Review of autoantigens in Sjögren’s syndrome: an update

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Abstract: Primary Sjögren’s syndrome (pSS) is an autoimmune disease characterized by inflammation in exocrine glands, resulting in reduced secretion of tears and saliva, manifesting as xerophthalmia and xerostomia, respectively. It is commonly associated with Sjögren’s syndrome type A (Ro) and Sjögren’s syndrome type B (La) antigens. However, in most patients, the identity of the triggering antigen is not known. Factors such as genetics of histocompatibility, dysregulation of T-cells, B-cells and viral infections have been implicated. Several important studies on autoantigens in pSS have been published since a review in 2012, and the aim of this review is to provide an update on further peer-reviewed original articles in this field. Oxidative damage of Ro60 antigen may explain the epitope spreading during the immune activation in pSS. Immune-mediated destruction of the muscarinic receptor-3-expressing cells has been associated with a reduction in parasympathetic function, which could cause reduced secretory function of exocrine glands. Such a process also activates reactive oxidative species and antioxidants, which are linked to the triggering of inflammatory responses. Elevated levels of kallikrein, yet another antigen present in the lacrimal gland and other tissues, are similarly involved in triggering an autoimmune T-cell response against target glands. Studying additional antigens, the platelet-selectin and vasoactive intestinal peptides, in patients with pSS can help to elucidate the origin and process of autoimmunity, or even lead to potential biomarkers. In conclusion, the understanding of autoantigens has led to exciting major advances in the biology of pSS and may influence diagnosis and management of pSS in future.

Keywords: Sjögren’s syndrome, review, ocular disease, autoimmune, autoantigen, inflammation

Autoimmunity and autoantigens

Under normal circumstances, self-antigens do not activate the immune system. When there is a conformational change in the epitopes of these self-antigens for any reason, eg., somatic mutations/injury (immunoediting concept), or when two antigens that had been spatially separated bind one other (hapten–carrier concept), immune reactivity against self-antigens can increase. Such breaks in immune tolerance may result in autoimmunity, mediated by T- and B-lymphocytes. The T-cells can mount effector cytotoxicity responses, and B-cells can differentiate into plasma cells that produce specific immunoglobulins against the autoantigens. Effector lymphocytes and antibodies then target the tissues expressing the self-antigens and cause damage in those tissues. As a consequence of the damage, other different antigens are likely to be subsequently targeted by secondary immune responses (epitope spreading), which can sustain the autoimmune disease.1
Based on these considerations, we hypothesize that specific molecular targets of autoantibodies deserve attention by researchers because the clinical manifestations of dysfunctional organs offer an opportunity to elicit the physiological role of the target antigen in the healthy state.

**Sjögren’s syndrome**

Sjögren’s syndrome is a chronic systemic autoimmune epithelitis involving the exocrine glands of the human body, ie, lacrimal and salivary glands. Primary Sjögren’s syndrome (pSS) is a disease entity that is unrelated to the presence of another known autoimmune disease. Secondary Sjögren’s syndrome refers to the autoimmune disease affecting the same exocrine glands but that exists as part of another known autoimmune systemic disease such as rheumatoid arthritis. The overall population prevalence rate of pSS in a meta-analysis was reported to be 6/10,000; pSS is known to be associated with significant morbidity, including psychological and social dysfunction. The autoimmune disease in pSS is triggered by B- and T-lymphocyte-mediated responses against certain antigens, although the nature of the primary antigen is not fully known, and may vary among individuals with pSS in different reports. More comprehensive reviews of autoantigens in Sjögren’s syndrome have been published in 2007 and 2012. At the same time, there was an article reviewing the autoantigens of Sjögren’s syndrome in 2017. However, it did not review recent original papers. Hence, we aim to list the more recently published research not covered by these reports, concerning the autoantigens in pSS (Tables 1 and 2). An initial literature search was also conducted using both PubMed and SciMed Central databases in order to ensure full coverage of all the recently available literature. In addition, based on these latest studies, we attempted to summarize the mechanisms through which autoantigens can lead to clinical disease in pSS (Figure 1). Apart from pSS, autoantigens in systemic lupus erythematosus and antiphospholipid syndrome have also received major research interest in recent years.

