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The presence of autoantibodies is the hallmark of systemic autoimmune diseases. During the past 30 years, intense clinical and basic research have dissected the clinical value of autoantibodies in many autoimmune diseases and offered new insights into a better understanding of the molecular and functional properties of the targeted autoantigens. Unraveling the immunologic mechanisms underlying the autoimmune tissue injury, provided useful conclusions on the generation of autoantibodies and the perpetuation of the autoimmune response. Primary Sjögren's syndrome (pSS) is characterized by the presence of autoantibodies binding on a vast array of organ and non-organ specific autoantigens. The most common autoantibodies are those targeting the Ro/La RNP complex, and they serve as disease markers, as they are included in the European–American Diagnostic Criteria for pSS. Other autoantibodies are associated with particular disease manifestations, such as anti-centromere antibodies with Raynaud's phenomenon, anti-carbonic anhydrase II with distal renal tubular acidosis, anti-mitochondrial antibodies with liver pathology, and cryoglobulins with the evolution to non- Hodgkin's lymphoma. Finally, autoantibodies against autoantigens such as alpha- and beta-fodrin, islet cell autoantigen, poly(ADP)ribose polymerase (PARP), NuMA, Golgins, and NOR-90 are found in a subpopulation of SS patients without disease specificity, and their utility remains to be elucidated. In this review, the molecular and clinical characteristics (divided according to their clinical utility) of the autoantigens and autoantibodies associated with pSS are discussed.

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Sjögren's Syndrome—Study of Autoantigens and Autoantibodies

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Abstract The presence of autoantibodies is the hallmark of systemic autoimmune diseases. During the past 30 years, intense clinical and basic research have dissected the clinical value of autoantibodies in many autoimmune diseases and offered new insights into a better understanding of the molecular and functional properties of the targeted autoantigens. Unraveling the immunologic mechanisms underlying the autoimmune tissue injury, provided useful conclusions on the generation of autoantibodies and the perpetuation of the autoimmune response. Primary Sjögren's syndrome (pSS) is characterized by the presence of autoantibodies binding on a vast array of organ and non-organ specific autoantigens. The most common autoantibodies are those targeting the Ro/La RNP complex, and they serve as disease markers, as they are included in the European–American Diagnostic Criteria for pSS. Other autoantibodies are associated with particular disease manifestations, such as anti-centromere antibodies with Raynaud's phenomenon, anti-carbonic anhydrase II with distal renal tubular acidosis, anti-mitochondrial antibodies with liver pathology, and cryoglobulins with the evolution to non-Hodgkin's lymphoma. Finally, autoantibodies against autoantigens such as alpha- and beta-fodrin, islet cell autoantigen, poly(ADP)ribose polymerase (PARP), NuMA, Golgins, and NOR-90 are found in a subpopulation of SS patients without disease specificity, and their utility remains to be elucidated. In this review, the molecular and clinical characteristics (divided according to their clinical utility) of the autoantigens and autoantibodies associated with pSS are discussed.

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Introduction

Sjögren's syndrome (SS) is a chronic autoimmune disease, characterized by the presence of a variety of autoantibodies directed against organ and non-organ specific autoantigens. Antinuclear antibodies (ANA), detected by immunofluorescence using Hep-2 cells, are present in the sera of 90% of patients. The most common of them are directed against two ribonucleoprotein antigens known as Ro or SSA and La or SSB. These autoantibodies are included in the European–American Diagnostic Criteria for Sjögren's Syndrome [1], but they can be also found in other autoimmune diseases, particularly systemic lupus erythematosus (SLE). High titer of antibodies to other immunoglobulins (known as rheumatoid factors) are also frequently found in SS. Apart from antibodies against salivary glands—found rather infrequently and in low titers—primary SS (pSS) sera contain many different autoantibodies against organ or tissue specific autoantigens, including acetylcholine receptors, the carbonic anhydrase and thyroid peroxidase. Finally, new autoantibodies directed against the cytoskeletal protein β-fodrin, and the muscarinic receptors M3, have also been described in primary Sjögren's syndrome.

During the last years, the pathogenetic mechanisms and the clinical utility of autoantibodies in pSS have been explored in detail [2]. Thus, it is now appreciated that the production of autoantibodies is an antigen-driven immune response, as (1) certain autoantibodies are disease specific, (2) contain multiple epitopes, and (3) the autoimmune response is perpetuated and augmented via intra- and in-

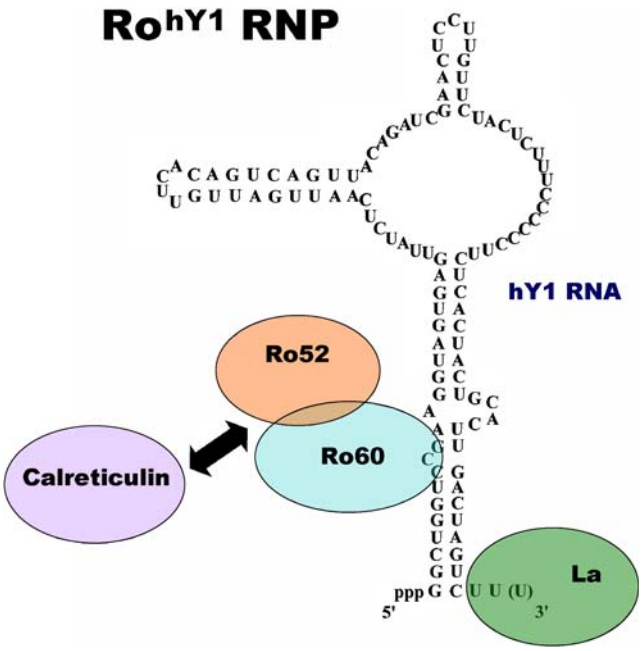

Fig. 1 Structure of the Ro/La ribonucleoprotein complex

termolecular spreading against the same or other autoantigens. It is still unknown if any of the autoantibodies have a pathogenic potential, or they are all parts of a secondary response to salivary glands already damaged by another process.

