Clinical implications of IgA rheumatoid factor subclasses

T Jónsson, H Thorsteinsson, S Arinbjarnarson, J Thorsteinsson, H Valdimarsson

Abstract

Objectives—To evaluate the diagnostic and pathogenetic significance of IgA rheumatoid factor (RF) subclasses in rheumatoid arthritis (RA).

Methods—Rheumatoid factors of the IgA class and IgA1 and IgA2 subclasses were measured by enzyme linked immunosorbent assay in 58 patients with RA, 31 patients with other rheumatic diseases, 30 non-rheumatic individuals with increased concentrations of IgA RF, and in 100 randomly selected healthy controls.

Results—Using a 95% cut off for the controls, 55% of the RA patients had increased total IgA RF, 64% IgA1 RF, and 60% IgA2 RF. RA patients with extra-articular manifestations more often had increased concentrations of IgA RF and both subclasses than patients without such manifestations ($p \leq 0.01$). Nearly all (31/32) RA patients with increased IgA RF had increases in both IgA RF subclasses, compared with 67% (20/30 of non-rheumatic symptom free individuals with increased IgA RF ($p = 0.002$).

Conclusion—Increased concentrations of the IgA2 RF subclass appears to be more specific for RA than increased IgA1 RF. Measurement of IgA RF subclasses may be clinically useful.

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Rheumatoid factors (RF) are antibodies directed against the Fc part of immunoglobulin G (IgG) molecules.¹ Small concentrations of RF can be detected in most healthy individuals, and these antibodies may have an immunoregulatory function.^{2,3} Increased concentrations of RF are characteristic of rheumatoid arthritis (RA), although increased RF can also be detected in some other rheumatic diseases, infections, and in a few apparently healthy individuals.^{1,4,5} Indeed, population studies have shown that the great majority of individuals with increased RF, including IgA RF, do not have clinical manifestations of RA or other rheumatic diseases.^{5,6} Individual RF classes and subclasses can now be measured by solid phase assays.^{7–9}

It has been reported that increased IgA RF is more specific for RA than are increases in other RF classes, and IgA RF has also been claimed to be an indicator of poor prognosis.^{6,10,11} Thus IgA RF has been associated with early development of bone erosions^{12–14} and with extra-articular manifestations,

especially from secretory glands and mucous membranes.^{15,16} To our knowledge, measurement of IgA subclasses in RA patients has been reported previously only by Otten *et al*,⁹ but their clinical significance was not evaluated.

We report the measurement of IgA1 and IgA2 in a group of RA patients with and without extra-articular manifestations, patients with other rheumatic diseases, and in a group of symptom free individuals with increased total IgA RF.

Patients and methods

PATIENTS AND SAMPLES

We tested 219 serum samples for total IgA RF and both IgA RF subclasses. The samples were taken from three groups of patients: 58 RA patients attending the University Hospital outpatient clinic; 31 patients with other rheumatic diseases, including 22 with systemic lupus erythematosus, three with mixed connective tissue disease, two with juvenile rheumatoid arthritis, three with psoriatic arthritis, and one with Reiter's disease; 30 non-rheumatic individuals with increased total IgA RF. As this was a hospital based study, the RA patients tended to have severe disease. Samples from 100 randomly selected healthy individuals, aged 31 to 50, were used as controls.

The non-rheumatic individuals with increased concentrations of IgA RF had previously participated in a population based study on the significance of increased RF.⁶ When the blood samples were collected, all participants answered a structured questionnaire about past and present rheumatic symptoms and diseases known to be associated with increased RF. Furthermore, they were assessed clinically and radiographs taken of their hands for determination of bone erosions. The individuals selected for this analysis did not have symptoms or signs associated with rheumatic diseases.

MEASUREMENT OF TOTAL IGA RF, IGA1 RF AND IGA2 RF

Total IgA RF activity was measured by an enzyme linked immunosorbent assay (ELISA) described previously.¹⁷ Briefly, microtitre plates (Dynatech Immulon I) were coated overnight at 4°C with a 40 µg/ml solution of purified rabbit IgG (Sigma), then serum samples (1/40) and serial dilutions (1/40 to 1/2560) of a local standard were incubated for two hours at room temperature. A 40 µg/ml solution of heat aggregated rabbit IgG was then added to block any free IgG binding sites on

Department of Immunology, National University Hospital, Reykjavik, Iceland
T Jónsson
H Thorsteinsson
S Arinbjarnarson
H Valdimarsson

Department of Rheumatology
J Thorsteinsson

Correspondence to:
Dr Thorbjörn Jónsson,
Department of Immunology,
National University Hospital,
Landspítaliinn, 101
Reykjavik, Iceland.

