Clin. exp. Immunol. (1985) **59**, 285–292.

Complement activating properties of complexes containing rheumatoid factor in synovial fluids and sera from patients with rheumatoid arthritis

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(Accepted for publication 28 September 1984)

SUMMARY

The relationship between complexes containing rheumatoid factor and complexes activating complement was examined in synovial fluids and sera from patients with rheumatoid arthritis (RA). In each case this was performed by quantifying the amount of rheumatoid factor bound by solid phase Fab'_2 anti-C3 and/or solid phase conglutinin. Both anti-C3 coated and conglutinin coated microtitre plates bound high levels of complexes containing rheumatoid factor from sera of RA patients with vasculitis. Unexpectedly, these complexes were detected in synovial fluids from only a minority of RA patients with synovitis. However, RA synovial fluids did contain other complexes as shown by the presence of complement consuming activity, C1q binding material and immunoglobulin attaching to conglutinin. It is considered that in RA synovial fluids the complexes containing RF and those activating complement are not necessarily the same whilst in vasculitic sera the complexes containing rheumatoid factor also activate complement.

Keywords rheumatoid arthritis rheumatoid factor complexes complement-fixation.

INTRODUCTION

Autoantibodies to IgG (rheumatoid factor) of both IgM and IgG isotype are found frequently in sera and synovial fluids from patients with rheumatoid arthritis (RA). In many synovial fluids the rheumatoid factors occur complexed with IgG. The titres of rheumatoid factor (RF) are increased after separating synovial fluid under conditions in which complexes are dissociated (Hannestad, 1968), and rheumatoid factor activity is found in complexes isolated by precipitation with C1q or IgM RF (Winchester, Agnello & Kunkel, 1970). Local complement activation also takes place in RA synovial fluid as complement levels are frequently decreased and their breakdown products increased (Winchester *et al.*, 1970; Zvaifler, 1969; Perrin *et al.*, 1977). Complexes containing IgG are thought to trigger the activation because there is a relationship between levels of high molecular weight IgG and anti-complementary activity in synovial fluid (Winchester *et al.*, 1970). In addition, analysis of the joint material which has bound complement shows it to be mainly IgG with some IgM (Male & Roitt, 1981). The apparent lack of other proteins in complexes isolated from synovial fluids (Male, Roitt & Hay 1980; Male & Roitt, 1981) together with the correlation between

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complement depletion and RF titres (Hedberg, 1967) has led a number of authors to infer that the complexes which are fixing complement consist of IgG and RF. It has been suggested that the ensuing complement activation liberates chemotactic factors which attract phagocytic cells into the joint. These phagocytose the complexes and discharge, from their lysosomes, hydrolytic enzymes which mediate the proliferative and destructive joint changes (Weissman, 1972; Zvaifler, 1973).

It has also been suggested that rheumatoid factor containing complexes which activate complement may be responsible for the development of vasculitis (Theofilopoulos *et al.*, 1974; Allen *et al.*, 1981). The evidence for the direct involvement of IgG RF in the pathogenesis of rheumatoid vasculitis is stronger than for its involvement with synovitis. Very high serum levels of IgG RF are closely associated with the symptoms of vasculitis but not synovitis in RA patients (Theofilopoulos *et al.*, 1974; Allen *et al.*, 1981). Studies of the temporal relationship of serum IgG RF levels to anti-complementary activity, C4 levels and clinical features of vasculitis revealed that the levels of IgG RF and anti-complementary activity rose while C4 levels fell with clinical relapse and that the levels returned to normal with clinical improvement (Scott *et al.*, 1981a, 1981b).

Whilst it may be true that RF containing complexes and complement fixing complexes co-exist in synovial fluid and vasculitic sera in RA, it is not proven that they are the same complexes. If, as has been argued, serum RF is merely an overflow from the joint then it would be expected that the same type of complex would be involved in both synovial and vasculitic inflamation. This prompted us to analyse the complement fixing ability of RF containing complexes in sera and synovial fluids from RA patients with synovitis and in sera from patients with RA complicated by vasculitis. The current report describes the application of two novel techniques to this problem. Evidence that one of the techniques is a valid measure for complement fixing rheumatoid factor containing complexes has been adduced already (Elson *et al.*, 1983).

