

IgG antiglobulins in rheumatoid arthritis and other arthritides: relationship with clinical features and other parameters

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SUMMARY A radioimmunoassay has been developed for measuring IgG antiglobulins using baboon IgG as antigen. Raised levels were virtually confined to the sera of patients with rheumatoid arthritis (RA) and not found in other seronegative arthritides. High levels were found in both seropositive and seronegative (as judged by latex slide and sheep cell differential agglutination for IgM antiglobulins) patients with RA and were associated with systemic disease but not synovitis. Very high levels (more than twice the upper limit of normal) showed a strong association with vasculitis.

The most consistent serological abnormality in rheumatoid arthritis (RA) is the presence of raised levels of antiglobulins (RF), leading to the suggestion that they may be intimately involved in the inflammatory lesions characteristic of this disease. The early techniques used to measure RF were based on the ability of sera containing RF to agglutinate IgG-coated particles, and patients were classified as seropositive (60 to 80%) or seronegative. Agglutination is about a thousand times more efficient for detecting IgM antibodies than IgG,¹ and so as more sensitive techniques for detecting IgG antibodies were developed IgG antiglobulins were also found in 'seronegative' patients. It is also reported that raised levels of IgG RF occur in patients with other seronegative arthritides such as ankylosing spondylitis, psoriatic arthritis, and gout,^{2,3} raising doubts as to the role of IgG RF in RA. We used a radioimmunoassay to study IgG RF in seropositive and seronegative RA and other seronegative arthritides. Baboon IgG (attached to plastic tubes) was used as antigen and radiolabelled baboon antihuman γ to measure the uptake of IgG RF. Baboon IgG was chosen as antigen because it resembles human IgG more closely than does rabbit IgG, while avoiding the problem of anti-Gm allotype specificity of some rheumatoid factors

which could be encountered if human IgG was used as antigen.⁴ The relationship between IgG RF and clinical features and other parameters was studied.

Materials and methods

PATIENTS AND CONTROLS

Blood was collected from a wide range of patients either attending routine rheumatology outpatient clinics or admitted to hospital for severe rheumatic disease. Sera were separated and stored at -20°C prior to estimation of IgG RF. Blood count and plasma viscosity done on the Coulter viscometer were performed routinely. IgM RF was measured by the latex slide test and sheep cell agglutination titre on the same serum sample as that used for IgG RF assay. Patients with RA fulfilled the American Rheumatism Association (ARA) criteria for classical or probable RA. Extra-articular manifestations were noted at the time of testing and included Sjögren's syndrome, fibrosing alveolitis, and Felty's syndrome. Vasculitis was diagnosed by clinical findings of typical nail fold, nail edge, or digital infarcts, deep cutaneous ulceration, or neuropathy. Histological evidence of vasculitis was found in most cases by either skin or rectal biopsy and was necessary for inclusion in the absence of typical clinical features. The activity of the arthritis (synovitis index) was assessed on a 3-point scale dependent on the number of joints involved and the degree of inflammation present by clinical assessment: a score of 0 represented inactive synovitis, 1 active,

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and 2 very active. Current drug therapy was noted and the results reported are from patients whose samples were taken before initiation of second-line drugs in at least 90% of cases.

Other seronegative arthritides defined by accepted clinical and radiological criteria were Still's disease (a systemic illness with fever and arthralgia in children and adults), and included in this group were some patients with juvenile chronic arthritis (JCA) who were negative for IgM RF, osteoarthritis including chondrocalcinosis, psoriatic arthritis Reiter's syndrome, ankylosing spondylitis, postyrsinial arthritis and systemic lupus erythematosus (SLE). Fifteen of 20 patients with ankylosing spondylitis had active peripheral joint synovitis and 3 iritis. These patients included 20 studied serially over a 3-month period with IgG RF estimations on 3 separate occasions. Sera from patients with postyrsinial arthritis were a gift (from Professor O. Laitinen and Dr M. Leirisalo, Second Department of Medicine, University of Helsinki). In Finland this disease is more prevalent than in other countries. Patients with SLE fulfilled at least 4 of the ARA criteria and had active peripheral joint involvement.

