Genes associated with rheumatoid arthritis and mild inflammatory arthritis. II. Association of HLA with complement C3 and immunoglobulin Gm allotypes

Alison H Puttick, D C Briggs, K I Welsh, Elizabeth A Williamson, R K Jacoby, Valerie E Jones

Abstract

Associations were sought between major histocompatibility complex (MHC) genes on chromosome 6 and the complement component C3 and immunoglobulin genes located on other chromosomes which might contribute to susceptibility to mild inflammatory arthritis (IA) or definite rheumatoid arthritis (RA). Frequencies of the complement C3F allele were raised in patients with IA but were normal in patients with RA and controls. When associations between C3F and MHC genes were sought frequencies of some MHC genes were greater in patients with C3F than in those without—for example, HLA-B8 and DR3 in patients with RA and DR2 in patients with IA. Conversely, DR4 frequency was lower in patients with IA with C3F than in those without. Thus the C3F allele may act independently or exert an epistatic effect on MHC genes to increase susceptibility or protect against disease.

The frequency of the immunoglobulin heavy chain allotype Gm(2) on chromosome 14 was increased in patients with RA but only in those with the phenotype Gm1,2,3,17;21,5; no significant associations were found between MHC genes and Gm phenotypes. Further, no associations of MHC, C3F, and immunoglobulin genes were shared by patients with RA and those with IA, indicating a different genetic basis for the two clinical entities.

In the accompanying paper¹ we confirmed the strong association of HLA-DR4 with definite rheumatoid arthritis (RA) and showed that patients with mild inflammatory arthritis (IA), which included patients who would be classified as 'possible' or 'probable' RA by earlier definitions,^{2,3} were not associated with DR4. HLA antigens associated with IA were found at the A and B loci, not at the DR locus, and major histocompatibility complex (MHC) associations with RA and IA did not appear to overlap.

Because the association of RA with DR4 and related MHC antigens is incomplete⁴ associations have been sought with genes on other chromosomes. Attempts to associate RA with complement component C3 genes, located on chromosome 19, and the immunoglobulin heavy chain genes (Gm) on chromosome 14, have produced conflicting results. These results were summarised by Thomson *et al*, who concluded that there is little evidence that C3 alleles predispose to RA, whereas genes linked to Gm may predispose to RA but only by interaction with HLA alleles.⁵

In this paper we have compared the frequencies of C3 and Gm alleles in patients with RA and IA and sought interactions of these alleles with the MHC genes associated with RA. Our aim was to discover if other genes as well as those in the MHC might (a) predispose to RA, (b) predispose to mild inflammatory arthritis, or (c) protect patients with IA from developing definite RA. In patients with RA we found some associations between the C3F allele of the complement component C3 and MHC genes; an association was also found between RA and the Gm allotype Gm(2). The C3F allele may also be relevant in patients with IA, particularly when associated with HLA-B62, DR4, or DR2.

Patients and methods

PATIENTS

The 61 patients with RA who were typed for MHC antigens, complement component C3, and Gm allotypes are described in the accompanying paper.¹ Thirty five additional patients with definite or classical RA^{2,3} were included in the study on Gm allotype frequencies; no MHC or complement allotypes were determined in these patients.

Similarly, 49 patients with IA typed for MHC, C3, and Gm allotypes, are also described in the accompanying paper.¹ Fifty six additional patients with IA were Gm allotyped; three of these were also typed for MHC and C3 allotypes.

CONTROLS

HLA frequencies were obtained from Exeter donors as already described.¹ Allotype frequencies of the complement component C3 were measured in 304 donors in the Tissue Typing Laboratory at Guy's Hospital and 34 of these were also tissue typed for HLA-A and B antigens. Frequencies of Gm allotypes but not HLA or C3 were measured in 94 Exeter donors.

