HUMAN AUTOANTIBODY-REACTIVE EPITOPES OF SS-B/La ARE HIGHLY CONSERVED IN COMPARISON WITH EPITOPES RECOGNIZED BY MURINE MONOCLONAL ANTIBODIES

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SS-B/La is a nuclear protein that is one of the target antigens of autoantibodies found in the sera of patients with SLE and Sjogren's syndrome (1, 2), and is transiently associated with a number of small RNA species in the form of ribonucleoprotein particles (3, 4). The SS-B-associated RNA species include RNA polymerase III transcripts such as precursors of tRNA and 5S RNA, VA RNA, EBER RNA, 4.5 RNA, 4.5I RNA, 7S RNA, Y RNA, and U6 RNA (3–11). In addition, it has been reported that SS-B/La binds to U1 RNA (12), an RNA polymerase II transcript, and to the vesicular stomatitis virus leader RNA (13). It appears that the 3′-oligouridylate tail of these small RNAs is required for interaction with the SS-B protein (7, 8, 10). The fact that SS-B/La is associated with the precursors of 5S RNA and tRNA but not with their corresponding mature species has been taken to imply that it may have a role in the maturation of these RNAs (3).

We have previously shown (14) that there are two protease-resistant domains in SS-B from HeLa cells. These domains are ~28 and 23 kD and have been provisionally called the X and Y domains, respectively. Domain X is rich in methionine but lacks phosphorylated amino acid residues, whereas domain Y contains little methionine but all of the detectable phosphorylated amino acids. The phosphorylated domain Y is not required for RNA binding, and UV-crosslinking experiments have demonstrated that the RNA binding site is located on domain X (15). Most human anti-SS-B autoantibodies react with both structural domains but some react only with one of the two domains (14). It is clear that there are at least two distinct autoantibody-reactive epitopes on the native SS-B protein, each located on a separate structural domain (14). Recent reports (16–20) have addressed the question as to whether autoantibodies to nuclear antigens such as SS-B/La, SS-A/Ro, and Sm/RNP are inducible in mice by immunization with these nuclear antigens. Some workers have been able to produce true autoantibodies by immunization (17–19), while others have been

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able to obtain only antibodies reactive with heterologous but not autologous proteins (16). In this study, we have established five anti-SS-B mAbs that were useful in defining differences between autoantibody-reactive epitopes and epitopes recognized by experimentally induced antibodies. The data might provide insights into the nature of epitopes on autologous proteins that are target antigens of spontaneously occurring autoantibodies.

Materials and Methods

Cell Culture and Labeling. HeLa (human), Vero (monkey), R9ab (rabbit), MDBK (bovine), 3T3 (mouse), BHK-21 (hamster), and PtK2 (rat kangaroo) cells were obtained from American Type Culture Collection (Rockville, MD) and grown in monolayer cultures with DMEM containing 10% calf serum at 37°C in a 10% CO_2 incubator. Mouse S49.1 cells (American Type Culture Collection) were grown as above except in suspension cultures. Rat 6m2 cell line was supplied by Dr. R. B. Arlinghaus (M. D. Anderson Hospital, Houston, TX) and was grown in DMEM with 15% calf serum. Cultures were supplemented with 2.5 μg/ml gentamycin sulfate and used at subconfluent densities. Monolayer cells were labeled with [^{32}P]orthophosphate (New England Nuclear, Boston, MA) or [^{35}S]-methionine (Research Products International Corp., Mount Prospect, IL) at 100 μCi/ml in phosphate-free medium or methionine-free medium (Flow Laboratories, Inc., McLean, VA), respectively, each supplemented with 2% calf serum and grown for 16 h. Cells were washed twice with PBS, harvested by scraping, extracted in Buffer A (150 mM NaCl, 10 mM Tris-HCl, pH 7.2, 0.5% Nonidet P-40); supernatants after centrifugation at 12,000 *g* for 15 min were used as the source of antigens in immunoprecipitation.

Affinity Column Purification of SS-B Antigen. IgG fraction from 10 ml of high titer anti-SS-B human serum was purified via a DEAE-Sephacel (Pharmacia Fine Chemicals, Uppsala, Sweden) column (35 x 2 cm) using conditions described (21). A 100 ml anti-SS-B affinity column was constructed by covalently crosslinking IgG to Sepharose CL-4B (Pharmacia Fine Chemicals) with the cyanogen bromide–coupling method (22) except for the following modifications. The concentration of cyanogen bromide was doubled and the coupling reaction was carried out in 100 mM sodium phosphate, pH 7.0, for 30 min at 15°C.

Extracts were obtained from calf thymus glands and fractionated by ammonium sulfate precipitation as described (14). The 60% ammonium sulfate–soluble fraction of the calf thymus extract (CTE)[1] was dialyzed in PBS and subsequently equilibrated with the affinity column overnight at 8°C. Unbound proteins were washed with 10 column volumes of PBS and then with 5 volumes of PBS also containing 0.5 M NaCl. Pure SS-B fractions were eluted with 3 M $MgCl_2$, dialyzed in PBS, concentrated with polyethelene glycol (PEG) 20,000–1 mg/ml, and stored frozen in small aliquots.

