Specific Concentration of Polynucleotide Immune

Complexes in the Cryoprecipitates of

Patients with Systemic Lupus Erythematosus

JOHN B. WINFIELD, DAVID KOFFLER, and HENRY G. KUNKEL

From The Rockefeller University, New York 10021, and the Department of
Pathology, Mount Sinai School of Medicine of the City University of
New York, New York 10029

A B S T R A C T Although the association of cryoglobu-linemia with hypocomplementemia and tissue injury in systemic lupus erythematosus is well recognized, the composition of cryoprecipitates in terms of circulating antigens and antibodies in this disease is less clear. To clarify this question, cryoprecipitates from patients with SLE were examined with sensitive assay techniques for certain antipolynucleotide antibodies and DNA antigen. DNA antibodies were highly enriched relative to serum levels in the majority of cryoprecipitates. DNA antigen was also demonstrable. Antibody to ribonucleo-protein, although less frequently present, was similarly enriched in certain cryoprecipitates. In contrast, anti-double strand RNA, which was commonly detectable in relatively high titer in serum, was only minimally concentrated in a minority of cryoprecipitates. Absorption experiments using red blood cells heavily coated with polynucleotide antigen indicated that a major proportion of the IgG in certain cryoprecipitates was specific antibody.

The data strongly suggest that the cryoprecipitates in systemic lupus erythematosus represent circulating immune complexes that are soluble at $37°C$ and come out of solution in the cold. The marked concentration of immune complexes in the cryoglobulin offers a simple and direct method for determination of the nature of the complexes. The accumulated evidence obtained in the present study indicates that these complexes closely reflect, in their composition, the circulating immune complexes which are most significant pathogenetically in renal tissue injury.

INTRODUCTION

Considerable evidence suggests that serum cryoprecipitation (cryoglobulinemia) may be a manifestation of immune complex formation (1). Apart from monoclonal gammopathies, cryoglobulins have been associated with persistent infections (2), hyperimmunization in animals (3, 4), and certain autoimmune diseases of man (5–9) and New Zealand mice (10, 11) in which circulating immune complexes are thought to have a pathogenetic role. In most of these clinical or experimental settings, intermittent antigenemia has been identified or suspected. IgM rheumatoid factor is often present and comprises varying proportions of the cryoprotein depending on the disorder involved (8, 9, 12–14). Cryoprecipitation also appears to involve fixation of C1q in certain sera of patients with systemic lupus erythematosus (SLE)[1] (12, 13).

The association of cryoprecipitation with hypocomplementemia and tissue injury in SLE is well known (7, 15, 13, 16). Several immune complex systems involving antipolynucleotide antibodies and certain nuclear antigens in serum have been related directly to nephritis in this disease (17–21). Although the presence of antigens and antibodies other than IgG and anti-IgG have been demonstrated in cryoprecipitates in SLE in certain cases, many aspects of their composition remain unclear (12–15, 22–25). To assess further the relationship between cryoprecipitation and the major im-

[1] Abbreviations used in this paper: ANA, antinuclear antibodies; dsRNA, double-strand RNA; ME, mercaptoethanol; nDNA, native DNA; PBS, phosphate-buffered saline; RBC, red blood cells; RNP, ribonucleoprotein; sDNA, single-strand DNA; SLE, systemic lupus erythematosus.

mune complex systems involved in tissue injury, cryo-precipitates from patients with SLE were analyzed for antibodies to polynucleotides as well as for DNA antigen by using sensitive assay techniques.

METHODS

Patients. 29 cryoprecipitates from 10 patients with active SLE were studied. Each patient met the preliminary criteria of the American Rheumatism Association for classification as SLE (26). Major system involvment included severe nephritis (three patients), severe skin disease (two patients), myositis (two patients), central nervous system disease (one patient). Hypocomplementemia was present in 27 of 29 serums.

