Autoantibodies

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SECOND EDITION

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32 SS-B (La) AUTOANTIBODIES

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ABSTRACT

Anti-La/SS-B antibodies are usually detected in patients with Sjogren's syndrome and Systemic Lupus Erythematosus. They target a 408 amino acid phosphoprotein – the La/SS-B autoantigen that associates with a variety of small RNAs transcribed by RNA polymerase III, protecting them from exonuclease digestion and regulating their downstream processing. La/SS-B also binds viral RNAs (e.g. adenovirus VA, Epstein Barr EBER), viral and human RNAs possessing IRES (internal ribosomal entry elements) as well as the RNA component of telomerase complex. A variety of methods including immunofluorescence (IF), immunobloting (IB), immunoprecipitation (IP), ELISA and counterimmunoelectrophoresis (CIE) have been applied for the detection of anti-La/SS-B antibodies. Among them RNA precipitation is considered as the "gold standard" method, but due to their availability CIE, IB and ELISA are used in every day routine. Anti-La/SS-B antibodies are closely associated with Sjogren's Syndrome, particularly with the presence of extraglandular disease (affecting kidney, lung and liver). Anti-La/SS-B antibodies are also found in the serum of mothers whose children had neonatal lupus; in systemic lupus erythematosus anti-La antibodies are typically present many years before the onset of the clinical disease. Additionally, anti-La/SS-B antibodies can be found in patients with rheumatoid factor, polyclonal hypergammaglobulinemia and cryoglobulinemia.

HISTORICAL NOTES

During the sixties and seventies a 46.7 kD protein reacting with autoantibodies derived from sera of patients with Systemic Lupus Erythematosus (SLE) and Sjogren's syndrome was identified. Several names have been allocated to this protein, like La, SS-B, Ha and SjT. In 1979, interlaboratory exchange of sera and antigen extracts showed that all these previously identified proteins are antigenically identical and correspond to the La/SS-B autoantigen [1].

Autoantibodies, 2/e
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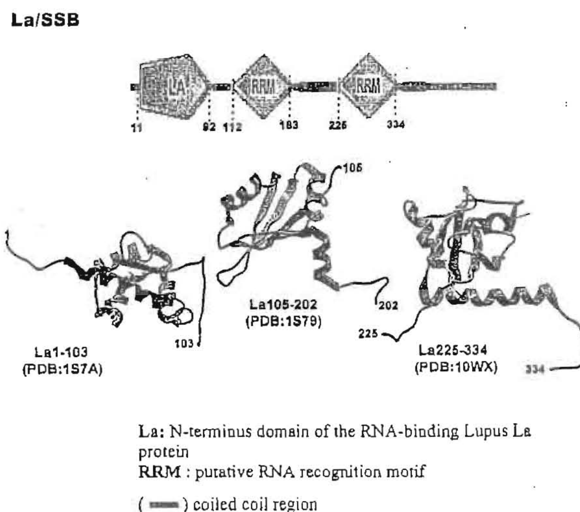
AUTOANTIGEN

Definition

The human La/SS-B gene is localized to chromosome 2 and encodes a phosphoprotein composed of 408 amino acid residues with a calculated molecular weight of 47 kDa, migrating at about 50 kDa in SDS-polyacrylamide gel electrophoresis (Figure 32.1). At least 8 isoelectric forms (pI: 6 to 7) can be distinguished by two-dimensional gel electrophoresis. La/SS-B associates with a variety of small RNAs, including 5S cellular RNA, tRNA, 7S RNA and hY RNAs, all transcribed by RNA polymerase III [2]. In molecular level, it associates predominantly with a short polyuridylate sequence that exists at the 3′ end of almost all nascent pol III transcripts. In addition, La binds viral RNAs (e.g. adenovirus VA RNA, Epstein Barr EBER RNA), viral and human RNAs possessing IRES (internal ribosomal entry elements) and RNA component of telomerase complex [3]. Human La is a multi-domain protein that contains the La motif in its N-terminal region, a typical RNA recognition motif (RRM) in its central part and an unusual RRM, encompassing residues 229–326. The latter is followed by a long, flexible polypeptide that contains a short basic motif (SBM), a regulatory phosphorylation site on Ser366 and a nuclear localization signal (NLS). It seems that both the La motif and the adjacent central RRM are required for high-affinity RNA binding, and that La motif provides specific recognition for poly(U) sequences. In addition, the C-terminal RRM, in conjunction with the SBM downstream, contributes to La interactions with non-poly(U) RNA targets such as viral RNAs and TOP mRNAs [4]. Recently, insights inro the three-dimentional structure of La/SS-B were gained by the structural



La: N-terminus domain of the RNA-binding Lupus La protein
RRM : putative RNA recognition motif
(▬) coiled coil region

FIGURE 32.1

analysis of the La-motif, the central RRM and the carboxyl-terminal RNA recognition domain of the autoantigen [5, 6]. The La motif folds into a winged-helix motif elaborated by the insertion of three helices. The central RRM consists of a four-strand β-sheet attached to two α-helices while the C-terminal domain folds to generate a five-stranded, antiparallel β sheet surface that is terminated by a long α helix (Figure 32.1). Although, all three domains possess RNA recognition structures, the La motif and the central RRM seem to be required for poly(U) RNA binding [5].

