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IgG rheumatoid factor, complement and immune complexes in rheumatoid synovitis and vasculitis: comparative and serial studies during cytotoxic therapy

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SUMMARY

IgG and IgM rheumatoid factors (IgG-RF and IgM-RF), complement and three assays for immune complexes were measured in 22 patients with rheumatoid arthritis (RA) complicated by either chronic active synovitis or vasculitis. Patients with vasculitis had relatively inactive arthritis but had higher titres of rheumatoid factors, especially IgG-RF, anticomplementary activity (ACA) and lower levels of C4 than those with synovitis. C1q-binding and platelet aggregation (PA) levels were similar in both groups. Serial measurements during cytotoxic therapy showed a close temporal relationship between the clinical features of vasculitis and levels of IgG-RF, ACA and C4 both with remission and with relapse. We suggest that immune complexes containing IgG-RF which activate complement and are detected by ACA are useful markers of rheumatoid vasculitis and may be important in its pathogenesis.

INTRODUCTION

Many immunological abnormalities are found in patients with rheumatoid arthritis (RA) but these have not been closely related to the particular organ involvement seen in this systemic disease. Vasculitis is one such manifestation which is an uncommon but serious complication of RA usually found in patients with severe seropositive erosive and nodular disease and often associated with other extra-articular manifestations (Epstein & Engleman, 1959; Schmid *et al.*, 1961; Bywaters & Scott, 1963). Comparative studies have shown many serological abnormalities in patients with systemic rheumatoid disease. Vasculitis has usually been included as one extra-articular feature signifying systemic disease except in a few cross-sectional studies which have shown specifically some association between cryoglobulins (Weisman & Zvaifler, 1975; Erhardt, Mumford & Maini, 1979), ACA (Bacon, 1979) and antibodies to extractable nuclear antigen (Venables, Erhardt & Maini, 1980) and rheumatoid vasculitis. We have recently shown a close relationship between high levels of IgG-RF and rheumatoid vasculitis in a large cross-sectional study (Allen *et al.*, 1980) and this has led us to undertake comparative studies in patients with RA selected for either active synovitis or vasculitis and serial studies during cytotoxic therapy.

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PATIENTS AND METHODS

Patients studied had either severe chronic active RA which had failed to respond to conventional second-line drugs or vasculitis. All patients fulfilled the ARA criteria for classical or definite RA.

Eleven patients had chronic active synovitis only (mean age 64 years; four male, seven female), and 11 vasculitis (mean age 64 years; four male, seven female); seven of these had systemic vasculitis and four nail fold vasculitis only.

Vasculitis was defined clinically by the presence of typical nail fold, nail edge or digital infarcts, deep cutaneous ulcers or neuropathy. Patients with less specific clinical evidence of vasculitis such as skin rashes and superficial or chronic cutaneous ulcers required confirmatory evidence of vasculitis by biopsy. Skin rashes were present for at least 2 weeks before treatment except in one case where persistent widespread digital, nodular and nail fold lesions preceded the rash. Cutaneous ulcers had been assessed for at least 4 weeks before treatment without improvement from local therapy and, in addition, one ulcer had failed to respond to plasma exchange and another to combined plasma exchange and oral azathioprine. Where digital or nail fold lesions were the sole clinical feature of vaculitis, these had to be either chronic, lasting for more than 4 weeks, or recurrent on at least two occasions within a 4-week period. Renal involvement was suggested by haematuria and proteinuria developing at the same time as other vasculitic features. Rectal biopsies were also undertaken in nine patients with vasculitis.

All patients were treated with intravenous methyl prednisolone, 750 mg, and cyclophosphamide, 15 mg/kg, on four occasions at weeks 0, 1, 4 and 7.

Synovitis was assessed before treatment and 2 weeks after completion of therapy by the Ritchie articular index (Ritchie *et al.*, 1968), a pain assessment using the 10-cm visual analogue scale (Huskisson, 1974) and a thermographic index of the hands, knees and ankles (Ring, 1976).

