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Current aspects of pathogenesis in Sjögren's syndrome

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***Abstract*:** Sjögren's syndrome is a chronic autoimmune process that primarily affects the exocrine glands and leads to their functional impairment. The exocrine gland involvement is characterized by a focal, mononuclear cell infiltrate which is accumulated around ducts and, in some patients, extends and replaces the secretory functional units. The mechanisms of this autoimmune 'exocrinopathy' are not fully understood. The immune attack that follows activation or apoptosis of glandular epithelial cells exposing autoantigens in genetically predisposed individuals may drive the immune-mediated tissue injury. Abnormalities related to the upregulation of type I interferon-regulated genes (interferon signature), abnormal expression of B-cell-activating factor (BAFF) and activation of the IL-23/TH17 pathway are among the immune mediators implicated in the pathogenesis of autoimmune lesions within the salivary glands. Such abnormalities demonstrate the complex interplay between innate and adaptive immunity that contributes to autoimmune 'exocrinopathy'.

***Keywords*:** Sjögren's syndrome, pathogenesis, cytokines, apoptosis, autoimmune exocrinopathy

Introduction

A chronic inflammatory process that primarily affects the exocrine glands, Sjögren's syndrome (SS) occurs either independently as a primary disease or as a secondary disease to other autoimmune rheumatic diseases [Ramos-Casals *et al.* 2005]. Affecting 0.5% of the general population, primary SS appears to be a common systemic autoimmune disorder whose prevalence is comparable to that of rheumatoid arthritis and whose ratio of 9:1 represents one of the highest female-to-male ratios among autoimmune diseases [Bowman *et al.* 2004]. Mounting clinical and laboratory evidence highlighting the central role of the epithelial cell in disease pathogenesis and evolution prompted the use of the term 'autoimmune epithelitis' as the aetiological name of this disorder [Moutsopoulos, 1994]. SS could, in general, be characterized as a chronic benign autoimmune condition displaying slow progression and low morbidity and mortality rates [Pertovaara *et al.* 2001; Martens *et al.* 1999]. However, lymphoma development, a serious complication of SS with an estimated prevalence of 5–10%, significantly increases the risk of premature mortality [Skopouli *et al.* 2000; Voulgarelis *et al.* 1999].

Clinical features

By and large, the course of SS is benign and relatively slow [Pavlidis *et al.* 1982]. With non-specific initial symptoms, it can commonly take 6 years before the condition is diagnosed. Periepithelial lymphocytic infiltration is a frequent characteristic of exocrine glands affected by SS, giving rise to functional impairment and diverse clinical manifestations. Being commonly affected and easily accessible, salivary glands are the most studied exocrine glands. The histopathological characteristics of minor salivary gland biopsy include: focal aggregates of at least 50 lymphocytes, plasma cells and macrophages adjacent to and replacing the normal acini; and the consistent presence of these foci in all or most of the glands in the specimen [Daniels, 1986]. Larger foci often display the formation of germinal centres (GCs). GC-like structures are found in 17% of minor salivary gland biopsies studied [Salomonsson *et al.* 2003]. Such pathological lesions are indicative of chronic lymphocytic sialadenitis. Moreover, B-cell hyperactivity, expressed by the presence of anti-SSA and anti-SSB autoantibodies, hypergammaglobulinemia and cryoglobulinemia, is a common finding in SS patients. Other autoantibodies found in

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50% of SS patients include autoantibodies against muscarinic M3 receptors that can induce decreased secretory function [Kovács *et al.* 2008]

Extraglandular manifestations in SS comprise two categories. The first refers to periepithelial organ involvement, such as interstitial nephritis, liver involvement and obstructive bronchiolitis, all of which manifest in the early stages of the disease and usually have a benign course [Papiris *et al.* 1999; Skopouli *et al.* 1994; Tu *et al.* 1968]. The second category includes extra-epithelial manifestations (palpable purpura, glomerulonephritis and peripheral neuropathy) that originate from an immune-complex deposition as a result of the ongoing B-cell hyperreactivity [Ramos-Casals *et al.* 2004; Tsokos *et al.* 1987]. This category is associated with increased morbidity and risk for lymphoma development [Skopouli *et al.* 2000; Voulgarelis *et al.* 1999].

