



Contents lists available at ScienceDirect

Journal of Autoimmunity

journal homepage: www.elsevier.com/locate/jautimm

Review

A comprehensive review of autoantibodies in primary Sjögren's syndrome: Clinical phenotypes and regulatory mechanisms

Nikolaos C. Kyriakidis, Efstathia K. Kapsogeorgou, Athanasios G. Tzioufas*

Department of Pathophysiology, School of Medicine, National University of Athens, Greece

ARTICLE INFO

Article history:

Received 5 November 2013

Accepted 13 November 2013

Keywords:

Sjögren's syndrome

Salivary gland epithelial cells

Anti-Ro/SSA

Anti-La/SSB

Autoantibodies

ABSTRACT

Sjögren's syndrome (SS) is a systemic autoimmune disease characterized by periepithelial lymphocytic infiltrates in affected tissues and the production of plethora of autoantibodies. Among them autoimmune responses against Ro/SSA and La/SSB are of major importance since their detection is routinely used for disease diagnosis and clinical characterization. Although the exact mechanisms underlying disease pathogenesis are not fully understood, the important role of salivary gland epithelial cells (SGEC) in the initiation and development of the local immune responses is well-established. SGECs are also capable to mediate the exposure of the Ro/SSA and La/SSB autoantigens to the immune system by elevated apoptosis and autoantigen release in apoptotic bodies and/or by the secretion of autoantigen-containing exosomes. The expression of these autoantigens in epithelial cells appears to be tightly regulated. Up-to-date, signaling of certain innate immunity receptors, such as TLR3, appear to be implicated in the regulation of Ro/SSA and La/SSB expression by SGECs, whereas the deregulated expression of certain miRNAs that are predicted to target them in SS patients suggests a regulatory feedback at the post-transcriptional level. In the periphery, the humoral autoimmune responses are further regulated by the development of an active network of idiotype–antiidiotype antibodies. The plethora of mechanisms suggests that autoimmune humoral responses in SS are tightly regulated. In this review, the major humoral autoimmune responses, recent advances on the role of epithelial cells in their development, as well as possible regulatory mechanisms will be discussed.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Sjögren's syndrome (SS) is a chronic systemic autoimmune disease characterized by a diverse clinical spectrum ranging from local exocrinopathy to extraglandular, systemic manifestations that affect several organs (eg. lungs, liver, kidneys, central nervous system). The destruction of affected tissues (mainly salivary and lacrimal glands) is associated with dense lymphocytic infiltrates. Another feature of the disease is B cell hyperactivity, as attested by abundant production of autoantibodies. The major autoantibodies in SS target the intracellular antigens Ro52/TRIM21, Ro60/TROVE2 and La/SSB, which are parts of ribonucleoproteins–RNA complexes [1].

Abbreviations: SS, Sjögren's syndrome; TLR, toll-like Receptor; SGEC, salivary gland epithelial cells; MSG, minor salivary glands; SLE, systemic lupus erythematosus; NL, neonatal lupus; CHB, congenital heart block; IVIG, intravenous immunoglobulin.

* Corresponding author. Department of Pathophysiology, School of Medicine, National University of Athens, 75 Mikras Asias street, Athens 11527, Greece. Tel.: +30 210 7462513; fax: +30 210 7462664.

E-mail address: agtzi@med.uoa.gr (A.G. Tzioufas).

The detection of autoreactive antibodies against intracellular autoantigens is essential for disease prognosis, diagnosis and clinical classification of patients with autoimmune disorders. Over the last fifteen years, emerging data led to the characterization of autoantibody production as an antigen-driven procedure, since: a) certain autoantibodies are disease specific, b) the majority of autoantibodies detected are of the IgG class, which is suggestive of an antigen-dependent T-cell help [2], b) autoimmune responses are mainly polyclonal, targeting multiple epitopes within the same or interacting autoantigens [2], and d) immunization of experimental animals with fragments of the autoantigens results in intra- and inter-molecular spreading of the immune response, similar to that observed after immunization with foreign antigens [3,4]. Despite extensive studies, the mechanisms operating for the exposure of certain intracellular autoantigens to the immune system remain rather elusive. The unraveling of the pathogenic mechanisms of SS revealed the crucial role of the epithelial cells in the initiation and regulation of autoimmune responses. The latter may also be implicated in the development of autoimmune responses against Ro52/TRIM21, Ro60/TROVE2 and La/SSB autoantigens.

In this review, recent advances on the diagnostic significance of autoantibodies in SS, the description of SS autoimmune lesions, the role of epithelial cells in disease pathogenesis and the candidate regulatory mechanisms for autoantibody production are discussed.

2. The pathologic lesion

Xerostomia (mouth dryness) and xerophthalmia (dryness of the eyes) owing to the dysfunction of the salivary and lacrimal glands, respectively, are the most common clinical manifestations of SS [1]. The dysfunction of the exocrine glands is commonly associated with the development of periepithelial lymphocytic infiltrates of variable degree. Studies investigating the inflammatory lesion of SS are mainly conducted in labial minor salivary glands (MSG). T and B cells constitute the vast majority of infiltrating mononuclear cells at the MSG inflammatory lesions of SS, with their prevalence varying according to the severity of the infiltrates. Thus, in mild lesions T cells predominate, whereas in severe B cells constitute the main population [5,6]. The majority of infiltrating T cells bear the CD4 phenotype and appear to be activated [5,7,8]. The prevalence of CD4⁺-T cells decreases with lesion severity, whereas the prevalence of CD8⁺-T cells remains unchanged [5]. Likewise, the prevalence of regulatory T cells associates with lesion severity, with the higher values to be observed at intermediate lesions [7]. NK-cells and professional antigen-presenting cells, such as macrophages and dendritic cells, comprise a small, but considerable portion of the infiltrating mononuclear cells, and their percentage correlates with the grade of the lesions [5,9–11]. Furthermore, in the majority of SS patients, the grade and the composition of the MSG inflammatory lesions are fully developed at the time of diagnosis, remaining largely unchanged thereafter. The only evolution observed involved the development of MALT-lymphoma in certain SS patients expressing adverse serological prognostic factors (i.e. C4 hypocomplementemia and cryoglobulinemia) [6]. These findings suggest that SS patients can be distinguished according to the predominant infiltrating mononuclear cell population into T-cell or B-cell driven autoimmune responses, which is of major importance for the proper treatment selection.

