

IgM rheumatoid factor (RF), IgA RF, IgE RF, and IgG RF detected by ELISA in rheumatoid arthritis

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SUMMARY One hundred patients with rheumatoid arthritis (RA), of whom 73 were seropositive by latex or Waaler-Rose (WR) assays, or both, 100 healthy subjects, and 102 diseased controls (22 patients with systemic lupus erythematosus (SLE) and 80 with bronchial asthma) were evaluated for the presence of IgM rheumatoid factor (RF), IgA RF, IgE RF, and IgG RF by an enzyme linked immunosorbent assay (ELISA). Ninety two per cent, 65%, 68%, and 66% of the patients with RA were found to be positive for IgM, IgA, IgE, and IgG respectively. A positive correlation existed between the levels of IgM RF and IgA RF on the one hand and disease activity on the other, and the levels of IgM RF and IgA RF correlated with the levels of circulating immune complexes as measured by a C1q binding assay. The presence of extra-articular features also correlated positively with the levels of IgA RF and IgE RF. Five out of six patients with Sjögren's syndrome had very high levels of IgA RF. Of 47 patients typed for HLA-DR, DR1 and DR2 were significantly more frequent in those with the highest levels of IgM RF. Conversely, DR3 was associated with low levels or absence of IgA RF and IgE RF. These results suggest that immune response genes may regulate the level of different RF isotypes. The frequencies of IgM, IgA, IgE, and IgG RF were 59%, 36%, 9%, and 27% respectively in SLE and 25%, 2.5%, 70%, and 59% in bronchial asthma.

Key words: systemic lupus erythematosus (SLE).

Standard assays for rheumatoid factor (RF), such as the latex or Waaler-Rose (WR) tests, favour the detection of the IgM RF, which is found in only 60-70% of RA sera.^{1,2} Immunofluorescent techniques, radioimmunoassay, and enzyme immunoassay enable measurement of the isotype specific IgM, IgA, IgG, and IgE RF.³⁻⁶ It is necessary to re-evaluate the notion of seronegative RA determined by these agglutination reactions in the context of the greater sensitivity of more recent techniques.

The aim of this study was the detection (using ELISA) of IgM, IgG, IgA, and IgE RF in 100 patients with RA, 22 with SLE, and 80 with bronchial asthma, to compare the results with the

latex and WR tests, and to consider the relation between RF isotypes and clinical findings such as disease activity, systemic manifestations, and HLA-DR antigens.

Patients and methods

PATIENTS

One hundred patients with RA were studied: 86 women and 14 men with ages ranging from 19 to 86 years. They fulfilled the American Rheumatism Association (ARA) criteria.⁷ Clinical evaluation of disease activity was performed on the basis of the duration of morning stiffness, the number of diseased joints, and erythrocyte sedimentation rate. The disease was thus graded as very (IV), moderately (III), slightly active (II), or inactive (I).^{8,9} Radiographic staging of RA was carried out according to the technique described by Stein-

brocker *et al.*¹⁰ Blood was drawn for estimation of erythrocyte sedimentation rate, isotype specific ELISA, latex and WR tests, circulating immune complexes by the C1q binding test,¹¹ antinuclear antibodies (indirect immunofluorescent techniques on blood smears), and anti-double stranded deoxyribonucleic acid antibodies (Farr assay). HLA-A, B, C, and DR typing was performed in 47 patients.

We also studied the sera of 22 patients with SLE (21 women and one man) with ages ranging from 18 to 80 years. They were diagnosed on the basis of the presence of at least four of the ARA revised criteria.¹² Eighty sera from young patients with bronchial asthma were also studied. Serum samples from 100 healthy subjects were used to determine the upper limit of normal activity for the different RF isotypes (mean (2 SD)).

DETERMINATION OF RF

Latex fixation tests were performed by the Singer and Plotz technique,¹³ a titre of >1/80 being considered positive. WR tests were determined by the technique proposed by Eyquem *et al.*¹⁴ and a titre of >1/32 was considered positive.