Nevertheless, anti-Ro and anti-La antibodies appear to participate in the local autoimmune response in the affected exocrine glands as: (1) autoantibodies to Ro and La are found in the saliva of patients [3, 4] and (2) B-cells infiltrating the salivary glands contain intracytoplasmic immunoglobulins with anti-Ro and anti-La activity [5–7]. In this case, ectopic lymphoid germinal centers that contain antigen presenting dendritic cells, T-cells, and B lymphocytes are found, providing a conducive microenvironment for the propagation of the autoimmune response [6]. Finally, (3) an increased mRNA production of La in acinar epithelial cells has been observed [8] and (4) translocation and membrane localization of the La protein has been observed in conjunctival epithelial cells of Sjögren's syndrome patients [9]. Recent studies have also shown that cultured epithelial cells from patients with Sjögren's syndrome constitutively secrete exosomes that contain the major autoantigens Ro and La [10]. This mechanism may represent a pathway whereby intracellular autoantigens are presented to the immune system.

On clinical ground, pSS is characterized by a variety of extraglandular manifestations involving epithelium (autoimmune epithelitis) and extra-epithelial tissues. Several autoantibodies may relate with particular disease subgroups or clinical manifestations.

Autontibodies Serving as Disease Markers

Ro/SSA and La/SSB The most common autoantibodies in patients with SS are the antibodies directed against the Ro/La RNP complex. These antibodies are detected in serum and can be produced locally in the affected salivary glands [6]. Anti-La antibodies are accompanied by anti-Ro antibodies, whereas anti-Ro antibodies can be found either with anti-La antibodies or solely. Structurally, human Ro/La RNP is composed of one of the four small, uridine rich hY RNAs (*h*uman c*Y*toplasmic *RNAs*) non-covalently associated with at least three proteins, the Ro52, La, and Ro60 autoantigens (Fig. 1) [11, 12]. Additional components of the complex have been recently identified as the proteins calreticulin [13] and nucleolin [14]. The localization of these complexes is mainly cytoplasmic. Their protein components, however, can be found in the nucleus as well. Nucleic Ro60, Ro52, and La autoantigens are not associated with hY RNA. After the assembly of the Ro/La RNP in the nucleus, the complex is rapidly and quantitatively transported to the cytoplasm [15, 16]. Under certain circumstances (e.g., stress, UV radiation, or viral infection), some of the protein components of the Ro/La RNP complex can be found on the cell surface.

Ro52 belongs to the tripartite motif (TRIM) or RING-B-box-coiled-coil (RBCC) protein family, thus, comprising an N-terminal RING, followed by a B-box and a coiled-coil region. The RING is a cysteine-rich Zn^{+2}-binding motif of the form C_3HC_4 [17], which binds two Zn^{+2} ions in a tetrahedral manner (Fig. 2) [18]. The RING is predominantly a protein–protein interaction motif, which also acts as a ubiquitin–protein isopeptide (E3) ligase in the ubiquitination pathway [19]. The B-box is the second Zn^{+2}-binding motif of Ro52 and has the form CHC_3H_2 [20]. Ro52 can also

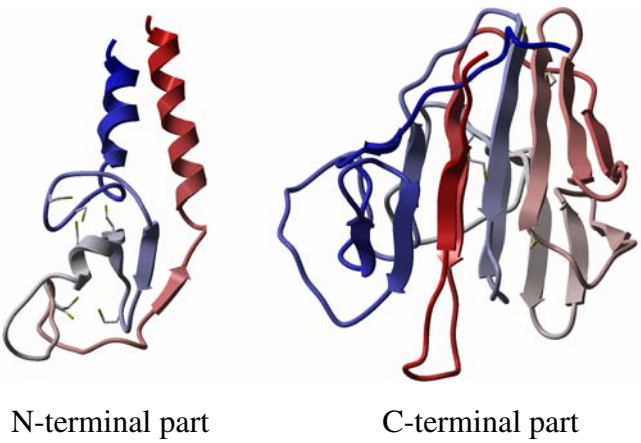

Fig. 2 Modeled structures of the N-terminal domain (1-84aa) and the C-terminal region (282-475aa) of human Ro52 autoantigen. These models were based on the structures of BRCA1 ring domain, Ubiquitin ligase protein RING2 and RAG-1 zinc binding domain (N-domain of Ro52) and Ret-finger protein like 1 (C-domain of Ro52)

homodimerize through its leucine zipper domain [21]. Several different proteomic functions have been suggested for Ro52, including DNA binding, protein interactions, and Zn^{+2}-binding. Overall, most probably, Ro52 functions as transcription modulator, due to its domain organization. In line with many other RING-containing proteins, Ro52 is involved in ubiquitination pathway [22]. Recent findings suggest that Ro52 autoantigen is a RING-dependent E3 ligase that is overexpressed in patients. In this regard, Ro52 may be directly involved in the reduced cellular proliferation and increased apoptotic cell death that is observed in Sjögren's syndrome and SLE [23].

