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INVITED REVIEW

Sjögren's syndrome: why autoimmune epithelitis?

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The expression 'autoimmune epithelitis' has been proposed as an alternative for Sjögren's syndrome (SS) based on data pointing out the central role of the epithelial cell in the pathogenesis of the syndrome. Clinically, apart from exocrine glands that are the main target, the epithelial component of the other organs such as kidneys, liver, lungs or thyroid is commonly affected resulting in various extraglandular manifestations. On the other hand, at the molecular and cellular level, the epithelial cell plays a major role in the initiation and perpetuation of the autoimmune lesion. Mechanisms such as antigen presentation, apoptosis, chemokine production or germinal center formation lie in the center of SS pathogenesis and the epithelial cell has a very important role. Herein, we present both aspects, review the data that support the proposed terminology and finally, suggest a unifying theory for the pathogenesis of SS.
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Keywords: Sjögren's syndrome; autoimmune epithelitis; extraglandular manifestations; epithelial cell; antigen-presenting cell; apoptosis

Introduction

Autoimmune attack of epithelial tissues is the main characteristic of Sjögren's syndrome (SS). Ocular and oral dryness are the main presenting symptoms as the lymphocytic invasion of salivary and lacrimal glands results in their functional impairment. SS is a relatively common chronic autoimmune disease affecting approximately 1–2% of total population and may occur either alone (primary SS) or along with other autoimmune diseases (mainly systemic lupus erythematosus or rheumatoid arthritis, secondary SS). Its progress is benign unless certain adverse prognostic factors are present, such as low C4 complement levels, mixed monoclonal cryoglobulinemia or palpable purpura. Patients with these factors are at high risk for B-cell (mucosa-associated lymphoid tissue; MALT) lymphoma and/or glomerulonephritis (GMN) leading to increased mortality (Skopouli *et al*, 2000).

Sjögren's syndrome has been characterized as a systemic disorder with features of both organ-specific and systemic autoimmunity (Kassan and Moutsopoulos, 2004). Indeed, apart from exocrine glands, other organs such as kidney, liver, lungs or thyroid may be involved with the primary target, their epithelial component. This observation has led to the alternative term, 'autoimmune epithelitis' (Moutsopoulos, 1994) pointing out the fact that SS may be considered as a cell-specific autoimmune disease.

In this review, we summarize the data that support the new terminology and organize them in two axes. The first focuses on the clinical and pathological studies regarding SS and the target organs; emphasis is given on extraglandular sites and their participation in the pathogenesis of the syndrome. Secondly, we review studies that reveal the key role of the epithelial cell in the initiation and perpetuation of the autoimmune lesion through specific molecular mechanisms. Finally, we propose a theory that unifies these data and addresses the question of the initiating offending factor that leads to epithelial dysfunction.

Epithelial cell as a target – clinical manifestations

Exocrine glands

Sjögren's syndrome is classically characterized by oral (xerostomia) and ocular dryness (xeropthalmia). In a recent analysis, the presence of both dry mouth and dry eyes classified patients with 93% sensitivity and 97.7% specificity (Al-Hashimi *et al*, 2001). According to the American–European consensus group, six criteria are proposed for the SS diagnosis (Table 1), four of which include subjective symptoms and objective signs of oral and ocular dryness (Vitali *et al*, 2002). Chronic persistence of decreased salivary flow leads to depapillation of the tongue, increased incidence of dental caries, superficial mouth ulcers and cheilitis (Figure 1a) (Ravald and List, 1998). Likewise, ocular manifestations may include erosions of the cornea, conjunctivitis and in severe cases corneal ulceration (Kassan and Moutsopoulos, 2004). The most important sequel of SS, although, is the

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Table 1 Classification criteria for Sjögren's syndrome (SS) as proposed by the American–European consensus group (Vitali *et al*, 2002)

1. Ocular symptoms
2. Oral symptoms
3. Ocular signs – positive Schirmer's test or rose bengal stain
4. Histopathology – positive minor salivary gland biopsy
5. Oral signs – positive whole salivary flow or parotid sialography or scintigraphy
6. Serology – positive anti- Ro/SS-A or anti-La/SS-B or both
Definite primary SS:
Presence of any 4 out of 6 (obligatory no. 4 or no. 6)
Presence of any 3 out of 4 objective (no. 3 to no. 6) criteria