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

Flat well microplates (Nunc) were coated with 100 µl of aggregated human IgG (25 µg/ml). Phosphate buffered saline (100 µl) containing 5% bovine serum albumin were used to coat the control without antigen. The plates were then incubated with test serum samples diluted in phosphate buffered saline/Tween-20 containing 5% bovine serum albumin in triplicate. Serum sample dilutions used were 1/40 for

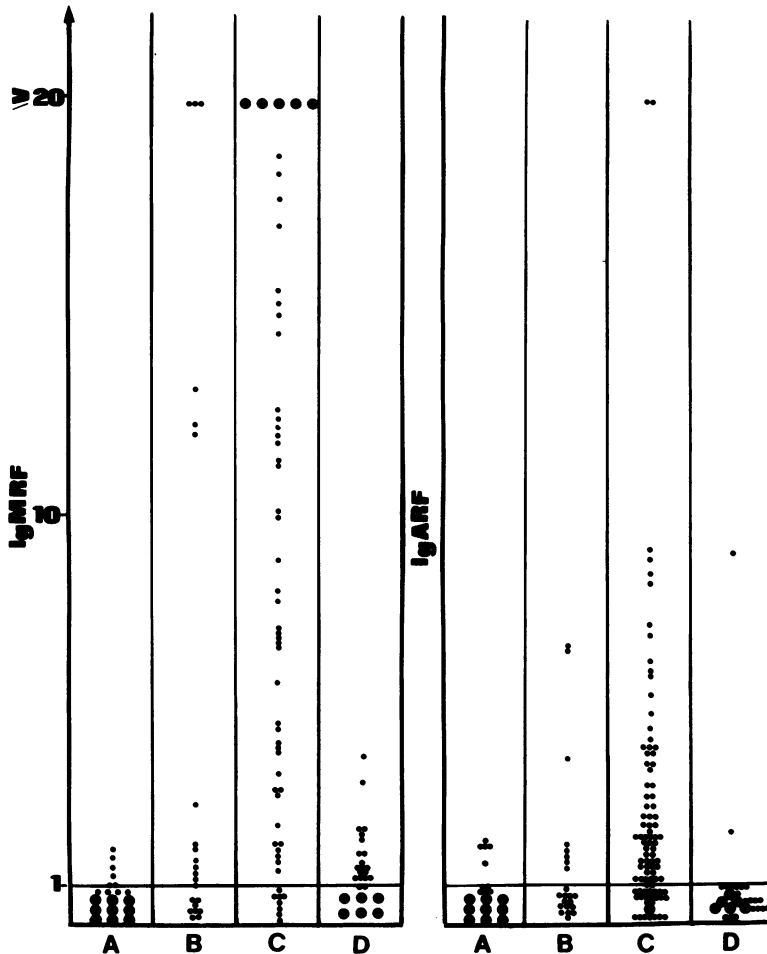


Fig. 1 IgM RF and IgA RF distribution in (A) 100 control sera, (B) 22 SLE sera, (C) 100 RA sera, and (D) 80 bronchial asthma sera by ELISA (OD patient sera/OD upper limit control sera). ●=one serum; ●=10 sera.

Table 1 Occurrence of IgM, IgG, and IgE RF in 100 patients with RA determined by an ELISA

ELISA		Latex/WR test		%
		Seropositive (n=73)	Seronegative (n=27)	
IgM RF	+	73	19	92
	-	0	8	
IgA RF	+	62	3	65
	-	11	24	
IgG RF	+	50	16	66
	-	23	11	
IgE RF	+	59	9	68
	-	14	18	

IgM RF and IgA RF, 1/6 for IgE RF, and 1/100 for IgG RF.

The plates were then incubated with antihuman immunoglobulin labelled with peroxidase (Institut Pasteur Production, Paris). The dilution used was 1/400 for the goat antihuman IgM and sheep antihuman IgA, 1/800 for sheep antihuman IgE, and 1/1000 for goat antihuman IgG. After addition of the substrate the plates were read with a spectrophotometer at 492 nm. The results were expressed as:

$$\frac{(\text{mean OD with Ag} - \text{mean OD without Ag}) \text{ test serum}}{(\text{mean OD with Ag} - \text{mean OD without Ag}) \text{ control serum}}$$

where OD=optical density, Ag=antigen.

Sera for which this fraction was >1 were considered as positive.

Before the IgG RF assay serum samples were treated with dithiothreitol (Sigma).

