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Intra-articular and circulating immune complexes and antiglobulins (IgG and IgM) in rheumatoid arthritis: correlation with clinical features

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SUMMARY Solid phase radioimmunometric methods have been used to assay immune complexes and IgG and IgM antiglobulins in paired samples of synovial fluid and serum from patients with rheumatoid arthritis (RA) or osteoarthrosis. Over 60% of RA patients had some increase in complexes in their sera, while nearly 90% had synovial fluid complexes. Moreover, the levels of complexes within the joint were much higher than in the serum. Both IgG and IgM antiglobulins were raised in most RA patients. The levels of IgG antiglobulins—and to a less extent IgM antiglobulins—were nearly always higher in synovial fluid than in the corresponding serum sample. A strong correlation was found between the levels of immune complex and IgG antiglobulin. A marked association was seen between the presence of subcutaneous nodules and increased IgG antiglobulins.

Antiglobulins (rheumatoid factors) present the most consistent serological feature in almost all patients with rheumatoid arthritis (RA). When both IgG and IgM antiglobulins are examined, even seronegative patients show higher than normal levels (Torrigiani, *et al.*, 1970; Hay *et al.*, 1975). Immune complexes are also often found in RA patients, particularly in the synovial fluid of inflamed joints. There is now good evidence that self-associated IgG-antiglobulins constitute a significant part of these complexes (Kunkel *et al.*, 1961; Winchester *et al.*, 1970), and analysis has now shown that they also contain appreciable amounts of C1q and other complement components (Male, Roitt and Hay, unpublished). It seems likely, therefore, that these complexes are involved in mediating inflammatory tissue damage through activation of the complement system and the release of lysosomal enzymes from granulocytes and monocytes.

To look further at the relationship between immune complexes and antiglobulins we have used solid phase radioimmunometric assays to determine the amounts of immune complexes and IgG and IgM antiglobulins in paired samples of synovial fluid and serum from patients with rheumatoid arthritis or osteoarthrosis. The degree of association between these parameters and the clinical status and treatment of these patients has been examined.

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Materials and methods

PATIENTS

Synovial fluid samples were obtained from 15 RA patients who were positive to the sheep cell agglutination test (SCAT) and 2 who were SCAT-negative, together with paired serum samples from all but 1 of the patients. The patients satisfied the American Rheumatism Association criteria for classical (C), definite (D), or probable (P) RA. There were 14 women and 3 men aged 29 to 81 years, mean 56 years. The duration of disease was from 4 months to 27 years with a mean of 8 years. Disease activity was classified as very active (A), moderately active (M), slightly active (S), or inactive or quiescent (O). Definite erosions were recorded in 13 cases. Rheumatoid nodules were present in 6, and 1 patient (No. 11) had Sjögren's syndrome in addition to nodules. None of the patients had detectable amyloidosis.

For comparison synovial fluid samples were obtained from 4 patients with osteoarthrosis (OA), with paired sera in 3 cases. These patients, 2 female

and 2 male, were aged from 49 to 64 years, mean 57 years; with clinically detectable disease from 4 months to 21 years, mean 8 years. Normal control sera were also obtained from 8 (3 female, 5 male, aged 26 years to 49 years, mean 34 years) laboratory workers within the Immunology Department.

16 of the RA and all OA patients were regularly taking aspirin or other anti-inflammatory drugs, 7 RA patients were receiving systemic steroids, one azathioprine, one penicillamine, and 3 sodium aurothiomalate. One RA patient (No. 2) was not receiving any treatment.

COLLECTION AND STORAGE OF SAMPLES

The synovial fluids were collected from knee effusions into plain sterile bottles and stored at −20°C. Blood samples were collected into plain bottles immediately after the fluid aspiration and the serum stored at −20°C.

HYALURONIDASE TREATMENT OF SYNOVIAL FLUID

Aliquots of synovial fluid were mixed with hyaluronidase (Hyalase, Fisons), 75 IU per ml synovial fluid, and incubated at 37°C for 30 minutes prior to testing (Hannestad, 1967).

SHEEP CELL AGGLUTINATION TEST

All serum samples were screened for classical rheumatoid factor with a sheep cell agglutination test kit (Rheumaton, Becton Dickinson).

