

# 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.<sup>1</sup>

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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.<sup>2</sup> 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.<sup>3</sup> The overall population prevalence rate of pSS in a meta-analysis was reported to be 6/10,000;<sup>4</sup> pSS is known to be associated with significant morbidity, including psychological and social dysfunction.<sup>5</sup> The autoimmune disease in pSS is triggered by B- and T-lymphocyte-mediated responses against certain antigens,<sup>6</sup> 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<sup>7</sup> and 2012.<sup>8</sup> At the same time, there was an article reviewing the autoantigens of Sjögren's syndrome in 2017.<sup>9</sup> 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.<sup>10,11</sup>

## 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.<sup>12–14</sup> These autoantibodies are used in routine clinical care to diagnose pSS and have been part of international classification criteria for pSS.<sup>15,16</sup> 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 sub-

serve highly conserved roles and are ubiquitous in vertebrate cells.<sup>13,14</sup>

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.<sup>17</sup> Autoantibodies to Ro and La were detected at 8 weeks of age in female mice models (non-obese diabetic mice NOD.H2<sup>h4</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.<sup>18</sup>

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.<sup>18</sup> More studies will be required to examine the site and timing of the initial antigen presentation, as well as how the naïve 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.<sup>19</sup> 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.<sup>20</sup> 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.<sup>21</sup> 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

Authors	Year	pSS number	Control number	Immune target	Tissues evaluated	Analytical techniques	Remarks
Gourzi et al <sup>47</sup>	2015	29	24*	Ro <sup>‡</sup> , La	Minor salivary gland, blood mononuclear cells	qRT-PCR	Certain miRNA correlated to levels of Ro and La.
Hirose et al <sup>48</sup>	2015	11	10*	Ro, La	Salivary gland tissues	Western blot, ELISA	Protein-conjugated acrolein higher in saliva of pSS, acrolein can confer greater activity to autoantibodies targeting Ro and La.
Katsiogiannis et al <sup>49</sup>	2015	8	4	Ro, La	Minor salivary gland tissues	ELISA	ER stress activated in salivary glands, causes apoptosis, leading to movement of SSA and SSB from cytosol to cell membranes.
Pertovaara et al <sup>50</sup>	2015	16	16	La	Plasma monocytes, T- and B-cells, salivary gland tissue	qRT-PCR, flow cytometry	Phosphorylation of STAT in blood macrophages more common in pSS than in controls.
Aqrabi et al <sup>51</sup>	2014	28	19	Ro52 <sup>‡‡</sup>	Salivary gland tissues, serum	ELISA	Little Ro52 secretion in saliva/serum but upregulated in ductal epithelium and mononuclear cell infiltrate in pSS patients
Zheng et al <sup>52</sup>	2014	289	Nil	Ro, La, ANA, ACA, RF	Serum	Not specified	Categorization of pSS based on clinical parotid gland features and serology may be useful. Ro60 may be associated with massive parotid glands.
Scofield et al <sup>53</sup>	2012	88	Nil	Ro, La	Serum	Immunodiffusion	Sensory neuropathy is associated with the presence of Ro and La autoantibodies in pSS patients.
ter Borg et al <sup>54</sup>	2011	65	Nil	Ro, La, ANA	Plasma	ELISA, Western blot	Presence of SSA autoantibodies predictor of extraglandular manifestations of pSS.
Zuo et al <sup>25</sup>	2016	24	23	M3R	Plasma	Western blot, in-cell Western assay	Higher M3R autoantibodies identified in pSS patients than in controls. M3R autoantibodies associated with SSA and SSB.
Wolska et al <sup>55</sup>	2016	235	50	TRIM-38	Serum	Quantitative immunoprecipitation	Anti-TRIM38 significantly associated with classical autoantigens of pSS and clinical signs such as high ocular staining and low Schirmer's scores.
Alam et al <sup>39</sup>	2016	102	53	Aq-5	Serum	IIFA, Western blotting	Anti-aq5 significantly associated with sera in SS patients than in controls. It is also related to the resting saliva flow in SS patients.
Hu et al <sup>42</sup>	2015	32	35	Platelet-selectin	Serum	ELISA	p-Selectin autoantibodies found elevated in pSS (with and without thrombocytopenia) compared to healthy controls
Suresh et al <sup>56</sup>	2015	89	50	SPI, CA6, PSP	Serum	ELISA	Antigens potentially able to be used to identify pSS in patients with low focus scores on lip biopsies.
Reina et al <sup>26</sup>	2015	25	25	M3R	Serum	ELISA	Summarized in main text
Hauk et al <sup>37</sup>	2014	38	16	VIP receptor 2	Monocytes from plasma	Flow cytometry, ELISA, qRT-PCR	Levels of VIPR differ between pSS and controls. Response of VIPR expression to LPS also differs.
El Annan et al <sup>43</sup>	2013	11	8	Kallikrein	Serum	Antibody capture assay	Anti-KLK11 can be used as a biomarker.
Shen et al <sup>40</sup>	2012	33 <sup>vs</sup>	23	SPI, CA6, PSP	Serum	ELISA	Antigens potentially able to identify patients in the early onset of SS and contribute in identifying patients who are negative for classical antigens.
Reina et al <sup>57</sup>	2011	17	15	M3R	Serum	ELISA	M3R induces the production of pro-inflammatory markers PGE <sub>2</sub> and MMP-3, which can be used as biomarkers.