**Ro and La autoantigens**

The Sjögren’s syndrome type A (Ro) and Sjögren’s syndrome type B (La) antigens in pSS have been researched for many years and their corresponding autoantibodies are known to be elevated in the plasma of patients with pSS. These autoantibodies are used in routine clinical care to diagnose pSS and have been part of international classification criteria for pSS. Ro52 is a transcription modulator and ubiquitin E3 ligase, Ro60 is an RNA-degrading protein, whereas the La antigen is an RNA-binding phosphoprotein. These subserve highly conserved roles and are ubiquitous in vertebrate cells.

Recently, a study has shed light on when autoantibodies first appear during the course of autoimmunity. During inflammatory states, lymphocytes can accumulate in certain organs, including exocrine glands. When this occurs, the lymphocytes infiltrate the tissues diffusely or as organized aggregates called lymphoid follicles. Autoantibodies to Ro and La were detected at 8 weeks of age in female mice models (non-obese diabetic mice NOD.H2<sup>+</sup> mice) of Sjögren’s syndrome. This time point was prior to the appearance of follicular lymphoid changes in the salivary glands, as well as prior to the time of elevation of the less-specific anti-double-stranded DNA (anti-dsDNA) autoantibody in the serum.

At 8 weeks, B-cells located in the germinal centers in the spleen proliferated, resulting in noticeable increases in the size of these structures, and since differentiated B-cells, ie, plasma cells, produce immunoglobulins (Igs), it is highly suggestive that these are the sources of the Ro and La autoantibodies, instead of the lymphoid follicles in the salivary glands. More studies will be required to examine the site and timing of the initial antigen presentation, as well as how the naive lymphocytic clone eventually is amplified to form the splenic germinal centers.

During chronic inflammation, the initial autoantigen may be further damaged due to ongoing immune activation. In an interesting recent study, oxidative (4-hydroxy-2-nonenal) modification of the Ro60 antigen has been shown to result in robust T- and B-lymphocyte activation in mice, compared to immunization with unmodified Ro60. The greater the extent of the oxidative modification, the more amplified were the observed T- and B-cell responses. This may be how epitope spreading occurs in some patients with pSS.

**Muscarinic receptors as autoantigens in Sjögren’s syndrome**

The muscarinic receptors are seven G-protein-coupled transmembrane glycoproteins that respond to acetylcholine. These receptors subserve neurotransmitter functions in the parasympathetic nervous system, as well as hormonal functions in nonneural tissues such as smooth muscles. There are, in total, five subtypes of muscarinic receptors (M1R–M5R). In the lacrimal glands, binding of the M3 receptor by acetylcholine leads to glandular secretion. In smooth muscles, M2R and M3R work in concert to initiate contraction. In myoepithelial cells, M3R facilitates the movement of tears in the lacrimal gland ducts. In conjunctival goblet cells, M2 and M3Rs have been implicated in secretion of...
Table 1 Clinical studies related to antigens in Sjögren’s syndrome since 2011