ANTIGLOBULIN ASSAY

Aliquots of serum or hyaluronidase-treated synovial fluid were incubated at 56°C for 30 minutes to inactivate complement. Duplicate 50 µl samples of serum of synovial fluid were then tested in a solid phase immunoradiometric assay (Hay *et al.*, 1975; Nineham, *et al.*, 1976). The assay involved the binding of antiglobulins to rabbit immunoglobulin linked to the surface of plastic tubes; the amount of antiglobulin bound was then determined by adding radiolabelled anti-human IgG or IgM.

The rabbit anti-human IgG employed as the radiolabelled reagent was prepared from a different pool of rabbit antisera from that used in previous studies. This new antiserum bound more avidly to human IgG; therefore the apparent values for IgG antiglobulins are higher in the present series. The ranking of patients and normal controls remained the same, however.

IMMUNE COMPLEX ASSAY

Immune complexes were determined as described previously (Hay *et al.*, 1976) with the following modifications. The assay involved the binding of the first component of complement C1q to plastic tubes. 1-ml volumes of human C1q solution 10 mg/l in PBS were incubated in polystyrene tubes (LP3, Luckham Ltd., Sussex) for 3 days at 4°C. After 3 washes with PBS the tubes were filled with 0·01% gelatin solution in PBS and incubated at room temperature for 2 h. After 3 more washes with PBS the tubes were

Table 1 *Immune complexes and antiglobulins*

	Synovial fluid			*Serum*											*Treatment*		
Patient no.	*Immune complex*	*IgG antiglobulin*	*IgM antiglobulin*	*Immune complex*	*IgG antiglobulin*	*IgM antiglobulin*	*Agglutination*	*ARA*	*Activity*	*Erosions*	*Nodules*	*Age*	*Sex*	*Duration (years)*	*Anti-inflam.*	*Steroids*	*Other drugs*
Rheumatoid arthritis																	
1	9·44*	3·42	2·10	2·99	2·32	2·18	Pos.	C	A	+	+	81	M	2	+	+	Pen.
2	7·61	2·02	1·78	1·44	1·60	1·96	Pos.	C	O	0	0	64	F	8	0	0	
3	7·37	2·62	1·82	1·26	2·32	2·02	Pos.	C	A	+	+	51	F	12	+	0	
4	5·31	3·00	2·02	2·04	1·48	1·96	Pos.	C	A	+	0	35	F	9	+	+	
5	4·66	2·64	2·16	n.t.**	n.t.	n.t.	Pos.	C	A	+	0	43	M	6	+	+	Gold
6	4·25	2·50	1·36	n.t.	1·00	1·18	Pos.	C	A	0	0	69	M	0·3	+	+	
7	3·83	2·98	1·84	0·89	2·60	0·86	Pos.	C	A	+	+	54	F	16	+	0	
8	3·07	2·10	1·92	2·04	2·62	0·86	Pos.	C	S	+	+	67	F	3	+	0	
9	2·65	1·88	1·46	1·49	1·36	1·36	Pos.	C	A	+	+	67	F	9	+	+	
10	2·42	2·42	1·86	2·16	2·02	1·48	Pos.	C	A	+	0	29	F	7	+	0	Gold
11	2·06	2·44	1·98	0·89	1·72	1·82	Pos.	C	M	+	+	42	F	6	+	+	
12	2·06	1·40	1·28	0·78	1·84	0·64	Pos.	C	A	+	0	50	F	11	+	+	Gold
13	1·59	1·08	0·80	1·08	0·96	0·40	Neg.	C	A	+	0	68	F	6	+	0	Aza.
14	1·42	1·18	1·16	n.t.	0·66	1·30	Pos.	C	A	+	0	54	F	27	+	0	
15	1·30	1·68	1·64	1·44	1·68	1·40	Pos.	D	O	0	0	57	F	8	+	0	
16	0·71	1·10	0·24	n.t.	0·72	0·34	Neg.	P	S	0	0	51	F	9	+	0	
17	0·54	1·36	1·56	0·78	1·06	1·32	Pos.	C	A	+	0	73	F	20	+	+	
Osteoarthrosis																	
18	0·71	1·08	0·40	n.t.	n.t.	n.t.	Neg.					53	M	9	+		
19	0·24	0·64	0·08	1·02	0·68	0·14	Neg.					49	M	0·3	+		
20	0·18	1·26	0·20	0·66	1·44	0·24	Neg.					64	F	21	+		
21	0·18	1·04	0·12	0·54	0·78	0·18	Neg.					61	F	0·5	+		

* = milligrams antibody bound per litre. ** Not tested.

used in the immune complex assay. 50 μl of test serum or synovial fluid were mixed with 100 μl Na_2 ethylene diaminetetra-acetic acid (EDTA), 0·2 M (adjusted to pH 7·5 with NaOH), and incubated for 30 min at 37°C. The mixture was then transferred to an ice bath (Zubler *et al.*, 1976). Duplicate 50μl samples were placed in the coated plastic tubes together with 950 μl of PBS containing 0·05% Tween 20 (PBS Tween).

