An Immunologic Precipitin System between Soluble Nucleoprotein and Serum Antibody in Systemic Lupus Erythematosus *

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Summary. Double diffusion in agarose was employed for the characterization of a soluble nucleoprotein antigen that gave precipitin reactions with sera from patients with systemic lupus erythematosus. The soluble antigen that was isolated from calf thymus nuclei was demonstrated by enzyme degradation and ultracentrifugation studies, and by immunologic analysis, to be a complex of deoxyribonucleic acid and a moiety susceptible to proteolytic digestion. Antibody to soluble nucleoprotein did not react with the DNA portion of the complex released after proteolytic digestion or with purified calf thymus DNA. Immunologic reaction of identity was obtained between the soluble nucleoprotein antigen and synthesized DNA-histone complex, suggesting that the protein moiety of soluble nucleoprotein might in part be composed of histone. Antibody to soluble nucleoprotein was present in 51% of systemic lupus erythematosus sera examined and was more common than antibody to deoxyribonucleic acid.

Introduction

A remarkable array of antibodies against different tissue components has been demonstrated in the sera of patients with systemic lupus erythematosus (SLE) by a number of immunologic systems. With the complement fixation technique, SLE sera were shown to react with different fractions of tissue extracts (1-5). The immunofluorescence technique has been employed to demonstrate that SLE sera react with tissue sections or cell smears to give different patterns

of nuclear staining (6-8), and precipitin techniques have demonstrated several different antigen-antibody precipitating systems (9-14). In recent years there has been increasing interest in the nature of the tissue antigens that react with these antibodies, but with the exception of DNA, most of the reactive antigens in these immunologic systems have not been physically or chemically characterized.

Recently a soluble nuclear component, provisionally called Sm antigen, has been isolated from tissue extracts and was identified as a 0.15 M saline soluble substance devoid of nucleic acids or nuclear histones (14). This report is concerned with another nuclear antigen that was isolated in soluble form. It was characterized as a DNA-protein complex and participated in immunologic precipitin reactions with sera from certain patients with SLE.

Methods

Preparation of tissue extract containing soluble nucleoprotein. Nuclei from fresh calf thymus glands were prepared in sucrose according to the method of Allfrey,

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Littau, and Mirsky (15), and only those preparations with less than 10% contamination with whole cells were used. All preparative procedures were carried out at 4° C. The isolated nuclei were extracted with an equal weight/volume of buffered saline (0.01 M phosphate, pH 7.0). This was performed by breaking up thymocyte nuclei in a Virtis homogenizer at medium speed for 4 minutes followed by further extraction with slow speed stirring for 60 minutes. The homogenate was centrifuged successively at 35,000 *g* for 30 minutes and 105,000 *g* for 60 minutes. The supernatant contained saline soluble components including the Sm antigen previously described. The sediment of the nuclear material was washed with the same volume of saline to remove any remaining saline soluble nuclear components. The washed nuclear material was then extracted with four to five times its volume of 1 M NaCl. The extraction was allowed to proceed overnight with vigorous stirring, and the resulting solution became a thick viscous fluid. The undissolved material was sedimented from the thick viscous solution by centrifugation at 35,000 *g* for 60 minutes. The supernatant represented nucleoprotein in 1 M NaCl and could be kept in this state for an indefinite time at -20° C. To obtain nucleoprotein in a form suitable for immunologic studies, samples were thawed and placed in Visking 23/32 tubing, and the solution in the dialysis sac was brought down by gradient dialysis to isotonicity with 0.015 M NaCl, 0.0015 M sodium citrate. The procedure of Huang, Bonner, and Murray (16) was followed; it consisted of dialysis against 0.4 M NaCl for 4 hours, 0.3 M NaCl for 1 hour, 0.15 M NaCl for 1 hour, and 0.015 M NaCl with 0.0015 M sodium citrate overnight. The precipitate that formed was cleared by centrifugation at 35,000 *g* for 60 minutes, and the supernatant was used for these studies. In two separate preparations the protein content of the extracts determined by the Folin procedure (17) averaged 2.10 mg per ml, and DNA determined by a modification of the diphenylamine procedure (18) averaged 2.30 mg per ml, giving an average ratio of protein to DNA of 0.91. The final material will be referred to as soluble nucleoprotein and given the abbreviation sNP.