Laboratory tests were carried out before treatment and at weekly intervals for the first 4 weeks and thereafter at 3-weekly intervals until treatment was completed. These included haemoglobin, differential white cell count, platelet count and plasma viscosity. Immunoglobulins G, A and M and complement levels C3 and C4 were measured by radial immunodiffusion.

IgM rheumatoid factor titre was measured by the sheep red cell differential agglutination test. IgG rheumatoid factor was measured by the radioimmunoassay based on the method of Klinman & Taylor (1969) and performed as described by Allen *et al.* (1980). Briefly, baboon IgG was attached to polystyrene tubes, incubated with the test sera and then with ^{125}I purified baboon anti-human gamma sera. The proportion of the total labelled antibody bound by test samples was calculated and the results expressed as mg labelled antibody bound/l serum. Normal levels were less than 0·2.

Assays for ACA, C1q binding and platelet aggregation were carried out as described by Verrier-Jones & Cumming (1977). The normal values in our laboratory are for ACA less than 1/2, for C1q binding less than 20% and for PA less than 1/16.

The data was analysed using the *t*-test, paired *t*-test, the signed rank sum test and the Mann–Whitney *U*-test where appropriate.

RESULTS

Synovitis and vasculitis

Clinical assessments of synovitis are shown in Table 1. Patients with widespread vasculitis had relatively inactive synovitis by articular index, pain score and thermographic index. The four patients who had nail fold vasculitis only showed active synovitis initially and had some improvement with treatment. Treatment had little effect on the arthritis of the patients with active synovitis, or those with more widespread or systemic vasculitis.

The clinical features of the patients with vasculitis are shown in Table 2. With treatment, complete healing or improvement occurred in 31 out of 38 lesions. This was most apparent for nail fold, digital and nodular vasculitis, skin rashes and peripheral gangrene. Healing or improvement was also seen in all ulcers considered to be vasculitic and in one ulcer where vasculitis was not

Table 1. Assessments of joint activity in patients with synovitis and vasculitis

	Synovitis (11)	Nail fold vasculitis only (4)	Systemic vasculitis (7)
Articular index	18·5±8·8 (16·8)	22·5±3·1 (13·8)	8·0±4·5 (9·0)
Pain score	5·1±2·0 (5·0)	5·8±1·9 (5·7)	3·4±2·4 (3·6)
Thermographic index	4·1±0·8 (3·7)	4·7±0·3 (4·2)	3·3±0·7 (3·5)

Mean levels (±s.d.) with post-treatment values in parentheses.

considered the primary cause. Sensory neuropathy cleared in one patient, improved in one, but remained unchanged in three. Significant improvement in lung function and breathlessness occurred in the one patient with fibrosing alveolitis. Scleritis improved in one patient but local treatment was also used. Two patients with vasculitis relapsed: one with Felty's syndrome (P.S.), in whom neutropenia dictated a lower dose of cytotoxics, developed new digital lesions shortly after the third injection and later also a cutaneous rash, and the other (E.L.) relapsed only after a delay in treatment but responded after treatment was restarted.

Rectal biopsies were performed in nine patients with vasculitis, and showed necrotizing vasculitis in four. After treatment, biopsies were repeated in these four patients only. Three were normal and one showed a healing vasculitis. Skin biopsies in three patients showed leucocytoclastic vasculitis but these were not repeated after treatment.

Laboratory and clinical associations

No significant differences were found between the two groups of patients for the initial levels of haemoglobin, plasma viscosity or immunoglobulins G, A or M. With treatment, plasma viscosity, IgG and IgA levels all fell significantly (Table 3).

