Decreased salivary sulphotransferase activity correlated with inflammation and autoimmunity parameters in Sjögren’s syndrome patients

Isabel Castro¹, Sergio Aguilera², Inka Brockhausen³, Cecilia Alliende¹, Andrew F. G. Quest¹,⁴, Claudio Molina⁵, Ulises Urzúa¹, Ulla Mandel⁶, Verónica Bahamondes¹, María-José Barrera¹, Marianela Sánchez¹, Sergio González⁵,⁷, Marcela Hermoso¹, Cecilia Leyton¹ and María-Julieta González¹

Abstract

Objectives. To determine the expression and enzymatic activities of sulphotransferases involved in mucin hyposulphation in labial salivary glands (LSGs) from SS patients and to correlate sulphotransferase activity with clinical parameters such as secretion, inflammation and serology.

Methods. LSG from 31 SS patients and 31 control subjects were studied. Relative mRNA and protein levels of Gal3-O-sulphotransferases (Gal3STs) and β1,3-galactosyltransferase-5 (β3GalT5) were determined by quantitative RT-PCR and western blotting, respectively. Enzymatic activities were quantified using radioactively labelled donor substrates and specific acceptor substrates. Products were purified by chromatography. Spearman’s correlation analysis was used to compare data.

Results. The levels of Gal3ST activity were significantly decreased in SS patients, without changes in mRNA and protein levels, while the enzymatic activities of glycosyltransferases involved in mucin glycosylation were similar in both groups. An inverse correlation was observed between Gal3ST activity and glandular function measured by scintigraphy, but not with unstimulated salivary flow. Gal3ST activity was inversely correlated with focus score, TNF-α levels and presence of the autoantibodies Ro/SS-A and La/SS-B.

Conclusion. The decrease in sulphotransferase activity provides an explanation for mucin hyposulphation observed in the LSGs from SS patients. The decrease in Gal3STs activity was not a consequence of reduced gene expression, but probably due to alterations in the enzyme activity regulation. Interestingly, the levels of sulphotransferase activity detected correlated well with secretory function, inflammation and serology. Finally, we postulate that pro-inflammatory cytokines induced by autoantibodies, such as Ro/SS-A and La/SS-B in SS patients, may modulate Gal3ST activity, thereby altering mucin quality and leading to mouth dryness.

Key words: Sjögren’s syndrome, salivary glands, glycosylation pathway, Gal3ST, mucin hyposulphation, dry mouth.
**Introduction**

SS is a systemic chronic autoimmune disease affecting mainly the exocrine glands. A high number of patients complain of mouth (xerostomia) and eye (keratoconjunctivitis sicca) dryness [1, 2]. Salivary hypofunction and xerostomia caused by Sjögren’s disease seriously affect the quality of life of SS patients [3]. A common histopathological finding in all affected organs is persistent infiltration of mononuclear cells [1, 2]. Also, levels of specific auto-antibodies (i.e. anti-Ro/SS-A and La/SS-B, anti-muscarinic receptors, among others) are suggested to correlate with the degree of lymphoid infiltration and loss of secretory capacity, both predictors of disease state and disease severity [4]. Scintigraphy data correlate with clinical and histopathological features of the salivary glands in patients with SS [5]. However, there is a great deal of variation in salivary flow, ranging from essentially normal rates to essentially none at all. The correlation between the focus score and salivary flow is poor, suggesting that these are two independent processes [6]. Moreover, data available in the literature indicate that a low correlation exists between salivary flow and the sensation of mouth dryness [7]. Thus, alterations not strictly linked to saliva volume need to be invoked to explain xerostomia.

The main therapeutic approach to reduce mouth dryness in SS patients is using secretagogues, such as cholinergic agonists, which bind to muscarinic receptors increasing the salivary flow, mainly by increasing water transport [8]. These treatments neither consider the quantity nor quality of the secretion products present in saliva, such as mucins, which are essential for lubrication of the oral epithelium.

