



Review

Oral dryness in Sjögren's syndrome patients. Not just a question of water

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ABSTRACT

Sjögren's syndrome (SS) is a chronic autoimmune disease of undefined etiology. Patients with this syndrome suffer from severe alterations in both the quality and quantity of saliva and tears, due to impaired function of the relevant exocrine glands. Prevalent symptoms experienced by SS-patients include a persistent dry mouth sensation (xerostomia) and dry eyes (keratoconjunctivitis sicca). Water content of saliva depends of acetylcholine levels, glandular innervation, M3R signaling, calcium tunneling and water release, among other factors. However, unstimulated salivary flow correlates only poorly with symptoms of mouth dryness, raising the question as to which other components of saliva may be involved in mouth dryness experienced by SS-patients? Salivary mucins are glycoproteins characterized by the presence of large oligosaccharide side chains attached to the protein backbone. These molecules are key saliva components that are required to sequester water and thereby moisturize, as well as lubricate the oral mucosa. In the labial salivary glands of SS patients, morphological and functional alterations are detectable that affect the maturation and trafficking of salivary mucins. In this review, we will focus the discussion on these aspects of reduced salivary flow and decreased quality of salivary mucins, since they are likely to be responsible for xerostomia in SS-patients.

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1. Introduction

Permanent dryness of the mouth (xerostomia) and eyes (keratoconjunctivitis sicca) is a common symptom affecting Sjögren's syndrome (SS) patients. These early warning symptoms are a consequence of functional impairment of salivary and lacrimal glands [1]. The standard aetiopathogenic model employed to describe SS disease suggests that in susceptible subjects [2], alterations in the immune system trigger a pathological response, which eventually destroys the exocrine glands [1,3].

More recent evidence, however, indicates that the severity of secretory dysfunction does not necessarily correlate with the degree of lymphocytic infiltration and glandular destruction. Dawson et al. proposed an alternative model of glandular dysfunction, referred to as the non-apoptotic model, in which mechanisms reducing or inhibiting water transport are thought to cause reduced salivary flow [4]. Nonetheless, unstimulated salivary flow (USF) also correlates poorly with symptoms of mouth dryness [5,6]. As a consequence, glandular dysfunction is not strictly linked to saliva volume and hence, does not explain the symptoms of xerostomia experienced by SS-patients due to the lack of saliva, which include sensations of burning, mucosal ulceration, difficulties in swallowing and a high incidence of both bacterial and fungal infections [7]. In this respect, salivary mucins are likely to be relevant, since they form a film that covers, lubricates and protects the oral epithelium against desiccation and environmental insults. Also, mucins interact with salivary proteins to provide protection against microorganisms [7–11].

Approximately 70% of salivary mucins are secreted by minor salivary glands [12]. Labial salivary gland (LSG) biopsy exhibits focal lymphocytic sialadenitis in SS-patients [13,14]. We have described that LSG of SS-patients show morphological and functional alterations that do not correlate with the number of proximal inflammatory cells [15,16]. These alterations modify secretory pathways and affect maturation, as well as trafficking of salivary mucins [17–20]. This review will focus on summarizing particularly data relevant to both reduced salivary flow and decreased quality of salivary mucins, which we believe are likely to be responsible for xerostomia symptoms in SS-patients.

2. The orthodox aetiopathogenic model of glandular hypofunction in SS-patients

Historically, glandular hypofunction in SS-patients has been correlated with the loss of acinar and ductal cells, as a consequence of cell atrophy and apoptosis. Soluble inflammatory molecules and the cytotoxic action of mononuclear cells infiltrating the glands were thought to represent the mediators of such damage [1,3,21,22]. The glandular destruction process is made persistent by the continued production of autoantigens and autoantibodies, secondary to apoptotic bleb formation or cell-death [23,24]. However, data concerning apoptosis in salivary gland acinar cells from SS-patients is controversial [25–28]. Mechanisms that favor either life or death of a cell are in a permanent state of equilibrium required to maintain cellular homeostasis. Unfortunately, however, the majority of studies available only evaluated apoptotic markers, hence providing partial information with respect to cellular homeostasis [26,27]. Perez et al. performed gene expression profiling of fractions enriched in epithelial cells from LSGs SS-patients and controls using cDNA microarrays. 528 genes were differentially expressed in SS-patients relative to controls. Among the up-regulated genes, 28 were pro-apoptotic and 15 were anti-apoptotic [29]. These findings suggest that the balance between death and survival signals is altered on a large scale in LSG from SS-patients. On the other hand, the severity of secretory dysfunction does not correlate with the degree of lymphocytic infiltration and glandular destruction in SS-patients [30]. Dawson et al. have proposed an alternative model of glandular dysfunction. This non-apoptotic model identified some mechanisms responsible for impairment of the secretory process [4].

