

Differences in immunochemical characteristics of cryoglobulins in rheumatoid arthritis and systemic lupus erythematosus and their complement binding properties

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SUMMARY Cryoglobulins isolated from sera of patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) were analysed for their immunoglobulin, antibody, and complement components. In both disease categories the cryoglobulins contained predominantly IgG with lesser amounts of IgM and IgA, but relative to serum more IgM was concentrated in the cryoglobulins. IgM rheumatoid factor was found in 65% of RA cryoglobulins but in only 17% of SLE cryoglobulins ($p < 0.02$), whereas SLE cryoglobulins contained more DNA binding activity than RA cryoglobulins ($p < 0.01$). C1q binding activity was detectable in the majority of SLE and RA sera and SLE cryoglobulins. Paradoxically only two out of 34 RA cryoglobulins bound C1q, although rheumatoid factor activity was present in both cryoglobulins and sera. When isolated from serum the rheumatoid factor fraction strongly bound C1q. Both RA and SLE cryoglobulins contained similar small amounts of C3 and C4. Differences in antibody composition and complement binding activity of cryoglobulins from RA and SLE sera may reflect properties of immune complexes which affect their tissue localisation and pathogenicity.

Sera from patients with RA and SLE contain circulating immune complexes detectable by a number of assays.¹ Circulating immune complexes detected by cryoglobulinaemia in patients with RA are associated with certain extra-articular disease features considered to have a vascular or granulomatous basis.^{2,3} In patients with SLE cryoglobulinaemia correlates with the presence of renal disease.^{4,5} The pathogenicity of immune complexes is determined by their ability to localise in target tissues and activate complement, which is in turn related to the size and shape of the immune complexes and their antigen and antibody composition.^{6,7} This paper compares the immunoglobulin, rheumatoid factor, DNA antibody, and C3 and C4 composition of cryoglobulins prepared from sera of patients with RA and SLE. It also reports on the ability of these cryoglobulins to bind C1q. Sera from patients with cryoglobulinaemic purpura, con-

taining high levels of serum cryoglobulins, were used as controls.

Materials and methods

Blood samples were collected from patients into prewarmed glass bottles and clotted at 37°C. The serum was separated at 37°C, and 10 ml were placed in a plastic tube containing approximately 2 mg of sodium azide for precipitation and quantitation of cryoglobulin as previously described.³ Another aliquot of serum was frozen and stored at -20°C for measurement of circulating immune complexes.

IgM rheumatoid factor was measured by a tube latex agglutination test employing the Rheuma-Wellcotest from Wellcome Diagnostics. Anti-DNA antibodies were quantitated by a Farr assay using the Amersham International kit.⁸ For measurement of cryoglobulin rheumatoid factor and DNA antibody titres the diluting buffer was prewarmed to 37°C. Antibodies to extractable nuclear antigens were detected by counterimmunoelectrophoresis and double diffusion as previously described.⁹

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The antiglobulin fraction from sera was partially purified by an affinity chromatography column. The immuno-adsorbent was Cohn fraction II coupled to cyanogen bromide activated Sepharose 4B. The coupling buffer was 0.1 M sodium bicarbonate, pH 8.3, and the eluting buffer 0.1 M glycine adjusted to pH 2.8 with hydrochloric acid, both buffers containing 0.5 M sodium chloride. Unoccupied reactive sites were blocked by incubation of the immuno-adsorbent in 1 M ethanolamine overnight at 4°C and passage of normal human serum before the rheumatoid serum was applied. The eluted antiglobulin fraction was dialysed against phosphate buffered saline, and its protein, immunoglobulin, and rheumatoid factor content were measured in the same way as that of cryoglobulins.