Pathology

Studies have shown that the main histopathological feature of SS is the periductal cellular infiltration of the salivary glands, predominantly by T lymphocytes, whilst B and plasma cells are commonly observed in more severe lesions. Although monocytes, macrophages, dendritic cells (DCs) and natural killer cells constitute less than 5% of the total population, they play an important role in those glands with ectopic GC formation [Vogelsang *et al.* 2006]. Approximately 60–70% of T lymphocytes bear the CD4 phenotype, the majority of which exhibit the memory and/or inducer marker (CD45 RO). The αβ T-cell receptor is expressed by most of the infiltrating T cells [Skopouli *et al.* 1991]. The latter are activated, as attested by the membrane expression of human leukocyte antigen (HLA) class II molecules, interleukin-2 receptor (IL-2R), lymphocyte function-associated antigen 1 (LFA-1), Fas (CD95) molecule and interleukin (IL-2) production [Fox *et al.* 1994; Skopouli *et al.* 1991]. Studies using an immunoperoxidase technique to assess the isotypes of intracytoplasmic immunoglobulins of the plasma cells infiltrating the salivary glands of SS patients showed that the IgG and IgM predominate, as opposed to the plasma cells of normal salivary glands, where the IgA is dominant [Bodeutsch *et al.* 1992]. Furthermore, B cells in the lesion contain intracytoplasmic immunoglobulins with anti-Ro (SSA) and/or anti-La (SSB) reactivity [Bodeutsch *et al.* 1992].

Sjögren's syndrome: genetic background

The high incidence of autoimmune diseases noted in families of SS patients suggests a genetic predilection. Predictably, class II genes of the major histocompatibility complex (MHC) determine the basic development of the immune system and ultimately the immune response and are consequently considered to be implicated in SS pathogenesis. Nonetheless, non-MHC genes and their products regulate most aspects of immune responses. The best identified genetic factors for SS are located within MHC class II genes, mainly HLA-DR and HLA-DQ. Patients of diverse ethnic origin carry different HLA susceptibility alleles. North and West European Whites and North Americans show a higher prevalence of B8, DRw52 and DR3 genes, whereas Scandinavians and Greeks show linkages predominantly to the DR2 and DR5 genes [Bolstad and Jonsson, 2002]. However, it has been found that HLA class II alleles are associated with specific subsets of autoantibodies rather than with the disease itself [Gottenberg *et al.* 2003; Miyagawa *et al.* 1998]. Worthy of mention is the association of gene polymorphisms outside the HLA locus in SS with specific elements of its pathogenesis. One study of SS genetics, using a candidate gene analysis, has confirmed the genetic association of IRF5 rs2004640 T allele with predisposition to SS [Miceli-Richard *et al.* 2007]. Another study has found a correlation between IRF5 (CGGGG indel, single nucleotide polymorphism [SNP] rs10488631) and STAT4 (SNP rs7582694) polymorphisms and SS development in a Swedish and Norwegian cohort [Nordmark *et al.* 2009]. These findings indicate that a genetic susceptibility favouring a higher interferon (IFN) response to different stimuli could be a key event in the onset of perpetuation of the disease.

