

## A 52-kD PROTEIN IS A NOVEL COMPONENT OF THE SS-A/Ro ANTIGENIC PARTICLE

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The SS-A/Ro autoantigen is a small intracellular RNA-protein complex that is the target of autoantibodies present in the sera of many patients with SLE and Sjogren's syndrome (SS)<sup>1</sup> (1, 2). The protein component(s) of SS-A/Ro have been variously described as polypeptides with molecular masses ranging from 50 to 150 kD (Table I). In human cells SS-A/Ro is associated with at least four RNAs (Y RNAs), ranging from 80 to 112 bases (3). The antigenic reactivity of SS-A/Ro is apparently independent of RNA since the precipitin activity of SS-A/Ro is not affected by RNase and since autoantibodies bind the protein after separation from the RNA (4). Immunological properties of the SS-A/Ro antigen extracted from human and other mammalian tissues were initially reported by Clark et al. (5), Mattioli and Reichlin (6), and Alspaugh and Tan (7). Recently, it has been the subject of more definitive biochemical evaluation using Western blot and ELISA techniques (8–10).

Despite our considerable knowledge of these particles, the number and identity of the proteins associated with the SS-A/Ro particle have not been well defined. Wolin and Steitz (3) reported that the major antigenic protein present in human HeLa cells was a polypeptide of 60 kD. Elkouss and Culhane (8) reported that antigenic determinants were identified on polypeptides of 50 kD and/or 57 kD. They suggested that the 50-kD species might be a degradation product of the 57-kD protein as has been shown for the 43-kD and 48-kD SS-B/La polypeptides (9, 11).

Recently we have examined sera from patients with Sjogren's syndrome for their profile of autoantibodies using several different immunochemical methods. We have detected a 52-kD protein in addition to the 60-kD polypeptide that appears to be a major component of the SS-A/Ro antigenic system. The present study was designed to characterize this protein and its relationship with the 60-kD SS-A/Ro antigen.

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<sup>1</sup> *Abbreviations used in this paper:* SS-A/Ro, nuclear antigen A of Sjogren's Syndrome; SS-B/La, nuclear antigen B of Sjogren's syndrome; PBS-T, phosphate-buffered saline with 0.5% Tween-20.

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TABLE I  
Reported Molecular Mass for SS-A/Ro

Author	Year	Mol mass	Method	Reference
		<i>kD</i>		
Clark et al.	1969	100–150	Gel filtration	5
Francoeur et al.	1982	90, 94	Immunoprecipitation	26
Venables et al.	1983	55	SDS-PAGE staining	29
Lieu et al.	1984	60	Immunoblotting	30
Wolin et al.	1984	60	Immunoprecipitation	3
Elkon et al.	1984	50, 57	Immunoblotting	8, 9
Deng et al.	1985	60	Immunoblotting	31
Herrera-Esparza et al.	1986	58	Immunoblotting	32
Bachmann et al.	1986	90, 94	Immunoblotting	25

### Materials and Methods

*Patients' Sera.* We studied sera from 61 patients with Sjogren's syndrome who were evaluated in the Rheumatology Division at Scripps Clinic and Research Foundation. Criteria for the diagnosis of Sjogren's syndrome were as previously described (12).

*Cell Extracts and Labeling (Preparation of Antigens).* HeLa (American Type Culture Collection, Rockville, MD), SAL-1 (human salivary gland tumor cell line) obtained from Dr. R. I. Fox (Scripps Clinic) were grown in monolayer cultures with DME containing 10% calf serum at 37°C in a 10% CO<sub>2</sub> incubator. The Raji cell line was obtained from Dr. A. N. Theofilopoulos (Scripps Clinic).

Cultures were supplemented with 2.5 µg/ml gentamicin sulfate and used at subconfluent densities. Cells were washed twice with PBS, and extracted in buffer A (150 mM NaCl, 10 mM Tris-HCl, pH 7.2, 0.5% NP-40) or in Empigen BB (Albright and Wilson, Old Tappan, NJ) and freed of cell nuclei by centrifugation at 12,000 *g* for 15 min. Wil-2 extract was prepared by sonication according to a previously described method (13). Cells were labeled for 16 h with [<sup>32</sup>P]orthophosphate (NEN Research Products, Boston, MA), or [<sup>35</sup>S]methionine (Research Products International Corp., Mount Prospect, IL) both at 100 mCi/ml in phosphate- or methionine-free media (Flow Laboratories Inc., McLean, VA), respectively, each supplemented with 2% calf serum. The cells were then harvested as described above.