Normal control sera were age-matched, either from healthy volunteers or from otolaryngology surgical patients who had no rheumatic disease.

IGG ANTIGLOBULIN ASSAY

The method was similar to that described previously.^{5,6} Baboon IgG was attached to polystyrene tubes (LF2: Luckham) by adding 200 μ l of a solution of 20 μ g/ml in phosphate buffered saline pH 7.2 (PBS) and incubating for 1 hour at 37°C and overnight at 4°C. After washing with PBS any free

reactive sites were blocked by a further incubation for 2 hours at 37°C with diluent (1% bovine serum albumin in PBS). Duplicate 10 μ l samples of sera were diluted 1:10 with diluent in the tubes and incubated for 1 hour at 37°C and $\frac{1}{2}$ hour at 4°C. The tubes were washed with PBS, incubated for 1 hour at 37°C and $\frac{1}{2}$ hour at 4°C with ¹²⁵I labelled purified baboon antihuman γ antibody (100 ng per tube in diluent), washed again, and counts made of the radioactivity bound to the tubes. Counts obtained from control tubes incubated with diluent alone followed by ¹²⁵I-labelled antibody were subtracted from each test sample count. The proportion of the total labelled antibody bound by test samples was calculated and the results expressed as mg labelled antibody bound per litre of serum.

Baboon IgG was prepared from normal baboon serum by precipitation with 20% sodium sulphate followed by elution from a diethylaminoethyl-sepharose column.

Baboon antihuman IgG (a gift from Dr D. R. Stanworth) was passed down human IgA and IgM sepharose immunoabsorbant columns before elution from a human IgG sepharose column with 1M ammonia. This antibody was specific for human γ as judged by the ability of human IgG but not IgM or IgA to inhibit the reaction of ¹²⁵I-labelled antibody with rheumatoid factor bound to baboon IgG coated tubes and by the binding of ¹²⁵I-labelled antibody to human IgG but not IgM or IgA coated tubes.

Results

Sera from normal subjects and 9 groups of rheumatic patients were tested for IgG antiglobulins (Fig. 1). Levels of antiglobulins significantly above

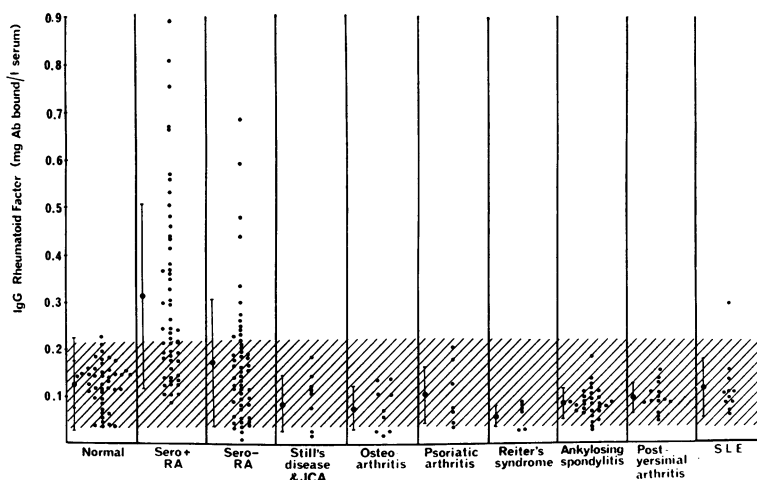


Fig. 1 Serum levels of IgG RF (mg Ab bound/litre serum) in normal subjects and patients with RA and other arthritides. The mean \pm SD for each group is shown. It should be noted that the individual values in the RA groups are markedly skewed in distribution, but according to the central limit theorem¹⁸ the group means should approximate to a normal distribution. Normal range (mean \pm 2 SD, 0.30 to 0.22 mg Ab bound/litre serum) is shaded.