Collection of serum and isolation of cryoprecipitates were performed as previously described (14). Serums were collected at 37°C. Cryoprecipitates were obtained by allowing 20 ml of serum to stand at 4°C for 1-7 days, followed by centrifugation at 1,000 *g* for 30 min in the cold. 1 ml of cold phosphate-buffered saline (PBS), pH 7.6 was layered over the cryoprecipitate allowing diffusion and dilution of the trapped serum. The saline was carefully drawn off after 30 min, and the cryoprecipitate was dispersed with a Pasteur pipette in 50 vol of distilled water and washed three times. This method minimized entrapment of serum proteins (estimated to be less than 3% of the protein in the cryoprecipitate) and reduced loss of cryoprotein during the washing steps. The cryoprecipitate was resolubilized in 1 ml of PBS by incubation with agitation for 1 h at 37°C or, for fractionation studies, in glycine acetate buffer, pH 3.5. Protein content of the solubilized cryoprecipitate was determined by the Folin-Ciacolteau method. Supernatant serums were inactivated by heating at 56°C for ½ h before use.

Fractionation of serum cryoprecipitates was performed by sucrose density gradient ultracentrifugation in glycine-acetate buffer, pH 3.5 (14). ^{125}I-IgM, which lacked antibody activity, was used as a 19S marker. IgM- and IgG-containing fractions were identified by quantitative radial immunodiffusion.

Quantitation of immunoglobulin in serum and cryoprecipitates. IgM and IgG in serums, in the whole resolubilized cryoprecipitates and in fractions obtained by sucrose gradient centrifugation, were quantitated by radial immunodiffusion in 1% agarose, Veronal buffer, pH 8.6, by using WHO standards (Meloy) and immunoglobulin class-specific rabbit antisera (27, 28). Determinations made at 25°C and 37°C were identical.

Anti-IgG antibody. Anti-IgG in the 19S fractions from acid density gradient centrifugation from serum and cryoprecipitates was determined by hemagglutination of chromic chloride-sensitized type O human erythrocytes coated with human IgG (Cohn fraction II) (29). The results are expressed as the log₂ of the reciprocal of the hemagglutination titer.

Antinuclear fluorescence. Antinuclear immunofluorescence was performed with serums and cryoprecipitates by using fluorescein isothiocyanate-conjugated rabbit anti-human immunoglobulin and frozen human liver cryostat sections.

Anti-DNA antibodies. Antibodies to single-strand DNA (sDNA) and native DNA (nDNA) were determined by hemagglutination of tanned, formalinized type O human erythrocytes coated with either native calf thymus DNA (Worthington Biochemical Corp., Freehold, N. J.) or heat-denatured DNA. The details of this method and the specificity of the reaction have been described in detail (19). Anti-DNA was also determined by a radioimmunoassay

(Farr) using [^{14}C]DNA from human KB cells [a] as previously described (30).

Antiribonucleoprotein (anti-RNP). A crude saline extract of calf thymus nuclei (31) was coated on tanned red cells for assay of anti-RNP antibodies.

Anti-double strand RNA (dsRNA). Poly A Poly U (Miles Laboratories, Inc., Elkhart, Ind.) was coated on tanned human erythrocytes and antibody was measured by hemagglutination (19).

Absorption studies. Certain 37°C resolubilized cryoprecipitates were absorbed twice at 25°C with equal volumes of tanned formalinized human type O erythrocytes heavily coated with sDNA, nDNA, or dsRNA. The amount of labeled antigen bound to 1 ml of packed red blood cells (RBC) was approx. 54 μg nDNA (incubation of 10 μg nDNA/ml 4% RBC for 60 min), approx. 105 μg nDNA (incubation of 50 μg nDNA/ml 4% RBC), and approx. 90 μg sDNA (incubation of 100 μg sDNA/ml of 4% RBC for 60 min).

The effect of absorption with antigen-coated RBC was determined by comparing residual immunoglobulin and antibody activity with levels in paired cryoprecipitates simultaneously absorbed with uncoated tanned cells.

Mercaptoethanol (ME) treatment was performed on selected serums and cryoprecipitates by treating serum with 0.2 M ME in 0.25 M Tris-HCl buffer pH 8.6 for 1 h at 37°C.

