Slowly Sedimenting Mercaptoethanol-resistant Antinuclear Factors Related Antigenically to M Immunoglobulins (γ_{1M}-Globulin) in Patients with Systemic Lupus Erythematosus *

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The human immunoglobulins currently recognized can be divided into three major classes: the 7 S γ_2-globulins (G immunoglobulins, IgG); the γ_{1M}-globulins (M immunoglobulins, IgM); and the γ_{1A}-globulins (A immunoglobulins, IgA)[1] (2). These proteins can be readily distinguished from each other with specific antisera directed against the antigenic determinants associated with the heavy chains characteristic of each class. In addition, M immunoglobulins have a sedimentation coefficient of 19 S (mol wt of about 1,000,000), and their antibody activity is destroyed by treatment with 2-mercaptoethanol or other thiol compounds, whereas G immunoglobulins have a sedimentation coefficient of 7 S (mol wt of about 160,000), and their antibody activity is not significantly affected by treatment with thiol compounds. Most A immunoglobulins sediment with the IgG fraction, although some have sedimentation coefficients ranging from 10 to 15 S, and their antibody activity is frequently destroyed by treatment with thiol compounds (3–5). Antibody activity has recently also been reported to be associated with certain low molecular weight urine γ-globulins (6) and with proteins with intermediate sedimentation coefficients whose precise nature remains to be determined (7, 8).

In patients with systemic lupus erythematosus (SLE), antibodies directed against nuclei have been described by many authors (9–12). Although these antinuclear factors (ANF) are usually G immunoglobulins, both M immunoglobulin (13–15) and A immunoglobulin ANF's have also been identified (15).

The present report describes an additional type of antinuclear globulin that cannot be classified as belonging to any one of the previously mentioned classes of immunoglobulins. It is a protein antigenically related to the IgM fraction. However, it resembles the IgG and IgA fractions in size as shown by density gradient centrifugation and Sephadex gel filtration. In addition, it differs from the usual IgM in not being inactivated by mercaptoethanol. This type of antibody has been found in the serum of eight of 53 patients with SLE.

Methods

Sera. Fresh and stored sera were studied from patients with typical systemic lupus erythematosus seen by the authors in the New York University Arthritis Clinic or the New York University Division of Bellevue or University Hospital. All patients had multiple system disease typical of SLE (16) and positive LE cell preparations at some time during the course of their disease.

Fluorescent antisera. Rabbits were immunized for prolonged periods of time with the following antigens in

Freund's adjuvant: *1)* rabbit no. 157, the fast fragment of IgG isolated from the urine of a patient with heavy chain disease (17), which carries the determinants specific for the major class of heavy chains (γ-chains) characteristic of IgG; *2)* rabbit no. 131, a pool of normal IgA prepared by the procedure of Heremans and colleagues (18); and *3)* IgM isolated from normal plasma, as described previously (19). Antisera to IgM produced in two rabbits (no. 133 and 134) were used, but the bulk of the studies was made with no. 133. Similar results, however, were obtained with no. 134 on several randomly selected fractions. The antibody-containing fractions of the rabbit antisera were precipitated with 40% $(NH_4)_2SO_4$, dissolved in 0.9% saline, and dialyzed against saline to remove the ammonium sulfate. The protein was diluted to a protein concentration of 20 mg per ml. Sixteen mg of fluorescein isothiocyanate in Celite [2] was added for each gram of protein (20). The mixture was stirred for 10 minutes and then centrifuged in a Spinco model L centrifuge at 10,000 rpm for 1 hour in a no. 40 rotor, and the precipitate was discarded. The conjugated antiserum was dialyzed against 0.0175 M phosphate buffer of pH 6.4 and applied to a column of DEAE cellulose, type 20,[3] which had been equilibrated with the same buffer. The protein was eluted with the same buffer made 0.1 M with sodium chloride and the absorption determined at 490 mμ for fluorescein and 280 mμ for protein. The ratio of fluorescein to protein ranged from 1:3 to 1:1. More heavily conjugated material remaining on the column required higher concentrations of salt for elution (21, 22). Only material coming off the column with the starting buffer was used in these studies. The conjugated antisera were then absorbed as follows to make them specific for only one class of immunoglobulin: *1)* the anti-IgM serum was mixed with serum from human umbilical cord blood added in small increments until it gave only a single line on Ouchterlony analysis and on immunoelectrophoresis with whole normal human serum and a single line with a macroglobulin from a pathologic serum (19); *2)* the anti-IgA serum was similarly absorbed with serum from human umbilical cord until it gave only a single line on agar diffusion and immunoelectrophoresis with whole normal serum and a single line with an A myeloma protein (23); *3)* the antiserum to the fast fragment from the subject with heavy chain disease reacted only with IgG and transferrin, but not with IgA, IgM globulins, or light polypeptide chains. It was used either as such or was absorbed with purified transferrin until it gave only a single precipitin line.