Preparation of soluble complexes of "synthetic" DNA-histone. Highly polymerized calf thymus DNA and calf thymus total histones were obtained commercially.¹ DNA was dissolved in 1 M NaCl at a concentration of 2 mg per ml and sonically disrupted in an ice bath with a 20-kc ultrasonic disintegrator at maximal output for 2 minutes. Polymerized DNA, which was previously a highly viscous solution, visibly became much less viscous after this treatment. Total histones were prepared at a concentration of 4 mg per ml in 1 M NaCl as the stock solution, and dilutions were made in 1 M NaCl. Equal volumes of DNA and histones were mixed, with DNA at a constant concentration of 2 mg per ml and histones at concentrations varying from 4 mg per ml to 1 mg per ml. The mixture in 1 M NaCl was stirred gently overnight at room temperature, and soluble DNA-

histone complexes suitable for precipitin studies were obtained from this mixture by gradient dialysis in 23/32 Visking tubing to isotonicity with 0.015 M NaCl, 0.0015 M sodium citrate according to the procedure used for preparation of sNP. Precipitates, which invariably formed in all the preparations, were removed by centrifugation at 2,500 rpm for 45 minutes. The clear supernatant, containing soluble DNA-histone complexes, was concentrated by pervaporation to one-fifth its original volume in order to demonstrate immunologic activity.

Immunologic techniques. For the demonstration of precipitin reactions between sNP and SLE sera, double diffusion in agarose² was employed according to the method previously described (14). The concentration of agarose was increased to 0.6% to produce a gel of firmer consistency.

Sucrose density gradient ultracentrifugation. This was performed in the manner previously described (19). Sucrose was dissolved in 0.015 M NaCl, 0.0015 M sodium citrate, and a gradient of 10 to 40% was employed. Centrifugation was carried out in the Spinco Sw 39 rotor at 35,000 rpm for 15 hours, and fractions were collected dropwise from the bottom of the tube.

Enzyme treatment of soluble nucleoprotein preparations. Pancreatic deoxyribonuclease (DNase), pancreatic trypsin, and yeast ribonuclease (RNase) were crystalline preparations obtained commercially.¹ The enzymes were used at a concentration of 0.1 mg in 1 ml of sNP with enzyme of substrate ratio of approximately 1:20 by weight. For DNase digestions, MgCl₂ was added to a final concentration of 0.003 mole per L in the mixture. All digestions were carried out at 37° C for 1 hour. Treatment of sNP with DNase resulted in an insoluble precipitate that was spun down by centrifugation, and the supernatant was used for the experiments. Where trypsin was employed, the enzyme was subsequently inactivated by adding an equivalent amount of soybean trypsin inhibitor. The various enzyme-treated preparations of sNP were employed in double diffusion studies as described above. To determine whether the enzyme-treated preparations contained residual enzyme activity that might interfere with the precipitating activity of serum antibody in agar, double diffusion reactions between enzyme-treated sNP and SLE sera were compared after incubation at room temperature and at 4° C. At the latter temperature, the enzymes employed have been shown to have minimal, if any, activity. Precipitin reactions at 4° C appeared 24 hours later than those developed at room temperature but were similar in all other respects.

Results

Identification of immunologically reactive components in soluble nucleoprotein preparations. When appropriate SLE sera were employed in double diffusion in agar, a number of immunologically reactive components were demonstrated

¹ Worthington Biochemical Corp., Freehold, N. J.

