

Anti-Ro(SSA) and Anti-La(SSB) Antibodies in Autoimmune Rheumatic Diseases

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Introduction

Antinuclear antibodies (ANA) are of importance to clinical medicine, inasmuch as they offer incomparable diagnostic help for a number of connective tissue diseases (1-5). Among them, impressive contributions to our understanding of the clinical relevance of anti-Ro(SSA) and anti-La(SSB) antibodies have been achieved over the past two decades. They have repeatedly been detected in patients with primary or secondary Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), and related disorders, and are now regarded as one of the hallmarks of these diseases. There has subsequently been a search for autoantibody fine specificity to define clinical subsets of SS and SLE. Background analysis of Ro(SSA) and La(SSB) ribonucleoprotein (RNP) complexes has now been completed and a series of studies has provided a vast body of new information about their nuclear structures.

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Ro(SSA) and La(SSB) RNP Complexes

RNPs

Historical Background

Much progress has been made since the original discovery of ANA to extractable nuclear antigens in 1958. The milestones in research on autoantibodies to Ro(SSA) and La(SSB) are shown in Table 1. Two groups of investigators (6,7) have detected the presence of precipitating autoantibodies in one-third of patients with SS, using saline extracts of salivary and lacrimal glands as substrate. From these studies, two autoantibody systems were defined and termed SjD and SjT, after the prototype sera (Table 2).

Another antibody was then described by Clark et al. (8) by gel double diffusion of SLE serum, together with saline homogenate of normal human spleen. Ultracentrifugation cell fractionation showed that the corresponding antigen, Ro, predominated in the cytoplasm. Mattioli and Reichlin (9) reported a distinct antibody directed toward a cytoplasmic component designated La. Later on, Alspaugh and Tan (10), using gel double diffusion of Wil 2 cell extracts, isolated two antibodies from the serum of SS patients, termed antisicca syndrome A (SSA) and antisicca syndrome B (SSB), of which the target was concentrated within the nucleus. Additional components were purified by Akizuki et al. (11) from calf thymus and liver, and rat liver. They showed identical immunological reactivities.

Suggestions arose that the two-letter system of designating nuclear antigens and their autoantibodies should be continued, and that autoantibodies should be named after the disease in which they have been found to be present with the highest incidence (12). Finally, an interlaboratory collaborative study (13) was carried out to demonstrate the identity of Ro and SSA on the one hand, and La, SSB, and Ha on the other.

Structure of RNPs

An important issue in the pathogenesis of connective tissue diseases is the definition of the targets of such restricted autoimmune responses. The Ro(SSA) and the La(SSB) antigens were originally characterized by precipitation in agar, and their nature as RNPs, i.e., RNA protein particles, was then established using liquid phase immunoprecipitation (14). The RNA moiety of the complex (see Fig. 1 on p. 256) includes precursors of 7S RNA, 5S RNA, t RNA, U6 RNA, as well as virally encoded RNAs, such as VA I and VA II RNAs, EBER 1, and EBER 2 RNAs, and leader RNAs of vesicular stomatitis and rabies viruses (15).

Table 1
Milestones in Research on Anti-Ro(SSA)
and Anti-La(SSB) Autoantibodies

Identification of antibodies to soluble whole cells
Differentiation of extractable nuclear antigens
Description of the Ro antigen
Description of the La antigen
Description of the SSA and SSB antigens
Description of the Ha antigen
Resolution of the antibody systems
Demonstration that antibodies are directed to RNA-associated proteins

Table 2
Common Antigenic Determinants of the Various
Extractable Nuclear Antigen Preparations

Antigens		
No. 1	No. 2	Authors
SjD	SjT	Anderson et al. (1961) (7)
Ro		Clark et al. (1969) (8)
	La	Mattioli and Reichlin (1974) (9)
SSA	SSB	Alsbaugh and Tan (1975) (10)
	Ha	Akizuki et al. (1977) (11)

With regard to the Ro(SSA) particle, a 60 kDa protein is believed to harbor most of the antigenicity. This is bound to the RNAs referred to as hY1–hY5 in HeLa cells (16). A second 52 kDa form of the antigen has been characterized (17), and a third 54 kDa protein, derived from human erythrocytes, has been shown to react with anti-Ro(SSA) antibodies (18). In fact, two additional proteins have been extracted from red blood cell extracts and it has been claimed that these proteins of 60 and 54 kDa were antigenically different from the corresponding 60 and 52 kDa proteins extracted from lymphocytes, since a number of sera bound each antigen exclusively. Thus, at least four distinct forms of Ro(SSA) peptides have been identified (Table 3). Some are unique to lymphocytes, whereas others are restricted to erythrocytes (19). Interestingly, the autoimmune response to these RNPs is directed to epitopes on the human antigen that are not conserved in evolution (20), which indicated that the human Ro(SSA) is the presumptive immunogen. The fraction of antibody that remained reactive with human Ro(SSA) after absorption with bovine spleen extract and recognition with the 60 and 54