Fluorescent antibody test. Snap frozen sections of mouse liver were cut at 4 μ on a cryostat. Slides were kept frozen for up to 3 weeks, but were usually used within 1 week of sectioning. Slides were fixed in acetone for 10 minutes and then washed with agitation in 0.02 M phosphate buffer of pH 7.05 for 2 minutes. After blotting around the tissue section, a drop of the ma-

terial to be tested was placed on the tissue and allowed to stand at room temperature for 30 minutes in a moist chamber. The slide was washed with agitation in the phosphate buffer with two changes of the buffer at 5-minute intervals. After blotting around the tissue, a drop of the fluoresceinated antiserum was placed on the tissue and allowed to stand at room temperature for 30 minutes in a moist chamber. After washing as before with buffer, the slides were mounted in pH 7 buffered glycerol and observed with an ultraviolet light source using a Leitz Orthlux microscope with a Leitz 200-watt mercury arc illuminator, UG 1 (2-mm thickness) excitor filter, and a Leitz UV absorbing barrier filter. In the absence of sufficient material for more precise quantitation by serial dilution studies, the degree of nuclear fluorescence was roughly graded from 0 to 6 +.

To avoid bias, slides prepared using material collected by density gradient centrifugation were read with no knowledge of the name of the patient or of the specific conjugated antiserum used to prepare the slides. The fractions obtained from the density gradient were read in order of collection. Fractions obtained by Sephadex gel filtration, as well as the native sera and sera treated with 2-mercaptoethanol, were read without knowledge of the patient, the number of the fraction, or the type of specific antiserum used.

The specificity of the nuclear staining produced with the conjugated antisera was established by blocking and cross-blocking experiments with unconjugated antisera specific for each class of immunoglobulin. A serum sample that showed nuclear fluorescence with all three specific conjugated antisera was used. To determine blocking by the unconjugated antisera, a drop of one of the unconjugated antisera was applied to the tissue for 30 minutes and then washed off before the addition of the conjugated antiserum. In each instance, complete blocking of fluorescence was achieved only with the corresponding antiserum. In addition, specificity of the conjugated antisera to IgM globulins was confirmed by complete removal of nuclear fluorescence following absorption with purified macroglobulins isolated from the sera of two patients with macroglobulinemia of Waldenström. These preparations gave only a single line on immunoelectrophoresis with an antiserum to normal human serum.

A similar blocking experiment with identical results was performed on each of the Sephadex fractions from two SLE sera, one with a typical IgM antinuclear factor and one with the unusual type.

Sucrose density gradient ultracentrifugation was carried out as described by Edelman, Kunkel, and Franklin (24), using a Spinco model L ultracentrifuge with a swinging bucket rotor SW 39 and a gradient of 12% to 37.5% sucrose. Centrifugation was allowed to proceed at 32,000 rpm for 18 hours at 4° C. The fractions were collected in a series of tubes through a small needle puncture in the bottom of the tube. Protein concentration was determined by the modified Folin technique (25). The fractions were dialyzed against 0.9% saline at 4° C for 18 hours before use.

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[2] General Biochemicals, Chagrin Falls, Ohio.
[3] C. Schleicher & Schuell Co., Keene, N. H.

TABLE I

Class of ANF in 53 patients with SLE*

Class of ANF	Patients	
	no.	%
IgG	50	94
IgM	44	83
IgA	29	55
IgG + IgM + IgA	29	55
IgG + IgM	12	23
Only IgG	9	17
Only IgM	3	6
Only IgA	0	0

* ANF = antinuclear factor; SLE = systemic lupus erythematosus.

Sephadex gel filtration, using Sephadex G-200,[4] was carried out on a 2.7 by 91.5-cm column. One-half to 1 ml of serum was applied and the column washed with 0.5 M saline. The eluate was collected in 6-ml samples (8). After the protein concentration had been estimated by measuring the absorbancy at 280 mμ, the tubes were pooled into four major fractions. The first consisted of the peak known to contain the IgM, the 19 S α_2-globulin fractions, and the lipoproteins. The second fraction consisted of the tubes containing material eluted between the first and second peaks. The third fraction encompassed the second peak that contained the IgG, and the fourth fraction was the albumin peak (8, 26). The pooled fractions were concentrated to a final volume of 1 to 3 ml. The concentrated fractions were dialyzed against 0.9% saline overnight and protein concentrations determined by the Folin technique.

Mercaptoethanol treatment was performed by allowing a 1:4 dilution of serum or the appropriate fractions

[4] Pharmacia, Uppsala, Sweden.

made 0.1 M with respect to 2-mercaptoethanol to stand at room temperature for 2 hours and then overnight at 4° C. Control samples of serum were similarly treated with phosphate buffer instead of 2-mercaptoethanol (27).

Latex fixation tests were carried out by the method of Singer and Plotz (28).

Immunoelectrophoresis and agar diffusion were performed as described previously (29).

