Clin. exp. Immunol. (1979) 38, 405–413.

The association of cryoglobulinaemia with nodules, vasculitis and fibrosing alveolitis in rheumatoid arthritis and their relationship to serum C1q binding activity and rheumatoid factor

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(*Accepted for publication* 29 *April* 1979)

SUMMARY

Two measurements of serum immune complexes, cryoglobulinaemia and ^{125}I-C1q binding, have been performed in patients with severe rheumatoid arthritis (RA) and compared with normal levels. Cryoglobulinaemia was present in 20 out of 28 patients (71%) with extra-articular disease (mean level 17 μg/ml) including nodules, digital vasculitis, cutaneous ulcers, rash, neuropathy, lung disease and scleritis, but in none of 32 patients with joint disease alone (uncomplicated RA) (mean level 3 μg/ml). Cryoglobulinaemia correlates with, but probably does not antedate, extra-articular disease, and may be useful in predicting morbidity and mortality in this group of patients. In contrast, serum ^{125}I-C1q binding was raised in patients with uncomplicated RA and those with extra-articular disease, although levels were higher in the latter group. Both tests showed a negative correlation with serum haemolytic complement and a positive correlation with IgM rheumatoid factor although there were some sera with raised levels of rheumatoid factor without cryoglobulinaemia. These results suggest that cryoglobulinaemia is a better test than C1q-binding for demonstrating the presence of circulating immune complexes involved in the pathogenesis of extra-articular lesions.

INTRODUCTION

Vascular, visceral and granulomatous lesions, in this study referred to as extra-articular manifestations, have been recorded in relation to rheumatoid arthritis (Sinclair & Cruickshank, 1956), the commonest lesion being rheumatoid nodules which occur in about 25% of patients. Circulating immune complexes have been implicated in the pathogenesis of some of these manifestations. For example, cryoglobulinaemia was found in patients with cutaneous vasculitis and neuropathy (Weisman & Zvaifler, 1975) and intermediate size (9–15S) complexes were detected by ultracentrifugation in patients with diffuse pulmonary fibrosis (Tomasi, Fudenberg & Finby, 1962) and hyperviscosity syndrome (Jasin, LoSpalluto & Ziff, 1970). Other workers, using a number of techniques, have demonstrated the presence of circulating immune complexes in the sera of rheumatoid patients without detailed reference to the presence of extra-articular disease (Winchester, Kunkel & Agnello, 1971; Zubler *et al.*, 1976). Hypercatabolism of circulating complement components has been documented in patients with vasculitis (Weinstein *et al.*, 1972; Nydegger *et al.*, 1977), and there is also a well recognized association between extra-articular disease and the presence of IgM rheumatoid factor (Mongan *et al.*, 1969). These findings raise the possibility that rheumatoid factors, immune complexes and complement are all involved in the pathogenesis of some systemic manifestations of RA.

In this study we have correlated two measurements of circulating immune complexes (cryoglobulinaemia and ^{125}I-C1q binding activity) with the clinical features of RA patients, with a view to establishing

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0099-9104/79/1200-0405$02.00

TABLE 1. Patients with extra-articular disease*

Patient number	1–7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27†	28
Features																						
Duration of disease (years)	3–27†	4	19	20	40	15	17	6	10	8	19	3	1	19	28	7	7	14	½	15	9	6
Subcutaneous nodules	±			+	+		+	+	+	±	+		+	+	+		+	+		+	±	±
Nail fold infarcts		+	+	+	+		+	+	+					+	+							
Necrotizing polyarteritis								+														
Leg ulcers						+	+	+		+												
Neuropathy:																						
Motor								+	+	+												
Sensory								+	±	+	+											
Lung disease																						
Fibrosing alveolitis												+	+	+	±	±	+					
Airways disease																+	+	±				
Nodules																			+	+		
Scleritis														+	±					+	+	±
Rash								+														+
Cryoprecipitate protein (μg/ml)	8–26	15	33	10	14	8	58	13	20	25	36	14	14	14	21	8	6	3	11	11	10	35

* The sign + indicates presence of clinical features at time of blood sample.
† Range in this group of seven patients.
‡ Patient no. 27 also had pericarditis and a pleural effusion.

whether circulating complexes detected by these tests are important in the pathogenesis of extra-articular lesions, and to what extent they are related to changes in serum complement and the appearance of IgM rheumatoid factor.