TISSUE TYPING

The major allotypes of the complement component C3—namely, F and S—were measured by the method of Alper and Propp.⁶ The rare C3 allotypes—namely, FO.5, SO.5, FO.8, and SO.25—were omitted from our analyses. Immunoglobulin Gm and Km allotypes were measured with reagents purchased from the Central Laboratory of the Netherlands, Amsterdam, using methodology described by van Loghem.⁷ Serum samples which reacted strongly with antibody coated erythrocytes were separated by gel filtration and the IgG fraction was

Postgraduate Medical School, University of Exeter, Barrack Road, Exeter, Devon EX2 5DW, United Kingdom
A H Puttick
E A Williamson
R K Jacoby
V E Jones

Tissue Typing Laboratory, Guy's Hospital, St Thomas's Street, London SE1 9RT, United Kingdom
D C Briggs
K I Welsh

Correspondence to:
Dr Jones.

Accepted for publication
6 June 1989

allotyped. Alternatively, serum samples were treated with dithiothreitol before allotyping. Thus no patients or controls were excluded because of anti-IgG activity. Typing of HLA-A, B, and DR antigens, complement components C4A, C4B, and Bf, and glyoxalase-1 allotypes was carried out as before.^{1,8}

STATISTICAL ANALYSIS

Differences in allele or phenotype frequencies between patient and control populations were assessed for statistical significance with the χ^2 test, using Yates's correction for small numbers. Numbers of individuals or chromosomes included in each analysis are given in the relevant table.

Gm NOMENCLATURE

Where reference is made to Gm phenotypes the immunoglobulin isotype of each Gm allotype has been omitted. Thus G1m(1,17); G3m(21) is expressed as Gm1,17;21.

Results

COMPLEMENT C3

In patients with RA frequencies of the C3 phenotypes FF, SF, and SS, and the F and S allotype and gene frequencies were similar to controls. In patients with IA the FF phenotype and C3F gene frequencies were raised, though not significantly (FF phenotype: IA 14.0%, RA 6.1%, controls 6.9%; C3F gene: IA 29.4%, RA 20.6%, controls 22.0%).

HLA AND C3

When patients were divided into those with and

those without C3F some significant differences emerged (table 1). In patients with RA the frequencies of B8 and DR3 genes were higher in C3F positive than in C3F negative patients. In patients with IA the frequency of DR2 was higher and of DR4 lower in C3F positive than in C3F negative patients. HLA gene frequencies in C3F positive patients with RA also differed from C3F positive patients with IA and from Exeter controls, though not all differences were significant (table 1). HLA gene frequencies were also measured in 34 donors from Guy's Hospital with known C3 allotypes (C3F positive=18 chromosomes; C3F negative=50 chromosomes); frequencies of HLA-B8 genes were 16.7% and 10.0%, for B40 were <5.6% and 4.0%, and for B62 were 11.1% and 6.0% respectively. Numbers were too few, however, for statistical analysis.

Analysis of MHC genes associated with IA—namely, A24, A25, B27, B35, C4A4 and DR5—in C3F positive and negative patients showed no interaction. For example, of the five patients with IA with HLA-A25, three were C3F positive and two were negative.

IMMUNOGLOBULIN Gm

When patients with homozygous Gm phenotypes were compared frequencies were similar in patients with RA, patients with IA, and controls (table 2). In heterozygous patients, however, the frequency of Gm1,2,3,17;21,5 was significantly greater in RA than in patients with IA and, presumably in compensation, the frequency of Gm1,3,17;21,5 was lower in RA than in patients with IA or controls. Thus the frequency of G1m(2) is significantly increased in RA, but only in patients with RA heterozygous for Gm allotypes. No other significant

Table 1: Interaction of HLA genes with the C3F antigen in patients with rheumatoid arthritis and inflammatory arthritis. The frequency (%) of HLA genes is shown