**Notes:** \*Controls with dry eye but without Sjögren's syndrome; <sup>‡</sup>Ro antigen; <sup>·</sup>La antigen; <sup>‡‡</sup>protein of Ro antigen epitopes; <sup>vs</sup>Sjögren's syndrome patients (inclusive of 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; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; PSP, parotid secretory protein; pSS, primary Sjögren's syndrome; qRT-PCR, qualitative reverse transcription- polymerase chain reaction; RF, rheumatoid factor; SPI, salivary protein-I; Ro, Sjögren's syndrome Type A; La, Sjögren's syndrome Type B; VIP, vasoactive intestinal peptide; VIPR, VIP receptor.

**Table 2** Studies related to antigens in Sjögren's syndrome (animal research)

Studies	Year	Species	Immune target	Tissues	Intervention
Szczerba et al <sup>58</sup>	2016	New Zealand mixed mice	Ro52 <sup>‡‡</sup>	Plasma, salivary gland tissue	Immunization
Ding and Zhang <sup>59</sup>	2016	Rabbits	La, ENA	Plasma	Immunization
Szymula et al <sup>60</sup>	2014	HLA-DR3 transgenic mice	Ro60	Plasma	Nil
Karnell et al <sup>18</sup>	2014	NOD mice <sup>⊖</sup>	Ro, <sup>‡</sup> La, <sup>‡</sup> Ds-DNA <sup>⊖</sup>	Salivary gland tissues	Nil
Al-Majdoub et al <sup>61</sup>	2013	Rabbits	Ro52	Plasma	Immunization
Kurien et al <sup>19</sup>	2011	BALB/c mice	Ro60 <sup>‡‡</sup>	Plasma	Immunization with HNE-Ro60
Yang et al <sup>27</sup>	2016	C57BL/6 Mice	M3R	Lacrimal gland	Immunization with M3R peptide
Hauk et al <sup>37</sup>	2014	NOD Mice	VIP receptor <sup>⊖</sup>	Plasma monocytes	Nil
Shen et al <sup>40</sup>	2012	NOD Mice, IL4 $\alpha$ TG Mice, C57BL/6 Mice	SPI CA6, PSP	Serum	Nil

**Notes:** <sup>‡</sup>Ro antigen; <sup>⊖</sup>La antigen; <sup>‡‡</sup>protein of Ro antigen epitopes; <sup>⊖</sup>Ds-DNA, double-stranded DNA antibody.

**Abbreviations:** CA6, carbonic anhydrase-6; ENA, extractable nuclear antigens; HLA-DR3, human leukocyte antigen – antigen D related-3; M3R, muscarinic receptor-3; NOD, non-obese diabetic mice; PSP, parotid secretory protein; SPI, salivary protein-I; Ro, Sjögren's syndrome Type A; La, Sjögren's syndrome Type B; VIP, vasoactive intestinal peptide.

their mucin contents, and in meibomian glands, muscarinic receptors have been found to regulate meibum secretion.<sup>22,23</sup>

Certain studies have implicated M3R as a potential autoantigen in pSS.<sup>20,24</sup> 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.<sup>25,26</sup> M3R peptides are able to activate interferon (IFN)- $\gamma$ -reactive T-cells, which then induce inflammation targeting the tissues expressing these peptides.