² SeaKem, Bausch and Lomb, New York, N. Y.

in the sNP preparation. In a typical experiment, illustrated in Figure 1, well 1 contained sNP and well 2 contained sonically disrupted calf thymus DNA (200 μ g per ml in saline buffered with 0.01 M phosphate, pH 7.5). Serum St, in the upper frame, reacted with sNP to form three precipitin lines and with purified DNA to form one line. The line in the St-sNP reaction that was farthest from the antigen well fused completely with the DNA line. Soluble nucleoprotein thus contained material antigenically related to DNA and at least two other antigenic components detected by St serum. Serum Ad reacted with sNP to give one precipitin line but did not react with DNA, suggesting that this reaction was not on the basis of material immunologically related to DNA. This was supported by the observation that the Ad-sNP precipitin line fused with the middle line of the St-sNP reaction but did not relate to the line farthest from the antigen well.

FIG. 1. DOUBLE DIFFUSION IN AGAR ILLUSTRATING THE DIFFERENT PRECIPITIN REACTIONS ENCOUNTERED BETWEEN SOLUBLE NUCLEOPROTEIN (sNP) AND SYSTEMIC LUPUS ERYTHEMATOSUS SERA. In the upper frame, serum St reacted with sNP to give three lines, the line farthest from the antigen well fused with the DNA line. Serum Ad did not react with DNA but reacted with sNP to give one line, which fused with the middle line of the St-sNP reaction. The results after absorption of the sera with DNA and sNP are illustrated in the lower two frames.

Further evidence that one of the precipitin reactions was with DNA and was distinct from the other precipitin reactions was achieved by absorption studies illustrated in the lower two frames of Figure 1. Serum St was absorbed at equivalence with calf thymus DNA, and as shown in the middle frame, the absorbed serum no longer reacted with DNA, and the related precipitin line in the sNP reaction was also abolished. The middle precipitin line was unaffected and continued to show identity with the Ad-sNP line. As illustrated in the lower frame of Figure 1, the precipitin reaction of serum Ad was abolished by absorption of this serum with sNP. Absorption of serum Ad with DNA did not remove the reaction with sNP.

Characterization of an antigen in sNP as a DNA-protein complex. The following studies were undertaken to identify the nature of the component in sNP that reacted with serum Ad and appeared to have different antigenic determinants from DNA. Soluble nucleoprotein was subjected to enzyme treatment, and alteration in precipitin activity was detected by immunologic double diffusion analysis. As illustrated in Figure 2, trypsin removed the precipitin line that was common to both St and Ad but did not affect the DNA precipitin line of St. Treatment with DNase removed the DNA line and also the line possessed in common by the two sera, whereas RNase had no effect on any of the lines. The data demonstrated that the serum factor possessed in common by St and Ad was reacting with a component in sNP that was susceptible to destruction by either trypsin or DNase. These studies have been repeated with two other sera of the St type and three other sera of the Ad type with identical results. The third reactant in sNP that gave the precipitin line with serum St nearest to the antigen well was also removed by treatment with either trypsin or DNase, as illustrated in Figure 2. This antigen has not been further characterized and will not be described in the subsequent studies.

The nature of the component in sNP that reacted with serum of the Ad type and was destroyed by either trypsin or DNase was further studied by sucrose gradient ultracentrifugation. Untreated sNP was shown to sediment in sucrose gradient with a broad asymmetrical distribution,

FIG. 2. EFFECT OF ENZYME TREATMENT ON THE PRECIPITATING ACTIVITY OF sNP. Either trypsin or pancreatic deoxyribonuclease (DNase) destroyed the antigenicity of the component in sNP that reacted with both St and Ad. Trypsin had no effect on the precipitin line between St and sNP identified as the DNA system. Yeast ribonuclease (RNase) treatment had no effect on any of the precipitin reactions.