The B-cell epitopes of Ro52 have been mapped in various studies with different methods. The major immunoreactivity of Ro52 kD autoantigen was localized, using recombinant Ro52 fusion proteins, in the middle coiled-coil region of Ro52 [24–26]. The 190-245aa region of the amino acid sequence was reactive with almost all anti-Ro52 positive sera and was independent of associated diseases [26]. An epitope spanning the 200-239aa of Ro52, which contains the complete leucine zipper motif, has been also identified in the same region [27]. Autoantibodies against this epitope were associated with neonatal lupus and congenital heart block. These autoantibodies have the potential to bind on the cell surface of cardiomyocytes in primary cultures and cause a dysregulation of the Ca^{+2}-homeostasis, which is followed by apoptosis [28]. Anti-Ro52 antibodies are also found in primary biliary cirrhosis associated with sicca syndrome. The anti-Ro52 antibodies in this setting are directed against a smaller epitope than in primary Sjögren's Syndrome [29].

The Ro60 antigen is found in virtually all vertebrate cells and the nematode *Caenorhabditis elegans* [30]. Its function is related with the quality control or discard pathway for nascent transcripts synthesized by RNA polymerase III (e.g., 5 S rRNA precursors). Thus, Ro60 binds misfolded small RNAs (e.g., 5 S RNA) and lead them to degradation [30]. Recently, the structure of the *Xenopus laevis* Ro60, 78% identical to human Ro60, was solved and found to consist of two distinct domains (Fig. 3) [31]. One domain resembles the von Willebrand Factor A (vWFA) domain, which is found in extracellular matrix proteins and proteins that function in cell adhesion. The other domain consists of a series of alpha-helical repeats (HEAT repeats) that are arranged orbicularly around an inner hole of 10–15 Å ("doughnut"-like structure). This hole most probably holds the 3′ ends of misfolded RNAs, while the YRNAs bind to conserved residues to the outside of the "doughnut". Another conserved role for the Ro60 in facilitating cell survival after ultraviolet irradiation has recently emerged from studies in radiation-resistant eubacterium *Deinococcus radiodurans* [32] and mammalian cells lacking Ro60 [33]. Studies of mice lacking the Ro60 kD protein suggest also that the normal function of Ro may be important for the prevention of autoimmune disease [33]. In these studies, mice lacking Ro were found to develop autoantibodies and membranoproliferative glomerulonephritis.

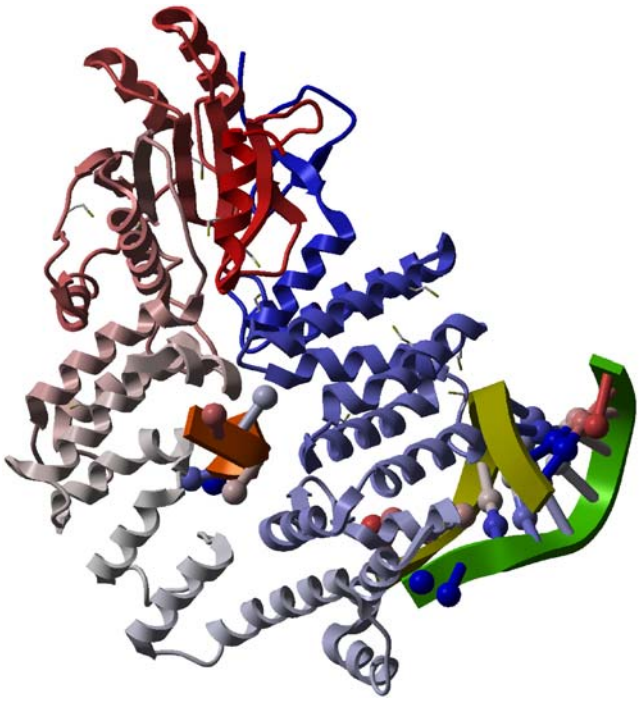

Fig. 3 Structure of *Xenopus laevis* Ro60, complexed with RNAs

Epitopes of Ro60 kD have been described by several authors using a variety of epitope mapping procedures [34, 35]. Initially, the major antigenic region of Ro60 kD identified within the central part of the molecule [36–38] (within 181–320aa, 139–326aa, and 155–295aa regions of the sequence, respectively). The fine localization of the antigenic determinants was revealed after the application of epitope mapping with synthetic peptides. Wahren et al. [35] identified a major epitope in synthetic peptide 216–245aa, Scofield and associates, identified numerous epitopes covering the entire length of Ro60 [39, 40] (presumptively, due to extended epitope spreading), and our group defined the antigenic regions of Ro60 kD in 169–190 and 211–232 parts of the antigen [41]. One of them, the 169–190 epitope, was found to share conformational and antigenic similarity with HLADR3 β-chain, an HLA class II allele, which was described to be highly associated with the anti-Ro60 response [42]. The same epitope was recently found to be the initial pre-disease target of autoantibodies in individuals, who developed SLE several years later [43]. This initial epitope has been reported to directly cross-react with a peptide from the latent viral protein Epstein–Barr virus nuclear antigen-1 (EBNA-1) [43, 44]. Recent studies suggest also that although the exact Ro epitopes were identified as small peptidic moieties, their recognition by autoantibodies is

conformation-dependent and is dramatically enhanced upon interaction with the molecular chaperone calreticulin [45].