PATIENTS AND METHODS

Studies were performed on sera from 77 patients with RA classified according to the American Rheumatism Association (1959) criteria as follows: classical 42, definite 31, probable 4. One or more extra-articular disease manifestations had been present in 28 of 60 patients for a variable period at the time of study (see Table 1). Digital infarcts, leg ulcers, neuropathy and rash were considered to be manifestations of rheumatoid vascular lesions (Glass, Soter & Schur, 1976), and one patient (No. 14) was proven to have necrotising arteritis on the basis of histologically observed lesions in liver and sural nerve biopsies. Fibrosing alveolitis was diagnosed in five patients (Nos 18–22) on the basis of persistent lung crepitations, chest X-ray appearances, a restrictive ventilation defect and reduced alveolar gas transfer. Patient No. 22 had had a lung biopsy which, in addition to changes of fibrosing alveolitis, showed a marked infiltration of the bronchial submucosa with mononuclear cells. Two additional patients (nos 23 and 24) were dyspnoeic on exertion, produced no sputum, had persistent lung crepitations and showed evidence of airways obstruction with normal alveolar gas transfer. The chest X-ray of one (No. 23) showed micronodular shadowing of the mid and lower zones, but patient No. 24 had a normal X-ray. These patients were considered to be suffering from bronchiolitis of the type described by Gosink, Friedman & Liebow (1973).

Rheumatoid inflammatory activity was assessed by enumerating joints with signs of synovitis and employing an articular index (Ritchie *et al.*, 1968). Schirmer's test for tear secretion was performed on 46 of the patients. The erythrocyte sedimentation rate (ESR) was measured in mm/hr by the Westergren method.

Blood from 26 healthy volunteers was used for control data.

Methods. Serum separated at 37°C was kept at 4°C for 7 days and the cryoprecipitate harvested by centrifugation at 1500 *g* for 30 min at 4°C. The precipitate was washed three times in 5 ml of cold normal saline and resolubilized in 1 ml of phosphate buffered saline at 37°C for 1 hr. The resolublized material was separated by centrifugation at 1500 *g* for 10 min at 37°C, the protein concentration measured by the method of Lowry *et al.* (1951), and the result expressed as μg/ml of original serum. Immunoglobulin classes and complement proteins were quantitated by single radial immunodiffusion on commercial plates (Behringwerke). Immunoglobulins accounted for more than half the soluble protein so we have followed the convention of terming the precipitates cryoglobulins.

Serum haemolytic complement (CH_{50}) was measured according to the method of Mayer (1964) the normal range being 36–72 units/ml in our laboratory. Serum ^{125}I-Clq binding activity (ClqBA) was measured by the method of Zubler *et al.* (1976). In this study the upper limit (mean+2 s.d.) of ClqBA of 30 normal sera was 11·4%. Rheumatoid factor was measured by sheep cell and latex agglutination tests (Cathcart & O'Sullivan, 1965). A differential agglutination titre of > 1:16 or a tube latex titre of > 1:80 was considered clinically significant.

Differences observed in measurements between groups of patients were examined statistically using the Mann-Whitney U test. Correlations between serological measurements were evaluated by calculating the correlation coefficient (r).

