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Pathophysiology of Sjögren's Syndrome

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The term Sjögren's syndrome refers to keratoconjunctivitis sicca and xerostomia due to lymphocytic infiltrates of lachrymal and salivary glands. The current used criteria for diagnosis of primary Sjögren's syndrome is the American–European consensus. Primary Sjögren's syndrome is an autoimmune disorder characterized by lymphocytic infiltrates and destruction of the salivary and lachrymal glands and systemic production of autoantibodies to the ribonucleoprotein particles SS-A/Ro and SS-B/La. The infiltrating cells (T- and B-cells, dendritic cells) interfere with glandular function at several points: destruction of glandular elements by cell-mediated mechanisms; secretion of cytokines that activate pathways bearing the signature of type 1 and 2 interferons; production of autoantibodies that interfere with muscarinic receptors; and secretion of metalloproteinases (MMPs) that interfere with the interaction of the glandular cell with its extracellular matrix, which is necessary for efficient glandular function. As the process progresses, the mucosal surfaces become sites of chronic inflammation and the start of a vicious circle. Despite extensive study of the underlying cause of Sjögren's syndrome, the pathogenesis remains obscure. In broad terms, pathogenesis is multifactorial; environmental factors are thought to trigger inflammation in individuals with a genetic predisposition to the disorder.

Key Words: Autoimmune, Pathophysiology, Sicca, Sjögren's syndrome.

Introduction

In 1932, the Danish ophthalmologist Henrik Sjögren reported the triad of keratoconjunctivitis sicca (KCS), xerostomia, and rheumatoid arthritis describing clinical and histological findings in 19 women, and then Sjögren introduced the term keratoconjunctivitis sicca for this syndrome, to distinguish it from dry eyes caused by lack of vitamin A (xerophthalmia) (1).

Sjögren's syndrome (SS) is one of the three most common autoimmune disorders (2). Primary Sjögren's syndrome (pSS) has a population prevalence of about 0.5% and a female preponderance ratio of 9:1 compared with men. There are two age peaks: the first after menarche (about 20–30 years old) and the second after menopause (mid-50s) (3,4).

The term SS refers to keratoconjunctivitis sicca and xerostomia due to lymphocytic infiltrates of lachrymal and salivary glands (5). Patients with SS also produce a diverse array of serum autoantibodies such as anti-Ro and anti-La, providing evidence of an autoimmune origin for this disease (6,7). pSS represents an idiopathic inflammatory exocrinopathy characterized by both organ-specific autoimmunity, in which immune cells chronically attack preferentially the salivary and/or lachrymal glands (8,9) and extraglandular systemic features, including fatigue, arthritis, pulmonary involvement, interstitial nephritis, peripheral neuropathy, and vasculitis. SS is also characterized by complications such as lymphoma, caries and other secondary infections, congenital heart blocks and neonatal lupus (10–12).

The first criteria used for diagnosis of SS was the Copenhagen criteria proposed in 1986 by Manthorpe et al. (13).

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The current used criteria is the American–European consensus published in 2002 (14) and involves lip biopsy, autoantibodies, and clinical features.

The purpose of this report is to review the proposed mechanisms and factors involved in the pathogenesis of SS.

Pathophysiology of Sjögren's Syndrome

Despite extensive molecular, histological and clinical studies, the underlying cause of SS and its pathogenesis remains unknown. In broad terms, pathogenesis is multifactorial, where several steps are necessary to establish the disease. It is thought that environmental factors trigger inflammation in individuals with a genetic predisposition. However, the factors driving autoimmunity leading to the differentiation of autoreactive lymphocytes into autoantibody-producing plasma cells remain largely unknown, although several epitope-mapping studies have suggested that autoimmunity in pSS is driven by autoantigens (9).

Fox and colleagues in two excellent reviews (3,10) have proposed the critical features in a model of SS that incorporates the histopathological, genetic, serological, and gene-profiling data available as follows: 1) an initial insult (either viral or non-viral) to the gland that leads to cellular necrosis or apoptosis with subsequent expression of the Sjögren's SS-A protein on the glandular-cell surface (the SS-A protein is known to form complexes with a double-stranded RNA [hYRNA], and this complex is found on the blebs of apoptotic cells); 2) failure to destroy autoimmune T-cells at the level of thymic selection; 3) production of cytokines by the injured gland that upregulate chemokines with subsequent homing of autoimmune lymphocytes and dendritic cells into the glands via high endothelial venules that express increased levels of cell adhesive molecules; 4) upregulation of major histocompatibility antigens and adhesive molecules on epithelial cells in the lacrimal and salivary glands leading to activation of lymphocytes within the glandular micro-environment, as a result of their interaction with epithelial cells that express HLA-DR(+), cell adhesion molecules, and other co-stimulatory factors; 5) production of antibodies to SS-A antigen by B lymphocytes under the influence of T-helper lymphocytes, formation of immune complexes containing anti-SS-A and ribonucleoprotein that bind to HLA-DR positive dendritic cells in the gland by their Toll receptor and their Fc-γ receptors; 6) secretion of pro-inflammatory cytokines by both lymphocytes and epithelial cells to perpetuate the inflammatory response and production of type 1 interferons by the dendritic cells, which further perpetuates the process of lymphocyte homing, lymphocyte and metalloproteinase activation, and apoptosis of glandular cells; 7) decreased secretion by the residual glandular acini as a result of their decreased neural innervation and defects in their post-signal transduction; 8) resistance of T-cells within the gland to undergo apoptosis, due to upregulation of bcl-2 and bcl-x (Figure 1).