IgM and IgG rheumatoid factor (IgM-RF and IgG-RF) levels are shown in Fig. 1. Despite the considerable overlap, mean initial IgM-RF titres were significantly higher in vasculitis (1/256) than synovitis (1/32) ($P<0·05$). Treatment was associated with a fall in titre in the vasculitis group to 1/32 ($P<0·05$) but with no change in the synovitis group. Differences in IgG-RF were more marked. All but one of the vasculitis group had initial levels greater than twice the upper limit of normal (mean 0·52) but none with synovitis had such high levels (mean 0·23; $P<0·01$). With treatment no change occurred in the synovitis group but in the vasculitis group the mean titre in patients fell to

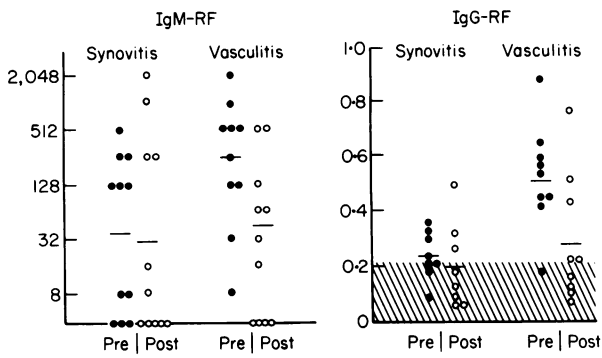

Fig. 1. Titres of IgM-RF and IgG-RF before (•) and after (o) treatment for patients with synovitis and vasculitis. The mean values are represented by the horizontal bars and the normal range by the shaded area. Significant differences were found between the two groups for IgM-RF ($P<0·05$) and IgG-RF ($P<0·01$) and with treatment in patients with vasculitis only, IgM-RF ($P<0·05$) and IgG-RF ($P<0·05$).

Table 2. Features of patients with vasculitis

	Patient											Before treatment (total, $n=11$)	After treatment		
	V.E. (8)*	R.G. (20)	N.J. (12)	C.J. (20)	I.J. (18)	W.L. (10)	E.L. (13)	P.M. (20)	P.S. (12)	A.W. ($1\frac{1}{2}$)	E.Y. (15)		Healed	Improved	No change
Subcutaneous nodules	+	+	+	+	+	+	+	+	+	+		10	—	—	10
Skin															
Nail fold, edge	+	+	+	+	+	+		+	+	+		9	8	1	—
Digital			+					+	+	+		4	4	—	—
Nodular			+					+	+	+		4	4	—	—
Ulcers†		(+)	+	(+)		(+)		+		+	+	4+ 3(+)	3+1(+)	1+	2(+)
Rash				+			+		+			3	2	1	—
Neuropathy (sensory)			+	+				+		+	+	5	1	1	3
Lung															
Fibrosing alveolitis											+	1	—	1	—
Pleurisy							+					1	1	—	—
Scleritis										+		1	—	1	—
Proteinuria							+					1	1	—	—
Others‡				Cardiac					Felty			2	—	—	2
Total												38	25	6	7
Biopsy															
Skin				+				+	+			3/3		No follow-up	
Rectum§	−		+	+	−		+	−	−	−	+	4/9	3/4	1/4	—

* Figures in parentheses express duration of disease in years.

† Ulcers: + = clinical diagnosis of vasculitis (deep, punched out, temporal relationship with other vasculitic features). (+) = Clinical diagnosis not vasculitis (shallow chronic, no temporal relationship with other vasculitic features).

‡ Cardiac = aortic incompetence.

§ Biopsy = rectal biopsy repeated only in four patients with positive biopsy before treatment.

Table 3. Effect of treatment on plasma viscosity and serum immunoglobulins

	Pretreatment	Post-treatment	
PV	2·02	1·90	$P<0·05$
IgG	14·4	10·3	$P<0·001$
IgA	3·8	3·1	$P<0·05$
IgM	1·8	1·6	n.s.

