Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 5401–5405, September 1982
Medical Sciences

Autoantibodies occurring in two different rheumatic diseases react with the same nuclear ribonucleoprotein particle

(Sm antigen/electroblot transfer/systemic lupus erythematosus/mixed connective tissue disease)

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Communicated by James Bonner, March 29, 1982

ABSTRACT **Patients affected with systemic lupus erythematosus and mixed connective tissue disease produce antibodies directed against two nuclear antigens, Sm and nuclear ribonucleoprotein (nRNP), respectively. The two antigens exhibit a relationship of partial identity in serologic assays, but the molecular basis of this relationship was not understood. This report describes the isolation of a nRNP particle containing both nRNP and Sm antigens. The particle was isolated by sucrose density gradient centrifugation of a rat liver nuclear extract followed by anti-nRNP affinity chromatography of a 14S gradient fraction. The protein moiety of the isolated particle consisted primarily of two polypeptides, P13 (M_r, 13,000) and P30 (M_r, 30,000). The immunoreactivity of P13 and P30 was demonstrated directly by transfer of these proteins from gels to nitrocellulose paper, followed by immunoautoradiography. Anti-nRNP sera reacted only with P30, whereas anti-Sm sera reacted with P13. Anti-nRNP sera were previously found to react with P13, but only in the presence of RNA [Douvas, A. S., Stumph, W. E., Reyes, P. R. & Tan, E. M. (1979) *J. Biol. Chem.* 254, 3608–3616]. From these observations it was concluded that P13 is the Sm antigen. The precipitating nRNP antigen is composed of P30–RNA complexes or P13–RNA complexes, with a RNA-independent reaction occurring with P30. The partial identity between Sm and nRNP antigens can be explained on the basis of a common reactivity to the P30–P13–RNA particle, with anti-Sm sera capable of binding additionally to RNA-free P13.**

The spontaneous production of antibodies directed against nuclear ribonucleoprotein (nRNP) complexes has been recognized for some time as an important diagnostic feature of mixed connective tissue disease (MCTD) (1, 2). Antibodies having apparently the same specificity occur with a lower frequency in yet another rheumatic disease, systemic lupus erythematosus (SLE) (3). In addition to anti-nRNP antibodies, patients with SLE produce antibodies to a second nuclear antigen, Sm, which on the basis of its resistance to DNase and RNase was presumed to be a protein (4). Sera from individual SLE patients may have antibodies to either the Sm antigen alone or to both Sm and nRNP antigens (5). An immunologic similarity between the Sm and nRNP antigens is suggested by two observations: (*i*) the antigens show partial identity in immunodiffusion assays (5); (*ii*) isolated preparations of nRNP antigen often, if not always, contain Sm activity (6). The chief distinction between the two antigens is that the ability of the nRNP antigen to precipitate with antibodies is sensitive to RNase A, whereas the Sm antigen is resistant to RNase A (7). Evidence for an immunologic similarity between the two antigens suggests that their respective diseases may have a common etiology.

The primary objective of the current study was to more exactly define the molecular composition of the nRNP antigen. Some important ambiguities, deriving from both technical and immunologic considerations, remained in previous efforts to characterize this antigen. One of the major technical obstacles has been the necessity of isolating the antigen from crude nuclear extracts, which are likely to form aggregates of different types of ribonucleoprotein particles *in vitro*. Immunoprecipitation from such extracts yields immune complexes containing non-antigenic as well as antigenic material. The contaminants may or may not appear to be selectively combining with antibodies. This problem was addressed in a previous study, in which we employed antibody affinity chromatography to isolate antigenic nRNP particles from rat liver nuclei (8). This study established that the antigen consisted of a ribonucleoprotein core composed of two polypeptides, P13 and P30, and RNA. However, in addition to the core particles, a considerable quantity of antigenically inactive ribonucleoprotein adhered to affinity columns. The question of whether this additional material represented contaminating ribonucleoprotein or was an integral part of the antigenic particle was not resolved in this study. The same ambiguity applies to subsequent investigations from other laboratories (6, 9, 10). A second problem has been that of demonstrating directly, by use of antibodies, which macromolecules are actually antigenic. Because the RNA is not itself antigenic, but depends on the presence of protein, evidence concerning its antigenicity is by necessity indirect.

