

Gm allotypes and HLA in rheumatoid arthritis patients with circulating antibodies to native type II collagen

P A SANDERS,¹ D M GRENNAN,¹ P S KLIMIUK,¹ R B CLAGUE,²
G G deLANGE,³ I COLLINS,² P A DYER⁴

From the ¹University of Manchester Rheumatic Diseases Centre, Hope Hospital, Salford; the ²Department of Rheumatology, Withington Hospital, Manchester; the ³Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam; and the ⁴Tissue Typing Laboratory, St Mary's Hospital, Manchester

SUMMARY HLA antigens and immunoglobulin heavy chain allotypes (Gm) were determined in 166 unrelated patients with rheumatoid arthritis (RA), 44 of whom had circulating antibodies to native type II collagen. Collagen antibody positive patients showed an association with HLA-DR3 and DR7 (68% compared with 39% of collagen antibody negative RA, $p < 0.005$), and with the Gm phenotype, Gm(zafngb). This contrasted with the collagen antibody negative RA patients where there was an association with HLA-DR4 and, in DR4 positive disease only, with the Gm allotype, G1m(x). The Gm(zafngb) phenotype was found in 26% of DR3 or DR7 positive patients overall and only 9% of RA patients negative for these DR antigens ($p < 0.005$), suggesting an interaction between HLA-DR3/7 and Gm(zafngb). The differing Gm associations for collagen antibody positive and negative RA provide further evidence for genetic heterogeneity in susceptibility to RA.

Key words: immunoglobulin heavy chain genes, interaction, immunogenetics, heterogeneity of rheumatoid arthritis.

RA is thought to be caused by a combination of genetic and environmental factors. There is a well recorded association between RA and HLA-DR4 in most populations studied¹ and a weaker association between RA and the immunoglobulin heavy chain constant region allotype, G1m(x), in DR4 positive disease only, suggesting that genes linked to HLA on chromosome 6 and to Gm on chromosome 14 may interact in disease predisposition.^{2,3}

A subgroup of patients with RA accounting for about 11% of RA patients seen in hospital has been shown to have circulating antibodies to native type II collagen and shows an association with HLA-DR3 or DR7 rather than DR4.^{4,5} Apart from a decreased frequency of seropositivity for rheumatoid factor and a later age of disease onset these patients are clinically indistinguishable from those with collagen antibody negative RA. It is uncertain what part the

collagen antibodies play in the pathogenesis of this form of RA. Native type II collagen is the major type of collagen in articular cartilage,⁶ and immunisation of rats and mice with native type II collagen induces a polyarthritis and an immune response.^{7,8} The results of a single study suggest that the Gm as well as the HLA associations may differ in collagen antibody positive and negative RA.⁹

The aim of this study therefore was to reinvestigate the relation between RA and Gm in a larger group of patients who had been tested for the presence of circulating antibodies to native type II collagen and to look for evidence of an interaction between HLA and Gm in this disease subset.

Patients and methods

One hundred and sixty six patients with classical or definite RA (by American Rheumatism Association criteria¹⁰) were studied. These were all unrelated Caucasians and were attending rheumatology outpatient clinics at Manchester Royal Infirmary

Accepted for publication 1 December 1986.

Correspondence to Dr P A Sanders, University of Manchester Rheumatic Diseases Centre, Hope Hospital, Eccles Old Road, Salford M6 8HD.

Table 1 *Gm phenotypes in patients with RA and controls*

<i>Gm</i> phenotype	Collagen antibody positive RA (n=44)		Collagen antibody negative RA (n=122)		Controls (n=101)	
	No	%	No	%	No	%
fmb	11	25.0	46	37.7	34	33.7
fb	3	6.8	10	8.2	5	5.0
zaxg	1	2.3	8	6.6	7	6.9
zag	2	4.5	5	4.1	3	3.0
zaxfngb	5	11.4	11	9.0	10	9.9
zaxfgb	2	4.5	6	4.9	4	4.0
zafngb	14*	31.8	17*	13.9	21	20.8
zafgb	5	11.4	15	12.3	15	14.9
Others	1	2.3	4	3.3	2	2.0

* $\chi^2=6.810$, $p=0.0093$ (see text).

(MRI) (85 patients) or Hope Hospital, Salford (81 patients). The RA group included 35 patients who were selected because they were known to be collagen antibody positive. A control population of 101 volunteer hospital staff unaffected by RA was also included for HLA and Gm typing.