MUC5B, the predominant mucin in saliva, is synthesized by mucous acini and variably glycosylated [9]. The synthesis of mucin oligosaccharides starts with the transfer of N-acetylgalactosamine (GalNAC) to serine or threonine residues of the mucin core. The oligosaccharides may be extended with galactose (Gal), N-acetylgalactosamine (GlcNAC), GalNAc, fucose or sialic acid (Neu5Ac) [10]. Each sequential step is catalysed by a different glycosyltransferase [10]. Modifications of mucin oligosaccharides include sulphation of Gal and GlcNAc. These reactions are catalysed by a different glycosyltransferase [10]. Modifications of mucin oligosaccharides include sulphation of Gal and GlcNAc. These reactions are catalysed by a different glycosyltransferase [10]. Modifications of mucin oligosaccharides include sulphation of Gal and GlcNAc. These reactions are catalysed by a different glycosyltransferase [10]. Modifications of mucin oligosaccharides include sulphation of Gal and GlcNAc. These reactions are catalysed by a different glycosyltransferase [10]. Modifications of mucin oligosaccharides include sulphation of Gal and GlcNAc. These reactions are catalysed by a different glycosyltransferase [10].

In labial salivary glands (LSGs) of SS patients mucins are hyposulphated [11]. The levels of sulfo-Lewis$a$ (SO$_2$Gal[1-3][Fuc$_1$-1-4][GlcNAC], an oligosaccharide exclusively attached to MUC5B in LSG, are notably decreased, thus resulting in a concomitant decrease of the number of sulfo-Lewis$a$-positive mucous acini [11]. Also, all of the SS patients studied experience oral dryness and reduced MUC5B sulphation, independently of whether unstimulated salivary flow (USF) was normal or reduced [11]. With this evidence in mind, we postulated that altered mucin quality rather than reduced water content of saliva leads to mouth dryness in SS patients. Currently, nothing is known about whether alterations in mucin glycosylation and sulphation pathways occur in LSG of SS patients.

Interestingly, for bovine synoviocytes exposed to TNF-α reduced sulphotransferase as well as changed glycosyltransferase activities and altered lectin-binding patterns are observed. These findings demonstrate that elevated levels of pro-inflammatory cytokines, as observed in RA and SS, can modulate glycosylation [12]. Reduced sulphation of mucins has also been described in inflammatory and neoplastic intestinal diseases [13]. Mucins in ulcerative colitis have shorter oligosaccharide chains and lower sulphate content than normal colonic mucosa [13]. Sulphomucins in colonic adenocarcinoma are notably lower than those in the adjacent normal mucosa [14, 15]. The synthesis of these Sulphomucins involves β[1,3-galactosyltransferase-5 ([β3GalT-5] and Gal3ST-2 [16-18]. Lower activity and reduced expression of these enzymes in non-mucinous adenocarcinoma compared with adjacent normal mucosa is thought to contribute to hyposulphation of mucins [17].

Given these observations, we sought to determine whether alterations in glycosyltransferase activities contribute to changes in mucins that explain the loss of their moisturizing and protective properties in SS patients. In this study, we evaluated the expression levels and enzymatic activities of glycosyltransferases and sulphotransferases that could be involved in hyposulphation of MUC5B in LSG from SS patients. The sulphotransferase activities were correlated with clinical parameters such as secretory function, inflammation and serology.

**Patients and methods**

**Primary SS patients and controls**

The selected group of patients included 62 non-smokers with good oral hygiene. Thirty-one individuals were diagnosed as having primary SS according to the American-European Consensus Group criteria [19], and 31 subjects were controls selected from individuals who did not fulfill the primary SS classification criteria. Also, they did not suffer systemic diseases and lip biopsy analysis revealed mild diffuse chronic sialadenitis. Evaluation of the salivary glands by scintigraphy was performed according to Schall et al. [20]. Scintigraphy data from all patients were classified in three groups: (i) normal, (ii) mild-to-moderate and (iii) severe-to-very severe. Table 1 shows demographic, serological and histological characteristics of the patients and controls. The subjects’s written consent was obtained according to the Declaration of Helsinki. The study was approved by the Ethical Committee of the Faculty of Medicine, University of Chile.