*Gel Double Diffusion (Ouchterlony).* Antibodies to the SS-A/Ro antigen were detected by using double immunodiffusion in 0.5% agarose. Precipitin lines were formed after interaction of Wil-2 extract (protein concentration 20 mg/ml) with 1:1 and 1:30 dilutions of sera and read after 48 h (14).

*Polyacrylamide Gel Electrophoresis (PAGE).* SDS-PAGE was performed following the method described by Laemmli (15), in 15% acrylamide gel slabs. The following protein standards were used to determine molecular weights: phosphorylase B, 92,500; BSA, 66,200; OVA, 45,000; carbonic anhydrase, 31,000; soybean trypsin inhibitor, 21,500; and lysozyme, 14,400.

*Western Blotting.* Electrophoretic blotting was performed using the method of Towbin et al. (16) with some modifications. Briefly, proteins were separated by SDS-PAGE and were electrotransferred to a nitrocellulose sheet for 3 h at constant voltage of 60 V. The nitrocellulose sheets were then incubated for 1 h in PBS with 0.5% Tween 20 (PBS-T) and 3% nonfat milk to block nonspecific binding sites. Then the nitrocellulose strips or sheets were incubated with a 1:100 dilution of sera, washed for 1 h in PBS-T. <sup>125</sup>I-protein A (ICN Biochemicals, Irvine, CA) was used to detect bound human Ig.

*Affinity Purification of Autoantibodies.* Anti-SS-A/Ro and anti-SS-B/La antibodies were affinity purified according to previously described methods (17, 18). Briefly, HeLa cell extracts (protein concentration 25 mg/ml) were electrophoretically separated on 30-cm lengths of 15% polyacrylamide gels and transferred to nitrocellulose paper as described

above. To identify the proteins, the outer lanes of the nitrocellulose sheet were blotted and detected by positive anti-SS-A/SS-B sera using  $^{125}\text{I}$ -protein A. The protein bands were cut out as horizontal strips from the remaining nitrocellulose, cut into smaller pieces ( $\sim 1\text{ mm}^2$ ) and washed in PBS-T and 3% nonfat milk for 1 h. Then they were incubated for 1.5 hours with a human anti-SS-A/SS-B autoimmune serum diluted 1:50 in PBS-T and 3% nonfat milk, followed by washing six times with PBS-T, twice in PBS, and once in 0.25 M KCl in PBS for 2 min. For elution of specifically bound antibodies, the nitrocellulose was incubated with 0.2 M glycine-HCl, pH 2.7, and 1 mg/ml BSA for 2 min. The eluate was neutralized immediately with 1 M Tris-HCl, pH 8.7, dialyzed against PBS overnight and concentrated by dialysis against PEG 20,000 (Kodak).

*Partial Proteolysis of SS-A/SS-B Antigens.* The procedure followed the method of Cleveland et. al. (19) with modifications. In the first dimension, 190  $\mu\text{l}$  of HeLa cell extract (25 mg/ml) in Laemmli sample buffer was loaded onto a 1-mm thick 15% polyacrylamide gel. After electrophoresis, the gel was cut into 10 slices of 0.8 cm and soaked in Laemmli sample buffer with 0.1% SDS for 15 min. Then the slices were loaded onto 1.5-mm thick 15% polyacrylamide gel. 300  $\mu\text{l}$  of a protease solution (*Staphylococcus aureus* V8 protease; Sigma Chemical Co., St. Louis, MO) at different concentrations (1–50  $\mu\text{g/ml}$ ) in Laemmli sample buffer with 0.1% SDS was layered on top of the gel slices. During the electrophoresis, proteolysis was allowed to occur in the stacking gel.

*Immunoprecipitation and SDS-PAGE Analysis of Labeled Cellular Antigens.* Protein A–Sephadex-facilitated immunoprecipitation of labeled cellular antigen by antisera or affinity-purified antibodies was performed as described (11). Typically, 10  $\mu\text{l}$  of antisera were added to 500  $\mu\text{l}$  buffer B (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) and 100  $\mu\text{l}$  of 10% (wt/vol) protein A–Sephadex beads in buffer B. After mixing for 1 h at 4°C the beads were washed three times in buffer B. Then 25  $\mu\text{l}$  of labeled extract, 20  $\mu\text{l}$  BSA (50 mg/ml), and 500  $\mu\text{l}$  buffer B were added and mixed for 1 h more at 4°C. The precipitates were washed five times with 1 ml buffer B. The protein was eluted by boiling in Laemmli sample buffer and analyzed by SDS-PAGE (15). For RNA analysis, precipitates were extracted with phenol and RNAs were precipitated with ethanol and electrophoresed in 7 M urea, 8% polyacrylamide gels.