The La antigen is a phosphoprotein that associates with a variety of small RNAs, including 5 S cellular RNA, tRNA, 7 S RNA, and hY RNAs, all transcribed by RNA polymerase III [46]. In molecular level, it binds a short polyuridylate sequence (poly-U) that exists at the 3 end of almost all nascent pol III transcripts. Moreover, La binds viral RNAs [e.g. adenovirus VA (virus associated RNA), Epstein Barr EBER (EBV encoded RNA)], viral and human RNAs possessing IRES (internal ribosomal entry elements), and RNA component of telomerase complex [47]. Structurally, human La is a multidomain 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). Recently, the three-dimensional structure of the La-motif, the central RRM, and the carboxyl-terminal RNA recognition domain of the autoantigen were solved (Fig. 4) [48, 49]. 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. It seems that both the La motif and the adjacent central RRM are required for high-affinity poly-U RNA binding and that 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 (terminal oligopyrimidine) mRNAs [50]. 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 an ATP-dependent helicase activity that melts RNA–DNA hybrids, unwinding ability of double-stranded RNA, association with telomerase and influencing telomere homeostasis, an RNA chaperone activity performed by transient bipartite (5′- and 3′- end) binding of nascent transcripts synthesized by polymerase III (e.g., tRNA precursors), the induction of cap-independent translation (La binds IRES to elements and promotes the internal, cap-independent initiation of translation).

During the last decade, the target epitopes of anti-La/SS-B autoantibodies have been mapped [34]. 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. Highly antigenic peptides were identified in the sequences: ^{147}HKAFKGSI^{154} (147–154aa) (located within central RRM motif), ^{291}NGNLQLRNKEVT^{302} (291–302aa), ^{301}VTWEVLEGEVEKEA-LKKI^{318} (301–318aa), and ^{349}GSGKGKVQFQGKKTKF^{364} (349–364aa) [51]. The most sensitive and specific epitope was 349–364aa, which showed a sensitivity and specificity of greater than 90%. Other epitopes have also been identified in other parts of the molecule using recombinant fragments of La/SS-B or synthetic peptides [2]. Their existence is believed to be correlated with extended intramolecular spreading of epitopes to the whole La/SS-B molecule.

Clinical significance of anti-Ro and anti-La antibodies Anti-Ro and anti-La antibodies are found in approximately 60–90 and 30–60% of patients with primary Sjögren's syndrome, respectively [52], depending on the method used for their detection. A variety of methods have been applied for the detection of anti-Ro and anti-La antibodies. Among them, RNA precipitation is considered as the gold standard method by various authors. However, this method cannot be used in everyday routine analysis, but it is useful as reference and confirmatory assay. More specifically,

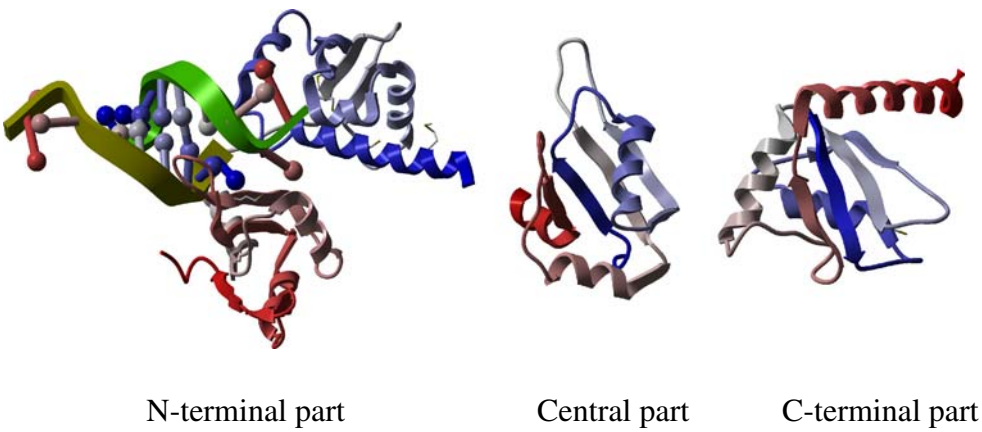

Fig. 4 Structures of the N-terminal RRM (5-189aa) (complexed with polyU RNA), the central RRM (107-193aa) and the C-terminal region (229-326aa) of human La autoantigen

counter-immunoelectrophoresis (CIE) and immunodiffusion (ID) are commonly used for the detection of anti-Ro and anti-La antibodies. However, in a small subpopulation of patient sera, precipitin-negative anti-La/SS-B antibodies can be found. These antibodies are believed to possess restricted epitope recognition and can be detected without problem using an anti-La enzyme-linked immunosorbent assay (ELISA) assay. Immunoblotting (IB) can be used for the detection of anti-La antibodies, but it lacks sensitivity for the detection of anti-Ro antibodies.

The presence of these antibodies in patients with suspected primary Sjögren's syndrome strongly supports the diagnosis. In patients with pSS, anti-Ro and anti-La antibodies are associated with a higher prevalence of extraglandular features, especially vasculitis and higher intensity of the lymphocytic infiltrates in the affected salivary glands [52]. Anti-Ro and anti-La antibodies are present in only 5–15% of patients with secondary Sjögren's syndrome associated with rheumatoid arthritis and 38.5% of secondary-SS/SLE patients [53]. Pregnancy in women with anti-Ro and anti-La antibodies may be complicated by the development of neonatal lupus in the fetus or neonate with increased risk for congenital heart block, the most serious manifestation of this disorder [54]. It is presumed that in this rare syndrome, maternal anti-Ro and anti-La 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 is required to become available for binding of autoantibodies. Such redistribution can be induced either by β-estradiol, viral infection, or apoptosis [55]. Recently, it was found that the presence of antiidiotypic antibodies to autoantibodies against La/SS-B may protect the fetus by blocking pathogenic maternal autoantibodies [56]. In this regard, sera from mothers that gave birth to a healthy child and without history of a child with NLS exhibit higher antiidiotypic antibody activity compared with mothers which gave birth to a child with NLS [56].