Serum ¹²⁵I C1q binding activity (C1qBA) was measured by the method of Zubler *et al.*¹⁰ The assay was performed on the whole serum, the cryoglobulin, and the cryoglobulin supernatant in the same assay to avoid interassay variation. Measurement of C1qBA of cryoglobulins and column fractions was performed after standardisation of their protein content by mixing 50 µl of the cryoglobulin solution with 50 µl of 0.2 M ethylenediamine tetra-acetic acid (EDTA) and 50 µl of normal human serum which had been dialysed against 0.2 M EDTA.¹⁰

Complement levels in cryoglobulins were measured by rocket immunoelectrophoresis using a modification of a standard method.¹¹ 0.8% agarose was used in 0.2 M barbitone buffer containing 0.01 M EDTA. Commercial anti-C3 and anti-C4 antisera (Behringwerke) were included in the gel and dilu-

tions of Human Serum Standard (Behringwerke) used for calibration.

The serum cryoglobulins examined in this study had protein concentrations greater than 10 µg/ml of original serum, which was the highest cryoglobulin protein found in 26 normal sera. The sera were obtained from patients with RA who fulfilled diagnostic criteria for classical or definite disease¹² and patients with SLE who also fulfilled diagnostic criteria.¹³ Four patients with cryoglobulinaemic purpura were included in the study. In addition to a purpuric rash one of these patients had Sjögren's syndrome, but systemic disease was absent in the other three patients. All four sera and cryoglobulins were negative for hepatitis B surface antigen and antibody.

Statistical analysis. The χ^2 test with Yates's correction and Wilcoxon's sum of ranks test were used.

Results

IMMUNOGLOBULIN COMPOSITION OF CRYOGLOBULINS

Cryoglobulin IgG, IgA, and IgM was quantitated by single radical immunodiffusion at 37°C. By this method the sum of IgG + IgA + IgM accounted for a mean of 73% (range 20–100%) of cryoglobulin protein from 66 rheumatoid arthritis sera and a mean of 52% (range 20–100%) of cryoglobulin protein from 13 SLE sera.

The concentrations of cryoprecipitable immunoglobulin are given in Table 1, which shows that the predominant immunoglobulin class which precipi-

Table 1 Characteristics of cryoglobulins in RA, SLE, and cryoglobulinaemic purpura

		RA	SLE	Cryoglobulinaemic purpura	
Percentage of patients with serum cryoglobulin > 10 µg/ml		38	67	100	
Cryo. Ig (µg/ml)*	G	11.4	8.3	248	
	M	6.3	6.8	163	
	A	0.8	1.1	61	
Cryo. Ig (µg/ml) × 10 ⁴	G	7	5		
	M	32	49	ND	
	A	2	3		
Serum Ig (µg/ml)					
	IgM rheumatoid factor	% positive	65	17	75
DNA binding		Median titre	1:40	0	1:2560
		% positive	57	95	25
ENA antibodies		Median (units/ml)	40	96	0
	C3		0	0	0
C4		% positive	57	92	100
		Mean (µg/ml)	0.07	0.10	3.1
Fall of serum C1qBA on cold incubation		% positive	43	50	100
		Mean (µg/ml)	0.09	0.08	0.6
Cryoglobulin C1qBA		Mean %	0	12	52
		Mean %	8	17	75

*Mean values. ND=not done.