and the peak material as measured by absorbancy at $260\text{ m}\mu$ and $280\text{ m}\mu$ was close to the bottom of the tube, as depicted in the lower frame of Figure 3. Serial samples of three fractions were pooled and concentrated, and the antigenicity of each of the concentrated fractions was determined by double diffusion analysis in agar. The antigenic material reacting with both St and Ad was detected only in the bottom three tubes of the gradient. Antigenic material reacting with St alone and not with Ad was present in tubes 4 to 9 and was shown to be identical with DNA by a precipitin line of identity with calf thymus DNA. When sNP was treated with trypsin, there was a significant shift of the absorbancy to a symmetrical peak in the middle portions of the tube. These fractions reacted with St and were shown to be identical with DNA but were not reactive with serum Ad. Deoxyribonuclease-treated sNP did not sediment to any significant extent in the sucrose gradient, and the fractions were not reactive with either St or Ad. These experiments have been repeated with a second preparation of sNP with similar results.

The data thus far have demonstrated that soluble nucleoprotein contained an antigenic complex composed of DNA or a subunit of DNA, and a component or components susceptible to trypsin digestion. The different specificities of antibody to DNA alone, and antibody to the DNA complex, were shown by immunologic analysis in agar diffusion. The data also showed that the DNA portion of the complex released after trypsin digestion was in a macromolecular form that retained the property of precipitating with DNA antibody.

Controls of the enzyme studies. It was important to determine whether the destruction by trypsin of the antigenicity of the DNA complex in sNP was due to contamination of the trypsin preparation with DNase or material with DNase-like activity. As an approach to this problem, the effect of trypsin on the sedimentation behavior of calf thymus DNA was studied. Highly polymerized calf thymus DNA, which did not contain detectable protein at a DNA concentration of 1 mg per ml , was used as the substrate. Trypsin was added to DNA at an enzyme-substrate ratio

FIG. 3. CHARACTERIZATION OF REACTIVE COMPONENTS IN sNP BY SUCROSE GRADIENT ULTRACENTRIFUGATION. With untreated sNP (lower curve), only material in the bottom three fractions of the tube reacted with both sera St and Ad. Material from tubes 4 to 9 reacted only with St and was identical with DNA. The effect of trypsin and DNase on the sedimentation and reactivity of sNP is illustrated in the upper two curves.

similar to that used for treatment of sNP and carried through similar digestion procedures. The trypsin-treated DNA was then compared with untreated DNA in the same run by sucrose density gradient ultracentrifugation. As shown in Figure 4, the bulk of untreated DNA sedimented with a peak in the fourth tube, and no DNA was detected after the seventh tube. Trypsin-treated DNA also exhibited a peak in the fourth tube, and no DNA was likewise detected after the seventh tube. A larger amount of material sedimented to the bottom of the tube in DNA treated with trypsin. However, the important finding was that treatment of DNA with trypsin did not result in the appearance of slower sedimenting material as was the case when sNP was treated with trypsin (see middle frame of Figure 3). Treatment of DNA with DNase, carried out in the same experiments, resulted in material that remained in the top three tubes of the sucrose

density gradient. The experiments have been repeated with a different batch of calf thymus DNA with identical results. These observations support the evidence obtained from agar diffusion analysis that the effect of trypsin was not on the DNA moiety of the deoxyribonucleic acid complex.

The converse experiments were done to determine whether the destruction of the antigenicity of the DNA complex in sNP by DNase was due to trace contamination with proteolytic enzymes. The method of Anson (20) employing denatured hemoglobin as the substrate was used to detect the presence of possible proteolytic activity in DNase. It was shown that DNase at a final concentration of 0.3 mg per ml did not demonstrate detectable proteolytic activity. This concentration of enzyme was three times the concentration of DNase routinely employed in the treatment of sNP. In separate experiments to determine the lowest concentration of either DNase or trypsin that was required to destroy the antigenicity of DNA complex in sNP, the end points were 0.04 mg per ml for DNase and 0.02 mg per ml for trypsin. A 50% contamination of DNase by trypsin would have been re-

FIG. 4. EFFECT OF TRYPSIN ON THE SEDIMENTATION OF HIGHLY POLYMERIZED THYMUS DNA. Trypsin treatment did not result in appearance of lighter sedimenting material, demonstrating that the observations illustrated in Figure 3 were not due to the effect of trypsin on DNA per se.

quired for the effect of DNase to be ascribed to tryptic activity.