*Immunofluorescence Studies.* Prefixed HEp-2 cell slides were obtained from Bion (Park Ridge, IL). Additional cell lines were grown in Lab-Tek tissue culture chambers (Miles Laboratories, Naperville, IL) following manufacturer's instructions and fixed in a mixture of acetone and methanol 1:3 at  $-20^\circ\text{C}$  for 2 min followed by air drying. Human monospecific sera as well as affinity-purified antibodies were tested. Antibodies were detected using fluorescein-labeled affinity-purified goat anti-human Ig A+G+M (H+L) antibodies (Calbiochem-Behring Corp., La Jolla, CA).

## Results

61 patients with Sjogren's syndrome were analyzed for anti-SS-A/Ro autoantibodies by Ouchterlony immunodiffusion using Wil-2 cell extracts as the antigen source. 42 patients (69%) possessed anti-SS-A/Ro antibodies as demonstrated by the presence of immunoprecipitin lines displaying identity to the anti-SS-A/Ro prototype and 27 (44%) had anti-SS-B/La activity. The fine specificities of the SS-A/Ro antigen-antibody system were further resolved into four classes using immunoblot analysis. 21 (50%) of the 42 sera reacted with two proteins of 60 and 52 kD, 13 sera (31%) reacted primarily with the 52-kD and not the 60-kD protein, two sera detected only the 60-kD antigen, and six sera were nonreactive with either protein. Noteworthy is the fact that the two sera reactive with the 60-kD and not with the 52-kD protein were from SLE patients and contained anti-Sm and other antibodies as well. Representative immunoblots of each of the three reactive groups of sera are shown in Fig. 1 where soluble

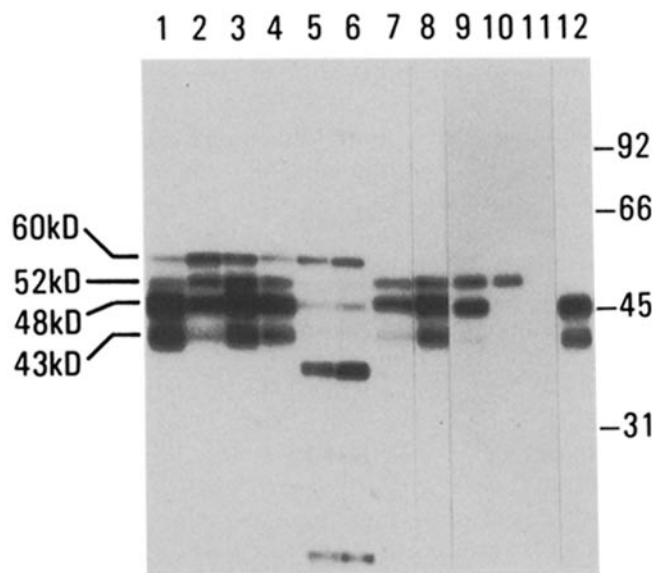


FIGURE 1. Immunoblotting analysis of reactivities of representative sera from patients with SS, using HeLa cell extract. Lanes 1-4 demonstrate the presence of antibodies to 60-, 52-, 48-, and 43-kD bands. Lanes 5 and 6 show antibodies to the 60-kD protein and not to the 52-kD antigen. Lanes 7-10 show antibodies to the 52-kD band but not the 60-kD polypeptide. Lane 11 represents normal serum and lane 12 is a monospecific anti-SS-B prototype serum. The 43-kD band is a degradation product of the 48-kD SS-B protein. Note that sera 5 and 6 derived from SLE patients also contained other antibodies. Serum 10 served as a monospecific anti-52-kD antiserum in subsequent studies.

HeLa cell proteins were used as antigen. Identical immunoblot results were obtained with Raji and Wil-2 cell extracts.