3. Salivary secretory process

Saliva is composed of over 99% of water plus sodium, potassium, chloride and bicarbonate as the main electrolytes. The salivary protein component includes soluble IgA immunoglobulins, digestive enzymes, such as amylase and lipase, antibacterial and antifungal proteins (lysozyme, lactoferrin, cystatins, histatins, among other), as well as mucins which lubricate and protect the oral mucosa [31,32].

Saliva is produced by the major (parotid, submandibular and sublingual) and minor salivary glands [31]. These glands are composed of secretory units called acini, which synthesize and secrete saliva constituents, and ducts which modify saliva osmolarity [32]. In acinar cells, apical–basal polarity depends on cell–cell and cell–extracellular matrix interactions and determines directionality of the secretory process [19,33]. The synthesis of secretory products is initiated in the rough endoplasmic reticulum (RER), which is localized in the basolateral region of acinar cells. Secretory products are then modified and stored in post-translational compartments (Golgi complex and secretory granules) which localized towards the apical pole. Exocytosis of salivary products occurs by fusion of secretory granules with the apical plasma membrane [19]. Salivary glands are innervated by sympathetic and parasympathetic fibers that control the secretion process. Thus, they express different types of receptors, including muscarinic M3, α - and β -adrenergic and peptidenergetic receptors. Acetylcholine stimulation of cholinergic receptors (M3) generates a saliva rich in water and electrolytes, but low in proteins and glycoproteins. In turn, adrenergic stimulation results in exocytosis mainly of protein and glycoproteins (i.e. mucins) with a low water and electrolyte content [32].

Cholinergic stimulation triggers ion fluxes through cell membrane channels that allow water transport [34]. Water flow into the acinar lumen is stimulated by an increase in cytosolic Ca^{2+} levels, which opens Ca^{2+} -sensitive chloride channels in the apical plasma membrane and Ca^{2+} -sensitive K^{+} channels in the basolateral plasma membrane of acinar cells [35]. Opening of these channels permits efflux of Cl^{-} and K^{+} to generate a transepithelial potential difference, leading to passive movement of cations through tight junctions to maintain the electrochemical balance. Luminal accumulation of these ions generates a transepithelial osmotic gradient that induces water movement by both paracellular and transcellular routes. Transcellular water flow is mediated by water channels known as aquaporins (AQPs), which increase the water permeability of the lipid bilayer [36]. The increase in cytosolic Ca^{2+} levels occurs as the consequence of IP_3 -receptor-mediated Ca^{2+} release from the endoplasmic reticulum after formation of the second messenger IP_3 . The generation of a global calcium signal depends on the activation of ryanodine receptors (RyR). RyR propagate and amplify Ca^{2+} signals through a mechanism referred as calcium-induced calcium release (CICR) [34,37]. Ca^{2+} signaling via RyR channels is controlled by the cyclic adenosine diphosphate ribose (cADPr) concentration, which is regulated by cyclic guanosine monophosphate (cGMP) levels. Remarkably, the levels of these molecules are also altered by Ca^{2+} -independent mechanisms, such as the production of nitric oxide (NO) which may increase cGMP levels by activating guanylate cyclases (GC) [4].

Calcium influx from the extracellular compartment is also important for water movement. Recent work has shown that store-operated channels (SOC) play a crucial role in this process. Release of Ca^{2+} from the RER activates SOC channels that allow reloading of the reticulum. Additionally, they have been identified as the channels responsible for mediating most of the functions regulated by Ca^{2+} in the cell [34,38].

4. Mechanisms involved in inhibition of the secretory process

4.1. Glandular denervation

A preliminary immunohistochemical study of LSG from SS-patients revealed that loss of nerve fiber immunoreactivity was most apparent

in areas of strong inflammation. These results suggested that denervation may induce glandular atrophy [39] and it was then hypothesized that lymphocytic infiltration could diminish the secretory function of acinar cells by promoting the loss of glandular innervation [39]. Dartt et al. analyzed the lacrimal glands of MRL/+ control mice and MRL/lpr mice, a murine model of SS, both in the lymphocytic pre-infiltrating stage (before 8 weeks) and in the infiltrating stage (up to 12 weeks). In this study, antibodies that specifically identified parasympathetic, sympathetic and sensory nerves were employed. Using this approach, no differences in the intensity or distribution of staining were detected after either 8 or 18 weeks [40]. Subsequently, Pedersen et al. characterized patterns of innervation of LSGs from SS-patients and controls using immunohistochemistry. The presence and distribution of nerve fibers, as well as markers for parasympathetic and sympathetic nervous systems were determined. In gland areas with normal or scattered inflammation, the same distribution of immunoreactivity markers was observed for SS-patients and control subjects [41]. These results suggest that the loss of glandular function in SS-patients is not likely attributable to denervation of the lacrimal and salivary glands.

