Class-specific rheumatoid factors, DR antigens, and amyloidosis in patients with rheumatoid arthritis

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SUMMARY Class-specific rheumatoid factors (RFs) were measured by enzyme immunoassay in 59 patients with rheumatoid arthritis complicated by systemic amyloidosis (RA+A), 47 patients with rheumatoid arthritis without amyloid (RA), 106 patients with other rheumatic diseases (juvenile rheumatoid arthritis, systemic lupus erythematosus, Sjögren's syndrome), and 55 blood donors. The patients with RA+A were characterised by a high prevalence of RF negativity; the IgM RF concentration was raised in only 18 of the 59 patients (31%, $p<0.001$ v RA), the IgG RF concentration in 20 of 59 (34%, $p<0.001$ v RA), and the IgA RF concentration in 24 of 59 (41% $p<0.001$ v RA). A higher prevalence of HLA-DR4 ($p<0.001$) and a lower prevalence of DR2 ($p<0.05$) were found among 48 tested patients with RA+A when compared with a control panel consisting of 500 blood donors. No significant differences in the prevalence of DR1-DR7 or B27 antigens were observed, however, between patients with RA with or without amyloid.

Key words: IgM rheumatoid factor, IgG rheumatoid factor, IgA rheumatoid factor, HLA-B27 antigen.

The mechanism of amyloidogenesis in amyloidosis associated with rheumatic diseases is not known in detail. Persistently high concentrations of the circulating lipoprotein associated precursor protein serum amyloid A and altered enzymatic degradation of the precursor and tissue amyloid A protein appear to be important factors.^{1,2} Only a small number of patients with these characteristics develop amyloid, however, suggesting the involvement of additional factors. Woo *et al* recently found that a DNA polymorphic site, 5' to serum amyloid P component gene, is significantly associated with amyloidosis in patients with juvenile arthritis.³ The role of genetic factors in adult rheumatoid arthritis is, however, unclear. Studies on HLA antigens have had somewhat conflicting results.⁴⁻⁶ We have previously observed that in adult rheumatoid arthritis low values of circulating IgM, IgG, and IgA rheumatoid factors are associated with the occurrence of

amyloidosis.⁷ This study examines more closely the rheumatoid factors, their isotypes and relation to inflammatory activity, and the prevalence of DR and B27 antigens in an extended patient population with rheumatoid arthritis and associated amyloidosis.

Subjects and methods

RHEUMATOID FACTOR STUDY

We studied 212 patients with various rheumatic diseases as follows: The RA+A patient group consisted of 59 patients (40 women, 19 men; mean age 54.4 years) with definite or classical rheumatoid arthritis⁸ complicated by secondary (reactive) amyloidosis proved by histological examination of renal or rectal biopsy specimens, or both. The mean duration of the rheumatoid arthritis was 17.3 years. Patients with ankylosing spondylitis, reactive arthritis, and psoriatic arthritis as well as patients with juvenile onset rheumatoid arthritis were carefully excluded from the RA+A patient group. The RA patient group consisted of 47 patients (33 women, 14 men; mean age 53.5 years) with definitive or classical rheumatoid arthritis⁸ with no clinical signs

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of amyloidosis. The mean duration of rheumatoid arthritis was 15.2 years. The JRA patient group consisted of 56 patients with juvenile rheumatoid arthritis (43 girls, 13 boys; mean age 10.2 years) without clinical signs of amyloidosis. The SS patient group consisted of 31 patients (26 women, five men; mean age 56 years) with primary (10 patients) or secondary (21 patients) Sjögren's syndrome. The SLE patient group consisted of 19 patients with systemic lupus erythematosus (18 women, one man; mean age 36.5 years). The healthy control subjects consisted of 55 blood donors (43 men, 12 women; mean age 37 years).

HLA ANTIGEN STUDY

The patient population consisted of a combination of a Heinola series and Helsinki series. The RA+A group comprised 48 patients with classical or definite rheumatoid arthritis⁸ (33 women, 15 men; mean age 50.8 years; mean duration of RA 18.4 years). The RA group consisted of 31 patients with classical or definite RA⁸ (26 women, five men; mean age 52 years; mean duration of RA 20 years). Amyloidosis was excluded in these patients with RA by a negative staining for amyloid in rectal biopsy specimens. The control panel consisted of 500 Finnish voluntary blood donors who had been typed at the Finnish Red Cross, Helsinki.

