

Relation between bone erosions and rheumatoid factor isotypes

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SUMMARY Total rheumatoid factor (RF) activity and individual RF isotypes were measured in 62 patients with rheumatic diseases. Retrospective analysis of available x rays showed an association between IgA RF and the occurrence of periarticular bone erosions in hands. In contrast, IgG RF and IgM RF did not show any significant association with erosions. Furthermore, a close correlation was observed between the RF isotype levels in simultaneously drawn serum and synovial fluid samples. The possible significance of IgA RF in the pathogenesis of bone erosions is discussed.

Key words: rheumatic diseases, bone resorption, IgA rheumatoid factor.

Rheumatoid factor (RF) is often present in the serum, synovial fluid (SF), synovial membrane, and other body fluids and tissues of patients with rheumatoid arthritis (RA). It also occurs in various other disorders as well as in healthy individuals and its role in the pathogenesis of RA is not clear. RF has been shown to exist in all immunoglobulin isotypes but their significance is not yet unequivocally established.¹

Periarticular bone destruction is a common feature of some rheumatic diseases, especially RA, where it occurs in most patients.² Such erosions can affect both seronegative and, usually more severely, seropositive patients,^{3,4} but the underlying pathogenetic mechanism is unknown. Furthermore, some patients with RA remain without erosions, whereas others develop erosions quite early in the course of their disease. Patients with RA who have not become erosive within three years of the onset of their disease tend to remain non-erosive.⁵

A correlation has been observed between the number of erosions and RF levels as determined by agglutination tests.² Tarkowski and Nilsson found in a retrospective study a correlation between raised levels of IgA RF or IgG RF, or both, and systemic manifestations, and also between increased levels

of IgA RF and articular destruction in female patients.⁶ Teitsson *et al* reported a prospective study indicating that raised levels of IgA RF early in RA predict the development of erosions.⁷ These findings have since been confirmed and extended.^{8,9}

It should be noted that RF positive systemic lupus erythematosus (SLE) is generally not an erosive disease.¹⁰ Conversely, seronegative arthritides may often have an erosive course.^{7,11} Thus development of erosions is neither confined to patients with positive RF, nor to RA as a clinical entity. The conventional RF agglutination tests, however, are frequently negative in patients with increases in a single RF isotype,¹² and RF isotype patterns have not been carefully analysed in rheumatic diseases other than RA. It therefore seemed logical to study the relation between RF isotype patterns and erosions without regard to conventional diagnostic criteria.

Materials and methods

SELECTION OF PATIENTS

Sixty two patients with various rheumatic diseases were admitted to the study on the basis of available x rays. Of these, 36 had RA, nine had psoriatic arthritis, five had ankylosing spondylitis, three had SLE, and nine had other or undiagnosed arthritides. The diagnoses were based on information from five rheumatologists. The group was also heterogeneous with respect to clinical course and treatment. The mean age was 51 years (range 15–84 years) and the

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mean duration of disease was 9.8 years (range 0.1–40 years). The male/female ratio was 0.44.

MEASUREMENT OF RF

RF was measured in the sera and SF by an enzyme linked immunosorbent assay (ELISA) system described in detail elsewhere.¹² Briefly, microtitre plates were coated with rabbit IgG, incubated with appropriate dilutions of the patients' samples, and then reacted with alkaline phosphatase conjugated monoclonal antibodies (AP Mab) specific against human κ chain or heavy chains to detect the total RF activity and individual isotypes respectively. This ELISA has been shown to be a more sensitive and specific screening system for RF than the conventional haemagglutination tests.¹² All AP Mab were obtained from Miles-Yeda Ltd. To minimise false positive reactions due to non-specific binding of the AP Mab the aggregated rabbit IgG was added before the monoclonal antibodies to saturate free RF.

An internal standard was calibrated against an international reference serum (State Serum Institute, Copenhagen) containing 100 units/ml of RF activity. A cut off level at 6 IU/ml for total RF activity was shown to be optimal for the detection of samples with raised RF isotypes. The upper 5% limits of normal, determined on 200 non-rheumatic Icelanders, were found to be 25 IU/ml for IgA RF and IgM RF but 150 IU/ml for IgG RF.

RF was also measured with two haemagglutina-

tion tests, the Rheumatoid slide test (Wampole Lab) and the RAHA system (Fujirebio Inc), which is a modified version of the Rose-Waaler test. A titre of 1/40 was considered positive in the RAHA system.

X RAY ASSESSMENT

All available x rays of joints were read independently and twice by each of two experienced radiologists. Erosions were always found in the metacarpophalangeal (MCP) joints or wrists of patients with erosive hands. It was therefore decided to score the erosions by counting eroded MCP joints (II-V) and the wrist of each hand, giving each eroded joint a score of one. Thus a maximum score of 10 could be obtained for both hands with this method. Other joints were registered only as positive or negative for erosions without regard to numbers or severity.