This vicious cycle that links the innate and acquired immune systems would occur in genetically predisposed individuals (i.e., positive for HLA DR3) who would generate an immune response to the SS-A antigen and thus give rise to immune complexes that stimulate Toll receptors to yield the characteristic interferon type 1 signature (3,5); therefore, the innate and acquired immune systems can be mutually costimulatory (15).

Environmental Factors

Environmental triggers include a viral infection of the glands or any intercurrent infection that stimulates dendritic or glandular cells to activate the HLA-independent innate immune system.

Several viruses, e.g., hepatitis C virus (16,17) and yet undefined retroviruses, have long been discussed as possible triggers in the pathogenesis of pSS and its malignant complications (18–22). Evidence to suggest an infectious agent as a co-factor in SS remains intriguing but still unproven. Elevated antibody titers in SS patients have been reported for a variety of Epstein-Barr virus (EBV) antigens, including BHRF1 (the viral homologue of bcl-2) and BMRF1 (an EBV DNA-binding protein) (23). The EBV genomes in SS have been detected in salivary glands (SG) and lachrymal glands by in situ hybridization, (24–26) polymerase chain-reaction methods, (27,28) and by culture of SG tissue

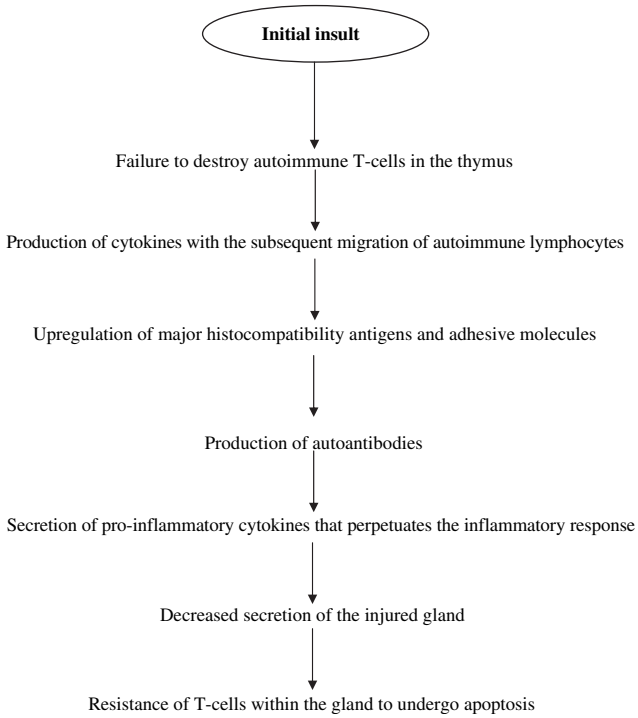

Figure 1. Proposed critical features in the pathophysiology of SS.

in severe combined immune deficiency (SCID) mice (29). The frequency of EBV-infected cells, however, is low (approximately 1 infected cell per 10^6 uninfected cells), and some studies failed to detect EBV genomes in SS SG biopsies (30).

Almstahl et al. (31) analyzed the microbial flora of patients with hyposalivation of different origins and found high numbers of mutans streptococci in patients with pSS. Another study (32) revealed a significant inverse relation between salivary flow and *Candida* sp. colony-forming unit counts in the saliva of patients with xerostomia, including a subgroup of patients with pSS. However, whether these agents are involved in chronic inflammation and salivary dysfunction or whether hyposalivation predisposes to changes in the oral flora is not clear. Interestingly, a case of sicca complex in a patient with intestinal *Tropherhyma whippleii* infection (Whipple's disease infectious agent) has been reported, introducing another infectious agent into the array of the potential causes of SS (33).