Autoantibodies Associated with Particular Disease Manifestations

Anti-centromere antibodies (ACA) Antibodies against centromere are found in patients with limited cutaneous sclerosis and a small percentage of patients with primary Sjögren's syndrome and idiopathic Raynaud's phenomenon [57] (Table 1). The SS patients with ACA are characterized by a lower incidence of parotid gland enlargement and anti-Ro anti-La antibodies [57]. Moreover, the ACA-positive patients were more likely to have Raynaud's phenomenon and sclerodactyly and less likely to have leukopenia, polyclonal hypergammaglobulinemia, and rheumatoid factor [58]. The main target of anti-centromere antibodies was recently defined as the centromere proteins (CENP). In a subset representing 15% of SS patients, anticentromere antibodies recognize exclusively CENP-C [59]. Anti-CENP-H antibodies were also found in patients with SS. Patients with anti-CENP-H antibodies had a lower frequency of rheumatoid factor (RF) and anti-Ro/SS-A and/or anti-La/SS-B antibody [60].

Table 1 Correlations of specific autoantibodies with disease manifestations

Disease manifestation	Related autoantibody
Raynaud's phenomenon and sclerodactely	Anti-centromere
Distal renal tubular acidosis	Anti-carbonic anhydrase II
Glandular hypofunction and reduced sensitivity to muscarinic stimulation	Anti-muscarinic receptors
Liver pathology and primary biliary cirrhosis	Anti-mitochondrial
Increased risk of lymphoma and indicator of severe salivary gland damage	Rheumatoid factors
Frequent evolution to malignant lymphomas and severe internal organ involvement	Cryoglobulins

Antibodies to carbonic anhydrase II Anti-carbonic anhydrase II antibody can be detected in a number of serum samples from patients with Sjögren's syndrome [61–63]. Carbonic anhydrase is an enzyme that catalyzes the reversible hydration of carbon dioxide to generate a proton and a bicarbonate ion, regulates the acid–base homeostasis in erythrocytes, the aqueous chamber of the eye, and the renal tubules [64]. Carbonic anhydrase II is the only soluble form of the enzyme and is found in the cytosol of both proximal and distal renal tubular cells [65]. When carbonic anhydrase II was used in immunization experiments, the immunized mice developed systemic exocrine gland inflammation similar to that observed in Sjögren syndrome [66].

Clinical significance of antibodies to carbonic anhydrase II Among patients with Sjögren syndrome, those with distal renal tubular acidosis had higher levels of anti-carbonic anhydrase II antibody than did those without renal tubular acidosis [67]. These results indicate that distal renal tubular acidosis in Sjögren syndrome may be caused, at least in some patients, by defective function of carbonic anhydrase II resulting from high plasma levels of carbonic anhydrase II autoantibodies.

Anti-muscarinic receptors It is known that acetylcholine (ACh) mediates glandular secretion, through a family of muscarinic receptor subtypes [68]. The muscarinic receptor family is encoded by five separate genes [69, 70], which

give five muscarinic gene products, designated M1R–M5R [71]. In the bladder and the colon, the muscarinic receptor population primarily comprises the M2R subtype (80% M2R and 20% M3R) [72], while in the parotid gland, the M3R represents the 93% of the muscarinic receptor population [73]. Studies with M3R- and M1R-knockout mice demonstrated that the M3R, but not the M1R, is essential for parasympathetic control of salivation [74]. In some patients with SS, autoantibodies directed against M3R acetylcholine receptors may block neuroglandular transmission, thereby, resulting in sicca symptoms [75–77]. In addition, antibodies raised against the second extracellular loop of the human muscarinic M3R receptor have been found to mimic functional autoantibodies in Sjögren's syndrome [78]. The most important evidence for the pathogenic role of anti-M3R antibodies was obtained by passive transfer experiments. In these experiments, transfer of SS IgG to mice have indicated that the recipient mice develop glandular hypofunction [77] and exhibit up-regulated M3R expression in bronchioles and marked hyperresponsiveness of bladder smooth muscle [79]. Furthermore, monovalent Fab fragment of IgG in patients with SS was found to inhibit cholinergic neurotransmission, indicating that the antimuscarinic antibody activity does not require receptor cross-linking [80]. However, this antimuscarinic antibody activity was neutralized in vitro by antiidiotypic antibodies in both pooled intravenous immunoglobulin (IVIG) and IgG from healthy individuals [80], suggesting the possibility that naturally occurring antiidiotypic antibodies may prevent the emergence of antimuscarinic autoantibodies. Data from immunofluorescence experiments using rat lacrimal glands revealed recognition of M3R by SS IgG. The immunofluorescent signal, in these experiments, could be quenched by preincubation of the SS IgG with a synthetic peptide corresponding to the second extracellular loop of M3R [81]. Moreover, the same M3R peptide could be used to detect anti-M3R IgG antibodies in SS sera [82] and IgA antibodies in SS saliva [83] by ELISA. Western blotting has also been reported as a suitable method for the detection of anti-M3R antibodies in SS sera, using crude lacrimal membrane fractions as a source of M3R [76]. However, in a more recent work of Dawson LJ and coworkers, it was found that there is no detectable anti-M3R activity in SS sera by Western blotting when membranes, obtained from Chinese hamster ovary (CHO) cells that had been stably transfected with functional human M3R, were used as antigen source [84]. In contrast, another study using M3R-transfected CHO cells for a flow cytometric assay, indicated that anti-M3R may be present in SS sera [85]. Therefore, further studies are needed to elucidate the discrepancies in these findings.