Pathogenesis: the implication of epithelial cells in the initiation and immunologic perpetuation

It has frequently been suggested over recent years that the activation and potential for antigen presentation of glandular or acinar epithelial cells play a central role in SS pathogenesis. Histopathological studies in newly diagnosed cases of SS showed that focal lymphocytic infiltration initiates around the ducts. Furthermore, staining of the labial salivary glands with anti-class II HLA monoclonal antibodies revealed that ductal and acinar epithelial cells inappropriately express these molecules [Moutsopoulos

et al. 1986]. Since both IFN-γ and tumour necrosis factor (TNF)-a trigger expression of histocompatibility antigen classes on the surface of epithelial cells and since both are produced by activated T cells, the question that arises is whether HLA-DR expression and possible antigen presentation by epithelial cells predates or is a consequence of the lymphocytic infiltration. Subsequent observations, however, suggest that the HLA class II molecule expression by epithelial cells may indicate a subtle activation of these cells. A study of proto-oncogene mRNA expression in the minor salivary glands of SS patients showed that the c-fos and c-jun were expressed by the epithelial glandular cells rather than by activated lymphocytes [Skopouli *et al.* 1992]. Owing to the restricted expression of c-myc, this phenomenon cannot be attributed to microenvironmental factors. Salivary gland epithelial cells also express B7 molecules that typically characterize classic antigen-presenting cells [Manoussakis *et al.* 1999]. These molecules play a critical role in the regulation of immune responses by providing activation or inhibitory signals to T cells through the ligation with CD28 or CTLA4 receptors, respectively. In a recent study, it was shown that B7 molecules expressed in the epithelial cells of primary SS patients are also functional, inducing costimulation signals in $CD4^+$ T cells [Kapsogeorgou *et al.* 2001b]. Furthermore, acinar epithelial cells express autoantigens on their membrane which, in conjunction with class II antigen expression and costimulatory signals, may potentially prime an autoimmune response. More specifically, translocation and membrane localization of the nuclear antigen La (SS-B) has been observed in conjunctival epithelial cells of SS patients [Yannopoulos *et al.* 1992]. Two possible mechanisms may be implicated, both of which play an important role in translocation, namely apoptotic phenomena (bleb formation, inside-out flip of the membrane) and also, according to a recent study, the expression of exosomes by cultured epithelial cells containing the major autoantigens Ro/SSA, La/SSB and SM. Expression of exosomes may constitute a novel pathway whereby intracellular autoantigens are presented to the immune system [Kapsogeorgou *et al.* 2005]. Epithelial cell lines derived from the salivary glands of SS patients spontaneously express the adhesion molecule ICAM-1, which seems to play an important role in the induction and maintenance of lymphocytic infiltrates of patients [Kapsogeorgou *et al.* 2001a]. Many studies have shown that chemokines such as CXCL13 and CCL21 are produced by the epithelial cells in the chronic inflammatory lesions of SS patients [Salomonsson *et al.* 2003, 2002; Xanthou *et al.* 2001]. These chemokines orchestrate leukocyte microenvironmental homing and contribute to the formation of lymphoid structures. The expression pattern of 'lymphoid' chemokine mRNA points further to the role of epithelial cells in the pathogenesis of SS and offers a new insight into the mechanisms that could potentially be involved in leukocyte homing and in the *in situ* formation of secondary lymphoid tissue structures.

Interestingly, all of the above-described autoimmune mechanisms linking the innate and adaptive immune system in SS could be induced by type I IFN interference. Gene expression analysis of minor salivary gland tissue from SS patients revealed enhanced IFN-inducible gene expression, whereas immunohistochemistry revealed plasmacytoid DCs in SS salivary glands, indicating that the persistence of type I IFN signature could be related to an inflammatory cycle associated with an inappropriate maturation of DCs, stimulation of autoreactive T cells, autoantibody production and increased endogenous INF-α production [Gottenberg *et al.* 2006; Bave *et al.* 2005]. The early presence of DCs in the salivary glands and their type I IFN production could be significant, since they can cause abnormal retention of lymphocytes in the tissues and the subsequent activation of lymphocytes and metalloproteinases [Ma-Krupa *et al.* 2004]. It has been proposed that viral infections or RNA-containing immune complexes acting through Toll-like receptors (TLRs) are responsible for plasmatocytoid DC activation [Bave *et al.* 2005]. The combination of apoptotic bodies from epithelial cells and anti-SSA antibodies in the salivary glands of patients with SS may trigger IFN-α production by cell infiltration which, in turn, could induce B-cell-activating factor (BAFF) expression by epithelial cells, leading to the stimulation of autoreactive B cells [Ittah *et al.* 2009, 2006; Bave *et al.* 2005]. However, a recent study demonstrated that repeatedly injecting NZB/W F1 mice with TLR3 ligand poly(I:C) incited a rapid induction of type I IFN and proinflammatory cytokines within the salivary glands, resulting in a significant and rapid loss of function [Deshmukh *et al.* 2009]. It has been suggested that the salivary epithelial cells activated by TLR3 ligand are the major source of

