Immune Complex Reactions in Systemic Lupus Erythematosus* **

JOHN STAIGE DAVIS, IV, M.D.

Professor of Internal Medicine, Chief of the Division of Rheumatology, University of Virginia School of Medicine, Charlottesville

We should first try to define systemic lupus erythematosus (SLE). Table 1 lists criteria proposed by a committee of the American Rheumatism Association (1). If four of these 14 major criteria are positive, then there should be about 98% specificity for SLE. There are clearly many facets to this disorder. Let us consider mechanisms that might account for a multisystem disease such as this.

A good model is that of experimental serum sickness. Though Germuth (2) provided much of the renewed impetus to study serum sickness in the '50's, it is Dixon (3) who has fully explored the variables of immune complexes and disease.

When a rabbit is given a single intravenous injection of bovine serum albumin (BSA) labelled with ^{131}I, one notes disappearance of the BSA in serum over a period of about 13 days. First there is a rapid fall, then an equilibrium develops, and then suddenly there is total disappearance of the injected BSA. Coincident with the rapid disappearance of BSA, one can measure antigen-antibody complexes. Finally, free antibody to BSA is detected. During the period when these immune complexes are found, the rabbits develop heart disease, arthritis, nephritis, and other manifestations. It is a self-limited disease, however, and when free antibody is found the disease clears up. Hemolytic complement (C) has been measured also; with the appearance of immune complexes, the C level falls, later returning to normal. This then is the model of experimental serum sickness in the rabbit; what about immune complexes in man?

We are postulating that many manifestations of SLE probably result from deposition of circulating immune complexes. Numerous data are now available which would support this concept. What can we now do to find these complexes and characterize them? If we can show what is in these complexes, we should know something more about pathogenesis.

Table 2 lists some of the methods that are available today. These methods are mostly limited to research laboratories. I have listed these methods as either analytical or preparative. Cryoprecipitation is both analytical and preparative; furthermore, cryoprecipitates are easily measured since all one needs is a refrigerator and a centrifuge. Recent studies by McIntosh et al. (4) suggest that cryoprecipitates correlate well with immune complex formation in rabbits; evidence in man is not as convincing since it is difficult to recover a specific pathogenetic antigen in SLE cryoprecipitates.

C1q precipitation was first described by Agnello et al. (5); here one utilizes purified C1q to precipitate with serum factors in double diffusion systems. Rheumatoid factor (RF) can be used in the same way. Efforts in our lab are now being directed toward an analysis of the characteristics of C1q reactive factors. It is of interest that C1q and RF seem to detect different substances in serum. Passive agglutination of RF-coated cells is another method which detects complexes, as is complement fixation (which is a way of interpreting "anticomplementary serum"). Recent work in England has shown complement fixation and C1q precipitins not only in

* Presented by Dr. Davis at the 45th Annual McGuire Lecture Series, November 8, 1973, at the Medical College of Virginia, Richmond.

** Supported in part by the USPHS Grant No. 5R01-AM11766 and a grant from the John A. Hartford Foundation, Inc.

TABLE 1

PRELIMINARY CRITERIA FOR CLASSIFICATION OF SLE

1. Facial Erythema (Butterfly Rash)
2. Discoid Lupus
3. Raynaud's Phenomenon
4. Alopecia
5. Photosensitivity
6. Oral or Nasopharyngeal Ulceration
7. Arthritis without Deformity. [One or more peripheral joints involved with any of the following in the absence of deformity: a) Pain on motion, b) Tenderness, c) Effusion or periarticular soft tissue swelling.]
8. LE Cells
9. Chronic False-Positive STS
10. Profuse Proteinuria
11. Cellular Casts
12. One or both of the following: a) Pleuritis, b) Pericarditis
13. One or both of the following: a) Psychosis, b) Convulsions
14. One or more of the following: a) Hemolytic anemia, b) Leukopenia c) Thrombocytopenia

SLE serum but in sera from patients with dermatitis herpetiformis, ulcerative colitis, regional enteritis, and coeliac disease (6, 7). Lymphocyte inhibition is another technique recently reported: B cells, which kill sensitized tumor cells, are inhibited by serum containing aggregates or immune complexes (8). Platelets can also be agglutinated by complexes.