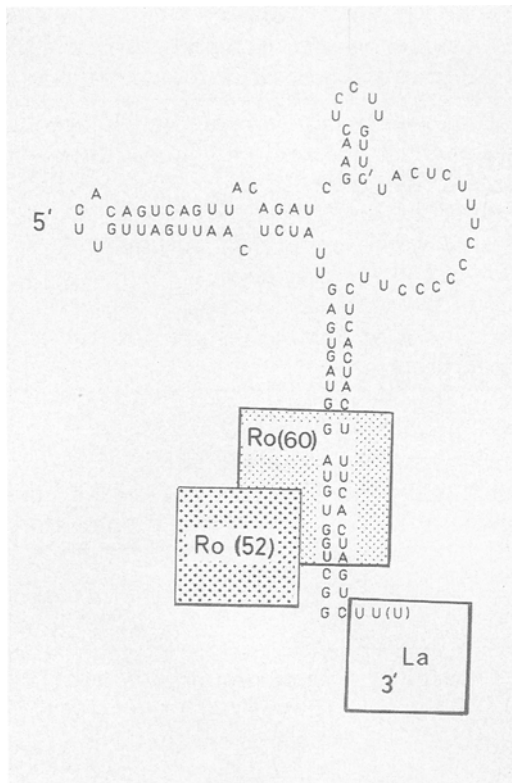


Fig. 1. Human Y1 ribonucleoprotein: The Ro(SSA)52 and 60 kDa and the La(SSB) proteins are attached to Y1 RNA.

Table 3
Heterogeneity of the Ro(SSA) Antigen

Cellular source	Molecular weight	Cell localization
Lymphocytes	52, 60 kDa	Nucleus + cytoplasm
Erythrocytes	54, 60 kDa	

kDa erythrocyte peptides was determined in SS and SLE patients (21). The sera containing anti-Ro(SSA) alone had the highest degree of reactivity with human Ro(SSA), whereas those containing Ro(SSA) and antinuclear RNP showed preferential reactivity with the 60 kDa erythrocyte-derived peptide.

Anti-La(SSB) sera react with a peptide of 46–48 kDa and its degradation products (22). Corresponding monoclonal antibodies have been obtained by several groups of investigators (23,24).

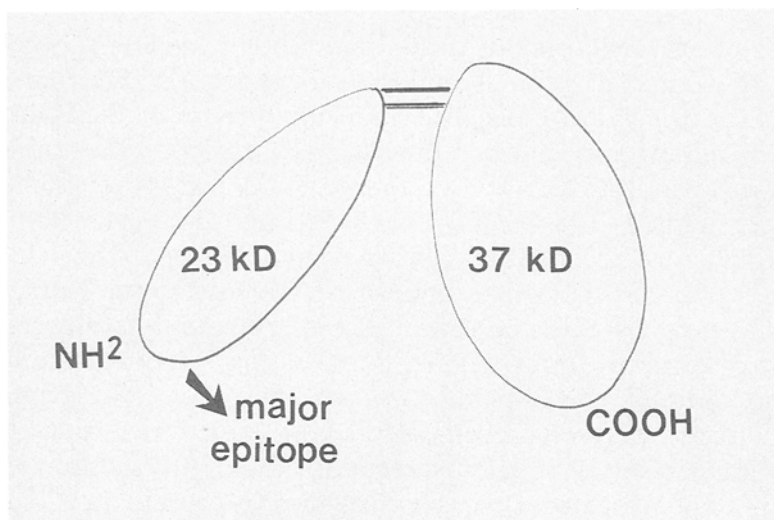


Fig. 2. Schematic model of the Ro(SSA)60 kDa peptide.

La(SSB) is required for efficient polymerase III function of host and viral origin (25).

Structure of Proteins

Recombinant clones for the human 60 kDa antigen have been isolated (26) and these code for a peptide containing 538 amino acid residues. A schematic model with a presumptive disulfide bond linking a 23–37 kDa domain has been proposed (Fig. 2). The amino terminus of the smaller domain contains a dominant epitope reactive with anti-Ro(SSA) antibodies (27). There have been major studies devoted to the characterization of the Ro(SSA) antigen, based on the cloning of the full-length complementary DNA encoding the 52 kDa component (28,29). Thus, the peptide has been unambiguously defined. A zinc finger and a leucine zipper motif were shown to be present, both having been implicated in nucleic acid and protein interactions. It was, however, possible that the 52 kDa form of the Ro(SSA) was a transcriptional or posttranscriptional product of the 60 kDa molecule. In fact, these peptides are encoded by distinct gene sequences. No similarity in the DNA or amino acid sequences were found between any of the present 52 kDa Ro(SSA) clones and the previous 60 kDa Ro(SSA) clones. The 52 kDa Ro(SSA) gene has been located on to chromosome 11 (30).

With regard to the anti-La(SSB) antibody, three approaches have been used to define antigenic sequences. One method used the controlled proteolytic degradation with *Staphylococcus aureus* V8 protease which allowed the description of two antigenically indepen-

dent sets of protease-resistant peptides (22). A second method was to clone the La(SSB) gene. This led several groups of investigators to define the B-cell epitopes on its peptidic products. St. Clair et al. (31) reported the binding of anti-La(SSB) antibody to fusion proteins encoded by La(SSA) complementary DNA fragments comprising amino acids 1–107 (LaA), 111–242 (LaC), and 242–408 (LaD) spanning the entire La(SSB)-coding region. The polymerase chain reaction has been utilized to generate a series of overlapping and nonoverlapping La(SSB) peptides (32) in order to demonstrate that here, the primary autoimmune response is restricted to an immunodominant epitope, the N-terminus LaA. However, over a period of time, all three major epitopes, including that encompassing the putative RNA binding motif, are recognized. A third method was the synthesis of peptides that were conjugated to protein carriers (33).

