nearby locus, such as a λ variable region gene. Adams and coworkers have proposed a scheme for involvement of immunoglobulin light chain V genes in the pathogenesis of other autoimmune disorders.¹⁴ This group has also shown that pathogenic autoantibodies to thyroid tissue are more likely to arise from immunoglobulin λ than κ clones.¹⁵ Our findings could represent an effect of particular immuno-

globulin light chain V genes increasing susceptibility to rheumatoid arthritis. A polymorphic immunoglobulin V λ DNA probe is available, and further studies with this probe would be of interest.¹⁶

These studies were supported by a research grant from the Nuffield Foundation. We are grateful to Professor Leder and his colleagues for providing the immunoglobulin C λ probe. The HLA typing was by courtesy of Dr P A Dyer.

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