MEASUREMENT OF RF ISOTYPES

Solid phase immunoassays were used to measure IgG, IgA, and IgM-type RFs. Polystyrene tubes were coated with swine IgG (Sigma Chemical Co, MO, USA) as follows: 1 ml swine IgG (Sigma Chemical Co, MO, USA), 4 mg/l in 50 mM phosphate buffer (0.5 g of NaN₃ per litre, pH 7.3), was incubated at 4°C overnight in polystyrene tubes. The tubes were then washed with distilled water, and 1 ml of serum diluted 100-fold with phosphate buffered saline containing 0.05% Tween 20 (PBS-Tween) was incubated in each tube at 4°C overnight to allow rheumatoid factors to bind to the surface adsorbed swine IgG. The alkaline phosphatase labelled, heavy chain specific swine antibodies to human IgG, IgA, or IgM (Orion Diagnostica, Espoo, Finland) (1 ml of 500-fold diluted in PBS-Tween) were added and incubated at 37°C for one hour. The amount of alkaline phosphatase fixed to the tubes was determined in diethanolamine-MgCl₂ buffer, pH 10, at 37°C with *p*-nitrophenyl phosphate as substrate. The concentration of rheumatoid factors is expressed as a change in absorbance (ΔA) at 406 nm during 30 minutes. In each assay a positive (Waller-Rose titre 1/1280; test dilutions of 1:100, 1:200, 1:400, and 1:800) and a negative (1:100 dilution) control serum were included. To eliminate

the possible interference of the non-RF IgG in the IgG RF assay we determined the IgG-type RFs both before and after the removal of IgG by pepsin digestion.⁹ The removal of non-RF IgG did not increase the amount of IgG RF in any of the sera. Therefore in this study the values of IgG RFs obtained without pepsin treatment are reported. Addition of purified non-rheumatoid factor IgG, IgA, and IgM to the RF positive and RF negative sera did not increase the concentration of respective RFs in any case by more than 5% (Hannonen P *et al*, unpublished data), indicating thus minimal non-specific binding of non-RF protein.

HLA TYPING

HLA typing was carried out with peripheral blood lymphocytes using the two stage microcytotoxicity assay described by Amos *et al*.¹⁰

STATISTICS

Statistical significances were assessed using the χ^2 test and Wilcoxon's ranking test for unpaired data. In this study only previously described HLA antigen associations were tested for statistical difference; hence the *p* values were not adjusted for the effect of multiple comparisons.¹¹

Results

Figs 1–3 show the concentrations of class-specific RFs in the rheumatic patients. When compared with patients with RA without amyloid, the patients with RA+A had significantly more often RF concentrations within the range of those of the normal subjects (IgM RF, *p*<0.001; IgG RF, *p*<0.001; IgA RF, *p*<0.001). With respect to IgG RF, the patients with RA+A did not differ significantly from the patients with JRA. Serum samples of patients with SLE frequently contained both IgG RF (7/19 (37%)) and IgA RF (9/19 (47%)).

There was no significant difference in C reactive protein (CRP) concentrations between the patients with RA+A and those with RA alone. When the patients with RA+A or RA were divided into two groups with differing inflammatory activity, as measured by CRP, no significant differences were found in the class-specific RF concentrations between patients with CRP concentrations ≥ 25 mg/l or <25 mg/l. (Table 1). Follow up of individual patients with RA+A showed some fluctuation in the RF concentrations. Fig. 4 illustrates the variations in class-specific RF concentrations in relation to the variation in Waller-Rose titre and CRP concentration in a Waller-Rose positive patient with RA+A, and Fig. 5 the variations observed in a Waller-Rose negative (titre <20) patient with

Fig. 1 IgM RF values in rheumatic diseases. ΔA =change in absorbance at 406 nm during 30 minutes.

Fig. 2 IgG RF values in rheumatic diseases. ΔA =change in absorbance at 406 nm during 30 minutes.

Fig. 3 IgA RF values in rheumatic diseases. ΔA = change in absorbance at 406 nm during 30 minutes.

Fig. 4 Changes in the serum concentrations of CRP and RF and in the Waaler-Rose titres during follow up of a Waaler-Rose positive patient with rheumatoid arthritis and amyloidosis.

Fig. 5 Changes in the serum concentrations of RF and CRP during follow up of a Waaler-Rose negative patient with rheumatoid arthritis and amyloidosis.

Table 1 IgM, IgG, and IgA RF values in patients with RA and RA+A with a serum C reactive protein (CRP) concentration of either ≥ 25 mg/l or < 25 mg/l

Patient group	CRP (mg/l)	Number of patients	CRP (mg/l)	Δ A RF		
			Median (range)	IgM Median (range)	IgG Median (range)	IgA Median (range)
RA	≥ 25	23	54 (25-210)	1.140 (0.160-3.585)	0.420 (0.120-0.813)	0.320 (0.015-1.580)
	< 25	24	10 (< 5 -24)	1.315 (0.030-2.710)	0.436 (0.070-1.446)	0.505 (0.015-2.075)
RA+A	≥ 25	28	45 (28-310)	0.073 (0.000-3.200)	0.096 (0.000-0.810)	0.044 (0.000-2.77)
	< 25	31	10 (< 5 -23)	0.135 (0.000-3.520)	0.095 (0.000-1.052)	0.100 (0.000-2.043)

The differences in RF values between the subgroups with CRP ≥ 25 mg/l or < 25 mg/l are not significant.

RA+A. In the latter patient the changes in IgG RF and CRP concentrations paralleled each other.