"Synthetic" soluble DNA-histone complexes. The elimination of the antigenic activity of the deoxyribonucleic acid complex in sNP by trypsin raised the possibility that the complex contained protein in addition to DNA. The following studies gave evidence to suggest that the non-DNA portion of the complex was, at least in part, composed of nuclear histones. Attempts were made to synthesize soluble DNA-histone complex that would react with SLE sera in the double diffusion system. It was difficult to produce a soluble complex with immunologic reactivity when highly polymerized calf thymus DNA and calf thymus histones were mixed, although there was a suggestion in some instances of a weak reaction near the antigen well. This might have been due in part to the low diffusibility in agarose of the large molecular complex. However, DNA, sonically disrupted into low molecular weight fragments, combined with histones to produce soluble complexes that were reactive with SLE sera. This was demonstrated by double diffusion studies depicted in Figure 5 employing sera St and Ad to determine immunologic antigenicity. Serum St reacted with sNP in well 1 to form the three precipitin lines previously characterized, the line farthest from the antigen well representing reaction with DNA and the middle line representing reaction with DNA complex. Serum St also reacted with material in well 2, which contained the supernatant from a mixture of sonically disrupted DNA and histones combined at a weight ratio of 2:1. Free DNA was present in this preparation as shown by the strong precipitin line farthest from the antigen well, which fused with the DNA line in sNP. In addition, a second precipitin line was formed that fused with the DNA complex present in sNP. When DNA and histones were mixed at a weight ratio of 2:2, the supernatant (well 3) consisted mainly of free DNA with a small amount of the second reactive component. The second precipitin line is not clearly visible in the photograph. With serum Ad, which reacts only with DNA complex, the DNA-histone solution in well 2 gave a precipitin line that fused with the sNP line. The solution in well 3 also reacted with serum Ad, but the reaction was much weaker, and there

FIG. 5. DOUBLE DIFFUSION IN AGAR TO ILLUSTRATE IMMUNOLOGIC IDENTITY BETWEEN "SYNTHETIC" DNA-HISTONE AND THE DNA-PROTEIN ANTIGEN PRESENT IN sNP. Well 1 = sNP. Well 2 = DNA-histone complexed at a ratio of 2:1. Well 3 = DNA-histone complexed at a ratio of 2:2. See text for details.

was a suggestion of two precipitin lines. Solutions of histones alone at different concentrations did not form precipitin lines with either St or Ad. Two other preparations of synthetic DNA-histone complexes were made and have been shown similarly to have the property of precipitating with SLE sera of the Ad type and to form a precipitin line of continuity with sNP. Free DNA was also present in these preparations.

It had been demonstrated earlier that the DNA-protein complex in sNP sedimented faster in sucrose gradient ultracentrifugation than the DNA moiety alone. If histones were complexed to DNA in the immunologically reactive "synthetic" DNA-histone preparations, these preparations might be expected to sediment more rapidly than DNA. A comparison of the density gradient

FIG. 6. SEDIMENTATION OF "SYNTHETIC" DNA-HISTONE COMPLEXES IN SUCROSE GRADIENT ULTRACENTRIFUGATION COMPARED WITH SEDIMENTATION OF DNA. Curve I represents DNA. Curves II and III represent DNA-histone complexes from material employed in wells 2 and 3, respectively, in Figure 5. The DNA-histone complexes contained components that sedimented more rapidly than uncomplexed DNA.

profile of DNA-histone with that of sonically disrupted DNA is depicted in Figure 6. Curve I was the sedimentation profile obtained with DNA alone. Curve II was the pattern with soluble DNA-histone derived from the 2:1 mixture, and curve III was the pattern from the 2:2 mixture described in Figure 5. The solutions containing the DNA-histone complexes sedimented more rapidly towards the bottom of the sucrose gradient than DNA alone. The sedimentation of the more reactive synthetic DNA-histone preparation represented by curve II differed from that of DNA by one tube and was not so significant as the difference observed between native soluble nucleoprotein and its DNA moiety (Figure 3). This slight difference in sedimentation between synthetic DNA-histone and DNA was observed in a second experiment with another preparation of DNA-histone.