These data indicate a substantial degree of heterogeneity in the autoimmune response against SS-A/Ro among individual patients. Further, the existence of sera with specificity directed against either the 52- or the 60-kD antigens suggested that the antibodies present in these sera might be recognizing distinct epitopes on these proteins.

The relationship between SS-A/Ro and SS-B/La is complicated by the fact that in most sera with anti-SS-B/La specificity there are also anti-SS-A/Ro antibodies. It was previously reported that SS-A/Ro and SS-B/La might share some common antigenic proteolytic degradation fragments (9). In view of the present identification of an apparently new 52-kD component of the SS-A/Ro system it was appropriate to reexamine the antigenic relationships among the 52-, 60-, and 48-kD proteins.

The first method used partial S.a.V8 protease digestion of HeLa cell extract. Fig. 2 shows that the 60-kD protein has a 44-kD degradation product, the 52-

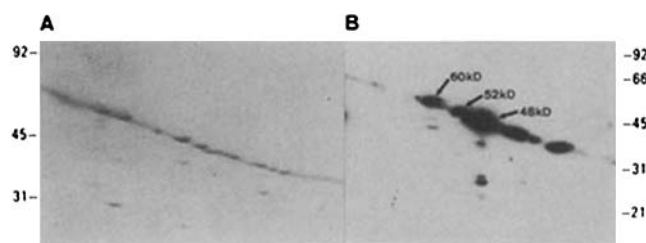


FIGURE 2. Partial proteolysis of HeLa cell extract by S.a. V8 protease. (A) Commassie blue staining of two dimensional 15% polyacrylamide gel of cell extract digested with 10 µg/ml protease. (B) Immunoreactive fragments of the different proteins 60 kD, 52 kD, and SS-B (48 kD), as detected by antibody and  $^{125}\text{I}$ -

labeled protein A. None of the degradation products of SS-A or SS-B have the same molecular weights. Note that the scales for the molecular weight markers in the two panels are different.

kD has a 38-kD fragment and the 48-kD SS-B/La has the 43-kD as well as X/X' and Y fragments (9). No common antigenic polypeptide fragments were observed among the 52-, 60-kD and SS-B/La 48-kD proteins even when a variety of protease concentrations were used (data not shown).

From Fig. 1 it is clear that some sera reacted only with the 52-kD and others only with the 60-kD polypeptide. However, we wanted to determine whether in sera reactive with both proteins the antibodies were crossreactive. Fig. 3 shows the reactivity of high titer sera that contained antibodies to 60-kD, 52-kD, and SS-B/La proteins (lanes 1-2). Affinity-purified antibodies from either the 60- or the 52-kD band reacted specifically with 60- and 52-kD antigens respectively (lanes 3-4). Affinity-purified antibodies from the 48-kD SS-B/La protein reacted with the 48-kD band and its degradation product of 43 kD (lane 5). These data indicate that the above antigens contained different and unique epitopes.

The lack of crossreactivity among affinity-purified antibodies demonstrated that the two antigens, 52 and 60 kD, were immunologically distinct. However, both proteins were simultaneously targeted for autoimmune response in 50% of the Sjogren's syndrome patients who were positive for SS-A antibody by immunodiffusion analysis. It was therefore of interest to examine the composition of antigens in the precipitin lines observed in immunodiffusion plates. SS-A precipitin lines were excised from Ouchterlony immunodiffusion plates, washed thoroughly, separated on SDS-PAGE and transferred to nitrocellulose. Analysis performed on precipitins isolated from three different sera using Western blotting demonstrated the presence of immunoreactive 52- and 60-kD proteins in these precipitin lines (data not shown). On the other hand, the SS-B precipitin lines analyzed in a similar fashion showed only the 48-kD protein and its 43-kD degradation product. The high frequency of combined anti-52-kD and anti-60-kD antibodies in anti-SS-A/Ro sera as well as the single precipitin line of anti-SS-A/Ro produced by both antibodies in Ouchterlony immunodiffusion led us