the normal range were virtually confined to sera from patients with RA. Raised levels were not found in any of the other arthritides, including 15 patients with ankylosing spondylitis who had active peripheral synovitis. In addition 20 of the patients with ankylosing spondylitis were followed up serially and had normal levels on each of 3 occasions. By contrast 29/52 patients (56%) with seropositive arthritis had raised IgG RF, and the mean value for the group was above the normal range. In seronegative RA the mean was within the normal range, but 12/52 (23%) had raised levels. Of these, 4 were only marginally raised but 8 had much higher levels (>0.25). All 8 of these patients were seropositive at some time before or after the collection of the sample tested for IgG RF, though the samples themselves were seronegative for IgM RF. In addition, 5 of these 8 showed extra-articular features: 3 nodules, 2 Sjögren's syndrome, 2 fibrosing alveolitis, and 1 vasculitis.

In view of these results we elected to consider all the cases of RA together in order to relate IgG RF levels to clinical features and other parameters. The 103 cases were divided into 3 groups according to their serum IgG RF levels: 57 with levels of IgG antiglobulins within the normal (N) range (<0.20 mg Ab bound/l), 30 with moderately raised, intermediate (I) levels (0.20 to 0.40), and 17 with high levels (H) (>0.40). Table 1 shows the mean values for plasma viscosity, synovitis index, and sheep cell agglutination test (SCAT) titre. The data were analysed by Student's *t* test to compare the mean for each parameter in each pair of groups (H/I, H/N, and I/N). No significant difference was found between the groups for plasma viscosity, synovitis index, or SCAT, despite the tendency for patients with high levels of IgG RF to have a relatively low synovitis index. The regression coefficient of SCAT (logs) on IgG RF for all the seropositive RA samples was also calculated but showed no correlation ($b=1.8 \pm 0.78$, $d=0.89$, $P>0.3$). The incidence of vasculitis, nodules, and other extra-articular disease activity (Felty's syndrome, Sjögren's syndrome and fibrosing alveolitis) in the 3 groups is shown in Fig. 2. 2×3 contingency analyses were performed between each of the extra-articular manifestations and the levels of IgG RF.

Table 1 Mean values \pm SD for plasma viscosity, synovitis index, and SCAT titre (\log_2) in normal intermediate and high groups for IgG RF

	Normal	Intermediate	High
Viscosity	1.87 \pm 0.15	2.21 \pm 0.29	2.01 \pm 0.22
Synovitis I	1.1 \pm 0.9	1.3 \pm 0.8	0.7 \pm 0.7
DAT (\log_2)	7.1 \pm 1.2	7.3 \pm 1.8	8.6 \pm 1.9

There was a striking association between the levels of IgG RF and vasculitis ($\chi^2=69.3$, $P<0.001$) and significant associations between the IgG RF levels and nodules ($\chi^2=13.4$, $P<0.001$) and between the IgG RF levels and other extra-articular disease activity ($\chi^2=10.6$, $P<0.01$). In the last 2 cases it was not self-evident which groups were significant, and so 2×2 contingency analyses of all possible combinations of 2 groups were made. The incidence of nodules differed between the high and normal groups ($\chi^2=11.7$, $P<0.001$) and between the intermediate and normal groups ($\chi^2=5.0$, $P<0.05$)