Results

RF ISOTYPES DETECTED BY ELISA IN RA
 IgM RF was present in 92/100 RA sera (73/73 RA sera seropositive by the latex or WR tests and 19 of the 27 seronegative RA) (Fig. 1, Table 1). The correlation coefficient was r=0.69, p<0.001 for the WR or the latex test.

IgA RF was present in 65/100 RA sera (Fig. 1).

IgG RF was found in 66/100 RA sera. The IgG RF levels were lower than the IgA RF and IgM RF levels. Furthermore, the IgG RF upper limit of normal was high because IgG RF was present in a high number of normal sera (Fig. 2).

Sixty eight of 100 RA sera contained low levels of IgE RF (Fig. 2).

Of 92 sera from patients with RA with IgM RF, 65 also had IgA RF. There was a significant correlation between the level of IgM RF and IgA RF (r=0.66, p<0.001), and between IgE RF and IgM RF (r=0.26, p<0.005), but none between IgM RF and IgG RF.

RF ISOTYPES DETECTED BY ELISA IN CONTROL SERA FROM SUBJECTS WITH OTHER DISEASES

IgM RF was present in the sera of 13/22 patients with SLE (59%), but the level was low by comparison with RA sera, as was the case for IgA RF which was present in 8/22 sera (36%), IgE RF in 2/22 sera (9%), and IgG RF in 6/22 sera (27%) (Table 2, Figs 1 and 2).

Twenty out of 80 (25%) patients with bronchial asthma had IgM RF, 2/80 (2.5%) IgA RF, 56/80 (70%) IgE RF, and 47/80 (59%) IgG RF. The mean levels of all these isotypes were lower than in RA (Figs 1 and 2).

RF ISOTYPES AND CLINICAL ACTIVITY IN PATIENTS WITH RA

No significant correlation was found between RF isotypes and age, sex, duration, or onset of the disease.

Using variance analysis IgM RF and IgA RF were shown to have significant correlations with the four grades of activity: p<0.0015 and p<0.02 respectively (Table 2). Eight patients with RA without IgM RF and IgA RF had either inactive (four cases) or very recent disease (six to 18 months) (four cases). The presence of circulating immune complexes also correlated with IgM RF and IgA RF: r=0.69, p<0.001. Antinuclear antibodies with the highest values (>1/4096) were significantly associated with high levels of IgM RF and IgA RF ($\chi^2=7.16$, p<0.01 and $\chi^2=5.35$, p<0.02 respectively). Radiographic stages did not correlate with the levels of RF isotypes. Of 25 patients with extra-articular features, 24 presented the four isotypes in their sera. IgM RF, IgA RF, and IgE RF levels were particularly high when compared with RA without extra-articular features ($\chi^2=1.1$, p=NS for IgM RF, $\chi^2=7.4$, p<0.01 for IgA RF, $\chi^2=10.0$, p<0.001 for IgE RF). There was no correlation between IgE RF levels and total IgE concentration in the sera. High levels of IgE RF were not associated with allergic symptoms.

The lymphocytes of 47 RA sera were tested for HLA-DR antigens. DR3 antigen, found in 11 patients, was present at a frequency significantly greater than that of the normal population when IgA RF or IgE RF were absent or present at a low level, whereas DR1 and DR2 antigens were associ-