This method was analysed with respect to intra- and interobserver error and consistency. There was a total agreement between the two observers with regard to the presence or absence of erosions in MCP and wrist joints and a good correlation regarding the total number of eroded joints ($r=0.844$, $p<0.001$). This also applied to the intra-observer score (data not shown). This scoring system was therefore considered satisfactory for the study. The correlation was not so good for the other joints studied, a discrepancy which was probably largely due to the more consistent technical quality of the x rays of hands.

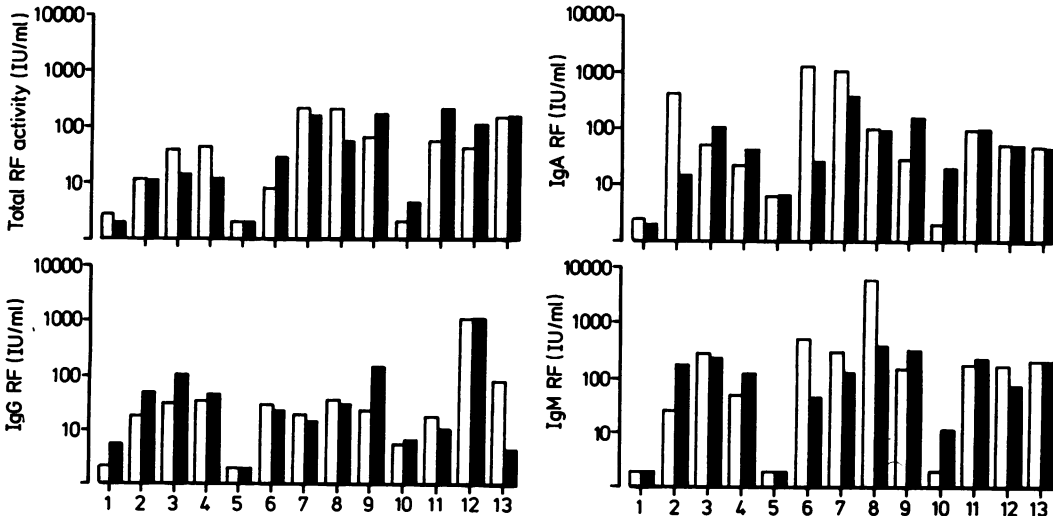


Fig 1 Rheumatoid factor levels in simultaneously drawn samples of sera and synovial fluid from 13 patients. ■=serum, □=synovial fluid. There was a significant correlation between RF levels in serum and synovial fluid for each patient (total RF activity $p<0.001$, IgA RF $p<0.02$, IgG RF $p<0.01$, and IgM RF $p<0.001$).

STATISTICAL EVALUATION

The association of RF levels and erosions was analysed with the Fisher's exact test. Where appropriate, the correlation coefficients were determined by Pearson's product moment and Spearman's ranking. The level of significance was set at $p < 0.05$.

Results

The question has often been raised how informative serum RF values are for ongoing immunological activities in joints. Fig. 1 shows the relation between the RF levels in simultaneously drawn serum and SF from 13 patients. A close relation was observed between the samples in each pair. This correlation was significant on a logarithmic scale for all RF types.

As shown in Table 1 13 patients who were RAHA negative had one or more RF isotype levels increased. Thirty two out of 36 patients with RA were RF isotype positive but only 23 were RAHA positive. More seropositive non-RA patients were also detected with the RF isotype specific ELISA (10/26) than the RAHA test (6/26). When individual RF isotypes were disregarded neither the RAHA nor the ELISA results showed a significant relationship with bone erosions (Fisher's exact test). Similarly, there was no linear correlation between the number of eroded joints and the levels of individual RF isotypes, including IgA RF. Table 2 shows the relation between raised RF levels and the presence of erosions in hands. Raised levels of IgA RF were significantly more frequently associated with erosions than were increases of the other isotypes. When all available *x* rays were evaluated, however, the interobserver difference became too high for meaningful analysis (Table 3). This discrepancy was probably due to the fact that only *x* rays of the hands were of sufficient quality for accurate evaluation of erosions.

Erosions can probably not be expected until after

Table 1 *Comparison of the RAHA and the ELISA systems in relation to the presence or absence of erosions*

	Number of patients	
	With erosions	Without erosions
RAHA+/RF isotype+*	25	4
RAHA-/RF isotype+	9	4
RAHA+/RF isotype-	0	0
RAHA-/RF isotype-	13	7

*Increases of one or more RF isotypes.

Table 2 *Number of patients with and without erosions in the hands in relation to RF levels**

	RF levels raised		RF levels not raised		p Value
	With erosions	Without erosions	With erosions	Without erosions	
Total RF activity	21	8	7	4	0.253
IgA RF	20	4	8	8	0.025
IgG RF	9	3	19	9	0.272
IgM RF	21	9	7	3	0.304

*There was complete agreement between the two radiologists regarding the presence or absence of erosions in every patient.

at least one year's duration of disease.¹³ When patients with shorter course were excluded the results for the remainder ($n=51$) showed a similar association between raised IgA RF and erosions in hands ($p=0.028$, $n=31$), but again the other RF types did not show such correlations.