Technical Problems

Screening of the Antibodies

The initial step in the detection of ANA is indirect immunofluorescence (IIF). It has, however, become increasingly clear that the correct antigenic substrate is crucial for the detection of anti-Ro(SSA) antibody. This antigen is present in undetectable amounts when mouse or rat tissues are used as IIF substrates. It is present in human epithelial cells, such as HEp-2 and KB cells. The IIF pattern is speckled nuclear staining (Fig. 3), that is typically more clumpy than the speckled pattern characteristic of anticentromere antibody binding (34). Agarose gel double-diffusion techniques (Fig. 4) employing a human spleen extract as a source of the Ro(SSA) antigen and rabbit thymic extract as a source of La(SSB) antigen has been extensively used in early studies (35). Antibody identification was performed according to full identify reactions of the test sera with precipitation lines produced by standard sera. Antibodies (36) can be faster and better identified by using counterimmunoelectrophoresis (CIE).

Identification of Anti-Ro(SSA) and Anti-La(SSB) Antibodies

Anti-Ro(SSA) antibodies are difficult to identify by Western blotting of cytoplasmic extracts, so that the 52 kDa protein is almost undetectable (37). The latter peptide migrates with the La(SSB) protein in dodecyl sulfatepolyacrylamide gel electrophoresis (see Fig. 5 on p. 260). Recently, Ben-Chetrit et al. (17) modified the crosslinking level of the gel to give better separation of the two proteins. Further-

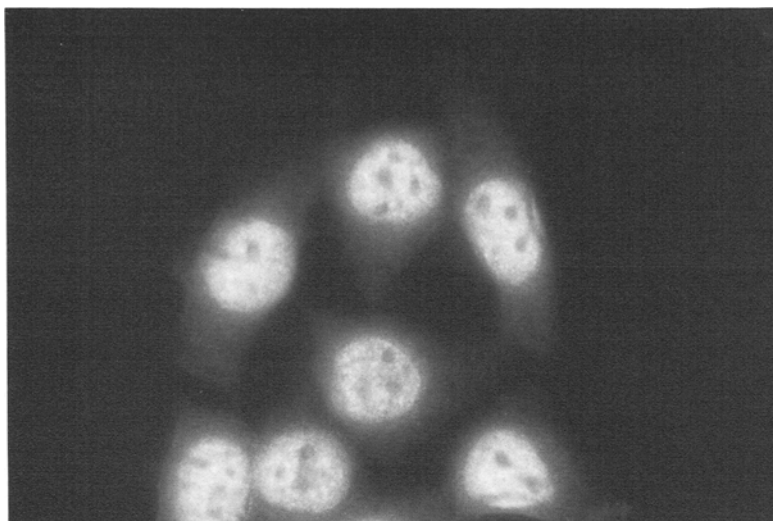


Fig. 3. Speckled nuclear staining of HEp-2 cells owing to anti-Ro(SSA) antibody.

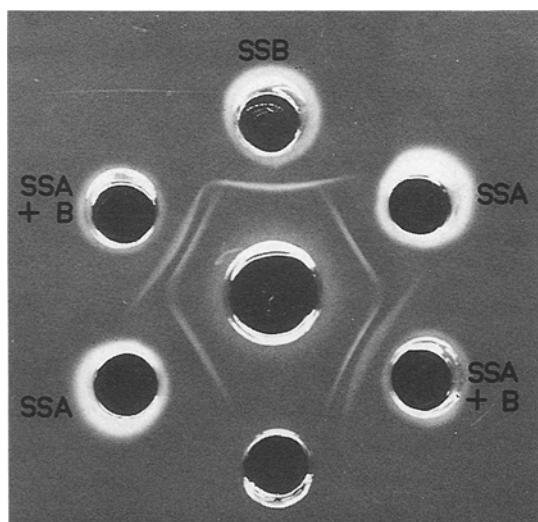


Fig. 4. Immunodiffusion: The central well contains extractable nuclear antigen prepared from human spleen and the peripheral sera are positive for anti-Ro(SSA) and/or anti-La(SSB) antibodies.

more, a sensitive RNA precipitation has been developed to detect very low levels of anti-La(SSB) antibody (38).

With the affinity-purification of Ro(SSA) and La(SSB), much more sensitive solid-phase assays have become feasible (39,40). Since the availability of recombinant Ro(SSA) and La(SSB) proteins (29,41), enzyme-linked immunosorbent assays (ELISAs) have been developed to determine the presence of the corresponding autoantibodies.

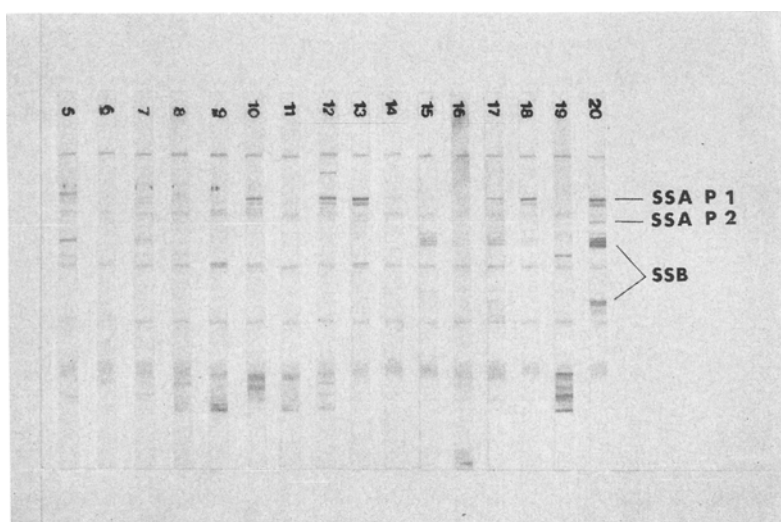


Fig. 5. Identification of anti-Ro(SSA) antibodies directed to the 52 kDa and/or the 60 kDa proteins and anti-La(SSB) antibodies in pathological sera using the Western-blot technique.