The essential question of which macromolecules actually bear antigenic determinants was approached in the current study by isolating the simplest possible ribonucleoprotein particle having a complete set of determinants as determined by immunodiffusion. This was accomplished by sucrose density gradient centrifugation followed by antibody affinity chromatography. The antigenicity of proteins forming this core particle was investigated by electrophoretic transfer from gels to nitrocellulose paper followed by immunoautoradiography. Sera from MCTD patients were employed that were effectively monospecific for the nRNP antigen (8).

The Sm antigen was found to coisolate with the nRNP antigen during the course of this study. Given the molecular homogeneity of the final nRNP product, it seemed likely that one of its major protein constituents was the Sm antigen. The relationship between these two antigens was explored by using two sera from SLE patients having anti-Sm antibodies. These sera were effectively monospecific for the Sm antigen in that they showed a single precipitin reaction identical to anti-Sm proto-

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Abbreviations: nRNP, nuclear ribonucleoprotein; MCTD, mixed connective tissue disease; SLE, systemic lupus erythematosus; hnRNP, heterogeneous nuclear ribonucleoprotein; PSS, progressive systemic sclerosis; NaCl/P_i, pH 7.5, 0.15 M NaCl/0.010 M sodium phosphate buffer, pH 7.5.

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types in immunodiffusion assays, and this reaction was not diminished by treatment with RNase A.

MATERIALS AND METHODS

Nuclear Fractionation. Nuclei were isolated from 200 g of rat livers (Pel-Freez) as described (8). Packed nuclei were resuspended in 50 ml of NaCl/P_i, pH 7.5 (0.15 M NaCl/0.01 M sodium phosphate buffer) to a protein concentration of approximately 20 mg/ml, then sonicated on ice in two 25-ml aliquots in 8–10 15-sec pulses with a Heat Systems sonicator at a setting of 4. The sonicate was fractionated as outlined in Fig. 1.

Sucrose Density Gradients. Linear sucrose gradients, 10–40% (wt/vol) in NaCl/P_i, pH 7.5, were loaded with 18–93 A_{260} units of A2 per gradient and centrifuged for 22 hr at 104,000 × g in a Beckman SW 28 rotor at 4°C. Markers of *Escherichia coli* 23S, 16S, and 4–5S ribosomal RNA, radiolabeled with ^{14}C,