Because the group was heterogeneous and the diagnosis had in some cases not been fully established it was not feasible to divide the group further on clinical grounds. Instead all seropositive patients, i.e., those having raised levels of at least one RF isotype, were analysed regardless of their diagnosis to determine whether any RF isotype in particular was more strongly associated with erosions than total RF activity alone. The results are shown in Table 4. Significant association was observed between increased levels of IgA RF and the presence of erosions in hands but not with the other isotypes or total RF activity. Elimination of patients with duration of disease of less than one year did not influence these associations (data not shown).

To study the significance of substantially raised RF levels the data were analysed using twofold normal limits for total RF activity and RF isotypes. A significant association was now found between total RF activity and erosions ($p=0.021$), but too

Table 3 *Correlation between raised RF values and erosions observed in all available x rays (n=62)**

	<i>x</i> Ray observer	
	AB	KS
Total RF activity	0.13	0.041
IgA RF	0.053	0.021
IgG RF	0.13	0.17
IgM RF	0.19	0.041

*Data shown as *p* values by Fisher's exact test.

Table 4 Number of seropositive* patients with and without erosions in hands in relation to RF level†

	RF levels raised		RF levels not raised		p Value
	With erosions	Without erosions	With erosions	Without erosions	
Total RF activity	21	8	3	1	0.445
IgA RF	20	4	4	5	0.035
IgG RF	9	3	15	6	0.310
IgM RF	21	9	3	0	0.370

*Seropositive indicates at least one RF isotype raised above upper limits of normal.

†There was complete agreement between the two radiologists regarding the presence or absence of erosions in every patient.

few patients had raised levels of individual RF isotypes beyond these limits for reliable analysis (data not shown).

A significant correlation was found between the duration of disease and number of eroded joints in hands according to both the x ray observers ($p < 0.02$ and $p < 0.01$), but significant correlation was not observed between the duration of the disease and levels of individual RF isotypes.

Discussion

The principal objective of this study was to investigate the association between individual RF isotypes and bone erosions in patients with various arthritides.

The production of individual antibody isotypes is subjected to distinct regulatory mechanisms and activates different effector pathways. Analysis of RF isotype patterns may therefore help to elucidate varying immunological processes which may operate in patients with clinically heterogeneous types of arthritis.

It has been argued that measurements of RF in serum may not reliably reflect immunological activities in the joints. We observed, however, a close relation between RF levels in simultaneously drawn sera and SF from 13 rheumatic patients. The upper limits of normal for RF isotypes have not been defined for SF, but in our system no RF activity could be detected in SF from five osteoarthritic patients (data not shown).

The isotype specific ELISA system detected RF in 13 patients who were RAHA negative (Table 1). Thus the availability of more sensitive and specific RF tests reduces the proportion of rheumatic patients who are judged seronegative. Yet several factors, including anti-idiotypic antibodies, can give

rise to false negative results, even when isotype specific tests are applied, but true seronegative RA patients probably do exist and may even become severely erosive.⁷

A significant association was seen between raised serum levels of the IgA RF isotype and the occurrence of erosions in hands. In contrast, neither total RF activity measured by RAHA agglutination or ELISA nor IgG RF and IgM RF showed significant association with erosions in this study.

A correlation was found, however, between erosions and marked increases of total RF activity as measured by the ELISA screening test. This is in agreement with previous observations of an association between erosions and RF levels measured by agglutination tests. We did not find a quantitative relationship between the number of erosions and the levels of individual RF isotypes, but the numbers involved were low.

It should be stressed that this is a retrospective study of a heterogeneous group of patients who had received various symptomatic and second line treatments. Several studies have indicated that both symptomatic and remitting drugs can affect the levels of IgG RF and IgM RF^{14, 15} and also of IgA RF (Withrington, personal communication). The findings in these 62 patients should therefore be viewed with the possibility in mind that previous treatments might have affected the RF isotype levels. This could explain why the association between IgA RF and erosions is stronger in a prospective study.⁷

These results support the view that IgA RF is related to the development of erosions. The production of IgA antibodies is believed to be T cell dependent,¹⁶ possibly induced by T cells with receptors for the Fc part of IgA.^{17, 18} It is therefore conceivable that IgA RF levels reflect the degree of direct T cell involvement in the pathogenesis of erosions. It should be noted in this context that T cells can produce osteoclast activating factor¹⁹ and may also be able to influence the osteoclasts by other means.²⁰

Non-aggregated IgA does not activate complement and can, furthermore, suppress IgG and IgM mediated complement activation.²¹ IgA has been reported to have a complement independent opsonic effect on phagocytosis by monocytes but not by neutrophils.²² The presence of IgA complexes could therefore selectively activate macrophages. Activated macrophages could in turn cause bone resorption by production of prostaglandin E^{23, 24} and interleukin 1,^{25, 26} both of which are potent promoters of osteoclastic activity.

The involvement of T cells and the direct role of IgA RF in the pathogenesis of erosions need not be mutually exclusive, but experimental evidence for

the involvement of these or other mechanisms remains to be established.

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