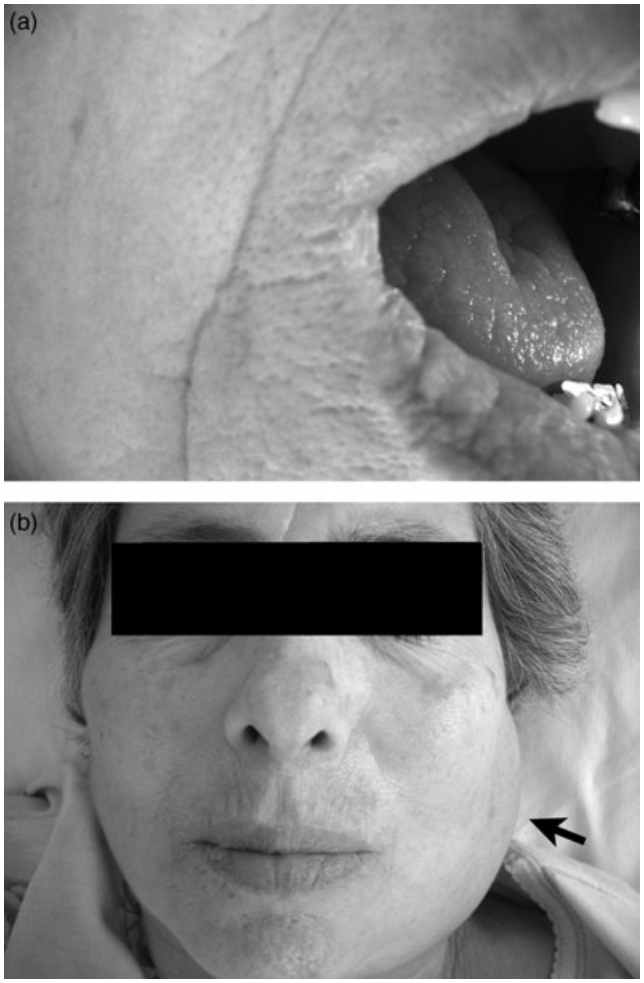

Figure 1 (**a**) A female patient with primary Sjögren's syndrome; angular cheilitis and dry tongue with atrophic papillae are evident. Note also that this patient lost many teeth while the rest are with fillings, (**b**) Primary Sjögren's syndrome patient with persistent left parotid gland enlargement (arrow)

progression of benign lymphoproliferation to B-cell lymphoma (mainly marginal zone lymphoma of the MALT); the relative risk is approximately 20 and major risk factors are low C4 levels, palpable purpura and the presence of mixed monoclonal cryoglobulinemia (Skopouli *et al*, 2000; Zintzaras *et al*, 2005). Clinically, the presence of significant salivary gland enlargement (Figure 1b), lymphadenopathy and skin vasculitis should raise the suspicion of lymphoproliferation (Table 2).

Table 2 Characteristics of lymphoma development in Sjögren's syndrome (Voulgarelis *et al*, 1999; Skopouli *et al*, 2000)

Clinical findings
Major (mainly, bilateral parotid) gland enlargement
Lymphadenopathy
Skin vasculitis
Peripheral nerve involvement
Low-grade fever
Splenomegaly
Laboratory findings
Mixed monoclonal cryoglobulinemia
Low C4 levels
Anemia/lymphopenia
Hypogammaglobulinemia

The hallmark of SS, as it appears in labial minor salivary gland (LMSG) biopsies, is the lymphocytic infiltration of the tissues (Figure 2a). In early lesions, the infiltrate primarily consists of activated T cells (increased $CD4^+$ to $CD8^+$ ratio) and tends to form around the ductal epithelium. In advanced chronic lesions, B cells predominate and the infiltrate extends to occupy the acinar epithelium as well. Macrophage and dendritic cells appear in intense lesions with germinal center (GC) formation (Xanthou *et al*, 1999). Decreased production of saliva is associated with ongoing accumulation of immune cells around the epithelia and the progressive destruction of the glandular architecture. Similar changes seem to be responsible for lacrimal gland dysfunction as well, as the lacrimal glands of SS patients are enlarged and prolapse with concomitant lymphocytic infiltration (Parkin *et al*, 2005).