DNA quantitation. DNA in the cryoprecipitates was determined by the method of Burton (32). This method could detect 0.5 μg of DNA.

RESULTS

Antipolynucleotide antibodies in SLE cryoprecipitates. In preliminary experiments, cryoprecipitates from SLE patients were examined for antinuclear antibodies (ANA) by indirect immunofluorescence using cryostat-sectioned human liver as substrate. Six of nine cryoprecipitates were positive for ANA. In order to define and quantitate this apparent antibody activity, further experiments were performed with a more sensitive hemagglutination system detecting antibodies to individual polynucleotides.

An analysis of antibody activity by hemagglutination in three representative cryoprecipitates and serums from patients with SLE is summarized in Table I. Considerable variation in the types of antibodies present in the different cryoprecipitates, and their relative amounts, is evident. In certain cases, antibody was present in the cryoprecipitate even though undetectable in serum, e.g., anti-RNP in Ha cryoprecipitate. Enrichment of antipolynucleotide antibodies in the cryoprecipitates over serum levels was demonstrated. This enrichment was often marked, particularly for antibodies to DNA. Antibody to tetanus toxoid, an exogenous antigen, although present in certain cryoprecipitates (Es, De) was not enriched over the serum level. Anti-tetanus toxoid titers were higher in the serum; the low titers

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[a] Gift of Dr. Thomas Caused, National Institute of Dental Research, National Institutes of Health, Bethesda, Md.

TABLE I
Antipolynucleotide Antibody and Enrichment over Serum Levels in Cryoprecipitates from Patients with SLE

Patient	Immunoglobulin		Anti-sDNA	Anti-nDNA	Anti-dsRNA	Anti-RNP	Anti-tetanus toxoid
	IgG	IgM					
	mg/ml						
Es							
Serum	12.1	1.2	4*	3	4	3	9
Cryo	1.0	0.2	7 (96×)‡	7 (192×)	4 (12×)	0	4 (0.4×)
De							
Serum	14.0	4.0	0	3	3	0	7
Cryo	0.4	0.1	0	3 (36×)	0	0	2 (1.1×)
Ha							
Serum	16.0	2.8	7	2	7	0	
Cryo	0.5	0.2	8 (56×)	3 (56×)	6 (14×)	5 (450×)	

* Antibody hemagglutination titer is expressed as $1/\log_2$.

‡ Enrichment of antibody in the cryoprecipitate relative to the serum level. This was calculated as follows:

$$\frac{\text{serum IgM} + \text{IgG}}{\text{cryo IgM} + \text{IgG}} \times 2^{(\text{cryo titer}-\text{serum titer})}.$$

noted in cryoprecipitates are consistent with approximately 3% "contamination" of the cryoprecipitates with noncryoprecipitable serum immunoglobulin.

Table II summarizes the data obtained concerning antipolynucleotide antibodies in all cryoprecipitates examined. Most of the cryoprecipitates contained anti-sDNA and anti-nDNA. Only 8 of 21 had anti-dsRNA activity, although this antibody was most frequently present in serum (20 of 23). Anti-RNP was found least commonly in both serums (4 of 13) and cryoprecipitates (3 of 13).

Each antipolynucleotide antibody could be shown to be specifically concentrated over the serum level in individual cryoprecipitates. The degree of enrichment, however, was quite different for the various polynucleotide antibodies. The data for 10 patients are given in Fig. 1. Each point represents the maximal antibody enrichment observed in a cryoprecipitate from a single

patient. A negative value indicates that no cryoprecipitate contained detectable antibody. Antibody to sDNA and nDNA, and infrequently, RNP was highly enriched in the cryoprecipitates. In contrast, the specific concentration of anti-dsRNA was uniformly low. Mean enrichment for each antipolynucleotide antibody calculated from data from all cryoprecipitates studied is shown in Table II.