RESULTS

Cryoglobulinaemia

The serum cryoglobulin protein concentrations of 26 sera from normal subjects were in the range 0–10 μg/ml, mean 3·8 μg/ml. Cryoglobulinaemia above 10 μg/ml was detected in 20 of the 60 patients described in Table 1. In the sera of 32 patients with uncomplicated RA the cryoglobulin concentration was in the range 0–10 μg/ml (mean 3·3 μg/ml), there being no significant difference from the normal population. Of the 28 sera from patients who had joint involvement together with extra-articular disease, 20 (71%) had cryoglobulin levels above 10 μg/ml, the mean value (16·9 μg/ml) being significantly higher than in uncomplicated RA ($P<0{\cdot}001$) (Fig. 1a).

Serum immunoglobulin levels, as measured by the sum (IgG+IgA+IgM), were higher in the patients with extra-articular disease than in uncomplicated RA, all three immunoglobulin classes being elevated. The immunoglobulin classes detected in the cryoprecipitates from 24 rheumatoid patients were IgG, IgM and IgA in 15; IgG and IgM in 8; and one serum had a cryoglobulin containing IgG and IgA. The concentrations of cryoprecipitable immunoglobulin are given in Table 2, which shows that the predominant immunoglobulin class in the cryoprecipitate was IgG. However, compared with serum levels, relatively more IgM than IgG was concentrated in the cryoprecipitates.

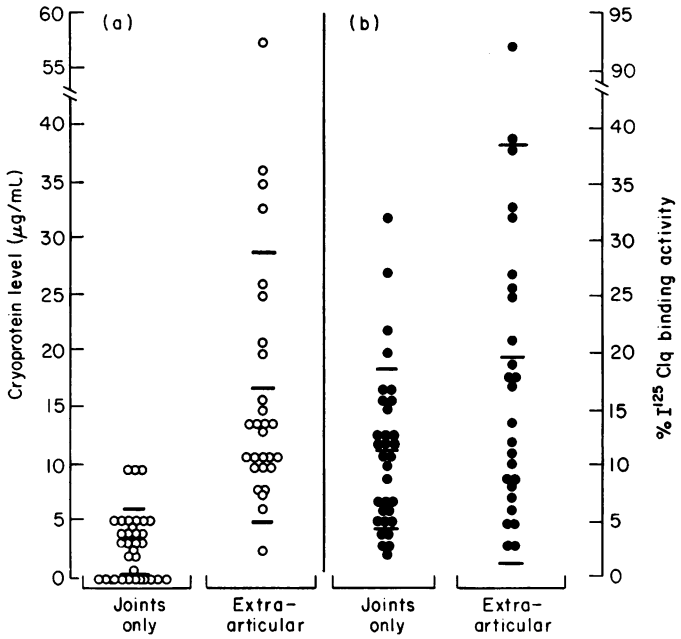

FIG. 1. (a) Serum cryoglobulin protein levels in rheumatoid arthritis, with and without extra-articular disease. (b) Serum ^{125}I-Clq binding activity in rheumatoid arthritis, with and without extra-articular disease. Bars show mean ± 1 s.d.

TABLE 2. Concentrations of immunoglobulins in cryoprecipitates

	Cryoprecipitable immunoglobulin* μg/ml serum			Cryoglobulin Ig μg/ml† ÷ serum Ig μg/ml	
	Mean	S.D.	Range	Mean (× 10⁴)	Range (× 10⁴)
IgG	11·4	11·3	0·9–55	6·5	1–29
IgM	6·3	4·5	0–23	32·2	12–182
IgA	0·8	1·1	0–6·4	2·0	0–8

* Results of 66 sera from 24 patients.
† Results of sera from 21 patients.

^{125}I-C1q binding activity of serum

Of the 60 sera examined for cryoglobulins, 58 were also examined for C1qBA. C1qBA was raised in 30 (52%) and was higher in patients with extra-articular disease (mean 19·5%, range 3–92) than in uncomplicated RA patients (mean 11·3; range 2–32) (not significant)(Fig. 1b). There was a positive correlation ($r = 0{\cdot}52$, $P > 0{\cdot}001$) between C1qBA and cryoglobulin (Fig. 2). From Fig. 2 it will be noted that 16 sera with raised C1qBA did not contain a significant cryoglobulin, and conversely three sera had a cryoglobulin without elevated C1qBA. Of the patients with extra-articular disease 71% had cryoglobulinaemia and 58% had raised C1qBA. Whereas no serum from patients with joint disease showed significant cryoglobulinaemia 47% of the same population had elevated C1qBA (Table 3). These results show that the presence of cryoglobulinaemia was a better index of extra-articular disease than C1qBA.