4.2. Inhibition of neurotransmitter release by cytokines

Having ruled out the possibility that lymphocyte infiltration affects gland innervation, further studies focused on evaluating neurotransmitter levels. Results obtained using MLR/lpr mice, a murine model of SS, demonstrated that acetylcholine release in response to depolarizing solutions (KCl) from nerves of salivary and lacrimal glands of these mice did not increase by 18 weeks, in contrast to what was seen for control MLR/+ mice. Furthermore, lacrimal glands of 18 weeks-MLR/lpr mice stimulated with KCl did not secrete peroxidase. Since the addition of exogenous secretagogue induced peroxidase secretion in lacrimal glands of MRL/lpr and MLR/+ [42], the above results were not attributable to a defect in the secretory process per se. The question became therefore, what mechanisms could explain the reduced release of neurotransmitters?

Considering the presence of both inflammatory cells and cytokines in SS-patients, it has been postulated that these molecules are responsible for the low neurotransmitter release. Cytokines are involved in the regulation of immunity and many studies show that there is a deregulation of these molecules in SS-patients [43]. Pro-inflammatory cytokines such as IFNs, IL-12, IL-18, TNF- α , IL-1 β , IL-6 and B-cell activating factor are elevated in salivary glands, saliva and serum of SS-patients whereas anti-inflammatory cytokines, such as TGF- β 1 are low, although this is not the case for IL-10 [44]. The inhibition of acetylcholine and norepinephrine release from myenteric nerves is mediated by pro-inflammatory cytokines such as IL-1 β , IL-6 and TNF- α [45–48]. In a rat model of inflammatory disease (acute colitis), IL-1 β was associated with reduced norepinephrine release induced by KCl [49]. TNF- α altered catecholamine release in cultures of sympathetic neurons [50]. Zoukhri et al., reported high levels of IL-1 β in lacrimal glands of a murine model of SS and the exogenous addition of cytokines inhibits secretion from these glands [51]. These observations suggest the existence of a connection between the immune system, the release of neurotransmitters and the process of secretion. All, together, these changes may result in glandular hypofunction.

4.3. Increased degradation of acetylcholine by cholinesterase

The previous data by Dawson et al., demonstrated increased levels of cholinesterase in salivary glands of SS mouse models, suggesting that a high rate of neurotransmitter degradation may modulate cholinergic signaling [52]. The same research group also detected increased levels of cholinesterases in saliva from SS-patients, which might taken to suggest that levels of this enzyme are increased in their salivary glands. Accordingly, the use of cholinesterase inhibitors, such as hydroxychloroquine [53] and muscarinic agonists, such as pilocarpine [54], does improve

the salivary flow of some SS-patients. However, this therapy has become controversial, since in some patients increased levels of disease markers, such as IgG, erythrocyte sedimentation rate, IL-6, ANA and RF, were detected after administration of these compounds [55–59].

4.4. Blockade of muscarinic acetylcholine receptor M3 (M3R)

The presence of auto-antibodies against the M3R has been reported in SS-patients [60] and inhibition of M3R function by auto-antibodies may lead to glandular hypofunction in these patients by reducing the water content of saliva [61]. These auto-antibodies may also contribute to the inflammatory reaction observed in SS-patients by activating phospholipase 2 (PLA2), which increases the levels of pro-inflammatory mediators, such as MMP-3 and PGE2 [62].

4.5. Altered calcium tunneling

Another important factor for an adequate secretory process is polarized calcium signaling. In secretory cells, calcium signals are initiated at the apical pole (local signal) and then extend toward the basal pole (global signal) [37]. This polarized signal is determined by proteins, such as G-protein coupled receptors (GPCR), ryanodine receptors (RyR), plasma membrane Ca²⁺ ATPase (PMCA), endoplasmic reticulum Ca²⁺ pumps (SERCA 2b) and transient receptor potential channels (TRPC) [63]. Alterations in any of these components could disturb polarized calcium signaling. Thus, it has been proposed that chronic exposure of human and mouse submandibular cells to nitric oxide (NO) leads to reduced sensitivity to acetylcholine stimulation, through a cGMP-independent mechanism. Instead, NO could alter the calcium-induced calcium release (CICR) mechanism by S-nitrosylation of RyR, as well as other components of the stimulus-secretion cascade, such as muscarinic receptors and ion channels involved in Ca²⁺ influx [64]. These results are consistent with the high NO levels and decreased sensitivity to acetylcholine stimulation observed in SS-patients [65,66].

Recently, Ambudkar et al., have described that mice with T-cell-targeted deletion of Stromal Interaction Molecule (STIM) 1 and STIM2, develop spontaneous and severe pSS-like autoimmune disease, displaying major hallmarks of the disease [67].