Immunization. 6–8-wk-old BALB/c mice were obtained from our own Animal Breeding Facility, Scripps Clinic and Research Foundation. The immunization protocols for five groups of mice are summarized in Table I. Basically, two types of immunogens were used: (*a*) 50-μl beads (protein A–Sepharose 4B, Pharmacia Fine Chemicals) coated with bovine SS-B. These were prepared by adding 4 gm protein from the 60% saturated ammonium sulfate–soluble fraction of CTE to 5 ml high titer human anti-SS-B serum and 50 ml of a 10% suspension of protein A–Sepharose 4B in PBS. The reaction mixture was incubated for 2 h at 8°C and the beads were washed extensively with PBS, resuspended to a 20% suspension in PBS, and injected intraperitoneally into mice. (*b*) 20 μg of gel-purified 46-kD bovine SS-B protein. The immunogen derived from affinity column–purified bovine SS-B and further purified via preparative SDS-PAGE and electroelution (23) was mixed in CFA, and injected intraperitoneally into mice. Mice were bled from the retrobulbar sinus at several time points and 2 wk after the last injection. Blood was allowed to clot at room temperature and the serum was recovered and stored at −20°C.

[1] *Abbreviations used in this paper:* BGG, bovine gamma globulin; CTE, calf thymus extract; PEG, polyethylene glycol.

TABLE I
Summary of Immunization Protocols

Group	Number of animals	Immunogen	Immunization protocols
I	5 (B1–B5)	Coated beads*	Weekly times 12
II	10 (C1–C10)	Protein‡	Biweekly times 6
III	6	Protein	Two times 1 mo apart
IV	4	Coated beads	Two times 1 mo apart
V	4§ (F1–F4)	Coated beads	Two times 1 mo apart

* Protein A–Sepharose 4B beads coated with bovine SS-B/La.
‡ Gel-purified 46-kD bovine SS-B/La.
§ All five hybridomas A1–5 were derived from mouse F1.

Cell Fusion. Hybridomas were produced by the fusion of splenocytes to the nonproducing mouse myeloma P3-X63-Ag8.653 using PEG as described (24) with the following modifications. Mice with the highest level of anti-SS-B antibodies were selected and they received two intravenous injections of 200 μg affinity-purified SS-B 10 d apart. 3 d after the last immunization, splenocytes (35×10^6) were harvested and fused with myeloma cells (7×10^6; i.e., 5:1 ratio) in 35% PEG 1450 (Eastman Kodak Co., Rochester, NY) for 1 min. After fusion, cells were diluted in 250 ml of DMEM supplemented with 15% FCS (HyClone Laboratories, Logan, UT), hypoxanthine, aminopterin, thymidine, glutamine, gentamycin sulfate, nonessential amino acids, sodium pyruvate, glucose, and 4×10^6/ml feeder thymocytes from 4-wk-old C3Heb/Fej mice (Animal Breeding Facility, Scripps Clinic and Research Foundation). The cell suspension was distributed into ten 96-well tissue culture plates, which were maintained in a humidified, 37°C, 10% CO_2 incubator. Wells containing growing hybridomas were screened for Ig production and antibody to SS-B by ELISA. Initial SS-B ELISA-positive supernatants were further tested by immunoblotting and by immunofluorescence using HEp-2 cells as substrate. Hybridomas producing anti-SS-B were subcloned by limiting dilution.

Immunofluorescence Techniques. 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 Inc., Naperville, IL) following manufacturer instructions, and they were fixed in a mixture of acetone and methanol (3:1) at −20°C for 2 min, then air dried. Mouse sera were tested at dilutions of 1/40 in PBS and hybridoma supernatants were screened without any dilution. Antibody was detected using fluorescein-labeled affinity-purified goat antibody to mouse IgA + G + M (H + L) (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD).

ELISA for mAbs and Isotype Analysis. ELISA for the detection of anti-SS-B mAbs was established with a standard method as described (25). Affinity-purified bovine SS-B was used for coating Immulon 2 microtiter plates (Dynatech Laboratories, Inc., Alexandria, VA) at a concentration of 1 μg/ml in PBS. Plates were incubated at 8°C for 6 h, blocked with 0.1% gelatin overnight, washed with PBS containing 0.05% Tween 20 (PBS-Tween), and incubated for 1.5 h with undiluted culture supernatants (100 μl). After incubation and washes with PBS-Tween, Ig was detected by peroxidase-conjugated goat anti–mouse IgG + M reagents (Caltag Laboratories, So. San Francisco, CA) and the substrate 2,2′-azinobis(3-ethylbenzthiazoline sulfonic acid) as described (25).

For the determination of Ig concentration, culture supernatants were diluted 50-fold with serum diluent containing 0.75 mg/ml bovine gamma globulin (BGG) and added to ELISA plates coated with affinity-purified goat anti–mouse IgG + M (Tago Inc., Burlingame, CA) at 1 μg/ml. mAb isotype was determined in a similar manner except using peroxidase-conjugated goat anti–mouse Ig class, subclass, and light chain–specific reagents (Caltag Laboratories).