Coated tubes containing 1 ml PBS Tween were used as background controls. The tubes were incubated for 1 h at 37°C and for 30 min at 4°C. Unbound proteins were then removed by washing 3 times with cold PBS. Immune complexes bound to the C1q-coated tubes were detected by incubating the tubes with 1 μg of purified radiolabelled anti-IgG in 1 ml PBS Tween at 37°C for 1 h and at 4°C for 30 min. Unbound labelled reagent was removed by 3 washes with cold PBS. The tubes were then counted in a gamma-ray spectrometer, the amount of radioactivity bound being a measure of the immune complexes in the patient's serum.

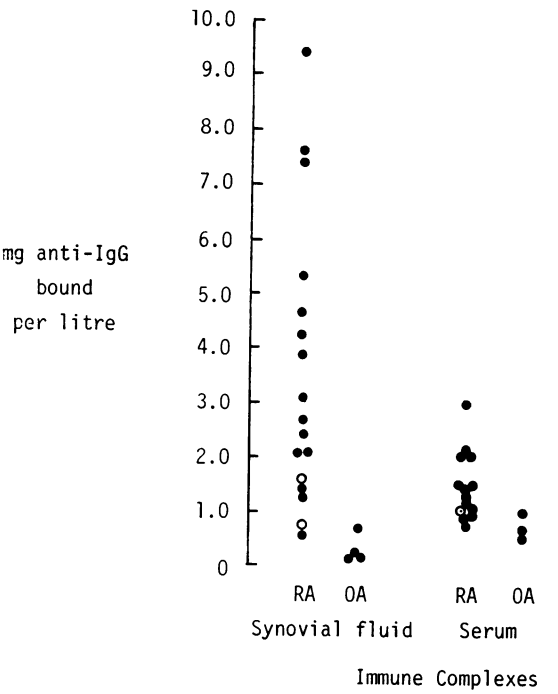


Fig. 1 *Immune complexes in synovial fluid and serum in patients with RA and OA. For the RA group open circles indicate SCAT-negative and closed circles indicate SCAT-positive patients. These symbols are used throughout all the Figures.*

Results

The individual results for immune complexes and IgG and IgM antiglobulins for all samples of synovial fluid and patients' sera are documented in Table 1. The mean values and percentage incidence of positive results in RA patients compared with OA synovial fluid or normal sera are summarised in Table 2.

SYNOVIAL FLUID

Immune complexes, IgG and IgM antiglobulins were all raised ($P<0·01$; Student's t test) in the synovial fluids of RA patients when compared with OA (Figs. 1 and 2). Values for immune complexes were elevated in 15/17 RA patients, IgG antiglobulins in 14/17, and IgM antiglobulins in 16/17. The 2 seronegative patients had low levels of each factor. There was a strong correlation between the levels of immune complex and IgG antiglobulin ($r=0·75$, $P<0·001$; Fig. 3) and between the amounts of the 2 classes of antiglobulins ($r=0·79$, $P<0·001$).

SERUM

Although immune complexes were raised in the RA group compared with OA sera and were above the normal range in 8/13 patients (upper limit of normality defined as mean of normal controls + 2 standard deviations = 1·18 mg/l), the increase was not significant ($P<0·1$) when compared with OA. However, it was significant when compared with the normal controls ($P<0·025$).

IgG antiglobulins were increased significantly ($P<0·025$) in RA (mean 1·62 ± 0·16 mg/l) compared with OA and were above the normal range in

Table 2 *Comparison between RA and OA*

	Immune complex		*IgG antiglobulin*		*IgM antiglobulin*	
		% positive		*% positive*		*% positive*
RA synovial fluid	3·60±0·64*	88	2·11±0·18	94	1·50±0·15	94
RA serum	1·48±0·18	61	1·47±0·19	69	1·32±0·14	87
OA synovial fluid	0·33±0·13	0	1·00±0·13	25	0·20±0·07	0
OA serum	0·74±0·14	0	0·73±0·03	33	0·19±0·01	0
Lab staff serum	0·83±0·06	0	0·76±0·06	0	0·45±0·03	0

*Milligrams anti-immunoglobulin bound/l. Values given are mean ± standard error.

