Gm and Km allotypes in rheumatoid arthritis

P A SANDERS,¹ G G DE LANGE,² P A DYER,³ AND
D M GRENNAN¹

From the ¹University of Manchester Rheumatic Diseases Centre, Hope Hospital, Eccles Old Road, Salford M6 8HD; the ²Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands; and the ³Department of Medical Genetics, St Mary's Hospital, Manchester M13 0JH.

SUMMARY Immunoglobulin heavy chain (Gm) and κ light chain (Km) allotype and phenotype frequencies were compared in 173 patients with rheumatoid arthritis (RA) and in 798 controls. No significant differences were found between allotype or phenotype frequencies in overall RA and control groups. However, the Gm(zaxfngb) phenotype and G1m(x) allotype were increased in HLA-DR4 positive RA patients compared with DR4 negative patients and controls, suggesting that immunoglobulin heavy chain genes interact with HLA in the pathogenesis of RA. All four patients with pulmonary fibrosis were Km(1) positive suggesting a possible role for immunoglobulin κ light chain genes in the pathogenesis of pulmonary fibrosis found in rheumatoid patients.

Key phrase: interaction between HLA and Gm in rheumatoid arthritis.

Rheumatoid arthritis (RA) is caused by an interaction between a polygenic susceptibility and unknown environmental factors.¹ Genes within the major histocompatibility complex (MHC) make a major contribution to inherited susceptibility as shown by the well reported association between RA and HLA-DR4² and the greater than random sharing of HLA haplotypes found in affected rheumatoid siblings.³ Family studies suggest that genes outside the MHC may also contribute to disease pathogenesis. Immunoglobulin heavy chain allotypes (Gm, A2m) and κ light chain allotypes (Km) are coded for by genes on chromosomes 14 and 2 respectively.⁴ Associations have been reported between particular Gm allotypes and other autoimmune diseases^{5–8} and between both Gm and Km variants and humoral immune responses.^{9–12} The results of previous studies of Gm allotypes in RA have provided conflicting results.^{13–17} We have compared Gm, A2m, and Km allotype frequencies in RA and control populations to look for evidence that either immunoglobulin heavy chain or κ light chain genes may influence disease susceptibility or complications.

Patients and methods

PATIENTS

One hundred and seventy three unrelated Caucasian patients with classical or definite RA¹⁸ who lived in Greater Manchester were studied. One hundred and seventy one of these were attending rheumatology outpatient clinics; the other two were probands in a parallel study of RA in families. One hundred and twenty one (69.9%) of the patients were female. The mean age was 55.5 years (range 24–82), and 148 (85.5%) were seropositive for rheumatoid factor. One hundred and twenty seven (73.4%) had received sodium aurothiomalate or D-penicillamine therapy.

The controls used for Gm typing were 798 Dutch volunteers from the Leiden region of the Netherlands who had been previously documented.¹⁹ There are no significant differences in Gm and Km allotype frequencies between English and Dutch Caucasian populations.²⁰ The controls for HLA typing were 163 healthy Caucasians living in Greater Manchester.

CLINICAL ASSESSMENT

Clinical features of all the RA patients were recorded in detail, and case notes were studied for the presence or absence of extra-articular disease

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Correspondence to Dr D M Grennan, University of Manchester Rheumatic Diseases Centre, Hope Hospital, Salford M6 8HD.

features and side effects of treatment with gold or D-penicillamine. Radiographs of the hands and feet were graded from 0 (normal) to 40 (most severe) by a modified Larsen's score.²¹

AUTOANTIBODIES

Sera from patients were tested for rheumatoid factor by the sheep cell agglutination test (SCAT) with RAHA Kit (Fujizoki Inc., Tokyo) and for antinuclear factor (ANF) with rat liver substrate and fluorescein-conjugated sheep antihuman immunoglobulin. Where serial SCAT results were available the highest titre was recorded.