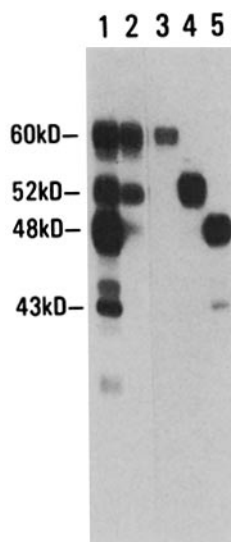


FIGURE 3. Immunoblotting analysis of the reactivities of whole sera and affinity-purified antibodies to 60-kD, 52-kD, and SS-B proteins, using HeLa cell extract. Lane 1 represents a serum from a patient with SS, containing antibodies to 60-kD, 52-kD proteins as well as to the 48-kD and 43-kD components of SS-B. Lane 2 shows reactivity of serum positive mainly for anti-60-kD and 52-kD antibodies. Lanes 3-5 demonstrate the affinity-purified antibodies to each component, 60-kD, 52-kD, and SS-B components, respectively. The serum in lane 1 served as a source for the purification of the affinity-purified antibodies.

to investigate the possibility that these proteins may coexist in the same subcellular particle.

Immunoprecipitation was used to determine whether the two SS-A associated antigens show any physical relationship in their native state. If the 52-kD and 60-kD antigens are tightly associated, they might be expected to coprecipitate even when monospecific or affinity-purified antibodies were used. Fig. 4 shows the results of an experiment in which either whole sera or affinity-purified antibodies were used to probe [<sup>35</sup>S]methionine-labeled HeLa cell extracts by immunoprecipitation. Affinity-purified anti-60-kD antibody precipitated a protein migrating at 60 kD (lane 5). Anti-52-kD antibodies precipitated a 52-kD component and, also, a 60-kD component (lane 6). The fact that anti-52-kD-specific antibodies precipitated both proteins suggests that at least some 52-kD antigen was complexed with 60 kD in the native state. In other experiments, where increasing amounts of affinity-purified anti-60-kD antibodies were added to [<sup>35</sup>S]methionine-labeled cells, it has been possible to show coprecipitation of a weaker 52-kD band. The possible implications of this finding are discussed below.

A complementary approach for examining the *in vivo* relationship between the 52- and 60-kD antigens is to analyze the RNAs species associated with each protein. For this purpose, we used different affinity-purified antibodies and several sera possessing various combinations of anti-SS-A/Ro and anti-SS-B/La antibodies. Fig. 5 shows that sera containing antibodies only against 52-kD protein precipitated the same spectrum of RNAs as did sera containing anti-52-kD and 60-kD antibodies. The affinity-purified anti-60-kD and anti-SS-B antibodies brought down the expected RNAs (lanes 9 and 10, respectively) and the affinity-purified anti-52-kD antibodies (lane 8) brought down the same species of RNAs as the affinity-purified anti-60-kD antibody, although the signals were weaker. The results with the monospecific sera as well as affinity-purified antibodies confirmed the notion that both antibodies are capable of precipitating the same RNA species, a fact that may suggest either that the two proteins share the same RNA binding sites or that some of the 60- and 52-kD proteins are in association with each other so that each of the antibody populations is capable of precipitating the same spectrum of RNAs.

Using a monospecific anti-52-kD serum and affinity-purified antibodies, we have analyzed the intracellular localization of the 52- and 60-kD proteins by

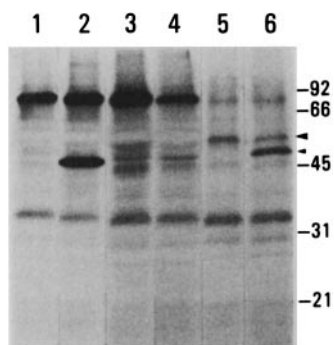


FIGURE 4. Immunoprecipitation of [<sup>35</sup>S]methionine-labeled HeLa cell extract. Lane 1 represents a normal serum control. Lane 2 shows 48-kD protein precipitated by monospecific anti-SS-B serum. Lane 3 represents serum that contained anti-60-kD and anti-52-kD antibodies, while lane 4 represents monospecific anti-52-kD antiserum. Lanes 5 and 6 demonstrate the immunoprecipitation by affinity-purified anti-60-kD and anti-52-kD antibodies, respectively. Noteworthy is the fact that affinity-purified anti-52-kD antibodies also precipitated a faint band of 60-kD protein (long arrow points to 60-kD and short arrow to 52-kD bands). The bands of 80 kD and 33 kD are not specific since they appear in the normal control. The additional band below the 52-kD protein in lane 3 was not identified.