Results

1) Class of antinuclear factor (ANF) in sera from SLE patients. Sera from 53 patients with SLE were tested with the three specific fluorescein conjugated antisera (Table I). The serum from 94% of the patients contained ANF that reacted with conjugated anti-IgG serum, 83% reacted with anti-IgM serum, and 55% reacted with anti-IgA serum. Staining with two or three antisera was present in 41 sera or 78% of the total tested. In view of the nature of the assay employed, the term "IgM ANF" will be used to describe antibodies detected with specific anti-IgM antisera regardless of their size. The molecular properties will be further defined by the results of ultracentrifugal studies and Sephadex gel filtration. "Typical" IgM antibodies sediment with the 19 S fraction and are inactivated by mercaptoethanol, whereas the "unusual" IgM antibodies sediment more slowly and are resistant to sulfhydryl reagents.

2) Effect of 2-mercaptoethanol. Since mercaptoethanol is known to inactivate IgM antibody ac-

TABLE II

Behavior of ANF reacting with antisera to IgM (IgM ANF) in density gradient centrifugation and on Sephadex gel filtration

Serum		Sucrose density gradient		Sephadex fractions		
		IgM ANF Tube no.†	Rheumatoid factor Tube no.	IgM ANF Fraction	Rheumatoid factor Fraction	IgM* Fraction
Mercaptoethanol-sensitive IgM ANF	Hu	1–3	0	I	0	I
	Se	1–4	1–5	I, II	I, II	I
	Ma	1–4	0	I, II	0	I
Mercaptoethanol-resistant IgM ANF	Br	7–10	1–5	III	I, II	I, II, III
	Ga	4–8	0	II	0	I
	Pe	6–9	0	II	0	I, II
	Ru	6–10	0	II	0	I, II, III
	To	1–9	0	III	0	I, II, III
	Ro	1–8	0	I, II, III	0	II
	Mu	1–8	1–5	I, II, III	I, II	I, II, III
	An	1–7	0	Not done	Not done	Not done

* Ouchterlony analysis.
† Numbered consecutively from bottom to top.

tivity (27), its effect on ANF that reacted with anti-IgM serum was studied. Mercaptoethanol, when added to whole serum, completely abolished or significantly reduced the nuclear staining in sera from 36 of the 44 patients. However, in eight sera (18% of the sera reacting with this antiserum) mercaptoethanol had little or no effect on the intensity of nuclear staining or the maximal dilution necessary for fluorescence. Serial dilutions of the eight sera and of ten mercaptoethanol-sensitive sera revealed no difference in titer of IgM ANF in the two groups (range,

FIG. 2. DENSITY GRADIENT ULTRACENTRIFUGATION OF SERUM SE TO ILLUSTRATE THE SIMILARITY IN SEDIMENTATION OF RHEUMATOID FACTOR AND "TYPICAL" IgM ANF IN THE SAME SERUM.

density gradient centrifugation. In each experiment, a serum containing the typical rapidly sedimenting IgM ANF was centrifuged at the same time as mercaptoethanol-resistant sera. In a number of instances, rheumatoid factor was present in the sera. This invariably behaved as a 19 S protein and was, therefore, available as an additional marker for the location of the 19 S fraction. Most of the sera studied also contained IgG and IgA ANF.

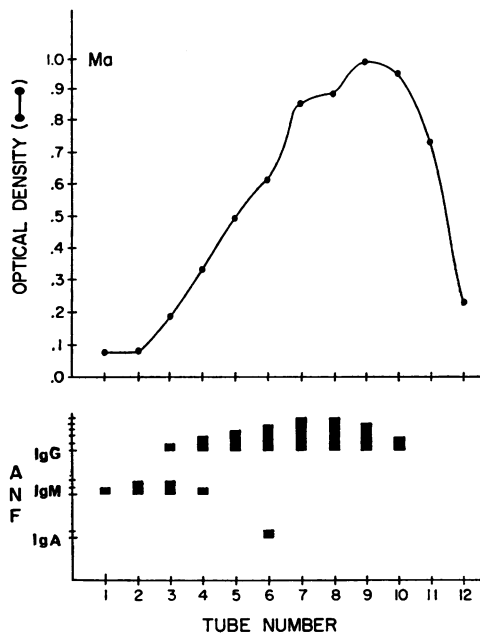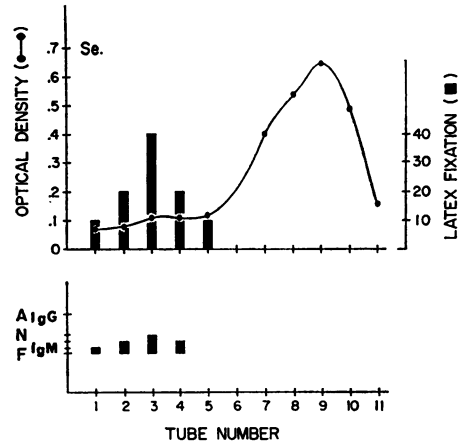As expected, mercaptoethanol-sensitive ANF,

FIG. 1. DENSITY GRADIENT ULTRACENTRIFUGATION OF SERUM MA CONTAINING A "TYPICAL" IgM ANF THAT SEDIMENTS IN TUBES 1 TO 4, WHILE IgG AND IgA ANF ACTIVITY SEDIMENTS MORE SLOWLY. Degree of positivity (1 to 5+) of ANF is indicated by height of vertical bar in all figures. OD reflects protein concentration.

