PROTEIN ANTIGENS OF THE RNA-PROTEIN COMPLEXES
DETECTED BY ANTI-SM AND ANTI-RNP ANTIBODIES
FOUND IN SERUM OF PATIENTS WITH SYSTEMIC
LUPUS ERYTHEMATOSUS AND RELATED DISORDERS*

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Recent clinical observations have assigned increased significance to the anti-Sm
and anti-RNP antibodies found in the sera of patients with systemic lupus erythematosus (SLE)1
and certain other related disorders. The anti-Sm antibodies are highly
specific for SLE, whereas the anti-RNP antibodies show a wider distribution and help
define a new syndrome termed “mixed connective tissue disease” (1, 2). Considerable
interest has centered on the character of the antigens recognized by these autoanti-
bodies because of their probable involvement in the processing of transcribed RNA.
It has been known for some years that they are present in the nuclei of a wide variety
of cells (1) and that RNP antigen is sensitive to RNase (3). Some work has been
carried out on the isolation of these antigens with emphasis on their size, charge, and
antigenicity (1, 3, 4). Very recently, considerably more insight has been gained from
the work of Lerner and co-workers (5-8). Their work has identified the RNA
precipitated by these two types of autoimmune sera, and differences in RNA com-
position were noted, although the same protein bands were precipitated by both types
of sera (5). These results are difficult to relate to earlier studies that suggested a role
for specific proteins in the differential reactivity of these sera. The present studies
indicate that both Sm and RNP coexist in a single complex that can be dissociated to
give clearly distinct antigenic proteins. The Sm determinants reside primarily on
proteins of 25,000 and 16,000 daltons, whereas the RNP determinants reside at least
in part on a protein of 19,000 daltons.

Materials and Methods

Antisera and Serological Analysis. Sm and RNP antibody titers of sera obtained from patients
with SLE were determined by passive hemagglutination as described by Tan and Peebles (9).
Several sera with anti-Sm and anti-RNP specificities were obtained from Dr. E. Tan (University
of Colorado Medical Center, Denver, CO) and Dr. V. Agnello (New England Medical Center
Hospital, Boston, MA). A mouse anti-Sm monoclonal antibody was generously donated by E.
Lerner (Yale University, New Haven, CT).

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‡ Abbreviations used in this paper: DTT, dithiothreitol; IAA, iodoacetamide; kd, kilodalton; PMSF,
phenylmethylsulfonyl fluoride; RNP, ribonucleoprotein; SDS, sodium dodecyl sulfate; SLE, systemic lupus
erythematosus; snRNP, small nuclear ribonucleoprotein.
IgG fractions of rabbit anti-human IgG serum, raised against myeloma protein, were labeled with $^{14}$C formaldehyde (40–60 mCi/mmol; New England Nuclear, Boston, MA) by reductive methylation (10), and the reaction was quenched by the addition of Tris buffer.

**Cell Growth and Labeling.** A human lymphocytic leukemia cell line, KE37, and a human IgG-secreting lymphoid cell line, 8866P (generously donated by Dr. Shu Man Fu, The Rockefeller University), were grown between 1 and $5 \times 10^5$ cells/ml in RPMI 1640 (Microbiological Associates, Walkersville, MD) supplemented with 10% fetal bovine sera (Grand Island Biological Co., Grand Island, NY), 2 mM glutamine, and antibiotics. A rat liver cell line, BRL 3A (CRL 1442, American Type Culture Collection, Rockville, MD) was grown on plastic substrates in Dulbecco’s Modified Eagle’s Medium (DME) (Grand Island Biological Co., Grand Island, NY) supplemented with 10% fetal bovine serum, 2 mM glutamine, and antibiotics. Cultures were passaged at confluency by detachment in 0.05% trypsin, 0.5 mM EDTA in Dulbecco’s phosphate-buffered saline and replating on new surfaces in fresh media. Cells were labeled with 50 mCi/ml $^{35}$S methionine (1,000 Ci/mmol, New England Nuclear) in methionine-depleted DME supplemented with 10% fetal bovine serum, 2 mM glutamine, and antibiotics.