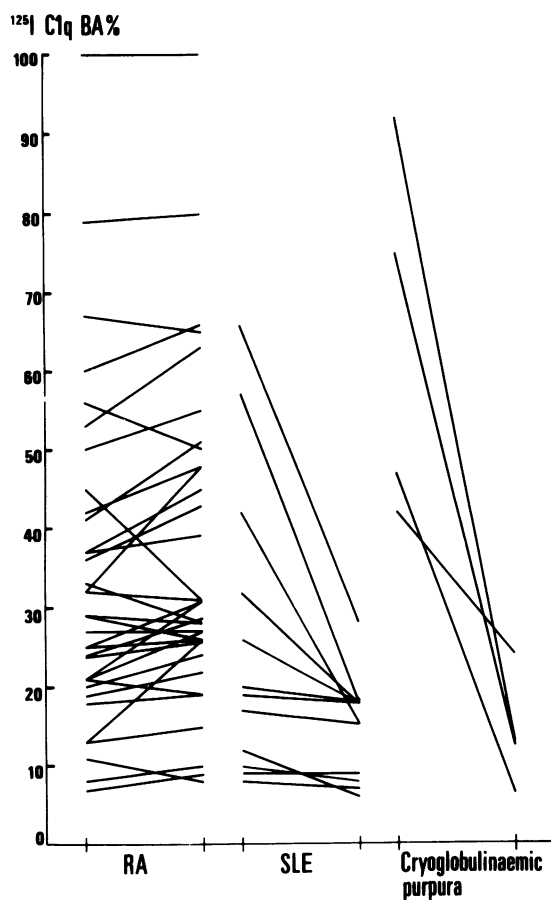


Fig 2 Serum ^{125}I -C1q binding activity before (left) and after (right) cold incubation and removal of the cryoglobulin.

The extent to which immune complexes containing rheumatoid factor accounted for the C1qBA of sera from four RA patients was investigated by an affinity column of aggregated human gammaglobulin bound to sepharose 4B. The results of these experiments, of which those in Table 2 are representative, showed that C1qBA was found mainly in the rheumatoid-factor-rich fraction. Affinity chromatography of a serum with a lower rheumatoid factor titre (1:160, C1qBA:24%) resulted in complete absorption of rheumatoid factor and C1qBA from the first drop-through fraction. The ratio of C1qBA to the sum of IgG and IgM was particularly high in the antiglobulin fraction, suggesting that much of the immunoglobulin in this fraction formed C1q binding immune complexes.

Seven out of 12 isolated cryoglobulins from SLE sera bound C1q (Fig. 1), and four of these sera

Table 2 Rheumatoid factor and C1qBA of serum fractions after affinity chromatography on Sepharose 4B-HAGG

	Whole serum	Effluent	Acid eluate
Latex reciprocal titre	> 40 960	1280	5120
Protein g/l	78.8	15.3	1.4
IgG g/l	15.8	3.6	0.18
IgA g/l	5.2	1.4	0.07
IgM g/l	4.3	1.6	0.66
% ^{125}I C1qBA	98	22	65
% ^{125}I C1qBA	4.9	4.2	77
IgG + IgM g/l			

showed a fall of C1qBA in the supernatant serum after the cryoglobulin was harvested (Fig. 2). All four isolated cryoglobulins from patients with cryoglobulinaemic purpura bound large amounts of C1q, and the C1qBA of the sera was markedly reduced by cold incubation.

Complement components C3 and C4 were quantitated by rocket immunoelectrophoresis in isolated cryoglobulins from 30 RA sera and 12 SLE sera with protein concentrations of greater than 10 $\mu\text{g}/\text{ml}$. Only small amounts of C3 and C4 were detected by this method (Table 1). C3 and C4 each accounted for a maximum of 1.5% of the serum cryoprecipitable protein. All three isolated cryoglobulins tested from patients with cryoglobulinaemic purpura contained both C3 and C4. Although cryoglobulins from these patients contained C3 and C4 in greater amounts than cryoglobulins from RA and SLE patients, the highest proportion of cryoglobulin accounted for by these complement components was 0.7%.

Discussion

This paper compares the characteristics of cryoglobulins isolated from the sera of patients with RA and SLE in order to ascertain whether there are differences which may explain the different clinical features seen in these two conditions.

In this study, which included only patients with cryoglobulinaemia, the amounts and ratios of the immunoglobulin classes precipitated from sera of patients with RA and SLE was approximately the same. IgG was the predominant immunoglobulin class which is in agreement with previous findings in SLE cryoglobulins,¹⁵ though in RA cryoglobulins Weisman and Zvaifler² found approximately equal amounts of IgG and IgM. In both RA and SLE there was relative concentration of IgM in the

cryoglobulins compared with the serum levels. It is likely that the IgM is concentrated in RA cryoglobulins by virtue of its antiglobulin activity,² which we also confirmed. The absence of rheumatoid factor from the majority of SLE cryoglobulins precludes this explanation for the enrichment of IgM in these cryoglobulins.