MATERIALS AND METHODS

Rheumatoid disease. All patients fulfilled the ARA criteria for classical or definite RA. Two groups of patients were studied. The first group (synovitis) consisted of 40 patients with active peripheral joint athritis but without vasculitis. Of these, 26 were female and 14 male with a mean age of 56·5 years and mean duration of RA of 6·2 years. Thirty-six of the patients were seropositive, as judged by the Rose Waaler test. The mean IgG RF levels of their sera was $1{\cdot}03 \pm 0{\cdot}52$ μg Ab/ml and of their synovial fluids was $1{\cdot}03 \pm 0{\cdot}49$ μg Ab/ml. The second group (vasculitis) consisted of 16 patients with rheumatoid disease complicated by active vasculitis. Of these, nine were female and seven male with a mean age of 59·2 years and mean duration of RA of 8·7 years. Fifteen of these patients were seropositive and their mean serum IgG RF level was $3{\cdot}26 \pm 1{\cdot}03$ Ab/ml. Vasculitis was defined clinically. The frequency and distribution of vasculitic lesions and other extra-articular features was: nail fold, nail edge or digital infarcts, 11; deep cutaneous ulcers, nine; cutaneous rash, three; peripheral gangrene, three; acute peripheral neuropathy, five (two mononeuritis multiplex); fibrosing alveolitis, two, episcleritis, two, pericarditis, two; aortic regurgitation, one. In addition, 10 patients had histological evidence of vasculitis (necrotizing arteritis at rectal biopsy, seven; small vessel vasculitis at skin biopsy, three).

Current drug therapy was noted in all patients with rheumatoid disease. Most patients were on regular non-steroidal anti-inflammatory drugs. At the time of study two patients from the synovitis group were on D-penicillamine. Of the vasculitis group, four patients were on corticosteroids, two on low doses of azathioprine, one on D-penicillamine and one on intramuscular gold.

Systemic lupus erythematosus (SLE). All patients fulfilled at least four of the ARA criteria for SLE. Of 20 patients studied, 18 were female and two male and the mean age was 36·4 years. Seven patients had active renal involvement (proteinuria, haematuria, casts, etc.), six had active skin disease and two severe pulmonary involvement.

Ankylosing spondylitis (AS). All patients fulfilled the New York criteria for AS. Of 20 patients studied, 18 were male and two female, and the mean age was 43·2 years.

Sera and synovial fluids. Synovial fluids were obtained following therapeutic aspiration of knee

joints of patients with rheumatoid arthritis. Within 2 h the samples were centrifuged to remove cells and stored at −25°C. Blood was collected from patients attending outpatients clinics or from patients admitted to hospital for severe rheumatic disease. It was allowed to clot at room temperature, the sera collected at once and stored at −25°C. Normal control sera were from healthy volunteers.

Estimation of RF activity bound by anti-C3. The procedure and specificity of the anti-sera for C3d has been described elsewhere (Elson *et al.*, 1983). Essentially, sera and synovial fluid samples were incubated in microtitre plates coated with Fab'_2 fragments of rabbit anti-human C3 or mouse monoclonal anti-C3d. After washing, any rheumatoid factor bound by the solid phase was detected by incubating overnight with ^{125}I-heat aggregated human IgG (Hagg).

Estimation of RF activity bound by conglutinin. The wells of microtitre plates (Linbro) were coated with bovine conglutinin (20 μg/ml) and free reactive sites blocked with 1% BSA in borate-buffered saline (BBS). Test serum (10 μl), fresh normal serum (10 μl) and 1% BSA in BBS (80 μl) were added to each well. After incubating at 37°C for 15 min, 5 μl of 20 mg/ml soya bean trypsin inhibitor was added to each well and the plates incubated at 4°C overnight. The plates were washed and ^{125}I-Hagg (1 μg/ml) added to the wells. The plates were incubated overnight at 4°C, washed and counts made of the radioactivity bound to each well. Counts obtained from control wells incubated with 1% BSA alone followed by incubation with ^{125}I-Hagg were subtracted from each sample count. The proportion of total radioactivity bound by test samples was calculated and the results expressed as μg Hagg bound per ml of sample.

In some experiments the total amount of IgG or IgM bound by conglutinin was measured. This was done by incubating samples in conglutinin coated microtitre wells and detecting bound immunoglobulins with radiolabelled (^{125}I) antibodies to human IgG or IgM. The preparation of these antibodies has been described previously (Jones *et al.*, 1981a).