HLA genes	Patients with RA*			Patients with IA*			Controls total (n=276)	Significance	
	Total (n=112)	C3F+ (n=40)	C3F- (n=72)	Total (n=104)	C3F+ (n=46)	C3F- (n=58)		χ^2	p
B8	18.8	32.5 ^a b	11.1 ^a	12.5	15.2	10.3	14.9 ^b	^a 7.25 ^b 7.68	<0.01 <0.01
B40	9.8	15.0 ^c	6.9	3.8	<2.2 ^c †	6.9	6.5	^c 5.29	<0.05
B62	15.2	20.0 ^d e	12.5	5.8	2.2 ^d	8.6	8.3 ^e	^d 5.48 ^e 5.52	<0.02 <0.02
DR2	7.1	5.0 ^f	8.3	16.3	26.1 ^f g	8.6 ^g	(n=252) 15.5	^f 5.51 ^g 4.52	<0.02 <0.05
DR3	10.7	20.0 ^h	5.6 ^h	15.4	19.6	12.1	15.1	^h 4.67	<0.05
DR4	48.7	55.0 ⁱ	44.4	26.9	13.0 ⁱ j	37.9 ^j k	21.0 ^k	ⁱ 15.29 ^j 6.86 ^k 7.34	<0.001 <0.01 <0.01

*RA=rheumatoid arthritis; IA=inflammatory arthritis; n=number of chromosomes.

†No patient carried this gene.

Table 2: Immunoglobulin Gm phenotype frequencies (%) in patients with rheumatoid arthritis and inflammatory arthritis

Gm phenotypes	Patients with RA* (n=96)	Patients with IA* (n=105)	Controls (n=94)	Significance	
				χ^2	p
Gm1,17;21	3.1	6.7	5.3		
Gm1,2,17;21	7.3	7.6	5.3		
Gm3;5	42.7	44.7	43.6		
Gm1,3,17;21,5	24.0	30.5	32.9		
Gm1,2,3,17;21,5	22.9 ^a	11.4 ^a	12.7	^a 3.93	<0.05
G1m(2) allotype	30.2 ^b	19.0	18.1 ^b	^b 4.18	<0.05

*RA=rheumatoid arthritis; IA=inflammatory arthritis; n=number of subjects.

differences in Gm frequencies were found, either between patients seropositive or seronegative for rheumatoid factor, or between male and female patients. There was a small but not significant increase in the kappa light chain allotype Kml in patients with RA compared with patients with IA and controls.

HLA AND Gm

Because differences were found in Gm heterozygous patients with RA, frequencies of HLA antigens and genes were compared in patients with Gm1,2,3,17;21,5 and Gm1,3,17;21,5 phenotypes. Differences in HLA frequencies were found but numbers of patients were too few for statistical significance. For example, the frequency of HLA-B7 in all patients with IA was similar to that in controls but none of five patients with IA with Gm1,2,3,17;21,5 had B7, whereas eight of 15 patients with IA with Gm1,3,17;21,5 carried B7, suggesting an association of B7 with Gm1,3,17;21,5 in patients with IA.

There was no significant difference between the frequency of DR4 genes in G1m(2) positive and negative heterozygous patients (RA 38.3% and 60.0% respectively) or between the frequency of the Gm1,2,3,17;21,5 phenotype in DR4 positive and negative patients (RA 23.9% and 45.5% respectively), but the results suggest that the Gm1,2,3,17;21,5 phenotype is more important in RA when DR4 is absent. The Gm1,2,3,17;21,5 phenotype plus DR4 may also increase susceptibility to RA in men. Eleven of the 12 male patients with RA were DR4 positive and six of these carried the Gm1,2,3,17;21,5 phenotype. In contrast, six of the 10 male patients with IA were DR4 positive but none carried G1m(2).

GLYOXALASE-1

Associations between frequencies of HLA antigens and glyoxalase allotypes were sought. Associations were also sought between C3 and Gm allotypes. No significant differences were found in patients with RA or IA in any comparison.