Kidney

One of the target organs that is very often involved in SS is the kidney (Table 3). The major clinicopathologic entity is interstitial nephritis (IN) which may appear early in the progress of the disease or even may precede the onset of subjective sicca symptoms (Bossini *et al*, 2001). Distal renal tubular acidosis (RTA) type I, decreased concentrating ability and RTA type II are the most frequent clinical presentations. However, it should be noted that overt renal disease due to IN is relatively infrequent ($< 3\%$) and follows a rather benign course (Goules *et al*, 2000). Histologically, IN resembles the lesion of LMSG (Figure 2b). Lymphocytic infiltration (mainly $CD4^+$) predominates around tubular epithelial cells, plasma cells and monocytes are present and tubular atrophy and interstitial fibrosis may ensue (Rosenberg *et al*, 1988; Goules *et al*, 2000). Moreover, apoptotic death of tubular epithelia seems to play important role (Matsumura *et al*, 1998a); this is another common feature with LMSG pathology, as will be discussed in detail later.

Apart from IN, to a lesser degree, GMN is present in patients with SS and renal involvement. Hypertension, mild proteinuria and microscopic hematuria are the major signs and in contrast to IN, tend to present late in the progress of the disease (Skopouli, 2001). The

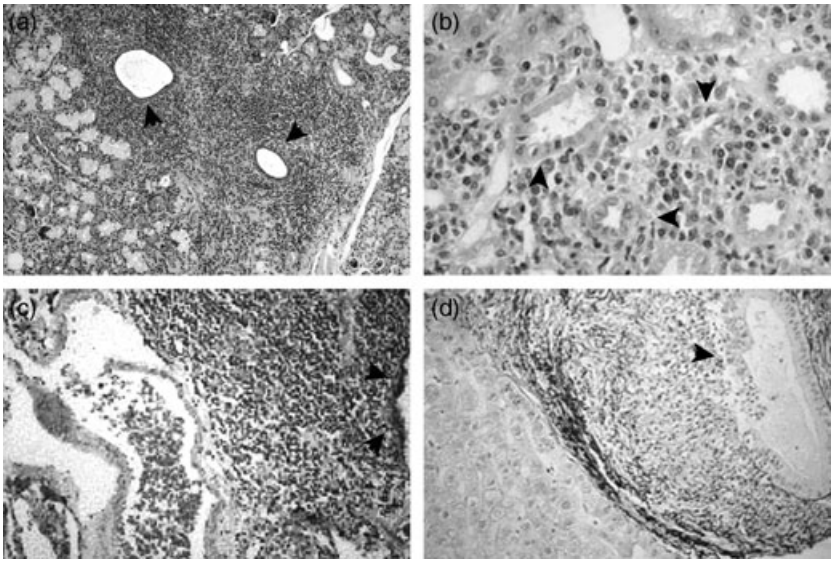

Figure 2 Lymphocytic infiltrates around the epithelial components (arrowheads) of various tissues, characteristic of Sjögren's syndrome. (**a**) Minor labial salivary gland biopsy, (**b**) tubular epithelia of a renal biopsy, (**c**) lung biopsy and (**d**) liver biopsy

Table 3 Extraglandular (systemic) manifestations of Sjögren's syndrome (Kassan and Moutsopoulos, 2004)

Fatigue
Hypothyroidism
Musculoskeletal involvement
Arthralgias
Myalgias
Fibromyalgia
Skin involvement
Dryness
Skin vasculitis
Raynaud phenomenon
Renal involvement
Tubulo-interstitial nephritis
Glomerulonephritis
Pulmonary involvement
Xerotrachea and chronic cough
Bronchiolitis (small airway obstruction)
Interstitial pneumonia (IP) – non-specific IP, lymphocytic IP, usual IP
Pulmonary lymphoma
Gastrointestinal involvement
Atrophic gastritis
Autoimmune cholangitis (similar to primary biliary cirrhosis stage I)
Autoimmune pancreatitis (commonly subclinical)
Peripheral neuropathy
Hematologic involvement
Lymphadenopathy, splenomegaly
Leukopenia, thrombocytopenia
Benign monoclonal B cell proliferation
Non-Hodgkin's lymphoma (low- or intermediate-grade B cell MALT lymphoma)

pathogenesis in this case seems to be completely different as it may involve immune complex deposition and complement activation (Moutsopoulos *et al*, 1978) as it is underlined by the strong association of GMN with low C4 levels and the presence of mixed monoclonal cryoglobulinemia (Skopouli *et al*, 2000). Of importance, the latter two are also strong predictors for lymphoma formation and, not surprisingly, GMN and lymphoma tend to coexist in SS patients.