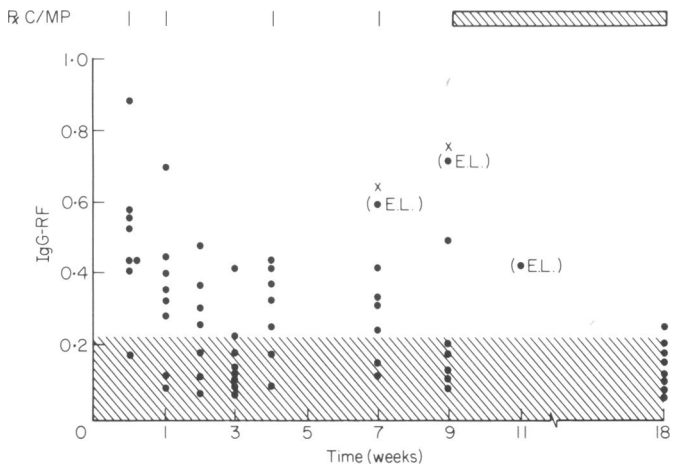

Fig. 2. Serial IgG-RF titres during treatment and at 18 weeks. Patient E.L.: values in parentheses at 7 and 9 weeks as treatment was delayed at 7 weeks and followed by relapse at 9 weeks; effect of restarting treatment also shown at 11 weeks. Treatment with C/MP = IV cyclophosphamide and methyl prednisolone followed by oral azathioprine. Patient A.S. who also relapsed: (×) values at 7 and 9 weeks.

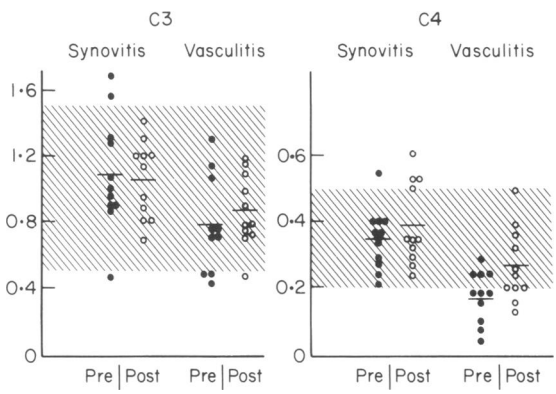

Fig. 3. C3 and C4 levels before (•) and after (○) treatment for patients with synovitis and vasculitis. The mean values are represented by the horizontal bars and the normal range by the shaded area. Significant differences were found between the two groups for C3 ($P<0·05$) and C4 ($P<0·001$) and with treatment in patients with vasculitis only for C4 ($P<0·01$).

0·28 ($P < 0·05$). Serial values (Fig. 2) showed a marked reduction in levels of IgG-RF in patients with vasculitis during the first 4 weeks but a later rise at the time of the final injection and at the final assessment was associated with clinical relapse in two patients and led to the overall reduction at the end of treatment being less significant. IgG-RF levels in the eight patients followed in detail showed almost normal levels by 18 weeks at a time when no patient had any clinical features of vasculitis. One patient with synovitis had a raised level of IgG-RF (0·52) after treatment. He had severe systemic symptoms with weight loss and lymphadenopathy but no definite evidence of vasculitis was found.

Initial complement levels (Fig. 3) were lower in the vasculitis group, especially C4 (0·17 *vs* 0·35; $P < 0·001$). Seven vasculitis patients, but none with synovitis, had C4 levels below normal. Treatment resulted in a significant rise in C4 in patients with vasculitis to a mean level of 0·28 ($P < 0·01$) and a smaller rise in C3. No significant changes occurred in patients with synovitis.

ACA, C1q-binding and PA levels are shown in Fig. 4. Anticomplementary activity (ACA) was present initially in eight patients with vasculitis but only one with synovitis. Treatment resulted in an overall fall in titre to 0 in five, reduction (of greater than 2 dilutions) in two and no change in one. A rebound rise occurred in the same two patients whose IgG-RF rose and whose vasculitis recurred. The patient with synovitis with ACA before treatment had clinical, X-ray and lung function evidence of fibrosing alveolitis. Treatment resulted in ACA returning to normal and also significant improvement in lung function. One patient with synovitis had a raised ACA after treatment with no apparent cause. C1q binding and platelet aggregation showed considerable overlap between the two groups both initially and after treatment. C1q levels were slightly higher in vasculitis but this difference was not significant. Treatment was not associated with any significant changes in either measurement. No significant correlations were found between IgG-RF and IgM-RF, platelet aggregation or C1q binding when the group was assessed as a whole. The regression coefficient of IgG levels and IgG-RF levels for the pretreatment samples was calculated but showed no correlation ($b = 0·00125 \pm 0·0091$, $d = 0·05$, $P < 0·9$).