1 : 16 to 1 : 128 in both groups). The studies with mercaptoethanol were repeated several times on each sample with identical results. Since this finding was contrary to expectation, attempts were made to characterize the antibodies further by density gradient ultracentrifugation and Sephadex fractionation.

3) Density gradient ultracentrifugation (Table II). Each of the sera containing the mercaptoethanol-resistant ANF that reacted with anti-IgM serum and six sera that showed mercaptoethanol-sensitive ANF were studied further by sucrose
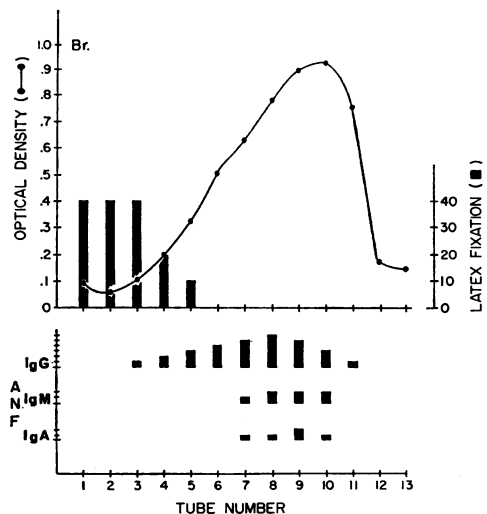

FIG. 3. DENSITY GRADIENT CENTRIFUGATION OF SERUM BR SHOWING "UNUSUAL" IgM ANF IN TOP FRACTIONS OF GRADIENT, WHICH ALSO HAVE IgA AND IgG ANF ACTIVITY, WHILE RHEUMATOID FACTOR SEDIMENTS MORE RAPIDLY.
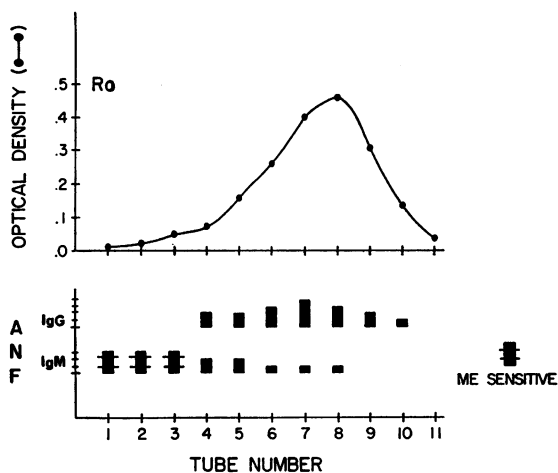
FIG. 4. DENSITY GRADIENT CENTRIFUGATION OF SERUM Ro HAVING IgM ANF IN TUBES 1 TO 8. Only the rapidly sedimenting antibody was inactivated by mercaptoethanol, while the "unusual" IgM ANF was not affected by thiol reagents.

in the top region and those reacting with anti-IgA serum were found in the mid to top portion (Figure 1). In serum Se (Figure 2), rheumatoid factor activity was present in the same portion of the gradient as the IgM ANF. In this serum mercaptoethanol completely abolished both rheumatoid factor activity and IgM ANF activity.

In contrast, in each of the sera with mercaptoethanol-resistant IgM ANF, ANF that reacted with anti-IgM serum was present in the more slowly sedimenting fractions, which are free of M immunoglobulins and contain IgA and IgG proteins. In serum Br (Figure 3), this activity was found only in the region of the gradient where most of the IgG ANF was present (tubes 7 to 10), and none was present in the rapidly sedimenting fraction (tubes 1 to 5). In this serum, rheumatoid factor activity, which was completely inactivated by 2-mercaptoethanol, was present only in the bottom region, thereby definitely establishing the location of the 19 S globulins. In sera from three other patients with mercaptoethanol-resistant ANF, which reacted with anti-IgM se-