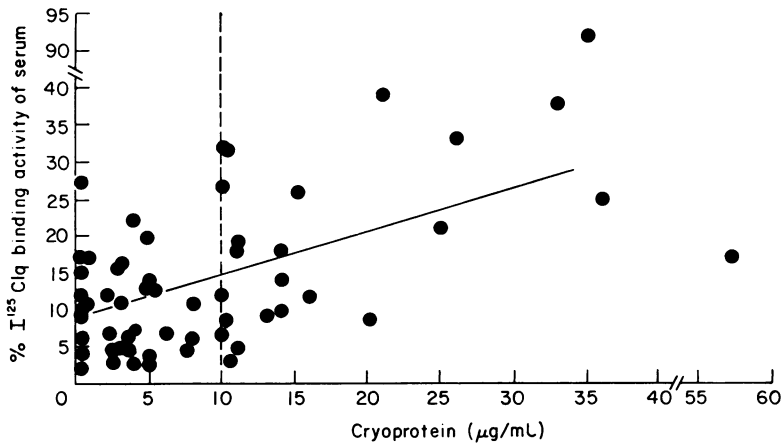

FIG. 2. Correlation between serum cryoglobulin in protein and serum ^{125}I-Clq binding activity. The upper limit of normal for cryoglobulin protein is shown by the dotted line. $n = 58$; $r = 0{\cdot}52$; $P < 0{\cdot}001$.

TABLE 3. Serum cryoprecipitate protein and Clq binding activity in rheumatoid arthritis

Clinical category	Sera with raised cryoprecipitate protein (> 10 g/ml)	Sera with raised Clq binding activity (> 11·4%)
Uncomplicated rheumatoid arthritis	0/32 (0%)	15/32 (47%)
Extra-articular disease	20/28 (71%)	15/26 (58%)

Complement and rheumatoid factor

In 38 out of 54 sera examined, CH_{50} was within the normal range for our laboratory. One serum from a patient with widespread cutaneous vasculitis had a value of less than 20 units/ml and 15 sera had values greater than 72 units/ml. The CH_{50} values of sera from patients with extra-articular disease (mean 58, range <20–76·9 units/ml) were lower than those of sera from patients with uncomplicated RA (mean 70·5, range 51·3–83·3 units/ml) ($P<0{\cdot}001$). There was a negative correlation ($r = -0{\cdot}56$, $P<0{\cdot}001$) between CH_{50} and cryoglobulinaemia (Fig. 3) and a similar negative correlation ($r = -0{\cdot}61$, $P<0{\cdot}001$) between CH_{50} and C1qBA. Levels of serum C4 and C3 were found to be negatively correlated with cryoglobulinaemia ($r = -0{\cdot}47$ and $r = -0{\cdot}31$ respectively) but not with C1qBA, indicating that hypercatabolism of complement correlates better with the former.

Sixty-two sera contained rheumatoid factor. Of the 60 patients in Table 1 rheumatoid factor (DAT and/or Latex test) was present in the sera of 20 out of 32 patients with joint disease alone, and in the sera of all 28 patients with extra-articular disease. The DAT showed a positive correlation with cryoglobulins ($r = 0{\cdot}39$, $P<0{\cdot}005$) (Fig. 4). However, there were a number of patients with raised titres of rheumatoid factor who did not have cryoglobulins, suggesting that cryoprecipitation is not merely related to the presence of IgM rheumatoid factor in the serum.