**Biopsies**

LSGs were obtained according to the method described by Daniels [21]. Following surgery, samples were frozen in liquid nitrogen and stored at −80°C until processed.
TABLE 1 Demographic, serological and histological characteristics of the patient and control groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls</th>
<th>SS patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender: female/male, n</td>
<td>27/4</td>
<td>29/2</td>
</tr>
<tr>
<td>Age, mean (s.d.) (range), years</td>
<td>42 (11)</td>
<td>46 (12)</td>
</tr>
<tr>
<td>Focus score, foci number/4 mm² of tissue</td>
<td>0</td>
<td>1 (n = 19), 2 (n = 5), ≥ 3 (n = 7)</td>
</tr>
<tr>
<td>USF, mean (s.d.) (range) ml/15 min</td>
<td>3.1 (1.7)</td>
<td>1.1 (1.4)</td>
</tr>
<tr>
<td>Scintigraphic data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ro antibodies</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>Ro/La antibodies</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>ANAs</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td>RF</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Glandular TNF-α levels, mean (s.d.) (range), AU/pixel</td>
<td>0.27 (0.11) (0.07–0.43)</td>
<td>0.53 (0.12) (0.36–0.74)**</td>
</tr>
<tr>
<td>Glandular IFN-γ levels, mean (s.d.) (range), AU/pixel</td>
<td>0.22 (0.11) (0.11–0.33)</td>
<td>0.39 (0.2) (0.13–0.69)</td>
</tr>
</tbody>
</table>

Scintigraphic data were classified according to Schall et al. [20] (see ‘Patients and Methods’ section). *P < 0.0001, **P < 0.0007. AU: arbitrary units.

Immunohistochemistry

Glandular TNF-α and IFN-γ levels were determined as previously described [22]. Images were captured under a Zeiss light microscope and quantified using the Image J 1.44 software (National Institutes of Health, MD, USA).

Quantitative RT-PCR

Total RNA from frozen LSGs was extracted with the RNeasy kit (Qiagen) as previously described [23]. Three micrograms of total RNA were reverse transcribed to a cDNA with Superscript II (Invitrogen) using oligo(dT) and random primers. The real-time PCR assays were performed with the Brilliant II SybrGreen QPCR Master Mix kit (Stratagene). The forward and reverse primer sequences were, respectively, 5’-CAGAGATACCTCCG GTCAT-3’ and 5’-GAAGCGGTAGAGGATGTGA-3’ for Gal3ST-2; 5’-ACCGTAAGCCTTCTCATCCA-3’ and 5’-CG GCAAAGCCAAAAGGATT-3’ for Gal3ST-3; 5’-CATGACC ATGGCGTGTCC-3’ and 5’-CTGGTCGCCGATTATGT GT-3’ for Gal3ST-4; 5’-ATCGAGCCACGATTCAACGCAA-3’ and 5’-ACGCAGCAAGAAACAGGA-3’ for β3GalT-5; and 5’-GATATGCTCATGTGGTGTTG-3’ and 5’-ATCAGGCAGCCATTCAGCAA-3’ as the mobile phase [12]. All enzyme assays were carried out in duplicate with <10% variation between assays.

Enzyme preparations

LSGs were homogenized in a 5-fold excess of 0.25M sucrose and stored at −80°C. The protein concentration was determined by the Bradford method [26].

Glycosyltransferase and sulphotransferase assays

Glycosyltransferase activities in LSG homogenates containing 70–85 μg protein were measured by Dowex (AG1×8) assays followed by reverse-phase HPLC separation using a C18 column or on Sep-Pak columns as previously described [27, 28]. Sulphotransferase activities were measured using Sep-Pak C18 and HPLC. HPLC separations were carried out in acetonitrile/water mixtures as the mobile phase [12]. All enzyme assays were carried out in duplicate with <10% variation between assays.