Discussion

Previous studies in Europe on the associations of complement C3 polymorphisms with RA have given conflicting results. Early reports suggested that the C3FF phenotype⁹ and C3F gene¹⁰ were important in RA, but later studies failed to confirm this association.^{11–13} Indeed, Thomson *et al* noted that in both early studies the C3F gene frequency, though raised, still lay within the expected range for normal European populations (0.14–0.257, summarised in ref 14).¹² The C3F gene frequencies in our patients with RA and controls were also within this range but the frequency in our patients with IA was higher. Discrepancies between the various studies might be explained by clinical ascertainment of RA because Brönnestam,¹⁰ for example, included 'probable' RA and a high proportion (38%) of patients seronegative for rheumatoid factor. Brönnestam found that the C3F gene

frequency was highest in patients without RA with the highest titres of rheumatoid factor but that the frequency in seropositive patients without RA was lower than that in controls; he therefore concluded that the C3F gene was associated with a clinical diagnosis of RA but not with the occurrence of rheumatoid factor. In our study a distinction was drawn between patients with RA (definite or classical), of whom 81% were seropositive, and patients with IA, who included those classified as 'possible' or 'probable' RA, of whom 92% were seronegative. Thus we suggest that the C3F gene is more likely to be a marker for mild self-limiting seronegative arthritis than for seropositive definite RA.

The associations found between HLA genes and C3F in patients with RA and those with IA are more difficult to interpret. Thomson *et al* compared the frequencies of C3 genetic variants in DR4 positive and DR4 negative patients but detected no associations of C3 with DR4 in RA.¹² A similar analysis of our patients also showed no associations, partly because too few of our patients with RA (12) were DR4 negative. The converse method of analysis—namely, comparison of the frequency of DR4 genes in C3F positive and C3F negative patients with RA—also failed to show an association between C3F and DR4. Further analysis of HLA gene frequencies in C3F positive and negative patients, however, showed several distinctive features. Firstly, HLA-B8 and DR3, which are in linkage disequilibrium and are associated with other autoimmune diseases, were not raised overall in patients with RA but were associated with C3F positive patients with RA. Secondly, DR2, which was low in our patients with RA and is considered to be protective in RA,⁴ was associated with IA in C3F positive patients. Thirdly, some gene frequencies—notably, B40, B62, and DR4—which were high in C3F positive patients with RA were unexpectedly low in C3F positive patients with IA; this suggests an inverse relation between RA and IA in their association of C3F with HLA genes and emphasises the genetic differences already reported between patients with RA and those with IA.¹ All three HLA genes are implicated in RA in their own right, irrespective of C3,¹⁵ therefore C3F may have an independent role in conferring susceptibility or protection in both clinical situations.

The significantly increased frequencies of both G1m(2) and the Gm phenotype Gm1,2,3,17;21,5 in patients with RA confirmed our preliminary study.⁸ Many, but not all, studies have reported associations of G1m(2) with RA and in three of these G1m(2) was associated with DR4 positive RA (summarised in ref 16). In these reports, however, frequencies of Gm phenotypes containing G1m(2) and their importance in RA vary considerably. Indeed one study on seropositive RA found that Gm1,2;21 was increased and Gm1,2,3;21,5 was decreased in RA,¹⁶ the converse of our results. The association of G1m(2) with DR4 positive RA was not confirmed in our patients because the sample size of DR4 negative patients was too small. Our results suggest the opposite, however, that G1m(2) and the Gm1,2,3,17;21,5

phenotype are more important in susceptibility to RA in the absence of other strongly predisposing factors such as DR4 and the female susceptibility gene. In patients with IA no associations of HLA genes with Gm markers were significant, though B7 may be important in the absence of G1m(2). In patients with IA, furthermore, C3F showed no interaction with any HLA-A and B locus genes associated with IA,¹ including the rare allele A25.

Now that RA has been redefined² the clinical distinction between RA and IA has been clarified, but in the earliest stages the clinical symptoms of RA and IA are often indistinguishable¹⁷ and genetic markers might aid disease prognosis. None of the genes studied here, however, is sufficiently strongly represented in either patient group to provide an accurate screening method for individual patients at onset of arthritis.