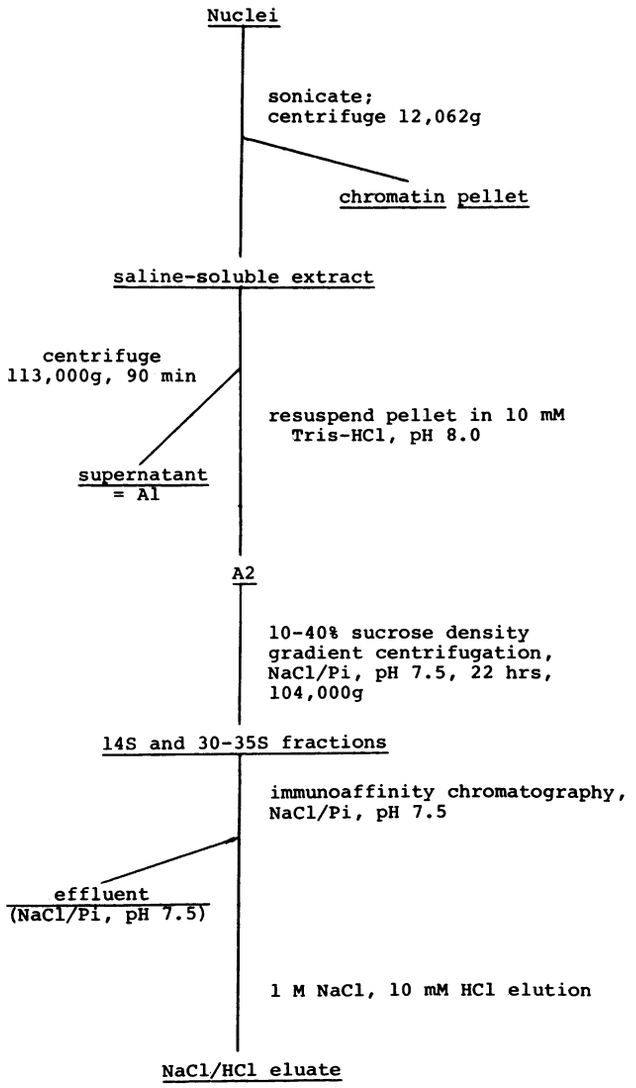

FIG. 1. Isolation of nRNP antigen from rat liver nuclei. Nuclei were sonicated at 0°C in NaCl/P_i, pH 7.5, at a protein concentration of 20 mg/ml. All subsequent procedures were performed at 4°C. A2 was resuspended in Tris·HCl buffer to a protein concentration of 7 mg/ml prior to sucrose density gradient fractionation. Gradient fractions were dialyzed extensively against NaCl/P_i, pH 7.5, prior to applying to affinity columns. Columns were loaded with 3.2–4.4 mg of either A2 or the 14S or 30–35S fractions, then washed extensively with NaCl/P_i, pH 7.5. Elution with 1 M NaCl/10 mM HCl, pH 2.0, resulted in >90% recovery of applied protein and nucleic acid.

were a gift of David Pettijohn. Gradients were collected in 1-ml fractions and the fractions were assayed for antigenic activity as described below.

Quantitative Assays. Protein concentrations were determined by the method of Sedmak and Grossberg (11). Nucleic acids were quantitated by a modification of the Schmidt–Tannhauser procedure for DNA (12) and by the orcinol method for RNA (13).

Sera and Immunologic Assays. Sera were obtained from the serum bank, Division of Rheumatic Diseases, University of Colorado Health Sciences Center. In a previous study (8) four anti-nRNP sera were characterized and found to be identical with respect to antibody specificity. Two of these sera and two prototype anti-Sm sera were employed in the current study. Sera from progressive systemic sclerosis (PSS) patients, having antibodies to another nuclear antigen, Scl 70, were used as autoimmune controls (14). Normal sera were obtained from laboratory personnel. Double diffusion assays were performed in glass plates containing 0.4% agarose in NaCl/P_i, pH 7.5, with 0.1% sodium azide added to retard bacterial growth.

Antibody Affinity Columns. Two anti-nRNP columns and one control column were constructed by linking 20 mg of IgG per column to cyanogen bromide-activated Sepharose 4B (Pharmacia) as described in detail previously (8). All antigen fractions were dialyzed against NaCl/P_i, pH 7.5, prior to chromatography.

Polyacrylamide Gel Electrophoresis. Slab gel electrophoresis, 15% polyacrylamide in $NaDodSO_4$, was performed as described (15, 16). For some purposes tube gels having the same composition as the slab gels were used. Slab gels were electrophoresed for 16 hr at 8 mA then either stained with Coomassie brilliant blue or subjected to electroblot transfer as described below. Tube gels were electrophoresed at 2 mA per gel for 5 hr.

Electroblot Transfer and Immunoautoradiography. Proteins were transferred electrophoretically at 60 V for 3 hr from unfixed slab gels to nitrocellulose paper as described (17, 18), using an E-C gel destaining apparatus (St. Petersburg, FL). Complete transfer of proteins was confirmed by staining the gel and test strips of nitrocellulose with Coomassie brilliant blue and amido black, respectively. Nitrocellulose strips containing protein were soaked in 1% bovine serum albumin (Sigma, RIA grade) in saline (0.9% NaCl/10 mM Tris·HCl, pH 7.4) then incubated with serum diluted ≥1:5,000 in bovine serum albumin/saline for 16 hr. After washing with saline, strips were incubated for 3 hr with ^{125}I-labeled goat anti-human IgG (a gift of Robert Rubin) at 20,000 cpm/cm^2. Strips were then washed with saline followed by distilled H_2O and air dried. Autoradiography was performed at −70°C for 24–84 hr with Kodak XRP-5 film.