Cryoprecipitation is, at the same time, a preparative method. Likewise C1q can be linked to Sepharose® columns through cyanogen bromide to facilitate removal of C1q precipitins from serum for analysis (9). Hopefully, the immune complexes and/or aggregates will stick and everything else will wash through; then the bound material can be eluted off with salt and the residue analyzed. Serum can also be run directly through agarose columns with exclusion of large molecular weight materials. In summary, the main point here is that many methods are being developed to find and characterize aggregates and complexes; the simplest method is probably cryoprecipitation.

TABLE 2

DETECTION OF CIRCULATING IMMUNE COMPLEXES AND/OR AGGREGATES

Analytical	Preparative
Cryoprecipitation	Cryoprecipitation
C1q detection in agarose gel	C1q precipitation
RF detection in agarose gel or reverse hemagglutination	Adsorption to columns of insoluble C1q
Complement fixation	Isolation by column chromatography
Lymphocyte inhibition	
Platelet agglutination	

Figure 1 shows various factors which we measured longitudinally in one SLE patient several years ago. The relationship between serum cryoglobulins and C levels is particularly noteworthy (our normal range for serum hemolytic complement is 34-48 CH5O units/ml) and one can clearly see the reciprocal relationship between C and cryoglobulin during the time span. This negative correlation is not always seen but usually is.

Figure 2 shows findings in another patient. Here the C level is first found in the normal range at a time when no cryoglobulins are seen. As the cryoglobulins appear, C levels begin to fall. It should also be noted that the antinuclear factor (ANF) remained fairly constant during this time period, though no titers were done. It has been our experience that when we use a 1:4 dilution of serum for the ANF test, little change in ANF can be noted between acute exacerbations and remissions of SLE. With prolonged remission, however, the staining may become less intense, though the ANF rarely disappears entirely in SLE patients. RF titers (usually low titers, when found) also tend to remain quite constant in SLE patients, though RF was not found in either of the patients shown in these figures.

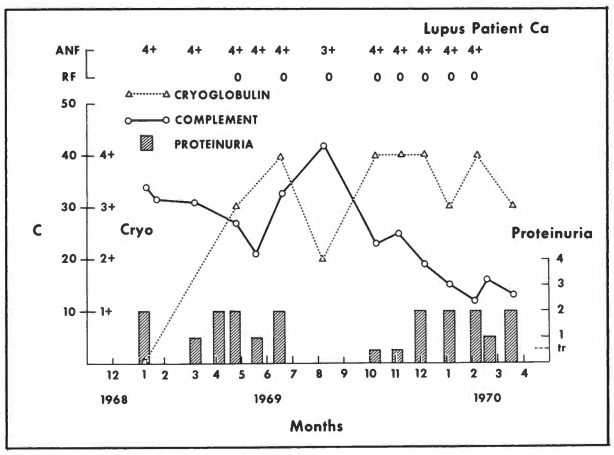

Fig. 1—Laboratory findings in a patient with SLE over a 16-month period. The reciprocal relationship between C and cryoglobulins is noteworthy. Antinuclear factor and proteinuria are relatively stable during this time.

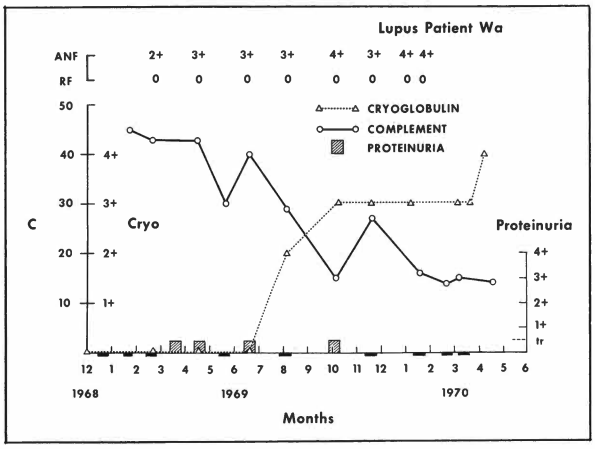

Fig. 2—Laboratory findings in another patient with SLE. Very little proteinuria is noted even when the C level is low and large amounts of cryoglobulin are found.

C1q precipitins have been found primarily in SLE patients with low serum C levels according to one report (10). In a group of 30 SLE patients with low C levels, 23 were positive for C1q precipitins; of those SLE patients who had normal C levels, none were positive for C1q precipitins. A few patients with miscellaneous diseases were positive (6/150).