The C1qBA of DAT positive sera (mean 18%, range 3–92%) was significantly higher than that of DAT negative sera (mean 7%, range 3–20%) ($P<0{\cdot}001$). In contrast to the previously published claim of Zubler *et al.* (1976) a good correlation was seen between C1qBA and DAT irrespective of whether all 77 sera ($r = 0{\cdot}43$, $P<0{\cdot}001$) or only the 55 DAT positive sera ($r = 0{\cdot}40$, $P<0{\cdot}005$) were analysed.

Essentially similar results were obtained when cryoglobulinaemia or C1qBA were correlated with latex titres.

The relationship of cryoglobulinaemia and C1qBA to clinical features

From Table 1 it will be seen that a variety of extra-articular features, singly or in combination, were associated with cryoglobulin values above 10 μg/ml. These included nodules (16 out of 22 positive), digital infarcts (8 out of 9), leg ulcers (3 out of 4) and all four patients with neuropathy. Fibrosing alveolitis was associated with a raised cryoglobulin protein in four out of six patients; the two patients with pulmonary nodules had elevated levels, but not the three with airways disease. Four out of five patients with scleritis had elevated values, as did both patients who had widespread cutaneous vasculitis, of whom one had necrotizing polyarteritis.

Of forty-six patients whose tear secretion was measured by Schirmer's test, cryoglobulinaemia occurred in six out of twenty (30%) with normal tear secretion and in nine out of twenty-six (35%) with diminished tear secretion, showing a lack of correlation between cryoglobulinaemia and this clinical feature.

The relationship between disease activity and circulating immune complexes was assessed by comparing cryoglobulinaemia and C1qBA with an articular index, the number of joints with clinical synovitis and the ESR. There was a significant correlation between serum C1qBA and the ESR ($r = 0{\cdot}44$, $P < 0{\cdot}005$) but not between cryoglobulinaemia and the ESR. No correlation was found between cryoglobulinaemia or C1qBA and the articular index or joint score.

DISCUSSION

In this study extra-articular manifestations of rheumatoid arthritis have been found to be strongly associated with cryoglobulinaemia. Weisman & Zvaifler (1975) have previously reported cryoglobulinaemia in seventeen (out of forty-three) rheumatoid patients and noted an association with extra-articular disease in the form of sensory-motor neuropathies and dermal infarcts in only eight patients. We have confirmed this association and find a wider spectrum of extra-articular lesions to be also associated with cryoglobulinaemia. Cryoglobulinaemia was invariably found in patients with neuropathies, occurred in almost all patients with digital vasculitis, scleritis and leg ulcers, and was present in four out of six patients with fibrosing alveolitis. However, in many patients more than one extra-articular feature was present which did not allow an assessment of an association between individual clinical features and cryoglobulinaemia.

The lower levels of protein in the cryoglobulins of our patients compared with those reported by Weisman & Zvaifler (1975) probably reflect differences in the technical aspects of the two methods. The lack of a generally accepted method of collecting, washing and re-dissolving serum cryoglobulins and expressing cryoglobulin protein concentration complicates inter-laboratory comparisons. Significant cryoglobulinaemia in our patients was defined by comparison with a normal population.

Immunoglobulin class measurements confirmed results by other workers that some rheumatoid sera contain mixed cryoglobulins, predominantly IgG and IgM (Weisman & Zvaifler, 1975; Brouet *et al.*, 1974). When serum concentrations of immunoglobulin classes were taken into account, a greater fraction of the serum IgM appeared in the cryoglobulin than of IgG, possibly due to the presence in the cryoglobulin of immune complexes rich in rheumatoid factor. Conversely, relatively low levels of IgA were seen in cryoglobulins in spite of raised serum IgA levels suggesting that this class of immunoglobulin is not important in forming cryoprecipitable immune complexes in rheumatoid arthritis. Our results support the concept that cryoglobulins are not merely reflecting the levels of circulating immunoglobulins but are concentrating immune complexes (McIntosh & Grey, 1976).