Sera. The anti-SS-B/La serum Ze was the Centers for Disease Control (CDC) (Atlanta, GA) reference serum (26) and has been shown (14) to contain antibodies reactive with both X and Y domains of SS-B/La. Normal human sera were obtained from our healthy laboratory personnel.

Immunoprecipitation and SDS-PAGE Analysis of Labeled Cellular Antigens. Protein A–Sepharose–facilitated immunoprecipitation of labeled cellular antigen with antisera or monoclonals was performed as described (6, 14, 15). Typically, to a 20–40 μl labeled extract we added 50 μl BSA (50 mg/ml), 500 μl 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), 10 μl rabbit anti–mouse Ig serum (Cederlane Laboratories Ltd., Westbury, NY), 10 μl mouse serum or 1 ml hybridoma culture supernatant, and 100 μl of 10% (wt/vol) Protein A–Sepharose beads in Buffer B. When the mixture contained human serum–positive control or mouse antisera, the rabbit antiserum was omitted. This suspension was mixed at 4°C for 1 h. The precipitated Protein A–Sepharose beads were washed five times with 1 ml of Buffer B. Proteins were eluted by boiling in Laemmli's sample buffer and analyzed in SDS-PAGE (27). For RNA analysis, precipitates were extracted with phenol and RNAs were precipitated with ethanol, and electrophoresed in 7 M urea–polyacylamide gels.

Immunoblot of SS-B and Fragments. Immunoblot detection of anti-SS-B antibodies in human and mouse sera was performed with a standard method (28). ^{125}I–Protein A and ^{125}I–goat anti–mouse κ chain were used to detect human and mouse Igs, respectively. Preparations of cell and tissue extracts and conditions for the generation of protease partial digests were described in detail elsewhere (14).

Results

Immunization. Initial immunization protocols (Table I, groups I and II) were carried out with multiple intraperitoneal injections over a 12-wk period. These lengthy immunization schedules were selected because we were concerned that a conserved protein such as SS-B/La might not be a good immunogen and multiple injections would increase the chance of producing antibody. It was apparent later that simple immunization protocols such as those of groups III–V were sufficient in the induction of mouse anti-SS-B/La antibodies.

Mouse antisera were tested initially by ELISA, immunoblotting, immunofluorescence, and immunoprecipitation with extracts from [^{32}P]phosphate-labeled HeLa cells. The results are summarized in Table II and Fig. 1 for mouse antisera obtained 8 wk after the first injection. Although at this time point the mice had gone through only two-thirds of the immunization protocol, it was clear that mice B1-5, C4-6, and C9 were producing humoral immune responses to bovine SS-B and HeLa SS-B. The immunoprecipitation results also indicated that anti-SS-B antibodies in these mouse sera were IgG since they were bound to protein A–Sepharose without requiring the rabbit-bridging antibody in the assay. There were strong correlations when comparisons were made among the reactivities of mouse antisera in ELISA, immunoblotting (Table II), and immunoprecipitation (Fig. 1), but the staining in indirect immunoflorescence by antisera varied from very weak to negative. It was interesting that mice C5, C8, and C10 immunoprecipitated a 95-kD HeLa phosphoprotein (Fig. 1), but these antibodies were apparently not linked inseparably to the SS-B immune response.

When the entire immunization protocol was completed, sera from all 5 mice in group I and 9 of 10 mice in group II reacted with SS-B by ELISA and immunoblotting. These reactivities were confirmed by immunoprecipitation where both the 48-kD SS-B phosphoprotein and its associated precursor tRNAs were precipitated (data not shown). Mouse C3 was the only one that did not

TABLE II
Reactivities of Mouse Antisera

Mouse sera*	ELISA OD‡	Immunoblotting HeLa extract
B1	2.58	+
B2	3.41	+
B3	3.84	+
B4	3.93	+
B5	4.06	+
C1	0.09	−
C2	0.10	−
C3	0.07	−
C4	1.40	+
C5	1.10	+
C6	0.98	+
C7	0.03	−
C8	0.04	−
C9	0.79	+
C10	0.12	−

* Mice B1–5 and C1–10 were from Table I, groups I and II, respectively. Sera were obtained at 8 wk, two-thirds through the course of the immunization protocol.
‡ Purified bovine SS-B was used as coating antigen. Absorbance at 405 nm were taken 2 h after the addition of substrate.

produce an antibody response to SS-B at the end of the immunization protocol. In subsequent studies with groups III, IV, and V, every mouse produced anti-SS-B/La antibodies detectable by immunoblotting and ELISA 2 wk after the last injection.

In view of the apparent crossreactivity of mouse antisera with bovine and HeLa SS-B as discussed above, we were interested to determine if the murine humoral immune response to bovine SS-B could be an autoimmune response in which antibodies were reactive with autologous protein. In Fig. 2, immunoblottings of SS-B from different species were compared using human SS-B serum Ze and mouse antisera from B5 and C4, which had the highest titer anti-SS-B antibody in groups I and II, respectively. It was clear that serum Ze reacted with SS-B from HeLa, mouse, bovine, and rabbit and the mouse antisera reacted with SS-B from human, bovine, and rabbit but not SS-B from mouse. This showed that the humoral immune responses of the mice were directed to bovine SS-B antigenic determinants that were also present in HeLa and rabbit SS-B but absent in mouse SS-B.