Prevalence of Autoantibodies

Prevalence and Association with Clinical Symptoms

Serum antibodies directed against a variety of cellular and extracellular antigens are of major importance for the diagnosis of autoimmune disorders. Much attention has therefore been paid to the identification of intracellular antigens and their association with certain disease states. Autoantibodies directed against the Ro(SSA) and La(SSB) antigens can be found in sera from patients with a variety of autoimmune disorders but do not seem to be entirely disease-specific. Differences between data from various sources can be partly accounted for by the use of distinct methods for the detection of the autoantibodies.

Since both anti-Ro(SSA) and anti-La(SSB) autoantibodies predominate in sera from patients with either SS or SLE, these two patient groups have been studied extensively in order to reveal possible disease associations. In SS (Table 4), anti-Ro(SSA) and anti-La(SSB) autoantibodies were found more frequently in those with the primary form than in those with SS associated with any connective tissue disease. Although anti-Ro(SSA) autoantibodies are not specific for SS, anti-La(SSB) autoantibodies seem to be characteristic of SS (42). In one study these were shown to be detectable years

Table 5
Prevalence of Anti-Ro(SSA) and Anti-La(SSB) Autoantibodies
in Patients with Systemic Lupus Erythematosus

Method of detection ^a	Anti-Ro (SSA), %	Anti-La (SSB), %	References
ELISA	55	24	Maddison et al. (61)
ID	51	6	Yamagata et al. (43)
ID	63	12	Boey et al. (70)
CIE	19–69	39–41	Speransky et al. (51)
WB	17	10	De Rooij et al. (71)
CIE, ID	35	15	Chan et al. (72)
CIE	12–28	18–45	Swaak et al. (50)
RNA	nd ^b	35	Vlachoyiannopoulos et al. (67)

^aID: immunodiffusion; WB: Western blotting; CIE: counterimmunoelectrophoresis; RNA: RNA precipitation.

^bnd: not determined.

association of anti-Ro(SSA) autoantibodies and lupus pneumonitis (52). There is also high incidence of anti-Ro(SSA) autoantibody in patients suffering from subacute cutaneous lupus erythematosus (SCLE) (53).

Mothers who are anti-Ro(SSA) positive display an increased risk for the fetus (54). Neonatal lupus erythematosus (NLE) is an autoimmune disease whose major findings are skin lesions and congenital heart block. Babies receive maternal anti-Ro(SSA) and anti-La(SSB) autoantibodies. Anti-Ro(SSA) are the predominant autoantibodies found in about 95% of the cases. Autoantibodies pass through the placenta from the mother to her child, skin disease resolves at about the time when maternal autoantibodies can no longer be detected, whereas heart block is almost always permanent. Individuals with NLE usually have healthy childhoods, but may develop autoimmune disease in adulthood. Mothers of babies with NLE usually develop symptoms of autoimmune disease later on (55).

Anti-Ro(SSA) and anti-La(SSB) autoantibodies are also found in patients with rheumatoid arthritis (RA) without secondary SS (56–58), although there is a high prevalence of secondary SS in RA with anti-Ro(SSA) and anti-La(SSB) autoantibodies, this diagnosis requires a positive lip biopsy and specific tests (59). Anti-Ro(SSA) autoantibodies seem to characterize a distinct group of RA patients who are almost exclusively female, express more activated B-cell function, have a high prevalence of SS features, and commonly develop side effects to D-penicillamine (60). Anti-Ro(SSA) and anti-La(SSB) autoantibodies can be found in sera of patients suffering

Table 4
Prevalence of Anti-Ro(SSA) and Anti-La(SSB) Autoantibodies
in Patients with Primary Sjögren's Syndrome

Method of detection ^a	Anti-Ro (SSA), %	Anti-La (SSB), %	References
ELISA	96	87	Harley et al. (46)
CIE,ID	63	40	Manoussakis et al. (45)
ID	83	25	Yarnagata et al. (43)
WB	38	77	De Rooij et al. (71)
CIE,ID	60	40	Chan et al. (72)
RNA	nd ^b	58	Vlachoyiannopoulos et al. (67)

^aID: immunodiffusion; WB: Western blotting; CIE: counterimmunoelectrophoresis; RNA: RNA precipitation.

^bnd: not determined.

prior to the confirmation of SS (43). On the other hand, recent data suggests predominance of IgG1 subclass of anti-Ro(SSA) but not anti-La(SSB) autoantibodies in primary SS (44). Their presence is associated with long disease duration, earlier disease onset, parotid or major salivary gland enlargement, intensive lymphocytic infiltration of the minor salivary glands, systemic manifestation as well as hypergammaglobulinemia, rheumatoid factor, and monoclonal type II cryoglobulin (42,45,46). Both anti-Ro(SSA) and anti-La(SSB) autoantibodies were found in the saliva of SS patients, but it was not clear whether they are produced locally or whether the presence of these autoantibodies in the saliva was owing to leakage from blood toward the saliva (47). Neither has it been demonstrated that the presence of anti-Ro(SSA) or anti-La(SSB) autoantibodies in the saliva accounts for the dry-mouth syndrome typical for patients with SS. There is no conclusive evidence relating pathogenetically these autoantibodies to tissue destruction as far as SS is concerned (42).