The factors or the cells implicated in the recruitment and accumulation of immune cells in the “inflamed” salivary glands have not been fully elucidated. Data that emerged over the last two decades, indicated that epithelial cells, the main target of local autoimmune responses are not “passive bystander targets”, but key regulators of local immune responses supporting that the disease is in fact an “autoimmune epithelitis” [1,12,13]. The first evidence about the epithelial role in SS pathogenesis was raised by clinical and histopathological observations, which indicated that the inflammatory lesions at the affected organs are developed around epithelia [13]. Immunohistochemical analyses of MSG tissues of SS patients revealed that epithelial cells proximal or distal to inflammatory lesions express numerous immune-modulatory molecules, such as cytokines or chemokines that mediate the recruitment, homing and differentiation of immunocytes, including B and T cells, as well as germinal center formation, adhesion molecules, MHC molecules, B7 and CD40 costimulatory molecules, molecules implicated in death due to apoptosis, as well as autoantigens and functional innate immunity receptors [1,14]. All the previous data suggested the significant role of epithelial cells in the regulation of immune responses that was further confirmed by subsequent findings obtained by the study of long-term cultured, non-neoplastic salivary gland epithelial cells (SGEC) established from SS patients and sicca-complaining control individuals, devoid of the inflammatory tissue microenvironment and the lymphocytic crosstalk [15]. Thus, long-term cultured SGEC from SS patients were shown to functionally express all the aforementioned molecules

implicated in both innate and adaptive immunity, such as cytokines and chemokines, adhesion and costimulatory molecules, death receptors, as well as receptors involved in innate immunity, including CD91 and various TLRs [1,12,16–18]. The constitutive expression of these molecules in SGEC from SS patients was found to remain stable even after prolonged cultivation and to be significantly higher than those obtained from non-SS sicca-complaining controls, a fact suggesting an intrinsic activation of salivary epithelia [reviewed in Ref. [12]]. Thus, SGEC seem to be equipped with the full armamentarium of professional antigen presenting cells, rendering them able to orchestrate the local autoimmune responses. Furthermore, SGEC were found able to mediate the exposure of intracellular autoantigens to the immune system by two pathways. First, the elevated cell-death due apoptosis and the subsequent release of autoantigen-containing apoptotic blebs [12,19–21], and second, by the secretion of exosomes, fully loaded with autoantigenic proteins, including Ro/SSA and La/SSB [22]. Taken together, these observations have led to the assumption that salivary glands are sites of autoantibody production, a hypothesis that will be extensively discussed later.

3. Serum autoantibodies and clinical phenotypes

B-cell hyperactivity is a major characteristic of SS and is predominantly manifested as severe hypergammaglobulinemia and production of autoantibodies against various autoantigens that are summarized at Table 1. Antinuclear antibodies (ANA) are present in the sera of 59–85% of patients [23], whereas antibodies against Ro/SSA and La/SSB autoantigens are the major autoantibodies in the syndrome and represent a diagnostic tool for the disease, as defined by the American-European classification criteria for SS [24].

Anti-Ro/SSA antibodies represent two distinct entities of autoantibodies that react with two non-homologous proteins, the Ro52/TRIM21 and Ro60/TROVE2, respectively. Ro52/TRIM21 is a tripartite motif containing protein that has been shown to act as an a) intracellular Fc-Receptor and b) E3-ubiquitin-protein ligase that is implicated in the regulation of cell proliferation and activation induced cell death, as well as the regulation of TLR signaling and subsequent IFN production by polyubiquitin-mediated degradation of IRFs [25]. Ro60/TROVE2 protein is a ring-shaped RNA-binding protein that participates in the quality control of nascent transcripts, including the recognition and leading of misfolded defective RNAs to degradation [26]. Furthermore, Ro60/TROVE2 has been shown to promote cell survival after ultraviolet (UV) irradiation, possibly by assisting in the decay of the UV-induced damaged RNA [26]. On the other hand, La/SSB appears to also be a RNA binding protein, involved in diverse aspects of RNA metabolism. It chaperones or binds to precursor RNA molecules, in order to protect them

Table 1
Prevalence of autoantibodies in SS patients.

Autoantibodies against	Prevalence (%)
Ro52/TRIM21	66.7
Ro60/TROVE2	52.1
La/SSB	49
U1RNP	2
Rheumatoid factor	36–74
Cryoglobulins	9–15
Centromere (ACA)	4–17
Mitochondria (AMA)	1.7–27
Smooth muscle	30
Cyclic citrullinated peptides (anti-CCP)	3–10
Calreticulin	20
Muscarinic 3 receptor	11
Carbonic anhydrases	12.5–20.8

from nuclease-mediated decay and to facilitate their correct processing, folding and maturation by specific ribonucleases [27]. It is also involved in the regulation of micro-RNAs (miRNAs) expression by protecting and stabilizing of pre-miRNAs from nuclease activity [28]. Other functions that have been attributed to the La/SS-B are a) enhancement of cap-independent translation of viral or human mRNAs that contain internal ribosome entry sites (IRES) or 5'-terminal oligopyrimidine motifs that encode ribosome subunits and translation factors, potentially through RNA chaperone activity, b) efficient RNAi, antiviral defense and transposon silencing by facilitating the release of cleaved mRNA from the RNA-induced silencing complex (RISC) and thus promoting the multiple-turnover of RISC catalysis, c) regulation of cell proliferation and tumor progression by promoting the IRES-dependent translation of the cyclin D1 (CCND1) protein [27–29].

Studies performed over two decades ago did not distinguish between anti-Ro52/TRIM21 and anti-Ro60/TROVE2 antibodies and although autoantibodies against both Ro52/TRIM21 and Ro60/TROVE2 proteins co-exist in a significant proportion of patients, distinct clinical features seem to be specifically associated with each specificity [30,31]. Anti-Ro52/TRIM21 seems to be the most common specificity in patients with SS (66.7%), whereas anti-Ro60/TROVE2 and anti-La/SSB antibodies are found in 52.1% and 49% of SS sera, respectively [25,32]. Detection of these autoantibodies in patients' sera has been correlated with longer disease duration before diagnosis, extensive lymphocytic infiltration of MSG, severe exocrine gland hypofunction and recurrent parotid gland enlargement. Moreover, they are associated with a higher prevalence of extraglandular features, the most noteworthy of which is vasculitis [33–35]. In addition, these autoantibodies have been strongly associated with the development of neonatal lupus syndrome, where they are thought to have direct pathogenic role. In this syndrome, maternal IgG autoantibodies pass through the placenta to the fetal circulation causing injuries to the affected organs [36].

Another set of autoantibodies with high prevalence in SS patients are rheumatoid factor and cryoglobulins with the former being present in 36–74% and the latter in 9–15% of SS sera, respectively [37–39]. Both types of autoantibodies are associated with extraglandular manifestations and younger age at diagnosis. The detection of cryoglobulins outlines a high risk for poor disease outcome and lymphomagenesis [40,41].

The anti-centromere antibodies (ACA), which have high value in the diagnosis of the limited form of systemic sclerosis, are also found in 4–17% of SS patients. ACA-positive SS patients seem to comprise a distinct clinical subgroup, characterized by overlapping clinical manifestations with systemic sclerosis and milder disease compared to both ACA-negative SS patients or patients with systemic sclerosis [42–45].

An additional specificity that requires attention is the autoantibodies against cyclic citrullinated peptides (anti-CCP). Although they are considered as specific markers for the diagnosis and prognosis of rheumatoid arthritis, their prevalence in SS patients has been estimated to range between 3 and 10% [46–49]. Anti-CCP reactivity is correlated with non-erosive arthritis in SS patients, whereas there is a controversy about its association with joint synovitis [47–50].

Anti-mitochondrial antibodies (AMA), which serve as a diagnostic serological marker of primary biliary cirrhosis (PBC), target the components of the ketoacid dehydrogenase complex and predominantly (in 95% of North American and European PBC sera) the pyruvate dehydrogenase subunit [51]. AMA are reportedly present in 1.7–13% of SS patients, with the rate rising to 3–27% depending on the diagnostic method applied [52]. AMA positivity in SS patients has been associated with liver involvement [52]. The histopathologic profile of liver and salivary gland lesions are

comparable. In both disorders, the tissue lesion is characterized by periductal lymphocytic infiltrates, which primarily consist of CD4⁺ T-cells. Moreover, in both entities, the histopathologic lesions present slow or no progression during disease course [53], whereas epithelial cells aberrantly express immunomodulatory molecules, suggesting that both manifest as “autoimmune epithelitis”.