4.6. Transcellular movement of water

A point of controversy in understanding disorders that affect water movement in SS-patients relates to the redistribution of water channels, specifically AQP5. AQP5 is predominantly expressed in parotid glands and normally localizes to the apical plasma membrane of acinar cells. In a recent study using isolated rat parotid acinar cells revealed that stimulation with M3R or α 1-adrenoceptors agonists increased AQP5 levels in apical plasma membrane [68]. The role of AQP5 in the SS pathology is unclear, since some studies did not observe changes in AQP5 localization when comparing SS-patients and controls [69], whereas others detected increased AQP5 presence in the basolateral membrane of salivary glands from SS patients, which would be expected to alter transcellular water transport into the acinar lumen [70]. Recent studies in submandibular glands from three SS animal models (IQI/JIC, r1 Δ T/r2n and non-obese diabetic mice), support the evidence favoring a redistribution of AQP5, since in all cases AQP5 relocation correlated with the degree of inflammation (Fig. 1) [71].

None-the-less, since USF correlates poorly with symptoms of mouth dryness [5,6], we propose that alterations not strictly linked to saliva volume need to be evaluated to explain xerostomia.

5. Salivary mucins in oral health

Salivary mucins are synthesized by submandibular, sublingual and numerous minor salivary glands distributed throughout the oral cavity (lip, palate, cheeks and tongue). Despite their minor contribution to

total saliva volume (10%), the minor salivary glands produce up 70% of the total mucins found in saliva [12]. Indeed, Eliasson et al. demonstrated that minor salivary glands affect the dry mouth sensation in a manner that is independent of whether saliva flow is normal or reduced [6]. The main properties of salivary mucins are their low solubility, high viscosity, high elasticity and strong adhesiveness. These characteristics of mucins are essential for lubricating the mouth in ways that facilitate chewing, swallowing and speech [31].

Mucins are complex O-linked glycoproteins composed of a protein backbone which contains at least one region of amino acid sequences rich in serine and threonine. These hydroxylated amino acids are sites for covalent attachment of a variety of oligosaccharides that can be sialylated and/or sulfated [72–74]. Due to their high content of anionic oligosaccharides, mucins can be considered hydrophilic polymers that bind water on the epithelial surface and thereby preserve mucosa humidity [7,17,20,74]. Three types of mucins are predominantly expressed in salivary glands: transmembrane mucins, such as MUC1, and two soluble secreted mucins, such as MUC7 and MUC5B. MUC7 is a monomeric mucin with a low molecular mass (~200 kDa) and MUC5B is a high molecular mass (> 1 MDa) polymeric mucin [9,74,75].

6. Altered secretion of salivary mucins in Sjögren's syndrome patients

6.1. Altered trafficking of mucins in Sjögren's syndrome patients

In our studies, we have recently reported on alterations in the secretory route and trafficking of mucins in LSGs of SS-patients. Significant increases in MUC1 protein levels and cytoplasmic accumulation in acinar

cells were detected in LSGs of SS-patients (Sung et al., manuscript in preparation). Likewise, MUC7 also accumulated in the cytoplasm of acinar cells from SS-patients where RAB3D expression or localization was altered [18]. Furthermore, MUC7 and MUC5B were detected in the extracellular matrix (ECM) of LSGs and we proposed that such changes in mucin destination may contribute to the inflammation in SS-patients [19].

Recently, also an association between M3R auto-antibodies and altered secretion of salivary mucins was reported [76]. In this study, slices of rat submandibular glands were incubated in Krebs ringer bicarbonate medium containing a serum IgG fraction from SS-patients or control individuals. The IgG fraction of SS-patients inhibited in a dose-dependent manner the production and release of mucins to the medium, as measured by Alcian Blue. This effect was neutralized by the M3R antagonist 4-DAMP, suggesting that functional IgG auto-antibodies produced by SS-patients interact with glandular M3R. However, the quantity of salivary mucins was not decreased in the saliva of SS-patients [77,78]. Consistent with this finding, we have not detected differences between the mRNA and protein levels of MUC5B in LSGs homogenates of SS-patients and control subjects [17]. For this reason, we propose that not only the quantity but also the quality of salivary mucins contributes to xerostomia in SS-patients.

6.2. Altered quality of salivary mucins in Sjögren's syndrome patients

MUC5B is the main mucin in saliva. The PANH2 antibody recognizes a partially deglycosylated region of MUC5B. In mucous acinar cells of control individuals, we detected MUC5B using PANH2 only in the basal