LABORATORY METHODS

Serum antibody levels to native type II collagen were measured either by a specific solid phase radioimmunoassay¹¹ (MRI patients) or by an enzyme linked immunosorbent assay method (Hope patients) developed from this technique. The normal range was calculated using 15 normal sera with each batch tested (these results did not differ significantly from those obtained using a larger group of 100 normal sera). Results greater than two standard deviations above the mean were considered to be positive.

HLA typing was carried out for A and B antigens using standard National Institute of Health methods. HLA-DR typing was carried out using B lymphocytes isolated by their adherence to nylon wool columns. All antisera were obtained locally or through mutual exchange with other laboratories and characterised using cell panels typed with international histocompatibility antisera.

Gm and Km allotyping was performed by the technique of haemagglutination inhibition.¹² The following allotypes were recognised: G1m(z, a, x, f), G2m(n), G3m(b0, b1, b3, b5, g, s, t, c3, c5), Km(1) and (3). G3m (b0, b1, b3, and b5) always occurred together and are henceforth referred to simply as G3m(b).

STATISTICS

Results were analysed using a χ^2 test or Fisher's exact test.

Results

Table 1 shows the distribution of Gm phenotypes in collagen antibody positive and negative patient groups and in controls. The Gm(zafngb) phenotype was increased in the collagen antibody positive RA patients when compared with collagen antibody negative RA ($p=0.0093$). As the phenotype Gm([z]afgb) has been previously reported to be associated with collagen antibody positive RA⁹ in a study in which the G2m(n) allotype was not tested for, there is a priori evidence to suggest that phenotypes Gm(zafngb) or Gm(zafgb) may be implicated in this type of RA so that the p value need not be corrected for the total number of phenotypes compared.¹³

Table 2 shows the distribution of Gm and Km allotypes in the two patient groups and controls. There were no significant differences in frequency between the groups for Gm allotypes. Km(1) was increased in the collagen antibody negative RA group compared with the controls, but the difference did not reach statistical significance after correction of the p value (which is necessary as there

Table 2 *Gm and Km allotypes in patients with RA and controls*

<i>Gm/Km</i> allotype	Collagen antibody positive RA (%) (n=44)	Collagen antibody negative RA (%) (n=122)	Controls (%) (n=101)
G1m(f)	90.9	88.5	89.1
G1m(z)	68.2	54.1	60.4
G1m(x)	18.2	22.1	21.8
G2m(n)	68.2	63.1	65.3
Km(1)	13.6	20.5*	9.9*

* $\chi^2=4.681$, $p=0.031$, $p_c=0.062$ (see text).

Table 3 HLA-DR4, HLA-DR3/7, and Gm(zafngb) in patients with RA and controls

HLA/Gm	Collagen antibody positive RA		Collagen antibody negative RA		Controls	
	No	%	No	%	No	%
DR3 or DR7*	28/41	68.3	44/113	38.9	49/101	48.5
DR4†	19/41	46.3	71/113	62.8	31/101	30.7
Gm(zafngb)	14/44	31.8	17/122	13.9	21/101	20.8
DR3/7+Gm(zafngb)‡	10/41	24.4	9/113	8.0	9/101	8.9
DR4+G1m(x)§	3/41	7.3	19/113	16.8	6/101	5.9

*Collagen antibody positive RA v antibody negative RA, $\chi^2=10.411$, $p<0.005$.

†Collagen antibody positive RA v controls, $\chi^2=4.600$, $p<0.05$.

‡Collagen antibody negative RA v controls, $\chi^2=21.084$, $p<0.0005$.

§Collagen antibody positive RA v antibody negative RA, $\chi^2=7.50$, $p<0.01$.

¶Collagen antibody positive RA v controls, $\chi^2=5.98$, $p<0.025$.

§Collagen antibody negative RA v controls, $\chi^2=6.11$, $p<0.025$.

is no a priori association of RA with Km allotypes).

In Table 3 we examine the possibility of an interaction between HLA and Gm in collagen antibody positive RA and show the relative frequencies of HLA-DR4, DR3, and DR7 in the patient groups. As expected there was a strong association between collagen antibody negative RA and HLA-DR4 and as previously shown an association between RA and G1m(x) bearing haplotypes in DR4 positive disease only.³ The collagen antibody positive subgroup was significantly associated with

HLA-DR3 or 7, or both. The combination of DR3 or 7 with the Gm(zafngb) phenotype was significantly increased in the collagen antibody positive compared with collagen antibody negative patients and controls.