At the time of sampling 35 of the 59 patients with RA+A had a Waaler-Rose titre < 20 ($p < 0.001$ v patients with RA). Retrospective review of previously measured Waaler-Rose titres showed that 26 of the 59 patients with RA+A had been Waaler-Rose negative during the whole disease period ($p < 0.05$ v RA group); in nine patients with RA+A a conversion of a Waaler-Rose positivity to negativity had occurred.

No significant differences were observed in the prevalence of the tested HLA antigens between patients with RA with or without amyloid (Table 2). A higher prevalence of DR4 ($p < 0.001$) and a lower prevalence of DR2 ($p < 0.05$) were found among the patients with RA+A as well as all patients with RA when compared with the normal population.

Table 2 HLA-DR and B27 prevalence (%) in patients with RA+A, patients with RA (combined Helsinki and Heinola series), all patients with RA, and in the Finnish voluntary blood donors

Antigen	Patients with RA+A (n=48)	Patients with RA (n=31)	All patients with RA (n=79)	Finnish blood donors (n=500)
B27	22.9	16.1	20.2	14.0
DR1	45.8	32.3	40.5	38.6
DR2	18.8*	25.8	21.5*	34.0
DR3	20.8	25.8	22.7	24.0
DR4	58.3**	48.4	54.4**	29.5
DR5	14.6	6.0	11.3	10.2
DR6	2.1	9.7	5.1	8.3
DR7	16.6	16.1	16.4	12.0

* $p < 0.05$ v control population; ** $p < 0.001$ v control population. The differences between the patients with RA+A and patients with RA are not significant.

Discussion

The present results show that the subset of patients with RA who have amyloidosis have lower concentrations of circulating IgM, IgG, and IgA RFs than the patients with RA with no clinical signs of amyloid disease. The RA+A and RA patient groups were comparable with respect to age, sex, duration of RA, and inflammatory activity as expressed by CRP concentrations, so these factors are not likely to have influenced the results. The patient groups were not directly matched for the use of drugs, but a review of the clinical records showed that the patients in both groups used, or had been using, the same type of drugs, including steroids and gold salts. As uraemia may be immunosuppressive the question arises whether the high prevalence of RF negativity among the amyloid patients may have resulted from the amyloid state itself. Many of the amyloid patients were, however, not uraemic, and retrospective review of previously measured Waaler-Rose titres showed that in only nine cases had a conversion from a seropositive to a seronegative state occurred. It is thus unlikely that the result can be explained on this basis only, though it appears possible that the amyloid state as well as differences in drug treatment^{13 14} may have influenced the absolute concentrations of circulating RFs.

Previous studies have demonstrated positive associations of HLA-DR4, Dw4, and DRw5 and negative associations of DR2 and DR7 with RA.¹⁵⁻¹⁹ Stronger associations were found in patient subsets with seropositivity, severe erosions, and extra-articular disease. In accordance with these studies we found a significantly higher prevalence of DR4 and a lower prevalence of DR2 in the patients with RA. The patients with RA+A, however, did not differ significantly from the patients with RA with respect to the tested DR antigens, suggesting that the low RF concentrations in the patients with amyloidosis are not related to DR status.

Arthropathies strongly associated with the occurrence of HLA-B27 are typically seronegative disorders. An increased prevalence of HLA-B27 has also been reported in Finnish patients with RA+A,^{4,5} in patients with RA,²⁰ as well as in the Finnish population in general.²⁰ Thus the possible relation between the low RF concentrations found in the patients with RA+A and HLA-B27 deserves consideration. Although there was a tendency towards accumulation of B27 among the patients with RA+A, the prevalence of B27 did not differ significantly from that in patients with RA without amyloidosis or that in the control population. Our findings are thus unlikely to be related to HLA-B27. This conclusion is also supported by the results of the RF concentrations among the control patients with RA, who were seropositive in about 80% of the cases, a finding which corresponds with those in patient series from countries with no increased prevalence of HLA-B27 among patients with RA. We do not know the reason for the discrepancies in the HLA-B27 results with respect to the patients with RA+A, but it is noteworthy that special effort was made to exclude patients with ankylosing spondylitis from the RA+A patient group.

The biological role of RFs is not known. They are low affinity antibodies with specificity for the antigenic determinants in the Fc region of IgG. RFs are found at low concentrations in normal sera and at high concentrations in several autoimmune and infectious diseases.²¹ RFs are part of the immunological network²² and may facilitate the clearance of immune complexes.²³ Although seropositivity at the onset of RA implies a poorer prognosis,^{24,25} several studies have failed to show a relation between IgM RF titres and disease activity.²⁶⁻³⁰ Demonstration of an association between amyloid disease and RF negativity in adult RA may be interpreted to mean some protective role of RFs, at least with respect to the development of amyloid, or, more likely, that seronegativity and the development of amyloid may be related to similar genetic factors yet unknown. The findings that about 30-40% of the patients with RA+A, depending on the immunoglobulin class of the RFs, were seropositive and that a few of them also had high concentrations of IgM, IgA, or IgG RF demonstrate that the amyloid disease is not directly related to RFs. It has previously been pointed out that seronegative RA represents a disease entity clinically and immunogenetically different from seropositive RA.³¹ This study strengthens that view.

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