IMMUNOGLOBULIN ALLOTYPING

Gm, A2m, and Km allotypes were detected by haemagglutination inhibition assay.²² Tests were made for the following allotypes: G1m(z,a,x,f), G2m(n), G3m(g,b0,b1,b3,b5,c3,c5,s,t), A2m(1,2), and Km(1,3). G3m(b0,b1,b3, and b5) usually occur together in Caucasians and are summarised as G3m(b).

HLA TYPING

HLA-DR typing was carried out in a subgroup of 98 RA patients selected at random. HLA-DR antigens were defined with B lymphocytes isolated from peripheral blood lymphocytes by their adherence to nylon wool columns. Antisera were obtained locally and were characterised by a cell panel typed with International Histocompatibility Workshop antisera.

STATISTICAL ANALYSIS

The significance of differences in phenotype and allotype frequencies was analysed by a χ^2 test or, where appropriate, by Fisher's exact test.²³ p values

were corrected for the number of statistical tests performed as indicated in the text (p_c).

Results

HLA typing in the 98 randomly selected RA patients showed that 63 (64.3%) were HLA-DR4 positive compared with 34% of local controls ($p<0.001$).

IMMUNOGLOBULIN ALLOTYPES

The frequencies of Gm phenotypes, allotypes, and G1m(f) or (z) homozygotes were not significantly different in the total RA and control groups (Table 1). The Gm(zaxfngb) phenotype was increased in the DR4 positive RA group compared with the DR4 negative group (Fisher's $p=0.0033$, $p_c=0.026$) and the control group ($\chi^2=6.497$, $p=0.017$, $p_c=0.136$).

The G1m(x) allotype (Table 2) was also more frequent in the DR4 positive RA group than in controls. ($\chi^2=8.477$, $p=0.004$, $p_c=0.016$) or the DR4 negative RA patients ($\chi^2=10.459$, $p=0.001$, $p_c=0.004$). There was a trend for a decrease in the G1m(x) allotype in DR4 negative RA patients compared with controls ($\chi^2=4.126$, $p=0.048$, $p_c=0.192$). The G1m(z) allotype was increased in DR4 positive patients and decreased in DR4 negative patients. These differences were not statistically significant (DR4 positive RA v controls $\chi^2=4.608$, $p_c=0.14$; DR4 negative RA v DR4 positive RA $\chi^2=5.047$, $p_c=0.10$) and are probably secondary to the increase in the G1m(x) allotype, as G1m(z) is present in all G1m(x) bearing haplotypes. No relationships were found between Gm phenotypes or allotypes and age of onset or any clinical feature. Nor was any phenotype or allotype associated with autoantibody titre or severity of radiological erosive change.

Table 1 Gm phenotype frequencies in RA and control groups

Phenotype	Total RA patients (n=173)		HLA-DR4 positive RA (n=63)		HLA-DR4 negative RA (n=35)		Controls (n=792)‡	
	Frequency	%	Frequency	%	Frequency	%	Frequency	%
Gm								
fmb	68	39.3	21	33.3	16	45.7	344	43.4
fb	11	6.4	2	3.2	5	14.3	50	6.3
zaxg	11	6.4	5	7.9	0	0	34	4.3
zag	6	3.4	3	4.8	0	0	36	4.5
zaxfngb	17	9.8	12*†	19.0	0†	0	72*	9.1
zaxfgb	11	6.4	5	7.9	2	5.7	45	5.7
zafngb	30	17.3	9	14.3	9	25.7	126	15.9
zafgb	15	8.7	5	7.9	3	8.6	66	8.3
Others	4	2.4	1	1.6	0	0	19	2.4

* $\chi^2=6.497$, $p=0.017$, $p_c=0.136$.

†Fisher's $p=0.0033$, $p_c=0.026$.

‡Six were excluded because of racial admixture.