Recent experimental data strongly indicate that the second extracellular loop of M3R is the target antigen in SS, but this has not been demonstrated conclusively. An epitope was recently identified in the 213–228aa region of this domain, and an ELISA system, which enables the measurement of anti-M3AChR213-228 antibody levels on a large scale has been developed [86]. Anti-M3AChR213-228 antibody positivity was observed in 90% of the pSS patients, 29% of sSS patients, 35% of RA patients, 32% of SLE patients, and none of the healthy controls [87]. However, all the antigenic regions of M3R are not currently known in detail, and it is apparently necessary for the future research on antimuscarinic antibodies to focus on identifying all epitopes recognized by SS sera. A starting point for determining additional epitopes may be derived from data demonstrating that the cleavage of M3R by granzyme B (during cytotoxic lymphocyte granule-induced cell death) results in the generation of novel fragments with pathogenetic potential [88].

Clinical significance of anti-muscarinic receptors Sjögren's syndrome has been described as "an autoimmune epithelitis" of the exocrine glands, which particularly involves the salivary and lacrimal glands. The secretory tissues in the affected glands are progressively destroyed and replaced by a lymphoreticular cell infiltrate, losing a significant amount of their function. It has been recently proposed that the pathology underlying the glandular hypofunction contributes inhibitory autoantibodies directed against muscarinic receptors. These antibodies may be found in both primary and secondary SS [89–91], and therefore, they would serve to unite the pathologies underlying the glandular hypofunction of both primary and secondary SS. In animal models, autoantibodies directed against salivary gland muscarinic receptors were found to decrease glandular secretion [77]. In SS patients, isolated salivary acinar cells remain functional in vitro [92, 93], but with a reduced sensitivity to threshold levels of muscarinic stimulation [93], suggesting that the lack of glandular function in many patients with SS is the result of a perturbation of acinar function [94, 95]. In addition, perturbation of muscarinic receptor function by the presence of antimuscarinic antibodies would account, in large part, for some of the reported extraglandular features of SS, such as bladder irritability [90, 96, 97], impairment of esophageal motor function [98], and microvascular responses to cholinergic stimulation [99], Adie pupil [100], and variable heart rate [101]. In one study, the antibody levels against the 213–228aa peptide of M3R correlated positively with the number of extraglandular organ manifestations [87].

Antimitochondrial antibodies Antimitochondrial antibodies (AMA) is a diagnostic marker for primary biliary cirrhosis (PBC), a chronic cholestatic liver disease predominantly affecting middle aged women [102]. In pSS, 7% of patients shows evidence of liver disease either subclinical (2%) or

asymptomatic (5%) with elevated liver enzymes. Moreover, 6.6% of SS patients possesses antimitochondrial antibodies (AMA). AMA is conventionally detected by immunofluorescence, and their major molecular targets have been identified to be dihydrolipoamide acyltranferases (E2 subunits) of the 2-keto acid dehydrogenase enzyme complex (mainly the E2 component of the pyruvate dehydrogenase complex) [103]. Ninety-two percent of the SS patients with AMA exhibit liver involvement with histological features of chronic cholangitis similar to stage I PBC [104]. Therefore, AMA appeared to be a sensitive indicator of underlying liver pathology in pSS patients. In a subsequent study, it was also concluded that although AMA is a rare finding in patients with SS, their presence predispose them to develop PBC upon a 5-year follow-up [105]. Thus, it seems that patients with SS and AMA are usually in an early asymptomatic stage of PBC.

Rheumatoid factors and cryoglobulins The rheumatoid factors were first identified by Waaler in 1940 [106], and these autoantibodies were named rheumatoid factor by Pike in 1949 [107] due to their association with rheumatoid arthritis (RA), before the understanding that they were antibodies. It is now known that these autoantibodies bind to the Fc portion of IgG in the γ2–γ3 cleft, but yet, many questions remain unanswered [108]. It has long been recognized that the RF response is transiently associated with many infectious diseases. In this case, the RF response may actually be beneficial as RF helps in the clearance of immune complexes by contributing to the formation of larger sized complexes, and therefore, facilitating their removal [109, 110]. In the rheumatic diseases, the existence of RF is associated with RA, where ~70% of patients are positive for RF [111], and pSS, where ~40–50% are positive for RF [112]. Approximately 20% of patients with Sjögren's syndrome have cryoglobulins in their sera [113]. Cryoglobulinemia is defined as the presence of circulating immunoglobulins that precipitate at temperatures below 37°C and redissolve on rewarming [114, 115]. According to cryoprecipitate composition, cryoglobulinemia is classified into three serological subsets: monoclonal cryoimmunoglobulinemia (type I) composed of single monoclonal immunoglobulin, mixed cryoglobulinemia containing a mixture of polyclonal IgG, and monoclonal (type II) or polyclonal (type III) IgM rheumatoid factor [114]. Type I cryoglobulinemia is frequently associated with well-known hematological disorders, while types II and III mixed cryoglobulinemia can be further classified as essential or secondary in the absence/presence of other well defined infectious, immunological, or neoplastic diseases [116].