Certain cryoprecipitates and serums were also examined for anti-DNA antibodies by an ammonium sulfate precipitation radioimmunoassay using [^{14}C]DNA (Farr assay), a quantitative technique relatively less influenced by the immunoglobulin class of the antibody than hemagglutination. [^{14}C]DNA binding curves and hemagglutination titers for a representative serum and cryoprecipitate (from patient Es) are shown in Fig. 2. The enrichment of anti-DNA in Es cryoprecipitate, as determined by the ratio of serum and cryoprecipitate

TABLE II
Antipolynucleotide Antibodies in SLE Cryoprecipitates

	Anti-sDNA	Anti-nDNA	Anti-dsRNA	Anti-RNP
SLE (10 patients)*				
Number positive	21/29	16/28	8/21	3/13
Mean titer, *per mg Ig*‡	6.0±0.5	5.2±0.3	4.4±0.5	5.3±0.7
Mean enrichment over serum level§	132× ±52	90× ±32	13× ±2	189× ±134

* The numbers of cryoprecipitates studied per patient were seven (one patient), five (one patient), three (four patients), two (one patient), and one (three patients).

‡ Titer is expressed as $1/\log_2$; mean of positive values ±SEM.

§ Enrichment was calculated as:

$$\frac{\text{serum IgM} + \text{IgG}}{\text{cryo IgM} + \text{IgG}} \times 2^{(\text{cryo titer}-\text{serum titer})}.$$
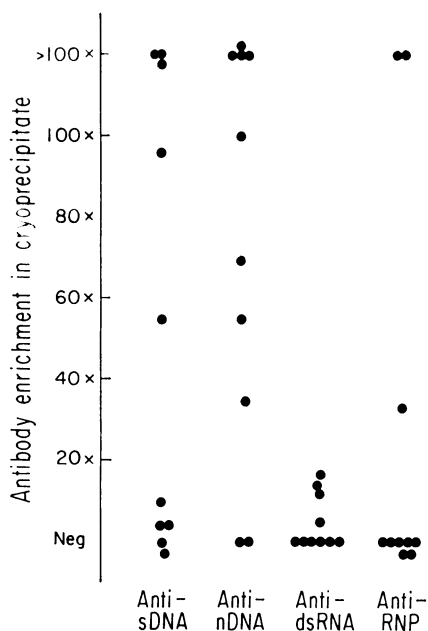
Values are means ±SEM for positive cryoprecipitates only.

Figure 1 Enrichment of antipolynucleotide antibodies in cryoprecipitates relative to serum levels. Each datum for each antipolynucleotide represents the maximum enrichment in cryoprecipitates from single, different patients. Enrichment was calculated from relative hemagglutination titers of serum and cryoprecipitates:

$$\frac{\text{serum IgM + IgG}}{\text{cryo IgM + IgG}} \times 2^{(\text{cryo titer—serum titer})}.$$

Titers in this calculation were expressed as $\log_2$ of the reciprocal.

immunoglobulins giving 30% antigen binding, was 28×. This value is in close agreement with enrichment determined by relative hemagglutination titers (40×). In general, a high correlation between anti-DNA en-
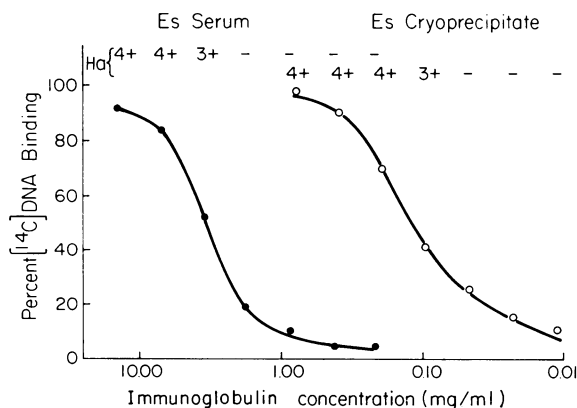

Figure 2 Comparison of anti-DNA quantitation by Farr assay and by hemagglutination in a representative cryoprecipitate. Serum (●); cryoprecipitate (○).

richment values determined by the two techniques was noted for other cryoprecipitates as well. The mean enrichment of anti-DNA for 10 SLE cryoprecipitates as determined by [^{14}C]DNA binding was 37× ±17 (±SEM) and by hemagglutination was 42× ±17.