In conclusion, renal involvement in the form of IN, although frequent, is largely subclinical for the majority of the patients with SS. Of those who present with overt renal disease (approximately 5% in a cohort of 471 patients) the percentages of IN and GMN are almost the same (Goules *et al*, 2000). The latter should be seen more as part of a generalized vasculitic syndrome, and due to the grave prognosis promptly receive the proper therapeutic intervention.

Lungs

The exact nature of pulmonary involvement in patients with SS has been a matter of debate over the past years. Depending on the methodology applied, pulmonary findings were evident in up to 75% of patients (Constantopoulos *et al*, 1985; Hatron *et al*, 1987; Matsuyama *et al*, 2003) but clinically significant disease approximates 10% (Constantopoulos and Moutsopoulos, 1986; Davidson *et al*, 2000). Dry cough is the most common and usually the only symptom and is a consequence of xerotrachea. Subclinically, abnormal pulmonary tests reveal small airway obstruction, while a detailed high-resolution CT scan detected predominantly wall thickening at the segmental bronchi (Papiris *et al*, 1999). Early studies using bronchoalveolar lavage showed increased numbers of $CD4^+$ lymphocytes (Wallaert *et al*, 1987; Dalavanga *et al*, 1991) and subsequent reports with bronchial biopsies focalized the inflammatory cells subepithelially, involving not only the glandular but the extraglandular tissues as well (Papiris *et al*, 1997). Once again, the characteristics of this peribronchial inflammation highly resemble the histological picture of LMSG biopsies of SS patients (Figure 2c).

The histological pattern of pulmonary lesion changes radically when the population under study has clinically evident lung involvement. In this case, interstitial expansion of the inflammatory process is frequent and may take various forms. Non-specific interstitial pneumonia seems to be the most frequent (Ito *et al*, 2005),

but lymphocytic IP and the usual IP are also present (Deheinzelin *et al*, 1996; Yamadori *et al*, 2002). Notably, these patterns are not clear-cut as they may coexist or evolve in the same individual along with the progress of the disease.

Whether IP comes on as a result of more severe bronchiolitis in those patients who finally become symptomatic is not known. It would be rational, however, to consider that an initial subclinical insult in the form of subepithelial peribronchial inflammation, in a selected minor percentage of patients, expands to the interstitial tissues and becomes clinically apparent.

Finally, ongoing lymphocytic proliferation may progress to pulmonary MALT lymphoma. It involves approximately 1–2% of primary SS patients (Cain *et al*, 1998) and as in the case of salivary glands the main histopathologic pattern is that of low-grade marginal zone lymphoma (Royer *et al*, 1997; Ito *et al*, 2005).

Liver

Liver involvement in patients with autoimmune diseases is not very common and, when present, it usually follows a benign progress. Moreover, even in the case of hepatic injury, it is very difficult to classify the lesion as primary with associated autoimmune manifestations or as an autoimmune disease with secondary liver involvement (Youssef and Tavill, 2002). This is especially prominent in the case of SS and primary biliary cirrhosis (PBC). In a study of 300 SS patients, approximately 7% had elevated liver enzymes and were positive for antimitochondrial antibodies and almost all had histological changes similar to stage-I PBC (Figure 2d). None, though, proceeded to liver damage compatible with cirrhosis (Skopouli *et al*, 1994). On the other hand, 38% of PBC patients were positive for antibodies against the SS antigen SS-B/La and 95% had histological changes in LMSG similar to focal sialadenitis (Hansen *et al*, 1988). Of special importance is the similarity of the histological lesion in the two diseases. Both are characterized by lymphocytic infiltrates (with predominance of $CD4^+$ cells) that initiate around ductal epithelium (salivary or bile ducts), while these epithelial cells inappropriately express HLA class II molecules (Abraham *et al*, 2004). Conclusively, in view of the preservation of hepatic architecture and the benign progress of the lesion, the hepatic involvement in SS can be characterized mainly as autoimmune cholangitis (AIC) (Moutsopoulos, 1994), especially given the fact that the immunohistochemical findings in AIC and PBC are practically identical (Kaserer *et al*, 1998).