For the purposes of genetic analysis in this and the accompanying paper¹ we have assumed that both RA and IA are homogeneous disease entities, but this assumption is valid only for clinical presentation. At present there is no strong evidence for or against the hypothesis that RA is induced by a single aetiological agent. But IA can be provoked by a number of different viruses and other agents—for example, we found evidence of B19 parvovirus infection in only five of our patients with IA.¹ Therefore the similar clinical features in patients with IA are not related to a single primary inducing agent. Nevertheless, our analysis does emphasise the difference in genetic background between mild self-limiting arthritis and the chronic severe arthritis of definite RA. In patients with RA, however, it is still not possible to distinguish between genetic markers for an aetiological agent and markers for risk of chronic arthritis.

- 1 Puttick A H, Briggs D C, Welsh K I, *et al.* Genes associated with rheumatoid arthritis and mild inflammatory arthritis. I. Major histocompatibility complex class I, II, and III allotypes. *Ann Rheum Dis* 1990; 49: 219–24.
- 2 Arnett F C, Edworthy S M, Bloch D A, *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; 31: 315–24.
- 3 Ropes M W, Bennett G A, Cobb S, Jacox R, Jessar R A. 1958 Revision of diagnostic criteria for rheumatoid arthritis. *Bull Rheum Dis* 1958; 9: 175–6.
- 4 Lanchbury J S S. Molecular genetics of the HLA-D region component of inherited susceptibility to rheumatoid arthritis. *Br J Rheumatol* 1988; 27: 171–5.
- 5 Thomson W, Dyer P A, Sanders P A, Grennan D M. C3, Gm and Pi polymorphisms in rheumatoid arthritis. *Dis Markers* 1986; 4: 145–50.
- 6 Alper C A, Propp R P. Genetic polymorphism of the third component of human complement (C3). *J Clin Invest* 1968; 47: 2181–91.
- 7 van Loghem E. Genetic studies on human immunoglobulins. In: Weir D M, ed. *Handbook of experimental immunology*. Vol 1. Oxford: Blackwell Scientific, 1978: 11, 1–16.
- 8 Puttick A H, Briggs D, Welsh K, Jacoby R K, Williamson E A, Jones V E. Extended haplotypes in rheumatoid arthritis and preliminary evidence for interaction with immunoglobulin genes. *Dis Markers* 1986; 4: 139–44.
- 9 Farhud D D, Ananthakrishnan R, Walter H. Association between C'3 phenotypes and various diseases. *Humangenetik* 1972; 17: 57–60.
- 10 Brönneham R. Studies of the C3 polymorphism. Relationship between C3 phenotypes and rheumatoid arthritis. *Hum Hered* 1973; 23: 206–13.
- 11 Dahlqvist R S, Beckman G, Beckman L. Bf and C3 complement types in rheumatoid arthritis. *Hum Hered* 1985; 35: 240–5.
- 12 Thomson W, Dyer P A, Sanders P A, Grennan D M. Genetic variants of complement component 3 (C3) in DR4 positive and DR4 negative rheumatoid arthritis. *Ann Rheum Dis* 1986; 45: 269–71.
- 13 Lanchbury J S S, Pal B, Papiha S S. Bf and C3 polymorphisms in rheumatoid arthritis. *Hum Hered* 1987; 37: 144–9.
- 14 Wyatt R J, Julian B A, Galla J H, McLean R H. Increased frequency of C3 fast alleles in IgA nephropathy. *Dis Markers* 1984; 2: 419–28.
- 15 Woodrow J C. Analysis of the HLA association with rheumatoid arthritis. *Dis Markers* 1986; 4: 7–12.
- 16 Zarnowski H, Mierau R, Werder D, Antons M, Genth E, Hartl P W. Increased frequency of Gm(1,2;21) phenotype in HLA-DR4 positive seropositive rheumatoid arthritis. *J Rheumatol* 1986; 13: 858–63.
- 17 Jones V E, Jacoby R K, Johnson P M, Phua K K, Welsh K I. Association of HLA-DR4 with definite rheumatoid arthritis but not with susceptibility to arthritis [Abstract]. *Ann Rheum Dis* 1983; 42: 223.