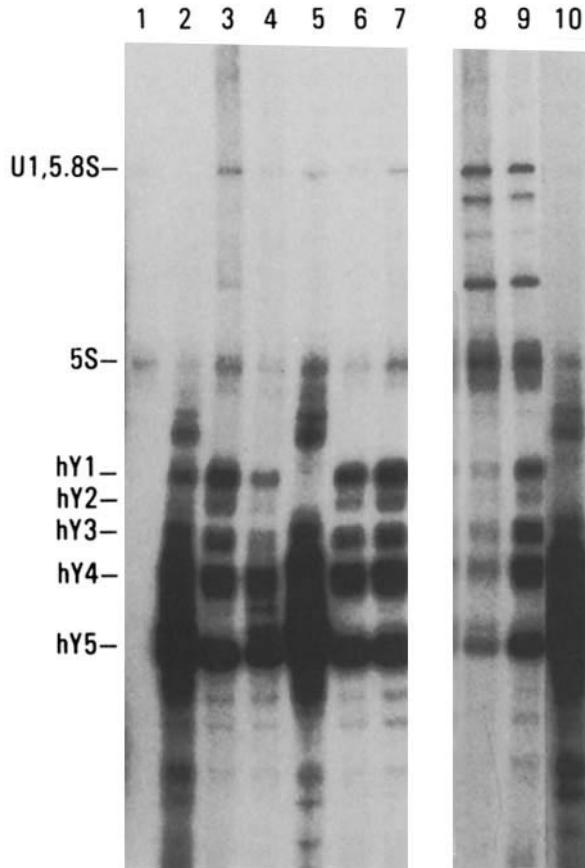


FIGURE 5 Immunoprecipitation of [ $^{32}$ P]phosphate-labeled HeLa cell extract by sera from patients with SS and by affinity-purified antibodies. RNA was extracted from the immunoprecipitate by phenol, precipitated with ethanol, and analyzed on 7M urea, 8% polyacrylamide gel. Lane 1 represents normal serum control. Lane 2 shows reactivity of serum containing anti-SS-A and anti-SS-B antibodies. Lanes 3 and 4 demonstrate the RNAs precipitated by sera containing both anti-60-kD and anti-52-kD antibodies. Lane 5 reveals reactivity of monospecific anti-SS-B antiserum. Lanes 6 and 7 represent sera containing anti-52-kD antibodies only. Lanes 8, 9, and 10 demonstrate the precipitation by affinity-purified anti-52-kD, anti-60-kD, and anti-SS-B antibodies, respectively. The spectrum of RNAs brought down by either whole serum containing anti-52-kD antibodies or by affinity-purified antibody are the same as that shown by anti-60-kD antibodies. The designation of the Y RNAs is based on the data of Hendrick et al. (24).

indirect immunofluorescence. In both HeLa and Hep-2 cells the patterns of intracellular distribution of the 52- and 60-kD antigens were indistinguishable, displaying the same patterns of strong punctate nuclear and slight cytoplasmic staining (Fig. 6).

Finally, the distribution and relative abundance of the 52-kD protein in various cell lines were examined by immunoblotting. The lymphocytic cell lines Raji, Wil-2, and Molt-4, as well as epithelial-derived cell lines, HeLa and SAL-1, all contain the 52-kD protein. Further, in each cell line the 52-, 60- (SS-A) and 48-kD (SS-B) polypeptides were present in similar quantitative ratios (Fig. 7).

### Discussion

Autoantibodies to a wide variety of cellular constituents have been reported in different rheumatic diseases. Among these are anti-SS-A/Ro and anti-SS-B/La antibodies detected mainly in Sjogren's syndrome and SLE (1-2). The identity of these antibodies was initially based upon immunofluorescence patterns and Ouchterlony double diffusion analysis (5-7). Subsequently, a variety of methods have been applied to define the molecular identity of the SS-A/Ro anti-