In SLE patients (Table 5) clinical disease manifestation associated with the presence of anti-La(SSB) and anti-Ro(SSA) autoantibodies are not definitely recognized. Wasicek and Reichlin (48) found a strong positive correlation between the presence of anti-Ro(SSA) autoantibodies alone and serious renal involvement. However, Maddison et al. (49) showed a negative correlation of the presence of anti-Ro(SSA) autoantibodies to nephritis, in agreement with Swaak et al. (50). These latter authors also reported a positive correlation of the presence of anti-La(SSB) autoantibodies with central nervous system complications. Additionally, Wasicek and Reichlin (48), as well as Speransky et al. (51) described the association of the presence of both anti-Ro(SSA) and anti-La(SSB) autoantibodies with skin rash in SLE patients. There are several reports describing the

from polymyositis (61), especially when a secondary SS is present (62). The presence of anti-Ro(SSA) autoantibody is associated with atrioventricular conduction disturbances (63,64).

In analyzing serological aspects of systemic autoimmunity in human immunodeficiency virus 1 (HIV-1) carrying individuals, antibodies to the 60 kDa Ro(SSA) antigens were found in 44–95% of sera (65). In those HIV-positive individuals with lymphocytic disorders resembling SS, such as bilateral parotid gland enlargement, xerostomia, and xerophthalmia, anti-Ro(SSA), or anti-La(SSB) autoantibodies were not detectable (66).

There are several examples of a correlation between drug toxicity and anti-Ro(SSA) and anti-La(SSB) autoantibody production, for instance, D-penicillamine toxicity in Greek patients with RA, which was found to be especially high in those RA patients positive for anti-Ro(SSA) antibody (60,67). Skin eruptions with features of SCLE occurred in patients treated with griseofalvin, suggested that photoactive drug may be synergistic with anti-Ro(SSA) and anti-La(SSB) autoantibodies to produce lesions of SCLE (68,69).

Precise Specificity of Autoantibodies

Several approaches have been required to map epitopes within Ro(SSA) and La(SSB) proteins. The most commonly used methods have consisted to test autoimmune sera with either overlapping synthetic peptides in ELISA or sequentially truncated recombinant fragments of the autoantigen in Western blot (70–72). Another approach has consisted to analyze, using an immunoprecipitation system, the reactivity of patient sera with recombinant fragments. As pointed out by several authors (73–75), none of these approaches is absolute and definitive in the complete mapping of autoepitopes of the protein. Several studies (both with autoimmune sera and with antibodies raised in animal to intact proteins) have shown that the antigenic reactivity of peptides is highly dependent on the assay format (76,77). In some cases, the free peptide used in solution as inhibitor is most active, whereas in other cases, antibodies react preferentially with immobilized peptides adsorbed onto a solid-phase or conjugated to a carrier. The length of the fragment tested can also represent a critical parameter. It is assumed that a long peptide will fold more easily than a short one into the proper orientation within the protein, and thus lead to a higher level of cross-reactivity with antibodies. However, it should be noted that in some cases, shorter peptides in solution have been shown to adopt a conformation that mimicked more closely the native protein (78). Finally, it is worth stressing that the presence of charged groups at the termini of peptides (recombinant or synthetic) can effect their

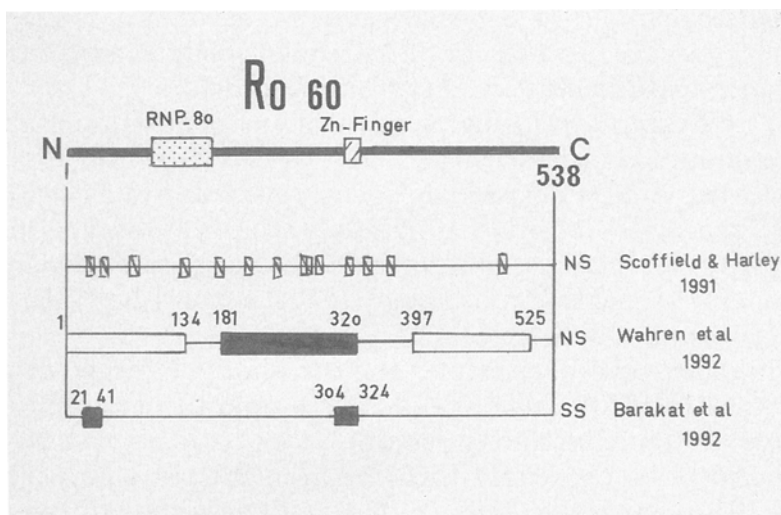


Fig. 6. Schematic representation of autoepitopes of the Ro(SSA)60 kDa protein, as described by authors quoted on the right. In the immunodominant region 181–320, Wahren et al. identified six epitopes in residues 181–200, 201–210, 216–235, 226–245, 246–265, and 301–320. The putative zinc finger is located in residues 305–323. (SS) Sjögren's syndrome; (NS) not specified.

reactivity when these peptides correspond to inner sequences of the protein (78–79). Conversely, the presence of the fusion partner of recombinant proteins (β -galactosidase, MS2-polymerase, and so on) that blocks the free amino group at the N-terminal end of the polypeptide may also alter the antigenicity of peptides (80). It must be recognized, therefore, that results reported in the various epitope mapping studies, should be considered complementary to each other, in an additive rather than in an exclusive manner.

