Human autoantibodies reactive with the signal-recognition particle
(autoimmunity/polyomyositis/ribonucleoprotein)

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ABSTRACT We describe autoantibodies reactive with the signal-recognition particle (SRP) in serum of a patient with polymyositis. The serum specifically immunoprecipitated the SRP from human erythroleukemia (K562) cell extracts. Analysis of immunoblots revealed that the serum contained autoantibodies specific for the 54-kDa protein of the SRP but had little or no reactivity with its other five proteins. Indirect immunofluorescence of human laryngeal carcinoma (HEp-2) cells confirmed that the ribonucleoprotein immunoprecipitated by this serum is found mainly in the cytoplasm. This autoimmune serum may be useful for studying the function of the 54-kDa subunit of the SRP in binding to signal sequences or in interacting with other components of the translational machinery.

The signal-recognition particle (SRP) is a cytoplasmic ribonucleoprotein consisting of six polypeptide chains, of 72, 68, 54, 19, 14, and 9 kDa, and one molecule of 7SL RNA (1, 2). The SRP recognizes the signal sequence of nascent secretory, lysosomal, and many integral membrane proteins (for review, see ref. 3). Recognition occurs as soon as the signal sequence has emerged from the ribosome (4) and involves the 54-kDa protein of the SRP (5, 6). In a cell-free system, recognition of the signal sequence by the SRP can cause a transient translocation arrest, which is released only after binding of the SRP to its cognate receptor in the endoplasmic reticulum (ER) (4, 7, 8). Thus, the SRP functions both in the recognition of ER signal sequences and in their subsequent targeting to the ER.

Sera from patients with systemic lupus erythematosus and related conditions frequently contain autoantibodies that react with various types of ribonucleoproteins (9). The discovery of these autoantibodies has led to major advances in our knowledge of the structure and function of small ribonucleoproteins (10–14). Because of the diversity of small nuclear ribonucleoproteins (snRNPs), small cytoplasmic ribonucleoproteins (scRNPs), and small nucleolar ribonucleoproteins recognized by certain human autoimmune sera, we anticipated that autoantibodies to the SRP might also be present in some patients’ sera. Such antisera may be valuable reagents for studying the functions of the SRP. In the present study, we have identified and characterized autoantibodies specific for the 54-kDa subunit of the SRP in serum of a patient with polymyositis, a collagen vascular disease affecting primarily striated muscle.

MATERIALS AND METHODS

Sera Sera were obtained from patients diagnosed and treated at The Rockefeller University Hospital. Serum from patient JB, a 50-year-old woman with typical polymyositis, was kindly provided by R. G. Lahita. Sera with the anti-To (Th) specificity were obtained from three patients with scleroderma and displayed typical intense nucleolar and weak cytoplasmic immunofluorescence, with immunoprecipitation of both 7-2 and 8-2 small RNAs (15). Anti-Ro/La, anti-U1 snRNP, and anti-Sm (reactive with U1, U2, U4, U5, and U6 snRNPs) reference sera were from the serum bank at Rockefeller University.

Analysis of Small RNAs. Human autoimmune sera were used to immunoprecipitate extracts of human K562 (erythroleukemia) cells labeled with [32P]orthophosphate (New England Nuclear) as described (10, 16). Immunoprecipitated RNAs were analyzed by electrophoresis in 7% polyacrylamide/7 M urea gels and detected by autoradiography.

Hybridization of RNA to 7SL Gene. Plasmid pT7-7SL carrying the gene for 7SL RNA has been described (17) and was a gift from P. Walter (University of California, San Francisco, CA). The pT7 plasmid in which the 7SL gene was inserted was purchased from United States Biochemical (Cleveland, OH). Plasmid DNAs (150 μg each of pT7-7SL or pT7) were boiled for 10 min and treated with 0.2 M NaOH at 80°C for 10 min before filtration onto a pre-cut nitrocellulose filter (Bethesda Research Laboratories), using a Hybrid-dot filtration apparatus (Bethesda Research Laboratories). The filter was baked for 2 hr at 80°C and prehybridized for 24 hr at 42°C in 50% (vol/vol) formamide/5× SSC/50 mM sodium phosphate, pH 6.5/sonicated denatured salmon sperm DNA (250 μg/ml)/1× Denhardt's solution (18). Hybridization was for 60 hr at 42°C in 50% formamide/5× SSC/yeast tRNA (500 μg/ml) (19) containing 20,000 dpm of RNA that had been 32P-labeled in vivo, immunoprecipitated by patient JB serum, and isolated by phenol/chloroform extractions and ethanol precipitation (10). After hybridization, the nitrocellulose filter was washed once with 50% formamide/5× SSC for 1 hr at 42°C and twice with 2× SSC, for 1 hr each time, at 42°C and then was autoradiographed.