Clinical significance of rheumatoid factors and cryoglobulins RFs potentially play an important role in the pathogenesis of pSS, as they have been shown to be an indicator of the severity of salivary gland damage [117]. In addition, there is an increased risk of lymphoma in SS [118] with an incidence of 12.2 per 1,000 person years [119]. The expansion of monoclonal RF has been demonstrated in a high percentage of the cases [120, 121]. In a study of a large cohort of patients with primary SS, individuals who developed lymphoma had mixed cryoglobulinemia both at initial diagnosis of SS and at follow-up, thus indicating that the mixed cryoglobulinemia was a detrimental prognostic event [119]. It has been proposed that RFs contribute in lymphomagenesis in pSS [122]. According to this theory, the first event is the chronic stimulation at the site of the disease of polyclonal B cells secreting rheumatoid factor (RF). Then, these RF-B cells may become monoclonal and disseminate in other organs. The monoclonal secreted RF complexed with polyclonal IgG may also cryoprecipitate. Afterwards, a chromosomal abnormality may confer to these cells a low-grade B cell lymphoma comportment. At last, an event (e.g., a mutation of p53) might transform this low grade B cell lymphoma into a high grade, large B cell lymphoma. These lymphoma B cells have been recently found to display RF activity, supporting the proposed hypothesis [121]. The presence of cryoglobulinemia seems to identify a particular clinical subset of Sjögren's syndrome, characterized by a poor prognosis due to more severe internal organ involvement and frequent evolution to malignant lymphomas [113, 123].

Other Autoantibodies

Anti-alpha-fodrin and anti-beta-fodrin Although no pathogenic role has yet been found for antibodies directed against alpha-fodrin and beta-fodrin, these antibodies are present in many SS patients. Alpha-fodrin is a 240-kDa protein forming a heterodimer with either beta-fodrin, a 235-kDa molecule that is homologous to alpha-fodrin, or with beta-spectrins [124]. These heterodimers can self-associate into tetramers [125], which are anchored to the plasma membrane and bind to actin, calmodulin, and microtubules [125]. Therefore, alpha-fodrin is a fundamental constituent of the membrane skeleton. In addition, alpha-fodrin has been shown to associate with membrane ion channels and pumps and appears to be involved in control of secretion from glands [126, 127]. Antibodies against alpha-fodrin have been shown to block nerve conduction in glutamate and other synapses present in salivary glands [128, 129]. From this point of view, antibodies against alpha-fodrin may interfere with the nerve impulses regulating secretions from the salivary and lacrimal glands providing a potential mechanism for the dysfunction of these glands that is observed in SS. In early reports, IgG antibodies against

alpha-fodrin were found in 95% (41/43) of the patients with primary Sjögren's syndrome (classified according to the Japanese criteria for Sjögren's syndrome) and 63% (5/8) with secondary Sjögren's syndrome, but none of patients with SLE, RA, or healthy individuals [130]. In subsequent studies, IgG antibodies against alpha-fodrin were detected in approximately 67 [131], 55 [132], 23 [133], or 2% [134] of SS patients, whereas IgA antibodies against alpha-fodrin were found in 64% of the SS patients [132]. Moreover, antibodies against alpha-fodrin can be found in 40% of patients with RA [135], 20% of patients with multiple sclerosis (MS) [135], and 47% of those with SLE without sicca symptoms [133]. Given that antibodies against alpha-fodrin are not characterized by the initially reported prevalence and disease specificity [130], their diagnostic value is questionable [136]. In this regard, Zandbelt et al. and Turkçapar et al. reported that measurement of anti-α-fodrin autoantibodies does not add much to the diagnosis of Sjogren's syndrome, as anti-Ro and anti-La autoantibodies are more sensitive than anti-alpha-fodrin for the diagnosis of SS and anti-La autoantibodies are also more specific [137, 138]. Beta-fodrin has also been shown to be an autoantibody target, associated with Sjögren's syndrome, and have been characterized in 51% of primary SS and 84% of secondary SS [139]. These antibodies were directed against the N-terminal domain of beta-fodrin, the only domain that is not homologous to alpha-fodrin. Insights for the potential role of alpha-fodrin in the pathogenesis of SS were gained from a mouse model of Sjögren's syndrome. In this model, NFS/*sld* mice were thymectomized on day 3 after birth, and thus, the CD4+CD25+ regulatory T cells were removed [130]. Later on, these mice developed lymphocytic infiltrates in the salivary glands as a histological sign of Sjögren's syndrome [130]. Analysis of the sera of these mice in immunoblots with organ extracts of the same mice revealed a 120-kDa band, which was subsequently sequenced and identified as a cleavage product of alpha-fodrin. The autoantibodies were recognized the cleavage product, but not the complete alpha-fodrin. This 120-kDa cleavage product of alpha-fodrin has been reported to be generated during apoptosis, by caspase 3 [140]. In line with this observation, treatment of the mice with inhibitors of caspases prevented induction of Sjögren's syndrome [141]. In SS patients, cleaved alpha-fodrin was detected in labial salivary glands and found to co-localize with PARP and caspase-3 along with DNA fragmentation [142]. Taken together, cleaved alpha-fodrin may be better antigenic substrate than intact alpha-fodrin for the detection of SS specific autoantibodies, but further studies are required to ascertain the specific association of cleaved alpha-fodrin with Sjögren's syndrome.

Clinical significance of anti-alpha-fodrin and anti-beta-fodrin Anti-fodrin antibodies are found not only in SS, but also in other autoimmune diseases such as RA and SLE. Therefore, they cannot serve as sensitive and specific markers for SS. On the other hand, follow-up of SS patients treated with antimalarials or low-dose glucocorticosteroids revealed that the concentration of antibodies against alpha-fodrin may fall within 3 months [135]. In addition, the titer of antibodies against alpha-fodrin is correlated to the degree of lymphocytic infiltration in the salivary glands [135]. Although, antibodies against alpha-fodrin appear to reflect the disease activity of SS, additional study of the clinical use of testing for anti-alpha-fodrin antibodies is needed to assess their role in the monitoring of the disease activity in Sjögren's syndrome.