B-Cell Epitopes of Ro(SSA)60

Autoepitopes of the 60 kDa Ro(SSA) protein have been described by several authors using different biochemical and molecular biological approaches. The location of autoepitopes of Ro(SSA)60 is illustrated in Fig. 6. By analysis of the antigenicity of the complete Ro(SSA)60 protein using 531 overlapping octapeptides synthesized on derivatised polyethylene pins with four anti-Ro(SSA) patient sera, Scofield and Harley identified 14 peptides that were antigenic (81). Interestingly, these authors showed that among the 531 peptides synthesized, 6 shared sequence identified with the nucleocapsid (N) protein from the Indiana serotype of vesicular stomatitis virus and that 5 of these 6 small peptides were recognized by anti-

Ro(SSA) antibodies. By using recombinant Ro(SSA)60 proteins encoded by both full-length and deletion clones in Western blot, Wahren et al. (79) identified a major antigenic domain located in the middle part of the protein (residues 181–320) and two other antigenic regions located in the N- and C-terminal ends of the protein (residues 1–134 and 397–525, respectively). The results obtained by immunoblotting with Ro(SSA)60 fragments successively truncated from the C-terminal end toward the N-terminus were further confirmed in ELISA by using overlapping synthetic peptides encompassing the region 181–320. Within this region, several epitopes were characterized in residues 181–200, 201–210, 216–235, 226–245, 246–265, and 301–320, respectively. The 42 patient sera tested in this study were selected on the basis of their anti-Ro(SSA) reactivity established by CIE and they were found to react individually to different peptides in the immunodominant region 181–320 of the protein (82). The heterogeneity in the immune response to the Ro(SS)60 protein was also described by Barakat et al. (83). These authors prepared five synthetic peptides corresponding to the N-, C-, and central domain of Ro(SSA)60. The activity of these five peptides was tested by ELISA with sera from 112 patients with SLE, 55 with primary SS, and 29 with RA. Peptide 21–41 was recognized by IgG antibodies of 57% of the primary SS patients and only 7% of the SLE patients, whereas 63% of primary SS sera and 46% of SLE sera tested in parallel possessed IgG antibodies reacting in ELISA with the purified Ro(SSA)60 protein. The central region covering residues 304–324 of the protein was recognized by IgG antibodies of 13% of patients with primary SS. The domain includes a putative zinc finger that may be involved in the protein–protein interaction in the Ro RNP particle. Recently, Bozic et al. (84) analyzed by immunoprecipitation several sera anti-Ro(SSA)-positive in CIE with wild type and deleted mutants of recombinant Ro(SSA)60 protein. They found that all sera had a strongly reduced reactivity with all Ro(SSA)60 N- or C- deleted mutants, extending previous studies that showed that most anti-Ro(SSA)60 autoantibodies recognized conformational epitopes on the Ro(SSA)60 antigen (85,86).

B-Cell Epitopes of Ro(SSA)52

The distribution of the epitope regions on Ro(SSA)52 kDa peptide as reported by Bozic et al. (84) and Ricchiuti et al. (85) is shown in Fig. 7. Bozic et al. used in precipitation assays recombinant Ro(SSA)52 fragments and patient sera positive with Ro(SSA) by CIEs. Most of these patients were from Slovenia, and they had SLE ($n = 22$), primary SS ($n = 20$), secondary SS associated with SLE ($n = 5$), RA, or another undefined disease ($n = 23$). Anti-Ro(SSA)52

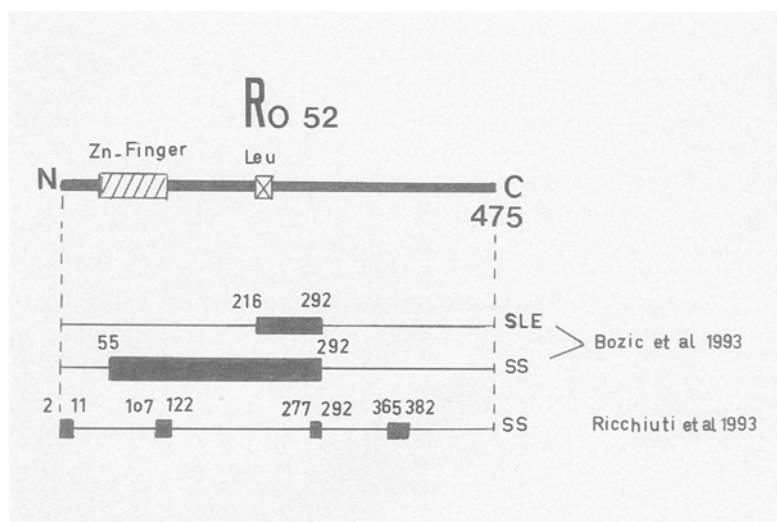


Fig. 7. Autoepitopes of Ro(SSA)52 kDa protein. See Fig. 6 for legend.