Thyroid

Since the first description of thyroid dysfunction in SS 40 years ago (Bertram and Halberg, 1965), many studies point out that autoimmune thyroid insult is relatively common, ranging from 10% to almost 50% (Karsh *et al*, 1980; Hansen *et al*, 1991; Perez *et al*, 1995; D'Arbonneau *et al*, 2003). Discrepancies regarding the exact percentages or even the relation between the two (Ramos-Casals *et al*, 2000) can be attributed to methodological and/or laboratory differences and more importantly, to the fact that both entities refer to the same population (that is middle-aged women). In any case, autoimmune thyroiditis (AT) (primarily, Hashimoto thyroiditis and to a lesser extend, Graves' disease) is the main clinical form and may precede or follow the SS diagnosis by many years. Primary finding is the presence of autoantibodies against thyroid peroxidase (anti-TPO), thyroglobulin (anti-TG), T3 and/or T4. The presence of anti-TPO/anti-TG or rheumatoid factor and anti-Ro/SS-A in the sera of SS patients can be used as primary indicators of those that are prone to develop thyroid disorders in the future (D'Arbonneau *et al*, 2003).

Histological studies of AT specifically in SS patients are very sparse (Hansen *et al*, 1991). The histological picture of Hashimoto thyroiditis *per se* however, is highly similar to that of SS (D'Arbonneau *et al*, 2003). The infiltrate consists primarily of $CD4^+$ T lymphocytes, the thyroid epithelial cells express HLA molecules class II and adhesion molecules while GC formation and progress to B cell MALT lymphoma is probable (Hanafusa *et al*, 1983; Aichinger *et al*, 1985; Tandon *et al*, 1992); Hashimoto thyroiditis may evolve to lymphoma in 0.5% of patients (Thieblemont *et al*, 2002). Moreover, one-third of patients with AT have SS features (Coll *et al*, 1997) and one of 10 ANA-positive AT patients shares the diagnosis of SS (Tektonidou *et al*, 2004). Therefore, as in the case of SS and PBC, SS and AT are two autoimmune entities closely related pathogenetically, as the target in both cases seems to be the epithelial cell.

Epithelial cell as a conductor – molecular mechanisms

As aforementioned, the main target organs of SS are exocrine and especially salivary glands. MLSG biopsies are routinely performed to establish the diagnosis (Vitali *et al*, 2002), they can be easily obtained and therefore the majority of the studies regarding the pathogenetic mechanisms of SS are carried out using this tissue.

Epithelial cell: a non-professional antigen-presenting cell

Professional antigen-presenting cells (APC) are dendritic cells, macrophages and B cells. These cells present antigen in the context of MHC molecules and express co-stimulatory and adhesion molecules to form immunologic synapsis with T cells (Montoya *et al*, 2002). Extensive data show that epithelial cells in SS are activated and capable to act as non-professional APC (Figure 3).

Indeed, salivary gland epithelial cells (SGEC) express MHC class I and MHC class II (HLA-DR) molecules as well as functional co-stimulatory B7.1 (CD80) and B7.2 (CD86) molecules (Moutsopoulos *et al*, 1986; Manoussakis *et al*, 1999; Kapsogeorgou *et al*, 2001a; Matsumura *et al*, 2001). Thus, they provide both signals necessary for T cell activation. B7 molecules bind CD28 on T cells (leading to activation), as well as CTLA-4, which is a negative regulator of T cells; the affinity of B7 for CTLA-4 is higher compared with CD28 and leads to