Yang et al<sup>27</sup> 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- $\gamma$ , which could have originated from the splenocytes that highly expressed these same cytokines. IFN- $\gamma$  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.<sup>27</sup>

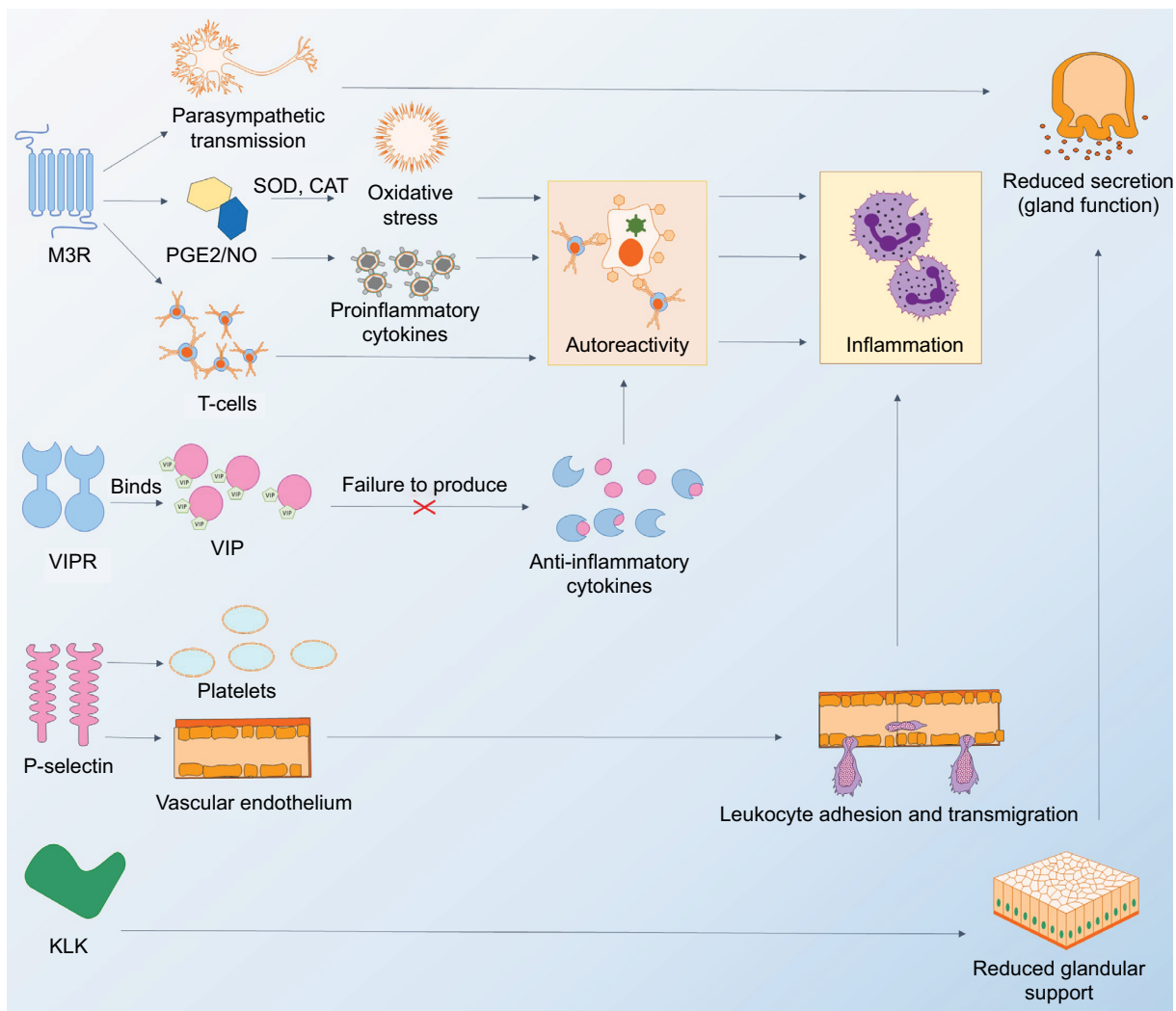
Activation of MNCs is highly dependent on growth factor signaling, such as that of the epidermal growth factor receptor (EGFR). The expression of antibodies blocking EGFR would reduce the number of activated MNCs. Immunization was performed with a peptide derived from EGFR-blocking