which reacted with anti-IgM serum, was found only in the bottom region of the gradient, while the ANF's, reacting with anti-IgG serum were
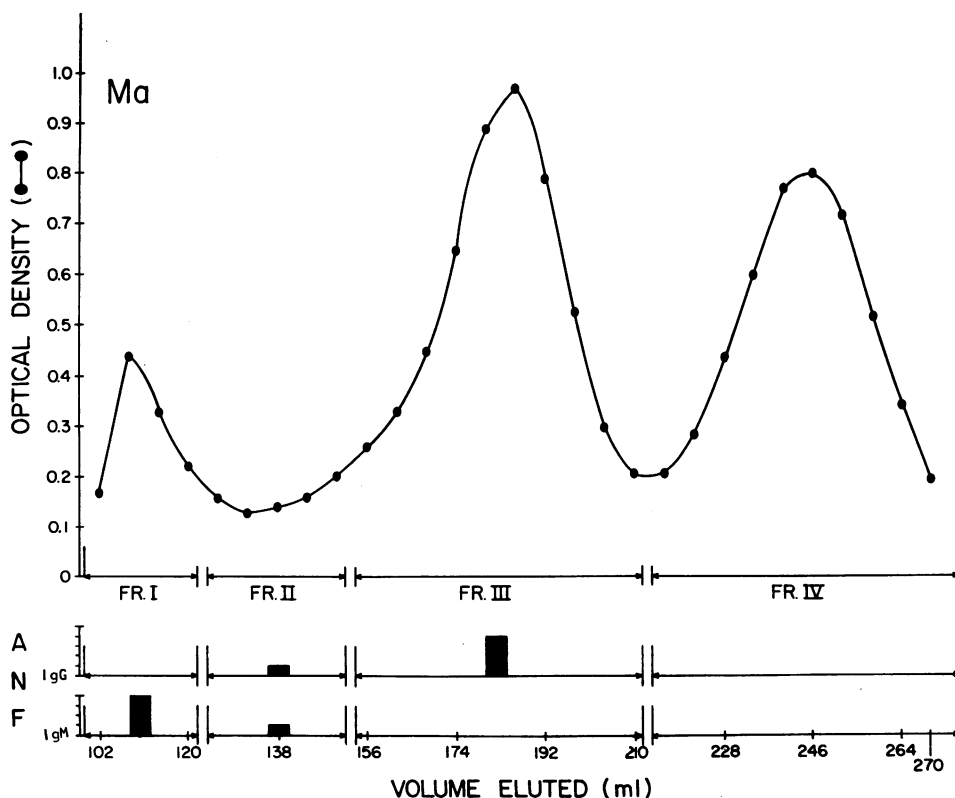

FIG. 5. SEPHADEX G-200 GEL FILTRATION OF SERUM Ma HAVING "TYPICAL" IgM ANF PRIMARILY IN FRACTION I, WHILE IgG ANF IS FOUND PRIMARILY IN FRACTION III.
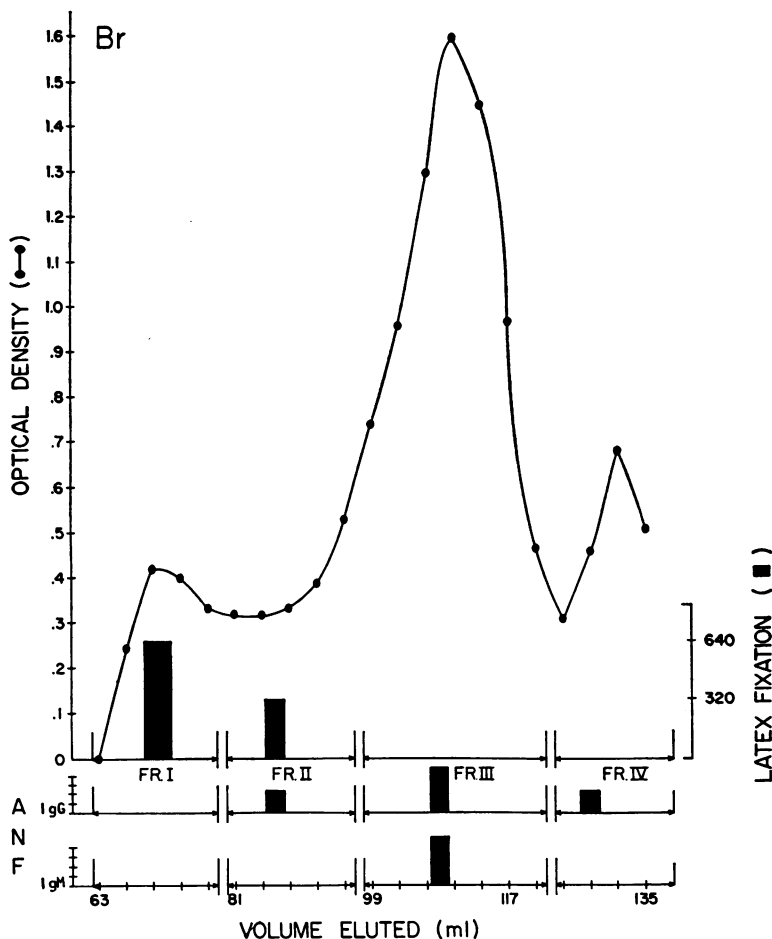
FIG. 6. SEPHADEX G-200 FILTRATION OF SERUM BR SHOWING RHEUMATOID FACTOR ACTIVITY IN FRACTIONS I AND II, WHILE THE UNUSUAL IgM ANF RESIDES IN FRACTION III, WHICH ALSO CONTAINS THE BULK OF IgG ANF.

rum, this ANF activity was exclusively located in the same region as the IgG ANF (Table II). In these sera, no ANF was present in the bottom fractions known to contain IgM globulins.

Density gradient centrifugation of three other sera with mercaptoethanol-resistant ANF, which reacted with anti-IgM serum, revealed the activity both in the bottom and middle regions of the gradient. In serum Ro (Figure 4), IgM activity was present in the bottom of the gradient (tubes 1 to 3) and also in the region of the IgG ANF (tubes 4 to 8). In serum Mu, IgM ANF was present in both the bottom and middle regions of the gradient, whereas rheumatoid factor activity, which was abolished by mercaptoethanol, was again present only in the bottom tubes (tubes 1 to 5). Mercaptoethanol treatment of fractions obtained from the sucrose density gradient of sera

Mu and Ro completely abolished nuclear fluorescence using conjugated anti-IgM serum in tubes 1 to 3, but did not abolish nuclear fluorescence in tubes 4 to 8, indicating the presence of two types of ANF with IgM antigenic determinants.