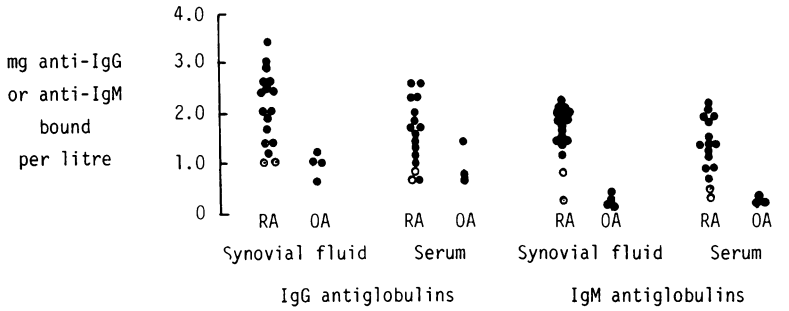


Fig. 2 *IgG and IgM antiglobulins in synovial fluid and serum in RA and OA patients.*

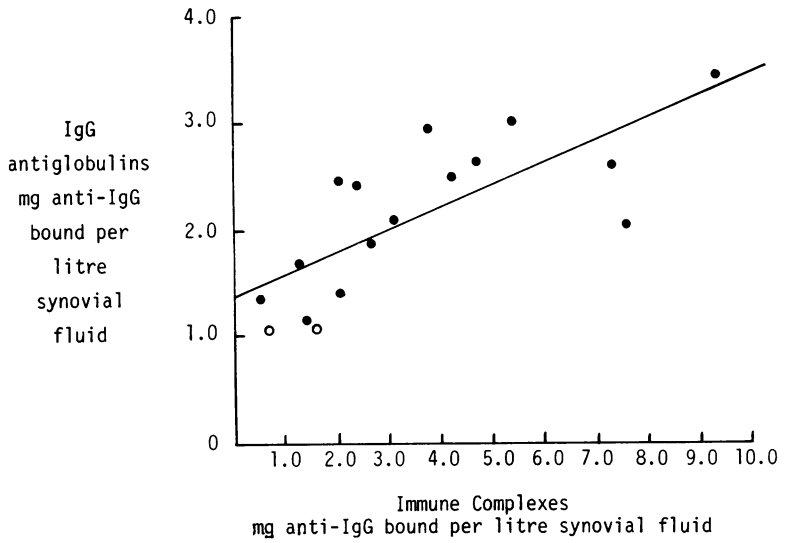


Fig. 3 *Correlation between IgG antiglobulins and immune complexes in synovial fluid of RA patients. The regression line is plotted for all the data.*

11/16 patients (mean + 2 SD normal controls = 1·09 mg/l).

IgM antiglobulins were also significantly elevated ($P<0·005$) in RA (1·32 ± 0·14 mg/l) compared with OA (0·19 ± 0·01 mg/l) and were above the normal range in 14/16 patients (mean + 2SD normals = 0·58 mg/l). In fact the only RA patients with normal values were the two found to be 'seronegative' by classical agglutination techniques.

The correlations between the levels of immune complex and IgG antiglobulins ($r=0·35$, $P<0·3$; Fig. 4) and between the 2 classes of antiglobulins ($r=0·29$, $P<0·3$) were much weaker than in synovial fluid.

COMPARISON OF SYNOVIAL FLUID AND SERUM

There was a correlation between immune complexes in synovial fluid and in serum ($r=0·56$, $P<0·05$), although the amounts were generally much higher in synovial fluid (Fig. 5). A stronger correlation existed for IgG antiglobulins ($r=0·66$, $P<0·01$), and the levels were comparable for paired samples of synovial fluid and serum (Fig. 6). Also there was a strong correlation between serum and synovial IgM antiglobulins ($r=0·75$, $P<0·001$; Fig. 7).

RELATIONSHIP BETWEEN CLINICAL STATUS AND LEVELS OF IMMUNE COMPLEXES AND ANTIGLOBULINS

There did not appear to be any direct relationship between the synovial fluid and serum levels of immune complexes or IgG or IgM antiglobulins, and the age or sex of the patient, the disease activity, duration of disease, or drug treatment. However, levels of IgG antiglobulins were significantly higher in the serum of RA patients with rheumatoid nodules than in those without extra-articular manifestations (Table 3).