Previous studies that detected antibodies in SS patients to retroviral gag proteins were probably detecting a cross-cellular protein (34). SG tissues from Caucasian SS patients were negative for HTLV-1 tax genes by DNA methods (35) and thus negates earlier findings of reactivity of this tissue with a monoclonal anti-HTLV-1 antibody (36). In comparison, genomic sequences homologous to HTLV-1 tax have been found in SS tissues in a subset of Japanese SS patients (37). In Table 1 we have summarized the proposed agents linked to the pathogenesis of pSS.

Genetic Factors

Very little is known about the genetics of SS. It has been reported that the major susceptibility loci for autoimmune sialadenitis resides on chromosomes 1, 4 and 10 in murine models. For instance, the chromosomal regions around Nss1 on chromosome 4 harbor a set of genes that are probably of importance for different kinds of autoimmune syndromes, including SLE and autoimmune hemolytic anemia (38). Interestingly, no association between sialadenitis in the non-obese diabetic (NOD) mice and collagen-induced arthritis was observed (38).

Table 1. Reports linking infection to the pathogenesis of SS

Report	Year	Pathogen
Brookes et al. (34)	1992	Retroviruses
Shattles et al. (36)	1992	HTLV-1
Pflugfelder et al. (28)	1993	Epstein-Barr virus
Sumida et al. (37)	1994	HTLV-1
Newkirk et al. (23)	1996	Epstein-Barr virus
Merne and Syrjanen (24)	1996	Epstein-Barr virus
Wen et al. (26)	1996	Epstein-Barr virus
James et al. (19)	2001	Epstein-Barr virus
Arrieta et al. (20)	2001	Hepatitis C virus
Torres et al. (32)	2002	*Candida albicans*
Bosman et al. (33)	2002	*Tropherhyma whippleii*
Ramos-Casals et al. (21)	2002	Hepatitis C virus
Almstahl et al. (31)	2003	Mutans streptococci
Kamimura et al. (22)	2005	Hepatitis C virus

Genetic studies using NOD mice have indicated that multiple immune as well as nonimmune genes are involved in the pathogenesis of sialadenitis (39,40). An important role for nonimmune genes in the sialidenitis of the NOD mouse is suggested by histologic abnormalities in the lachrymal and salivary glands. In NOD and SCID mice, which lack functional T-cells or B-cells (40). Only when T-lymphocytes infiltrate the gland, the salivary flow rate in NOD mice is significantly decreased and even in the absence of a competent immune system degenerative changes occur in the salivary glands. In fact, commonly observed features of these mice include organized lymphocytic foci, composed of CD4+ and CD8+ T-cells, in both the salivary and the lacrimal glands. However, only the diabetic mouse model (NOD) undergoes a corresponding loss in exocrine gland function related to the presence of lymphocytic infiltrates (39). It is possible that cytokines released by these lymphocytes contribute to an early decrease in the neural innervation of the lacrimal gland epithelium (41) as well as decreased signal transduction in response to neural signals (42).

Previous analyses of autoimmune B cells focused almost exclusively on productive V gene rearrangements and therefore could not discern the impact of molecular and selective influences (9). Recent analysis of the nonproductive V gene repertoire in patients with pSS and SLE has documented minimal abnormalities in the nonproductive VH, Vκ and Vλ gene repertoires indicating that IgV gene usage in the nonproductive repertoire is not significantly different from normal but this does not rule out the possibility that abnormalities in the usage of specific VH gene might contribute to autoimmunity (43−46).

Gene-profiling studies on cytokine production in samples of salivary gland suggest an important role for type I and type II interferons in this perpetuation of the immune response (47,48).

It has also been reported that X-chromosome-linked factors might influence apoptosis in salivary and lacrimal glands in patients with SS, therefore decreasing its secretion (49).

Strong linkage disequilibrium among the loci of transporters associated with antigen processing-2 and HLA-DQB1 was found in Colombian pSS patients (50). The formation of autoantibodies against B-cell epitope analog of SS-B/La in European patients with pSS seemed to be dependent on the presence of a permissive HLA−DQ heterodimer, most prominently represented by HLA−DQA1*0501/DQB1*0201. As a result of this finding, a model of HLA-restricted presentation of SS-B/La peptide determinants has been suggested (51). Font et al. (52) found an abnormal

distribution of interleukin-10 promoter haplotypes (i.e., a predominance of the GCC haplotype) in pSS patients compared with healthy control subjects that did not correlate with a different immunologic pattern but with an earlier onset of pSS in GCC allele carriers. The importance of individual HLA class II genes in controlling the immune responses to human Ro60 has been implicated by studies in mice lacking murine class II molecules but carrying distinct HLA genes. The results show that human Ro60 induces strong T- and B-cell responses in DR2, DR3, and DQ8 mice as well as a differential pattern of dominant T- and B-cell epitopes of the human Ro60 molecules (53).