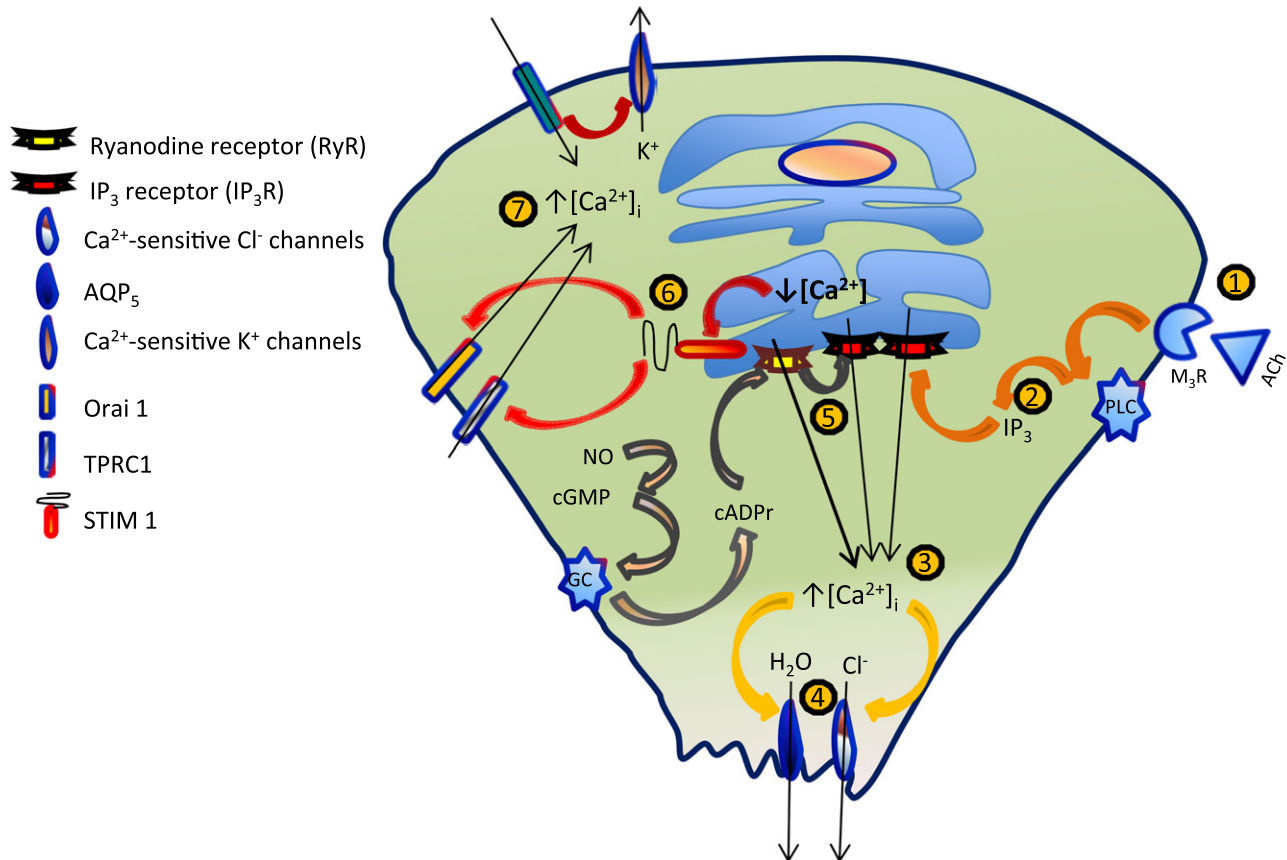


Fig. 1. Calcium signaling regulates the salivary flow. 1. Acetylcholine (ACh) binds to muscarinic acetylcholine receptors type-3 (M₃R) localized in the basolateral membrane of the acinar cells; 2. ACh stimulates production of inositol 1,4,5-trisphosphate (IP₃) via phospholipase C (PLC) activation; 3. IP₃ binds IP₃R initiating Ca²⁺ release from the endoplasmic reticulum and increasing the [Ca²⁺]_i in the acinar cell apical pole. 4. Increased [Ca²⁺]_i opens Cl⁻ channels sensitive to Ca²⁺ and allows transcellular movement of water through aquaporin 5 (AQP₅); 5. IP₃ and cyclic ADP ribose, the natural agonists of RyR, allow Ca²⁺ signal propagation by means of Ca²⁺-induced-Ca²⁺ release (CICR); 6. Activation of store-operated calcium entry (SOCE) by Ca²⁺ depletion in the endoplasmic reticulum (ER); 7. Ca²⁺ influx mediated by store-operated calcium (SOC) channels (STIM 1, Orai 1 and TRPC1) modulates Ca²⁺ signals and regulates salivary flow.