Antibodies to muscarinic receptors, detected by functional methods or the use of synthetic peptides, have been described in SS patients. Muscarinic receptors (MRs or mAChRs) are acetylcholine receptors that form G protein-receptor complexes in the plasma membranes of certain neurons or other cells. Their functions are numerous, the most noteworthy being their function as the main receptors stimulated by acetylcholine released from postganglionic fibers in the parasympathetic nervous system [54,55]. Muscarinic receptors received their name from their higher affinity to muscarine than nicotine and have been further categorized in five subgroups (M1R–M5R) depending on their reactions with several selective agonists and antagonists. Two decades ago, it was demonstrated that agonist-mediated stimulation of M3R in rat salivary glands led to saliva production [56] and subsequent studies revealed that M3R, rather than M1R, was fundamental for the parasympathetic regulation of saliva secretion [57]. Further studies attempting to associate the role of anti-M3R autoantibodies in SS sera with defective saliva production were based on functional rather than solid phase assays. It was shown that anti-M3R antibodies in SS sera functionally regulate the M3R mediated saliva production [58]. Further functional studies led to the assumption that anti-M3R antibodies contribute to sicca manifestations, potentially via direct blockade of neurotransmission [59]. Subsequent functional analyses utilizing SS sera [60], the employment of bioinformatics and chemically synthesized peptides led to the identification of possible antigenic targets on M3R molecule; thus, characterizing the second extracellular loop of M3R as the most prominent target of anti-M3R autoimmune responses [28,61]. Nevertheless, discrepancies in solid-based assays (ie. ELISA) [62] and the difficulties observed in detecting anti-M3R antibodies by conventional immunologic techniques [63] failed to provide a consensus for the association of anti-M3R occurrence with clinical manifestations.

Carbonic anhydrases comprise a family of enzymes that catalyze the conversion of carbon dioxide and water to bicarbonate and protons and vice versa. They are classified as metalloproteases, since their active site contains a zinc ion and they are mainly involved in the regulation of acid base balance in blood and other tissues, and in aiding carbon dioxide transport out of tissues. Autoantibodies targeting carbonic anhydrase II (anti-CAII) are present in 12.5–20.8% of SS patients [64,65]. Furthermore, experimental immunization of PL/J mice with human carbonic anhydrase II evoked experimental autoimmune sialadenitis in mice, suggesting that anti-CAII antibodies could have a role in SS pathogenesis [66]. The occurrence of anti-CAII antibodies has been associated with distal renal tubular acidosis [67].

Finally, another set of autoantibodies that can be found in SS patients are the anti-smooth muscle antibodies (ASMA). Their prevalence in SS is around 30% [68]. In general, ASMA along with ANA positivity are indicative of type-1 autoimmune hepatitis [69], however, their clinical value in SS is not clear. It has been reported that ASMA positivity in SS patients correlates with autoimmune hepatitis [70–72], but this was not corroborated [23]. The aforementioned autoantibodies and their associations with the expression of SS are summarized at Table 2.

4. Regulation of the autoimmune response

Similarly to immune reactions against foreign antigens, autoimmune responses are tightly regulated. The aforementioned data,

Table 2
Association of autoantibodies with clinical features of SS patients.

Autoantibodies against	Clinical associations
Ro52/TRIM21	• Younger age at diagnosis and longer disease duration • Exocrine gland hypofunction as attested by functional tests
Ro60/TROVE2	• Severe infiltration of salivary glands • Salivary gland enlargement • Extraglandular manifestations
La/SSB	• Hypergammaglobulinemia, Cryoglobulinemia • Neonatal lupus-congenital heart block
U1RNP	Overlapping syndrome with MCTD
Rheumatoid factor	• Younger age at diagnosis • Extraglandular manifestations
Cryoglobulins	• Younger age at diagnosis • Salivary gland enlargement • Extraglandular manifestations • MALT lymphoma • Hypocomplementemia • Hypergammaglobulinemia
Centromere (ACA)	• Overlap with systemic sclerosis • Milder disease • Negative correlation with anti-Ro/SSA, anti-La/SSB antibodies
Mitochondria (AMA)	Primary biliary cirrhosis
Smooth muscle	Autoimmune hepatitis
Cyclic citrullinated peptides (CCP)	Articular manifestations
Muscarinic 3 receptor	Sicca symptoms
Carbonic anhydrases	Renal tubular acidosis

as well as pathways that will be discussed in this section, provide evidence for the operation of several potential regulatory mechanisms in the development of autoimmune responses. These include either mechanisms mediated exclusively by components of the immune system, such as the formation of idiotype–antiidiotype network or elements of innate immunity, namely TLR expression by inflamed epithelia, or wide cellular mechanisms that regulate molecule expression, in this case the expression of autoantigens, such as miRNAs.

4.1. Salivary glands are sites of autoantigen presentation and initiation of the immune response

Despite intensive studies, the mechanisms involved in the generation, as well as the sites of autoantibody production are not defined. The affected exocrine glands appear to serve as a major site of autoantibody formation. This is supported by the following findings: a) lymphocytic infiltrates in approximately 20% of SS patients form germinal center-like aggregates in the salivary glands [73,74] and contain autoreactive B cells, b) the saliva of SS patients contains high levels of IgG, as well as anti-Ro/SSA and anti-La/SSB autoantibodies, and c) many of the infiltrating plasma cells contain intracytoplasmic immunoglobulins with anti-Ro/SSA activity [75]. Finally, it has been reported that nuclear autoantigens translocate to the cytoplasm and the cellular membrane during ductal cell apoptosis [20]. In line with the local autoantibody production in the salivary glands, is the elevated epithelial expression of BAFF and various chemokines that regulate B cell survival and differentiation by SGEc. As mentioned earlier, SGEc demonstrate properties of antigen-presenting cells, thus being able to coordinate local immune responses. An additional important role that has been attributed to SGEc is the exposure of intracellular antigens and their presentation to the immune system. It appears that this is achieved by two processes, the apoptotic cell death and the release

of exosomes. Apoptotic cell death has been shown to result in the exposure of Ro52/TRIM21, Ro60/TROVE2 and La/SSB autoantigens through the release of autoantigen-loaded apoptotic blebs [12,20]. Although cultured SGEc have been found resistant to Fas-mediated apoptosis, they were rendered sensitive by a second signal, such as CD40 signaling [19,76], whereas they have been shown to undergo detachment-induced apoptosis (termed as anoikis) in response to TLR3 stimulation [14]. The in-vitro data supporting that epithelial cells die through apoptosis are further supported by numerous studies showing elevated apoptosis of the salivary gland epithelia in SS patients [reviewed in Ref. [1]]. Exosomes are small membrane vesicles of endocytic origin with a diameter between 30 and 100 nm that are secreted by almost every cell type. They are involved in diverse physiological processes, including the exchange and discharge of intracellular material. They have been proven to play a key regulatory role in immune responses by mediating T-cell activation and the transport of antigens to antigen-presenting. Cultured SGEc release exosomes that contain the major autoantigens Ro/SSA, La/SSB and Sm and express on the surface MHC molecules, indicating that they are capable to activate the immune system [22].