RESULTS

Purification and Size of the Antigenic nRNP Particle. The strategy employed in these experiments was to isolate the nRNP antigen, then analyze the purified antigenic particle for the presence of both Sm and nRNP determinants. This analysis was then extended to cruder nuclear fractions. Rat liver nuclei were partitioned as outlined in Fig. 1 to yield a fraction, A2, containing all of the nRNP antigen and most of the Sm antigen (8). A2, which contains 11% of the nuclear protein and 38% of the RNA, is identically active against MCTD sera containing anti-nRNP antibodies (8). Application of A2 to 10–40% sucrose gradients resulted in the profile shown in Fig. 2. The bar graph indicating nRNP antigenic activity was constructed by assaying each fraction against anti-nRNP serum by immunodiffusion. By assaying gradients having different initial loads of A2, the relative intensity of nRNP antigenic activity was determined. The

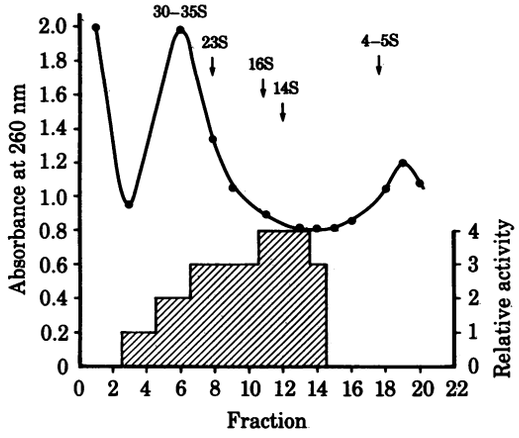

FIG. 2. Sucrose density gradient fractionation of A2. Linear 10–40% sucrose density gradients were run with loads of A2 protein ranging between 1.3 and 9.1 mg per gradient. ^{14}C-Labeled *E. coli* RNA fractions were run as markers either in a separate bucket or mixed with A2, with the same results. Gradients were collected dropwise and individual fractions were quantitated by spectrophotometry, then assayed for immunologic activity by immunodiffusion in agar as described in the legend of Fig. 3. A relative activity was assigned to each fraction on the basis of the strength of its precipitin reaction against anti-nRNP antibodies. The histogram was composed by evaluating fractions from four different gradients having different initial loads of A2. An activity of one implies that the fraction showed a visible reaction only in the most heavily loaded gradient (9.1 mg of A2 protein).

peak of activity was concentrated at a position of 14 S. In heavily loaded gradients, such as the one shown in Fig. 2, antigenic activity extended as far as the 30–35S peak, consisting of heterogeneous nuclear ribonucleoprotein (hnRNP) particles. However, there was no evidence of a bimodal distribution of antigenic activity at either light or heavy loads that would be indicative of a selective association of smaller antigenic particles with the larger hnRNP particles.

To determine if identical nRNP antigenic determinants were present in every active gradient fraction, a comparison was performed by immunodiffusion. Fig. 3 demonstrates complete immunologic identity of all active fractions and of unfractionated A2, indicating that no dissociation of the antigen occurred during this procedure. The immunologic identity of A2 to all nuclear fractions from which it was derived was demonstrated previously (8). All nuclear and sucrose gradient fractions con-