Sulphotransferase assays

Gal3ST activity was measured in a total volume of 40 μl with 2 mM Gal[1-3(6-deoxy)GalNAcα-benzyl] as acceptor substrate, 2 mM ATP, 50 mM MES pH 7.0, 10 mM NaF, 10 mM 2,3-dimercapto-1-propanol, 2.5 mM MnCl2, 0.1% Triton X-100, 6.5 μM 3'-phosphoadenosine 5'-phosphosulphate (PAP[35S]) (993 cpm/pmol) and 10 μl of enzyme homogenate. Assay mixtures were incubated for 1 h at 37°C and passed through Sep-Pak (C18) cartridges. After five washes with water (A–E fractions), products were eluted with methanol (F–J fractions). The collected fractions (F–J) were pooled, evaporated with a flash evaporator and resuspended in 120 μl of water. Eighty microlitres were injected on a C18 HPLC column and eluted in acetonitrile/water (10/90). Fractions were collected and counted in scintillation fluid. The standard compound Gal[1-3]-GalNAcα-benzyl was eluted at 24 min, the enzyme product eluted at 12–18 min, while PAPS and sulphate were eluted in earlier fractions (2–10 min). The results of endogenous assays, performed without acceptor substrate, were subtracted from the results of assays containing the acceptor substrate.
Sialyltransferase assays

Core 1 (Galβ1-3GalNAc) α3-sialyltransferase (ST3GalT) activity was measured in a total volume of 40μl with 1 mM Galβ1-3(6-deoxy)GalNAcβ3-benzyl as specific acceptor substrate, 10 mM AMP, 125 mM MES pH 7.0, 0.125% Triton X-100, 7.9 mM CMP-[3H]sialic acid (1500 cpm/nmol) and 10 µl of enzyme homogenate. Assay mixtures were incubated for 1 h at 37°C and passed through Sep-Pak (C18) cartridges followed by elution of product with methanol. Enzyme product was also confirmed by HPLC analysis.

Polypeptide GalNAc-transferase assays

Polypeptide GalNAc-transferase (ppGalNAcT) activity was assayed in a total volume of 40 μl containing 0.2 mM AQPTPP peptide as acceptor substrate, 10 mM AMP, 125 mM MES pH 7.0, 0.125% Triton X-100, 10 mM MnCl2, 0.91 mM UDP-[3H]GalNAc (3,800 cpm/nmol) and 10 μl of enzyme homogenate. The product was isolated by chromatography using AG1x8.

Galactosyltransferase assays

[β3- and β4-Gal-transferase (GalT) activities were measured in a total volume of 40μl with either 1 mM GalNAcβ3-benzyl [for core 1 Gal-transferase (C1GalT)] or 1 mM GlcNAcβ3-benzyl [β3 and β4 Gal-transferases (β3/β4 GalT)] as acceptor substrates, 10 mM AMP, 5 mM γ-galactonolactone, 125 mM MES pH 7.0, 0.125% Triton X-100, 10 mM MnCl2, 1 mM UDP-[3H]Gal (6100 cpm/nmol) and 10 μl of enzyme homogenate. Mixtures were incubated for 1 h at 37°C and passed through AG1x8 columns. Eluates were lyophilized and subjected to HPLC analysis. β3-GalT activity was measured similarly, but in the presence of 1 mM β4-GalT inhibitor N-butyryl-glucosamine-S-naphthyl.

GlcNAc-transferase assays

Core 2 β6-GlcNAc-transferase (C2GnT) activity was measured in a total volume of 40μl with 2 mM Galβ1-3GlcNAcβ3-benzyl as acceptor substrate, 125 mM GlcNAc, 10 mM AMP, 125 mM MES pH 7.0, 0.125% Triton X-100, 0.91 mM UDP-[3H]GlcNAc (1600 cpm/nmol) and 10 μl of enzyme homogenate. Mixtures were incubated for 1 h at 37°C and passed through AG1x8 columns. Eluates were lyophilized and subjected to HPLC analysis. Core 4 β6-GlcNAc-transferase (C2GnT) activity was measured similarly using GlcNAcβ1-3GalNAcβ3-p-nitrophenyl acceptor substrate. Core 3 β3-GlcNAc-transferase (C3GnT) was measured similarly, except for the presence of 10 mM MnCl2, and GalNAcβ3-benzyl as substrate.