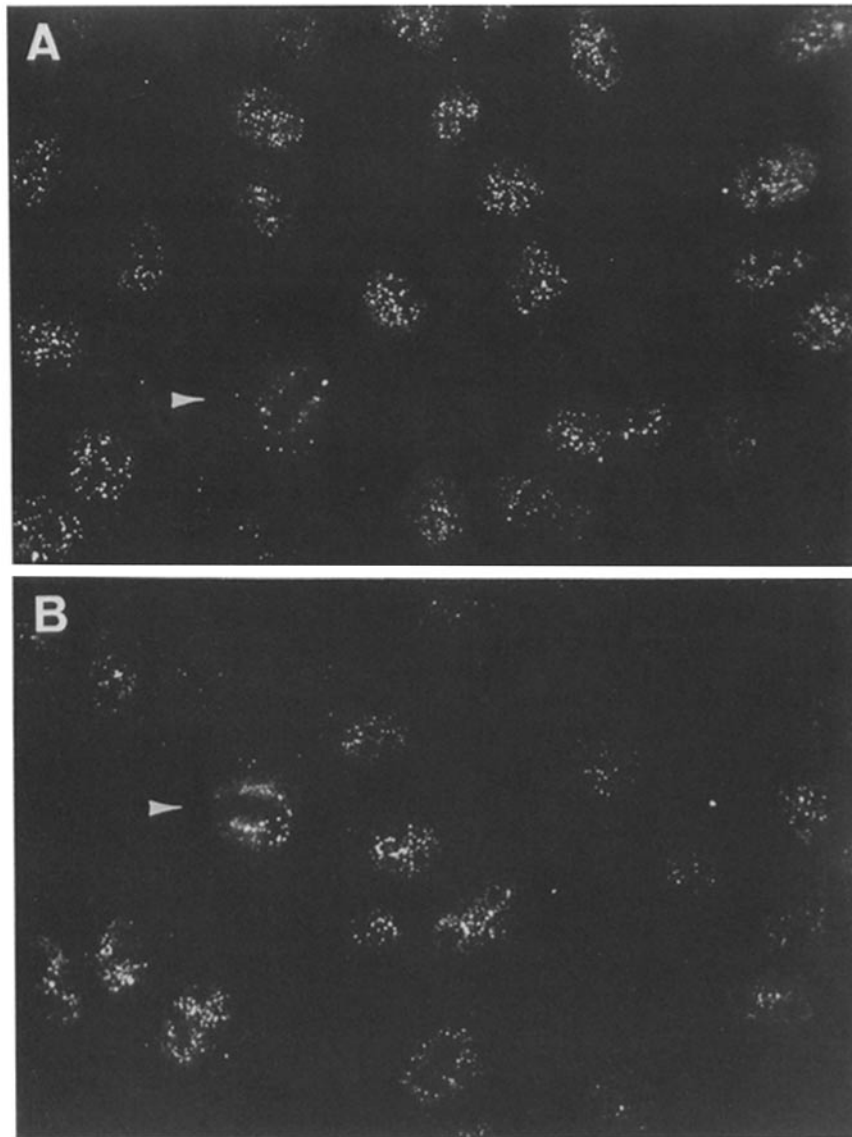


FIGURE 6. Indirect immunofluorescence of prefixed HEp-2 cells. (A) Affinity-purified anti-60-kD antibodies; (B) the serum is monospecific for anti-52-kD antibodies. Note the similar punctate staining patterns in both cases and the absence of localization in condensed chromosomes of the mitotic cells in prophase (*arrows*).

gen(s). The 60-kD protein appears to represent the major component of the SS-A/Ro antigen system in several reports but there still is a confusing array of other candidate proteins (Table I). This variation in results could be due in part to differences in experimental methods and sera used to identify the SS-A/Ro antigens.

In this work we have used immunoblotting and immunoprecipitation to inves-



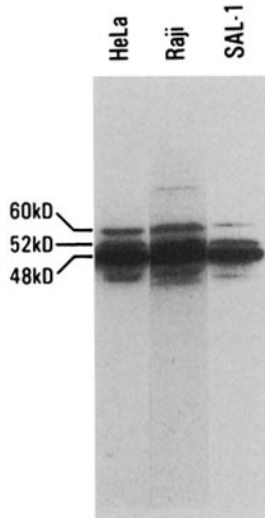


FIGURE 7. Immunoblotting analysis of extracts of different cells. Both 60-kD and 52-kD polypeptides are present in all three cell lines (HeLa, Raji, and SAL-1). Other cell lines showing similar results were Molt-4 and Wil-2.

tigate the antigens recognized by 42 SS-A/Ro<sup>+</sup> sera. We have consistently detected a 52-kD band in >80% of SS-A/Ro precipitin-positive sera, strongly suggesting that this protein is, with the previously described 60-kD antigen, an additional component of the SS-A/Ro autoantigen system. This 52-kD polypeptide displays antigenic differences from the previously described 60-kD protein since each can react with a unique antibody population (Fig. 1). The possibility that the 52-kD protein is a degradation product of the 60 kD is unlikely based on the lack of crossreactivity between antibodies affinity purified from each protein as well as the lack of common protease-resistant fragments in the partial proteolysis of these proteins.