type I IFNs within the gland. This study further reinforces the notion that glandular epithelial cells play an important and active role in the disease pathogenesis and, moreover, that chronic viral infection of salivary epithelial cells bears the potential for initiation of salivary inflammation. All of these suggest that epithelial cells have a key role in autoimmune lesions in SS and more specifically their potential to produce locally a broad variety of B-cell targeted cytokines and survival factors including BAFF, demonstrating that the interplay between epithelial cells, lymphocytes, and DCs prompts chronic B-cell hyperactivity, the prominent immunoregulatory abnormality in SS [Vogelsang *et al.* 2006; Lavie *et al.* 2004].

Clinically and laboratorially, B-cell deregulation is manifested by the presence of circulating immune complex, hypergammaglobulinemia, alterations in peripheral B-cell subpopulations, oligoclonal B-cell expansion and the well-described increased risk of lymphoma development [Fox, 2005; Zintzaras *et al.* 2005]. Ectopic GC-like structure formation is considered the histologic trait of the abnormal B-cell proliferation [Fox, 2005]. Proposed as the locus of autoantibody production, newly formed ectopic GCs have been implicated in the SS-associated lymphomagenesis [Salomonsson *et al.* 2003]. Interestingly, ectopic GC formation in SS is also associated with a higher degree of glandular inflammation, elevated titres of rheumatoid factor, increased levels of autoantibodies and increased IgG levels [Le Pottier *et al.* 2009; Jonsson *et al.* 2007]. Szodoray and colleagues, using a broad-spectrum bead-based immunoassay and multivariate analysis, discovered reliable biomarkers that identified the presence of GC in SS patients, amongst which CCL11 (Eotaxin), BAFF and IFN-γ proved to have the strongest discriminatory capacity. This study indicates that GC formation is a complex process that may be regulated by an array of cytokines, chemokines, adhesion molecules and lymphoid-stimulating factors [Szodoray *et al.* 2005].

Mounting evidence supports that BAFF is an important mediator in the neogenesis of GC in SS. The abundant BAFF expression resulting from reduced levels of apoptosis in SS salivary gland cells, amplifies B-cell signalling and promotes their regional proliferation and differentiation to autoantibody producing plasma cells [Sutherland *et al.* 2006; Mariette *et al.* 2003]. BAFF transgenic mice develop a lupus-like disease but at a later stage they display salivary gland infiltration reminiscent of SS. A feasible explanation for SS-like disease in BAFF transgenic mice lies in the excessive survival signals to autoreactive B cells, overcoming the critical tolerance checkpoint while maturing in the spleen [Groom *et al.* 2002]. Taking into consideration that BAFF can be secreted by human salivary gland epithelial cells following type I IFN stimulation and that viral infection directly induces BAFF secretion by epithelial cells, it has been suggested that epithelial cells not only express and present autoantigens but can also concomitantly activate B cells by the local secretion of BAFF [Ittah *et al.* 2009, 2006]. It is important to note that the promising results of B-cell depletion therapy in SS, support the important role of B-cell deregulation in the pathogenesis of the disease. Therefore, in addition to rituximab trial other types of B-cell depletion therapies should be assessed including anti-BAFF agents.