Immunohistochemical Findings and Immune System in SS

Ocular and Oral Mucosal Surface Abnormalities in SS

The mucosal surfaces of the eye or mouth are heavily innervated by unmyelinated fibers that carry afferent signals to the lacrimatory or salivatory nuclei located in the medulla. These medullary nuclei, which are part of the autonomic nervous system, are influenced by higher cortical inputs including taste, smell, anxiety, and depression. The efferent neurons innervate both glandular cells and local blood vessels. The blood vessels provide not only water for tears and saliva, but also growth factors including hormones (e.g., insulin) and matrix proteins (e.g., fibronectin and vitronectin) in the perivascular space of the lacrimal and salivary glands. In response to neural stimulation through muscarinic M3 receptors and vasoactive intestinal peptide (VIP) receptors, glandular acinar and ductal cells secrete water, proteins, and mucopolysaccharides (mucins). This complex mixture forms a hydrated gel that lubricates the ocular surface (tears) and the oral mucosa (saliva).

Glandular destruction in SS is mediated mainly by primed CD4+ T lymphocytes (54). However, salivary and lacrimal glands from SS patients have an impaired secretory function, even during the initial phase of inflammatory/lymphocytic destruction. Despite loss of glandular elements, destruction of neural innervation, and absence of muscarinic receptors on glandular cells, the release of proinflammatory cytokines by both lymphocytes and glandular cells, induction of matrix metalloproteinases (55), as well as impaired release of and response to neurotransmitters may be involved in the loss of adequate secretory function of the residual glandular cells in pSS (56). In the simplest model of pSS the lacrimal or salivary gland is incapable to respond adequately to neural signals as a consequence of local immune infiltrates and their derived cytokines (57). A recent immunohistological study (58) noted a decrease in the phosphokinase C (PKC) isoform beta pi in acinar epithelial cells and the zeta isoform in myoepithelial cells of minor SG biopsies of SS patients, this alteration in PKC isoforms would decrease the secretory response to cholinergic stimulation. Moreover, antibodies against muscarinic M3 receptors have been found in SS patients and may compete with acetylcholine stimulation of glandular ductal cells by interferon-γ in the presence of matrix and leads to differentiation and induction of HLA-DR (59). However, in the absence of matrix, similar stimulation leads to apoptosis (60).

Cell–matrix interactions are important for cellular functions of the epithelial cell, including response to growth factor signals and ability for cellular regeneration. In lacrimal gland cells grown *in vitro*, cell–matrix interactions are necessary for secretory responses to muscarinic M3 agonists and are further augmented by novel non-matrix proteins such as BM180 (61,62). The epithelial cells express a family of specific receptors including integrins to bind these matrix proteins, as well as receptors for cyclic AMP (cAMP), growth factors, estrogens, and cytokines. These receptors are expressed at increased levels in SS patients (5). Moreover, the extracellular matrix in SS glands may be modified by collagenases (63) and other metalloproteinases (64). Also, cytokines including IL-1, IL-6, IL-8, and TNF-α are transcribed in increased amounts by SS conjunctival epithelial cells (65). Local production of cytokines by mononuclear cells and also epithelial cells might contribute to the immune-mediated destruction of exocrine glands in pSS (66). pSS has therefore been called autoimmune epithelitis (67), emphasizing that epithelial cells are thought to be important in the immunopathogenesis.

It is now evident that the interaction of Fas with FasL regulates a large number of pathophysiological processes of apoptosis including autoimmune diseases (68). Accumulated evidences suggest an important role of apoptosis in the pathogenesis of SS (69). Glandular destruction could occur by means of perforin and granzyme A as well as Fas/Fas ligand mechanisms (70). Because it was reported that Fas expression was observed in the salivary gland cells in human SS patients (71), it was likely that Fas-mediated apoptosis may contribute to tissue destruction in the salivary glands with pSS. However, only partial destruction of the gland is noted in most patients, and local production of cytokines, autoantibodies, and metalloproteinases probably leads to dysfunction of the residual glandular tissue (3). All these factors might interact and negatively influence the transport of aquaporin in lacrimal and salivary glands (72).

The initial steps in pathogenesis probably involve glandular vascular endothelial cells, the glandular epithelial cells, or their underlying stromal and dendritic cells (73). The earliest and important changes involve the development of small capillaries into high endothelial venules that secrete chemokines and express adhesive molecules that promote the migration of immune cells into the glands that perpetuate the cycle of homing into the gland of lymphocytes and dendritic cells (3,74,75). The glandular infiltration in pSS is composed mainly of CD4+ T lymphocytes

(76) but usually also contains a substantial number of B cells and plasma cells (77,78). The infiltration of lymphocytes into glandular aggregates apparently has a crucial role in the tissue pathology of SS. This process seems to be tightly regulated at least in part by chemokines and the local expression of their receptors (9).