It seemed possible that IgM antibody to nuclear antigens, or possibly IgM anti-IgG which is also usually present, might be responsible in part for the relatively high DNA hemagglutination titers observed with certain cryoprecipitates. To clarify this question eight cryoprecipitates from four patients were treated with ME, and the anti-DNA hemagglutination titers of specimens so treated were compared with the titers of untreated controls (incubated with buffer alone). ME did not significantly decrease the titer of five cryoprecipitates. A decrease in titer of three tubes was observed with three. Thus, for Ha cryoprecipitate, the original anti-sDNA hemagglutination titer of 6 was decreased to 3 after ME treatment. When Ha cryoprecipitate was fractionated by sucrose gradient centrifugation at pH 3.5, the peak IgM fraction was shown to contain both anti-IgG (titer/milligram IgM = 7) and anti-sDNA (titer/milligram IgM = 4, 30% [^{14}C]DNA binding capacity = 0.25 mg IgM). The peak IgG fraction did not contain detectable anti-sDNA by hemagglutination, although 20% [^{14}C]DNA binding was observed. 46 and 28% of Ha IgM were absorbed with insolubilized IgG and sDNA-coated RBC, respectively. IgM and IgG fractions from a cryoprecipitate from patient Es, which showed no reduction in the anti-DNA hemagglutination titer after ME treatment, were analyzed in similar fashion (Table III). The IgM fractions contained IgM anti-IgG but no anti-DNA. Anti-nDNA was detected only in the IgG fractions by both hemagglutination and [^{14}C]DNA binding.

In other experiments, 100 μg of monoclonal IgM anti-IgG (isolated from the cryoprecipitate of a patient

Table III

Anti-nDNA and Anti-IgG Antibodies in Es Cryoprecipitate Immunoglobulin Fractions Obtained by Sucrose Density Gradient Ultracentrifugation at pH 3.5

Gradient fraction	Immunoglobulin		Anti-nDNA		Anti-IgG titer
	IgM	IgG	Hemagglutination titer	Percent binding	
	mg/ml		*1/log₂*		*1/log₂*
1	0	0	0	6	0
2	0.25	0	0	3	4
3	0.30	0.03	0	0	6
4	0.05	0.11	0	46	2
5	0	0.89	4	78	0
6	0	0.30	2	59	0
7	0	0.03	0	7	0
8	0	0.02	0	0	0

TABLE IV

Absorption of Cryoprecipitates with nDNA-Coated Erythrocytes

Cryoprecipitates absorbed with:	SLE						Non-SLE					
	Es			De			M1			Do		
	IgG	IgM	Anti-nDNA*	IgG	IgM	Anti-nDNA	IgG	IgM	Anti-nDNA	IgG	IgM	Anti-nDNA
	mg/ml		$1/\log_2$	mg/ml		$1/\log_2$	mg/ml		$1/\log_2$	mg/ml		$1/\log_2$
Tanned RBC	0.31	0.04	6	0.26	0.07	5	0.06	0.03	<2	0.18	0.09	<2
nDNA-50 RBC‡	0.17	0.05	<2	0.17	0.07	2						
nDNA-100 RBC‡	0.17	0.05	<2	0.15	0.07	3	0.07	0.04	<2	0.19	0.09	<2

* Titer of anti-nDNA.

‡ Tanned, formalinized type O human RBC were incubated with nDNA, 50 or 100 µg/ml, 4% RBC for 60 min. The cells were washed and centrifuged. Absorptions were carried out twice with equal volumes of packed antigen-coated RBC and resolubilized cryoprecipitate at 25°C.

with mixed cryoglobulinemia) was added to SLE serums giving low anti-DNA hemagglutination titers. The anti-DNA titer of this mixture was then determined in parallel with that of the SLE serum alone at 25°C and at 4°C. Addition of the IgM anti-IgG did not enhance the hemagglutination titer at either temperature. Similarly, recombination of SLE cryoprecipitate IgM (containing anti-IgG activity) and IgG fractions isolated by sucrose density gradient centrifugation at pH 3.5 did not give hemagglutination titers greater than that observed with the 7S fractions alone.