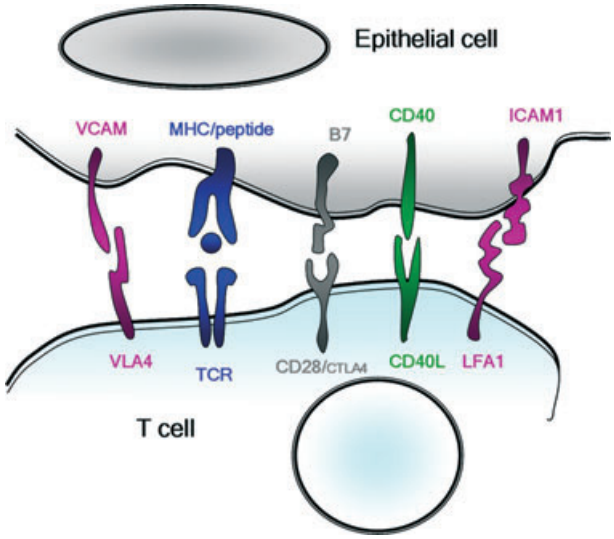

Figure 3 Epithelial T-cell interaction. The epithelial cell expresses all the molecules necessary for optimal T-cell response and therefore is a potent non-professional antigen-presenting cell

self-limitation of immune reaction. Interestingly, B7.2 molecules expressed by SGEC were found to have higher affinity for CD28 compared with the negative regulator CTLA-4 (Kapsogeorgou *et al*, 2001a). Intercellular adhesion molecule-1 (ICAM1) and vascular cell adhesion molecule (VCAM) are expressed on APCs and ensure stabilization of the APC/T cell synapsis. Epithelial cells on LMSG biopsies and cultured SGEC lines from SS patients express both ICAM1 and VCAM (Kapsogeorgou *et al*, 2001b; Tsunawaki *et al*, 2002). Moreover, SGEC express functional CD40 molecules and CD40L is highly expressed on the infiltrating lymphocytes (Dimitriou *et al*, 2002; Ohlsson *et al*, 2002a). The consequences of this interaction are not fully clarified but are thought to augment the inflammatory response either by directly activating T cells or by enhancing co-stimulatory and adhesion molecules, such as ICAM1, on the APC (Grewal and Flavell, 1998). Toll-like receptors (TLRs) recognize distinct pathological molecular patterns, and upon binding, lead to cytokine production and upregulation of co-stimulatory and adhesion molecules. In this way, they link innate to adaptive immunity (Takeda *et al*, 2003). Recent data from our group indicate that SGEC express functional TLRs, further implicating the epithelial cell in the local inflammatory responses (Spachidou *et al*, 2005). Finally, SGEC produce proinflammatory cytokines such as tumor necrosis factor-α, interleukin (IL)-1 and IL-6, significantly contributing to the inflammatory milieu (Boumba *et al*, 1995).

Interestingly, similar properties are identified for tubular epithelial cells. Indeed, B7.2 as well as ICAM and VCAM were present in renal biopsies from SS patients with IN (Matsumura *et al*, 1998b, 2001), linking the extraglandular manifestations with epithelial activation. Whether this activation results from intrinsic capacity of the epithelial cell (including a possible infectious agent) or is dictated by the extrinsic autoimmune reaction is an open question. The answer is probably both. SGEC from SS patients, even after long-term cultures *in vitro*, keep a constitutively high expression of the molecules mentioned above, indicative of intrinsic activation. On the other hand, various factors seem to control their expression; e.g. cytokines such as IFN-γ consistently upregulate both adhesion and co-stimulatory molecules.

Chemokines and germinal center formation

As discussed elsewhere, salivary gland dysfunction is related to the ongoing accumulation of lymphocytes and destruction of glandular architecture. In approximately 20% of patients (Xanthou *et al*, 1999; Salomonsson *et al*, 2003) the perpetuation of the lesion involves the formation of ectopic GC-like structures with evidence of clonal B-cell proliferation (Moutsopoulos *et al*, 1990). Chemokines are key regulators in these processes.