Conglutinin. This was prepared using methods described by Lachmann, Hobart & Aston (1973). The preparation gave a line of gel diffusion against an anti-conglutinin serum raised in rabbits and this line gave a reaction of identity against a preparation of conglutinin kindly donated by Professor P. J. Lachmann. Wells of microtitre plates coated with the conglutinin preparation bound labelled Hagg only if incubated with fresh normal serum, indicating that immunoglobulin uptake by conglutinin was complement-dependent.

Immune complex assays. The assay for complement consumption was performed as described previously (Harkiss & Brown, 1979). It involved the precipitation of complexes from sera with 2·5% polyethylene glycol 6000 (PEG) and the assay of the solubilized precipitate's consumption of complement (Grace & Elson, 1982). The results are expressed as the highest serum dilution consuming 50% of the complement (fresh normal human serum) added. The C1q binding test was carried out as described by Verrier-Jones & Cummings (1977).

RESULTS

RF activity of material bound by anti-C3

Sera and synovial fluids from patients with RA and other connective tissue diseases were examined for their capacity to bind ^{125}I-Hagg after incubation on (rabbit) anti-C3 coated plates (Fig. 1). Binding was detected with only six of 29 synovial fluids from patients with RA and most of these had low activity as compared with sera from patients with rheumatoid vasculitis. Sera from 20 patients with AS and 20 patients with SLE showed little or no activity. Similar results were obtained on plates coated with IgM monoclonal anti-C3d antibodies.

Possibly unbound complement breakdown products in synovial fluid could prevent the binding of C3 containing complexes by anti-C3. To examine this synovial fluids were tested for RF fixing C3 and the levels compared with those found in PEG (2·5%) precipitates of the same samples. Three of 21 synovial fluids had detectable binding PEG precipitation. After PEG precipitation the same three synovial fluids were positive and the others remained negative.

RF activity of material bound by conglutinin

It could be argued that the failure to detect complement activating RF containing complexes in

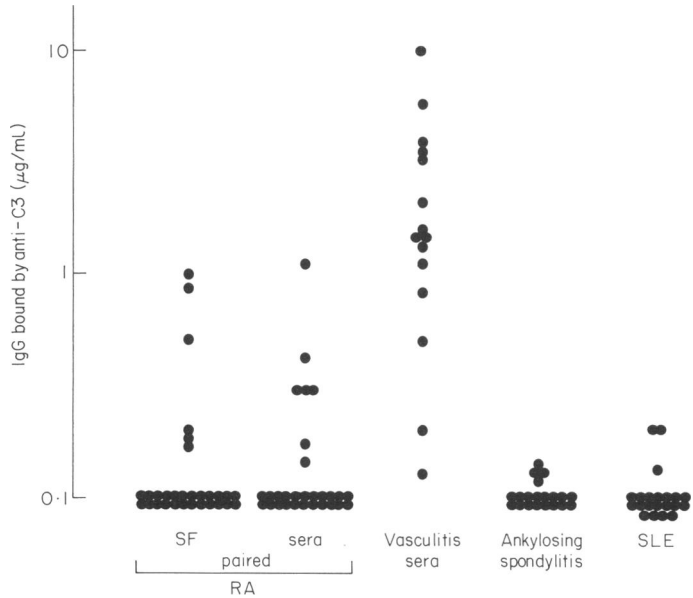

Fig. 1. Estimate of anti-C3 binding of RF from synovial fluids and sera of RA patients and sera from patients with AS and SLE.

synovial fluids was because insufficient complement was available in the fluids. Accordingly, a method was devised to detect complement activating RF-containing complexes which depends on the ability of complexes to convert added complement. Fig. 2 shows the uptake of ^{125}I-Hagg by conglutinin after incubation with synovial fluids from patients with RA or sera from patients with a range of rheumatological disorders. Again, binding was detected with only a minority (14 of 39) of synovial fluids and most of these had low activity by comparison with sera from patients with

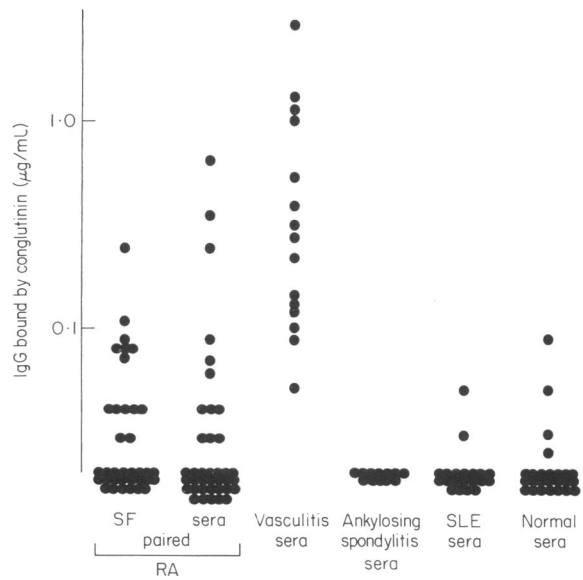

Fig. 2. Estimate of conglutinin binding of RF from synovial fluids and sera of RA patients and sera from normal subjects and other arthritides.

rheumatoid vasculitis. Sera from healthy volunteers, patients with AS and SLE showed little or no activity.