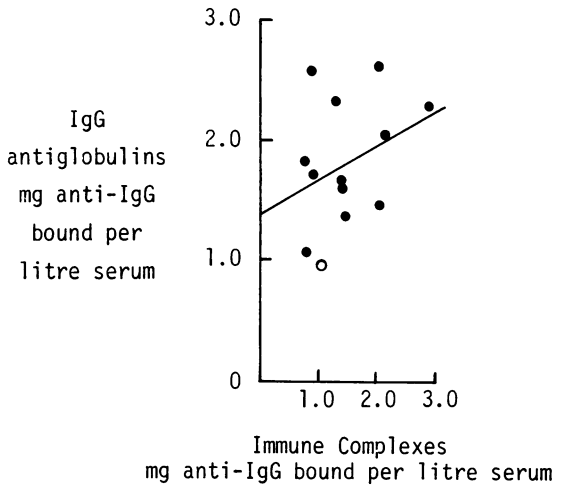


Fig. 4 *Correlation between IgG antiglobulins and immune complexes in serum of RA patients. The regression line for all the data is shown.*

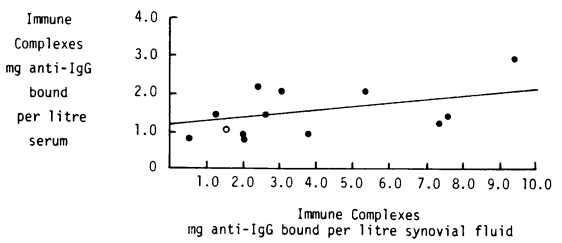


Fig. 5 *Correlation between serum and synovial fluid levels of immune complexes in RA patients. The regression line for all the data is shown.*

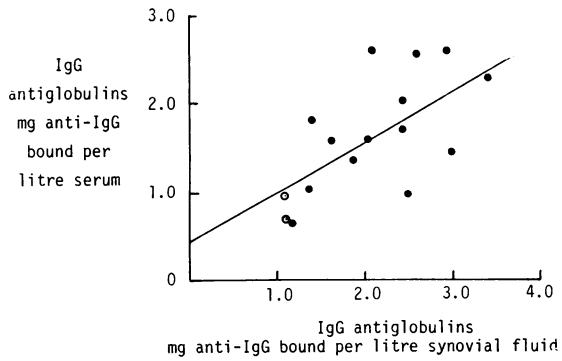


Fig. 6 *Correlation between serum and synovial fluid levels of IgG antiglobulins in RA patients. The regression line for all the data is shown.*

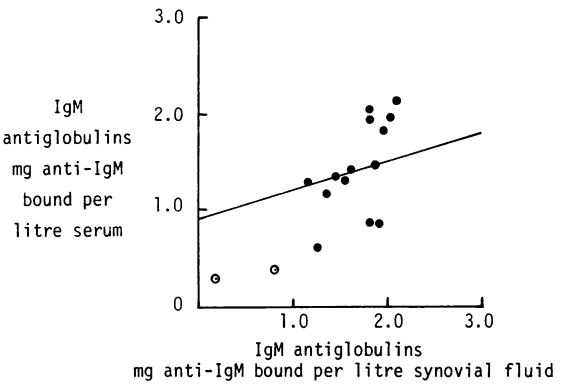


Fig. 7 *Correlation between serum and synovial fluid levels of IgM antiglobulins in RA patients. The regression line for all the data is shown.*

Table 3 *Antiglobulins and immune complexes in RA patients with and without nodules*

	RA with Nodules	*RA without Nodules*	*P*
Serum IgG antiglobulin	2·16±0·51*	1·30±0·48	<0·005
Serum IgM antiglobulin	1·52±0·58	1·20±0·58	<0·4
Serum immune complex	1·60±0·80	1·39±0·55	<0·6
Synovial fluid IgG antiglobulin	2·57±0·57	1·85±0·69	<0·05
Synovial fluid IgM antiglobulin	1·85±0·21	1·44±0·56	<0·2
Synovial fluid immune complex	4·81±3·02	2·94±2·29	<0·2

*Mean ± standard deviation, mg/l.