For RNA blot analysis, unlabeled K562 cell extract (2.5 × 109 cell equivalents per lane) was immunoprecipitated with 15 μl of serum from patient JB or normal human serum or with anti-Ro/La, anti-RNP, or anti-Sm reference serum as above. RNA was isolated from the immunoprecipitates and electrophoresed in a 7% polyacrylamide/7 M urea gel. The RNA was localized by staining with ethidium bromide and then transferred electrophoretically for 4 hr at 4°C to nylon membrane (Zeta Probe, Bio-Rad), following the manufacturer's directions. The filter was baked for 2 hr at 80°C, prewashed for 1 hr at 65°C in 0.1× SSC/0.5% NaDodSO4, and prehybridized for 14 hr at 42°C in 50% formamide/5× SSC/50 mM sodium phosphate, pH 6.5/10× Denhardt’s solution/sonicated denatured salmon sperm DNA (1 mg/ml). Hybridization was for 24 hr at 42°C in 50% formamide/5× SSC/25 mM sodium phosphate, pH 6.5/2× Denhardt’s solution/sonicated denatured salmon sperm DNA (100

Abbreviations: SRP, signal-recognition particle; ER, endoplasmic reticulum; RNP, ribonucleoprotein; snRNP, small nuclear ribonucleoprotein; scRNP, small cytoplasmic ribonucleoprotein.

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µg/ml containing nick-translated pT7-7SL plasmid DNA. After hybridization, the filter was washed twice for 1 hr each in 2× SSC/0.1% NaDodsO₄ at 22°C, once for 30 min in 0.1× SSC/0.1% NaDodsO₄ at 22°C, and twice for 30 min each in 0.1× SSC/0.1% NaDodsO₄ at 55°C before autoradiography.

**Indirect Immunofluorescence.** The intracellular distribution of antigens bound by patient JB autoantibodies was determined by indirect immunofluorescence using methanol-fixed human HEp-2 (laryngeal carcinoma) cells (20). Cells were incubated for 30 min with serum diluted 1:20, washed, and incubated for 30 min with a 1:60 dilution of rhodamine-conjugated goat anti-human IgG antibodies (Tago, Burlingame, CA). After washing, the cells were examined in a Leitz Ortholux II epifluorescence microscope.

**Analysis of Proteins.** Human autoimmune sera were used to immunoprecipitate extracts of [³²P]methionine-labeled K562 cells as described (16, 21). The extraction buffer was 150 mM NaCl/50 mM Tris Cl, pH 7.5/2 mM EDTA/5 mM iodoacetamide/0.5 mM phenylmethylsulfonyl fluoride. Immunoprecipitated proteins were electrophoresed in NaDodsO₄/12.5% polyacrylamide gels, which were stained, de-stained, and fluorographed (22).

**Immunoblots of Purified SRP Proteins.** Highly purified canine SRP, kindly provided by R. Gilmore (University of Massachusetts, Worcester, MA) and P. Walter (University of California, San Francisco, CA), was electrophoresed in a NaDodsO₄/12.5% polyacrylamide gel and immunoblotted for 12 hr onto nitrocellulose paper (0.45 µm pore size, from Schleicher & Schuell) in a buffer containing 20% methanol, 25 mM Tris base, 0.2 M glycine, and 0.1% NaDodsO₄. The immunoblot was incubated in 1% bovine hemoglobin in phosphate-buffered saline (PBS: 150 mM NaCl/20 mM sodium phosphate, pH 7.2) for 1 hr followed by patient JB serum at a 1:25 dilution for 12 hr at 4°C. After washing with PBS, the immunoblot was incubated for 3 hr with [³²P]-labeled protein A (sp. act. 7.2 µCi/µg, from New England Nuclear; 1 Ci = 37 GBq) at concentration of 0.35 µCi/ml in PBS. The immunoblot was washed three times over 2 hr with PBS, dried, and autoradiographed.