Relationship of RF bound by anti-C3 to RF bound by conglutinin

Thirty-six sera (from RA patients undergoing treatment for vasculitis) were selected because they had detectable activity either for RF bound by anti-C3 and/or RF bound by conglutinin. Fig. 3 compares the results obtained by the two tests on these sera. A regression coefficient analysis showed a significant ($r = 0{\cdot}80$, $P < 0{\cdot}001$) correlation between the tests.

Complex assays

It is possible that the synovial fluids investigated contained no complexes, or at least none capable of activating complement. Fig. 4a shows that 35 of 39 of the synovial fluids tested had C1q binding of

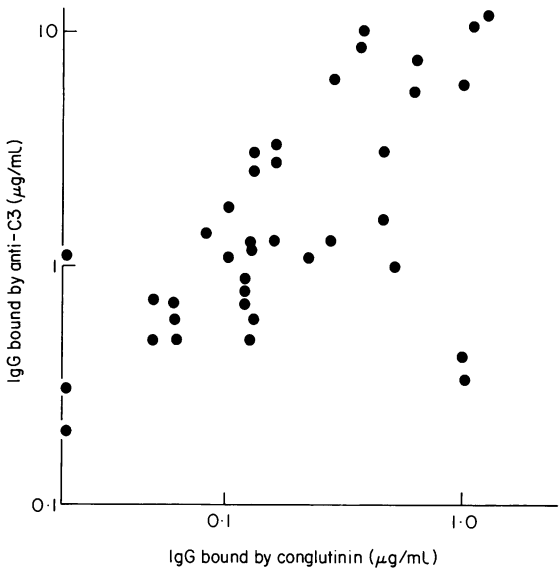

Fig. 3. Relationship between RF bound by anti-C3 and RF bound by conglutinin.

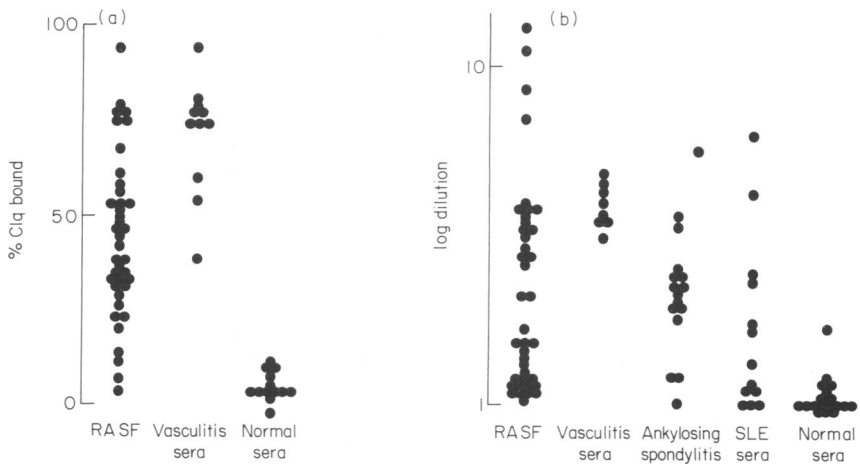

Fig. 4. Comparison of C1q binding activity (a) and complement consuming activity (b) of synovial fluids from rheumatoid patients with synovitis, sera from rheumatoid patients with vasculitis and normal sera.

20% or more and Fig. 4b shows that all the synovial fluids tested had some complement-consuming activity. Of these 27 of 39 had complement consuming activity outside the range (1·3 ± 0·5 log dil.) for normal sera. In addition many of the synovial fluids had C1q binding and complement-consuming activity comparable to that of vasculitic sera. Of the 16 synovial fluids with the highest complement consuming activity only 7 had detectable levels of RF bound by conglutinin.