Table 2 Gm allotype frequencies in HLA-DR4 positive and negative RA patients and control groups

Allotype	DR4 positive RA (n=63)		DR4 negative RA (n=35)		Controls (n=792)	
	Frequency	%	Frequency	%	Frequency	%
G1m(f)	55	87.3	35	100	718	90.7
G1m(z)	40†	63.5	14†	40.0	391	49.4†
G1m(x)	22*	34.9	2*	5.7	154*	19.4
G2m(n)	43	68.3	25	71.4	556	70.2

*DR4 positive RA v controls $\chi^2=8.477$, 1df, p=0.004, p_c=0.016.
DR4 positive RA v DR4 negative RA $\chi^2=10.459$, 1df, p=0.001, p_c=0.004.
DR4 negative RA v controls $\chi^2=4.126$, 1df, p=0.048, p_c=0.192.
†DR4 positive RA v DR4 negative RA $\chi^2=5.047$, 1df, p=0.025, p_c=0.100.
DR4 positive RA v controls $\chi^2=4.608$, 1df, p=0.034, p_c=0.136.

Table 3 Frequency of Km(1) allotype in RA and control groups

Disease group	Number tested	Number Km(1) positive	% Km(1) positive
Controls	798	143†	17.9
RA overall	173	27	15.6
DR4 positive RA	63	8*	12.7
DR4 negative RA	35	9*	25.7
Early onset RA (<30 years)	25	3	12.0
Nodular RA	45	8	17.8
Vasculitic RA	8	0	0
RA + pulmonary fibrosis	4	4†	100
RA + high titre SCAT (≥1/1024)	35	5	14.3
RA + positive ANF (≥1/40)	47	7	14.9
RA + high radiological score (≥21/40)	27	4	14.8
RA + gold/D-penicillamine nephropathy (proteinuria ≥0.5 g/24 h)	5	1	20.0

* $\chi^2=2.661$, 1df, p>0.05.

†Fisher's p=0.001.

The Km(1) allotype was found in 15.6% of patients and 17.9% of controls (Table 3). 25.7% of DR4 negative RA patients were Km(1) positive ($\chi^2=2.66$, p>0.05). Recent chest radiographs were reviewed in 60 RA patients and definite, non-apical, pulmonary fibrosis was seen in four of these. In three the fibrosis was idiopathic and in one patient was due to coal-miner's pneumoconiosis. All four patients were Km(1) positive (Fisher's p=0.001) versus controls).

There were no significant differences between A2m(1) or (2) allotype frequencies in RA and control groups.

Discussion

Gm, A2m, and Km allotype and phenotype frequencies were similar in total RA and control groups so we have been unable to show any independent effect of immunoglobulin heavy chain

genes or of κ light chain genes on susceptibility to RA. However, in the DR4 positive RA group there is a statistically significant increase in the Gm(zaxfngb) phenotype and a highly significant increase in the G1m(x) allotype. These findings are in keeping with those of Probert and coworkers²⁴ and suggest that immunoglobulin heavy genes interact with DR4 or its linked susceptibility gene in the pathogenesis of RA. Class I HLA antigens and Gm have previously been shown to interact in the determination of humoral immune responses²⁵ and in the pathogenesis of chronic active hepatitis.²⁶ The biological significance of the trend for a decrease in the G1m(x) allotype in the fewer DR4 negative patients investigated is uncertain. There was a trend for the Km(1) allotype to be increased in DR4 negative patients, which if confirmed in a larger patient group would suggest a role for immunoglobulin light chain genes. No differences in terms of clinical, radiological, or immunological disease fea-

tures were noted when DR4 positive and negative patients were compared. The difference in associations with Gm or Km allotypes between DR4 positive and negative RA groups therefore suggests genetic rather than clinical heterogeneity and may have important implications in terms of future investigation of disease pathogenesis.

Previous associations have been described between the G1m(x) allotype and immune-mediated forms of renal disease.²⁷ No such association was noted in the present study, though only five patients with gold or D-penicillamine induced nephropathy were studied. We were surprised to find that all four patients with radiological signs of pulmonary fibrosis (non-tuberculous) were Km(1) positive, suggesting that immunoglobulin κ light chain genes may predispose to pulmonary disease. There is a well documented association between certain alpha-1-antitrypsin phenotypes (Pi, linked to Gm on the 14th chromosome)²⁸ and pulmonary disease in RA,²⁹ but we know of no such previously recorded association between pulmonary fibrosis and Km.

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