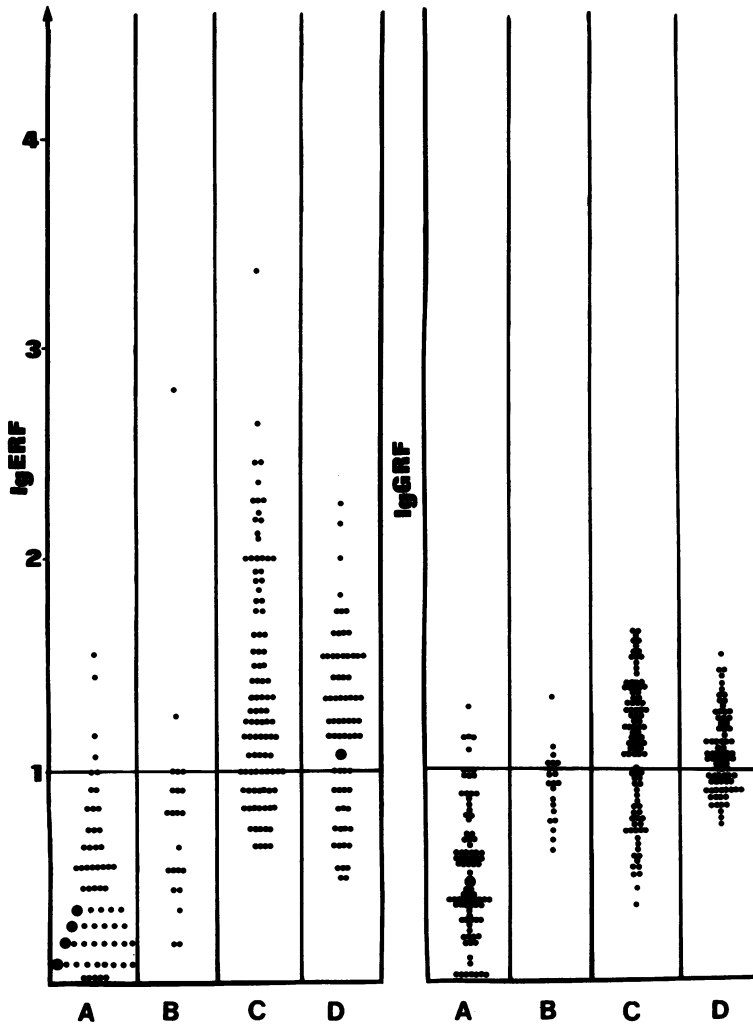


Fig. 2 *IgE RF and IgG RF distribution in (A) 100 control sera, (B) 22 SLE sera, (C) 100 RA sera, and (D) 80 bronchial asthma sera by ELISA (OD patient sera/OD upper limit control sera). ●=one serum; ●=10 sera.*

Table 2 *RF levels in 100 patients with RA and relations between RF levels and clinical disease activity. RF levels in 22 patients with SLE and 80 with bronchial asthma*

	<i>n</i>	<i>IgM RF</i>	<i>IgA RF</i>	<i>IgE RF</i>	<i>IgG RF</i>
RA	100	13.53 (7.60)*	2.42 (3.15)	1.39 (0.53)	1.13 (0.41)
Disease activity					
I	13	10.53 (7.57)	0.96 (0.83)	1.30 (0.57)	1.22 (0.33)
II	29	13.97 (8.46)	1.60 (2.74)	1.17 (0.38)	1.00 (0.35)
III	39	23.56 (6.30)	2.75 (2.40)	1.33 (0.47)	1.04 (0.28)
IV	19	23.62 (5.72)	3.70 (4.19)	1.62 (0.46)	1.18 (0.26)
		<i>p</i> <0.0015	<i>p</i> <0.02	NS	NS
SLE	22	5.66 (7.44)	1.44 (1.89)	0.79 (0.54)	0.92 (0.15)
Asthma	80	0.88 (0.56)	0.31 (0.12)	1.33 (0.37)	1.08 (0.16)

*Values are mean (SD).

Table 3 HLA-DR antigens in 47 RA sera. IgM, IgA, IgE, and IgG RF levels by ELISA

HLA-DR	n	IgM RF	IgA RF	IgE RF	IgG RF
DR1	15	24.73 (5.78)*	2.76 (2.54)	1.61 (0.45)	1.10 (0.20)
2	11	19.29 (7.93)	2.78 (2.03)	1.34 (0.47)	1.11 (0.24)
3	11	15.45 (8.37)	1.12 (1.75)	1.09 (0.36)	1.03 (0.35)
4	23	13.40 (8.40)	1.12 (1.02)	1.32 (0.53)	1.07 (0.36)
		p<0.06 (NS)	p<0.01	p<0.04	NS

*Values are mean (SD).

ated with the highest levels of IgM, IgA, IgE, and IgG RF (Table 3).