While the 52-kD protein is distinct from the 60-kD antigen, several lines of evidence suggest that in their native state they may exist as a complex or as associated particles. In Ouchterlony analysis, antibodies against the 52-kD protein form a precipitin line indistinguishable from that of the 60-kD antigen. Further, using immunoprecipitation, affinity-purified anti-52-kD antibodies coprecipitate the 60-kD antigen. Moreover, the profiles of small RNAs immunoprecipitated by antibodies specific for either protein are indistinguishable (Fig. 5). In indirect immunofluorescence, both proteins share a similar intracellular localization pattern (Fig. 6). Western blotting under nonreducing conditions did not show a slower migrating protein band, suggesting that the association between the 52- and 60-kD proteins is not through disulfide bonding (data not shown).

For the Sm antigen-antibody system it has been shown that there exist two forms of antigen, one that is free and another that is bound in a complex of Sm and U1-RNP (20). In fact, earlier immunological studies had already indicated the existence of free Sm and of Sm bound to nuclear U1-RNP (6, 21). Recent studies suggest that the interaction between Sm and U1-RNP might be even more complex, since biochemical separation methods demonstrate three to four fractions, some of which contain Sm antigen alone and others Sm associated with U1-RNP (22). A similar interaction may exist for SS-B/La and SS-A/Ro

antigens (23–24). The data presented here suggest that some, if not most of the 52-kD protein may be complexed with the 60-kD protein. However, since large amounts of affinity purified anti-60-kD antibodies were required to coprecipitate the 52-kD protein, the data might be interpreted to reflect a situation analogous to Sm and U1-RNP, that is, that the 60-kD antigen may exist in both the free form and as a complex with the 52-kD protein.

It is difficult to place in the proper context the relationship of the 52-kD antigen described here with many other antigens of somewhat similar size described by others (3, 9, 25–27). The distinguishing features in this report are the demonstration that the 52-kD antigen contains a distinctive epitope and is a separate protein species from the 60-kD protein and the possible physical interaction between the 52- and 60-kD proteins.

In summary, the work described here defines a new component of the SS-A/Ro antigen particle as a 52-kD protein present in the sera of 80% of patients with detectable SS-A/Ro precipitins. While the biological function of the SS-A/Ro antigen-containing RNP particle is not precisely known, it has been proposed that it plays a role in regulating the distribution of cytoplasmic mRNA into translationally active or inactive forms (28). Further characterization of the novel 52-kD protein using biochemical and molecular biological techniques is being pursued in the hope of gaining a better understanding of the physiological function of the SS-A/Ro antigens and the abnormal events that result in their behavior as immunogens in autoimmune diseases.

### Summary

Anti-SS-A/Ro autoantibodies are found in the sera of patients with Sjogren's syndrome (SS) and SLE. In the course of analyzing 61 SS patients for their autoantibody profiles, we found that 42 were positive for anti-SS-A by double diffusion in agarose and demonstrated precipitin lines identical to that produced by a prototype anti-SS-A serum. Further analysis of these SS-A antibody-positive sera by Western blotting of cell extracts revealed that 21 sera reacted with two proteins of 60 and 52 kD, 13 sera reacted with 52-kD protein, two detected only 60 kD, while six were nonreactive. Affinity-purified anti-60-kD and anti-52-kD antibodies reacted exclusively with their corresponding antigens. Partial proteolysis of these proteins did not reveal common degradation fragments. Thus the 52- and 60-kD proteins were found to be antigenically and apparently structurally distinct from each other. They were also distinct from 48-kD SS-B/La protein. In immunoprecipitation using labeled cell extracts, affinity-purified anti-52-kD antibodies brought down the 52-kD protein as well as the 60-kD band. In [<sup>32</sup>P]orthophosphate-labeled HeLa cell extract both antibodies precipitated the same spectrum of small RNAs (hYI-5). In indirect immunofluorescence, anti-52-kD and anti-60-kD antibodies immunolocalized in similar subcellular structures and showed similar punctate nuclear staining patterns. Western blot analysis revealed that both proteins were present in lymphocytic as well as epithelial human cell lines tested. The data above define a new antigen of 52 kD which is another component of the SS-A particle and is associated in complex formation with the previously reported 60-kD protein.

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