Cell Fusion. Six earlier fusions were carried out with splenocytes from different mice immunized with SS-B. Numerous Ig-secreting hybridomas were obtained but none reacted with SS-B. In the last and successful fusion, 35×10^6 cells were recovered from the spleen and fused with myeloma cells at a ratio of 5:1. Hybridoma supernatants from 193 wells were tested for anti-SS-B activity by ELISA. Seven positive supernatants were initially detected and were further tested by immunoblotting with HeLa cell extracts. Five of the seven clones reacted with the 48-kD SS-B protein. These five positives were subcloned twice

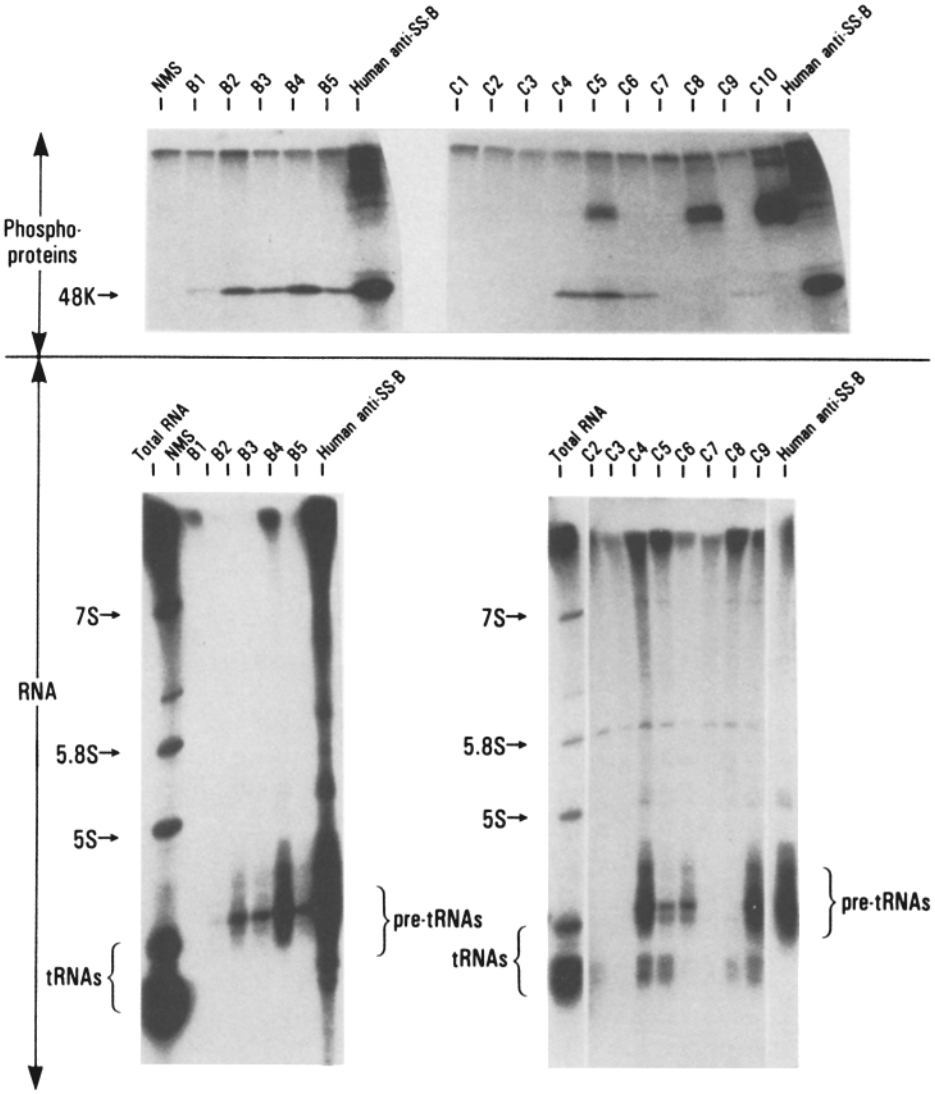

FIGURE 1. Immunoprecipitation of extracts from [^{32}P]phosphate-labeled HeLa cells with mouse antisera and serum Ze. (*Top*) Immunoprecipitate digested with RNase and the remaining phosphoprotein analyzed on standard SDS-PAGE. (*Bottom*) RNAs of the immunoprecipitates were phenol extracted, ethanol precipitated, and analyzed on 7 M urea/10% polyacrylamide gel. There was good correlation between the amount of 48-kD protein and pre-tRNA precipitated. B1–5 and C1–10 mouse antisera were obtained from group I and II mice used for immunization with bovine SS-B. NMS, normal mouse serum.

by limiting dilution and labeled A1 to A5. All five subcloned mAbs were found to be IgG1κ.