antibodies of SLE patients recognized predominantly an antigenic determinant located in residues 216–292, whereas anti-Ro(SSA)52 antibodies from 55 patients reacted with several epitope regions in the domain 55–292. Significant differences between the regions of Ro(SSA)52 that are recognized by either SLE or SS sera were also found by Ricchiuti et al. (85) who used a different methodological approach for mapping Ro(SSA)52 autoepitopes. Thirty-nine synthetic peptides (10–24 residues long) covering the whole molecule of the Ro(SSA)52 protein and overlapping each other through 1–10 residues were tested in ELISA for reactivity with sera from patients suffering from primary SS ($n = 89$), secondary SS ($n = 57$), SLE ($n = 141$), and other rheumatic diseases ($n = 202$). Only four peptides in residues 2–11, 107–122, 277–292, and 365–382, respectively, were immunoreactive. Reaction with Ro(SSA)52 peptides was essentially observed with sera from SS patients. Less than 20% of SLE sera reacted with at least one Ro(SSA)52 peptide. Furthermore, significant differences were found in the reactivity with Ro(SSA)52 peptides according to the geographical origin of patients. For example, raised levels of IgG antibodies reacting with peptide 277–292 were found in primary SS sera collected in France, whereas raised levels of antibodies reacting with peptide 365–382 were found in primary SS sera collected in England and Poland. Also noticeable is the fact that although primary SS sera collected in the United States reacted strongly with recombinant Ro(SSA)52 (76%; $n = 21$) as primary SS sera collected in the other countries (52–80%), very few sera from the United States reacted with Ro(SSA)52 peptides.

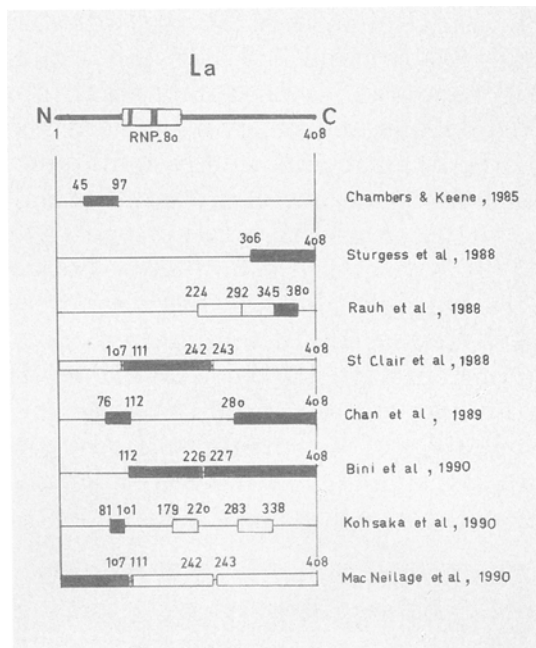


Fig. 8. Autoepitopes of La(SSB) protein. See Fig. 6 for legend. (SLE) systemic lupus erythematosus.

B-Cell Epitopes of La(SSB)

Epitopes of the La(SSB) antigen have been mapped using both recombinant proteins and enzymatic degradation of natural La(SSB) protein (Fig. 8) (38). In 1985, Chambers and Keene (86) identified an autoepitope within a partial cDNA product of La(SSB), later identified in the full-length sequence in residues 45–97. Chan et al. (22) described the presence of autoepitopes on two fragments of 29 and 23 kDa, termed X and Y, obtained by limited proteolytic cleavage of La(SSB) and then found as corresponding to the N- and C-terminal domains of the protein (87). Sturgess et al. (88), utilizing a cDNA encoding 87% of La(SSB), identified a major epitope within the C-terminal 103 residues of the molecule (residues 306–408). Rauh et al. (89) defined three independent antigenic regions in residues 284–292, 293–345, and 346–383, the latter corresponding to an immunodominant region. St. Clair et al. (31) found activity in three cDNA fragments comprising residues 1–107, 111–242 (showing the

highest level of binding with autoantibodies), and 242–408. Bini et al. (90) identified two immunodominant epitopes in residues 112–126 and 226–408, and Kohsaka et al. (91) identified two major epitopes in residues 81–101 and 283–338 and a minor one in residues 179–220. All these studies used only small numbers of anti-La(SSB) sera. McNeilage et al. (32), by using sera from 156 patients containing anti-La(SSB) antibodies and recombinant La(SSB) polypeptides, confirmed the existence of a dominant epitope in the N-terminal domain 1–107 in addition to epitopes in the fragments 111–242 and 243–408. A striking homology between the region 93–100 and a viral gag protein has been reported. Similarities between the La(SSB) region 81–101 and immunodominant epitopes of the HIV-capsid protein have also been described (for a review, see ref. 92). Interesting also was the finding by McNeilage et al. (32) that in the very early stage of the anti-La(SSB) response, the immunodominant N-terminal fragment 1–107 was recognized preferentially and, with time, the response broadens to include other epitopes.

Associations with HLA Alleles

In primary SS, as well as SLE, the production of anti-Ro(SSA) and La(SSB) antibodies is associated with the human leukocyte antigen (HLA) class I and class II phenotype (93,94). The HLA-B8/DR3/DRwS2a/DQw2 haplotype is often found in patients with primary SS displaying anti-Ro(SSA) and anti-La(SSB) activity (46). The patients positive for anti-Ro(SSA) and anti-La(SSB) antibody are primarily suffering from SLE and present the HLA-DR2/DQw1 (DRw6) haplotype. Whatever the underlying disease, the HLA-DQw1/dQw2 heterozygotic patients are particularly prone to the autoantibody production.

Moreover, the frequency of the HLA-DR3 allele is significantly increased in anti-Ro/SSA positive mothers of infants with NLE, but that of the HLA-DR2 allele is increased in anti-Ro/SSA positive mothers of normal infants, irrespective of infant HLA phenotype (94). Perhaps Ro/SSA autoantibodies from HLA-DR3 mothers are more likely to cause NLE because they react with different Ro/SSA epitopes than the antibodies produced in HLA-DR2 mothers or because they have a higher affinity for the same epitope.