The temporal relationship between clinical and laboratory features is illustrated by the two cases who relapsed (Fig. 5). One patient (E.L.) had a recurrent purpuric skin rash, general malaise and weight loss. Proteinuria and haematuria were present and her creatinine clearance was reduced (36 ml/min). IgG-RF was high (0·89), C4 low (0·08) and ACA positive (1/256), C1q-binding raised (92%) but platelet aggregation normal (1/4). Treatment resulted in the disappearance of the skin rash, clearing of the haematuria and proteinuria and reduction in the levels of IgG-RF and ACA and a later rise in C4, but little change in C1q binding or IgM-RF. A gap in treatment was associated

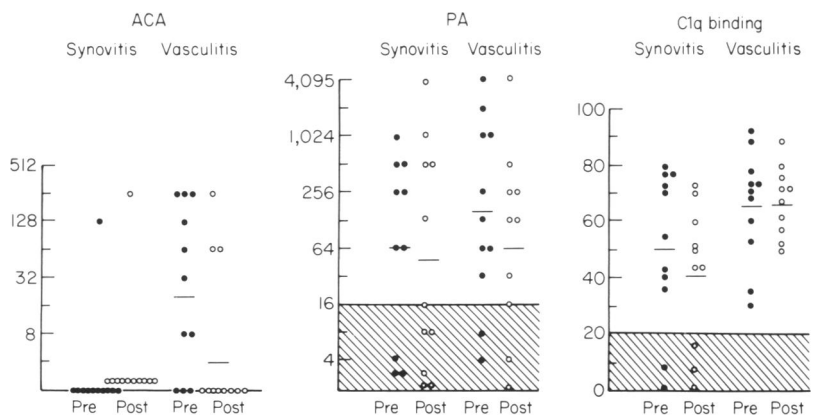

Fig. 4. ACA, PA and C1q binding before (•) and after (o) treatment for patients with synovitis and vasculitis. The mean values are represented by the horizontal bars and the normal range by the shaded area. No significant differences were found between the two groups or with treatment for platelet aggregation (PA) or C1q binding. Anticomplementary activity (ACA) was positive in eight of 11 patients with vasculitis (mean 1/16) before treatment which was associated with a fall to zero in five; significant reduction in titre in two and unchanged in one (mean 1/4).