**Cell Lysis and Immunoprecipitation.** $^{35}$S methionine-labeled cell cultures were harvested by centrifugation, washed three times in Dulbecco’s phosphate-buffered saline, and lysed either (a) by addition of 0.5% sodium dodecyl sulfate (SDS), 50 mM Tris pH 7.5, 150 mM NaCl, 2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride (PMSF), and 5 mM iodoacetamide (IAA) followed by sonication and addition of Triton X-100 to 2.5% final concentration, or (b) by swelling 10 min at 0°C in 10 mM Tris pH 7.5, 10 mM KCl, 1 mM EDTA, 0.5 mM PMSF, and 5 mM IAA and five strokes with a Dounce homogenizer followed by addition of NaCl to 200 mM final concentration. Each lysis condition was performed at $10^6$–$10^7$ cells/ml, and each immunoprecipitate was derived from ~5 × $10^5$ cells.

After cell lysis, samples were centrifuged at 12,000 g for 5 min and the supernatants incubated with either serum from patients with SLE or normal control serum for 1 h at room temperature. Protein A sepharose was then added, incubated for 1 h, washed five times with 0.5% SDS, 2.5% Triton X-100, 50 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, and 0.25 M sucrose. The washed immunoprecipitates were eluted by incubation at 100°C for 3 min in 0.25 M sucrose, 0.005% bromphenol blue, 2% SDS, 0.1 M Tris pH 7.5, 5 mM EDTA (loading buffer), and 0.1 M dithiothreitol (DTT) and immediately loaded onto 10–15% gradient polyacrylamide gels (14). Gels were fluorographed (11) after staining in Coomassie Blue and destaining (12).

**Sample Preparation for Electrophoresis and Blot Transfer of Protein.** Rat liver nuclei were isolated as described by Blobel and Potter (13) and stored until use at −20°C in 50% glycerol, 50 mM triethanolamine pH 7.6, 25 mM KCl, 5 mM MgCl. The protease inhibitors, 0.5 mM PMSF and 5 mM IAA, were included during all steps of the isolation procedure. When needed, nuclei were centrifuged from the glycerol suspension, resuspended in loading buffer, and sonicated or vortexed to reduce viscosity. Samples were either incubated at 100°C for 5 min and loaded onto polyacrylamide gels or loaded directly without boiling. Rat and rabbit tissues were collected from freshly killed animals, frozen immediately in liquid nitrogen, and held at −80°C until use. Before use, frozen tissues were powdered under liquid nitrogen with a geologist’s hammer, immediately mixed into loading buffer which contained 0.5 mM PMSF and 5 mM IAA, and vortexed or sonicated to reduce viscosity. Samples were then loaded onto SDS gels as described above. Cell pellets were washed with Dulbecco’s phosphate-buffered saline, resuspended in loading buffer containing PMSF and IAA, mixed, and loaded onto SDS gels as described above. Rabbit thymus extract was prepared according to Tan and Peebles (9) from rabbit thymus acetone powder (Pel-freeze Biologicals, Rogers, AR). The extract was diluted with 0.1 volume of 10 × loading buffer and treated as described above. Blot transfer of proteins from SDS gels to nitrocellulose paper and the subsequent identification of antigens were performed as described by Fisher et al. (14).

**Results**

**Immunoprecipitation of Cell Extracts.** Previous studies (5) have reported that anti-Sm and anti-RNP sera precipitated similar small nuclear ribonucleoprotein particles (snRNP) that could not be differentiated on the basis of protein composition. These
experiments were carried out on nondenatured nuclear extracts of Ehrlich ascites cells. We have also found that both anti-Sm and anti-RNP sera qualitatively precipitate similar sets of proteins from nondenatured cell extracts of a human cell line KE37 (Fig. 1A). After SDS gel electrophoresis, a total of eight proteins can be detected in the anti-Sm precipitate (lane 1, Fig. 1A), and in the anti-RNP precipitate (lane 2, Fig. 1A), which are not found in the precipitate with normal human sera (lane 3, Fig. 1A). Quantitatively, the anti-Sm precipitate contains larger quantities of band a and smaller quantities of band d when compared with the anti-RNP precipitate. Similar results were obtained with a number of different anti-Sm and anti-RNP sera and with lysates prepared from the human lymphoid cell line 8866P.

Immunoprecipitation after SDS Denaturation of Cells. If the labeled KE37 cells were

Fig. 1. (Left) Immunoreactive polypeptides precipitated from [35S]methionine-labeled KE37 cell lysates with anti-Sm or anti-RNP sera in the absence (panel A) or the presence (panel B) of SDS. The immunoprecipitates were analyzed by SDS gel electrophoresis and fluorography as described in Materials and Methods. Lane 1, immunoprecipitate obtained by anti-Sm serum; lane 2, immunoprecipitate obtained by anti-RNP serum; lane 3, immunoprecipitate obtained by normal human serum. Sm- and RNP-specific bands in panel A are indicated by letters a-h. Molecular weight markers × 10^3 are indicated at the margin of panel B. They are BSA, ovalbumin, chymotrypsinogen, and myoglobin.