Biological Function

The specific binding of La to precursor RNA molecules protects them from exonuclease digestion and thereby regulates their downstream processing. La also serves to retain precursor RNA molecules in the nucleus. Other cellular functions of La/SS-B autoantigen include: (i) an ATP-dependent helicase able to melt RNA–DNA hybrids, (ii) unwinding of double-stranded RNA that leads to inhibition of double-stranded RNA-dependent activation of the protein kinase PKP, (iii) association with telomerase thus influencing telomere homeostasis in vivo, (iv) an RNA chaperone effect capable of transient bipartite (5′- and 3′-end) binding of nascent transcripts synthesized by polymerase III (e.g. tRNA precursors), (v) induction of cap-independent translation (La binds to the 5′ untranslated region of viral or human mRNAs possessing IRES elements and promotion of internal, cap-independent initiation of translation at the correct AUG). Phosphorylation of serine-366 has been shown to be essential in La functions (e.g. La phosphorylated at this position appears to be transcriptionally inactive whereas dephosphorylated La is active).

Origin/Sources

Mammalian cells contain about 2×10^7 copies of La/SS-B protein. These molecules reside predominantly in the nucleus, but can also be found in the cytoplasm. Early assays, used purified bovine La, as derived by conventional cell-francrionation procedures and affinity purification, for the detection of anti-La/SS-B antibodies. Later on, bacterially expressed recombinant human La/SS-B was used as the antigenic substrate. Until today La/SS-B homologues have been characterized in human (Uniprot: La_human), bovine (Uniprot: La_bovin), murine (Uniprot: La_mouse), rat (Uniprot: La_rat), rabbit (Uniprot: La_rabit), *Xenopus laevis* (Uniprot: Laa_xenla & Lab_xenla), *Drosophila melanogaster* (Uniprot: La_drome) and *Aedes albopictus*/ Forest day mosquito (Uniprot: La_aedal).

Methods of Purification

La/SS-B autoantigen can be purified from cultured cells or homogenized tissues by conventional cell-fractionation followed by affinity chromatography with anti-La/SS-B antibodies. Recombinant La/SS-B has also been produced by cloning and expression of human La/SS-B cDNAs in *E. coli* either as fusion or non-fusion proteins. If recombinant La/SS-B is produced as a non-fusion protein, it is purified conventionally by affinity chromatography with anti-La/SS-B antibodies. Otherwise it is purified taking advantage of the specific tag that it is attached to the La/SS-B molecule. Phage-displayed La/SS-B antigen can also be used as a diagnostic reagent. In this case the phage-displayed antigen is obtained by precipitation of the phage particles from the bacterial culture supernatant with polyethylene glycol.

AUTOANTIBODY

Definition

Anti-La/SS-B antibodies are usually accompanied by anti-Ro/SS-A antibodies, whereas anti-Ro/SS-A antibodies can be found alone in many autoimmune sera. During the last decade, the target epitopes of anti-La/SS-B autoantibodies have been mapped [7]. Early efforts to identify epitopes on the La antigen used enzymatic digestion of the native protein. In this instance, antigenic sites covering the larger part of La autoantigen were identified. These sites were called LaA (amino acids 1–107), LaC (amino acids 111–242) and LaL2/3 (amino acids 246–408). Later, more detailed and analytical epitope mapping revealed the exact localization of its antigenic determinants. Some of the La epitopes were found to reside in functional regions of the autoantigen, like the central RNA recognition motif (RRM) and the ATP binding site. However, the interaction of hYRNA with the RRM motif did not affect the autoantibody binding in the same region. In contrast, the interaction of the ATP binding site with ATP abolished the autoantibody binding at the same part of the protein. B-cell epitope mapping of La/SS-B was also performed using synthetic peptides covering the whole sequence of the protein. Highly antigenic peptides were those spanning the sequences: ^{147}HKAFKGSI^{154} (147–154aa) (located within central RRM motif), ^{291}NGNLQLRNKEVT^{302} (291–302aa), ^{301}VTWEVLEGE-VEKEALKKI^{318} (301–318aa) and ^{349}GSGKGKVQFQGKKTKF^{364} (349–364aa). The most sensitive and specific epitope was 349–364aa, which showed a sensitivity and specificity of greater than 90%. This epitope was attached to a tetramer sequential oligopeptide carrier SOC_4 used for immunoassay development. Approximately 90% of anti-La positive sera were reactive with both the synthetic peptide pep349–364 and the recombinant La protein. Therefore, this epitope analogue exhibited comparable reactivity with the recombinant La/SS-B and anti-La/SS-B antibodies obtained from autoimmune patients. Other epitopes have also been identified in other parts of the molecule using recombinant fragments of La/SS-B or synthetic peptides (see Figure 32.2). Their existence is believed to be correlated with extended intramolecular spreading of epitopes to the whole La/SS-B molecule, as previously described.