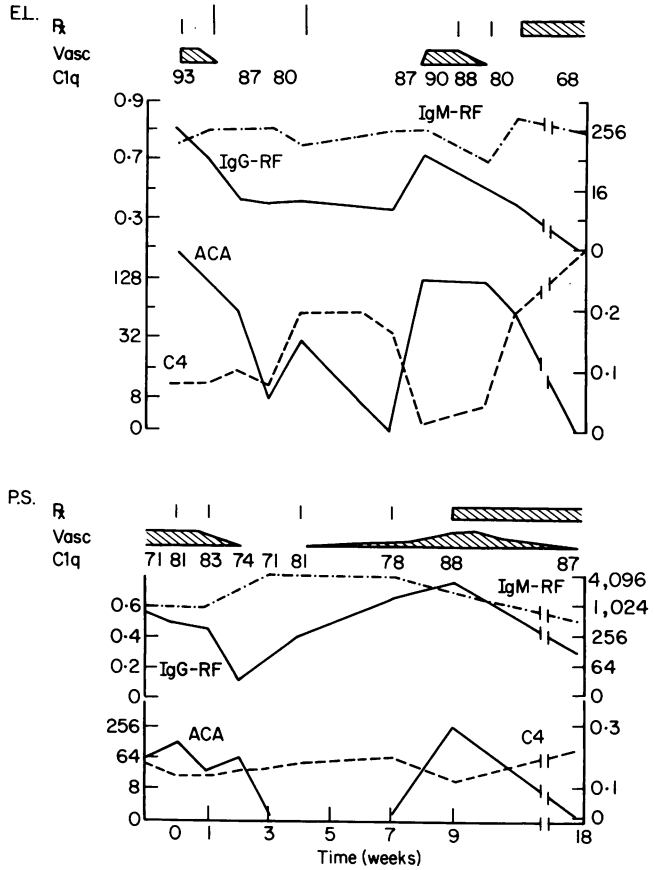

Fig. 5. Serial results in two patients who relapsed (see text). Treatment: cyclophosphamide and methyl prednisolone (C/MP) followed by oral azathioprine. Vasc = clinical features of vasculitis. At the time of vasculitis and at relapse both patients had high levels of IgG-RF, raised levels of ACA and low C4. C1q binding and IgM-RF levels showed little significant change.

with recurrence of vasculitis, together with the development of pleurisy. IgG-RF and ACA rose and C4 fell, but without significant alterations in C1q binding. Restarting treatment resulted in clinical improvement together with normalization of the laboratory features described and at the end of the 14-week period shown, she had no clinical evidence of vasculitis, no proteinuria or haematuria and an improved creatinine clearance of 56 ml/min. IgG-RF and C4 levels had returned to normal, ACA was negative and C1q binding was still raised at 68%. Throughout the entire period there was little change in the titres of IgM-RF. Rectal biopsy showed necrotizing vasculitis before treatment and at the time of relapse but was normal after treatment was completed. A similar pattern was seen in the other patient (P.S.) who had Felty's syndrome and who was treated with a lower dose of cytotoxics.

DISCUSSION

The clinical features of vasculitis and its association with the variety of extra-articular manifestations seen in our RA vasculitis patients have been previously well documented in the classical descriptions of RA vasculitis (Schmid *et al.*, 1961; Bywater & Scott, 1963).

Digital, nail fold and nodular infarcts may resolve spontaneously but are often recurrent. Cutaneous ulcers complicating RA are often chronic and fail to respond to local or systemic therapy

(Allison & Bettley, 1957; Wilkinson & Kirk, 1965; Anderson, 1967). The healing of deep cutaneous ulcers and the disappearance of other cutaneous lesions, together with improvement in other parameters such as lung function, suggest an active healing process with treatment.

The initial serological tests showed a significant association between high titres of IgM-RF, IgG-RF, low levels of C4 and ACA with clinical vasculitis. Serial results showed this association to be most marked for C4, ACA and IgG-RF suggesting that these factors may indeed have pathogenetic relevance. The IgG-RF results confirm and extend our previous study which had shown an association between high levels of IgG-RF (greater than twice normal) and rheumatoid vasculitis (Allen *et al.*, 1980). Associations between clinical vasculitis and IgM-RF or C1q binding and platelet aggregation were much less clear.

IgG-RF has been described in patients with RA and extra-articular manifestations including vasculitis (Theophilopoulos *et al.*, 1974). Relationships have been described between IgG-RF and the presence of immunoglobulin and complement in the blood vessels of skin in patients with RA with or without clinical vasculitis (Ullman *et al.*, 1979) and immune deposits have been found in both skin and sural nerve biopsies of patients with vasculitis (Conn, McDuffie & Dyck, 1972; Conn, Schroeter & McDuffie, 1976).

The mechanisms by which IgG-RF could participate in the pathogenesis of vasculitis are speculative. It presumably participates in immune complex formation and it has been suggested that IgG may be the sole antigen in RA (Male, Roitt & Hay, 1980). In addition it may affect coagulation mechanisms. Platelet aggregation *in vitro* is enhanced by immune complexes containing IgG-RF but inhibited by IgM-RF (Fink *et al.*, 1979). It is possible that immune complexes containing IgG-RF may aggregate platelets *in vivo* leading to the development of vascular damage.