Fig. 2. (Right) Sequential immunoprecipitation of nondenatured KE37 cell lysates by anti-Sm and anti-RNP sera. [35S]methionine-labeled KE37 cells were lysed in the absence of SDS. The cell lysates were subjected to immunoprecipitation and the immunoprecipitates were analyzed by SDS gel electrophoresis and fluorography as described in Materials and Methods. Lane 1 displays an immunoprecipitate obtained by anti-Sm serum. Immunoprecipitation supernatant, obtained from three times the amount of immunoprecipitate shown in lane 1, was divided into three equal aliquots and reprecipitated with anti-Sm serum (lane 2), anti-RNP serum (lane 3), or normal human serum (lane 4), demonstrating that all of the RNP antigen can be precipitated by anti-Sm serum. Lane 5 displays an immunoprecipitate obtained by anti-RNP serum. Immunoprecipitation supernatant, obtained from three times the amount of immunoprecipitate shown in lane 5, was divided into three equal aliquots and reprecipitated with anti-Sm serum (lane 6), anti-RNP serum (lane 7), or normal serum (lane 8), demonstrating that some of the Sm antigen is not precipitated by anti-RNP serum. All lanes represent equivalent volumes of cell lysate. Note that the particular Sm serum used here (lanes 1, 2, and 7) not only precipitated bands a-h but also two major bands (indicated by arrows) of ~80 and 68 kd, which are not related to the Sm antigen.
lysed in the presence of SDS before immunoprecipitation, anti-Sm and anti-RNP sera could be distinguished on the basis of the immunoprecipitated proteins. Fig. 1 B shows these immunoprecipitates after analysis by SDS polyacrylamide gels. Anti-Sm sera precipitate two proteins (lane 1, Fig. 1 B), in the 25-kilodalton (kd) range, that correspond to bands b and c in Fig. 1 A. If the rat liver cell line BRL was substituted for the KE37 cells, only one band in the 25-kd range, corresponding to band c, was found after immunoprecipitation, and a 16-kd protein was seen that corresponded by molecular weight to band e in immunoprecipitates of nondenatured extracts (data not shown). With either cell line, anti-RNP sera precipitate only one protein of 19 kd (lane 2, Fig. 1 B), corresponding to band d seen in Fig. 1 A. None of these bands are precipitated by sera of normal individuals (lane 3, Fig. 1 B) or by SLE sera that lack these antibodies in serological analysis. SLE sera containing both of these specificities precipitated mixtures of these proteins (data not shown). These results were observed with all anti-Sm and anti-RNP sera tested. Immunoprecipitation of SDS-treated cell extracts with an anti-Sm mouse monoclonal antibody precipitated bands b, c, and e, showing that the e band is antigenically similar to the b-c bands.

Sequential Immunoprecipitates. Immunoprecipitation with either anti-Sm or anti-RNP sera was performed on nondenatured KE37 cell lysates. The supernatant from these immunoprecipitates was then reprecipitated with anti-Sm, anti-RNP, and normal serum. In Fig. 2 (lane 1), an immunoprecipitate by an anti-Sm serum can be seen. The supernatant of this immunoprecipitate was divided into three equal aliquots, and each was reprecipitated with anti-Sm (lane 2, Fig. 2), anti-RNP (lane 3, Fig. 2), or normal serum (lane 4, Fig. 2). Because reprecipitation with either serum does not bring down any of the previously identified proteins, all immunoreactive Sm and RNP proteins were effectively removed during the first precipitation with anti-Sm serum. When the supernatant from an anti-RNP precipitation (lane 5, Fig. 2) was reprecipitated with an anti-RNP serum (lane 6, Fig. 2) no bands were observed. However, anti-Sm serum (lane 7, Fig. 2) brought down bands a–c, f–h. Reduced quantities of band e were visible after longer exposures of the autoradiograph, but none of the band d characteristic of RNP was seen. These results suggest that at least two classes of particles exist, one class which reacts with both anti-Sm and anti-RNP and another class, lacking the band d, reacts only with anti-Sm and not with anti-RNP.