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Total IgA rheumatoid factor (RF), IgA1 RF and IgA2 RF concentrations in patients and controls. Group 1 = 100 randomly selected controls; group 2 = 58 patients with RA; group 3 = 31 patients with other rheumatic diseases; group 4 = 30 non-rheumatic symptom free individuals with increased total IgA RF. The 95% cut off for the control group is indicated by the broken horizontal line. AU = Arbitrary units.

the solid phase bound RF. After two hours of incubation with mouse monoclonal anti-human IgA (Oxoid, clone 2D7), an appropriate dilution of alkaline phosphatase conjugated rabbit anti-mouse IgG was added. Finally, a 1 mg/ml solution of para-nitrophenylphosphate substrate solution was incubated and the colour reaction read in a microplate reader (Flow Laboratories) when absorbance of the 1/40 dilution of the local standard was approximately 1.6–1.8. Results were expressed in arbitrary units (AU/ml) according to a local standard (see below). IgA1 RF and IgA2 RF were measured in the same manner, except that mouse monoclonal anti-IgA1 (Nordimmune, clone NI 69-11) or anti-IgA2 (Nordimmune, clone NI-512) antibodies were used.

A local standard was prepared from a serum pool collected from 11 patients with RA and large concentrations of RF. This standard was titrated against the International Reference Preparation of Rheumatoid Arthritis Serum (Statens Serum Institute, Copenhagen, Denmark). The local standard was found to contain 3.75 times more of total IgA RF than the International Standard and was therefore given an arbitrary value of 375 units (AU/ml). Similarly, the local standard was given a value of 375 AU/ml for both IgA RF subclasses.

RF values above the 95% cut off level for 100 randomly selected controls were considered to be increased. Thus the upper limit of normal was 25 AU/ml for total IgA RF activity, 11 AU/ml for IgA1 RF, and 23 AU/ml for IgA2 RF.

MEASUREMENT OF IgM RF

IgM RF was measured by ELISA as previously described for IgA RF, except that mouse monoclonal anti-human IgM antibodies (Sigma, clone MB-11) were used for detection of the solid phase bound RF.¹⁷ Fewer than 5% of randomly selected controls were positive for IgM RF by this method.

STATISTICAL EVALUATION

The results of the study were evaluated with the chi-square test and Spearman's rank coefficient of correlation. The level of significance was set at $p < 0.05$.

Results

The figure shows the concentrations of IgA RF, IgA1 RF, and IgA2 RF in the patients and control subjects. Using a 95% cut off for the healthy controls, increased concentrations of IgA RF were found in 55% (32/58) of the RA patients; 64% (37/58) had increased IgA1 RF, and 60% (35/58) had increased IgA2 RF. In contrast, only 10% (3/31) of the patients with other rheumatic diseases had increased IgA RF; 13% (4/31) had increased IgA1 RF and 6% (2/31) had increased IgA2 RF ($p < 0.0001$).

Table 1 shows the prevalence of increased IgM RF, IgA RF, and both IgA RF subclasses

Table 1 Patients with increased IgM rheumatoid factor (RF), IgA RF, and its subclasses in patients with rheumatoid arthritis (RA) and other rheumatic diseases

Increase in	RA (n = 58)		Other rheumatic diseases (n = 31)	
	No	%	No	%
IgM RF	40	69	8	26
IgA RF	32	55	3	10
IgA1 RF	37	64	4	13
IgA2 RF	35	60	2	6*

*p = 0.038 compared with IgM RF.

in patients with RA and other rheumatic diseases. Among these patients, 26% (8/31) had increased IgM RF, while only 6% (2/31) had increased IgA2 RF (p = 0.038).

Twenty five of the 58 RA patients (43%) had some extra-articular manifestations, including 21 with rheumatoid nodules, seven with sicca symptoms, two with rheumatoid lung, one patient with vasculitis and one with Felty's syndrome. Compared with the RA patients who did not have extra-articular manifestations, significantly greater numbers of these patients had increased concentrations of IgA RF (p = 0.006), IgA1 RF (p = 0.010), and IgA2 RF (p = 0.008) (table 2). This difference was not attributable to the RA patients with sicca syndrome, as they did not have greater concentrations of IgA RF or its subclasses than patients with other extra-articular manifestations (data not shown).