In Table 4 we consider the relative risks for HLA and Gm linked markers found in association with the respective patient groups. The relative risks for HLA-DR3/7, Gm(zafngb), and the combination of these markers were significantly higher in the collagen antibody positive than in the collagen antibody negative RA group. Although there are trends with this method of analysis for interactions between HLA-DR3/7 or DR4 and the Gm(zafngb) phenotype or G1m(x) allotype in collagen antibody positive and negative RA groups respectively, the 95% confidence limits for the combinations of markers overlapped with those for HLA and Gm linked markers alone.

In the above search for an interaction between HLA and Gm the numbers of collagen antibody positive patients were relatively small. In Table 5, therefore, we have looked for evidence of an interaction between HLA-DR3/7 and the Gm(zafngb) phenotype in the RA population overall and irrespective of collagen antibody status. As shown there was a statistically significant increase in frequency of this Gm phenotype in DR3 and DR7 positive RA patients.

Table 4 Relative risks (RR) for HLA and Gm markers in RA

HLA/Gm	Collagen antibody positive RA		Collagen antibody negative RA	
	RR	95% Conf. limits	RR	95% Conf. limits
DR3/7	2.24	[1.65-3.04]	0.66	[0.57-0.77]
DR4	1.95	[1.46-2.60]	3.82	[3.23-4.52]
Gm(zafngb)	1.78	[1.28-2.47]	0.62	[0.48-0.80]
G1m(x)	0.80	[0.52-1.22]	1.02	[0.83-1.26]
DR3/7+				
Gm(zafngb)	3.74	[1.94-7.22]	0.58	[0.34-0.99]
DR4+G1m(x)	2.00	[0.75-5.34]	4.62	[2.72-7.86]

Table 5 Interaction between HLA-DR3/7 and Gm(zafngb) phenotype frequencies in all patients with RA subdivided according to DR3/7 status

	HLA-DR3+ or DR7+		HLA-DR3- and DR7-	
	No	%	No	%
Gm(zafngb)+	19/72	26.4	7/82	8.5
Gm(zafngb)-	53/72	73.6	75/82	91.5

$\chi^2=8.706$, $p<0.005$.

Discussion

In previous studies a subgroup of patients with RA characterised by persisting antibodies to native type II collagen was shown to have an HLA association with HLA-DR3 or DR7 rather than with HLA-DR4, the DR antigen associated with RA patients overall.^{4,5} The antigens HLA-DR3 and DR7 are in very strong linkage disequilibrium with DQw2. It is

therefore possible that DQw2 is a better HLA marker for this subset of rheumatoid patients rather than the DR antigens themselves. In the present study we have shown that collagen antibody positive and negative RA subgroups also have different Gm associations, and these findings are in keeping with those of a previous study of Gm in collagen antibody positive RA.¹² These differing associations for HLA and Gm markers in different RA subgroups add further evidence to a growing concept of genetic heterogeneity in susceptibility to RA (unpublished data).^{14 15}

Previous evidence for an interaction between HLA and Gm linked genes has been provided by the demonstration of an increased frequency of the Gm allotype, G1m(x), in DR4 positive but not DR4 negative RA.³ In the present study there is a trend for a comparable interaction between genes in linkage disequilibrium with HLA-DR3/7 and Gm in the collagen antibody positive RA subgroup, and this interaction appears statistically significant irrespective of collagen antibody status when the 154 patients typed for HLA and Gm overall are analysed. (This group, however, contained a high percentage of collagen antibody positive patients, and we would not expect to find evidence for an interaction between these HLA and Gm markers in an unselected RA population.) The evidence for an interaction between HLA and Gm linked genes predisposing to RA is in keeping with the findings of previous studies showing that HLA and Gm may interact in influencing humoral responsiveness to bacterial antigens¹⁶ as well as in predisposing to other autoimmune disorders such as chronic active hepatitis and systemic lupus erythematosus.^{17 18}

How 14th and 6th chromosomal genes interact is unknown. One mechanism suggested by a study of collagen induced arthritis in mice is that major histocompatibility complex (MHC) linked genes may regulate the level of overall immunoglobulin synthesis but that non-MHC linked genes (possibly immunoglobulin heavy chain genes) may determine the proportion of this response devoted to a particular autoantibody.¹⁹ The site of the RA susceptibility gene(s) on the 14th chromosome is similarly uncertain and could be accounted for by immunoglobulin heavy chain constant region genes themselves or by linked V region genes which map telomeric to those for the immunoglobulin constant region on chromosome 14.²⁰

We are grateful to the North West Regional Health Authority for financial support.