Statistical analysis

Normalized data of mRNA, protein and enzymatic activity were processed to calculate mean values and s.d. The Mann–Whitney U test and Spearman’s correlation were used. P < 0.05 was considered statistically significant.

Results

Levels of Gal3ST activity

Mucin sulphation is reportedly reduced in LSG from SS patients [11], suggesting that the levels of sulphotransferase activity are decreased in LSG from such patients. To evaluate this possibility, we compared Gal3ST activities using Galβ1-3(6-deoxy)GalNAcβ3-benzyl as acceptor substrate in LSG homogenates from controls (n = 10) and SS patients (n = 7). Fig. 1 shows that the levels of Gal3ST activity were significantly lower in LSG from SS patients [1.25 (0.8) pmol/h/mg of protein] than those in control samples [6.5 (4) pmol/h/mg of protein] (P = 0.0004). Moreover, Table 2 shows a Spearman’s rank correlation analysis between Gal3ST activity levels and clinical parameters of SS patients and controls. The sulphotransferase activities correlated significantly with dryness symptoms (mouth, r = -0.4880 and eye, r = -0.8660), scintigraphy (r = -0.6890), focus score (r = -0.8271), auto-antibodies (Ro, r = -0.6281, La, r = -0.5636 and ANA, r = -0.7856) and glandular TNF-α levels (r = -0.7204), but not with USF (r = 0.2205).

Relative mRNA and protein levels of Gal3STs

To assess whether reduced activity of Gal3STs was a consequence of a down-regulated expression of these proteins, we evaluated the relative mRNA levels of Gal3ST-2, Gal3ST-3 and Gal3ST-4 in LSG extracts from control individuals and SS patients. No significant differences in relative mRNA levels were observed between SS patients and control individuals for Gal3ST-2 and Gal3ST-4 (P = 0.8 and 0.96, respectively) (data not shown). Transcripts of Gal3ST-3 were almost undetectable in the LSG extracts, while in a sample of human thyroid this transcript was readily detected. We also evaluated the protein levels of Gal3ST-2 and Gal3ST-4 relative to β-actin. No significant changes in relative protein levels were observed between control subjects and
TABLE 2 Correlation coefficients between Gal3ST activity and clinical parameters of SS patients

<table>
<thead>
<tr>
<th></th>
<th>Dryness</th>
<th></th>
<th></th>
<th>Serology</th>
<th></th>
<th></th>
<th>Cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Age</td>
<td>Eye</td>
<td>Mouth</td>
<td>USF</td>
<td>Scintigraphy</td>
<td>Ro</td>
</tr>
<tr>
<td>Gal3ST activity</td>
<td>0.2409</td>
<td>-0.8660*</td>
<td>-0.4880*</td>
<td>0.2205</td>
<td>-0.6080*</td>
<td>-0.6281*</td>
<td>-0.5636*</td>
</tr>
</tbody>
</table>

For scintigraphic data, we used lower values to indicate better glandular function, then, lower values of Gal3ST activity are associated with poor glandular function. *P < 0.05.

Fig. 2 Relative protein levels of Gal3ST-2, Gal3ST-4 and β3GalT-5. (A, C and E) correspond to western blots for each protein. Specific bands are shown for the indicated number of representative SS patients and controls. (B) Relative Gal3ST-2 levels are shown as box plots for a total of 18 controls and 14 SS patients. (D) Relative Gal3ST-4 levels are shown as box plots for a total of 19 controls and 21 SS patients. (F) Relative β3GalT-5 levels are shown as box plots for a total of six controls and six SS patients. β-actin was used for normalization. Results are representative of three independent experiments. C: controls, P: SS patients.