region, where the mucin is synthesized (RER). In LSGs from SS-patients, we observed PANH2-positive staining in both the apical and basal regions of mucous acinar cells, suggesting a reduced glycosylation of this mucin in post-translational compartments, including the RER, the Golgi complex and secretory granules [17]. Alcian Blue (AB) staining is employed to identify sulfated oligosaccharides. Using this technique, we observed a significant decrease in AB staining intensity in sections of LSGs from SS-patients compared with controls ($p=0.04$). Decreased staining for AB in LSGs from SS-patients correlated with diminished immunoreactivity for laminin in the disorganized basal lamina (Fig. 2). In SS-patients, altered signaling through basal lamina receptors could lead to acinar cell de-differentiation and alterations in post-translational processing of glycoproteins (i.e. mucins) [17]. Moreover, a decrease in the content of the sulfo-Lewis^a (SO₃-Galβ1-3GlcNAc) (Fig. 3) residues and a reduced number of mucous acini positive for this antigen were observed in LSG of SS-patients. Additionally, in this study, all SS-patients complained of mouth dryness, which correlated low levels of sulfo-Lewis^a, independent of whether USF was normal (≥ 1.5 mL of saliva/15 min) or diminished. These results link hyposulfated MUC5B to reduced mucin hydration and xerostomia in SS-patients [17].

To address the possible causes of MUC5B hyposulfation, enzymatic activities of glycosyltransferases involved in the biosynthesis of oligosaccharides bound to mucins of LSG were compared between SS-patients and control subjects. Our results showed that polypeptide-GalNAc transferase, core 1 galactosyltransferase, core 2 GlcNAc transferase, β3/β4 galactosyltransferases and core-1 α3-sialyltransferase were active in LSG. Moreover, the enzymatic activities that synthesize core 3 and core 4 were very low, but clearly detectable by HPLC analysis. These observations indicate that LSGs can synthesize the four mucin O-glycan-type core structures (1–4), which is in agreement with previous reports that identified O-glycans containing uncommon core 3 and 4 structures in human salivary mucins [82]. Here, it should be noted that no significant changes in glycosyltransferase activities were detected when comparing SS-patients and control subjects (Fig. 3) [20].

Sulfotransferase activity was assayed with core 1 (6-deoxy) α-benzyl as an acceptor substrate and radioactive phospho-adenosine-phospho-sulfate (PAPS) as a donor. Our results showed that the activity of Gal3-O-sulfotransferase (Gal3ST) was significantly lower in LSGs from SS-patients ($p=0.0004$). No changes in sulfotransferase mRNA and protein levels were observed. Alternatively, an inverse correlation between Gal3ST activity and dryness symptoms was detected in SS-patients. Additionally, low Gal3ST activity correlated inversely with gland function as measured by scintigraphy, but did not correlate with USF. These findings confirm our previous observations indicating that MUC5B hyposulfation in SS-patients does not correlate with USF [17]. Moreover, Gal3ST activity also correlated inversely with lymphocyte infiltration and glandular levels of pro-inflammatory cytokines, suggesting that inflammation might inhibit this activity. Decreased sulfotransferase activity in the Golgi complex of acinar cells due to inflammation may then lead to MUC5B hyposulfation and thereby contributes to xerostomia in SS-patients [20]. Given that inhibition of Golgi complex glycosylation interferes with glycoprotein trafficking to the cell surface [79], it would be interesting to evaluate whether additional changes in the molecular machinery lead to post-translational modifications of salivary mucins that may cause their accumulation and altered maturation in LSGs of SS-patients.

7. Conclusions

Xerostomia is a consequence of both reduced salivary flow and decreased quality of salivary mucins. These changes lead to a variety of oral and dental disorders that affect the quality of life of SS-patients [80]. The main therapeutic approach to reduce mouth dryness in SS patients involves the use of secretagogues. These cholinergic agonists bind to muscarinic receptors and increase the salivary flow, mainly by enhancing water release [81]. These treatments neither consider the quantity nor quality of the secretory products present in saliva, such as mucins, which are complex O-linked glycoproteins with sialylated and/or sulfated oligosaccharides attached to their protein backbone [72–74].