**RESULTS**

Serum from a patient (JB) with polymyositis was found to contain autoantibodies that immunoprecipitated a single small RNA molecule from human K562 (erythroleukemia) cell extracts (Fig. 1, lane 2). In 7% polyacrylamide/7 M urea gels this small RNA migrated slightly slower than 7-2 small RNA and faster than 8-2 small RNA, which are immunoprecipitated by sera with the anti-To (15) or anti-Th (23) specificities (Fig. 1, lane 3). An immunoprecipitate obtained using a serum with anti-Sm/RNP, anti-Ro, and anti-La specificities is shown (lane 4) for comparison. Anti-Sm/RNP antibodies immunoprecipitate U1, U2, U4, U5, and U6 small RNAs (10); anti-Ro antibodies immunoprecipitate Y1–Y5 small RNAs from human cell extracts (12); and anti-La antibodies immunoprecipitate precursor RNAs synthesized by RNA polymerase III, including the precursors of 7-2, 7SL, 5S, Y1–Y5, and tRNAs (14, 23, 24). An immunoprecipitate obtained using serum from a normal individual is also shown in Fig. 1 (lane 1).

The relative intensities of the radioactively labeled small RNAs immunoprecipitated by patient JB serum and reference serum containing anti-Sm/RNP, anti-Ro, and/or anti-La antibodies suggested that the RNA immunoprecipitated by JB serum is less abundant or is synthesized more slowly than U1 and U2 RNAs but is more abundant or is synthesized more rapidly than 7-2 and 8-2 RNAs immunoprecipitated by anti-Th (Th) antisera. The possibility that the RNA immunoprecipitated by JB serum is a relatively abundant species was also supported by immunoprecipitation analysis of unlabelled K562 cell extract with an excess of antiserum, followed by electrophoresis and ethidium bromide staining of the immunoprecipitated small RNAs (Fig. 2A).

From the mobility of the RNA immunoprecipitated by JB serum in 7% polyacrylamide/7 M urea gels, we concluded that this RNA was either 7SL or 7SK RNA (25, 26). 7SL and 7SK are distinct molecular species that do not cross-hybridize with one another (26). To distinguish between the two possibilities, we hybridized RNA immunoprecipitated by JB serum with plasmid pT7-7SL, which carries the 7SL gene (17). Cell extract from 2.5 × 10⁷ cells was immunoprecipitated with normal human serum (Fig. 2, lane 1) or patient JB serum (lane 2) or with anti-Ro/La (lane 3), anti-U1 RNP (lane 4), or anti-Sm (lane 5) reference serum. The RNA isolated from the immunoprecipitates was visualized by staining with ethidium bromide (Fig. 2A). As was observed using [³²P]-labeled RNAs (Fig. 1), JB serum immunoprecipitated a single RNA molecule (arrow) that comigrated with a weaker band in the anti-La immunoprecipitate. The RNAs were transferred to nylon membrane and hybridized with nick-translated pT7-7SL (Fig. 2B). The probe hybridized with the RNA molecule immunoprecipitated by JB serum (lane 2, arrow) and with the weaker band of nearly the same mobility that was immunoprecipitated by anti-Ro/La reference serum (lane 3). There was no detectable hybridization to the other RNA polymerase III transcripts immunoprecipitated by anti-La antibodies (lane 3) or to U1 (lane 4) or U1, U2, U4, U5, and U6 RNAs (lane 5) immunoprecipitated by anti-U1 snRNP or anti-Sm antibodies, respectively. Thus, in addition to hybridizing with 7SL RNA (the precursor of 7SL RNA) in the anti-La immunoprecipitate, the probe hybridized intensely with the small RNA immunoprecipitated by JB serum.

![Fig. 1. Immunoprecipitation of 32P-labeled RNA. Small RNAs were labeled in vivo with [³²P]orthophosphate, immunoprecipitated using autoimmune sera (lanes 2-4) or normal human serum (lane 1), and analyzed on a 7% polyacrylamide/7 M urea gel. Patient JB serum (lane 2) immunoprecipitated a single radioactive band (asterisk). Immunoprecipitates using reference serum with anti-To/Th specificity (lane 3) or anti-Sm/RNP plus anti-Ro/La specificities (lane 4) are shown for comparison. Positions of prominent small RNA bands are indicated at right.](image-url)
We also used in vivo-labeled RNA immunoprecipitated by patient JB serum to perform dot hybridizations with either pT7-7SL or pT7 (vector) DNA (Fig. 3). In agreement with the RNA blot analysis, the purified RNA hybridized specifically with pT7-7SL but not with pT7 DNA, confirming that the immunoprecipitated RNA is 7SL rather than 7SK.