The pivotal role of epithelia in autoantibody production has been recently shown in an experimental mouse model lacking the transcriptional regulator IκB-ζ [77]. More particularly, IκB-ζ deficient mice, which are prompted to epithelial apoptosis, were found to spontaneously develop an SS-like inflammation, manifested as dacryoadenitis associated with the development of lymphocytic infiltrates in the affected tissue and production of high titers of the SS-associated autoantibodies Ro/SSA and La/SSB. This phenotype was found to specifically correlate with increased apoptosis of epithelial, but not the immune, cells. Furthermore, it was significantly reversed by treatment with caspase inhibitors, a finding that strengthens the role of epithelial apoptosis in autoimmune responses and provides a mechanistic insight on how the apoptosis of epithelial cells can initiate local inflammation, lymphocytic recruitment and autoantibody production, characteristic of SS.

4.2. Antiidiotypic antibodies targeting autoantibodies: possible suppressors of autoimmunity

One of the major immunologic breakthroughs proposed by Nobel laureate N.K. Jerne four decades ago was the idiotype–antiidiotype network theory [78]. Jerne suggested that the antibodies recognizing a specific epitope on an antigen are also capable to induce an immune response against them, which in turn leads to the production of antiidiotypic antibodies that neutralize the former antibodies. According to this theory, the proportion of idiotype and antiidiotypic antibodies in the circulation is balanced, forming a network of idiotype–antiidiotypic antibodies and resulting in the neutralization of idiotype antibodies by the anti-idiotypes, leading eventually to downregulation of the humoral responses.

This property of the immune system is obviously very important in autoimmune diseases, since it provides a regulatory mechanism to neutralize the harmful effects of autoantibodies. However, the study of the idiotype–antiidiotype network has some inherent difficulties arising from the following reasons: 1) the autoimmune response is heterogeneous, directed against multiple epitopes within various autoantigens, 2) the antiidiotypic antibodies are blocked and neutralized by autoantibodies, 3) the purification methodologies for antiidiotypic antibodies cannot, so far, disclose a homogeneous antiidiotypic antibody population to be used for further studies.

In our laboratory we used complementary peptides to major epitopes of La/SSB, spanning amino acids (aa) 349–364 of the

protein. The complementary peptides can bind the anti-idiotypic antibodies of anti-La/SSB (349–364aa) autoantibodies and, therefore, are useful tools to evaluate the idiotype–anti-idiotypic network. Experiments on this subject revealed the following [reviewed in Ref. [79]]:

1. Sera of patients with SS contain an active idiotype–anti-idiotypic network of the autoantigen La/SSB.
2. Immunization of animals with either epitopes or their complementary peptides results in the production of both idiotype and anti-idiotypic antibodies.
3. A substantial population of anti-La/SSB (349–364aa) is blocked and hidden in the conventional immunoassays, by the anti-idiotypic antibodies.

The latter observation prompted us to study mothers who gave birth to children with neonatal lupus syndrome (NLS). In studies performed blindly in sera of anti-La positive mothers, it was shown that mothers who gave birth to a healthy child had higher titers of anti-idiotypic antibodies, compared with mothers who gave birth to a child with NLS, leading to the assumption that anti-idiotypic antibodies are protective, most probably by binding to pathogenic anti-La/SSB antibodies, thereby blocking their entrance in the fetal circulation [80].

4.3. TLRs as regulators of the local autoimmune responses

TLRs are a family of highly conserved membrane receptors that are considered as major innate immunity sensors and list 10 members in humans and 12 in mice [81]. They recognize certain pathogen-associated molecular patterns (PAMPs), which are small molecular motifs conserved within a class of pathogens and mediate the production of several proinflammatory and immunomodulatory cytokines that are required for effective acquired responses. Of particular note is TLR3 that recognizes double-stranded RNA, which can be delivered locally from viruses or damaged cells, and upon ligation promotes the production of type-I interferons [82]. TLR3 appears to play a significant role in SS pathogenesis, since its signaling has been shown to reduce salivary gland function in several mouse strains and to significantly accelerate sialoadenitis in the NZB/NZW mouse model of SS [83–85]. Moreover, its constitutive expression on SGEc is high and localized on the cell-surface, which is indicative of a remarkable activation status, whereas TLR3 signaling on SGEc by polyI:C treatment has been shown to induce the expression of immunomodulatory molecules, interferons, as well as the apoptotic cell death via anoikis [14,86,87]. On the other hand, autoantigenic proteins have been shown capable of activating TLRs through the RNA molecules involved in the formation of their RNA-ribonucleoprotein complexes [88]. Furthermore, Ro52/TRIM21 autoantigen, with its E3 ubiquitin-protein ligase function has been implicated in the regulation of TLR3, TLR7 and TLR9 mediated inflammation [25]. Under this perspective, we recently sought to investigate whether TLR3 signaling could participate in the regulation of Ro52/TRIM21, as well as Ro60/TROVE2 and La/SSB, expression [89]. We found that indeed, TLR3, but not TLR4, signaling significantly upregulates the expression of Ro52/TRIM21 mRNA expression in SGEcs, and that this increment is achieved in two steps, one early evident from 6-hrs and one late at 24–48-h. The late increase of Ro52/TRIM21 mRNA expression that was induced by TLR3 signaling in SGEcs was accompanied by protein redistribution in the nucleus, from nucleolar-like to several dots spanning throughout nucleus. The TLR3-induced late Ro52/TRIM21 mRNA increase and protein redistribution were found to be largely mediated by IFN β production. Furthermore, TLR3 signaling in SGEcs was found to slightly,

but significantly induce the expression of Ro60/TROVE2 and La/SSB mRNAs [89]. These findings suggest that TLRs might be implicated in the regulation of the expression of autoantigens by non-immune tissues, by creating large quantities of autoantigens within epithelial cells, which under sustained ligation are led to apoptosis and autoantigen exposure to the immune system.

4.4. MicroRNAs targeting autoantigens

MicroRNAs (miRNAs) are small non-coding single-stranded RNA molecules (approximately 22 nucleotides long) which function as post-transcriptional regulators of gene expression in plants and animals [90]. Recent data from *in vitro* and *in vivo* experiments implicate miRNAs in aberrant immune responses and consequently autoimmune manifestations [91]. Despite rising interest in miRNA implication in autoimmunity, only a few studies have investigated their participation in SS etiopathogenesis. Alevizos et al. explored miRNA signatures in salivary glands from SS patients [92] and has reported 94 miRNAs that are differentially expressed between salivary glands obtained from SS patients and non-SS controls. Furthermore, the expression profile of these miRNAs can discriminate different SS patients regarding to salivary hypofunction and lymphocytic infiltration severity.