When similar sequential immunoprecipitates were made on SDS denatured KE37 cell lysates, different polypeptides were precipitated with the two types of antisera, independent of the order in which the antisera were used. Using supernatant from an anti-Sm immunoprecipitate (lane 1, Fig. 3), we found extensive depletion of bands b and c by the first precipitation (lane 2, Fig. 3) but band d remained in the supernatant to be precipitated by anti-RNP serum (lane 3, Fig. 3). When supernatant from an anti-RNP immunoprecipitate (lane 5, Fig. 3) was reprecipitated with anti-RNP (lane 6, Fig. 3), band d was almost completely removed by the initial anti-RNP precipitation, but bands b and c were left in the supernatant to be removed by anti-Sm sera (lane 7, Fig. 3). These data, taken together with the sequential immunoprecipitation of nondenatured lysates, indicate that SDS treatment of whole cells disrupts the antigenic particles allowing distinctions to be made between anti-Sm and anti-RNP sera.

Nitrocellulose Blot Analysis. To confirm the antigenicity of the proteins identified by
FIG. 3. (Left) Sequential immunoprecipitation of SDS-denatured KE37 cell lysates by anti-Sm and anti-RNP sera. [35S]methionine labeled cells were lysed in the presence of SDS. The lysate was subjected to immunoprecipitation and the immunoprecipitates were analyzed by SDS gel electrophoresis and fluorography as described in Materials and Methods. Lane 1 represents an immunoprecipitation obtained by anti-Sm serum. Immunoprecipitation supernatant, obtained from three times the amount of immunoprecipitate shown in lane 1, was divided into three equal aliquots and reprecipitated with anti-Sm serum (lane 2), anti-RNP serum (lane 3), and normal serum (lane 4), thus demonstrating the physical dissociation of Sm- and RNP-specific bands after SDS treatment. Lane 5 represents an immunoprecipitate obtained by anti-RNP serum. Immunoprecipitation supernatant, obtained from three times the amount of immunoprecipitate shown in lane 5, was divided equally into three aliquots and reprecipitated with anti-RNP serum (lane 6), anti-Sm serum (lane 7), and normal human serum (lane 8), confirming the SDS induced dissociation of snRNP. All lanes represent immunoprecipitates from equal volumes of cell lysate.

FIG. 4. (Right) Nitrocellulose blot analysis of purified rat liver nuclei with anti-Sm and anti-RNP sera. Purified rat liver nuclei were solubilized with SDS and subjected to gel electrophoresis. Polypeptides were then transferred to nitrocellulose paper as described in Materials and Methods. Molecular weights were determined by mobility relative to radioactive standards. Two antigens, one at 25 and one at 16 kd, were identified by incubation of the blot with anti-Sm sera and subsequently by 14C-labeled rabbit anti-human IgG antibodies. Nitrocellulose strips were then fluorographed. Lanes 1–5, individual anti-RNP sera; lanes 6–10, individual anti-Sm sera.

Immunoprecipitation, we also examined anti-Sm and anti-RNP reactivity by nitrocellulose blot procedures. Isolated rat liver nuclei, rabbit thymus extract, whole rat and rabbit kidney, spleen, thymus, and liver, as well as KE37 and BRL cells were solubilized in SDS, and subjected to SDS gel electrophoresis. The polypeptides were then blot transferred to nitrocellulose paper, as described in Materials and Methods.

When isolated rat liver nuclei were examined with various anti-Sm and anti-RNP sera, two major bands were visualized with each anti-Sm serum tested (lanes 6–10, Fig. 4), whereas anti-RNP sera did not consistently label any band with high intensity (lanes 1–5, Fig. 4). The two bands reacting with anti-Sm sera corresponded by molecular weight to bands b, c (25 kd) and band e (16 kd), identified by immunoprecipitation of non-denatured cell lysates. The other faint bands visualized on the nitrocellulose blots by anti-Sm sera were also recognized by anti-RNP sera as well as by SLE sera, which were negative for anti-Sm and anti-RNP antibodies in serological
analyses, but not with normal human sera, indicating that they are not related to the Sm antigen. These results for the Sm system were clear cut and relate well to the immunoprecipitation results. The RNP antibodies did not consistently label any band which could be correlated to the presence of anti-RNP antibodies.