The relative amounts of sera and synovial fluid IgG bound by conglutinin are shown in Table 1. Little conglutinin binding IgG was detected in normal sera whereas large amounts were found in RA synovial fluid and vasculitic sera. There was no difference in the amount taken up between synovial fluids and vasculitic sera. With IgM, again more was bound by conglutinin from synovial fluid ($P < 0·02$) and vasculitic sera ($P < 0·001$) than from normal sera. However, the amount of IgM taken up from vasculitic sera was greater ($P < 0·001$) than from synovial fluids.

DISCUSSION

The aim of this study was to determine the complement fixing ability of RF containing complexes in sera and synovial fluids from RA patients. The assays required complement fixing complexes to be bound by solid phase anti-C3 or solid phase conglutinin and then that any rheumatoid factors in these complexes would bind radiolabelled IgG. The latter was favoured by using heat-aggregated IgG as the radiolabelled probe as this would compete successfully with monomeric IgG already bound to RF. A possible drawback of the assay employing solid-phase anti-C3 is that there may have been reduced complement levels in some samples, giving low readings of complement fixing RF. This was overcome in the assay using solid phase conglutinin in that fresh normal human sera was added to the wells so that complexes could activate the additional complement. Despite the significant correlation between the two tests, it was thus not unexpected that some sera should be weakly positive by one test but not by the other, nor that occasional sera should be strongly positive by the anti-C3 test but not by the conglutinin test. The latter could result from the anti-C3 binding of RF from complexes dissociated by the alternative complement pathway. Such immunoglobulins would have bound C3 and so would be taken up by anti-C3 but would be dead end in that they are incapable of activating the classical or alternative complement pathways (Takahashi, Takahashi & Hirose, 1980). Consequently, they would be unable to generate C3bi and hence would not bind to conglutinin.

Both the assays showed high levels of RF containing complexes which fixed complement in most vasculitic sera but not in the majority of synovial fluids. However, there were high levels of complexes in the synovial fluids as judged by complement consuming activity and C1q binding levels. In addition, similar amounts of IgG bound by conglutinin were found in vasculitic sera and synovial fluids. This suggests that RF–IgG complexes are not the sole agent responsible for the generation of phlogistic complement components in synovial fluid. The corollary of this is that complement may be activated by antibody complexing with an as yet unidentified antigen. Others too, have concluded that complexes not containing RF occur in synovial fluid because complexes appeared before RF levels increased in synovial fluids from patients with early RA (Jones *et al.*, 1981b, 1984). Perhaps the stimuli for RF synthesis in RA are such complexes as RF production can be stimulated by seemingly any antigen–antibody complex (Nemazee and Sato, 1983).

Table 1. Estimate of conglutinin-bound IgG and IgM from sera and synovial fluid (mean ± s.d.)

Test sample	Number	μg anti-IgG bound/ml by conglutinin	μg anti-IgM bound/ml by conglutinin
Normal sera	11	<0·02	0·13 ± 0·11
RA synovial fluid	33	0·15 ± 0·14	0·29 ± 0·27
RA vasculitic sera	10	0·14 ± 0·14	0·82 ± 0·50

There remains the problem of explaining how it is that whilst both RA synovial fluid and vasculitic sera may have complexes containing RF, those from the latter appear to fix complement more effectively. The answer may lie in the recent work of Nardella, Teller & Mannik (1981) which showed that a normal IgG molecule is effectively a monovalent antigen for RF. This is because after RF has attached to one epitope the other unoccupied epitope is rendered unavailable. By contrast, the IgG in self-associating IgG RF complexes appeared to retain its divalency. It is also known that much higher levels of IgG RF are found in vasculitic sera than synovial fluid (Elson *et al.*, 1984) and in vasculitic sera this IgG RF is frequently associated with IgM RF (Jones *et al.*, 1981a). Together these findings lead us to suggest that the bulk of RF containing complexes in synovial fluid include normal IgG whereas in vasculitic sera they include self-associating IgG RF (either alone or with IgM RF bound to it) with only the latter type of complex being sufficiently stable to activate complement. The differences in the complexes may be due to the local production of rheumatoid factor in the joints of synovitis patients whereas the large amounts of circulating RF in vasculitic patients are produced systemically rather than being an overflow from the joints. Such a mechanism would explain why RA patients with vasculitis have relatively inactive synovitis (Scott *et al.*, 1981a).

This work was supported by grants from the Nuffield Foundation, Medical Research Council and Arthritis and Rheumatism Council.

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