IgM-RF is usually present in high titres in patients with RA vasculitis (Epstein & Engleman, 1959; Bywaters & Scott, 1963; Mongan *et al.*, 1969). Despite the inhibition of platelet aggregation described above, negative correlations between IgM-RF titre and platelet aggregation in the platelet aggregation test for immune complexes have not been found either by us or other workers (Fink *et al.*, 1979). IgM-RF may still be important in the production of vasculitis in other ways. *In vitro*, sera containing high titres of IgM-RF causes decreased solubilization of immune complexes (Naylor *et al.*, 1979) which may be necessary for deposition of such complexes in the walls of blood vessels.

Complement levels are often raised in patients with active synovitis as part of an acute-phase response (Schubart *et al.*, 1965). Active complement consumption occurs in synovial fluid (Ruddy & Austen, 1970) but low complement levels are rarely found in the serum. Hunder & McDuffie (1973) detected only 16 patients with low C4 in RA out of a total of 365 over a 3-year period. All 16 had extensive extra-articular disease, especially vasculitis. A low C4 is thus associated with severe complicated RA in agreement with our findings. C4 is a sensitive indicator of complement activation by the classical pathway (Ruddy & Austen, 1973). C3, IgG and IgM have been found in the blood vessels of patients with vasculitis (Conn *et al.*, 1972) suggesting that classical pathway complement activation by immune complexes may be involved in the vascular damage.

C1q binding is found in up to 80% of patients with RA and titres are said to reflect the degree of synovitis (Halla, Volkanakis & Schrohenloher, 1979) and the presence of extra-articular disease (Zubler *et al.*, 1976). Complexes containing IgG and IgM of varied sizes are detected by C1q binding but intermediate complexes, less than 19S in particular, are found in patients with RA (Lambert & Casali, 1978). Our patients with vasculitis did have high levels of C1q binding but there was considerable overlap with synovitis. It is possible that immune complexes responsible for vasculitis are a subpopulation of those detected as C1q binding and as such are not detected separately. Platelet aggregation detects IgG complexes over a wide size-range and is found in 40% of patients with RA usually at low titres (Lambert & Casali, 1978), which may be due to the inhibitory effect of IgM-RF. We found a wide variety of levels of platelet aggregation in both groups but were unable to correlate titres with clinical or other serological parameters. ACA is a less sensitive test which is reported to detect large IgG- and IgM-containing complexes greater than 19S and is found in a variety of vasculitic disorders (Johnson & Mowbray, 1977; Verrier-Jones *et al.*, 1979) including RA vasculitis (Bacon, 1979). ACA has been reported in 70% of patients with systemic vasculitis but less frequently with nail fold changes only (Bacon, 1979). The rapid change in titre which was associated

with clinical improvement in our patients suggested that it detects complexes important in the production of vascular damage.

Patients with RA vasculitis have high titres of rheumatoid factors, especially IgG-RF, together with raised levels of ACA and low C4 when compared with patients with synovitis. Treatment with cyclophosphamide and methyl prednisolone resulted in little change in synovitis, possibly related to the short length of the study and the long interval between injections. By contrast there was marked improvement of vasculitis. The close temporal relationship between the improvement of vasculitis and the return to normal of these serological abnormalities underlines their usefulness in diagnosis and monitoring treatment and also suggests they may be important in the aetiology of the vasculitis. The results suggest that large complexes of greater than 19S that fix complement and contain IgG-RF may be responsible for the development of vasculitis. Dissociation of the material detected by the IgG-RF assay has shown that some of the IgG-RF detected is complexed to IgM-RF but retains its RF properties after separation (Jones *et al.*, 1980). The large complexes important in the development of vasculitis may therefore contain both IgG-RF and IgM-RF.

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