Pathogenic Role

Anti-La/SS-B antibodies are directly involved in the pathogenesis of the neonatal lupus syndrome, characterized by transient skin rash, liver and hematological

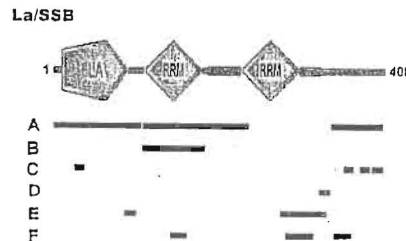


FIGURE 32.2

features and fetal heart conduction abnormalities. This rare syndrome occurs in newborns of mothers with anti-Ro and anti-La antibodies. It is presumed that maternal IgG autoantibodies pass through the placenta to the fetal circulation and cause tissue injury to the heart and skin. It is thought that that redistribution of Ro and La autoantigens to the surface of myocardial cells induced either by β-estradiol, viral infection or apoptosis. Anti-La/SS-B antibodies, along with anti Ro/SSA antibodies have also been detected in skin biopsies from patients with subacute cutaneous lupus erythematosus (SCLE). In Sjogren's syndrome La/SS-B appears to participate in the local autoimmune response, in the affected exocrine glands since: (1) autoantibodies to La/SS-B are found in the saliva of patients, (2) B-cells infiltrating the salivary glands, contain intracytoplasmic immunoglobulins with anti-La/SS-B activity, (3) an increased mRNA production of La/SS-B in acinar epithelial cells has been observed and (4) translocation and membrane localization of the protein has been observed in conjunctival epithelial cells of Sjögren's syndrome patients. Recent studies have shown that cultured epithelial cells from patients with Sjögren's syndrome constitutively secrete exosomes that contain the major autoantigens Ro/SSA, La/SS-B and Sm. This mechanism may represent a pathway whereby intracellular autoantigens are presented to the immune system.

Genetics

The presence of anti-La/SS-B antibodies is associated with HLA DR3 and/or DQ2, similarly to the presence of anti-Ro antibodies. In addition, DQα alleles from anti-La positive patients possess a glutamine residue at position 34 of the outer domain, while the DQβ alleles possess a leucine residue at position 26. These amino acids map to the floor of the peptide binding groove of HLA molecules. Antibodies targeting the major epitope of La/SS-B, 349–364aa, were also associated in European patients with DR2, DQ2 HLAs and DQA1*501, DQB1*02 alleles.

Methods of Detection

A variety of methods have been applied for the detection of anti-La/SS-B antibodies. Among them RNA precipitation is considered the gold standard method. However, this method cannot be used in everyday routine analysis, but it is useful as a reference and confirmatory assay. More specifically, the methods used for the detection of anti-La/SS-B antibodies include:

1. *Immunofluorescence* (IF): This technique is applied in cultured cells of different origin. The staining pattern that is usually produced by anti-La/SS-B autoantibodies, is fine speckled with fine grainy staining in a uniform distribution. In some rare cases anti-La/SS-B antibodies have been reported to produce a homogenous pattern and a nucleolar staining.
2. *Counterimmunoelectrophoresis* (CIE) and immunodifussion (ID): Anti-La/SS-B antibodies can be efficiently detected by ID and CIE. However, a distinct subpopulation of patient sera contains precipitin-negative anti-La/SS-B antibodies. These antibodies possess restricted epitope recognition, targeting one or a few epitopes on the autoantigen.
3. *Immunobloting* (IB): IB provides an efficient and sensitive method to detect anti-La/SS-B antibodies. Both nuclear and cytoplasmic extracts from many cell lines have been used successfully for the detection of these autoantibodies.

The fact that La/SS-B is rather sensitive to proteolysis and that La/SS-B co-migrates with one of the Ro proteins (Ro52) in conventional SDS-PAGE may complicate the interpretation of IB data.