The presence of IgG anti-DNA in several cryoprecipitates was demonstrated by absorption experiments using tanned human erythrocytes heavily coated with either sDNA or nDNA (Table IV). 45 and 42% of Es and De cryoprecipitate IgG, respectively, were removed by absorption with nDNA. sDNA-coated RBC absorbed a lesser amount of IgG (35 and 23%). IgM levels were not decreased by these absorptions. As controls, two cryoprecipitates from patients with diseases other than SLE and which had no anti-DNA by hemagglutination were similarly absorbed. Neither IgG nor IgM were decreased.

DNA in cryoprecipitates. DNA was determined directly by a diphenylamine assay in 17 cryoprecipitates from seven patients. DNA was detectable in all, ranging in amount from 2.2-109.8 µg/mg protein. No correlation between the concentration of DNA and the anti-DNA titers was apparent. DNA was also sought by hemagglutination inhibition. Although this indirect method will detect approximately 3 µg/ml of free DNA, antigen was not demonstrable in either whole cryoprecipitates or fractions obtained by sucrose density gradient ultracentrifugation at pH 3.5. In further experiments, four serums from patients with SLE which contained free DNA antigen by hemagglutination inhibition were fractioned under conditions identical to

those for the cryoprecipitates. In each case, DNA was localized exclusively to the 7S region. This finding suggests that the failure to demonstrate DNA by hemagglutination in the cryoprecipitate fractions was due to reformation of DNA-anti-DNA complexes after the pH of the fractions was restored to 7.2.

DISCUSSION

Specific concentration of antipolynucleotide antibodies in the cryoprecipitates from patients with SLE was demonstrated by hemagglutination techniques. Antibodies to sDNA and nDNA were present in highest incidence in the cryoprecipitates. Anti-dsRNA and anti-RNP were present in a minority of cryoprecipitates although anti-dsRNA was encountered most frequently in serum in the group of patients studied. Specific concentration of anti-sDNA and anti-nDNA and anti-RNP relative to serum levels was often high. Enrichment of anti-dsRNA was minimal. Antibody specific for an exogenous antigen, tetanus toxoid, was not concentrated in cryoprecipitates over the serum level and its presence in certain cryoprecipitates could be explained by minimal (approximately 3%) contamination with noncryoprecipitable serum immunoglobulins. This strongly suggests that the observed titers for the polynucleotide antibodies, which were relatively much higher in the cryoprecipitates than in the serums, were not due to noncryoprecipitable serum immunoglobulin contamination of the cryoprecipitates.

Several lines of evidence indicate that the antipolynucleotide antibodies determined by hemagglutination reflect closely the actual amount of antibody present in the cryoprecipitates. Considerable effort was made to examine the extent to which IgM antibody either specific for polynucleotides or for IgG might artifactually enhance the hemagglutination titers observed with the cryoprecipitates. Treatment of cryoprecipitates

with ME failed to reduce the original titer in the majority of cases. Addition of IgM with rheumatoid factor activity to either SLE serum or SLE cryoprecipitate 7S IgG did not increase the anti-DNA hemagglutination titer above that observed with serum or cryoprecipitate IgG alone, suggesting that the partial reduction in titer seen with several cryoprecipitates after MS treatment was largely due to the presence of IgM antipolynucleotide antibodies. There was good agreement generally between the anti-DNA activity determined by hemagglutination and by [^{14}C]DNA binding (Farr assay). The latter technique is relatively independent of the immunoglobulin class of antibody or the presence of IgM anti-IgG. Finally both fractionation and specific absorption experiments localized anti-DNA activity to the IgG component in most cases tested.