Sjögren's syndrome: lessons from animal models

Although controversy surrounds the use of animal models, we cannot dismiss their value in confirming the pathogenetic aspect of human disease. In this regard, studies using various mouse models of SS have begun to provide new insights into the mechanisms pivotal to the understanding the disease pathogenesis. It is already known that salivary glands of the nonobese diabetic (NOD) mouse model of SS are characterized by the presence of multiple alterations in glandular homeostasis even before the onset of lymphoid salivary infiltration. Such abnormalities include altered cell proliferation at the time of birth, upregulated apoptosis of acinar tissues, proteolysis of secreted proteins and increased expression of IFN-γ [Cha *et al.* 2002]. Aberrant proteolysis may play a role in the generation of otherwise hidden cryptic antigens, thereby priming the immune system for an autoimmune response [Casciola-Rosen *et al.* 1995]. An analysis of these data indicates the presence of underlying nonimmune components associated with regulation of glandular homeostasis that may initially trigger the disease independently of immune cells. SS patients have also been reported to display abnormal glandular homeostasis. Compared with normal controls, biopsies from SS patients showed significant increases in laminin protein and laminin mRNA expression preceding lymphocytic infiltration, suggesting that an alteration in basement

membrane synthesis is an early event associated with salivary gland pathology in SS [McArthur *et al.* 1997]. Interestingly, the human salivary gland cell line showed upregulation of laminin and metalloproteinase-9 (MMP-9) expression when treated *in vitro* with IFN-γ [Daniels *et al.* 1999; Wu *et al.* 1997]. Elevated levels of MMP concentration in the saliva of SS patients could account for the increased remodelling of basement membrane proteins, such as laminin, and/or structural destruction of the basement membrane scaffolding in the salivary glands [Konttinen *et al.* 1998]. These observations highlight the implication of the intrinsic properties of salivary glands (availability of antigens) in the breakdown of peripheral tolerance and the activation of autoreactive immune cells.

Sex hormones influence humoral and cell-mediated immune responses and oestrogen is one of the factors responsible for gender immunologic dimorphism. In a model of SS (RbAp48-Tg mice), the overexpression of retinoblastoma-associated protein 48 (a gene specific for oestrogen deficiency-dependent apoptosis) in the exocrine glands caused an age-dependent SS-like autoimmune exocrinopathy [Ishimaru *et al.* 2008]. The salivary epithelial cells of these transgenic mice behaved as antigen-presenting cells, upregulating MHC class II, secreting IFN-γ and IL-18, leading to $CD4^+$ T-cell activation and glandular inflammation. This organ-specific autoimmunity was successfully achieved by an adaptive transfer of lymph node T cells from the above mice into oestrogen-deficient $Rag2^{-/-}$ mice. These findings strongly suggest that the upregulation of RbAp48 is responsible for the oestrogen deficiency-related association of the salivary epithelial cell potential to present an endogenous autoantigen to $CD4^+$ T cells that induces lesions in the salivary glands resembling human SS. Most importantly, consistent with the findings in RbAp48-Tg mice, salivary epithelial cells from SS patients simultaneously express RbAp48 and IFN-γ or IL-18 suggesting that this molecule presents an interesting reference connecting oestrogen deficiency and autoimmune exocrinopathy.

Similarly, another study that evaluated the effects on antigen cleavage in oestrogen-deficient healthy C57BL/6 (B6) mice demonstrated that a significantly increased apoptosis in the salivary glands through caspase-1 activation was associated with a α-fodrin cleavage [Ishimaru *et al.* 2003]. Inflammatory lesions that developed exclusively in the salivary glands after the adaptive transfer with α-fodrin-reactive T cells in ovariectomized B6 mice suggest that the α-fodrin cleavage during oestrogen deficiency-induced apoptosis plays an important role in the development of autoimmune exocrinopathy in SS. Moreover, since it has been reported that Fas is observed in the salivary epithelial cells of SS patients, it is likely that Fas-mediated apoptosis and autoantigen cleavage through caspase cascade may reveal immunocryptic epitopes that could potentially trigger an autoimmune response [Kong *et al.* 1997]. These findings are of particular interest since SS patients are mostly postmenopausal woman with oestrogen deficiency, which guides our attention toward sex hormone deregulation in the pathogenesis of SS.