Both 19 S (rheumatoid factor) and 7 S (IgG ANF) globulins were localized in their usual position in all of these gradients (Table II). Experiments were repeated several times with most of the sera with similar results each time.

4) Sephadex gel filtration. This is an alternative technique to characterize the size of the serum proteins that takes advantage of the molecular sieving effect of Sephadex G-200. To check the results of density gradient centrifugation, gel filtration in Sephadex G-200 was performed on three sera containing IgM ANF, which sedimented as 19 S globulins and were mercaptoethanol sen-

sitive, and in seven of the eight sera with slowly sedimenting IgM ANF (Table II). In the three mercaptoethanol-sensitive sera, IgM ANF activity was found only in the first peak (Fraction I), and the IgG ANF activity was located in the second peak (Fraction III). Small amounts of IgM and IgG ANF activity were also found in the intermediate fraction (Fraction II) in serum Ma (Figure 5). Agar double diffusion studies (Table II) confirmed the presence of IgM globulin only in the first peak and its absence from the second peak (Fractions II and III). Rheumatoid factor activity in serum Se (Table II) was present in the first peak in a titer of 1 : 160 and in the intermediate fraction (Fraction II) in a titer of 1 : 20, whereas no rheumatoid factor activity was present in the second peak (Fraction III). In this serum, IgM ANF was present in the same fractions as rheumatoid factor, and both activities were completely abolished by treatment with 2-mercaptoethanol.

The results obtained by Sephadex gel filtration of seven sera containing mercaptoethanol-resistant ANF that reacted with anti-IgM serum were quite different (Table II). In the four sera where sucrose density centrifugation showed only slowly sedimenting ANF that reacted with anti-

IgM serum, no IgM ANF activity was found in the first peak. In serum Br (Figure 6), all IgM ANF activity was located in the second peak (Fraction III) where IgG ANF activity was also present, while the mercaptoethanol-sensitive rheumatoid factor activity was present only in Fractions I and II. In the other three sera having only slowly sedimenting IgM antibodies (sera Ga, Pe, and Ru), ANF activity, which reacted with anti-IgM serum, was not found in the first peak, but was present instead in Fraction II from the valley between the two peaks. This indicates the absence of 19 S ANF and the presence instead of smaller molecules possessing an antigenic determinant of the IgM type and confirms the results obtained by sucrose density gradient centrifugation. In serum To, which contained ANF that reacted with anti-IgM serum both in the rapidly and slowly sedimenting regions of the sucrose gradient, this activity was present only in the second peak eluted from the Sephadex. In sera Ro and Mu (Figure 7), which had IgM antibody activity in the rapidly and slowly sedimenting fractions on sucrose density gradient ultracentrifugation, ANF that reacted with anti-IgM serum was present in the first and second peaks, indicating the presence of IgM antigenic determinants
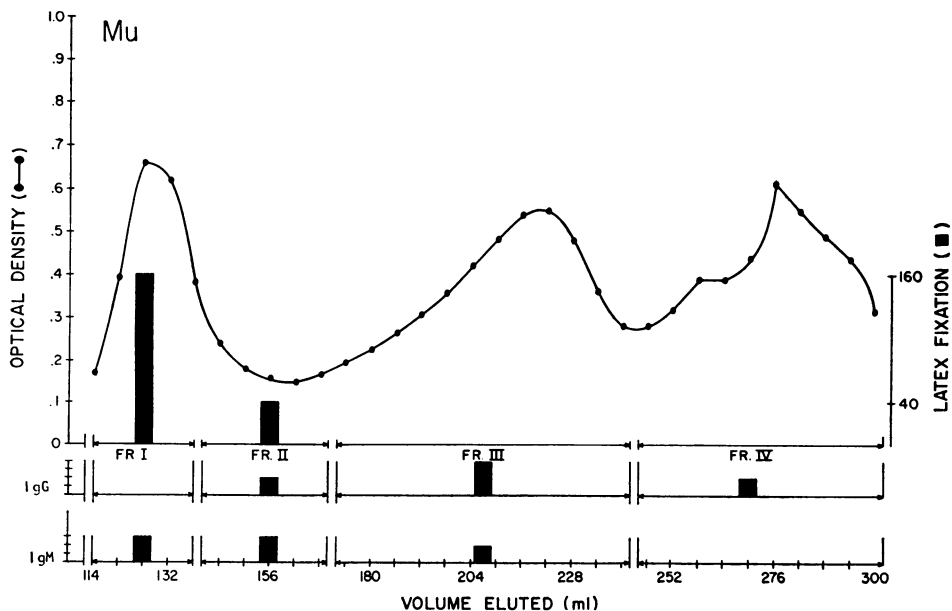