T-lymphocytes infiltrating the SG exhibit CD41, memory T-cell phenotype and cytokine profile similar to Th-1 cells (79,80). CD41 T-cells eluted from the SG of SS patients are resistant to apoptosis after stimulation by anti-CD3 or anti-Fas antibody stimulation; this resistance may result from increased levels of bcl-278 or bcl-x (3). Bcl-x is a member of the bcl-2 family, which contains binding sites for protooncogenes that resist apoptosis (81). Significant proportions of acinar and ductal epithelial cells were found to exhibit apoptotic markers (82).

In pSS, the coexpression of the p53 tumor suppressor gene and its transcription factor p21 has been suggested to represent a defensive mechanism in ductal salivary cells surrounding lymphoid foci that prevents apoptosis but is not expressed in acinar cells, thereby accounting for their sensitivity to damage (83). The T-cells may promote the apoptosis of glandular epithelial cells by secretion of TNF-α85 as well as by production of granzyme A (84).

α-Fodrin is a ubiquitous, calmodulin-binding protein (85) cleaved by calcium-activated protease (calpain) in apoptotic T cells and by calpain and caspase 3 (86) in anti-Fas-stimulated Jurkat cells (87). The observation that ubiquitously expressed autoantigens (e.g., α-fodrin, La, and nuclear mitotic apparatus protein) in SS are specifically cleaved by granzyme B strongly suggests that a common biochemical event (novel autoantigen cleavage during granule-induced epithelial cell death) is responsible for selecting the unconnected group of molecules (88).

Local production of nitrous oxide may also contribute to apoptosis (89). In contrast, production of protective cytokines such as transforming growth factor beta (TGF-β) may be reduced in the SS SG (90,91). Furthermore, it has been suggested that TGF-β, an important immunoregulatory cytokine whose absence can lead to systemic autoimmune disease, might be deficient in SS (9). In this regard, reduced levels of TGF-β have been found in SS glands with intense lymphocytic infiltrates (92). In contrast to these findings, Koski et al. (93) found increased levels of TGF-β in salivary glands. They concluded that the localization and level of expression of TGF-β 2 indicate its involvement in local tissue fibrosis and may reflect attempts at immunosuppression.

Recently, a group from Italy (94) analyzed minor salivary gland biopsy specimens from 20 healthy subjects and 18 patients with primary SS and demonstrated that CXCR3, in particular, the B form of this receptor, is constitutively expressed by human salivary gland epithelial cells; thus, these findings suggest that epithelial CXCR3 may be involved in postsecretion regulation of chemokine bioavailability. They also support a critical role for CXCR3 in the pathogenesis of SS and identify its agonists as potential therapeutic targets.

There is increasing evidence that the cascade of caspases is a critical component of the cell death pathway (95,96), and a few proteins were cleaved during apoptosis. These include poly(ADP-ribose) polymerase, a small U1 nuclear ribonucleoprotein, and α-fodrin, which were subsequently identified as substrates for caspases (97,98).

The development of autoimmune exocrinopathy in SS appears to be dependent on autoantigen cleavage through caspase cascade (99). Other murine models for SS indicate that prevention and induction of autoimmune exocrinopathy is dependent on presentation and cleavage of α fodrin. Thus, the specific inhibition of the caspase cascade and the cysteine protease cathepsin S have been implicated as potential new therapeutic options to reduce tissue damage (100−103).

Cell clusters resembling germinal center (GC)-like structures have been reported in the focal lymphocytic sialadenitis of the minor (labial) salivary glands in patients with pSS (104). It has been proposed that such potentially functional B-cell aggregates can be induced in several extrafollicular tissues in autoimmune disorders (104−106) and the formation of these aggregates seems to be clearly dependent on the interaction of chemokines and their receptors (107,108). Similar ectopic GC-like reactions have also been observed in the synovium in rheumatoid arthritis, as well as in a variety of other diseases such as spondylarthopathies, myasthenia gravis and thyroiditis (9).

Although local autoantibody production in the glands has been suspected and autoantibodies have been found in the saliva, the pathogenetic role of the glandular B lymphocytic infiltrates remains largely unknown. In this regard, it is notable that autoantibodies to the M3 type of the muscarinergic acetylcholine receptor might function to inhibit salivary flow (109) in a manner comparable to the antibody-mediated blockage of nicotinergic receptors in patients with myasthenia gravis. Of potential importance is the fact that enhanced levels of B lymphocyte stimulator (BLyS; also known as B-cell activating factor belonging to the TNF family [BAFF] or transmembrane activator and CAML interactor [TACI]) have been demonstrated in patients with SS (110). In addition, expression of BLyS was markedly enhanced in the inflamed salivary glands, indicating that activation of B-cells might take place in the parotids (111).