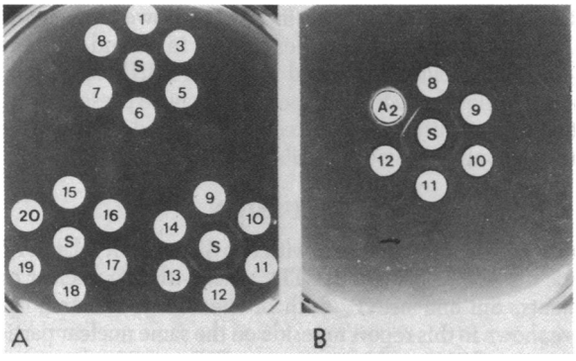

FIG. 3. Evaluation of sucrose density gradient fractions by immunodiffusion. Immunodiffusion was performed in glass plates containing 0.4% agar. Each well contained 150 μl of either serum (S) or antigen. The anti-nRNP serum shown was diluted 1:20 in NaCl/P_i, pH 7.5. (*A*) Undiluted sucrose gradient fractions 1–20. (*B*) Comparison of gradient fractions 8–12 to A2 (7 mg/ml). Reactions were allowed to develop for 18 hr at room temperature.

taining the nRNP antigen also reacted with anti-Sm sera from SLE patients. Normal control sera were unreactive.

To test the hypothesis that the heterogeneity of material binding to antibodies depends on the relative purity of applied antigen, three fractions were chromatographed over antibody affinity columns: A2, the 14S sucrose gradient pool (which is 20-fold enriched for antigen relative to A2), and the 30–35S hnRNP pool (which contains only trace amounts of antigen). The three fractions, which contain identical nRNP determinants (Fig. 3), were loaded on antibody affinity columns under conditions that ensure complete retention of all antigenic determinants (8). In brief, samples were applied to anti-nRNP columns in NaCl/P_i, pH 7.5, and the effluents, when assayed by immunodiffusion in agar, were found to be totally devoid of antigenic activity. Two anti-nRNP and one normal human IgG column were employed, and effluents from the normal IgG column were fully active. To quantitatively recover bound ribonucleoprotein, columns were eluted with 1 M NaCl/10 mM HCl, pH 2.0 (Fig. 1). Although this step results in dissociation of RNA–protein complexes as well as antigen–antibody complexes, it allows for full recovery of the molecules of RNA and protein that associate to form the immunologically complete antigen. The RNA-to-protein mass ratios in 14S, 30–35S, and A2 column eluates were 0.28:1, 0.28:1, and 0.25:1, respectively. The protein moieties were resolved by polyacrylamide gel electrophoresis as discussed below.

Protein Composition of the nRNP Antigen. Fig. 4 shows the polypeptide composition of ribonucleoprotein retained by anti-nRNP antibody affinity columns after application of the 14S pool, the 30–35S pool, or A2. Lanes A and B contain the NaCl/HCl eluates obtained from chromatography of the 14S pool over two different anti-nRNP columns, constructed with antibodies from two different patients. The effluent corresponding to