Nitrocellulose blot analysis of rabbit thymus extract prepared from rabbit thymus acetone powder widely used in serological studies gave results that were slightly different from those obtained with rat liver nuclei. Anti-Sm sera reacted with bands of 70 and 16 kd (lanes 5–8, Fig. 5), whereas anti-RNP sera reacted with a 70-kd band (lanes 1–4, Fig. 5). The 25-kd protein seen after probing blots of rat liver nuclei with anti-Sm sera was totally absent in blots of rabbit thymus extract, whereas the 70-kd band seen after probing rabbit thymus extract blots with either sera was never seen in blots of rat liver nuclei with anti-Sm sera. In contrast to the conventionally used saline extract, if the thymus tissue or the acetone powder was directly solubilized in SDS and examined on blots, the 25-kd band could be detected by anti Sm serum. We feel that the 25-kd Sm band is degraded during saline extraction of the acetone powder.

![Fig. 5](image)

**Fig. 5.** (Left) Nitrocellulose blot analysis of rabbit thymus extract with anti-Sm and anti-RNP sera. Rabbit thymus extract was incubated with SDS and subjected to SDS polyacrylamide electrophoresis. Polypeptides were transferred to nitrocellulose and probed with anti-Sm and anti-RNP sera as described in Materials and Methods. Molecular weights were determined by mobility relative to radioactive standards. Lanes 1–4, representing blots probed with anti RNP sera, show labeling of a 70-kd band. Lanes 5–8, representing blots probed with anti-Sm sera, demonstrate labeling of the 70-kd and a 16-kd band.

**Fig. 6.** (Right) Immunoprecipitation of polypeptides that are not related to the Sm and RNP antigens by individual SLE sera. Sera from three SLE patients were used for immunoprecipitation exactly as described in Fig. 1A except that cell lysates were prepared from [35S]methionine human lymphoid cell line 8866P. Analysis by SDS gel electrophoresis demonstrated the presence of previously undescribed autoantigens (arrows). Lanes 1–3, SLE sera immunoprecipitates; lane 4, normal human sera immunoprecipitate. Molecular weight markers × 10⁻³ are shown in the margin.
In view of the differences noted above, the species, and tissue distribution of these antigens were studied by similar blot analysis on liver, kidney, spleen, and thymus from rat and rabbit, as well as cultured rat and human cell lines (data not shown). The same anti-Sm bands seen in rat liver nuclei were visualized in the other rat tissues and the rat liver cell line. Additional bands were also often visualized that seemed to be either cytoplasmic or tissue specific and not clearly related to Sm and RNP antigens. Kidney and liver seem to contain weaker Sm bands than spleen or thymus, probably reflecting differences in the ratio of nucleus to cytoplasm. Similar results were obtained when rabbit tissues were examined. When rat tissues or the rat liver cell lines was compared to human cells, some differences were noted. Human cells contained two immunoreactive bands in the 25-kd region of the blot and a minor band in the 16-kd range, whereas rat tissues and cells contained only one band in the 25-kd range and a stronger 16-kd band. These differences were also noted in the immunoprecipitation studies.

The nitrocellulose blot experiments did not significantly help in elucidating the nature of the RNP antigen. Anti-RNP sera did appear to recognize a number of bands that did not correlate well to the level of anti-RNP antibodies detected by serological analysis. One band of ~68 kd seemed to be labeled consistently by anti-RNP sera when whole tissues were examined. The relationship of this band to the 70-kd band labeled by anti-Sm and anti-RNP sera in rabbit thymus extract is not known.

Detection of Other Autoantibodies. In the course of these studies, it was noted that several SLE sera gave bands that did not relate to the content of anti-Sm or anti-RNP antibodies in these sera or to any known antibody system including the Ro and La systems of Reichlin. Fig. 6 shows immunoprecipitates of nondenatured cell lysates using sera from three different SLE patients (lanes 1–3). Several bands are specifically precipitated by each serum and not by control serum (lane 4). Corresponding bands were observed in other SLE sera; the lower band in lanes 1 and 2 has been observed in 6 of 30 SLE sera studied. Studies are currently underway to characterize the antigens involved.