Specificity of mAbs. When the supernatants from the subcloned hybridomas were allowed to react with HeLa cell extracts in immunoblotting (Fig. 3*A*), all five mAbs reacted with the 48-kD SS-B protein. The human serum Ze reacted strongly with the 48-kD protein and a spontaneous 43-kD degradation product,

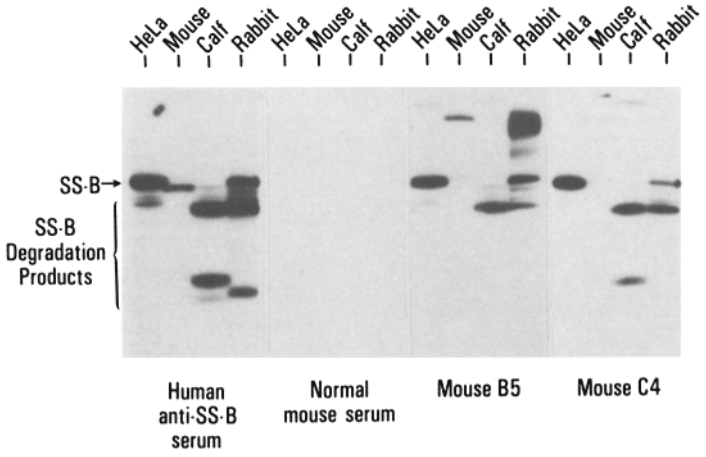

FIGURE 2. Immunoblotting analysis of the reactivities of mouse antisera and human serum Ze using extracts from HeLa cell, S49.1 cell (mouse), calf thymus gland, and rabbit thymus acetone powder. It was apparent that both mouse sera B5 and C4 reacted with SS-B from HeLa, calf thymus, and rabbit thymus but were not reacting with SS-B from mouse S49.1 cells.

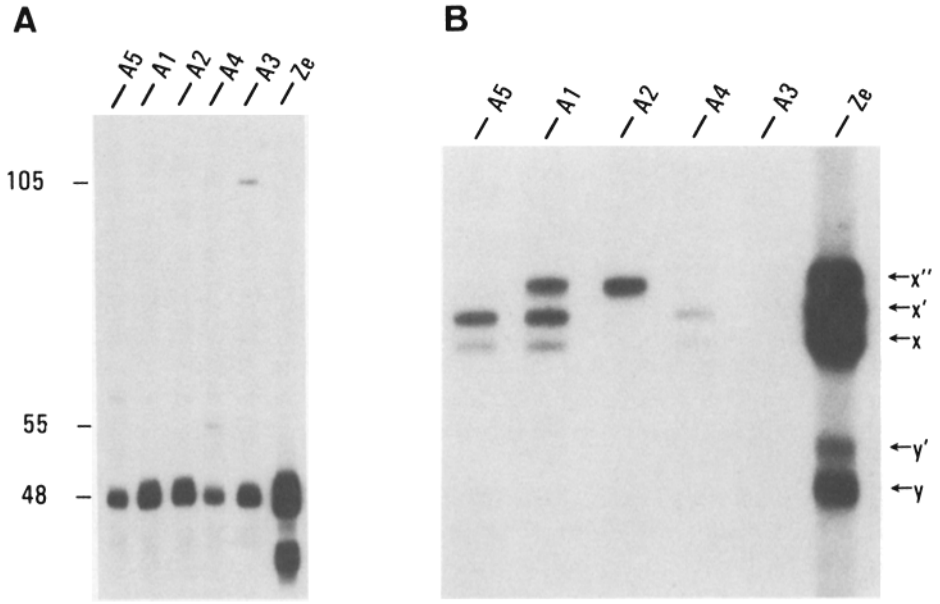

FIGURE 3. Immunoblotting analysis of the reactivities of mAbs using HeLa cell extract (*A*) and HeLa cell extract partially digested with S.a.V8 protease (*B*).

whereas the mAbs did not react with the 43-kD fragment. In further blotting experiments not shown here, A1 and A2 reacted with the spontaneous 43-kD fragment but the intensity was always lower than that of human serum. In addition to the reactivity with the 48-kD SS-B protein, A3 and A4 also reacted with additional HeLa proteins of 105 and 55 kD, respectively. On the other hand, A1, A2, and A5 appeared to be highly specific for the HeLa SS-B protein.

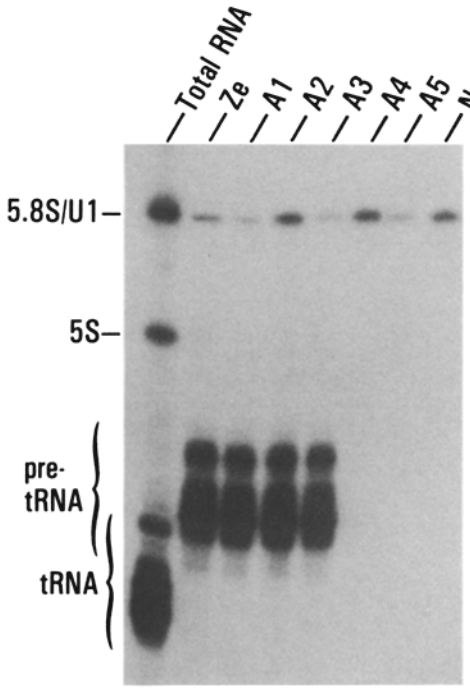

FIGURE 4. Immunoprecipitation of [^{32}P]-phosphate-labeled HeLa cell extract with mouse mAbs and serum Ze. RNA was extracted from the immunoprecipitate with phenol, precipitated with ethanol, and analyzed on 7 M urea/8% polyacrylamide gel.