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>pSS number</th>
<th>Control number</th>
<th>Immune target</th>
<th>Tissues evaluated</th>
<th>Analytical techniques</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gourzi et al</td>
<td>2015</td>
<td>29</td>
<td>24*</td>
<td>Ro, La</td>
<td>Minor salivary gland, blood mononuclear cells</td>
<td>qRT-PCR</td>
<td>Certain miRNA correlated to levels of Ro and La.</td>
</tr>
<tr>
<td>Hirose et al</td>
<td>2015</td>
<td>11</td>
<td>10*</td>
<td>Ro, La</td>
<td>Salivary gland tissues</td>
<td>Western blot, ELISA</td>
<td>Protein-conjugated acrolein higher in saliva of pSS, acrolein can confer greater activity to autoantibodies targeting Ro and La.</td>
</tr>
<tr>
<td>Katsiougiannis et al</td>
<td>2015</td>
<td>8</td>
<td>4</td>
<td>Ro, La</td>
<td>Minor salivary gland tissues</td>
<td>ELISA</td>
<td>ER stress activated in salivary glands, causes apoptosis, leading to movement of SSA and SSB from cytosol to cell membranes.</td>
</tr>
<tr>
<td>Pertovaara et al</td>
<td>2015</td>
<td>16</td>
<td>16</td>
<td>La</td>
<td>Plasma monocytes, T- and B-cells, salivary gland tissue</td>
<td>qRT-PCR, flow cytometry</td>
<td>Phosphorylation of STAT in blood macrophages more common in pSS than in controls.</td>
</tr>
<tr>
<td>Aqrawi et al</td>
<td>2014</td>
<td>28</td>
<td>19</td>
<td>Ro52‡‡</td>
<td>Salivary gland tissues, serum</td>
<td>ELISA</td>
<td>Little Ro52 secretion in saliva/serum but upregulated in ductal epithelium and mononuclear cell infiltrate in pSS patients</td>
</tr>
<tr>
<td>Zheng et al</td>
<td>2014</td>
<td>289</td>
<td>Nil</td>
<td>Ro, La, ANA, ACA, RF</td>
<td>Serum</td>
<td>Not specified</td>
<td>Categorization of pSS based on clinical parotid gland features and serology may be useful. Ro60 may be associated with massive parotid glands.</td>
</tr>
<tr>
<td>Scofield et al</td>
<td>2012</td>
<td>88</td>
<td>Nil</td>
<td>Ro, La</td>
<td>Serum</td>
<td>Immunodiffusion</td>
<td>Sensory neuropathy is associated with the presence of Ro and La autoantibodies in pSS patients.</td>
</tr>
<tr>
<td>ter Borg et al</td>
<td>2011</td>
<td>65</td>
<td>Nil</td>
<td>Ro, La, ANA</td>
<td>Plasma</td>
<td>ELISA, Western blot</td>
<td>Presence of SSA autoantibodies predictor of extraglandular manifestations of pSS.</td>
</tr>
<tr>
<td>Zuo et al</td>
<td>2016</td>
<td>24</td>
<td>23</td>
<td>M3R</td>
<td>Plasma</td>
<td>Western blot, in-cell Western assay</td>
<td>Higher M3R autoantibodies identified in pSS patients than in controls. M3R autoantibodies associated with SSA and SSB.</td>
</tr>
<tr>
<td>Wolska et al</td>
<td>2016</td>
<td>235</td>
<td>50</td>
<td>TRIM-38</td>
<td>Serum</td>
<td>Quantitative immunoprecipitation</td>
<td>Anti-TRIM38 significantly associated with classical autoantigens of pSS and clinical signs such as high ocular staining and low Schirmer’s scores.</td>
</tr>
<tr>
<td>Alam et al</td>
<td>2016</td>
<td>102</td>
<td>53</td>
<td>Aq-5</td>
<td>Serum</td>
<td>IIFA, Western blotting</td>
<td>Anti-aq5 significantly associated with sera in SS patients than in controls. It is also related to the resting saliva flow in SS patients.</td>
</tr>
<tr>
<td>Hu et al</td>
<td>2015</td>
<td>32</td>
<td>35</td>
<td>Platelet-selectin</td>
<td>Serum</td>
<td>ELISA</td>
<td></td>
</tr>
<tr>
<td>Suresh et al</td>
<td>2015</td>
<td>89</td>
<td>50</td>
<td>SP1, CA6, PSP</td>
<td>Serum</td>
<td>ELISA</td>
<td>Antigens potentially able to be used to identify pSS in patients with low focus scores on lip biopsies. Summarized in main text</td>
</tr>
<tr>
<td>Reina et al</td>
<td>2015</td>
<td>25</td>
<td>25</td>
<td>M3R</td>
<td>Serum</td>
<td>ELISA</td>
<td>Levels of VIPR differ between pSS and controls. Response of VIPR expression to LPS also differs. Anti-KLK11 can be used as a biomarker.</td>
</tr>
<tr>
<td>Hauk et al</td>
<td>2014</td>
<td>38</td>
<td>16</td>
<td>VIP receptor 2</td>
<td>Monocytes from plasma</td>
<td>Flow cytometry, ELISA, qRT-PCR, Antibody capture assay</td>
<td></td>
</tr>
<tr>
<td>El Annan et al</td>
<td>2013</td>
<td>11</td>
<td>8</td>
<td>Kallikrein</td>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shen et al</td>
<td>2012</td>
<td>33</td>
<td>23</td>
<td>SP1, CA6, PSP</td>
<td>Serum</td>
<td>ELISA</td>
<td>Sensory neuropathy is associated with the presence of Ro and La autoantibodies in pSS patients.</td>
</tr>
<tr>
<td>Reina et al</td>
<td>2011</td>
<td>17</td>
<td>15</td>
<td>M3R</td>
<td>Serum</td>
<td>ELISA</td>
<td>M3R induces the production of pro-inflammatory markers PGE2 and MMP-3, which can be used as biomarkers.</td>
</tr>
</tbody>
</table>