Recent evidence in animal models and SS patients demonstrates the complex interaction between innate and adaptive immunity in salivary glands. Notably, one study revealed that the upregulation of inflammatory caspase-1 in macrophage and DCs at 8 weeks of age in C57BL/6.NOD-Aec1Aec2 mice concurred with increased apoptosis in the salivary glands [Bulosan *et al.* 2009]. The caspase-1, which cleaves pro-IL-1β and IL-18 to produce mature forms of IL-1β and IL-18, was associated with an elevated level of apoptotic cell death in the neighbouring epithelial cells. It has been indicated that IL-18 produced by phagocytic cells may up-regulate FasL on natural killer cells and Fas on epithelial cells through caspase-1 activation, resulting in subsequent apoptosis in the epithelial cells. It should be noted that IL-18, originally described as an IFN-γ inducing factor, is a potent inflammatory stimulant known to enhance antigen-specific expansion of IFN-γ producing T cells. Consequently, it is considered that synergistic effects between cytokines IL-1β and IL-18 in the salivary gland microenvironment induced by macrophages and/or DCs, as well as IFN-γ produced by salivary epithelial cells, form part of an essential process of chronic inflammation that precedes the $CD4^+$ T lymphocytic infiltration of the affected glands. The duration of DCs stimulation of lymphocytes may influence the outcome of immune response. Moreover, DC apoptosis could favourably affect their life span to preserve self-tolerance. Interestingly, a recent report indicated that blocking DC apoptosis in a Bim deficiency mouse model led to a robust T-cell activation, autoantibody production and

systemic autoimmunity [Chen *et al.* 2007]. Therefore, if we consider that IL-18 expression by macrophages is strongly and positively correlated with glandular lymphoid infiltration and bear in mind that IL-1β and IL-18 have been reported to be upregulated in both sera and the salivary glands of SS patients, it becomes apparent that the potentially critical role of macrophages and DC population in the early pathogenesis of SS should not be underestimated [Manoussakis *et al.* 2007; Bombardieri *et al.* 2004; Szodoray *et al.* 2004].

So far, studies have focused less on the alteration in the function of DCs in patients with SS and more on plasmacytoid DCs, the main cellular population for secretion of type I IFN. On the other hand $Id^{-/-}$ mice, a model which develops many disease symptoms found in SS patients, offers a wonderful opportunity to study in detail the relationship between the various cells of the immune system and autoimmunity occurring in the context of a single genetic lesion [Li *et al.* 2004]. Id3 an inhibitory transcription factor of the basic helix–loop–helix family inhibits the development of plasmacytoid DCs [Heemskerk *et al.* 1997]. Notably, plasmacytoid DCs have been claimed to induce tolerance against environmental antigens in the lung [de Heer *et al.* 2004]. Furthermore, data indicate that plasmacytoid DCs have a pivotal role in autoimmunity. Therefore, it can be hypothesized that abnormalities in plasmacytoid DCs caused by Id3 deficiency lead to aberrant presentation of autoantigen, a process capable of stimulating highly responsive autoreactive T cells which had escaped thymic selection. Regardless, of the initiating trigger, type I IFN constitutes an important link between innate and adaptive immune response through induction of various cytokines, upregulation of MHC class I, II and costimulatory molecules on antigen-presenting cells.