A recent study from our lab revealed a different aspect in the pathophysiologic role of miRNAs in SS. In an attempt to identify possible regulators of Ro52/TRIM21, Ro60/TROVE2 and La/SSB expression, we selected by a computational approach a panel of eleven miRNAs that are predicted to target Ro52/TRIM21, Ro60/TROVE2 and/or La/SSB mRNAs and analyzed their expression in salivary glands (SG) tissues, peripheral blood mononuclear cells (PBMC) and SGEc of SS patients and non-SS controls [93]. Seven miRNAs, including let-7b, miR-16, miR-181a, miR-200b, miR-200b*, miR-223 and miR-483-5p were found to be expressed in SGs, PBMCs and SGEcs. Among these, miR-181a, miR-200b and miR-223 were found to be significantly upregulated in SGs, SGEcs PBMCs, respectively, from SS patients compared to non-SS controls. It is noteworthy that miR-181a and miR-16 overexpression in SG tissues of SS patients has been previously correlated with decreased salivary function [92]. Lastly, let-7b miRNA expression was down-regulated in SGEcs established from SS patients that were anti-Ro/SSA positive and/or anti-La/SSB positive, as compared to anti-Ro/SSA and anti-La/SSB negative SS patients. The rest of miRNAs investigated were not found to be differentially expressed in the aforementioned two SS subgroups [93]. However, it should be noted that the multiplicity of miRNA targets and the complexity of their regulation render extremely difficult the interpretation of data concerning their role in autoimmune responses.

In summary, the autoimmune responses in SS appear to be extremely regulated. This is indicated by a) the complex pathways involved in the expression of intracellular autoantigens by non-immune tissues, such as glandular epithelia, that include the cross-talk of innate immunity signals and post-transcriptional regulation and b) the mechanisms involved in the exposure of these autoantigens in the immune system and the development of autoimmune reactions, with special attention to the apoptotic cell death and the release of autoantigen-loaded exosomes by SGEcs (Fig. 1). Further studies are needed to elucidate the regulation of autoantigen expression, as well as the autoantibody production and pathogenicity in SS, and to enable the development of therapeutic interventions.

The above-mentioned data were presented at the 12th International Symposium on Sjögren's Syndrome that was held at Kyoto, Japan on October 9–12, 2013. Along with the other studies presented in this special issue of the Journal of Autoimmunity, sheds a light on the novel clinical, pathogenic and therapeutic aspects of the disease.

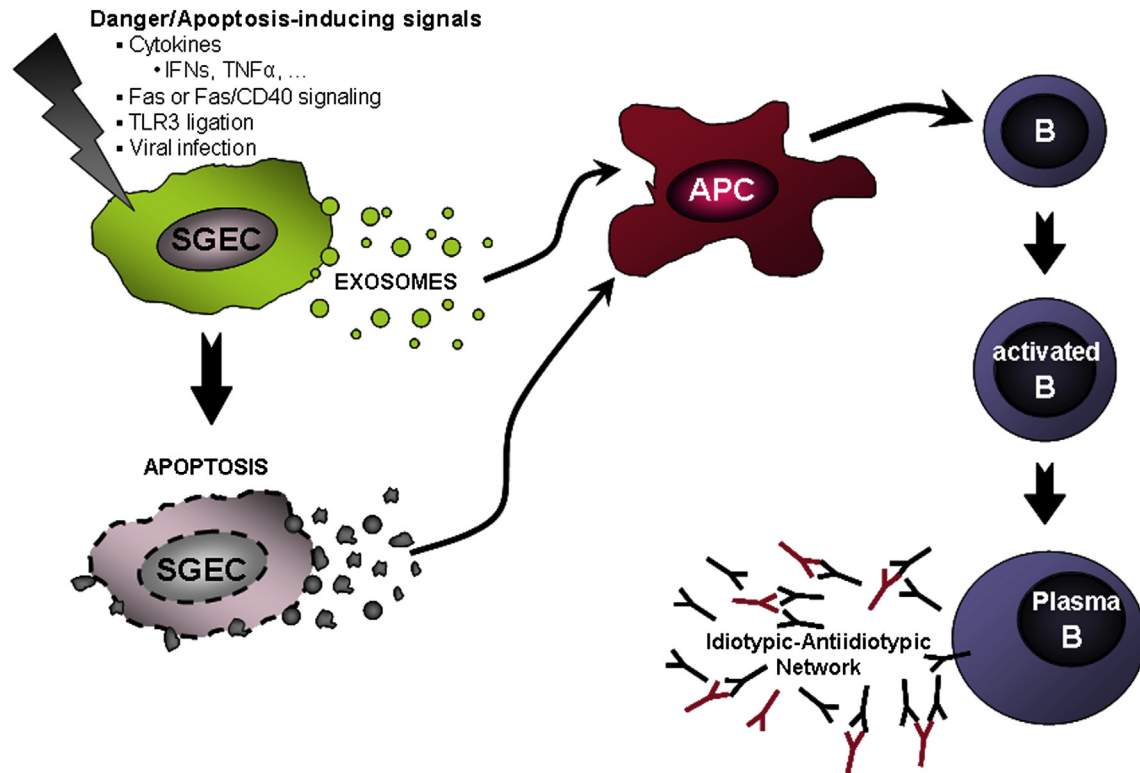


Fig. 1. Schematic representation of the development and regulation of humoral autoimmune responses in SS. At inflamed salivary tissues, exposure to danger or apoptosis-inducing signals leads glandular epithelial cells (SGECs) expressing the intracellular autoantigenic proteins to discharge them to the microenvironment through exosomes or apoptotic bodies. These autoantigen-loaded vesicles are processed by antigen-presenting cells (APCs) and result to the activation of B cells and the generation of autoantibody-producing B cells. The detection and pathogenicity of the produced autoantibodies depends on the balance of idiotype/antiidiotype antibodies and the development of an idiotype–anti-idiotype network, which neutralizes the pathogenic autoantibodies.