The comparison performed in this paper showed the presence of DNA binding activity in cryoglobulins from patients with RA and SLE and from one patient with cryoglobulinaemic purpura. The incidence and level of DNA binding was higher in SLE cryoglobulins than in the other two conditions. Both DNA and DNA binding activity have previously been shown to be concentrated in cryoglobulins from patients with SLE.^{14, 16} We also found a high incidence of DNA binding in RA cryoglobulins in contrast with the normal values obtained in sera. Our findings suggest that DNA antibodies are concentrated in RA cryoglobulins, though the possibility that DNA binds to non-immunoglobulin molecules such as C1q^{17, 18} and lipoprotein¹⁹ cannot be excluded. It is noted that specific DNA antibodies have been found in the cryoglobulins of non-SLE patients in another study in which they were considered to be a ubiquitous constituent of circulating immune complexes in many disorders.^{20, 21} In view of the frequent occurrence of antibodies to extractable nuclear antigens (ENA) in SLE sera²² and their correlation with extra-articular manifestations of RA⁹ we also performed a preliminary examination of cryoglobulins for the presence of these antibodies. However, ENA antibodies were not detected in the cryoglobulins of our patients with SLE, RA, or cryoglobulinaemic purpura. This is in keeping with the low incidence of ENA antibodies in cryoglobulins from SLE sera.¹⁴

One half of the SLE cryoglobulins tested bound significant amounts of C1q, as did all the cryoglobulins isolated from patients with cryoglobulinaemic purpura. This finding, together with the fall of C1qBA in the supernatant sera, is consistent with the concept of enrichment of immune complexes in cryoglobulins. Furthermore, the presence of C1qBA, C3, and C4 in cryoglobulins suggests that these immune complexes bind and activate complement. In contrast to the findings in patients with SLE and cryoglobulinaemic purpura the C1qBA of RA sera was retained in the supernatant after cold incubation and was detected in only two cryoglobulins. In some RA sera there was an increase in C1qBA on cold incubation, probably resulting from the formation of C1q binding immune complexes which remained soluble in the cold. The following considerations make the lack of C1qBA by RA cryoglobulins surprising: (1) The correlation of

cryoglobulinaemia with extra-articular features in which complement is thought to be implicated.^{2, 3}

(2) Direct evidence of hypercatabolism of C3 in RA patients with extra-articular disease.^{23, 24} (3) The C1qBA of rheumatoid factor which was demonstrated in the cryoglobulins. (4) The presence of C3 and C4 in RA cryoglobulins,^{2, 25} which we have quantitated and shown to be present in similar amounts as found in SLE cryoglobulins.

We have considered a number of reasons for our inability to demonstrate C1qBA in RA cryoglobulins. Technical factors may play a part—for example, the concentration of immune complexes in RA cryoglobulins may be too low. The importance of cryoglobulin protein concentration was suggested by the high C1qBA of cryoglobulins from patients with cryoglobulinaemic purpura. However, this does not fully explain the differences between RA and SLE cryoglobulins because six RA cryoglobulins with protein concentrations greater than 50 µg/ml did not bind C1q and SLE cryoglobulins with similar protein concentrations did. Another factor which might explain the differences of C1qBA between RA and SLE cryoglobulins is differences in their temperature requirements; the optimum binding of complement by cryoglobulins is temperature dependent.²⁶ A third alternative is that there are at least two distinct populations of immune complexes, of which the complement fixing variety remains soluble in the cold in RA sera but forms cryoglobulins in SLE sera. Nevertheless there is strong evidence that cryoglobulinaemia in RA is a marker of pathogenic immune complexes.^{2, 3}

The differences in the antibody composition and complement binding properties of immune complexes in RA and SLE that we have documented may explain differences in the patterns of tissue localisation and tissue pathology observed in patients. Factors affecting localisation of immune complexes include their size,²⁷ the nature of the antigen,²⁸ the prior deposition of antigen,²⁹ and possibly cross-reaction of the antibody component.³⁰ The importance of these and other variables in the pathogenesis of RA and SLE remains to be determined.

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