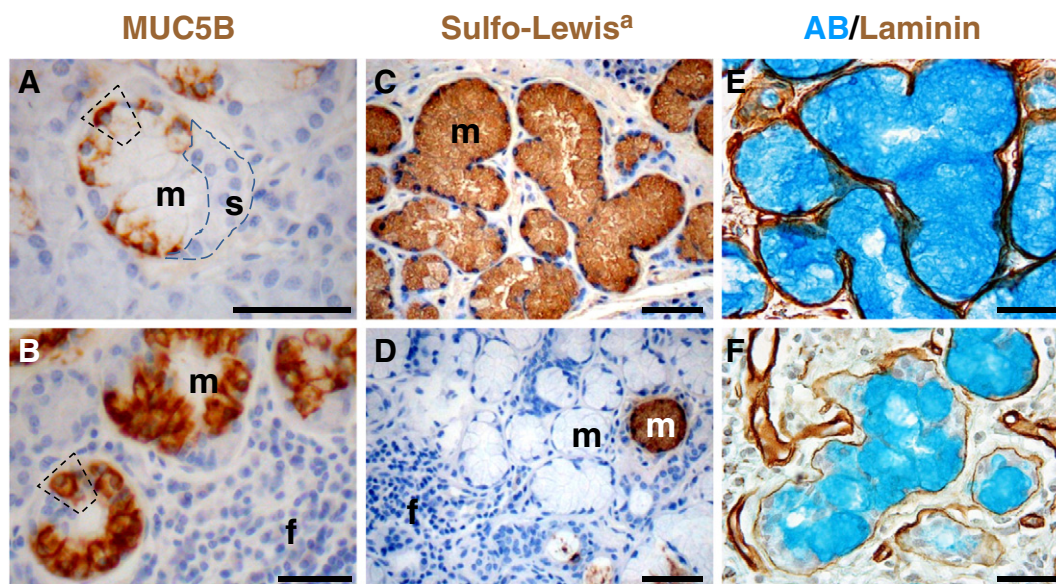


Fig. 2. Sections of labial salivary glands from control individuals (A, C, E) and SS-patients (B,D,F). A–B. Immunohistochemistry of MUC5B. Gland section showing MUC5B in the basal region of mucous acinar cells (A). In SS-patients, MUC5B was observed both in basal and apical cytoplasm of mucous acinar cells (B). C–D. Sulfo-Lewis^a antigen immuno-detection. Mucous acini showed sulfo-Lewis^a immunoreactivity in the whole cytoplasm (C). Low abundance of mucous acini showing immunoreaction for sulfo-Lewis^a in SS-patients was observed (D). E–F. Double staining with Alcian Blue (AB) and immunohistochemistry for laminin. Positive AB staining was stronger in control individuals (E) than in SS-patients (F). Decreased AB staining correlated with decreased laminin immunoreactivity in SS-patients (F). Bars: 50 μm, m: mucous acini; s: serous acini; f: inflammatory focus. Images reproduced with permission of Allende et al. [17].