The protein content of the sucrose density gradient fractions represented by the DNA-histone preparations in curves II and III of Figure 6 was determined with the Folin phenol reagent. In separate studies, it was determined that this reagent did not detect DNA alone at the concentrations present. The protein content of the density gradient fractions is represented by the 700-m μ absorbance curve in Figure 7. The ma-

jor protein peak correlated with the region of maximal 260-m μ absorbancy in tubes 7 and 8, with a minor protein peak in tube 11. It was determined in two different experiments that total histones, carried through similar stepwise dialysis as the DNA-histone mixtures, sedimented in sucrose gradient ultracentrifugation in tubes 10, 11, and 12 with the peak in tube 11 and with no detectable protein in other tube fractions. The presence of protein in tubes 7 and 8 of Figure 7 suggested that the added histones were sedimenting with DNA and associated with it in some form.

Incidence of the serum factor reacting with soluble nucleoprotein in SLE sera. Evidence has been presented that the DNA complex in soluble nucleoprotein prepared from calf thymus nuclei was composed of DNA in association with a protein moiety that was, at least in part, nuclear histones. The serum factor that precipitated with this soluble DNA-protein complex has been identified by immunoelectrophoresis to migrate in the γ -globulin region. Reactive sera gave immuno-

FIG. 7. DETERMINATION OF THE PROTEIN CONTENT OF THE SUCROSE GRADIENT FRACTIONS OBTAINED FROM ULTRACENTRIFUGATION STUDIES OF DNA-HISTONE COMPLEXES DEPICTED IN FIGURE 6. The protein peaks (Folin reaction curve) coincided with the 260-m μ peaks. Residual uncomplexed histone was present in the top fractions of the tubes.

TABLE I

Precipitating antibodies in systemic lupus erythematosus (SLE) to soluble tissue antigens

Antibody to	No. positive/ No. patients studied	Incidence
		%
DNA	15/65	23
Soluble nucleoprotein	33/65	51
Sm antigen	37/65	57

logic quantitative precipitin reactions, and the serum proteins eluted from precipitates were shown to be identical with γ G-globulin by double diffusion in agar. A survey was made of the incidence of this antibody in the sera of 65 patients with SLE, which was compared with incidence of precipitating antibody to two other defined nuclear components, DNA and Sm anti-

TABLE II

Precipitating antibodies to soluble tissue antigens in non-SLE sera

Category	Precipitating antibodies to	No. positive/ No. patients studied
Rheumatoid arthritis	DNA	1/16
	sNP*	2/16
	Sm	0/16
Dermatomyositis and polymyositis	DNA	0/21
	sNP	0/21
	Sm	0/21
Scleroderma	DNA	1/12
	sNP	0/12
	Sm	0/12 (4)†
Hypertension treated with hydralazine	DNA	0/32
	sNP	3/32
	Sm	0/32
Idiopathic thrombo- cytopenic purpura	DNA	0/18
	sNP	0/18
	Sm	0/18 (4)†
Autoimmune hemolytic anemia	DNA	0/21
	sNP	0/21
	Sm	0/21 (7)†
Sjögren's syndrome	DNA	0/4
	sNP	0/4
	Sm	0/4
Blood donors	DNA	0/20
	sNP	0/20
	Sm	0/20

* sNP = soluble nucleoprotein.