Notes: *Controls with dry eye but without Sjögren’s syndrome; ‡Ro antigen; ‡‡protein of Ro antigen epitopes; ‡‡‡proteins of Ro antigen epitopes; §secondary Sjögren’s syndrome.

Abbreviations: ACA, anti-centromere antibody; ANA, anti-nuclear antibody; Aq-5, aquaporin-5; CA6, carbonic anhydrase-6; ELISA, enzyme-linked immunosorbent assay; ER, endoplasmic reticulum; IIFA, indirect immunofluorescence assay; KLK, kallikrein; LPS, lipopolysaccharide; M3R, muscarinic receptor-3; PGE2, prostaglandin E2; PSP, parotid secretory protein; pSS, primary Sjögren’s syndrome; qRT-PCR, qualitative reverse transcription-polymerase chain reaction; RF, rheumatoid factor; SP1, salivary protein-1; Ro, Sjögren’s syndrome Type A; La, Sjögren’s syndrome Type B; VIP, vasoactive intestinal peptide; VIPR, VIP receptor.
their mucin contents, and in meibomian glands, muscarinic receptors have been found to regulate meibum secretion.22,23

Certain studies have implicated M3R as a potential autoantigen in pSS.20,24 Recently, two clinical studies have shown that the immune responses stimulated by M3R could cause the failure of M3R signaling pathways, ultimately leading to the development of pSS.25,26 M3R peptides are able to activate interferon (IFN)-γ-reactive T-cells, which then induce inflammation targeting the tissues expressing these peptides.

Yang et al27 described an interesting model of Sjögren’s syndrome in mice induced by immunization of a mixture of peptides derived from extracellular loops of M3R. These mice displayed features of inflammation in the lacrimal glands, salivary glands and the intestines. The inflammation was characterized by an increase in CD4 and CD8 T-lymphocytes in the salivary gland and the intestines but by an increase in non-CD4+/non-CD8+ mononuclear cells (MNCs) in the lacrimal glands. Plasma cells were elevated in all three tissues. The inflammation in the salivary glands and intestines, but not the lacrimal glands, was mediated by the cytokines interleukin (IL)-17 and IFN-γ, which could have originated from the splenocytes that highly expressed these same cytokines. IFN-γ may have increased the activation of MNCs or reduced the apoptosis of MNCs, and either scenario would explain the greater infiltration of MNCs into the lacrimal gland.27

Activation of MNCs is highly dependent on growth factor signaling, such as that of the epidermal growth factor receptor (EGFR). The expression of antibodies blocking EGFR would reduce the number of activated MNCs. Immunization was performed with a peptide derived from EGFR-blocking antibodies (mimotope immunization [MI]) as well as M3R peptides. This caused the resulting MNCs in the lacrimal gland to reduce in number, and so did the T-lymphocytes in the salivary glands. In these animals, the serum antibodies against EGFR were elevated on Day 38, and their splenocytes produced similar antibodies. At the same time, there was increased Fas expression in MNCs, which could mediate apoptosis and reduce the number of MNCs in the lacrimal gland. The splenocytes obtained from these mice had down-regulation of IL-17. Since IL-17 normally stimulates MNC proliferation, this may also explain the reduction of MNCs in the lacrimal glands.27