Table 3 shows our results: Of 908 total sera tested for C1q precipitins, 19% were positive. (These sera came from patients who had a variety of diseases and were mainly suspected of having a connective tissue disease or vasculitis.) Of the lupus *sera,* 15% were positive and of the lupus *patients,* 43% were positive on at least one occasion. Sera from patients with other diseases were positive 27% of the time, the 28% positivity of nonlupus *patients* reflecting the fact that many of these patients were only studied by this technique on one occasion. Of particular note is the finding of C1q precipitins in other diseases besides SLE—a finding supported by other investigators (6, 7, 10).

TABLE 3

C1q PRECIPITATION

	Total	# Positive	% Positive
Total Sera	908	174	19
Lupus Sera	601	90	15
Lupus patients	95	41	43
Other Sera	307	84	27
Other patients	216	61	28

TABLE 4

C1q PRECIPITINS VS. CRYOGLOBULINS

	# Positive	% Positive
Total Sera (106)	16	15
Lupus Sera (74)	11	15
Other Sera (32)	5	16
Cryoglobulins (106)	0	0
Cryo. Supernatants (106)	24	23
Lupus (74)	18	24
Other Sera (32)	6	19

Results of the C1q precipitin test as it relates to sera with cryoglobulins are shown in Table 4. One notes that of the 160 sera tested here, 16 were positive for C1q precipitins; and of these 16 sera, 11 were SLE sera and 5 were other disease sera. Only some of these sera had cryoglobulins, but we treated them all as if they did—that is, they were all centrifuged after 48 hours in the cold and the supernatant transferred to another tube for testing against C1q. The cryoprecipitates, whether visible or not, were resolubilized at a ten-fold concentration and also retested against C1q. No cryoprecipitates showed a precipitin line, suggesting that 1) there was not enough material, 2) the material did not react with C1q, or 3) that the cryoprecipitate was already saturated with bound C1q. Of more importance, however, was the finding that of the 106 supernatants, 24 (instead of 16) were now positive. All of the 16 sera which were positive originally remained positive when the supernatant was checked and eight sera which were originally thought to be negative were now positive after removal of any cryoprecipitates. These studies have suggested to us that cryoglobulins have properties, at least in part, distinctive from C1q precipitins. Possibly the cooling and centrifugation of certain sera lead to aggregation of small complexes that do *not* spin down as cryoglobulins, but *do* subsequently react with C1q, even at room temperature!

For several years, we have been interested in how RF might modify IgG aggregates or immune complexes in human disease (11, 12). We have wondered whether RF might affect the deposition or metabolism of complexes by changing their size or their physicochemical properties in some other way. Small, soluble complexes might be made insoluble by RF; this might serve a protective function or a pathogenetic function depending on the circum-

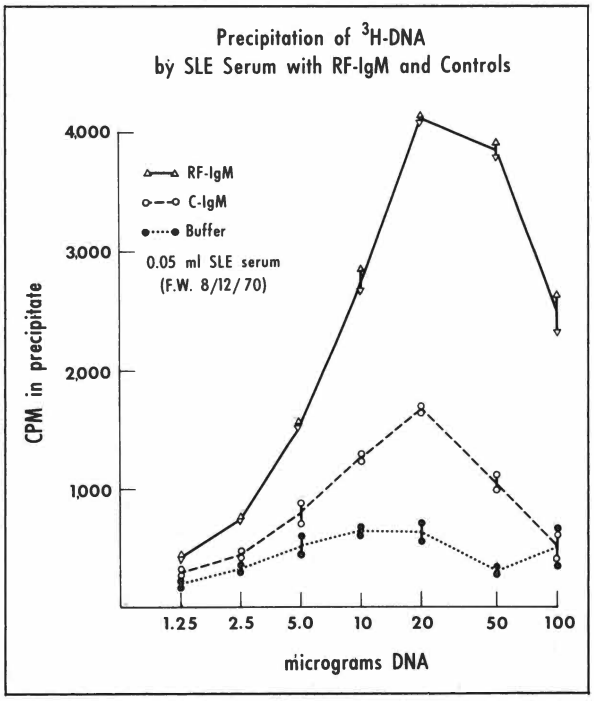

Fig. 3—Effect of IgM rheumatoid factor on DNA-anti-DNA complexes. Constant amounts of IgM [either control (C) or containing rheumatoid factor (RF)] are added to constant amounts of anti-DNA (SLE) serum and increasing amounts of DNA. Much more radioactive DNA is found in the precipitate when RF is added than in appropriate control systems.