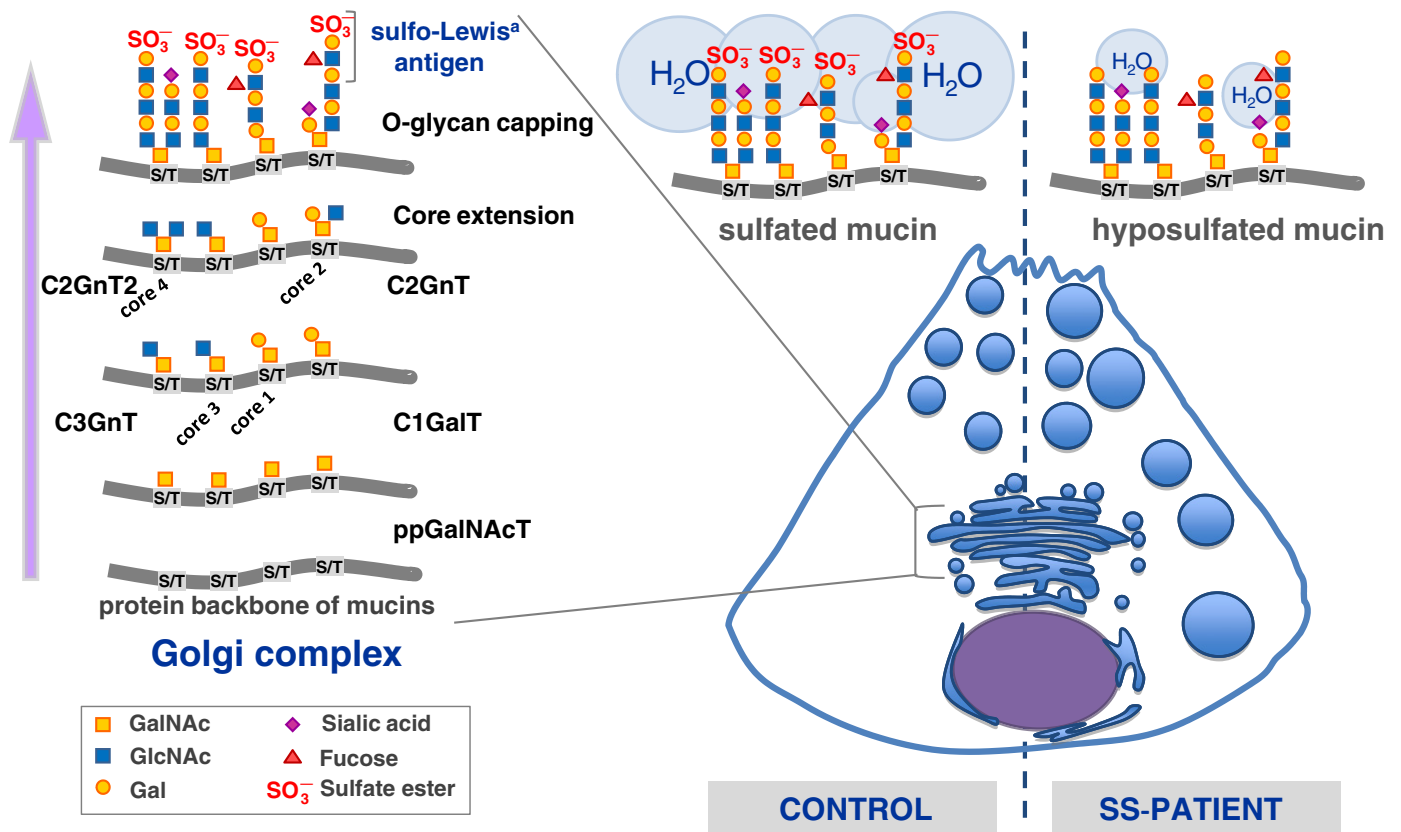


Fig. 3. O-glycosylation of mucins in salivary acinar cells. In the Golgi complex, ppGalNAcT initiates O-glycosylation by addition of GalNAc to the hydroxyl group of either serine (S) or threonine (T) residues of the mucin protein backbone. C1GalT adds a Gal residue to synthesize the core 1 structure which can be branched by C2GnT forming core 2. The core 3 structure is synthesized by C3GnT that adds GlcNAc to GalNAc. Core 3 can be branched by C2GnT2 to form core 4. All core structures can be further extended, branched and modified to form complex O-glycans. Terminal GlcNAc residues can be used as the basis for the attachment of Lewis determinants. Sulfo-Lewis^x antigen is shown. Sulfated mucins retain large amounts of water on the epithelial surface. In SS-patients, the hyposulfated mucins lose hygroscopic properties contributing to mouth dryness sensation.

This characteristic allows mucins to bind large amounts of water and lubricate the oral epithelium. In salivary glands of SS-patients, altered trafficking and maturation of salivary mucins were observed [17–20]. These alterations are likely to contribute to the dryness sensation in SS-patients. A better characterization of the molecular mechanisms that cause these alterations will favor the development of more effective therapies to treat xerostomia in SS-patients.

Take-home messages

- SS-patients commonly complain of xerostomia, which seriously affects oral health and their quality of life.
- In salivary glands of SS-patients, altered cholinergic signaling, calcium tunneling, among other factors, have been detected that may result in reduced water release. However, mouth dryness correlates only poorly with the USF.
- The lubricating properties of saliva depend on both salivary flow and the quality of secretory products like mucins. Thus, altered maturation of salivary mucins, observed in salivary glands of SS-patients, may contribute to the mouth dryness symptoms in these patients.

Conflict of interest

The authors have declared no conflicts of interest.

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Immunogenicity and safety of the 2009 non-adjuvanted influenza A/H1N1 vaccine in a large cohort of autoimmune rheumatic diseases

Despite the WHO recommendation that the 2010–2011 trivalent seasonal flu vaccine must contain A/California/7/2009/H1N1-like virus there is no consistent data regarding its immunogenicity and safety in a large autoimmune rheumatic disease (ARD) population. 1668 ARD patients (systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), ankylosing spondylitis (AS), systemic sclerosis, psoriatic arthritis (PsA), Behçet's disease (BD), mixed connective tissue disease, primary antiphospholipid syndrome (PAPS), dermatomyositis (DM), primary Sjögren's syndrome, Takayasu's arteritis, polymyositis and Granulomatosis with polyangiitis (Wegener's) (GPA)) and 234 healthy controls were vaccinated with a non-adjuvanted influenza A/California/7/2009(H1N1) virus-like strain flu. Subjects were evaluated before vaccination and 21 days post-vaccination **Saad CG, et al. (*Ann Rheum Dis* 2011;70:1068-73)**. The percentage of seroprotection, seroconversion and the factor increase in geometric mean titre (GMT) were calculated. After immunisation, seroprotection rates (68.5% vs 82.9% $p<0.0001$), seroconversion rates (63.4% vs 76.9%, $p<0.001$) and the factor increase in GMT (8.9 vs 13.2 $p<0.0001$) were significantly lower in ARD than controls. Analysis of specific diseases revealed that seroprotection significantly reduced in SLE ($p<0.0001$), RA ($p<0.0001$), PsA ($p=0.0006$), AS ($p=0.04$), BD ($p=0.04$) and DM ($p=0.04$) patients than controls. The seroconversion rates in SLE ($p<0.0001$), RA ($p<0.0001$) and PsA ($p=0.0006$) patients and the increase in GMTs in SLE ($p<0.0001$), RA ($p<0.0001$) and PsA ($p<0.0001$) patients were also reduced compared with controls. Moderate and severe side effects were not reported. The novel recognition of a diverse vaccine immunogenicity profile in distinct ARDs supports the notion that a booster dose may be recommended for diseases with suboptimal immune responses. This large study also settles the issue of vaccine safety.