† These numbers denote patients whose sera gave precipitin lines with saline extract of calf thymus nuclei not identifying with Sm antigen.

gen. The characterization of antibody to sNP was established by precipitin reactions of identity with sera standardized according to the methods described here and that of antibody to the Sm antigen with sera standardized according to methods previously described (14). Antibody to DNA could be determined specifically by a precipitin line with DNA in double diffusion in agarose under the conditions described. The patients with SLE were an unselected group at different levels of disease activity and, with the exception of five patients, had had positive LE cell tests at some stage of their illness but not necessarily at the time the serum was studied. Five patients with negative LE cell tests had clinical features of SLE with pathological changes in kidney biopsies supporting the clinical diagnosis. The results presented in Table I therefore reflect the relative incidence of different antibodies under these circumstances. Antibody to soluble nucleoprotein (51%) was more common than antibody to DNA (23%) but slightly less common than antibody to Sm antigen (57%). The incidence of precipitating antibodies in other diseases and in blood donors is presented in Table II. The only positive sera encountered were in one patient with rheumatoid arthritis and one patient with scleroderma, who had antibodies to DNA, and in two patients with rheumatoid arthritis and three patients with hypertension treated with hydralazine, who had antibodies to sNP.

In the 65 SLE patients studied, 54 or 83%

TABLE III

Characteristics of SLE sera with DNA antibody

Patient	Antibody to		
	DNA	sNP	Sm
St	+	+	—
Ma	+	+	+
Ro	+	+	—
Re	+	+	+
Lo	+	+	—
Mo	+	+	+
Jo	+	+	—
Ag	+	+	+
Ev	+	+	+
Pr	+	+	+
Bi	+	+	—
Sw	+	+	+
Sa	+	+	+
Me	+	—	—
He	+	—	—

had precipitating antibodies to at least one of the three soluble tissue antigens described. Of the 54 positive sera, 27 (50%) had antibodies to more than one tissue antigen, 15 (28%) had antibody to Sm antigen alone, and 10 (18%) to sNP alone. Sera with antibody to DNA alone, to the exclusion of antibodies to the other tissue antigens, were uncommon; only two such sera were encountered. The characteristics of sera with DNA antibody, presented in Table III, illustrate that the large majority of these sera also contain antibody to sNP and about half contain antibody to Sm antigen.

Discussion

A precipitating system between antibody in SLE sera and soluble nucleoprotein has been described. The characteristics of this precipitin system were established by the following criteria: 1) Antibody to soluble nucleoprotein did not react with either DNA alone or histones alone. 2) In sera that contained antibodies to both DNA and soluble nucleoprotein, the different specificities of these antibodies were demonstrated by separate precipitin reactions. 3) The antigenicity of soluble nucleoprotein was destroyed by either deoxyribonuclease or trypsin.

The soluble nucleoprotein antigen was identified as a complex of macromolecular DNA and a component susceptible to proteolytic digestion. The non-DNA component of this complex might in part be composed of nuclear histones since synthetic DNA-histone complexes and soluble nucleoprotein reacted with SLE sera to give precipitin lines of identity.

These studies have confirmed and extended the observations of Holman and Deicher (21) and of Hijmans and Schuit (22). These authors demonstrated that LE cell factor reacted with particulate deoxyribonucleoprotein and showed that the particulate antigen after treatment with deoxyribonuclease or trypsin was incapable of removing LE cell factor from serum. Holman and Deicher further demonstrated that nuclear histone was an integral moiety of particulate deoxyribonucleoprotein. In the studies reported here, double diffusion analysis in agar was employed for the detection of reactive antigens. This technique offers the advantage of the reso-

lution and identification of multiple antigen-antibody reactions in systems under study. It was thus possible to demonstrate that the soluble nucleoprotein employed here contained at least three reactive components, two of which have been identified as DNA and a DNA-protein complex. The third component that reacted with serum St and a number of other SLE sera was not further characterized. It is likely that yet other antigenic components are present in deoxyribonucleoprotein, some of which might be present in an insoluble form. These considerations raise the possibility that the earlier investigators using particulate deoxyribonucleoprotein were also dealing with a number of different antigenic components and not with a single antigen-antibody reaction.