Discussion

Although immunoprecipitation of nondenatured cell lysates by anti-Sm and anti-RNP sera gave similar sets of protein bands on polyacrylamide gels, treatment of the cell lysates with SDS to break up the complex before immunoprecipitation proved to be effective in distinguishing the two types of sera. All anti-Sm and anti-RNP sera used in immunoprecipitates of SDS-treated cells revealed the presence of different sets of proteins that corresponded to the specificity of the sera. Anti-Sm sera precipitate 25- and 16-kd proteins (bands b, c, and e), whereas anti-RNP sera only precipitate a 19-kd protein (band d). These proteins are subsets of those seen after immunoprecipitation of cell lysates before SDS treatment. Patient’s sera, which contained antibodies of both specificities precipitated mixtures of the proteins found after precipitation with prototype anti-Sm and anti-RNP sera. These data suggest that the proteins found after immunoprecipitation of SDS-solubilized cells represent, in part at least, the antigenic determinants of Sm and RNP. Although the proteins found after immunoprecipitation of SDS-treated cell lysates may contain the Sm and RNP antigenic sites in their entirety, we cannot rule out that the SDS solubilization
denatured other potentially immunoreactive proteins (e.g., bands f, g, and h). It is clear that the SDS treatment before immunoprecipitation abrogates the interaction of the immunoreactive proteins with each other and the other proteins in the snRNP particles. Immunoprecipitation of SDS-treated cell extracts with a mouse anti-Sm monoclonal antibody confirmed the assignment of Sm determinants to the 25- and 16-kd proteins and suggested that these proteins are immunologically related.

The results of experiments in which immunoprecipitations were performed sequentially with both sera suggest that the antigenic snRNP can be subdivided into two classes. One class, which is precipitated by both sera, contains the RNP antigen in association with the Sm antigen. The other class is precipitated only by anti-Sm sera, and thus contains no RNP antigen. These results are consistent with the relationship of Sm and RNP lines seen in gel diffusion analysis, and with chromatographic characterization of crude nuclear extracts (3, 4). Takano et al. (15) drew conclusions similar to those reported here by successively passing nuclear extracts over immunofinity columns of anti-Sm and anti-RNP antibodies and analysing the eluates by gel diffusion analysis.

SDS polyacrylamide gel analysis of these two classes of particles show only one difference in protein composition. The class of particles which are not precipitated by anti-RNP sera lack the 19-kd band (band d). The class of particles that is precipitated by both anti-Sm and anti-RNP sera contains the full complement of proteins (band a–h) seen in immunoprecipitates of either sera. This difference supports the view that the 19-kd band represents, at least in part, the RNP antigen. In addition, the sequential immunoprecipitations suggest that bands f, g, and h may be closely related to the Sm antigen because they are found in anti-Sm immunoprecipitates after removal of the RNP antigen.

Lerner and Steitz (5) have suggested that the snRNP immunoprecipitated by anti-Sm and anti-RNP sera are distinguished only on the basis of their RNA. Their anti-RNP immunoprecipitates quantitatively recovered the U1a and U1b snRNA. Our data predict that the supernatant of their anti-RNP immunoprecipitate, which contains U2, U4, U5, and U6 RNA, will lack the 19-kd band (band d), while still retaining the other proteins of the particles. This is band c by their lettering system.

Nitrocellulose blot analysis, using anti-Sm sera, confirmed that the 25-kd proteins (bands b and c) bear at least part of the Sm determinant. These experiments also indicated that the 16-kd band (band e) carries part of the Sm determinant. The presence or absence of the 16-kd band in immunoprecipitates obtained from SDS-solubilized cells or in nitrocellulose blots seems to vary with the source of the material. Much more of the 16-kd band was visible in rat tissues and cells when compared, using either technique, with human cells. Preliminary pulse chase experiments using the human cell line KE37 suggest that a precursor product relationship between the 25- and 16-kd proteins does not exist. In addition, the presence of protease inhibitors during all steps subsequent to cell lysis and varying extraction conditions makes unlikely the possibility that these differences reflect proteolysis during the procedures.

The fact that anti-Sm sera react with the 25- and 16-kd bands even after boiling in SDS confirms that their reactivity is not dependent on their association with RNA. Boiling in SDS certainly dissociates all noncovalent RNA-protein interaction. These proteins, as well as the other proteins of the snRNP, do not contain any associated nucleic acid when immunoprecipitates of cells, labeled with $^{32}$P phosphate, are
examined by SDS gel electrophoresis (unpublished observation).