Evaluation of cytokine mRNA expression in the salivary glands of SS patients and animal models favoured an increase in several pro-inflammatory cytokines, including IL-1β, IL-6, IL-7, IL10-, IFN-γ and TNF-α [Kolkowski *et al.* 1999; Robinson *et al.* 1998a, 1998b]. On the other hand, Th-2 cytokine mRNA, such as IL-4 and IL-5, were occasionally detected in association with strong B-cell accumulation in the salivary glands of SS patients [Ohyama *et al.* 1996]. Generally, Th2 cytokines are thought to dominate the early phase of human SS, as opposed to Th1 cytokines that are associated with a later stage of the disease [Mitsias *et al.* 2002]. In contrast, studies of NOD mice revealed that the decrease in salivary flow, following the emergence of glandular inflammation, may be associated with Th2 cytokines [Nguyen *et al.* 2007; Brayer *et al.* 2001]. While the above observations suggest the implication of both Th1 and Th2 cell-associated functions in the onset of clinical disease, a recent study identified the presence of $CD4^+$ Th17 memory cells within the lymphocytic foci of the salivary and lacrimal glands of SS-susceptible C57BL/6.NOD-Aec1Aec2 mice, indicating a much greater complexity [Nguyen *et al.* 2008]. It has been suggested that the process of autoimmune attack against the exocrine gland tissues is characterized by reduced Treg1 and IL-27-dendritic cell function that would normally negatively regulate $CD4^+$ Th17 cells, allowing their aberrant activation [Bettelli *et al.* 2008]. Potential candidate genes located within the redefined Aec2 interval could account for this Treg1 cell abnormality [Baum *et al.* 1994]. Based on this assumption, it could be hypothesized that a pathway from the Tnfsf4 gene encoding the OX40 ligand influences Treg1 function [Ito *et al.* 2006]. Since Tnfsf4 expression is downregulated during the development of the disease, the imbalance of Th17/Treg1 ratio presents an interesting basis for a better understanding of the autoimmune process in SS.

The implication of IL-23/Th17 pathway in SS pathogenesis has been further supported by recent data from $Ro52^{-/-}$ mice that developed systemic autoimmunity, characterized by enhanced production of pro-inflammatory cytokines regulated by interferon regulatory factor (IRF), including the T17 [Espinosa *et al.* 2009]. Interestingly, loss of IL-23/IL-17 by genetic deletion of IL-23/p19 in the $Ro52^{-/-}$ mice conferred protection from systemic autoimmunity suggesting that a defective Ro52 function can lead to tissue inflammation and systemic autoimmunity through the IL-23/IL-17 pathway. Similarly, SS patients display increased serum IL-17 levels as well as increased Th17 cells and related cytokines that are found to be dominant in salivary glands and to be strongly correlated with the histological focus score [Katsifis *et al.* 2007]. Several mouse models have further demonstrated that the salivary gland is a target of the multiorgan autoimmune inflammatory syndrome associated with Treg1 cell-deficient mouse strains [Sharma *et al.* 2006]. According to recent data, Treg1

cell population in minor salivary gland lesions of SS patients correlates with histological focus score and certain risk factors for lymphoma development [Christodoulou *et al.* 2008]. While in early and moderate infiltrations a compensatory control of Treg1 against Th17 expansion seems to occur, in advanced lesions Treg1 fail to control immune-mediated tissue injury [Katsifis *et al.* 2007]. Although these observations intimate the important role of Treg1 in the pathogenesis of SS, the precise mechanism of Treg1 implication in the whole process still eludes us.

Conclusions

The aetiology and pathogenesis of SS are not clearly understood. The hallmark of this disease is an immunologically mediated inflammatory exocrinopathy that is initially characterized by periductal infiltration of the salivary tissue by lymphocytes and plasma cells. Epithelial cell apoptosis may provide cellular proteins as autoantigens which subsequently drive the autoimmune response in SS. It is plausible that lymphocyte infiltrates may represent secondary phenomenon rather than being the main orchestrator of the disease. Sex hormone deregulation, abnormalities of glandular homeostasis, Treg1 deficiency and plasmacytoid DC deregulation comprise an important network in the breakdown of peripheral tolerance and activation of autoreactive immune cells. Eventually, these lymphocytes promote autoantibody production and further damage of epithelial cells by cytotoxic mechanisms.

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Conflict of interest statement

None declared.

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