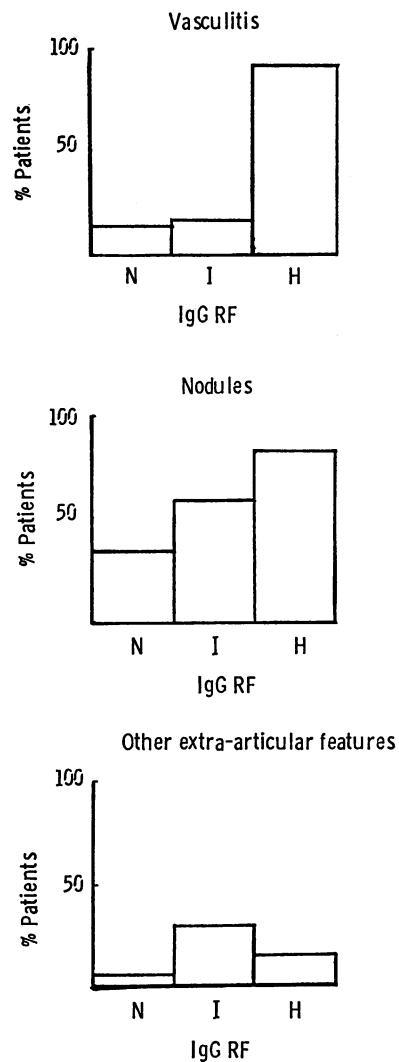


Fig. 2 Incidence of vasculitis, nodules, and other extra-articular disease in RA patients with IgG RF levels in the normal (N), intermediate (I), and high (H) range.

but not between the high and intermediate groups ($\chi^2=2.5$, $P>0.05$). The only significant difference in the incidence of other extra-articular manifestations between any 2 age groups was between the intermediate and normal group ($\chi^2=10.1$, $P<0.01$).

Discussion

Raised levels (here defined as those above the levels found in 95% normal sera) of IgG antiglobulins were found to be virtually confined to the sera of patients with rheumatoid arthritis and did not occur in other arthritides. This is in agreement with a recent report⁷ but contrasts with earlier work.^{2,3} The latter groups assayed IgG RF by a method which involved adsorption of rheumatoid factors on to insolubilised IgG, followed by elution with a disassociating buffer, and immunodiffusion against class-specific antisera,⁸ whereas Pope and McDuffy⁷ used a radioimmunoassay which was similar to that used here except that rabbit IgG was used as antigen. In our view the discrepancy is likely to be due to the technical difficulties associated with the adsorption-elution method.^{9,10}

If IgG RF is important in the pathogenesis of rheumatoid arthritis, then increased levels of IgG RF should correlate with some aspect of disease activity. The results show that the most striking clinical association with IgG RF was RA vasculitis. RA vasculitis usually accompanies severe erosive nodular disease and previously has been associated with such serological abnormalities as raised levels of IgM RF, 7S IgM, and IgG RF by absorption-elution.¹¹ We have also noticed the temporal relationship of serum IgG RF levels with anti-complementary activity, C₄ levels, and clinical features of vasculitis. IgG RF levels and anti-complementary activity rose, while C₄ levels fell with clinical relapse, and the levels returned to normal with clinical improvement,¹² suggesting that IgG RF-containing complexes which activate complement might be responsible for the development of vasculitis. Immune complexes containing only IgG RF are reported not to activate complement,¹³ but, if IgG RF (as opposed to normal IgG) is bound by IgM RF, it is possible that such complexes, if stable, enable IgM RF to activate complement via the classical pathway. Recently we have isolated such complexes from RA sera using an anti- μ immunoabsorbant column and shown that the IgG attached to IgM RF is itself RF, because it retains RF activity after dissociation from IgM RF.¹⁴

The reason for the low incidence of other extra-articular manifestations in the group with high IgG RF is not clear. Many of the extra-articular manifestations described are associated with vasculitis,

especially in the early stages, and it has been suggested that vasculitis is the cause of most if not all extra-articular rheumatoid disease.¹⁵ RA vasculitis is usually relatively short-lived and often intermittent. By contrast other extra-articular manifestations are more chronic, and it may be that high levels of IgG RF occur at their initiation or early in their development but that, by the time they have fully developed, IgG RF levels have fallen.

Recently we have shown that patients with systemic vasculitis have relatively inactive synovitis.¹⁶ This is consistent with the association of increased levels of IgG RF with vasculitis but not synovitis. Had the IgG RF found in vasculitis been produced in joints and merely overflowed into the circulation then the degree of synovial and vascular movement should be related. As this is not the case, we consider that the site of IgG RF production at least during vasculitis is that usually associated with antibody production, namely, spleen, lymph nodes, and bone marrow. The fact that IgM RF synthesising cells occur frequently among bone marrow cells sets a precedent for this suggestion.¹⁷

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