Abnormalities of dendritic cells have also been suggested as an important feature of organ-specific lymphocytic localization. These cells are proposed to cause abnormal retention of lymphocytes in the tissues and production of type 1 interferon that subsequently activates lymphocytes and metalloproteinases (3). The infiltrating cells (T- and B-cells, dendritic cells) interfere with glandular function at several points: destruction of glandular elements by cell-mediated mechanisms, secretion of cytokines that

activate pathways bearing the signature of type 1 and 2 interferons, production of autoantibodies that interfere with muscarinic receptors, and secretion of metalloproteinases that interfere with the interaction of the glandular cell with its extracellular matrix, which is necessary for efficient glandular function. As the process progresses, the mucosal surfaces become sites of chronic inflammation with corneal abrasions and rampant dental loss (3,10).

In comparison to the decreased apoptosis of SG T-cells, peripheral blood T-cells of SS patients have an increased rate of apoptosis in comparison to peripheral blood of normal controls; (112) this rules out a global genetic defect leading to deficient apoptosis in all lymphocytes in SS patients, such as noted in the MRL/lpr mouse model of SS (113).

After migration to the gland in response to chemokines and adhesion to specific vascular adhesive molecules, when lymphocytes enter the gland they interact with dendritic cells and epithelial cells (114). Within the glands (and in other lymphoid tissues), activation of T and B lymphocytes occurs by means of HLA-DR-restricted antigen-presenting cells in the presence of costimulatory molecules. This acquired immune system perpetuates immune response with memory lymphocytes and autoantibodies (115). Therefore, it has been suggested that T helper type 1 (Th1) cytokines, such as IFN-γ and IL-2, as well as IL-10, IL-6 and TGF-β, might be important in the induction and/or maintenance of pSS (107), whereas Th2 cytokines, detected in some cases in association with a striking B-cell accumulation in the labial salivary glands, might be involved in the progression of the disease (9). The most prominent cytokines detected in affected salivary glands of patients with pSS are IL-1, IL-6, IL-10, TGF-β, interferon IFN-γ, and TNF.

Fox et al. (116) found that salivary gland CD4+ T cells of patients with SS produced over 40-fold more IL-2, IFN-γ, and IL-10 than peripheral blood CD4+ T cells from patients with SS and controls. Moreover, salivary gland epithelial cells produced 40-fold more IL-1α, IL-6, and TNF mRNA than epithelial cells from individuals with histologically normal salivary glands (9). Chemokines and the expression of chemokine receptors by the inflamed tissue as well as by lymphocytes are therefore likely to be of importance in the evolution of tissue pathology in pSS (9).

In conclusion, the main features in pSS are well described by Fox (3) as follows: Infiltrating cells (T- and B-cells, dendritic cells) interfere with glandular function at several points: destruction of glandular elements by cell-mediated mechanisms, secretion of cytokines that activate pathways bearing the signature of type 1 and 2 interferons, production of autoantibodies that interfere with muscarinic receptors, and secretion of metalloproteinases that interfere with the interaction of the glandular cell with its extracellular matrix, which is necessary for efficient glandular function. As the process progresses, the mucosal surfaces become sites of chronic inflammation and a vicious circle (3) (Figure 2).

In 1998, Hanemaaijer et al. (117) determined the gelatinase-B (MMP-9) activity present in saliva from patients with SS. Using a general gelatinase assay with radioactively labeled gelatinated collagen, it was observed that gelatinase activity was slightly, though not significantly, increased in patients: general gelatinase activity in patients vs. healthy controls: 17.0 ± 4.9 vs. $12.2 \pm 2.5 \times 10^4$ cpm/mL ($p > 0.05$, and 44.0 (4.0 vs. $36.1 \pm 1.9 \times 10^4$ cpm/mL ($p > 0.05$), for active and latent gelatinase, respectively. However, using the immunocapture activity assay (using modified urokinase), MMP-9 activity was specifically measured, which was significantly increased in saliva from patients compared to healthy controls: MMP-9 (already active): patients 8.9 ± 2.5 U/mg, controls 1.0 ± 0.5 U/mg ($p = 0.002$); latent plus active MMP-9: patients 53.1 ± 9.8 U/mg, controls 16.5 ± 2.6 U/mg ($p = 0.01$). Another excellent work was done that year by Konttinen et al. (118). They studied the implication of MMP-9 in the pathogenesis of SS and concluded that SS saliva contains increased concentrations of MMP-9, which is of glandular origin in part. This is probably mediated by the most potent pro-MMP-9 activator found *in vivo* thus far, namely, trypsin-2. Therefore, the MMP 9/trypsin-2 cascade may be responsible for the increased remodeling and/or structural destruction of the basement membrane scaffolding in salivary glands in SS. During recent years, much has been investigated about the role of MMPs in the pathogenesis of SS, for instance, Azuma et al. (119) studied the suppression of TNF-α-induced MMP-9 production by the induction of a super-repressor form of inhibitor of NF-κB-α, they concluded after their results that their observations indicate that suppression of TNF-α-induced MMP-9 production by the introduction of srIκBα cDNA corrected the aberrant *in vitro* morphogenesis of acinar cells grown on type IV collagen.