In brief, CXCL13 (or B-cell attractant, BCA1) attracts naïve B cells and certain T cells through the receptor CXCR5 (or BLR1) and is important for GC formation. Epithelial cells are implicated in the production of CXCL13 (Xanthou *et al*, 2001; Salomonsson *et al*, 2002, 2003) as well as endothelial cells and monocytes (Amft *et al*, 2001; Barone *et al*, 2005). Normal mechanisms such as transcytosis (capture and subsequent presentation of molecules by cells that do not synthesize them) or experimental differences may be the reason for such discrepancies. Either way, the expression of CXCR5 by infiltrating cells (Amft *et al*, 2001; Salomonsson *et al*, 2002) points out the importance of CXCL13 for cellular trafficking in LMSGs of SS patients. The same applies for CCL21 (or secondary lymphoid tissue/T-cell attractant, SLC/TCA), a chemoattractant for T cells and dendritic cells highly expressed by high-endothelial venules (HEVs). Whether epithelial (Xanthou *et al*, 2001; Salomonsson *et al*, 2003) or endothelial (Barone *et al*, 2005) cells are the source, CCL21 seems to lead to a higher degree of organization within the aggregate through the formation of peripheral lymph node addressin (PNAd)-positive HEVs (Barone *et al*, 2005). In addition, three other potent T-cell chemoattractants have been studied in SS lesions; CXCL9 (or monokine induced by INF-γ, Mig), CXCL10 (or IFN-γ inducible 10 kDa protein, IP10) and CXCL12 (or stromal-cell derived factor, SDF1). Epithelial cells from SS patients produce CXCL9 and CXCL10 while most of the $CD3^+$ lymphocytes in periductal foci express CXCR3 (common receptor for CXCL9 and CXCL10) (Ogawa *et al*, 2002). On the other hand, CXCL12 is constitutively expressed by both SS and controls, whereas all three chemokines are consistently upregulated by IFN-γ (Amft *et al*, 2001; Ogawa *et al*, 2002). Finally, B-cell activating factor (BAFF) is a cytokine that plays an important role in various developmental stages of B cells and seems to be especially important for the survival of autoreactive B cells (Thien *et al*, 2004) and autoantibody production (Pers *et al*, 2005). BAFF is present in salivary glands of patients with SS and although unclear, epithelial cells seem to participate in its production (Groom *et al*, 2002).

The initiation and perpetuation of the autoimmune lesion in SS, the organization in ectopic GCs and probably the progression to lymphoma is largely attributed to factors that favor lymphocytic homing in LMSG. Epithelial production of chemokines is crucial in these processes.

Epithelial apoptosis and exosomes

Detection in the patients' sera of antibodies against the autoantigens Ro/SS-A and La/SS-B is one of the criteria for SS diagnosis (Vitali *et al*, 2002). The Ro/La ribonucleoprotein complexes are protein-RNA complexes formed by the association of the Ro52 kDa, Ro60 kDa and La proteins with small cytoplasmic RNA (hyRNA) (Slobbe *et al*, 1992; Fabini *et al*, 2000). The Ro/La proteins are intracellular and therefore not accessible to the immune system. It takes specific mechanisms, such as apoptosis and exosomes in order for them to be disclosed and become immunogenic.

Apoptosis is a mechanism of utmost importance during embryogenesis and leads to elimination of damaged cells throughout life. The initial stimulus may be either intrinsic to the cell (e.g. uncorrectable DNA damage) or delivered extrinsically by other cells. The most important pathways of the second category are the Fas (CD95)/Fas ligand (FasL) and that of perforin and granzynes (Figure 4). Elevated apoptotic death of the epithelia from SS patients compared with the controls has been repeatedly reported, indicating that these pathways are functional and lead to epithelial destruction (Polihronis *et al*, 1998; Manganelli and Fietta, 2003). Fas and FasL is expressed by acinar and ductal epithelial cells, as well as by the infiltrating mononuclear cells of LMSGs and lacrimal glands from SS patients (Kong *et al*, 1997; Polihronis *et al*, 1998; Matsumura *et al*, 2000; Abu Helu *et al*, 2001; Tsubota *et al*, 2003). Perforin and granzyme B expression was observed in the mononuclear cell infiltrates of SS patients, but not in biopsies from control individuals