We have previously shown (14) that there are two protease-resistant domains in HeLa SS-B based on the reactivity with human sera. S.a.V8 protease can reproducibly cut HeLa SS-B protein to generate fragments X/X′/X″ (28/29/30 kD) and Y/Y′ (23/24 kD) derived from domains X and Y, respectively. Fig. 3*B* shows the results of the analysis of the fine domain specificity of these mAbs. A1, A2, A4, and A5 reacted with X/X′/X″ fragments of the X domain but their reactivities varied with respect to different fragments. A3 did not react with either X or Y fragment. These results suggested that A1, A2, and A3 were recognizing different epitopes from each other and from A4 and A5, which are both reactive with the X/X′ fragments. Taking into account the further reactivity of A4 with the 55-kD HeLa protein, we concluded that A5 was recognizing a different epitope from A4.

Immunoprecipitation of mAbs. SS-B/La protein is associated with precursor tRNAs in HeLa cell, and human sera specific for SS-B can immunoprecipitate not only the SS-B protein but also pre-tRNAs. Fig. 4 shows the urea gel analysis of the RNA present in the immunoprecipitate between mAbs and extracts from ^{32}P-labeled HeLa cells. Three of the five anti-SS-B/La mAbs could immunoprecipitate pre-tRNAs that appeared to be identical to those of serum Ze. The band running around 5.8S/U1 RNA is apparently nonspecific because it was also present in the reaction with normal human serum (N). The same three mAbs also precipitated the 48-kD phosphoprotein of SS-B as expected (data not shown). A4 and A5, which did not immunoprecipitate RNA or protein, were probably reacting with epitopes only present in the denatured SS-B protein as demonstrated in the immunoblot results.