References

- [1] Tzioufas AG, Kapsogeorgou EK, Moutsopoulos HM. Pathogenesis of Sjögren's syndrome: what we know and what we should learn. *J Autoimmun* 2012;39:4–8.
- [2] Routsias JG, Vlachoyiannopoulos PG, Tzioufas AG. Autoantibodies to intracellular autoantigens and their B-cell epitopes: molecular probes to study the autoimmune response. *Crit Rev Clin Lab Sci* 2006;43:203–48.
- [3] Routsias JG, Kyriakidis N, Latreille M, Tzioufas AG. RNA recognition motif (RRM) of La/SSB: the bridge for interparticle spreading of autoimmune response to U1-RNP. *Mol Med*;16:19–26.
- [4] Yiannaki E, Vlachoyiannopoulos PG, Manoussakis MN, Sakarellos C, Sakarellos-Daitsiotis M, Moutsopoulos HM, et al. Study of antibody and T cell responses in rabbits immunized with synthetic human B cell epitope analogues of La (SSB) autoantigen. *Clin Exp Immunol* 2000;121:551–6.
- [5] Christodoulou MI, Kapsogeorgou EK, Moutsopoulos HM. Characteristics of the minor salivary gland infiltrates in Sjögren's syndrome. *J Autoimmun* 2010;34:400–7.
- [6] Kapsogeorgou EK, Christodoulou MI, Panagiotakos DB, Paikos S, Tassidou A, Tzioufas AG, et al. Minor salivary gland inflammatory lesions in Sjögren syndrome: do they evolve? *J Rheumatol* 2013;40:1566–71.
- [7] Christodoulou MI, Kapsogeorgou EK, Moutsopoulos NM, Moutsopoulos HM. Foxp3+ T-regulatory cells in Sjögren's syndrome: correlation with the grade of the autoimmune lesion and certain adverse prognostic factors. *Am J Pathol* 2008;173:1389–96.
- [8] Skopouli FN, Fox PC, Galanopoulou V, Atkinson JC, Jaffe ES, Moutsopoulos HM. T cell subpopulations in the labial minor salivary gland histopathologic lesion of Sjögren's syndrome. *J Rheumatol* 1991;18:210–4.
- [9] Jonsson MV, Skarstein K. Follicular dendritic cells confirm lymphoid organization in the minor salivary glands of primary Sjögren's syndrome. *J Oral Pathol Med* 2008;37:515–21.
- [10] Manoussakis MN, Boiu S, Korkolopoulou P, Kapsogeorgou EK, Kavantzias N, Ziakas P, et al. Rates of infiltration by macrophages and dendritic cells and expression of interleukin-18 and interleukin-12 in the chronic inflammatory lesions of Sjögren's syndrome: correlation with certain features of immune hyperactivity and factors associated with high risk of lymphoma development. *Arthritis Rheum* 2007;56:3977–88.
- [11] Xanthou G, Tapinos NI, Polihronis M, Nezis IP, Margaritis LH, Moutsopoulos HM. CD4 cytotoxic and dendritic cells in the immunopathologic lesion of Sjögren's syndrome. *Clin Exp Immunol* 1999;118:154–63.
- [12] Manoussakis MN, Kapsogeorgou EK. The role of intrinsic epithelial activation in the pathogenesis of Sjögren's syndrome. *J Autoimmun* 2010;35:219–24.
- [13] Moutsopoulos HM. Sjögren's syndrome: autoimmune epithelitis. *Clin Immunol Immunopathol* 1994;72:162–5.
- [14] Manoussakis MN, Spachidou MP, Maratheftis CI. Salivary epithelial cells from Sjögren's syndrome patients are highly sensitive to anoikis induced by TLR-3 ligation. *J Autoimmun* 2010;35:212–8.
- [15] Dimitriou ID, Kapsogeorgou EK, Abu-Helu RF, Moutsopoulos HM, Manoussakis MN. Establishment of a convenient system for the long-term culture and study of non-neoplastic human salivary gland epithelial cells. *Eur J Oral Sci* 2002;110:21–30.
- [16] Bourazopoulou E, Kapsogeorgou EK, Routsias JG, Manoussakis MN, Moutsopoulos HM, Tzioufas AG. Functional expression of the alpha 2-macroglobulin receptor CD91 in salivary gland epithelial cells. *J Autoimmun* 2009;33:141–6.
- [17] Kapsogeorgou EK, Manoussakis MN. Salivary gland epithelial cells (SGEC): carriers of exquisite B7-2 (CD86) costimulatory molecules. *J Autoimmun* 2010;35:188–91.
- [18] Katsiogiannis S, Kapsogeorgou EK, Manoussakis MN, Skopouli FN. Salivary gland epithelial cells: a new source of the immunoregulatory hormone adiponectin. *Arthritis Rheum* 2006;54:2295–9.
- [19] Abu-Helu RF, Dimitriou ID, Kapsogeorgou EK, Moutsopoulos HM, Manoussakis MN. Induction of salivary gland epithelial cell injury in Sjögren's syndrome: in vitro assessment of T cell-derived cytokines and Fas protein expression. *J Autoimmun* 2001;17:141–53.
- [20] Ohlsson M, Jonsson R, Brokstad KA. Subcellular redistribution and surface exposure of the Ro52, Ro60 and La48 autoantigens during apoptosis in human ductal epithelial cells: a possible mechanism in the pathogenesis of Sjögren's syndrome. *Scand J Immunol* 2002;56:456–69.
- [21] Polihronis M, Tapinos NI, Theocharis SE, Economou A, Kittas C, Moutsopoulos HM. Modes of epithelial cell death and repair in Sjögren's syndrome (SS). *Clin Exp Immunol* 1998;114:485–90.
- [22] Kapsogeorgou EK, Abu-Helu RF, Moutsopoulos HM, Manoussakis MN. Salivary gland epithelial cell exosomes: a source of autoantigenic ribonucleoproteins. *Arthritis Rheum* 2005;52:1517–21.
- [23] Nardi N, Brito-Zeron P, Ramos-Casals M, Aguilo S, Cervera R, Ingelmo M, et al. Circulating auto-antibodies against nuclear and non-nuclear antigens in primary Sjögren's syndrome: prevalence and clinical significance in 335 patients. *Clin Rheumatol* 2006;25:341–6.
- [24] Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, et al. Classification criteria for Sjögren's syndrome: a revised