The close association between cryoglobulinaemia and extra-articular diseases invites comment on those eight patients with extra-articular features but without cryoglobulinaemia. Firstly, some extra-articular features of rheumatoid arthritis may not be a consequence of deposition of circulating immune complexes, but may result from other forms of hypersensitivity reactions, e.g. mediated by cytotoxic

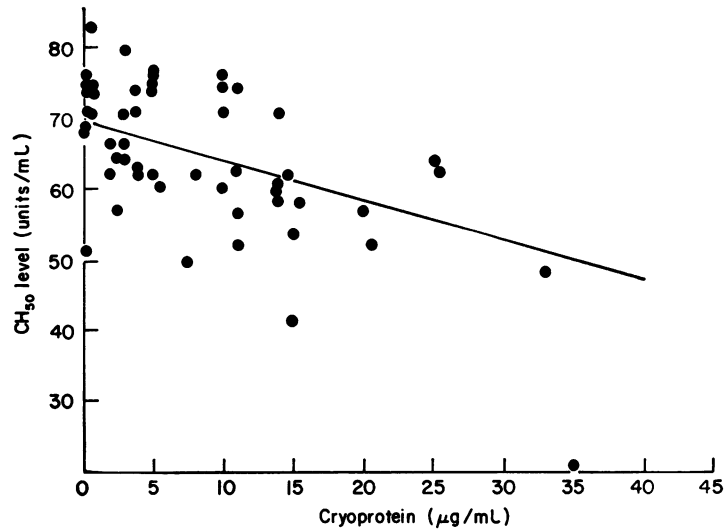

FIG. 3. Negative correlation between serum cryoglobulin protein and haemolytic complement (CH_{50}). The normal range for CH_{50} is 36–72 units/ml. $n = 54$; $r = -0{\cdot}56$; $P < 0{\cdot}001$.

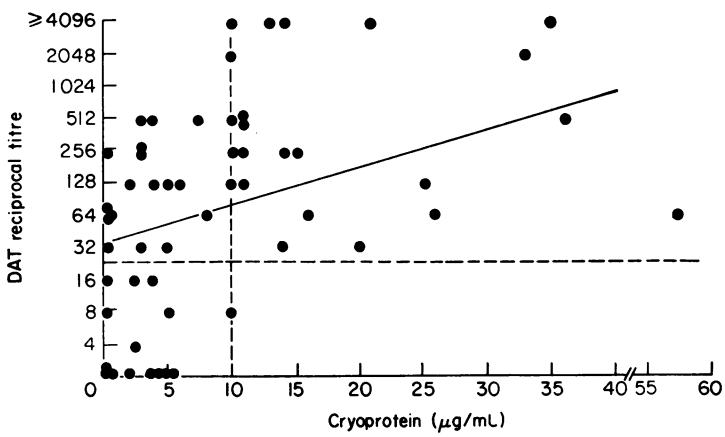

FIG. 4. Correlation between serum cryoglobulin protein and differential agglutination titre. The upper limits of normal for these tests are shown by the dotted lines. $n = 57$; $r = 0{\cdot}39$; $P < 0{\cdot}005$.

antibodies or by cellular immune mechanisms. In this category might be included the development of kerato-conjunctivitis sicca (Sjogren's syndrome), since in this study no association was observed between diminished tear secretion and either of the two measurements of circulating immune complexes. Likewise, the absence of cryoglobulinaemia or C1qBA in the two patients with bronchiolitis (Nos 23 and 24), and another patient (No. 22) whose lung biopsy showed severe chronic bronchiolitis and interstitial inflammation, suggest involvement of other pathogenetic mechanisms. Secondly, immune complexes may only appear in the circulation intermittently during the course of active disease, or disappear as a result of therapy. Our study was based on a single-point observation and no patient was excluded on the grounds of previous treatment. D-Penicillamine may effectively abolish circulating complexes in treated patients (Norberg & Gedda, 1977), and may have done so in three (Nos 6, 23 and 27) whose signs of extra-articular disease were still evident, but who did not have cryoglobulins. Thirdly, persistence of extra-articular lesions may reflect irreversible structural changes, for example as demonstrated by Dreisin

et al. (1978) who showed that patients with idiopathic interstitial pneumonia whose lungs were fibrotic, had no circulating complexes. Fourthly, chronic tissue lesions may be initially triggered by deposition of immune complexes but perpetuated by a locally autonomous process. Fifthly, the assays used may not detect all types of immune complexes.