Discussion

ELISA was first used by Vejtorp *et al*¹⁵ and Maiolini *et al*¹⁶ for the detection of IgM RF and enables the determination of IgM RF with a greater sensitivity and specificity than agglutination reactions.^{17, 18} All seropositive RA sera (with latex or WR tests, or both) used in our study were positive in ELISA, and furthermore, 19/27 (70%) 'seronegative' RA sera were positive in ELISA. This assay recognised a positivity of 92% for our RA population, whereas this was only 73% in latex or WR tests.

In addition, ELISA and radioimmunoassay are able to measure the different isotypes of RF.^{9, 19-25} The results have been contradictory in the case of IgG RF because of technical problems: IgG RF is present in normal subjects^{20, 21} and the control antigen gives a high value as the antigen is an aggregated IgG recognised by the anti-IgG conjugate (McDougal *et al* eliminated this difficulty by using Fc as antigen and anti-Fd as conjugate²²). The IgG RF frequency in patients with RA varies between 36% to 59%.^{9, 19, 23-25} Our results show low IgG RF values in 66% of patients with RA (58% of seronegative RA and 68% of seropositive RA).

IgA RF was present in 65% of our RA sera, whereas Bampton *et al* reported IgA RF in 88% of their RA sera,¹⁹ and Tarkowski and Nilsson in only 19%.⁹

Mizushima *et al*,²⁶ Zuraw *et al*,²⁷ and Youinou *et al*²⁸ who studied IgE RF using ELISA, radioimmunoassay, and immunofluorescent techniques respectively, detected them in 16%, 79%, and 34% of the RA sera studied. IgE RF was present in 68% of our RA sera with levels close to those of Zuraw *et al*.²⁷ IgM RF was present in 59% of SLE sera, compared with 31% found with agglutination methods. Figs 1 and 2 illustrate the low values detected in SLE (compared with RA sera) for IgM RF, IgA RF, IgG RF, and IgE RF and lower frequency (59%, 36%, 27% and 9%). IgA RF was rare in sera from

patients with bronchial asthma, whereas IgE RF was present in 70% of these patients. Zuraw *et al* found IgE RF in only 3/32 patients with bronchial asthma.²⁷

Our study emphasises the clinical value of measuring individual isotype IgA RF and IgM RF levels. These have a significant correlation with the clinical activity of the disease and the levels of circulating immune complexes as determined by C1q binding assay.

Nishimaki *et al*⁸ and Tarkowski and Nilsson⁹ reported a positive correlation with the activity staging and RF whatever the isotype considered (IgM, IgG, or IgA). Pope and McDuffy showed a close association between high levels of RF (IgM or IgG) and circulating immune complexes when detected by C1q binding assay.²⁹

Some authors^{9, 30-33} have reported an association between IgA RF and RA with systemic symptoms and poor prognosis. We also found IgA RF in clinically and biologically active RA and also in 80% of RA with extra-articular features (5/6 with Sjögren's syndrome). Carson *et al*,¹ McDougal *et al*,²³ Pope and McDuffy,²⁹ Quismorio *et al*,³⁴ and Lessard *et al*³⁵ found an association between IgG RF and disease activity vasculitis, but, like Wernick *et al*,³⁶ we found that IgG RF levels were independent of the clinical pattern.

The clinical significance of IgE RF was studied by Mizushima *et al*,²⁶ who associated these factors with vasculitis (p>0.01). Youinou *et al*, however, considered that although RA with IgE RF indicated a poor clinical and biological prognosis, IgE RF was not associated with extra-articular features.²⁸ In our study IgE RF levels were significantly higher in active progressive RA with extra-articular features.

Interestingly, the determination of HLA-DR antigens enabled us to show a significant association between low IgA RF levels and DR3 and DR4 antigens, whereas DR1 and DR2 antigens were associated with high levels of IgA RF, IgM RF, and IgE RF. The association of HLA-DR4 antigen and IgM RF remains very controversial.³⁷⁻³⁹ We found

low IgM RF levels in HLA-DR4 RA subjects, but this result did not appear to be significant. Alarcon *et al* have reported that HLA-DR3 was not present in white RA subjects with high IgA RF (11 RA).⁴⁰ Only one of our 11 DR3 positive RA patients had raised IgA RF levels, but this subject was also DR2 positive, this latter antigen being associated with high IgA RF levels (9/11 RA).

A study of larger populations will be necessary to confirm the role of genetic factors in the regulation of RF isotype level production.

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