Interestingly, enzyme-linked immunosorbent assay (ELISA) studies showed that increased serum antibodies in mice with additional MI could bind M3R peptides. This may explain why coimmunization with MI and M3R peptides did not significantly improve saliva secretion compared to animals immunized with M3R. The beneficial effects of less immune cell infiltration into the salivary glands may be partly obliterated by the detrimental effect of the cross-reacting antibodies blocking M3R signaling for saliva production.27

In another murine study, the antibody to M3R has been reported to increase levels of antioxidants, ie, superoxide dismutase (SOD) and catalase (CAT).26 Since dry eye disease in humans has been linked to abnormal antioxidant status,28 Reina’s study provides a mechanistic link between the M3R autoantigen and the disease mechanism in dry eye disease.

Notwithstanding these considerations, there is controversy concerning the mechanism and effects of autoantibodies on M3Rs in pSS. Some research has found that autoantibodies act like muscarinic agonists, producing effects
like the natural ligands of the receptor. Overstimulation of the M3R increases nitric oxide synthase activity, and the resulting increase in nitric oxide, if excessive, can induce cytotoxicity in the lacrimal gland. Studies have also shown that these autoantibodies act like M3 antagonists and inhibit acetylcholine release. In a mouse model of Sjögren’s syndrome, there was retention of goblet cell secretions as well as reduced M3R expression in these cells, suggesting that the autoantibodies played an M3R antagonistic role or indirectly opposed muscarinic receptor signaling by reducing the numbers of M3Rs.

**Other autoantigens in Sjögren’s syndrome**

Vasoactive intestinal peptide (VIP) is a neuropeptide and hormone from the glucagon family that binds to VIP receptor (VIPR) 1 and 2 on target cells, ie, immune cells, smooth muscles, as well as lung and intestinal epithelial cells.
By binding to VIPR, VIP is also able to induce immunomodulatory effects on monocytes, macrophages, and T-cells. It has anti-inflammatory effects, as it inhibits proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and IL-6,13-15 and upregulates the anti-inflammatory cytokine IL-10.14-16 However, a recent study17 has shown that exogenous VIP did not change TNF and IL-10 levels expressed by cultured MNCs that had phagocytosed apoptotic salivary gland cells obtained from pSS patients. This study did not evaluate cytokine changes in MNCs from pSS patients under other conditions.17

The VIPRs are found in lacrimal glands, where they promote the secretion of proteins, water, and electrolytes. They are translated in the endoplasmic reticulum and are then modified and stored in the Golgi body within endoplasmic granules. When specific signals in the form of second messengers are activated, these granules migrate to the cell membranes, enabling functioning VIPRs to be incorporated in the cell surface. VIPRs are also co-regulated by cholinergic agonists.18 Although the function of VIPRs is unclear in meibomian glands,22,23 exogenous VIP can stimulate increase in intracellular calcium.22 The expression of VIPR2 was recently found to be increased in monocytes from pSS patients, but unfortunately, VIPR levels were not assessed in lacrimal and meibomian glandular tissues.17

Lipopolysaccharide (LPS) stimulates cells via innate toll-like receptors to influence many immune genes.36 In such activated monocytes derived from pSS patients, VIPR2 was observed to be upregulated, compared to activated monocytes from healthy controls. More interestingly, the levels of VIPR2 in pSS monocytes were restored to normal when VIP was added to the activated monocytes.37 This reduction of VIPR2 in pSS may potentially reduce any downstream cytokine effects triggered by VIP, including those that exert anti-inflammatory effects.