SS patients (P = 0.92 and 0.085, respectively) (Fig. 2A and C). Thus, reduced sulphotransferase activity did not correlate with protein levels of Gal3ST-2 (r = 0.19) and Gal3ST-4 (r = −0.24). In order to determine whether reduced levels of sulfo-Lewis a (SO₄Gal[1-3](Fucα1-4)GlcNAc) in LSG from SS patients could be in part caused by altered expression of glycosyltransferases involved in Lewis a antigen synthesis, we evaluated the relative mRNA and protein levels of β3GalT-5, an enzyme that catalyses the formation of type 1 carbohydrate chains, the scaffold for Lewis a. No significant changes in relative mRNA (P = 0.7) (data not shown) and protein levels (P = 0.39) (Fig. 2E) were observed between SS patients and control individuals.

Activity levels of glycosyltransferases

To determine the enzymatic activity of glycosyltransferases involved in O-glycosylation pathway in human LSGs, we measured the activities of ppGalNAcT (in six SS patients and seven control individuals), C1GalT (in seven control individuals and four SS patients), C2GnT (in seven control individuals and four SS patients) and β3-GalT and β4-GalT (in five SS patients and four control individuals). No significant changes in glycosyltransferases activities were observed between SS patients and control subjects for any of the analysed enzymes (P = 0.53, 0.1, 0.52 and 0.73, respectively) (Fig. 3A–D). In addition, we determined the enzymatic activities of α3-sialyltransferase (ST3GalT), using Galβ1-3(6-deoxy)GalNAc>benzyl as acceptor substrate (using LSG homogenates from 7 SS patients and 10 control individuals). The α3-sialyltransferase activity was lower in SS patients [4.45 (3) nmol/h/mg of protein] compared with control individuals [7.42 (3) nmol/h/mg of protein], but this difference was not significant (P = 0.08) (Fig. 3E).
The activity of C2GnT2 that synthesizes the O-glycan Core 4 (Fig. 4) was also high in LSG homogenates and varied between 1.1 and 19.0 nmol/h/mg. The activity that synthesizes Core 3, C3GnT, was very low but detectable by HPLC assays in LSG homogenates (0.01-0.16 nmol/h/mg). In order to estimate the relative activities of β3-GalT and β4-GalT, we added the β4-GalT inhibitor N-butyrylglucosamine-S-naphthyl to the assays [29]. The GalT activity was reduced by 77-95%, indicating that most activity measured was done in β4-GalT in LSG from both normal and SS patients.

Discussion
To our knowledge, these results identify for the first time the O-glycosylation pathways in LSG and suggest a mechanism for the decrease in sulphated oligosaccharides in mucins from LSG of SS patients. Glycosyltransferase activities involved in these pathways were compared between SS patients and controls. We showed that LSGs are capable of synthesizing all four mucin type O-glycan core structures 1-4 (Fig. 4). In addition, sialyl- and sulphotransferases that can modify these core structures were active. These pathways resemble those found in the human colon [30] and are consistent with the O-glycans found in human salivary mucins that have the rare Core 3 and 4 structures [9].

The Gal3ST activity we observed in LSG homogenates was likely due to either Gal3ST-2 or Gal3ST-4 but not Gal3ST-3, since high Gal3ST-2 and Gal3ST-4 mRNA levels but very low Gal3ST-3 mRNA levels were observed in LSG. These results agree with previous data showing ubiquitous Gal3ST-2 and Gal3ST-4 expression, while Gal3ST-3 expression was restricted to thyroid, heart, kidney and spinal cord [17]. A significant decrease was detected in Gal3ST activity levels in LSG from SS patients (P=0.0004). Interestingly, no difference was found both in mRNA and protein levels of Gal3ST-2 and Gal4ST-4 between SS patients and controls. This suggests that the reduction in Gal3ST enzyme activity was not due to gene down-regulation, but may have been due to a yet unrevealed regulation of enzyme activity. We cannot exclude that other unknown factors, such as a possible increase in endogenous inhibitors, a decrease in activators or factors that affect conformation and/or activity are responsible for decreased Gal3ST activity in LSGs from SS patients.