4. *Immunoprecipitation* (IP): When [^{32}P]-labeled cell extracts are used for I IP with anti-La/SS-B antibodies, a characteristic set of small RNAs is precipitated, representing all newly synthesized RNA polymerase III transcripts. This method, is highly specific and sensitive to confirm the presence of anti-La/SS-B antibodies.
5. *ELISA*: Recombinant and purified La/SS-B have been used in a variety of ELISAs for the detection of anti-La/SS-B antibodies with high specificity and sensitivity. Similarly the ^{349}GSGKGKVQ-FQGKKTKF^{364} epitope of La/SS-B in attachment with a tetramer sequential oligopeptide carrier SOC_4 utilized for the development of an ELISA with >90% sensitivity and specificity. Recent studies demonstrated that anti-La/SS-B antibodies are highly masked by anti-idiotypic antibodies targeting their antigen recognition site. In this regard, a specific procedure has been developed for the release of anti-La/SS-B antibodies from their anti-idiotypic counterpart, allowing their detection in a conventional ELISA assay [8].

CLINICAL UTILITY

Disease Association

Anti-La/SS-B antibodies are closely associated with Sjogren's Syndrome, since they are detected more frequently than SLE. Moreover, anti-La/SS-B antibodies are more frequently detected in patients with rheumatoid factor, polyclonal hypergammaglobulinemia and cryoglobulinemia, independently of the autoimmune disease. Anti-La/SS-B antibodies are also frequently seen in mothers whose children had neonatal lupus.

Disease Prevalence

The presence of anti-Ro and anti-La autoantibodies is included in the classification criteria suggested by the European Community Study Group on Diagnostic criteria for SS (not defined and used). Using different methods anti-La/SS-B antibodies are detected in about 25–50% of patients with Sjogren's syndrome and 10–15% of patients with systemic lupus erythematosus and 5–10% of patients with rheumatoid arthritis. Patients with RA or SLE and anti-La/SS-B autoantibodies, usually develop secondary Sjogren's syndrome. The prevalence of anti-La/SS-B antibodies in mothers of children with neonatal lupus is around 60%.

Diagnostic Value

The presence of anti-Ro/SSA and/or LaSS-B autoantibodies is associated with earlier disease onset, longer disease duration, recurrent parotid gland enlargement, splenomegaly, lymphadenopathy, vasculitis and high intensity of minor salivary gland infiltration in primary Sjögren's syndrome patients.

Specificity

RNA precipitation shows the highest specificity of the methods used for the detection of anti-La/SS-B antibodies. Among the assays used for the every day clinical practice, the IB and CIE are the most specific.

Sensibility

Both RNA precipitation and ELISA are highly sensitive techniques for the detection of anti-La/SS-B antibodies. However, the specificity of anti-La/SS-B ELISA is highly depended on the source of antigen (affinity purified, recombinant, phage-displayed, synthetic epitopes etc.), ranging from 15 to 90%.

Prognostic Value

Recent studies suggested that anti-Ro, and anti-La antibodies are typically present many years before the diagnosis of SLE and can be detected earlier than anti-Sm and anti-RNP antibodies (a mean of 3.6 years before the diagnosis vs. 1.2 years) [9]. It seems that the appearance of autoantibodies in patients with SLE tends to follow a predictable course, with anti-Ro and anti-La antibodies to appear first, followed by a progressive accumulation of lupus specific autoantibodies (e.g. anti-Sm, anti-DNA) before the onset of the clinical disease.

In Sjogren's syndrome La/SS-B autoantibodies correlate with the appearance of extraglandular disease (affecting kidney, lung and liver) with an odds ratio of about 6 [10].

Disease Activity

There is not association of anti-La/SS-B autoantibodies with disease activity in SLE.

Organ Involvement/Damage

A number of studies have suggested that in neonatal lupus, maternal anti-La/SS-B autoantibodies pass from mother to the embryo and cause conduction abnormalities to the fetal heart.

Clinical Utility of the Different Methods of Detection

The detection of anti-La/SS-B is not showing many problems. The ELISA, IB and CIE generally show good concordance rate and comparable sensitivity (over 90%). Among them, ELISA and CIE are generally considered as safe, rapid, sensitive and specific techniques for the detection of La/SS-B antibodies. The latter methods are most frequently used in diagnostic laboratories.

TAKE-HOME MESSAGES

- Anti-La/SS-B antibodies are usually detected in patients with Sjogren's syndrome.
- Anti-La/SS-B antibodies are mostly accompanied by anti-Ro/SS-A antibodies.