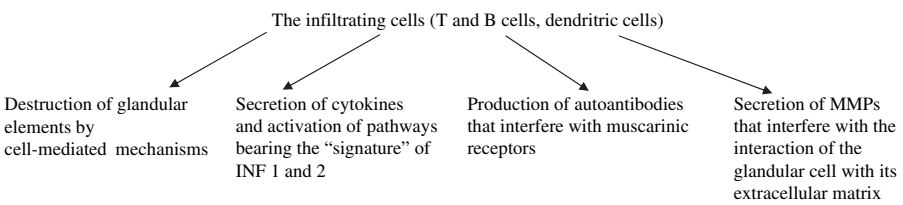

Figure 2. Main mechanisms of gland-induced dysfunction in pSS. INF, interferon; MMP, metalloproteinase.

Another excellent work done in Latin America (120) by a group in Chile investigated the enzymatic activity and cellular localization of MMPs 2, 3, and 9 in labial salivary glands from patients with different degrees of severity of pSS. They found that MMP-3 and MMP-9 expression, as well as MMP-9 catalytic activity, were increased in tissue samples from SS patients in a manner that correlated with the severity of the disease. Most important, increased MMP activity stemmed from exocrine epithelial cells and was not due to infiltrating lymphocytes. Thus, changes in salivary glands as a consequence of proteolysis may lead to severe glandular destruction. Finally in 2003, the same group (121) investigated the effect of MMP activity from the labial salivary glands of SS patients on proteins of the extracellular matrix that form the basal lamina and stroma, and they compare this effect with the structural integrity of acini and ducts as well as the functionality of the labial salivary glands. The results provide new evidence that acinar and ductal cells from the labial salivary glands of SS patients display a molecular potential, with increased capacity to markedly disorganize their extracellular matrix environment and, thus, damage their architecture and functionality.

MMPs modulate the basement membrane. Two important observations about the role of MMPs and base membrane modelation have been published: Royce et al. (122) in 1993 showed that a cell with intercalated duct cell phenotype differentiates into acinar cells upon contact with basement membrane containing laminin-1, which a decade later has been shown to be present in the normal acinar basement membrane but to be absent in patients with SS (123).

Biomolecular Markers in SS

Autoantibodies are features of most systemic autoimmune diseases, including pSS. The production and persistence of autoantibodies in autoimmune conditions is considered to occur because of immune dysregulation with a resultant break in tolerance, regardless of whether these autoantibodies are pathogenic (124).

There is a close overlap in autoantibody profile between SS and a subset of SLE (i.e., the group positive for antibody to the Sjögren's syndrome-related antigen A [SS-A]) (125). Several studies have shown that particular profiles of autoantibodies are more closely associated with extended HLA DR haplotypes than with clinical manifestations (126), for example, HLA-DR3 in pSS and sarcoidosis (127).

The SS-A, a 52-kD antigen, has an alternatively spliced form that is expressed in fetal heart from 14 to 18 weeks of gestation (127) and has been proposed as a target for maternal anti-52-kD antibodies that cross the placenta (128). An altered form of SS-B has also been reported and antibodies against SS-B (but not SS-A) cross-react with laminin leading to a proposal that anti-SS B antibody is a cause for congenital heart block (129).

It has been proposed that SS-A and SS-B antigens may escape the normal tolerance processes by serving as cryptic antigens, that is, binding with relatively low affinity to self-MHC molecules in the thymus, thus avoiding negative selection (130). Both SS-A and SS-B antigens are found in the blebs of apoptotic cells and do not undergo proteolysis during apoptosis (131,132).

It is possible that increased levels of cell death (either apoptotic or necrotic) or aberrant clearance and processing of antigens derived from dying cells may lead to the accumulation of potentially immunogenic forms of autoantigens (133). Thus, under the appropriate genetic background these could amplify and maintain T-cell−dependent responses by an autoimmunization process.