In our study, extra-articular rheumatoid disease was diagnosed when overt clinical signs were detectable, but it is known that sub-clinical manifestations, for example, digital vascular occlusion (Laws, Lillie & Scott, 1963) and abnormal lung function tests (Leading article, 1978) may be much more commonly present. If circulating immune complexes are responsible for the evolution of such lesions, then it would seem likely that cryoglobulinaemia correlates only with the more severe pathological lesion leading to obvious clinical signs. The higher frequency of positive C1qBA assay in our rheumatoid patients could relate to sub-clinical lesions, but the relationship has not been investigated.

At least some of the extra-articular lesions which we have found to be associated with cryoglobulinaemia are thought to arise due to vascular injury in which complement is activated (Nydegger *et al.*, 1977). As reported by others (Franco & Schur, 1971; Hunder & McDuffie, 1973) the mean CH_{50} value of patients with extra-articular disease was significantly lower than the mean value of patients with joint disease alone, although the catabolic rate was insufficient to lower CH_{50} levels below the normal range. There may also be increased production of complement components as reflected by raised CH_{50} levels in fifteen of the patients.

This study has provided evidence of heterogeneity of circulating immune complexes in rheumatoid arthritis by the finding that not all sera with raised C1qBA form cryoglobulins, and that there is a better correlation of extra-articular disease with cryoglobulinaemia than with C1qBA. A correlation between rheumatoid factor and both tests for immune complexes may indicate that the immune complexes we detected contained rheumatoid factor. Complexes of IgG and IgM-rheumatoid factor (Franklin *et al.*, 1957) and self-associating IgG rheumatoid factor (Pope, Teller & Mannik, 1974), have been described in RA sera. It is possible that such complexes form an appropriate lattice structure which *in vivo* results in tissue injury, and *in vitro* leads to precipitation in the cold.

There was no significant relationship between joint inflammation (as measured by an articular index or joint score) and cryoglobulinaemia or C1qBA, and it is not our contention that circulating immune complexes perpetuate joint disease. However, there was a positive correlation between C1qBA and the ESR which suggests that circulating immune complexes which bind to C1q rise simultaneously with factors (e.g. fibrinogen and globulins) which influence the ESR. The ESR is frequently raised in patients with active rheumatoid joint inflammation and in such patients the joints may be rich in immune complexes, some of which escape into the circulation. In the extra-articular group cryoglobulinaemia may predict those with a higher morbidity and mortality. Six patients (not all in Table) have died during follow-up of whom five (including patients Nos 3, 19 and 21) had cryoglobulinaemia and the sixth was patient No. 27 with constrictive pericarditis. The lack of a significant number of patients with cryoglobulinaemia who did not have extra-articular disease does raise the possibility that cryoglobulinaemia occurs secondarily to the extra-articular lesions. Cryoglobulinaemia levels have been shown to fall following treatment with cyclophosphamide of patients with severe rheumatoid arthritis (Weisman & Zvaifler, 1975) and may be useful in monitoring such therapy.

Cryoglobulins isolated from the sera of patients with several diseases have been demonstrated to contain antigens and/or antibodies probably involved in the pathogenesis of that disease (Wands *et al.*, 1975; Davis, Godfrey & Winfield, 1978). Although in rheumatoid arthritis the amount of cryoglobulin which forms is usually smaller, the procedure could be used to isolate complexes for further investigation of their composition.

We should like to thank The Arthritis and Rheumatism Council for financial support and Dr J. T. Scott for allowing us to include some of his patients in this study.

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