FIG. 7. SEPHADEX G-200 GEL FILTRATION OF SERUM MU DEMONSTRATING SIGNIFICANT IgM ANF IN FRACTIONS I TO III, WHILE RHEUMATOID FACTOR ACTIVITY IS PRIMARILY IN FRACTION I AND IgG ANF IN FRACTIONS II, III, AND IV.

in both large and small molecules and thus confirming the results of the sucrose density centrifugation. Table II compares the results of the two methods used to characterize the proteins. In general, there was excellent agreement. Minor inconsistencies may reflect differences in the concentration of the molecules in the fractions or may be due to technical factors inherent in these fractionation procedures or differences in the sensitivity of different assays. The insignificant change in nuclear fluorescence after treatment with mercaptoethanol of sera with both the classical and unique IgM antibodies may be because intensity of nuclear fluorescence is difficult to quantitate in undiluted sera, and serial twofold dilutions of sera are not sufficiently sensitive to detect inactivation of antibody activity in the presence of significant residual antibody by these techniques.

Immunologic characterization by agar double diffusion with specific antiserum to IgM globulin (Figure 8) proved difficult in the case of density gradient fractions due to the small amounts of protein remaining after the fluorescence studies. However, double diffusion studies were performed with the Sephadex fractions from seven of the sera; the results are listed in Table II. The three sera with rapidly sedimenting mercaptoethanol-sensitive ANF's contained protein reacting with the anti-IgM antiserum only in the first fraction. Similarly, two of the eight mercaptoethanol-resistant sera contained IgM globulins detectable by the technique only in Fraction I. In contrast, three of the four remaining sera, where enough material was available for testing, contained material reacting with an antiserum to IgM in each of the first three fractions, and the fourth had IgM material in the first two fractions only. It would appear from these findings that a major fraction of the IgM proteins fell in the low molecular weight fraction in these four sera. In the other two, the slowly sedimenting IgM ANF was apparently not present in sufficient quantity to be detected by this technique, which is significantly less sensitive.

5) Clinical characteristics. The clinical data on the eight patients whose sera contained the unusual ANF are compared with data on the 45 other SLE patients in Table III. Most striking is the high incidence of male patients in this group. In the total of 53 patients studied, there

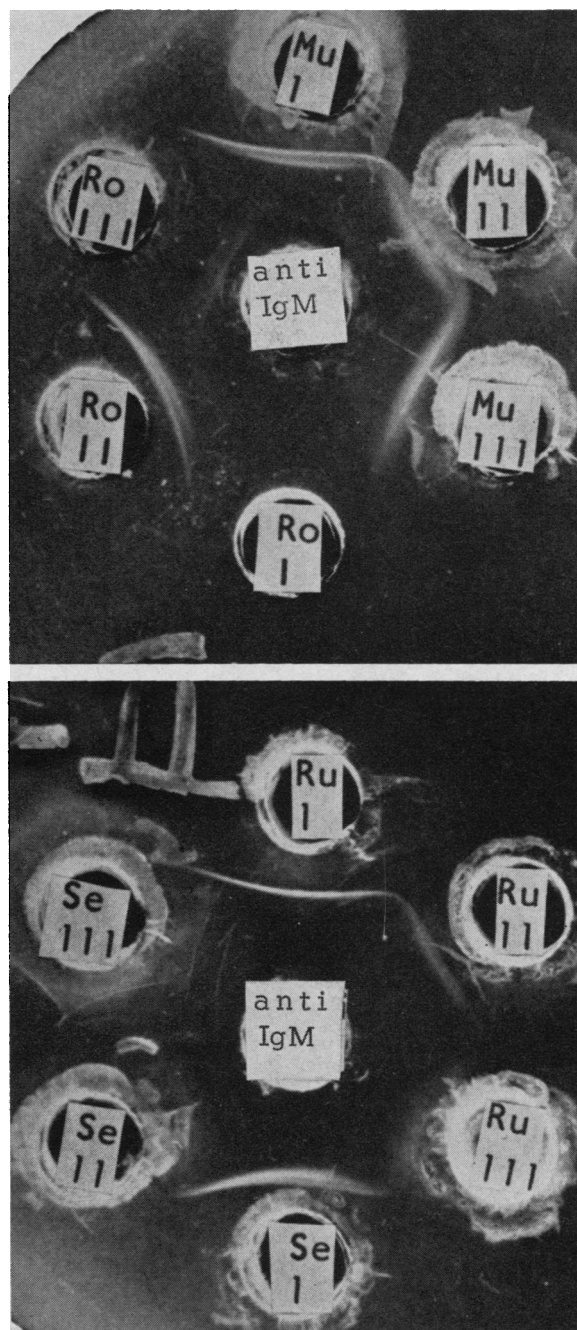FIG. 8. OUCHTERLONY STUDY OF SEPHADEX FRACTIONS FROM ONE SUBJECT (SE) WITH TYPICAL IgM ANTIBODY AND THREE OTHERS WITH "UNUSUAL" ONES. IgM is present only in Fraction I of Se, but it appears in II; I and II; or I, II, and III in the other three.