Table 3 shows that all RA patients with increased total IgA RF had also increased concentrations of the IgA1 RF subclass, and 97% (31/32) had increased IgA2 RF. Of the non-rheumatic individuals with increased total IgA RF, 97% (29/30) had increased IgA1 RF, but only 67% (20/30) had increased IgA2 RF. Thus 97% (31/32) of the IgA RF positive RA patients had increases in both IgA RF subclasses, while 33% (10/30) of non-rheumatic individuals with increased total IgA RF had an isolated increase in IgA1 RF (p = 0.002). The non-rheumatic individuals with increased IgA2

Table 2 Rheumatoid arthritis (RA) patients with and without extra-articular manifestations (EAM) in relation to increased concentrations of IgA rheumatoid factor (RF) and its subclasses

Increase in	RA patients				p†
	With EAM (n = 25)		Without EAM (n = 33)		
	No	%	No	%	
Total IgA RF	19	76	13	39	0.006
IgA1 RF	21	84	17	52	0.010
IgA2 RF	20	80	15	45	0.008

†Evaluated with the chi-square test.

Table 3 Increases in concentrations of IgA rheumatoid factor (RF) subclasses in patients with rheumatoid arthritis (RA) and non-rheumatic symptom free individuals with increased total IgA RF

Increase in	RA (n = 32)		Non-rheumatic individuals (n = 30)		p†
	No	%	No	%	
IgA1 RF	32	100	29	97	NS
IgA2 RF	31	97	20	67	0.002

†Evaluated with the chi-square test.
NS = Not significant.

Table 4 Correlation between IgA rheumatoid factor (RF) and its subclasses in patients with rheumatoid arthritis (RA) and non-rheumatic symptom free individuals with increased total IgA RF

	RA		Non-rheumatic individuals	
	Correlation†	p	Correlation†	p
Total IgA RF v IgA1 RF	0.853	0.0001	0.828	0.0001
Total IgA RF v IgA2 RF	0.629	0.0005	0.206	NS
IgA1 RF v IgA2 RF	0.736	0.0001	0.352	NS

†Results are given as Spearman's coefficient of correlation (Rho).
NS = Not significant.

RF did not have greater concentrations of IgA RF than those who did not have increased IgA2 RF (data not shown).

In addition, a significant positive correlation was noted between total IgA RF and both subclasses in patients with RA, while in the non-rheumatic individuals there was a positive correlation only between IgA RF and IgA1 RF (table 4).

Discussion

Although increased RF, as measured by conventional agglutination tests, is found in the majority of RA patients, it is also frequently found in patients with other rheumatic and non-rheumatic diseases. Agglutination tests mainly detect IgM RF and do not discriminate between patients with isolated increases in IgM RF and those with increases in both IgM RF and IgA RF. We have previously demonstrated that a concomitant increase in concentrations of IgM RF and IgA RF is much more specific for RA than increase in IgM RF or IgG RF alone.¹⁸

This study indicates that increased IgA2 RF is markedly more common among RA patients than among patients with other rheumatic diseases, or symptom free individuals with increased total IgA RF. Furthermore, increased IgA2 RF seems to be more specific for RA than increased IgM RF (table 1).

Otten *et al*⁹ reported a prevalence of increased IgA1 RF in RA patients similar to the one we observed in this study (73% v 64%). However, they detected increased IgA2 RF concentrations in only 36% of their patients, compared with 60% of the RA patients in our study. This cannot be explained by different criteria used for defining the upper limit of normal, as a 95% cut off was used in both studies. No clinical details are presented in the report by Otten *et al* and it is therefore possible that their patients had less severe disease; however, 45% of our patients who had no evidence of extra-articular manifestations had increased IgA2 RF. A more likely explanation for the discrepancy may be that Otten's group used human IgG as antigen in their ELISA system, while we used rabbit IgG. It has been claimed that a stronger association has been found between increased IgA RF and bone erosions when rabbit IgG, rather than human IgG, has been used as antigen.¹⁹ The pathophysiological significance of this phenomenon is not known, but it is conceivable that IgG molecules in individuals susceptible to RA

share epitope(s) with rabbit IgG. It has indeed been shown that RF reacts more strongly with IgG from RA patients than with IgG from healthy subjects.²⁰

Otten *et al* did not analyse the relationship between IgA RF subclasses and disease manifestations in RA.⁹ Our findings that RA patients with extra-articular manifestations had greater concentrations of both IgA RF subclasses than patients without such manifestations are consistent with several previous studies,^{15 16 21 22} but also show that there is no real difference between the subclasses in this respect.

It has been reported that increase in RF concentration often precedes the clinical onset of RA by months or even years, and this may be particularly true for increases in IgA RF.^{6 23 24} Thus it is possible that healthy individuals with increases in both IgA RF subclasses are at greater risk of later developing RA than those individuals who have increases in only the IgA1 RF subclass. It is intended to follow the IgA RF positive non-rheumatic individuals presented in this study in order to evaluate this possibility.

This study has indicated that measurement of IgA RF subclasses may be diagnostically useful, but larger prospective studies are required to elucidate fully the prognostic and pathogenic significance of increased IgA1 and IgA2 RF.

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