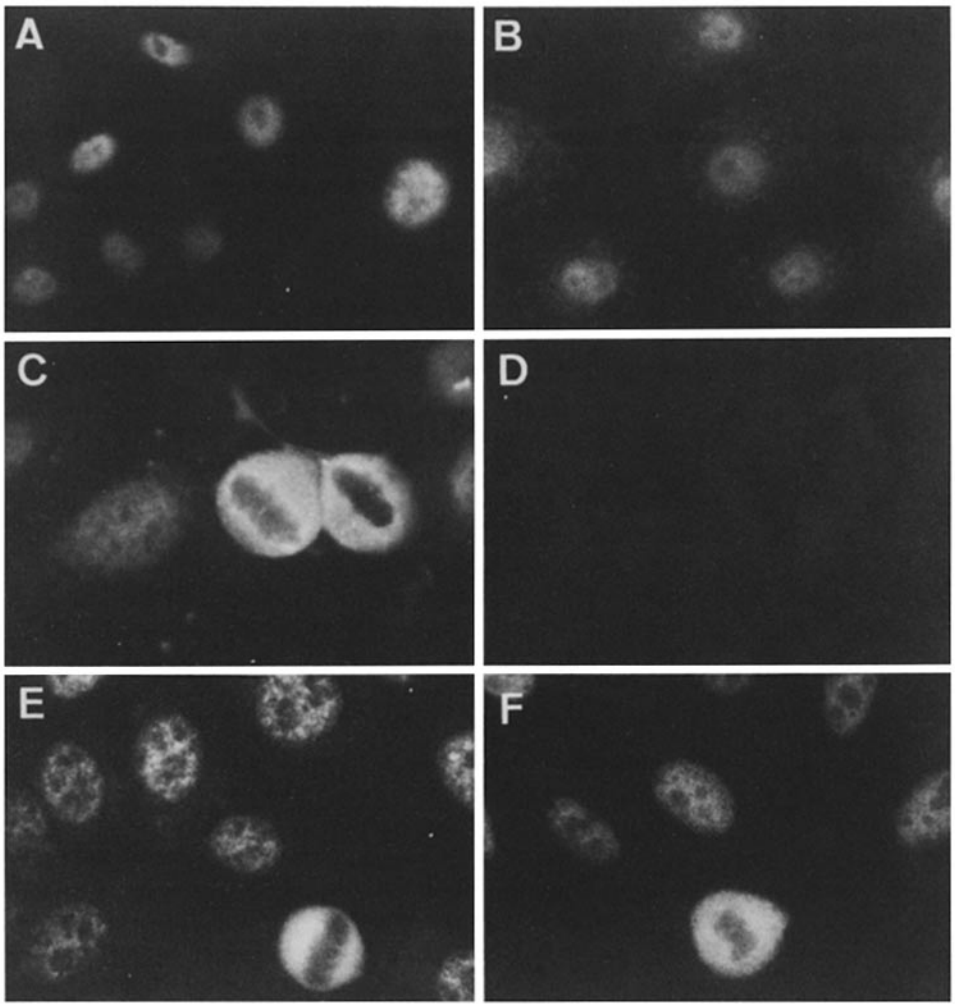

FIGURE 5. Immunofluorescent staining of anti-SS-B antibodies. Bovine MDBK cells were grown on slides, fixed with acetone/methanol, and stained with serum Ze (*A*) and serum from the mouse F1 used for the production of hybridomas (*B*). Pre-fixed human HEp-2 cells were stained with same mouse antiserum F1 (*C*), with a normal mouse serum (*D*), with human serum Ze with antibodies specific for SS-B (*E*), and murine anti-SS-B mAb A1 (*F*). mAbs A2 and A3 also gave identical staining pattern as A1 and human serum Ze. Original magnification, × 500.

Immunofluorescence. Fig. 5, *B* and *C*, shows the staining patterns of the antiserum from mouse F1 that was used for the production of these hybridomas. F1 serum gave fine speckles in the nucleoplasm, which excluded the nucleolus, and this pattern was identical to that of the CDC human anti-SS-B reference serum Ze both for MDBK and HEp-2 cells (Fig. 5, *A* and *E*). Normal mouse serum did not give any staining (Fig. 5 *D*). As expected from the immunoblotting of mouse antisera described above, the F1 serum did not give any staining with mouse 3T3 cells (data not shown).

Initial examination of the mAbs showed that A1 and A2 gave similar patterns

TABLE III
Species-specific Reactivities of mAbs as Detected by Immunofluorescence

Species	Cell line origin	Immunofluorescence*					
		Murine mAbs					Human serum
		A1	A2	A3	A4	A5	Ze‡
Human	HEp-2, larynx	+	+	+	−	−	+
Human	HeLa, cervix	+	+	+	−	−	+
Human	Raji, Burkitt lymphoma	+	+	+	−	−	+
Monkey	Vero, kidney	+	+	+	−	−	+
Rabbit	R9ab, lung	+	+	+	−	−	+
Bovine	MDBK, kidney	+	+	+	−	−	+
Hamster	BHK-21, kidney	−	−	−	−	−	+
Rat	6m2, kidney	−	−	−	−	−	+
Mouse	3T3, fibroblasts	−	−	−	−	−	+
Rat kangaroo	PtK2, kidney	−	−	−	−	−	+

* Cells were grown in Lab-Tek tissue culture chambers and fixed in a mixture of acetone and methanol (3:1) at −20°C for 2 min.
‡ Ze serum is the CDC reference serum for anti-SS-B/La specificity.

of fine nuclear speckles as the human serum with commercial Bion HEp-2 cells (Fig. 5, *E* and *F*), and A3, A4, and A5 did not stain. In view of the positive immunoblotting results as discussed above, we decided to determine the effect of other fixation methods on the reactivity of the mAbs. In addition to the standard acetone/methanol fixation method as outlined in Materials and Methods, we tried three other methods: (*a*) 95% ethanol, 0°C, 10 min; (*b*) 100% methanol, −20°C, 4 min; (*c*) acetone/ethanol (1:1), 0°C, 2 min. A1 and A2 mAbs and serum Ze gave nuclear staining under all of these conditions, but A4 and A5 did not give any staining with any of these fixation methods. However, A3 gave fine nuclear speckles when either acetone/methanol or acetone/ethanol fixation was used. Based on double-staining experiments, the staining patterns of A1, A2, and A3 were identical to those of human autoantibodies.

Unlike the human SS-B autoantibodies that reacted with SS-B from many species, the mAbs A1, A2, and A3 react with SS-B in a more species-restricted manner. As summarized in Table III, A1, A2, and A3 gave typical nuclear-staining patterns with three human cell lines, a monkey, a rabbit, and a bovine cell line, but gave no staining with a hamster, a rat, a mouse, and a rat kangaroo cell line. These data in Table III provide further support for the view that the mAbs were reacting with epitopes different from those recognized by the human SS-B autoantibody.

Discussion

Normal BALB/c mice were successfully immunized to produce IgG antibody to bovine SS-B. These mouse antisera were reactive with SS-B from bovine, human, and rabbit but did not contain antibodies that were reactive with homologous SS-B. Five hybridomas secreting IgG1κ mAbs to SS-B were cloned from the spleen cells of one of these immunized mice. Data from immunoblotting, immunoprecipitation, and immunofluorescence showed that these mAbs reacted

TABLE IV
Characteristics of mAbs

Antibody	Subclass	HeLa immunoblot	Domain specificity*	Cell staining‡	Immunoprecipitation§
A1	IgG1κ	+	Domain X	+	+
A2	IgG1κ	+	Domain X	+	+
A3	IgG1κ	+		+	+
A4	IgG1κ	+	Domain X	−	−
A5	IgG1κ	+	Domain X	−	−

* Defined by immunoblot reactivities with HeLa SS-B fragments generated by limited S.a.V8 protease digestion.
‡ Human HEp-2 cell as substrate.
§ Labeled extracts from HeLa and MDBK cells.