were nine males, four of whom had the unusual ANF. All eight patients with the unusual ANF had positive LE cell preparations on the same sample of blood, whereas only 16 of the 45

TABLE III

Clinical characteristics of patients with and without unusual ANF

	Total patients (53)		Patients with usual ANF (45)		Patients with un- usual ANF (8)	
	no.	%	no.	%	no.	%
Males	9	17	5	11	4	50
+ LE cell preparation	24	45	16	36	8	100
Active disease	29	55	22	49	7	88
Disease duration of 1 year or less	13	25	6	13	7	88

other patients had positive LE cell preparations on the same sample of blood. Evidence of active disease (30) was present in seven of the eight patients with the unusual antibody, whereas only 22 of the 45 other patients had active disease at the time the blood was obtained. A very striking characteristic of the patients with the unusual antibody was the short duration of their disease. Seven of the eight patients had been ill for one year or less, whereas only six of the other 37 patients had been ill for less than 1 year. Thus, the unusual antibody was present in seven of 13 patients whose disease was of short duration.

Discussion

By techniques similar to those we have employed, most human serum antibodies (13–15), including ANF's, have been classified into one of the three groups of immunoglobulins (IgG, IgA, and IgM) (2). A few exceptional antibodies with intermediate sedimentation properties have been reported recently (4, 5, 7, 8, 26). In previous reports on ANF's, relatively small numbers of sera have been studied, and only limited attempts have been made to characterize them further. Baum and Ziff (14) found that IgM ANF activity in five sera was completely abolished by treatment with mercaptoethanol, and Barnett, Condemi, Leddy, and Vaughan (15) obtained similar results with mercaptoethanol treatment of 17 sera that contained IgM ANF. Since we found only eight of 53 sera possessing the unusual antibody, its discovery may require the analysis of a sufficiently large sample.

Analysis of the clinical characteristics of the eight patients in whom the unusual ANF was present revealed that these individuals were, in general, young patients whose disease was of short duration. In addition, a surprisingly high number of these patients were males. A 50% incidence of males has not been reported in previous clinical or serologic studies of patients with SLE (11, 16, 31).

The unusual antibody characterized in these patients differs from all previously described antibodies in the following ways. It has the antigenic characteristics of an IgM and is detected with an antiserum specific for it. Unlike the usual IgM antibodies, however, this antibody is resistant to mercaptoethanol. In this respect, it resembles the artificially produced incomplete Rh agglutinin reported by Chan and Deutsch (32), who converted a 19 S Rh agglutinin into an incomplete antibody detected only by the Coombs test by treatment with mercaptoethanol. Similarly, Jacot-Guillarmod and Isliker have reported that certain 19 S isoagglutinins, when treated with sodium borohydride (NaBH$_4$), yield fragments that retain their antibody activity, as detected by the Coombs test (33). The unusual antibody reported here differs from the classical IgM globulins further in that both in the ultracentrifugation studies and on Sephadex, it migrates with fractions generally devoid of IgM globulins and with fractions rich in the IgG globulins or those containing molecules with intermediate sedimentation characteristics. This combination of properties clearly distinguishes these antibodies from classic IgG, IgA, and IgM globulins and suggests also that they differ from the intermediate antibodies of Rockey and Kunkel (7) and Flodin and Killander (8), which have not been characterized further.

The presence in fresh and stored sera of the unusual antibody may be explained in various ways. This material may represent the monomeric unit or the heavy chain of the IgM globulins that have not been incorporated into the completely synthesized M immunoglobulin. On the other hand, the unusual antibody may be a product of *in vivo* or *in vitro* breakdown of normal IgM globulin. A third possibility is that the unusual antibody represents a new class of immune globulins that has not previously been recognized in man. An analogous situation appears to exist in horses immunized with meningococci that appear to have large amounts of 7 S molecules with antigenic characteristics of M immunoglobulins (34). To date

there is no evidence allowing a choice between the three alternative hypotheses. The data currently available do not allow any statement as to the valence of these antibodies. The possibility naturally exists that this, like most other antibodies, is multivalent and consequently that it could be detected by direct techniques. Definite proof of this will have to await the isolation of these antibodies free of G immunoglobulins. Such studies are currently in progress. It is also conceivable, however, that this unusual antibody may be a univalent antibody that can be identified only by the use of indirect techniques such as the Coombs test or the indirect fluorescent technique with specific antisera to M immunoglobulins used in the present study. If this were the case, it would have escaped detection by more usually employed techniques, which detect only bivalent antibodies. A search for similar antibodies to other antigens may help to answer this question.

Summary

An unusual antinuclear antibody in sera from eight patients with systemic lupus erythematosus has been reported. The antibody is a small molecule that is antigenically related to the M immunoglobulins but sediments slowly in a sucrose density gradient and is eluted after the first peak from a Sephadex G-200 column. The antibody retains its activity after treatment with mercaptoethanol.

The unusual antibody is present in a high proportion of male patients and is most frequently found in sera from patients with active disease of short duration.

The possible significance of the unusual antibody has been discussed, and alternative explanations for its presence in the sera from patients with systemic lupus erythematosus have been proposed.

Acknowledgments

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