stances (13). Figure 3 shows that RF has the capability of precipitating partially soluble DNA–anti-DNA complexes formed in vitro when one adds increasing amounts of labelled DNA to an SLE serum. It can be seen that much more precipitate is formed (based on counts of radioactive DNA in the precipitate) in the presence of RF than when the complexes are formed in the presence of control-IgM or buffer alone. We interpret these findings to mean that RF can interact with and precipitate DNA–anti-DNA complexes, thus converting these partially soluble complexes into insoluble complexes. As mentioned above, such a function for RF in vivo could be either protective or damaging.

Figure 4 shows effects of RF on cryoprecipitates. It would appear that RF has the potential for either increasing or decreasing cryoprecipitation, probably depending on characteristics of the cryoprecipitate. Conceivably one might be dealing with a system analagous to an antigen-antibody complex which would precipitate at equivalence but would resolubilize in both antigen and antibody excess.

Table 5 shows the sort of data being accumulated currently on patients seen at the University of Virginia Lupus Clinic. In the serum of this patient before treatment, a C1q precipitin was found, along with 3+ cryoglobulins, strong anti-DNA activity, a low C level, and strong ANF staining. After institution of steroid treatment, the C1q precipitin disappeared, but then we found material in the serum reacting with RF! (This often-seen reciprocal relation is of great interest to us, but we don't understand it.) Cryoglobulins disappeared (as did the anti-DNA antibodies) and at the same time the serum C level returned to normal. Since starting treatment, the patient has done well, and we have continued to be on the lookout for the reappearance of those factors associated with disease activity as we slowly lower the steroid dose.

Table 6 is an analysis of serum material bound to C1q on a cyanogen bromide Sepharose® column.

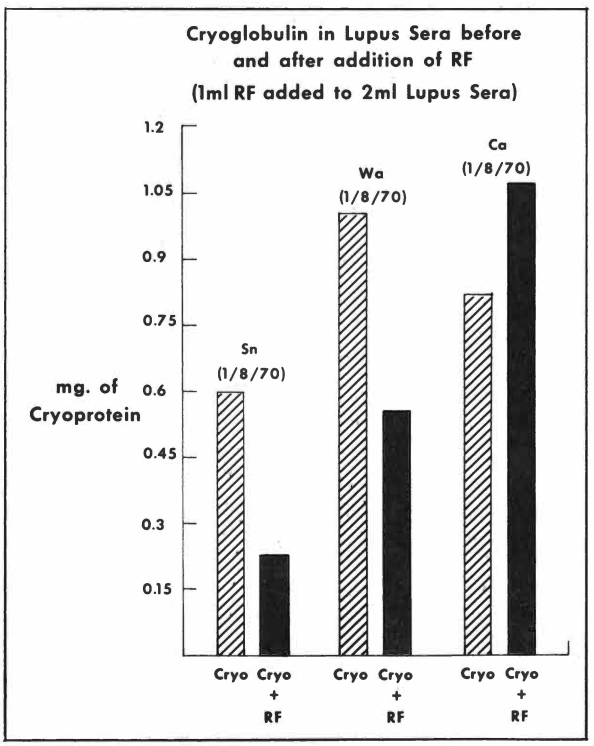

Fig. 4—Effect of IgM rheumatoid factor on three SLE sera containing cryoglobulins. After addition of RF to fresh sera Sn and Wa, less cryoglobulin was detected after 48 hours at 4°C; when RF was added to serum Ca, however, more cryoprotein was detected.

TABLE 5

S. P. (SLE)

	Complexes					
	C1q	RF	Cryo	Anti-DNA	ANF	CH50
4/6/72	+	−	3+	3+	4+	10
Treatment Started						
4/11/72	−	+	ND*	3+	ND	13
4/13/72	−	+	ND	2–3+	ND	ND
4/17/72	−	−	ND	2–3+	ND	ND
4/21/72	−	−	1–2+	2+	1–2+	ND
5/2/72	−	−	2+	−	ND	40
6/13/72	−	−	−	−	3+	53
7/25/72	−	−	−	−	3–4+	48
11/21/72	−	−	−	−	3–4+	45

* ND = Not Done

One notes the presence in the two eluates of IgG and IgM but no anti-DNA or DNA. Currently, we are analyzing these eluates by a variety of techniques including animal immunizations (to elicit antibodies to any infectious agents which might be there). Characteristics of C1q precipitins are also being compared with characteristics of cryoglobulins. It should be noted at this point that normal serum also contains significant amounts of material which bind to the C1q adsorbent.