Recent data confirm that HLA-DQ genes are better candidates than DR β 1 or DR β 3 genes to confer susceptibility to Ro(SSA) and La(SSB) autoantibody production. DQw1.2 (DQw6) and DQw2.1 (linked to DR3) appear to bring about the strongest risk factors, especially when they occur together. Other DQ (or DR alleles) might also carry the same or similar sequences that promote this response, especially in blacks, since DQw1.2 (DQw6) and DQw2.1 were not

found in all patients. DQw3 might be relatively protective since it occurs less frequently. It should be noted that DQw1 occurs in linkage disequilibrium with DR1, DR2, DRw6, and DRw10, and DQw2 with DR3 and DR7. Thus, effects from both of HLA haplotypes should be important in the generation of the Ro(SSA) and La(SSB) autoantibodies.

Pathophysiology of the Autoantibody Production

Autoimmunogenicity

The demonstration that only a few proteins are targeted, notably the Ro(SSA) and La(SSB) peptides, suggests that autoantibody production is antigen-driven. To examine this hypothesis, lymphocytes from normal volunteers were stimulated with Ro(SSA). Surprisingly, a striking inhibition of the autologous mixed lymphocyte reaction occurred, indicating that Ro(SSA) peptides may interfere with recognition of self-antigens by autologous T-cells (95).

We have focused on epithelial cells in SS (96) and have taken advantage of the accessibility of conjunctival cells. Our findings of La(SSB) membrane-expression (Fig. 9) demonstrated that the autoantigenic peptides may be expressed on the cell membrane. This observation confirms the results of Baboonian et al. (97) who induced the same phenomenon by infecting HEp-2 cells with adenovirus. Keratinocyte membrane expression of nuclear antigens Sm, RNP, and Ro(SSA) has also been obtained through ultraviolet light exposure of the cells (98) on stimulation of serum-starved cells with 10% fetal calf serum (99). Transformed keratinocytes (100) and SLE patient keratinocytes (101) have been subsequently shown to expose nuclear antigens on the cell surface. It is of interest that SLE-derived keratinocytes were more susceptible than control keratinocytes to ultraviolet-induced intracellular and surface expression of soluble intracellular antigens.

Once made accessible to T-lymphocytes, the autoantigen still has to be presented within an immunogenic context. It has indeed been shown that major histocompatibility complex class II molecules are present on diseased epithelial cells. Mobilization of nuclear peptides is an intriguing phenomenon, but the presentation of these autoantigens is accomplished through HLA class II molecules.

Pathogenicity

NLE is a productive model for the study of anti-Ro(SSA) and anti-La(SSB) antibody pathogenicity. Titers of autoantibodies in serum from babies with congenital heart block are lower than in the mothers, suggesting that they are removed from the maternal circu-

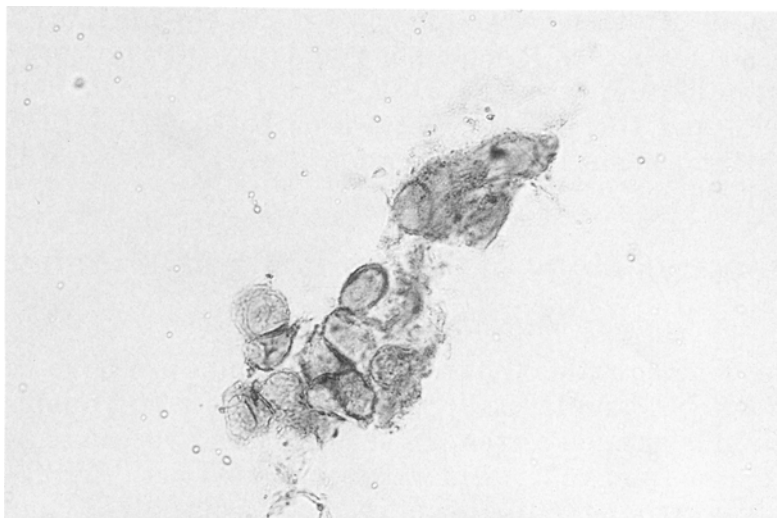


Fig. 9. The La(SSB) antigen is expressed on the membrane of epithelial conjunctival cells from patients with primary Sjögren's syndrome.

lation and deposited in the baby's tissue (102). Unaffected children have autoantibody titers comparable to maternal levels (103). Skin biopsies have yielded positive IIF of skin lesions in infants with NLE (104), and such studies on cardiac tissue from a baby born with congenital heart block has also been performed (105). Using human sera monospecific for Ro(SSA), this antigen was found to be present in the nuclei of myocardial cells and cells of the cardiac conduction system (106). Ro(SSA) might be temporarily expressed on the cell surface of conduction system cells during cardiac embryopogenesis. Using monoclonal and affinity-purified antibodies to La(SSB) and affinity-purified anti-Ro(SSA) antibodies, Horsfall et al. (107) identified both antigens on the surface of the fibers of the affected heart. Surface coexpression of immunoglobulin, complement, and class II antigen, consistent with a local immune response, was also found.

A great deal of knowledge has thus become available over the last 20 yr, owing to the availability of new immunological and molecular techniques. Much further work is needed to bridge the gap between the functions of Ro(SSA) and La(SSB) antigens and the clinical manifestations of connective tissue diseases.

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