with different SS-B epitopes. As summarized in Table IV, A1, A2, and A3 appeared to recognize epitopes on the native protein as it is constructed in a ribonucleoprotein particle and thus were able to immunoprecipitate both protein and RNA and gave nuclear staining in immunofluorescence. A4 and A5 were negative in both assays but were able to detect the 48-kD protein and the X/X′ fragments in immunoblot; this suggested that they recognized epitopes that were probably hidden in the native protein but were probably exposed after PAGE and transfer to nitrocellulose. The species-restricted reactivity of these mAbs demonstrated the difference between immunogenic epitopes recognized by mAbs and the more highly conserved epitopes recognized by human autoantibodies. This is the first report of anti-SS-B/La mAbs capable of precipitating human SS-B/La particles including the associated RNAs. Previously, a single anti-SS-B antibody specific to bovine SS-B was shown to precipitate SS-B and its RNAs from Madin-Darby bovine kidney (MDBK) cells (16).

The evidence that immunogenic epitopes recognized by mAbs A1–5 are not the targets of human autoantibodies is supported by additional evidence from blocking experiments showing that human serum Ze failed to inhibit mAb reactivity with SS-B. When either nitrocellulose strips or ELISA wells coated with SS-B were first incubated with serum Ze, washed, and later incubated with different mAbs, reactivities of mAbs were not inhibited (data not shown). The reciprocal blocking experiment of human SS-B sera by mAbs also showed no inhibition.

The highly conserved nature of the SS-B/La epitopes recognized by human autoantibodies is by no means unique to this autoantigen-antibody system. Most if not all of the systemic rheumatic disease–associated autoantibodies are directed to ubiquitous cellular components such as DNA, histones, ribosomes, Sm/RNP, Scl-70 (DNA Topoisomerase I), RNA polymerase I, PCNA/cyclin (auxiliary protein of DNA polymerase δ), and U3-RNP, which subserve important functions in eukaryotic cells.

Published data show that human autoantibodies are able to inhibit many of these cellular functions where they have been tested. Such examples include inhibition of precursor mRNA splicing (29–31), relaxation of supercoiled DNA (32), transcription of ribosomal RNA (33), and aminoacylation of transfer RNAs

(Dang, C. V., E. M. Tan, and J. A. Traugh, submitted for publication). Such information, together with the data provided in the current experiments, has led us to consider the possibility that the conserved epitopes recognized by human autoantibodies might also be the functional or active sites of these intracellular proteins. Recent experiments carried out in collaboration with other laboratories provide preliminary evidence to support this hypothesis. Human autoantibody to PCNA/cyclin was shown to recognize different epitopes from two immunization-induced murine mAbs and from rabbit antibody to a synthetic NH_2-terminal peptide of PCNA (34). In an in vitro assay system for demonstrating auxiliary protein-dependent DNA polymerase δ function, only the human autoantibody inhibited function (Tan, C. K., K. F. Sullivan, E. M. Tan, K. M. Downey, A. G. So, submitted for publication). In another study, human autoantibody to threonyl-tRNA synthetase obtained from a patient with polymyositis inhibited the catalytic threonyl charging activity, whereas an experimentally induced rat antibody to the purified enzyme did not (Dang, C. V., et al., submitted for publication). It thus appears that the conserved epitopes on many ubiquitous intracellular proteins that invoke autoimmune responses might also be the active or catalytic sites of these molecules. If this proves generally to be the case, elucidation of the mechanism whereby these epitopes are rendered immunogenic might provide insights into certain aspects of the autoimmune process.

Summary

SS-B/La, an ubiquitous nuclear protein of 46–48 kD, is a target antigen of autoantibodies in SLE and Sjogren's syndrome and is involved in the maturation of RNA polymerase III transcripts such as 5S RNA and tRNAs. We have previously shown (14, 15) that SS-B consists of two protease-resistant domains of 23 and 28 kD, with the latter containing the RNA binding site. The epitopes of SS-B/La reactive with human autoantibodies are conserved among several mammalian species examined. BALB/c mice immunized with affinity-purified calf thymus SS-B produce IgG anti-SS-B/La antibodies, which reacted with bovine, human, and rabbit SS-B but not with mouse SS-B/La. The spleen of a mouse with the highest antibody titer was selected for fusion with P3 myeloma. Five IgG1κ mAbs (A1–5) were selected by ELISA and immunoblotting. All except A3 reacted with the 28-kD domain. A1, A2, and A3 were capable of immunoprecipitating the 48-kD SS-B protein and its associated RNAs. A1, A2, and A3 also gave fine nuclear speckled staining on human, monkey, bovine, and rabbit cells that was similar in appearance to that with human autoantibodies, but in contrast to staining with human autoantibodies, they did not stain cells from rat, mouse, or rat kangaroo. It appears that human autoantibodies target highly conserved epitopes that can be distinguished from epitopes recognized by immunization-induced murine mAbs. Taken together with other data, it appears that human autoantibodies may be recognizing epitopes that are active or catalytic sites of molecules subserving important cellular functions.

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