Since 7SL RNA is a component of the SRP, a cytoplasmic ribonucleoprotein, we examined the intracellular distribution and protein components of the antigen bound by patient JB autoantibodies. By indirect immunofluorescence using methanol-fixed human HEP-2 cells, the serum stained the cytoplasm, but not the nucleus, in a speckled/granular distribution (Fig. 4). Normal human serum showed no cytoplasmic or nuclear staining, and anti-Sm/RNP reference serum displayed only speckled nuclear immunofluorescence (not shown). This provided further evidence that JB serum immunoprecipitates 7SL rather than 7SK small ribonucleoproteins, since 7SL is a cytoplasmic ribonucleoprotein, whereas 7SK is thought to be nuclear (25).

The polypeptide components of the scRNPs immunoprecipitated by patient JB autoantibodies were determined by immunoprecipitation of [35S]methionine-labeled K562 cell extracts (Fig. 5). The serum immunoprecipitated several heavily labeled proteins, including proteins of 72, 68, and 56 kDa, as well as less heavily labeled proteins of 19.5, 16, and 9 kDa (indicated by dots in Fig. 5A; the 9-kDa protein has run off this 12.5% gel). In addition to these proteins, the serum also immunoprecipitated two larger proteins at >205 kDa and ~110 kDa (Fig. 5A), as well as proteins of 33 kDa and 11 kDa (Fig. 5B, lane 1, arrows). The 33- and 11-kDa proteins do not appear to be physically linked to the other immunoprecipitated proteins, since sera from other patients immunoprecipitate the 33- and 11-kDa proteins but not the >205-, 110-, 72-, 68-, 56-, 19.5-, 16-, or 9-kDa protein (Fig. 5B, lane 2). The significance of the coprecipitating >205-, 110-, 33-, and 11-kDa proteins remains to be determined.

Analysis of immunoprecipitated proteins (Fig. 5) is useful for determining the protein constituents of ribonucleoproteins, since each protein that is physically linked to the

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**Fig. 2.** RNA blot analysis of immunoprecipitated small RNAs. Unlabeled cell extract was immunoprecipitated with normal human serum (lane 1), patient JB serum (lane 2), anti-Ro/La reference serum (lane 3), anti-U1 RNP reference serum (lane 4), or anti-Sm reference serum (lane 5) and analyzed on a 7% polyacrylamide/7 M urea gel. (A) Ethidium bromide staining of the immunoprecipitated small RNAs. (B) Hybridization of [32P]-labeled pT7-7SL, carrying the gene for 7SL RNA, to these RNAs blotted onto nylon membrane (Zeta probe). Arrow in A and in B indicates position of the small RNA immunoprecipitated by patient JB serum.

**Fig. 3.** DNA blot analysis of immunoprecipitated small RNAs. Plasmids pT7-7SL (carrying the 7SL gene) and pT7 (without insert) were immobilized on nitrocellulose membrane and probed with 20,000 dpm of small RNA labeled in vivo with [32P]orthophosphate and immunoprecipitated by patient JB serum. Autoradiograph was exposed for 7 days.

**Fig. 4.** Indirect immunofluorescence pattern of HEP-2 cells stained with JB serum autoantibodies. HEP-2 cells were grown on coverslips and fixed with methanol at −20°C for 10 min. Cells were stained with serum at a dilution of 1:20 followed by rhodamine-conjugated goat anti-human IgG antibodies at a dilution of 1:60.
complex appears in the immunoprecipitate, regardless of which protein(s) is recognized by the precipitating antisera. To determine which of the proteins immunoprecipitated by patient JB serum is recognized by the patient's autoantibodies, we used the patient's serum to probe immunoblots of highly purified SRP from dog pancreas on immunoblots, but a high concentration (~1:25 dilution) was necessary for detection. In contrast, the serum reacted well at 1:1000 dilution with a protein of approximately the same molecular mass in crude human cell extracts (data not shown); in addition, we cannot presently rule out weak reactivity with the 68- or 72-kDa proteins of human SRP (unpublished observation). Thus, the antisera appears to recognize the 54-kDa protein of the SRP, but reacts much more strongly with human SRP than with canine SRP.