Discussion

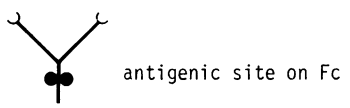
There is now good evidence that immune complexes and antiglobulins are present in both the serum and synovial fluid of patients with rheumatoid arthritis and play an important role in the pathogenesis of the synovial inflammatory response (Ziff, 1974). In the present study over 60% of patients had some increase in complexes in their sera, while nearly 90% had synovial fluid complexes. Not only did more patients have joint complexes, but the levels were markedly higher than in the serum. However, despite these differences there was a correlation between the amounts in synovial fluid and serum. Similar incidences of serum and synovial complexes have been found by Zubler *et al.* (1976) and Gabriel and Agnello (1977). Care must always be taken in interpreting immune complex results, as the detection rate is very dependent on the test system used. Using monoclonal rheumatoid factor Luthra *et al.*

(1975) found 26% of patients had raised serum complexes and only 22% showed any increase in the synovial fluid values. Certainly most methods including the solid phase C1q assay used in this study, have different sensitivities depending on the size of the complex, and various tests have greater affinity for complexes in certain diseases (Lambert *et al.*, 1977). Even within one disease such as rheumatoid arthritis complications may arise in comparing synovial with serum complexes because of possible differences in size and composition of the complexes in different sites.

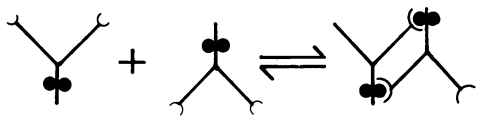
In accord with earlier findings (Hay *et al.*, 1975) both IgG and IgM antiglobulins were raised in most RA patients. Although the differences were not as striking as those found for immune complexes, the levels of IgG antiglobulins—and to a somewhat less extent IgM antiglobulins—were nearly always higher in synovial fluids than in the corresponding serum sample. This strongly points to the joint tissue as the major site of antiglobulin synthesis, with most, if not all, of the increased serum rheumatoid factor being attributable to 'spillover'. The correlation between serum and synovial fluid values would be consistent with this view. There is certainly ample evidence for local synthesis from the studies of Munthe and Natvig (1972) which showed that a major proportion of the plasma cells in the inflamed rheumatoid synovium are engaged in the synthesis of antiglobulins.

Intra-articular production of antiglobulins, in particular those of the IgG class, could account for the better correlation between IgG antiglobulins and complex levels in the joint rather than serum, and also the more marked elevation of complexes relative to antiglobulins in the synovial fluid. An IgG antiglobulin is a unique molecule in the sense that its antibody specificity is directed to a site on its own Fc fragment, thereby endowing it with the ability to self-associate (Pope *et al.*, 1974). As shown in Fig. 8, high local concentrations in the joints would lead to the formation of large self-associated aggregates, whereas in the serum, with its much higher relative concentration of normal immunoglobulin, the equilibrium is shifted towards the formation of small complexes.

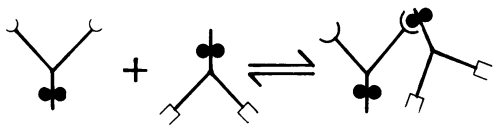
Whereas the large articular complexes would be potent activators of complement and macrophages, these small serum complexes are probably relatively harmless as evidenced by the lack of renal involvement compared with SLE. Certainly there is little evidence of serum complement activation (Winchester *et al.*, 1971) except in the presence of systemic disease (Hunder and McDuffie, 1973). However, Nydegger *et al.* (1977) have found increased levels of circulating C3d, but this could possibly be an overflow from inflamed joints or extra-articular disease sites.



(a) IgG antiglobulin



(b) Strong self-association of IgG anti-globulin through the extra binding forces of multipoint attachment (bonus effect of multivalency). Larger aggregates may be stabilized by IgM anti-globulins and C1q which are polyvalent binding agents with respect to IgG Fc.



(c) Weak association between IgG anti-globulin and an irrelevant IgG molecule forming a complex held by only one bond.

Fig. 8 *Complex formation by IgG antiglobulins. Local production in joints encourages the build up of aggregates by reaction (b). In serum, competition from irrelevant IgG will lead to complexes of smaller size and lower concentration through the dominance of reaction (c).*

Six of the 15 RA patients had subcutaneous nodules. There was a tendency for IgM antiglobulins and immune complex levels to be higher in this group but the presence of nodules correlated most significantly with raised IgG antiglobulins. Without excluding a role for IgM rheumatoid factors, this would be consistent with the notion that local production of IgG antiglobulins could be a major factor in the generation of these granulomatous structures (Nowoslawski and Brzosko, 1967).

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