Nitrocellulose blot analysis with anti-RNP sera was much less informative concerning the nature of this antigen. Band d, which the immunoprecipitation studies suggest carries the RNP determinant, was never labeled during the nitrocellulose blot procedures. A 70-kd band was consistently labeled in blots of rabbit thymus extract by the anti-RNP sera. A less prominent but nevertheless consistently labeled band at 68-kd was also seen when whole tissues were probed with anti-RNP sera, but not when isolated rat liver nuclei were examined. The relationship, if any, between the 70-kd band in rabbit thymus extract and the 68-kd band in whole tissues is not understood, nor is either of these bands' relationship to the 19-kd protein (band d) known. The possibility that either band represents the 19-kd band in association with other components of the snRNP is unlikely, as boiling in SDS does not diminish the intensity of either the 70- or 68-kd bands.

Several explanations can be offered for lack of anti-RNP reactivity on blots of isolated rat liver nuclei. First, long incubation (~15 h) in the presence of SDS during electrophoresis may irreversibly denature the RNP antigen. The loss of RNP antigenicity after SDS treatment and later analysis in gel diffusion has been noted (E. Tan, personal communication). Second, Zieve and Penman (16) and Lerner and Steitz (5) have pointed out that the anti-RNP reactive snRNP containing U1 RNA are extracted from the nucleus under very mild conditions. It is possible that we have lost these particles during isolation of the rat liver nuclei.

Several other groups have published reports dealing with differences in the protein composition of Sm and RNP. Douvas et al. (17), using anti-RNP immunoaffinity columns, found proteins of 30 and 13 kd bound specifically to the columns. Although their 13-kd protein, based on migration in SDS gels relative to histones, could be identical to our 16-kd band e, recognized by anti-Sm sera, the preparation has no Sm activity when assayed by gel diffusion. Takano and co-workers (15) have also isolated an antigenic fraction from nuclear extracts by immunoaffinity chromatography. Their RNP preparation contained bands of 65, 30, and 13 kd, whereas their Sm preparation contained multiple bands in the 13-kd region. The proteins of their RNP preparation contained proteins similar in size to those of Douvas et al. (17). The Sm preparation of Tanako and co-workers (15) contains bands which, based on mobilities relative to cytochrome c, probably correspond to our bands e, f, g, and h seen in immunoprecipitates of native cell extracts. White and Hoch (18) examined Sm and RNP peptides in rabbit and calf thymus extract. They identified proteins at 13, 40, and 70 kd reactive with anti-RNP sera and a 13-kd protein reactive with anti-Sm sera. More recent data from their laboratory (19) suggest that anti-RNP reactivity primarily is with 68- and 70-kd proteins. Our results differ from those of the above authors in the presence of major 25-kd proteins reactive with anti-Sm sera in all detection protocols used. They more closely resemble those in a preliminary report of Hinterberger et al. (20) concerning the Sm antigens.

During these studies, it became evident that a number of additional antibodies are present in SLE sera that precipitate polypeptides found in the cell extracts used here and that do not correlate to the Sm and RNP antibody systems or other known serologically detected antibodies. The nature of the antigens detected by these sera has not been determined. Attempts are currently underway to detect these new systems serologically and to study their significance.
Evidence has been obtained previously indicating that the antigens reacting with the anti-Sm and anti-RNP sera are present as a large complex, and similar protein bands are obtained with both types of sera. In the present study, it proved possible to break up this complex using SDS treatment before immunoprecipitation. After such treatment, different protein bands were immunoprecipitated by the two antisera; Sm determinants resided primarily in proteins of 25 and 16 kd, whereas the RNP determinant resided, at least partially, in a 19-kd protein. Sequential immunoprecipitation with and without prior SDS treatment provided further evidence for these specificities and suggested that two classes of particles exist in different tissues, one containing proteins immunoreactive with the Sm and RNP antisera and the other containing proteins immunoreactive only with the Sm antisera. The latter particle contained all the bands seen with the first type except for the absence of the 19-kd band. Nitrocellulose blot analyses confirmed the assignment of the 25- and 16-kd polypeptides to Sm antigenic determinants; analyses for RNP proved less informative by this technique. Some differences in the banding patterns were obtained using cells from different species: the 25-kd Sm band was usually double in human cells and single in rat and rabbit tissue. Methods of extraction also caused some differences, which was especially true for the rabbit thymus extract widely used for Sm and RNP studies. Additional immunoreactive bands at 68 and 70 kd also were detected when the Sm and RNP antisera were used in nitrocellulose blot analyses. Furthermore, evidence was obtained for a number of other antibodies in lupus sera which have not as yet been detected by serological methods.

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