DISCUSSION

In the present studies, we found autoantibodies reactive with the SRP in serum of a patient with polymyositis. The serum immunoprecipitated a single small RNA molecule with mobility corresponding to that of 7SL RNA (Fig. 1). Further, the RNA hybridized to 7SL DNA on RNA (Fig. 2) and DNA (Fig. 3) blots. The serum also immunoprecipitated proteins of approximately 72, 68, 56, 19.5, 16, and 9 kDa (Fig. 5), which agree well with the reported molecular weights of the six SRP polypeptides (1). In addition, the serum gave a cytoplasmic rather than nuclear pattern of immunofluorescence, consistent with the cytoplasmic location of the SRP (25). The autoantibodies bound specifically to the 54-kDa subunit of purified SRP from dog pancreas (Fig. 6) and much more strongly to a protein of approximately the same molecular mass in human cell extracts (data not shown). The latter finding agrees with the studies of Mimori et al. (27), who described autoantibodies that specifically immunoprecipitate a 55-kDa protein along with 7SL RNA. The failure to immunoprecipitate the 72-, 68-, 19-, 14-, and 9-kDa proteins in those studies and the appearance of these proteins in our immunoprecipitates may reflect different experimental conditions. In the present studies, both the RNA and the protein immunoprecipitations were performed in the presence of 150 mM NaCl and 2 mM EDTA. Under these conditions, the SRP would be expected to unfold but not to dissociate, since previous studies have shown that the particle remains intact in the presence of 500 mM KOAc and 10 mM EDTA (28).

The relationship of the autoantibodies described here to those described by Kole et al. (29) is uncertain. In those studies, a 68-kDa protein that binds to the 5' end of the Alu RNA was immunoprecipitated by certain sera from patients with systemic lupus erythematosus. Although this protein is similar in size to the 68-kDa SRP protein, the latter does not bind to the Alu sequences of 7SL RNA (5, 30). Also, as pointed out by the authors, the sera with specificity for Alu RNA did not immunoprecipitate 7SL RNA, making it unlikely that these sera react with the 68-kDa subunit of the SRP.
It is notable that patient JB and one of the two patients reported by Mimori et al. (27) have clinical polymyositis and that all three sera react predominantly with autoantigenic epitopes on the 54-kDa subunit of the SRP. The reason that a single polypeptide is apparently "selected" for this autoimmune response is not known but is reminiscent of the specific recognition of three of the nine components of U1 snRNPs by anti-U1 snRNP autoantibodies in sera of patients with mixed connective tissue disease (21). The recognition of only a small subset of ribosomal proteins by anti-ribosomal antibodies in sera of patients with lupus is another example of the same phenomenon (31). An understanding of the reasons for the selectivity of these autoimmune responses may provide insights into the mechanisms of autoimmune diseases.

The selectivity of the autoimmune responses mounted by patients with certain collagen vascular diseases may also make these sera more valuable as reagents for studies of the structure and function of ribonucleoproteins. The specificity of the autoantibodies in the serum of patient JB for the 54-kDa polypeptide of the SRP may make the serum useful for examining the function of this protein. The 54-kDa protein is the only component of the SRP that does not bind directly to 7SL RNA (5, 28). Rather, it is linked to the particle through an association with the 19-kDa polypeptide. The 54-kDa protein appears to bind ER-targeted signal sequences (6), and rabbit antisera against the denatured 54-kDa protein allowed SRP-dependent elongation arrest of preprolactin to occur but interfered with the release of the elongation arrest by SRP receptor (32). The autoantibodies described here also appear to react with the denatured 54-kDa protein, since they react on immunoblots (Fig. 6), but by analogy with antibodies found in other autoimmune sera (33), reactivity with determinants of the native protein might also be expected. If such antibodies are found, the serum may be particularly useful for mapping the location of sites on the 54-kDa protein responsible for its interaction with the SRP receptor or the 19-kDa subunit.

Finally, the presence of other reactivities in this patient's serum (in addition to anti-SRP antibodies) is noteworthy. Although the proteins immunoprecipitated by these additional autoantibodies (Fig. 5) remain to be characterized, they are probably cytoplasmic proteins, since the serum reacted with HEP-2 cells in a cytoplasmic distribution, without detectable nuclear immunofluorescence (Fig. 4). Because individual autoimmune sera frequently react specifically with several components of the same macromolecular structures (16, 21, 34, 35), the possibility that these autoantibodies react with other SRP-associated components should be investigated.

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