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.<sup>27</sup>

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.<sup>27</sup>

In another murine study, the antibody to M3R has been reported to increase levels of antioxidants, ie, superoxide dismutase (SOD) and catalase (CAT).<sup>26</sup> Since dry eye disease in humans has been linked to abnormal antioxidant status,<sup>28</sup> 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



**Figure 1** Diagram showing autoantigens and how disease processes in Sjögren's syndrome depend on autoreactivity against these autoantigens.

**Notes:** The M3R plays a role in parasympathetic functions by acting as a receptor to neurotransmitters in the nervous system. The dysfunction of this parasympathetic transmission can cause reduced function in gland secretion. The M3R autoantigen, when expressed abnormally, is able to increase levels of PGE2 and NO. It also increases levels of antioxidants such as SOD and CAT. Activated enzymes SOD and CAT are able to induce oxidative stress, causing the destruction and damage of salivary glands in rats, leading to inflammation. Abnormal release of T-cells and/or proinflammatory cytokines induces an autoimmune reaction when T-cells attack host cells (such as acinar cells in salivary or lacrimal glands), resulting in increased inflammation. The VIP binds specifically to its receptor, VIPR, which during normal function, is able to stimulate the production of anti-inflammatory cytokines. The failure to produce these cytokines can also result in an autoreactive response. P-selectin induces platelet activation and is crucial for cell adhesion on the vascular endothelium. Finally, immune-mediated responses against KLK could cause damage to acinar cells in exocrine glands, causing reduced glandular support and secretion. Not shown in figure: carbonic anhydrase-6 and aquaporin-5 may be directly involved in fluid production from the salivary and lacrimal glands, and autoimmune damage is likely to cause reduced secretion.

**Abbreviations:** CAT, catalase; KLK, kallikrein; M3R, muscarinic receptor subtype 3; NO, nitric oxide; P, platelet; PGE, prostaglandin E; SOD, superoxide dismutase; VIP, vasoactive intestinal peptide; VIPR, vasoactive intestinal peptide receptor.

like the natural ligands of the receptor.<sup>62</sup> 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.<sup>24,29</sup> Studies have also shown that these autoantibodies act like M3 antagonists and inhibit acetylcholine release.<sup>30</sup> 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.<sup>31</sup>

## 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.<sup>32</sup>

By binding to VIPR, VIP is also able to induce immunomodulatory effects<sup>32</sup> on monocytes, macrophages and T-cells. It has anti-inflammatory effects, as it inhibits proinflammatory cytokines, such as tumor necrosis factor (TNF)- $\alpha$  and IL-6,<sup>33-35</sup> and upregulates the anti-inflammatory cytokine IL-10.<sup>34-36</sup> However, a recent study<sup>37</sup> 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.<sup>37</sup>

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.<sup>38</sup> Although the function of VIPRs is unclear in meibomian glands,<sup>22,23</sup> exogenous VIP can stimulate increase in intracellular calcium.<sup>22</sup> 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.<sup>37</sup>

Lipopolysaccharide (LPS) stimulates cells via innate toll-like receptors to influence many immune genes.<sup>36</sup> 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.<sup>37</sup> 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.<sup>39,40</sup> 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.<sup>39</sup> Aq-5, in particular, has recently been emphasized due to its link to the clinical manifestations of Sjögren's syndrome.<sup>39</sup> 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.<sup>40</sup>

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.<sup>41</sup> Recently, p-selectin autoantibodies have been found to be elevated in pSS patients (with and without thrombocytopenia) compared to healthy controls.<sup>42</sup> 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 pSS<sup>43</sup> and experimental dry eye.<sup>44</sup> 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.<sup>43</sup> KLK11 is located in the Golgi apparatus of saliva-producing acinar cells in the salivary glands.<sup>45</sup> 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.<sup>46</sup>

Sex hormones may play a role in regulation of KLKs, but the changes may or may not be occurring through hormone receptors.<sup>46</sup> 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,<sup>37,43</sup> 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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