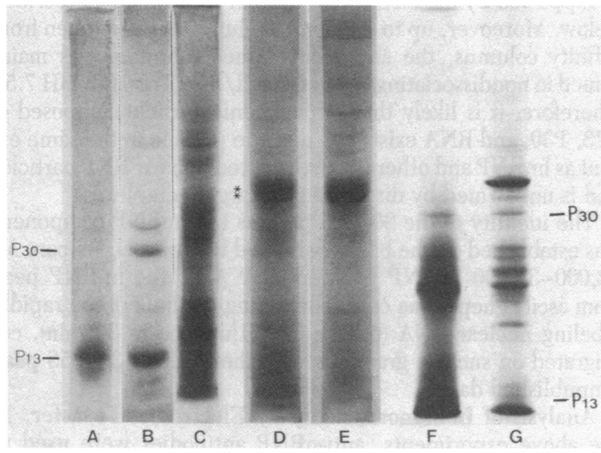

FIG. 4. Polyacrylamide gel electrophoresis of antibody affinity column fractions. Electrophoresis was in 15% polyacrylamide gels, in 0.8 × 13 cm glass tubes as described (8). Molecular weights were calculated relative to the mobilities of the following molecular weight markers: bovine serum albumin, 68,000; pyruvate kinase, 57,000; heavy chain of human IgG, 50,000; ovalbumin, 43,000; DNase I, 31,000; trypsin, 23,000; histone H2A, 14,000; and histone H4, 11,282. Lane A, 60 μg of NaCl/HCl eluate from anti-nRNP column 1 loaded with 14S ribonucleoprotein; lane B, 60 μg of NaCl/HCl eluate from anti-nRNP column 2 loaded with 14S ribonucleoprotein; lane C, 100 μg of NaCl/P_i effluent, same column as lane A; lanes D and E, 60 μg of NaCl/HCl eluate and NaCl/P_i effluent, respectively, from anti-nRNP column 1 loaded with 30–35S ribonucleoprotein; lanes F and G, 100 μg of NaCl/HCl eluate and NaCl/P_i effluent from anti-nRNP column 1 loaded with A2. The two asterisks to the left of lane D indicate the positions of core hnRNP proteins (M_r 34,000 and 32,000, respectively).

NaCl/HCl eluate A appears in lane C (the effluent from the second column was indistinguishable from that in lane C and is not shown). The heterogeneity of the effluent contrasts with the homogeneity of the NaCl/HCl eluate, which is composed of P13, P30, and a few minor polypeptides. The composition of the minor polypeptides was not reproducible with the exception of a M_r 40,000 polypeptide that was found to be immunologically inactive (see Fig. 5). When the antigenically less enriched 30–35S and A2 fractions were subjected to affinity chromatography, the NaCl/HCl eluates were correspondingly more heterogeneous in polypeptide composition (lanes D and F). A comparison of lanes D and E reveals that whereas P13 is selectively retained by affinity columns, there is no apparent selectivity in the distribution of other hnRNP polypeptides. The P30 band is too minor to follow reliably in heterogeneous mixtures of protein. Some selectivity is apparent in the distribution of A2 polypeptides between eluate and effluent (lanes F and G), but both fractions are heterogeneous with respect to polypeptide composition. In a previous study, ribonucleoprotein that bound to antibody affinity columns after application of A2 was recovered in two fractions: a 1 M NaCl (pH 7.5) eluate, and a 10 mM HCl eluate representing more tightly bound material (8). The 1 M NaCl eluate contained 84% of the total ribonucleoprotein retained by affinity columns but was devoid of antigenic activity. The 10 mM HCl eluate, which was antigenically active, had a protein moiety consisting largely of P13 and P30. In the current study, the ribonucleoprotein binding to affinity columns was recovered quantitatively in a single step, a combined NaCl/HCl elution, and had a protein moiety consisting of P13 and P30. Because a complete set of antigenic determinants was applied and bound to affinity columns in the current study, it follows that the great reduction in quantity and heterogeneity of ribonucleoproteins over previous experiments reflected a loss of nonantigenic ribonucleoprotein. This analysis is supported by the electroblot transfer experiments described below. Moreover, up to the point of elution of the antigen from affinity columns, the antigenic ribonucleoprotein was maintained in nondissociating buffers (NaCl/P_i or Tris·HCl, pH 7.5). Therefore, it is likely that the antigenic particle composed of P13, P30, and RNA exists as a discrete particle to the same extent as hnRNP and other *in vitro* isolated nuclear RNP particles and is not formed by dissociation of larger complexes.

The identity of the 30–35S peak as the hnRNP component was established on the basis of size and the presence of core M_r 32,000–34,000 hnRNP polypeptides. Also, the hnRNP peak from ascites hepatoma cells, containing the bulk of the rapidly labeling nuclear RNA (uptake of [^{3}H]uridine in 30 min), comigrated on sucrose gradients with the rat liver 30–35S peak (unpublished data).

Analysis of Immunoreactivity by Electroblot Transfer. In the above experiments, anti-nRNP antibodies were used to isolate an antigen containing a complete set of determinants as assayed by immunodiffusion in agar. This assay scores only those interactions between antigen and antibodies that result in the formation of a precipitate. In order to more directly investigate the reactivity of isolated nRNP antigen components, polypeptides were transferred from gels to nitrocellulose paper, then incubated with either anti-Sm or anti-nRNP sera. NaCl/HCl eluates and effluents from anti-nRNP columns that had been loaded with the 14S sucrose gradient pool were subjected to this analysis. Fractions from two different anti-nRNP columns were analyzed against all anti-nRNP, anti-Sm, and control sera with the same results. A representative subset of the results is presented.