When soluble nucleoprotein was treated with trypsin, the DNA moiety of the complex sedimented more slowly than highly polymerized DNA. This suggests that soluble nucleoprotein might represent a subunit of larger or more highly polymerized nucleoprotein present in its native state in the cell nucleus. The subunit might have resulted from the action of intracellular enzymes during the preparative procedure or might have been caused by the mechanical manipulations of the procedure itself. A further possibility to be considered is that certain segments of polymerized deoxyribonucleoprotein might be more liable to breakage than others and that the soluble nucleoprotein studied here represented selected segments with antigenic reactivity.

The exact nature of the non-DNA moiety of soluble nucleoprotein will require chemical characterization. Although synthetic complexes of DNA and histones were demonstrated to react with SLE sera in a manner antigenically identical with soluble nucleoprotein extracted from the cell nucleus, synthetic complexes differed from the native material in at least two respects. They were not so antigenically reactive and did not sediment in sucrose gradient so rapidly as the native material. In the native state, deoxyribonucleoprotein exists in cell nuclei principally in chromatin, where it is frequently associated with other nuclear components, such as phosphoprotein, phospholipid, and nonhistone residual proteins (23). It remains to be determined whether the latter type of nuclear components was present in the soluble nucleoprotein complex studied here,

and whether this might account for the differences observed.

The antigenic determinant of soluble nucleoprotein has not been elucidated in these studies. It is conceivable that complexing with protein altered the tertiary structure of DNA resulting in a new antigenic determinant. A similar situation could be postulated for the protein moiety of the complex. Other investigators have suggested that the antigenic site might be related to the DNA-histone junction (24). These questions could be approached by determining whether substitution of other negatively charged molecules for DNA, or of other positively charged proteins for histones, would alter the antigenicity of soluble nucleoprotein. Studies by previous investigators using particulate deoxyribonucleoprotein as the substrate have demonstrated that the reactivity of the particulate antigen was destroyed by some such substitutions (21).

It has been shown in this and previous studies (14, 25) that precipitating antibodies to three nuclear components, DNA, Sm antigen, and soluble nucleoprotein can be identified by double diffusion analysis in agarose. The characteristic differences between antibody to soluble nucleoprotein and antibody to DNA have been described in this paper. The distinction between antibody to soluble nucleoprotein and antibody to Sm antigen can be determined by precipitin lines of nonidentity in double diffusion studies. Furthermore, the antigenicity of soluble nucleoprotein is destroyed by treatment with deoxyribonuclease or trypsin, whereas the Sm antigen is resistant to digestion with either of these enzymes as previously described (14).

The nature of the serum antibody or antibodies that induce the LE cell phenomenon has been a subject of considerable interest. It is widely accepted that antibody to nucleoprotein, demonstrated by a variety of techniques, can induce LE cell formation (21, 22, 26, 27), but some question remains about the ability of DNA antibody to induce this phenomenon. Holman and Deicher (21) observed that DNA antibody showed weak ability to form LE cells, whereas Seligmann (28) is convinced that DNA antibody is capable of this activity. Both groups of investigators have also observed that the LE factor in some sera could not be abolished by the addition of DNA to

sera containing DNA antibody. This observation can be explained by the data presented in Table III where it is shown that the majority of sera containing DNA antibody also contain sNP antibody. The removal of DNA antibody from such sera would still leave behind sNP antibody in the majority of cases. In this study, all sera that contained antibody to sNP gave a positive LE cell reaction. This was the case whether the serum contained sNP antibody alone or sNP antibody in conjunction with antibodies to DNA or Sm antigen. The two patients (Table III, Me and He) with DNA antibody alone, to the exclusion of antibodies to sNP and Sm, also gave positive LE cell reactions. With a two stage indirect LE cell technique (29), these two sera absorbed with DNA gave negative LE cell tests, whereas sera St and Ro (Table III) gave positive LE cell reactions before and after absorption of DNA antibodies. Sera containing antibody only to Sm antigen were not capable of inducing LE cell formation.

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