Our data indicate that mucin hyposulphation reported by Alliende et al. [11] in LSG from SS patients is the result of decreased sulphotransferase activity, but not the consequence of a general impairment of the synthesis of underlying structures that form scaffolds for sulphation (see details in Fig. 4). Moreover, LSGs are rich in glycosyltransferase activities that synthesize Core 1 and 2 structures of mucin O-glycans, and were also shown to have low activities that synthesize Core 3 and 4 structures. The major mucin type O-glycans are therefore expected to have Core 1 and 2 structures.

An inverse correlation between Gal3ST activity and oral and eye dryness was found in SS patients. Similarly, Gal3ST activity was inversely correlated with gland function, measured by scintigraphy, but not with USF (Table 2). These findings support previous observations showing decreased amounts of sulphated

Fig. 3 Levels of glycosyltransferase activities. LSG extracts from controls and SS patients were assayed for (A) ppGalNAcT, (B) C1GalT, (C) C2GnT, (D) (β3/β4 GaIT) and (E) α3-sialyltransferase (ST3GalT) activity. Assays were carried out in duplicate as described in the ‘Patients and Methods’ section. P < 0.05 was used as threshold.

![Image of glycosyltransferase activities](http://rheumatology.oxfordjournals.org/Downloaded from Universidad de Chile on May 15, 2012)
oligosaccharides and sulfo-Lewis a residues in SS patients, either having normal or low USF [11]. Despite such differences in USF, all SS patients in this study had complained of oral dryness [11]. Mucins are hydrophilic polymers with sulphate and sialic acid groups that bind salt and water molecules, thereby preserving the humidity of the mucosa. Therefore, the quality of salivary mucin determines oral mucosal lubrication. In SS patients, hypo-sulphation due to decreased Gal3ST activity may result in a mucin with decreased water-binding capacity, contributing to the dry mouth sensation.

No differences between β3GalT-5 mRNA, protein levels or activities were detected. This enzyme catalyses the backbone synthesis for addition of Lewis a, sulphate or sialic acid [30]. Our results suggest that decreased mucin sulphation in SS is not the consequence of a decreased concentration of sulphate acceptor substrates, but is related to decreased Gal3ST activity. Results from our laboratory indicate that the amount of sialyl-Lewis a remains unchanged in SS patients (Yoon-Jeoung Kwon, unpublished work), a finding that agrees with normal β3GalT-5 expression reported here. Conversely, in colonic adenocarcinoma, mucin hyposulphation is accompanied by decreased β3GalT-5 and Gal3ST-2 mRNA and enzyme activity levels [16-18]. Therefore, the differences in regulation observed for β3GalT-5 and Gal3STs depend on the type of disease.

Levels of Gal3ST activity were inversely correlated with lymphocyte infiltration and glandular levels of pro-inflammatory cytokines. An intriguing question is how inflammatory mediators modulate sulphotransferase activities. A decrease in the synthesis of the sulphate donor substrate PAPS can also result in a decrease in protein sulphation [31]. In addition, PAPS transporters might represent key factors regulating PAPS availability within the Golgi apparatus [32]. Two PAPS transporters, PAPST-1 and PAPST-2, have been described in human salivary glands [33, 34]. Analysis of the PAPST-1 promoter using a bioinformatics approach identified potential IFN-response elements that bind IFN regulatory factor 1 (IRF1) and IRF7. Accordingly, these transcription factors are increased in LSGs from SS patients [35, 36]. Particularly, IRF1 may suppress the transcription of several genes, such as BP230 and ARPC5 [37]. Thus, in a pro-inflammatory environment, both PAPST-1 and PAPST-2 expression as well as activity are likely to be modified. This, in turn, would affect Gal3STs activities, as well as the sulphation of many proteins in the LSGs of SS patients.