- version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002;61:554–8.
- [25] Oke V, Wahren-Herlenius M. The immunobiology of Ro52 (TRIM21) in autoimmunity: a critical review. *J Autoimmun* 2012;39:77–82.
 - [26] Yang K, Shi HX, Liu XY, Shan YF, Wei B, Chen S, et al. TRIM21 is essential to sustain IFN regulatory factor 3 activation during antiviral response. *J Immunol* 2009;182:3782–92.
 - [27] Wolin SL, Cedervall T. The La protein. *Annu Rev Biochem* 2002;71:375–403.
 - [28] He J, Guo JP, Ding Y, Li YN, Pan SS, Liu Y, et al. Diagnostic significance of measuring antibodies to cyclic type 3 muscarinic acetylcholine receptor peptides in primary Sjogren's syndrome. *Rheumatology (Oxford)* 2011;50: 879–84.
 - [29] Sommer G, Dittmann J, Kuehnert J, Reumann K, Schwartz PE, Will H, et al. The RNA-binding protein La contributes to cell proliferation and CCND1 expression. *Oncogene* 2011;30:434–44.
 - [30] Itoh K, Itoh Y, Frank MB. Protein heterogeneity in the human Ro/SSA ribonucleoproteins. The 52- and 60-kD Ro/SSA autoantigens are encoded by separate genes. *J Clin Invest* 1991;87:177–86.
 - [31] Schulte-Pelkum J, Fritzler M, Mahler M. Latest update on the Ro/SS-A autoantibody system. *Autoimmun Rev* 2009;8:632–7.
 - [32] Routsias JG, Tzioufas AG. Sjogren's syndrome—study of autoantigens and autoantibodies. *Clin Rev Allergy Immunol* 2007;32:238–51.
 - [33] Manoussakis MN, Tzioufas AG, Pange PJ, Moutsopoulos HM. Serological profiles in subgroups of patients with Sjogren's syndrome. *Scand J Rheumatol Suppl* 1986;61:89–92.
 - [34] Mavragani CP, Tzioufas AG, Moutsopoulos HM. Sjogren's syndrome: autoantibodies to cellular antigens. Clinical and molecular aspects. *Int Arch Allergy Immunol* 2000;123:46–57.
 - [35] Tzioufas AG, Wassmuth R, Dafni UG, Gualis A, Haga HJ, Isenberg DA, et al. Clinical, immunological, and immunogenetic aspects of autoantibody production against Ro/SSA, La/SSB and their linear epitopes in primary Sjogren's syndrome (pSS): a European multicentre study. *Ann Rheum Dis* 2002;61: 398–404.
 - [36] Friedman DM, Llanos C, Izmirly PM, Brock B, Byron J, Copel J, et al. Evaluation of fetuses in a study of intravenous immunoglobulin as preventive therapy for congenital heart block: results of a multicenter, prospective, open-label clinical trial. *Arthritis Rheum* 2010;62:1138–46.
 - [37] Fauchais AL, Martel C, Gondran G, Lambert M, Launay D, Jauberteau MO, et al. Immunological profile in primary Sjogren syndrome: clinical significance, prognosis and long-term evolution to other auto-immune disease. *Autoimmun Rev* 2010;9:595–9.
 - [38] Martel C, Gondran G, Launay D, Lalloue F, Palat S, Lambert M, et al. Active immunological profile is associated with systemic Sjogren's syndrome. *J Clin Immunol* 2011;31:840–7.
 - [39] Ramos-Casals M, Solans R, Rosas J, Camps MT, Gil A, Del Pino-Montes J, et al. Primary Sjogren syndrome in Spain: clinical and immunologic expression in 1010 patients. *Medicine (Baltimore)* 2008;87:210–9.
 - [40] Bournia VK, Vlachoyiannopoulos PG. Subgroups of Sjogren syndrome patients according to serological profiles. *J Autoimmun* 2012;39:15–26.
 - [41] Voulgarelis M, Tzioufas AG. Pathogenetic mechanisms in the initiation and perpetuation of Sjogren's syndrome. *Nat Rev Rheumatol* 2010;6:529–37.
 - [42] Katano K, Kawano M, Koni I, Sugai S, Muro Y. Clinical and laboratory features of antientromere antibody positive primary Sjogren's syndrome. *J Rheumatol* 2001;28:2238–44.
 - [43] Kitagawa T, Shibasaki K, Toya S. Clinical significance and diagnostic usefulness of anti-centromere antibody in Sjogren's syndrome. *Clin Rheumatol* 2012;31: 105–12.
 - [44] Nakamura H, Kawakami A, Hayashi T, Iwamoto N, Okada A, Tamai M, et al. Anti-centromere antibody-seropositive Sjogren's syndrome differs from conventional subgroup in clinical and pathological study. *BMC Musculoskelet Disord*;11:140.
 - [45] Bournia VK, Diamanti KD, Vlachoyiannopoulos PG, Moutsopoulos HM. Anti-centromere antibody positive Sjogren's Syndrome: a retrospective descriptive analysis. *Arthritis Res Ther*;12:R47.
 - [46] Barcelos F, Abreu I, Patto JV, Trindade H, Teixeira A. Anti-cyclic citrullinated peptide antibodies and rheumatoid factor in Sjogren's syndrome. *Acta Rheumatol Port* 2009;34:608–12.
 - [47] Gottenberg JE, Mignot S, Nicaise-Rolland P, Cohen-Solal J, Aucouturier F, Goetz J, et al. Prevalence of anti-cyclic citrullinated peptide and anti-keratin antibodies in patients with primary Sjogren's syndrome. *Ann Rheum Dis* 2005;64:114–7.
 - [48] Iwamoto N, Kawakami A, Tamai M, Fujikawa K, Arima K, Aramaki T, et al. Determination of the subset of Sjogren's syndrome with articular manifestations by anticyclic citrullinated peptide antibodies. *J Rheumatol* 2009;36: 113–5.
 - [49] Tobon GJ, Correa PA, Anaya JM. Anti-cyclic citrullinated peptide antibodies in patients with primary Sjogren's syndrome. *Ann Rheum Dis* 2005;64:791–2.
 - [50] Atzeni F, Sarzi-Puttini P, Lama N, Bonacci E, Bobbio-Pallavicini F, Montecucco C, et al. Anti-cyclic citrullinated peptide antibodies in primary Sjogren syndrome may be associated with non-erosive synovitis. *Arthritis Res Ther* 2008;10:R51.
 - [51] Bogdanos DP, Komorowski L. Disease-specific autoantibodies in primary biliary cirrhosis. *Clin Chim Acta* 2011;412:502–12.
 - [52] Selmi C, Meroni PL, Gershwin ME. Primary biliary cirrhosis and Sjogren's syndrome: autoimmune epithelitis. *J Autoimmun* 2012;39:34–42.
 - [53] Hatzis GS, Fragoulis GE, Karatzaferis A, Delladetsima I, Barbatis C, Moutsopoulos HM. Prevalence and longterm course of primary biliary cirrhosis in primary Sjogren's syndrome. *J Rheumatol* 2008;35:2012–6.
 - [54] Eglen RM. Muscarinic receptor subtypes in neuronal and non-neuronal cholinergic function. *Auton Autacoid Pharmacol* 2006;26:219–33.
 - [55] Ishii M, Kurachi Y. Muscarinic acetylcholine receptors. *Curr Pharm Des* 2006;12:3573–81.
 - [56] Iwabuchi Y, Masuhara T. Sialogogic activities of SNI-2011 compared with those of pilocarpine and McN-A-343 in rat salivary glands: identification of a potential therapeutic agent for treatment of Sjogren's syndrome. *Gen Pharmacol* 1994;25:123–9.
 - [57] Nakamura T, Matsui M, Uchida K, Futatsugi A, Kusakawa S, Matsumoto N, et al. M(3) muscarinic acetylcholine receptor plays a critical role in parasympathetic control of salivation in mice. *J Physiol* 2004;558:561–75.
 - [58] Bacman S, Sterin-Borda L, Camusso JJ, Arana R, Hubscher O, Borda E. Circulating antibodies against rat parotid gland M3 muscarinic receptors in primary Sjogren's syndrome. *Clin Exp Immunol* 1996;104:454–9.
 - [59] Li J, Ha YM, Ku NY, Choi SY, Lee SJ, Oh SB, et al. Inhibitory effects of autoantibodies on the muscarinic receptors in Sjogren's syndrome. *Lab Invest* 2004;84:1430–8.
 - [60] Koo NY, Li J, Hwang SM, Choi SY, Lee SJ, Oh SB, et al. Functional epitope of muscarinic type 3 receptor which interacts with autoantibodies from Sjogren's syndrome patients. *Rheumatology (Oxford)* 2008;47:828–33.
 - [61] Kovacs L, Marczinovits I, Gyorgy A, Toth GK, Dorgai L, Pal J, et al. Clinical associations of autoantibodies to human muscarinic acetylcholine receptor 3(213–228) in primary Sjogren's syndrome. *Rheumatology (Oxford)* 2005;44: 1021–5.
 - [62] Roescher N, Kingman A, Shirota Y, Chiorini JA, Illei GG. Peptide-based ELISAs are not sensitive and specific enough to detect muscarinic receptor type 3 autoantibodies in serum from patients with Sjogren's syndrome. *Ann Rheum Dis* 2011;70:235–6.
 - [63] Dawson LJ, Allison HE, Stanbury J, Fitzgerald D, Smith PM. Putative anti-muscarinic antibodies cannot be detected in patients with primary Sjogren's syndrome using conventional immunological approaches. *Rheumatology (Oxford)* 2004;43:1488–95.
 - [64] Itoh Y, Reichlin M. Antibodies to carbonic anhydrase in systemic lupus erythematosus and other rheumatic diseases. *Arthritis Rheum* 1992;35:73–82.
 - [65] Ono M, Ono M, Watanabe K, Miyashita Y, Inagaki Y, Ueki H. A study of anti-carbonic anhydrase II antibodies in rheumatic autoimmune diseases. *J Dermatol Sci* 1999;21:183–6.
 - [66] Nishimori I, Bratanova T, Toshkov I, Caffrey T, Mogaki M, Shibata Y, et al. Induction of experimental autoimmune sialoadenitis by immunization of PL/J mice with carbonic anhydrase II. *J Immunol* 1995;154:4865–73.
 - [67] Takemoto F, Hoshino J, Sawa N, Tamura Y, Tagami T, Yokota M, et al. Autoantibodies against carbonic anhydrase II are increased in renal tubular acidosis associated with Sjogren syndrome. *Am J Med* 2005;118:181–4.
 - [68] Manthorpe R, Permin H, Tage-Jensen U. Auto-antibodies in Sjogren's syndrome. With special reference to liver-cell membrane antibody (LMA). *Scand J Rheumatol* 1979;8:168–72.
 - [69] Bogdanos DP, Invernizzi P, Mackay IR, Vergani D. Autoimmune liver serology: current diagnostic and clinical challenges. *World J Gastroenterol* 2008;14: 3374–87.
 - [70] Csepregi A, Szodoray P, Zeher M. Do autoantibodies predict autoimmune liver disease in primary Sjogren's syndrome? Data of 180 patients upon a 5 year follow-up. *Scand J Immunol* 2002;56:623–9.
 - [71] Lindgren S, Manthorpe R, Eriksson S. Autoimmune liver disease in patients with primary Sjogren's syndrome. *J Hepatol* 1994;20:354–8.
 - [72] Ramos-Casals M, Sanchez-Tapias JM, Pares A, Forns X, Brito-Zeron P, Nardi N, et al. Characterization and differentiation of autoimmune versus viral liver involvement in patients with Sjogren's syndrome. *J Rheumatol* 2006;33: 1593–9.
 - [73] Jonsson MV, Szodoray P, Jellestad S, Jonsson R, Skarstein K. Association between circulating levels of the novel TNF family members APRIL and BAFF and lymphoid organization in primary Sjogren's syndrome. *J Clin Immunol* 2005;25:189–201.
 - [74] Salomonsson S, Jonsson MV, Skarstein K, Brokstad KA, Hjelmstrom P, Wahren-Herlenius M, et al. Cellular basis of ectopic germinal center formation and autoantibody production in the target organ of patients with Sjogren's syndrome. *Arthritis Rheum* 2003;48:3187–201.
 - [75] Tengner P, Halse AK, Haga HJ, Jonsson R, Wahren-Herlenius M. Detection of anti-Ro/SSA and anti-La/SSB autoantibody-producing cells in salivary glands from patients with Sjogren's syndrome. *Arthritis Rheum* 1998;41: 2238–48.
 - [76] Ping L, Ogawa N, Sugai S. Novel role of CD40 in Fas-dependent apoptosis of cultured salivary epithelial cells from patients with Sjogren's syndrome. *Arthritis Rheum* 2005;52:573–81.
 - [77] Okuma A, Hoshino K, Ohba T, Fukushi S, Aiba S, Akira S, et al. Enhanced apoptosis by disruption of the STAT3-IkappaB-zeta signaling pathway in epithelial cells induces Sjogren's syndrome-like autoimmune disease. *Immunology* 2013;38:450–60.
 - [78] Jerne NK. Towards a network theory of the immune system. *Ann Immunol (Paris)* 1974;125C:373–89.
 - [79] Routsias JG, Tzioufas AG. B-cell epitopes of the intracellular autoantigens Ro/SSA and La/SSB: tools to study the regulation of the autoimmune response. *J Autoimmun* 2010;35:256–64.