Alternative mechanisms that might expose immunocryptic epitopes in autoantigens include structural alterations caused by abnormal protein−protein interactions during aberrant cell death, mutations, and interactions with toxins, chemical or foreign antigens derived from microorganisms such as viruses (5).

Anti-neutrophil cytoplasmic antibodies (ANCA) are relatively uncommon in patients with pSS, and when present they are usually p-ANCA (perinuclear) antibodies. Caution must be used in interpreting the ANCA in SS patients as false-positive findings may result from the presence of other anti-nuclear antibodies (ANA) (134).

Deep vein thrombosis may occur in SS patients and should stimulate search for anticardiolipin antibodies (AcL). AcL are found in a subset of SS patients and are generally IgA isotype, with lower incidence of thrombosis than found in SLE patients (135).

Based on a recent long-term outcome study in pSS patients, the presence of palpable purpura and low C4 levels have been proposed as predictive factors distinguishing patients at high risk for developing lymphoma from patients with an uncomplicated disease course (136). Moreover, analysis of peripheral B-cells has demonstrated an enhanced frequency of CD27+ B-cells in pSS patients with lymphoma in contrast to patients with pSS without lymphoma (137).

A recent investigation by Lovgren et al. (138) studied the ability of systemic lupus erythematosus (SLE) autoantigen- and SS autoantigen-associated U1 small nuclear RNA (U1 snRNA) and hY1RNA to induce IFN-α production. They concluded that their finding that U1 snRNA and hY1-RNA have IFN-α-inducing capacity indicates that immune complexes containing such RNA, for example, U1 snRNP particles, can be at least partly responsible for the ongoing IFN-α production seen in SLE and SS. These results may help to explain the molecular mechanisms behind the pathogenesis of these and other autoimmune diseases in which autoantibodies to RNA-binding proteins occur.

One important issue about SS is that female dominance (9:1) cannot be explained by only extrinsic factors, chemokines and immune responses; therefore, Valtysdottir et al.

(139) tried to assess the hypothalamic-pituitary-adrenal (HPA) and thyroid axes in women with pSS. They found that the results showed that women with pSS had intact cortisol synthesis but decreased serum concentrations of dehydroepiandrostenidione sulfate (DHEA-S) and increased cortisol/DHEA-S ratio compared with healthy controls. The findings may reflect a constitutional or disease-mediated influence on adrenal steroid synthesis. Another study about female dominance in SS and the role of hormonal control was done by Sullivan et al. (140) and investigated whether women with SS had a deficiency in total androgens. They found that concentrations of 5-androstene-3β,17β-diol (5-diol), DHEA, dihydrotestosterone (DHT), androsterone-glucuronide (ADT-G), and androstane-3a,17β-diol-G (3β-diol-G) were all significantly reduced in SS sera relative to controls. In contrast, SS was not associated with significant alterations in the serum concentrations of testosterone, androstenedione, estrone, or 17β-estradiol. Thus, this reflects that women with SS are androgen-deficient and is supported by the fact that the meibomian gland is an androgen target organ and that androgen deficiency may promote meibomian gland dysfunction and evaporative dry eye (141).

Clinical Implications of SS Pathophysiology

A common misconception about SS is that sicca symptoms result from the total immune destruction of the lacrimal or salivary gland. The degree of glandular destruction and symptoms of dryness do not seem to be directly related to the number of infiltrating lymphocytes. Indeed, the mechanism of glandular damage remains incompletely delineated, although a role for CD4+ T-cells has been proposed, either directly or through the action of secreted cytokines (9).

Morphometrical analysis of SS biopsies shows that almost half of the acinar cells remain histologically intact in patients with long-standing sicca symptoms (142). Failure of residual acini in SS glands to function adequately may result partly from the loss of neural innervation, as indicated by decreased neural axon-specific protein 9.5 and synaptophysin by immunohistological methods (143). Acetylcholine is required for acinar secretion and VIP for glandular homeostasis. The release of cytokines (particularly TNF-α and IL-1) may be toxic to local nerves or acini (144,145). Cytokines IL-1 or TNF-α (amounts similar to the levels found in SS glands or saliva) are toxic to nerve cells grown *in vitro* or in mice expressing these transgenes (146).

Dryness of the mouth cannot simply be attributed to the total destruction of the gland in most biopsy samples from patients with SS. The residual glandular elements in the salivary gland appear dysfunctional even though they maintain their neural innervation (147,148) and upregulation of their muscarinic receptors (149). In patients with SS, the local environment of the inflamed gland leads to dysfunction of the residual glandular units owing to release of cytokines, MMPs, and autoantibodies (3).

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