Gal3ST activity correlated inversely with glandular TNF-α levels and the presence of serum autoantibodies directed against the ribonucleoprotein antigens Ro/SSA and La/SSB. When A-253 cells, derived from a human epidermoid carcinoma of the submaxillary gland, were treated with anti-Ro and anti-La IgG autoantibodies from SS patients, TNF-α induction was observed [38]. Conversely, cells treated with IgG from healthy individuals produced TNF-α amounts comparable to those of

![Fig. 4 O-glycan biosynthesis in LSG of SS patients. Enzymes responsible for the synthesis of four O-glycan structures are indicated. ppGalNAcT initiates O-glycosylation by transferring GalNAc to Ser or Thr residues. C1GalT adds a Gal residue to synthesize the Core 1 structure, which can be branched by C2GnT to form 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. These structures can be further elongated, sialylated or sulphated. Black arrows show normal activity. The red fragmented arrow indicates reduced activity in SS patients.](http://rheumatology.oxfordjournals.org/)
untreated control cells [38]. Additionally, anti-Ro autoantibodies stimulate production of the pro-inflammatory cytokines IL-6 and IL-8 in healthy human salivary gland epithelial cells [39]. In vitro studies in bovine synoviocytes exposed to TNF-α demonstrated a decrease in Gal3ST activity [12]. In RA, a decrease in proteoglycan sulphation, among other factors, would contribute to loss of the morpho-functional integrity of joints [40], suggesting that impairment of Gal3ST activity is a recurrent problem in autoimmune diseases. We postulate that pro-inflammatory cytokines induced by circulating auto-antibodies in SS patients modulate Gal3ST activity.

In summary, decreased sulphotransferase activity in the Golgi apparatus of epithelial cells from LSG of SS patients decreases mucin sulphation, explaining the dryness symptoms in these patients. As many other proteins are sulphated in the Golgi apparatus, we cannot rule out that additional proteins, when desulphated, may also contribute to functional LSG alterations. In this context, syndecan-1 and syndecan-4 localized in the acinar plasma membrane of mammary gland cells are sulphated in Golgi apparatus and thus bind growth factors (i.e. bFGF). Removal of sulphate chains of these syndecans resulted in a complete loss of bFGF binding [41]. It has been suggested that mammary cell surface proteoglycans take part in anchorage of the cell to the extracellular matrix via sulphated chains. Our previous studies in LSG from SS patients reported on the deattachment of acinar cells from their basal lamina and modifications in the biological function of these components [42]. Thus, the alterations described here could affect not only the secretory function, but also many other features of epithelial cells, including cell–ECM interactions and immunological cell–cell interactions. The present study constitutes the first to evaluate Golgi sulphation in LSG from SS patients. A further understanding of this process should allow us to identify new mechanisms in SS pathogenesis, as well as possible therapeutic targets that modulate inflammation and thereby alleviate dryness symptoms of SS patients.

References

9 Thomsson KA, Prakobphol A, Leffler H et al. The salivary mucin MG1 (MUC5B) carries a repertoire of unique oligosaccharides that is large and diverse. Glycobiology 2002; 12:1–14.

Acknowledgements

The authors thank Dr John Schutzbach for advice and Dr Juan Carlos Aguillón for kindly donating antibodies to IFN-γ and TNF-α. I.C. was supported by a fellowship granted by the Department of Post grade and Post title, Vice-Presidency of Academic Affairs, University of Chile. All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr M.-J.G. had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design was by I.C., I.B., S.A. and M.-J.G. Acquisition of data by I.C., I.B., S.A., C.A., C.M., M.H., C.L., M.-J.B., M.S., V.B., S.G., U.U. and M.-J.G. Analysis and interpretation of data by I.C., I.B., U.M., S.A., U.U., A.F.G.Q., C.M. and M.-J.G.

Funding: Supported by grants (to M.-J.G, S.A. and C.M) FONDECYT-CHILE 1080006, A.F.G.Q. by FONDAP 15010006 and the Canadian Cystic Fibrosis Foundation (to I.B.).

Disclosure statement: The authors have declared no conflicts of interest.


24 Pfaffi MW. Quantitative mRNA analysis in molecular endocrinology. Weihenstephaner: Chair of Physiology DoAS, Center of Life and Food Sciences, 2003:41.