In summary, we can draw very few firm conclusions about immune complexes in patients with SLE. SLE patients during episodes of disease activity often show both cryoglobulins and C1q precipitins in their sera, but in our experience, the sera may show only one of the factors and occasionally neither one is found. When cryoglobulins are removed from sera, C1q precipitins not only remain in the supernatant but occasionally may be found in the supernatant for the first time. This finding suggests that cryoglobulins and C1q precipitins are at least in part distinct. RF may interact with DNA–anti-DNA complexes and cryoprecipitates at least in vitro. Studies are in progress to further characterize the material found in these complexes. Hopefully, a particular antigen or a particular characteristic of SLE complexes will be found which will lead to a better understanding of the primary cause and intermediate mechanisms involved in the serious disease known as SLE.

TABLE 6

ANALYSIS OF ELUATES BOUND TO INSOLUBLE C1Q

	E.F. (SLE)*	J.D. (N)**
IgG (mg)	3.12	0.73
IgM (mg)	0.49	0.31
IgA (mg)	0	0
C1q	+	Trace
DNA	−	−
Anti-DNA	−	−
RF	1:8	1:8
Pptns. vs C1q	+	+

* SLE = Lupus serum eluate
** N = Normal serum eluate

REFERENCES

1. ARA COMMITTEE OF DIAGNOSTIC AND THERAPEUTIC CRITERIA: Preliminary criteria for the classification of systemic lupus erythematosus. *Bull Rheum Dis* 21:643, 1971.

2. GERMUTH FG JR: A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type. *J Exp Med* 97:257, 1953.

3. DIXON FJ: The role of antigen-antibody complexes in diseases. *The Harvey Lectures,* Series 58:21, 1962-63.

4. MCINTOSH RM, GROSS M, LAPLANTE M, KAUFMAN DB, KULVINSKAS C: Cryoproteins in immune complex disease: Role of nephrectomy and implications in recurrent glomerulonephritis in human transplants. *Exp Med Surg* 29:108, 1971.

5. AGNELLO V, WINCHESTER RF, KUNKEL HG: Precipitin reactions of the C1q component of complement with aggregated γ-globulin and immune complexes in gel diffusion. *Immunology* 19:909, 1970.

6. MOWBRAY JF, HOLBOROW EJ, HOFFBRAND AV, SEAH PP, FRY L: Circulating immune complexes in dermatitis herpetiformis. *Lancet* 1:400, 1973.

7. DOE WF, BOOTH CC, BROWN DL: Evidence for complement-binding immune complexes in adult coeliac disease, Crohn's disease, and ulcerative colitis. *Lancet* 1:402, 1973.

8. JEWELL DP, MACLENNAN ICM: Circulating immune complexes in inflammatory bowel disease. *Clin Exp Immunol* 14:219, 1973.

9. FATHMAN CG: Characterization of circulating antigen-antibody complexes in patients with systemic lupus erythematosus. *Clin Res* 21:577, 1973 (abstract).

10. AGNELLO V, KOFFLER D, EISENBERG JW, WINCHESTER RJ, KUNKEL HG: C1q precipitins in the sera of patients with systemic lupus erythematosus and other hypocomplementemic states: Characterization of high and low molecular weight types. *J Exp Med* 134:2285, 1971.

11. DAVIS JS, TORRIGIANI G: Effects of rheumatoid factor on *in vivo* distribution of aggregated human IgG and antigen-antibody complexes. *Proc Soc Exp Biol Med* 125:772, 1967.

12. GOUGH WW, DAVIS JS: Effects of rheumatoid factor on complement levels *in vivo*. *Arth Rheum* 9:555, 1966.

13. DAVIS JS: A hypothetical common mechanism in systemic lupus erythematosus and rheumatoid arthritis. *Arth Rheum* 9:631, 1966.