Islet cell autoantigen Islet cell autoantigen 69 (ICA69) is a 69-kD protein that is present in salivary and lacrimal glands and pancreatic beta cells and tissue of the nervous system. In one study, elevated levels of autoantibodies to this protein were frequently found in the serum of patients with primary SS (eight of nine patients), but not in patients with SLE (zero of six) or in healthy controls (0 of 12) [143]. In a murine model of SS (the nonobese diabetic or NOD mouse), in which spontaneous lymphocytic infiltration of the lacrimal and salivary glands occurs, animals that did not express the ICA69 protein had a markedly slower progression of glandular lymphocytic infiltration than wild-type or heterozygous ICA69 knockouts [143]. However, larger studies are required to confirm the differential presence in SS vs SLE, or other rheumatic disorders, so that testing for these antibodies may find a role in clinical practice.

Poly(ADP)ribose polymerase Poly(ADP)ribose polymerase (PARP) is a chromatin-bound, DNA-dependent enzyme that catalyzes the ADP-ribosylation of nuclear acceptor proteins by using NAD^+ as a substrate [144]. Proteins that are covalently modified by poly(ADP)ribose polymerase include DNA topoisomerases I, II, DNA polymerases α and β, RNA polymerase II, histones H1 and H2B, and lamins [145]. PARP displays a three-domain structure, which can be further broken down into modules A-F [146]. The N-terminal 42 kDa DNA-binding domain also comprises the nuclear localization signal of the protein and is adjacent to a central 16 kDa automodification domain. The 55 kDa catalytic domain, which includes the active site, is located at the C-terminus. The N-terminal region of PARP binds to single- or double-strand breaks with high affinity via two zinc fingers. Non-B DNA structures, such as DNA hairpins, cruciforms, and stably unpaired regions are all effective activators of PARP leading to poly(ADP)-ribosylation of substrates like histone H1 [144]. Sera with autoantibodies to PARP recognize the NAD-binding domain of the enzyme, as demonstrated by either immunoblotting or immunoprecip-

itation of partially proteolyzed ADP ribose polymerase [147]. These autoantibodies are identified in SS and related rheumatic diseases and found to inhibit the catalytic activity of PARP, as measured by the transfer of ADP-ribose from ^{32}P-NAD to either histones or to PARP itself [147, 148]. Negri et al. reported that only a few patients with SS actually possess anti-PARP antibodies [149]. Muller et al. identified a 44-mer peptide epitope in the second zinc finger of the DNA-binding domain of PARP. This epitope was recognized by 42% of pSS and 56% of sSS sera [150]. Recently, it was reported that cleaved PARP together with activated caspase 3 is elevated in ductal and acinar cells of SS salivary glands, but not in normal salivary glands [151].

NuMA In some cases "anti-mitotic spindle" autoantibodies, staining mitotic poles, and spindles of Hep-2 cells in indirect immunofluorescence (IIF), are identified during laboratory routine. These autoantibodies most commonly target type-1 nuclear mitotic apparatus (NuMA-1) [152]. In one study, autoantibodies against NuMA-1 were identified in patients, who had clinical and minor salivary gland biopsy findings compatible with Sjögren's syndrome at 53% [153]. However, in a more recent study [154], anti-NuMA antibodies were not found to prevail in any defined rheumatic disease. None of the patients in this study fulfilled the criteria for Sjögren's syndrome [154].

Golgins Anti-Golgi complex autoantibodies were first identified in the serum of a Sjögren's syndrome patient with lymphoma [155]. These autoantibodies have been identified primarily in patients with Sjögren's syndrome and systemic lupus erythematosus, although they are not restricted to these diseases [156]. The Golgi complex is an organelle with a prominent function in the processing, transporting, and sorting of intracellular proteins subsequent to their synthesis in the rough endoplasmic reticulum. Structurally, the Golgi complex is localized in the perinuclear region of most mammalian cells and is characterized by stacks of membrane-bound cisternae [157]. Autoantibodies against this complex commonly target autoantigens like giantin [156] and golgin-97 [158]. Antibodies to golgin-97 have been identified in SS patient sera [158].

90-kDa nucleolar organizer region protein Autoantibodies to nucleolar transcription factor NOR 90/hUBF (anti-NOR 90) were detected in about 10% of sera. The majority of these sera (78%) is reported to belong to patients with SS [159]. More recent data suggest that anti-NOR90 is a rare autoantibody specificity, associated with Reynaud phenomenon [160, 161].

Lipocalin Navone R et al. screened a random peptide library with pooled IgG immunoglobulins derived from patients with primary SS. Among the identified peptides, one was recognized by the majority of patients' sera, but not by sera of normal donors and of patients with other autoimmune diseases. This peptide (SS-peptide) showed homology with Epstein Barr Virus (EBV) derived early antigen protein D, with tear lipocalin and with alpha-fodrin [162]. Lipocalin is a protein highly expressed in tears and saliva and account for 15–33% of the amount of proteins in tears. Its function is to lubricate the eyelids, to form a smooth and even layer over the corneal surface, and to create an antimicrobial system for the ocular surface [163]. It may also act as a scavenger of lipophilic, potentially harmful substances protecting the epithelium [164]. Lipocalin was specifically recognized by anti-SS-peptide antibodies, affinity purified from patients' sera. The same antibodies also recognized the viral early antigen protein D and alpha-fodrin, providing a potential link between viral infection, apoptosis, and disruption of lipocalin's protective function in tears and saliva [162].

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