The data regarding the relative enrichment of the different antipolynucleotide antibodies in the cryoprecipitates bear a striking resemblance both quantitatively and qualitatively to those obtained by analysis of antibody activities in glomerular eluates from patients with SLE nephritis (20, 21). Glomerular eluates have been shown to contain anti-DNA most commonly, anti-RNP occasionally, but not anti-dsRNA. Enrichment of these antibodies in the eluates relative to the serum levels is roughly that observed for cryoprecipitates in the present study (33). The evidence is thus compelling that the cryoprecipitates represent, in terms of antibody, the same circulating immune complex systems shown previously to be of primary importance in tissue injury. Further preliminary data supporting this concept has been obtained in a collaborative investigation with Dr. Frank Dixon and co-workers (unpublished results), using the Raji cell system for estimation of circulating immune complexes (34). The level of such complexes was strongly correlated with hypocomplementemia and antipolynucleotide antibody activity in both sera and cryoprecipitates from three patients included in the present investigation.

Some confusion exists in the literature concerning the presence of DNA is cryoprecipitates from patients with SLE. Using the diphenylamine assay, Lee and Rivero (22) and Forsen and Barnett (23) demonstrated DNA in certain cryoprecipitates, findings in agreement with the present data. Limited studies by others were negative (12, 15). DNA was not found when indirect techniques depending upon detection of DNA antigenically were used. Failure to detect DNA in unfractionated cryoprecipitates by indirect antigen-antibody inhibition might be expected because antigenic sites on the DNA would be blocked by specific antibody in excess. Somewhat surprising was the inability to demonstrate DNA in individual fractions of cryoprecipitates obtained by

sucrose density gradient centrifugation at acid pH. A reasonable explanation derives from fractionation experiments of SLE sera having high levels of DNA antigen but undetectable antibody. The DNA was localized exclusively to the 7S region, strongly suggesting that even after fractionation of the cryoprecipitates, which contained IgG anti-DNA, DNA antigen was blocked by antibody in marked excess.

Previous investigation has shown IgM anti-IgG to be a significant component of the cryoprecipitates in SLE (12-14) and to be enriched relative to the serum level (13, 14). The present data concerning the presence of antipolynucleotide-polynucleotide complexes in cryoprecipitates suggest the strong possibility that, in serum, IgM rheumatoid factor interacts with such complexes, thereby promoting their precipitation in the cold. Indeed, DNA, anti-DNA, and IgM rheumatoid factor were all demonstrable in the cryoprecipitate from patient Es. The hypothesis has been advanced that such interaction of IgM rheumatoid factor with circulating complexes might potentiate their deposition and result in tissue injury (13). Certain evidence has been obtained in support of this hypothesis. With the idiotype as a marker of IgM anti-IgG, this antibody was localized directly to both immunoglobulin deposits in the glomerulus and to the cryoprecipitate of a patient with SLE nephritis (13).

Cryoprecipitates from patients with SLE have been shown to contain Clq as well as rheumatoid factor (12). This observation was extended by the demonstration of Clq precipitins in serum and the specific concentration of Clq in cryoprecipitates over serum levels (13). Cryoprecipitation was considerably diminished by inactivating Clq or by the reduction and alkylation of rheumatoid factor. More recently Clq was found in tissue sections from kidneys of certain patients with SLE and seemed to be associated with the presence of sDNA (33). It is conceivable that DNA or other polyanionic substances might interact directly with Clq. However, it appears more likely that antigen, specific antibody, and complement, as well as IgM rheumatoid factor, form the serum complexes having optimum potential for tissue injury. The present data would suggest that the constituents of certain cryoprecipitates in SLE might reflect this particular pathogenetic combination.

In disorders such as SLE the presence of a cryoglobulin not only represents direct evidence for the presence of circulating immune complexes but also offers a ready means of isolating and determining the type of complex involved. Numerous other methods for the determination of circulating immune complexes are currently becoming available. However, many problems remain with

these techniques and proof that they measure immune complexes in specific instances is required. Such proof for the cryoglobulins in SLE was obtained in the present study and the nature of at least some of the complexes determined.

ACKNOWLEDGMENTS

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