- [80] Stea EA, Routsias JG, Clancy RM, Buyon JP, Moutsopoulos HM, Tzioufas AG. Anti-La/SSB antiidiotypic antibodies in maternal serum: a marker of low risk for neonatal lupus in an offspring. *Arthritis Rheum* 2006;54:2228–34.
- [81] Kawai T, Akira S. Toll-like receptors and their crosstalk with other innate receptors in infection and immunity. *Immunity* 2011;34:637–50.
- [82] Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-kappaB by toll-like receptor 3. *Nature* 2001;413:732–8.
- [83] Nandula SR, Dey P, Corbin KL, Nunemaker CS, Bagavant H, Deshmukh US. Salivary gland hypofunction induced by activation of innate immunity is dependent on type I interferon signaling. *J Oral Pathol Med* 2013;42:66–72.
- [84] Nandula SR, Scindia YM, Dey P, Bagavant H, Deshmukh US. Activation of innate immunity accelerates sialoadenitis in a mouse model for Sjogren's syndrome-like disease. *Oral Dis* 2011;17:801–7.
- [85] Deshmukh US, Nandula SR, Thimmalapura PR, Scindia YM, Bagavant H. Activation of innate immune responses through toll-like receptor 3 causes a rapid loss of salivary gland function. *J Oral Pathol Med* 2009;38:42–7.
- [86] Ittah M, Miceli-Richard C, Gottenberg JE, Sellam J, Eid P, Lebon P, et al. Viruses induce high expression of BAFF by salivary gland epithelial cells through TLR- and type-I IFN-dependent and -independent pathways. *Eur J Immunol* 2008;38:1058–64.
- [87] Spachidou MP, Bourazopoulou E, Maratheftis CI, Kapsogeorgou EK, Moutsopoulos HM, Tzioufas AG, et al. Expression of functional Toll-like receptors by salivary gland epithelial cells: increased mRNA expression in cells derived from patients with primary Sjogren's syndrome. *Clin Exp Immunol* 2007;147:497–503.
- [88] Kelly KM, Zhuang H, Nacionales DC, Scumpia PO, Lyons R, Akaogi J, et al. "Endogenous adjuvant" activity of the RNA components of lupus autoantigens Sm/RNP and Ro 60. *Arthritis Rheum* 2006;54:1557–67.
- [89] Kyriakidis NC, Kapsogeorgou EK, Gourzi VC, Moutsopoulos HM, Tzioufas AG. RO52/TRIM21 expression in salivary gland epithelial cells (SGECs) is up-regulated by TLR3-signaling. In: *Annual European Congress of RHEUMATOLOGY*, Madrid, Spain: Annals of the Rheumatic Diseases 2013. p. 78.
- [90] Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell* 2009;136:215–33.
- [91] Carissimi C, Fulci V, Macino G. MicroRNAs: novel regulators of immunity. *Autoimmun Rev* 2009;8:520–4.
- [92] Alevizos I, Alexander S, Turner RJ, Illei GG. MicroRNA expression profiles as biomarkers of minor salivary gland inflammation and dysfunction in Sjogren's syndrome. *Arthritis Rheum* 2011;63:535–44.
- [93] Kapsogeorgou EK, Gourzi VC, Manoussakis MN, Moutsopoulos HM, Tzioufas AG. Cellular microRNAs (miRNAs) and Sjogren's syndrome: candidate regulators of autoimmune response and autoantigen expression. *J Autoimmun* 2011;37:129–35.