As shown in Fig. 5, anti-nRNP sera reacted with P30 (lanes A and C) and anti-Sm sera reacted with P13 (lanes E and F).

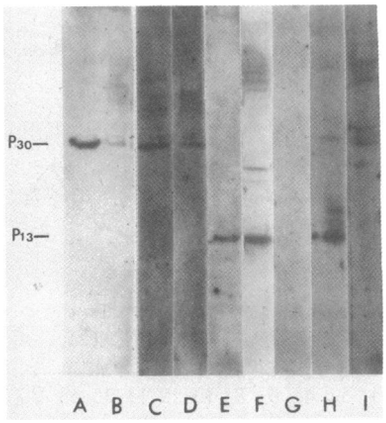

FIG. 5. Electroblot transfer and immunoautoradiography of affinity column fractions and A2. Polyacrylamide slab gel electrophoresis was conducted in 15% acrylamide, and proteins were transferred to nitrocellulose. To confirm transfer of proteins and identify reactive species, nitrocellulose strips corresponding to each of those shown were stained with amido black. Lanes A and B, NaCl/HCl eluate and NaCl/P_i effluent from anti-nRNP column 1 allowed to react with anti-nRNP serum 1; lanes C and D, the NaCl/HCl eluate and effluent from anti-nRNP column 2 allowed to react with anti-nRNP serum 2; lane E, the same NaCl/HCl eluate shown in lane A, allowed to react with anti-Sm serum 1; lane F, the same NaCl/HCl eluate as in lane A allowed to react with anti-Sm serum 2; lane G, the same NaCl/P_i effluent as in lane B, allowed to react with anti-Sm serum 1; lane H, A2 allowed to react with anti-Sm serum 1; lane I, A2 allowed to react with anti-nRNP serum used at a dilution of 1:5,000. Reactions with control normal sera and anti-Scl-70 autoimmune controls were negative and are not shown.

As shown in Fig. 4, both P30 and P13 were present in the NaCl/HCl eluate, with only trace amounts leaking into the effluent (lanes B, D, and G). Autoimmune controls from PSS patients, having antibodies to the chromosomal protein Scl-70 (14), and normal sera were unreactive with all fractions (not shown).

The above results and those presented in a previous communication (8) demonstrate that the determinants responsible for the precipitin reaction between anti-nRNP antigen and antibodies are contained in the particle composed of P13, P30, and RNA. Sm determinants are also found there. To determine if other, non-precipitating determinants are present in cruder nuclear fractions, the protein moieties of A1 and A2 (see Fig. 1) were transferred to nitrocellulose paper and allowed to react with anti-Sm and anti-nRNP sera. An insignificant amount of additional reactivity was observed when either A1 or A2 reacted with anti-Sm sera (A2 is shown in lane H). However, in addition to P30, three other polypeptides present in A1 and A2, but not in NaCl/HCl eluates, reacted with anti-nRNP sera (lane I). These additional reactive polypeptides may be present in crude extracts in quantities greatly exceeding P30 and may be reacting with relatively low-avidity antibodies.

DISCUSSION

Autoantibodies from patients with two distinct but related rheumatic diseases, SLE and MCTD, react with two nuclear components, Sm and nRNP. Both of these nuclear components were shown in this report to reside on the same nuclear particle, consisting of RNA and two proteins, P13 and P30 (Fig. 4). P13 was identified as the Sm antigen on the basis of the demonstration that anti-Sm antibodies react primarily or exclusively with P13 either in crude nuclear extracts or in the purified particle (Fig. 5). Although anti-nRNP antibodies were used to isolate the particle, these antibodies failed to react with RNA-free P13 (Fig. 5). This observation is consistent with either of two