Recently, original articles studying the role of aquaporins (Aqs) and carbonic anhydrases (CAs) have been published.39,40 Aqs are a group of water channels found in various cell types, including the lacrimal and salivary acinar cells, which suggest that autoimmune targeting of these membrane proteins may cause reduced tear and saliva production, respectively.39 Aq-5, in particular, has recently been recently emphasized due to its link to the clinical manifestations of Sjögren’s syndrome.39 The resulting reduction of exocrine functions as mentioned earlier is highly suggestive of the dysfunction of Aq-5. The CA enzymes are critical for ionic exchange in acinar cells. These convert carbon dioxide and bicarbonate to maintain pH, and in the renal tubules, they promote water loss through urine. In pSS, it is likely that damage to CA-6, a member of the CA family, reduces fluid transport into the acinar cavity and therefore the secretion of saliva or tear fluid.40

Another potential autoantigen is platelet (p)-selectin, a 140 kDa protein found in the granules of platelets and vascular endothelial cells. This protein can bind to sialylated Lewis blood group carbohydrate antigens or high-affinity receptors of p-selectins on immune cells such as neutrophils and monocytes. During inflammation, p-selectin can be released from the platelet granules into the plasma.41 Recently, p-selectin autoantibodies have been found to be elevated in pSS patients (with and without thrombocytopenia) compared to healthy controls.42 This suggests that even in pSS patients without platelet deficiency, p-selectin could play a role in the disease. It is possible that p-selectin increased the adhesion of immune cells to the walls of blood vessels within exocrine glands and facilitated leukocyte trafficking out of blood vessels during inflammation.

A class of small plasma peptidases called kallikreins (KLKs) has also been implicated in pSS43 and experimental dry eye.44 A recent study has shown that a particular subtype of KLKs, KLK11, is elevated in humans with Sjögren’s syndrome compared to dry eye patients without Sjögren’s syndrome, or compared to age- and gender-matched healthy controls. However, in this study, the proportion of pSS patients in the Sjögren’s syndrome group has not been reported. What is interesting is that KLK11 is not one of the many KLK subtypes reported in previous pSS patients and mouse models of dry eye.45 KLK11 is located in the Golgi apparatus of saliva-producing acinar cells in the salivary glands.45 Immune-mediated targeting of KLK11 would therefore destroy the subcellular structure that secretes saliva. Anti-KLK antibodies may thus destroy sufficient glandular acini to reduce secretion. It is unclear at the moment which subtype of KLKs can be used as a marker to determine the severity and/or presence of pSS.46

Sex hormones may play a role in regulation of KLKs, but the changes may or may not be occurring through hormone receptors.46 It is not known whether the hormonal effects are relevant to pSS, which is more common in females. KLKs activate bradykinin by cleaving kininogen, and this is a very important process in inflammation, but it is unclear whether KLKs play such a role in pSS. KLKs may just be secondary antigens resulting from epitope spreading.

**Limitations and future directions**

Future challenges include the evaluation of the autoantibodies against the proposed autoantigens in larger samples of
patients. Many of the cited studies only evaluated salivary glands,\textsuperscript{37,43} so more work should be done involving lacrimal and meibomian glands, in addition to analyzing the secretory function of these glands. Future studies should also be performed with epitope mapping of the members of the KLK protein family and to explain the involvement of specific subtypes of KLKs. In terms of using autoantibodies as diagnostic markers for pSS in clinics, more work should be done on the performance of test panels that include these assays compared to the conventional assays against Ro and La autoantibodies.

**Conclusion**

Patients with pSS battle a systemic autoimmune disease triggered by a variety of autoantigens. Despite advances in the past few years, the sequence of activation of immunity against these antigens and the relative importance of these antigens remain unknown. It is difficult to determine the primary event from secondary processes in a patient with well-established pSS.

Whenever new autoantigens are discovered or implicated in pSS, it is important to elucidate their physiological function and signaling role in healthy persons. This may lead to a more comprehensive understanding of disease development and progression. Understanding the biological pathways in disease development will help to develop more precise and accurate diagnostic tools and more effective and targeted therapies for these patients.

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**Disclosure**

The authors report no conflicts of interest in this work.

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Classical and novel autoantigens in Sjögren’s syndrome