References

- 1 Stastny P, Association of the B-cell alloantigen DRw4 with rheumatoid arthritis. *N Engl J Med* 1978; **298**: 869-71.
- 2 Probert D N, Kay P, McCluskey J, Zilko P J, Mathews J. Immunoglobulin allotypes in rheumatoid arthritis. In: Dawkins R L, Christiansen F T, Zilko P J, eds. *Immunogenetics of rheumatoid arthritis*. Amsterdam: Excerpta Medica, 1983; **313**: 127-9.
- 3 Sanders P A, deLange G G, Dyer P A, Grennan D M. Gm and Km allotypes in rheumatoid arthritis. *Ann Rheum Dis* 1985; **44**: 529-32.
- 4 Dyer P A, Clague R B, Klouda P T, Firth S, Harris R, Holt P J L. HLA antigens in patients with rheumatoid arthritis and antibodies to native type II collagen. *Tissue Antigens* 1982; **20**: 394-6.
- 5 Klimiuk P S, Clague R B, Grennan D M, Dyer P A, Smeaton I, Harris R. Autoimmunity to native type II collagen—a distinct genetic subset of rheumatoid arthritis. *J Rheumatol* 1985; **12**: 865-70.
- 6 Miller E J. Isolation and characterization of the cyanogen bromide peptides from the 1(II) chain of chick cartilage collagen. *Biochemistry* 1971; **10**: 3030-5.
- 7 Trentham D E, Townes A S, Kang A H. Autoimmunity of type II collagen: an experimental model of arthritis. *J Exp Med* 1977; **147**: 857-67.
- 8 Courtenay J S, Dallman M J, Dayan A D, Martin A, Mosedale B. Immunisation against heterologous type II collagen-induced arthritis in mice. *Nature* 1980; **283**: 666-8.
- 9 Collier D H, Kerwar S S, Brown R, Stabo J D, Schanfield M S. Immunoglobulin G heavy chain (Gm) allotypes and the immune response to types I and II collagen in rheumatoid arthritis. *Dis Markers* 1985; **3**: 91-102.
- 10 Ropes M W. Diagnostic criteria for rheumatoid arthritis. 1958 revision. *Ann Rheum Dis* 1959; **18**: 49-53.
- 11 Clague R B, Firth S A, Holt P J L, Skingle J, Greenbury C L, Webley M. Serum antibodies to type II collagen in rheumatoid arthritis: comparison of 6 immunological methods and clinical features. *Ann Rheum Dis* 1983; **42**: 537-44.
- 12 Van Loghem E. Genetic studies on human immunoglobulins. In: Weir D M, ed. *Handbook of experimental immunology*. 3rd ed. Oxford: Blackwell, 1978; **I**: 11.1-11.16.
- 13 Svejgaard A, Ryder L P. Associations between HLA and disease. Notes on methodology and a report from the HLA and disease registry. In: Daussett J, Svejgaard A, eds. *HLA and disease*. Copenhagen: Munksgaard/Baltimore: Williams & Wilkins Co, 1977: 46-53.
- 14 Grennan D M, Sanders P A, Dyer P A, Harris R. HLA haplotype sharing by siblings with rheumatoid arthritis: evidence for genetic heterogeneity. *Ann Rheum Dis* 1986; **45**: 126-9.
- 15 Grennan D M, Sanders P A, Dyer P A. Rheumatoid arthritis: inheritance and association with other auto-immune diseases. *Dis Markers* 1986; **4**: 157-62.
- 16 Whittingham S, Mathews J D, Schanfield M S, et al. Interactive effect of Gm allotypes and HLA-B locus antigens on the human antibody response to a bacterial antigen. *Clin Exp Immunol* 1980; **40**: 8-15.
- 17 Whittingham S, Mathews J D, Schanfield M S, Tait B D, Mackay I R. Interaction of HLA and Gm in autoimmune chronic active hepatitis. *Clin Exp Immunol* 1981; **43**: 80-6.
- 18 Whittingham S, Mathews J D, Schanfield M S, Tait B D, Mackay I R. HLA and Gm genes in systemic lupus erythematosus. *Tissue Antigens* 1983; **21**: 50-7.
- 19 Watson W C, Townes A S. Genetic susceptibility to murine collagen II autoimmune arthritis. Proposed relationship to the IgG2 autoantibody subclass response, complement C5, major histocompatibility complexes (MHC) and non-MHC loci. *J Exp Med* 1985; **162**: 1878-91.
- 20 Johnson M J, Natali A M, Cann H M, Honjo T, Cavalli-Sforza L L. Polymorphisms of a human variable heavy chain gene show linkage with constant heavy chain genes. *Proc Natl Acad Sci USA* 1984; **81**: 7840-4.