interpretations: (*i*) P13 is entirely unreactive with anti-nRNP antibodies but binds to affinity columns through an association with RNA and P30; (*ii*) P13–RNA complexes, but not free P13, are reactive. Although the antigenic status of P13–RNA complexes is pending, it is clear that determinants composing the precipitating nRNP antigen are contained in the isolated particle. This conclusion is based on the complete retention of precipitating determinants by antibody affinity columns (*Results* and ref. 8) and the quantitative recovery of determinant-bearing molecules from these columns. The efficiency of retention of determinants by these columns was demonstrated in immunoautoradiography experiments presented in Fig. 5. Thus the precipitating nRNP antigen, which is highly susceptible to RNase A (8), is composed either of P30–RNA complexes or both P30–RNA and P13–RNA complexes.

It was not possible to separate P13 from P30 in 14S fractions from sucrose gradients short of treatment with RNase A. However, the partial identity between Sm and nRNP antigens seen in cruder nuclear extracts suggests that P13 exists free of RNA *in vitro*. A bimodal distribution of the Sm antigen has been observed in gel filtration and sucrose density gradient fractions of crude extracts, with the larger component coinciding in position with the nRNP antigen (5). Further, the partial identity between Sm and nRNP antigens indicates that P13–RNA and P30–RNA complexes do not exist as separate entities. Given the lack of reactivity of anti-Sm antibodies to P30, the separate existence of the two types of particles would result in a relationship of nonidentity in immunologic assays.

The relative simplicity of the antigenic particle described here contrasts with the more heterogeneous protein moieties isolated by some investigators (6, 9, 10). The advantages of the protocol employed in the current study were: (*i*) the use of preparative sucrose density gradient centrifugation to size the antigenic particles and minimize artifactual aggregation of ribonucleoprotein during isolation; (*ii*) direct analysis of the antigenic status of isolated proteins by electroblot transfer. The experiments summarized in Fig. 4 and in a previous publication (8) demonstrate that a considerable quantity of nonantigenic ribonucleoprotein will adhere to affinity columns in the absence of efforts to minimize aggregation in the applied material. Our original assessment that P13 and P30 are the antigenically important protein constituents of affinity-isolated ribonucleoprotein was confirmed here by electroblot transfer analysis (Fig. 5). Some higher molecular weight proteins, contained in A2 but not the purified particle, reacted with anti-nRNP antibodies (Fig. 5). These proteins were not present in 14S sucrose gradient fractions, because they appeared in neither affinity column effluents nor eluates (Fig. 5). The immunologic identity between A2 and 14S gradient fractions in immunodiffusion assays (Fig. 3) indicates that the higher molecular weight proteins do not contain major determinants of the precipitating antigen.

The Sm and nRNP antigens are two of a limited number of nuclear elements reacting with autoantibodies from rheumatic disease patients. In the entire spectrum of rheumatic diseases approximately 15 to 20 immunologically distinct nuclear components have been identified as antigens (for a review, see ref. 19). The fact that these nuclear antigens are limited in number suggests that they may belong to functional units in the nucleus that are vulnerable to attack by specific agents (such as drugs, viruses, or hormones) that interact directly with the nucleus to alter cellular function. The nRNP particle described here may represent such a functional unit, which has been rendered immunogenic by a perturbing stimulus. The presence of two different antigens on the same particle suggests that the same perturbing stimulus may be the etiologic agent in both diseases. The RNA moiety of the antigenic particle has not yet been characterized. Preliminary data indicate that a >20S RNA molecule is the primary nucleic acid component of the 14S particle, and that small nuclear RNAs are selectively lost during purification. This finding suggests that small nuclear RNAs may be part of the nonantigenic ribonucleoprotein that can adhere to the antigen in the course of isolation *in vitro*. Alternatively, the bimodal distribution of the Sm antigen reported elsewhere (5) may reflect P13 associated with larger (>20S) and smaller nuclear RNAs, rather than an RNA-associated and RNA-free protein.

This research was supported by National Institutes of Health Grant AM 21839.

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