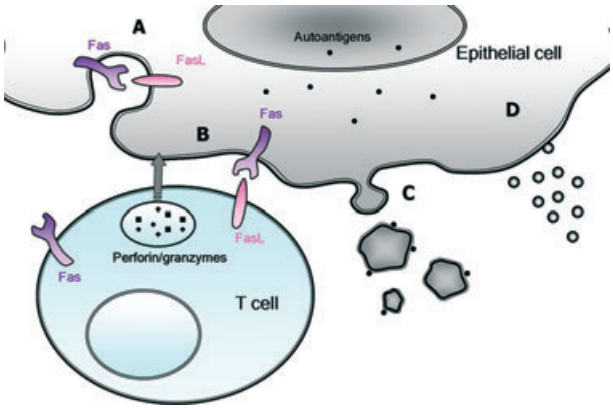

Figure 4 Apoptosis and exosomes. An initial, probably pauci-immune phase of epithelial destruction (A) is followed by a lymphocyte-dependent autoimmune aggression involving Fas/FasL and perforin/granzymes (B). This leads to typical apoptotic characteristics including bleb formation, inside-out flip of the membrane and translocation of intracellular antigens to the surface (C). Exosomes also may be vectors of autoantigens (D)

(Polihronis *et al*, 1998). Furthermore, IFN-γ induces apoptosis by at least three ways: first, upregulates Fas expression by epithelial cells (Kong *et al*, 1997; Matsumura *et al*, 2000; Abu Helu *et al*, 2001), secondly, upregulates CD40 expression which in turn downregulates c-FLIP (anti-apoptotic molecule downstream of Fas) (Ping *et al*, 2005) and thirdly, through STAT-1 mediated upregulation of caspase-8 expression (Fulda and Debatin, 2002). The study of intracellular components of the apoptotic pathway points to the same direction. The balance between apoptotic (e.g. Bax) and anti-apoptotic (e.g. Bcl-2, Bcl-xL) molecules dictates cellular fate, and several studies show Bax predominance in epithelial cells. In contrast, anti-apoptotic molecules are mainly expressed by infiltrating lymphocytes partly explaining the chronicity of the disease (Kong *et al*, 1998; Humphreys-Beher *et al*, 1999).

Key characteristics of apoptosis include bleb formation, inside-out flip of the membrane and translocation of intracellular components to the membrane (Martin *et al*, 1995). Indeed, it has been clearly shown that induction of apoptosis leads to the redistribution of La from the nucleus to the cytoplasm, while both Ro and La fill apoptotic blebs and then are exposed on the surface of the cell (McArthur *et al*, 2002; Ohlsson *et al*, 2002b). Therefore, apoptotic death not only diminishes the total number of epithelia and the functional capacity of the gland but also leads to presentation of intracellular antigens to the immune system evoking an autoimmune reaction.

Exosomes are small (30–100 nm) membrane vesicles, which result from the fusion of endosomes/lysosomes with the membrane. Dendritic cells, cytotoxic T and B lymphocytes, platelets, reticulocytes and neoplastic intestinal epithelial cells produce exosomes. Largely unclear, the biological functions of exosomes include the exclusion of obsolete proteins and membranes, while recent studies indicate that they may have immunologic functions, such as the stimulation or inactivation of T cells, directly or indirectly by the transfer of antigens to the dendritic cells (Thery *et al*, 2002). Recently, our group has shown that, in long-term cultures, non-neoplastic SGEC constitutively release exosomes *in vitro*. Notably, the exosomes secreted by SGEC contain the autoantigens Ro/SS-A, La/SS-B and Sm that are major targets of immune responses in several autoimmune disorders (Kapsogeorgou *et al*, 2005) and therefore seem to be a novel cell-free antigen presentation mechanism in SS (Figure 4D).

The viral hypothesis

The pathogenesis of autoimmune diseases is multifactorial and includes genetic, hormonal and environmental factors. On the other hand, a link between autoimmunity and infectious agents, namely a virus, has been speculated for a long time but unquestionable data are still lacking (Zinkernagel, 2002). HIV, HTLV-1 and HCV infections in a percentage of patients present with symptoms resembling SS (Talal *et al*, 1990; Haddad *et al*, 1992; Ohyama *et al*, 1998). None of these agents, however, can reproduce the full spectrum of

clinical and laboratory manifestations of SS, while the vast majority of confirmed SS patients have no other evidence of HIV or HTLV-1 infection. Chronic HCV infection warrants special reference because sialadenitis is accompanied by lymphocytic infiltrate of the LMSG, highly similar to that of SS. HCV RNA is present in salivary epithelial cells in patients with chronic HCV infection (Arrieta *et al*, 2001). Moreover, mixed cryoglobulinemia and B-cell lymphoma are probable sequelae of both entities (Ramos-Casals *et al*, 2005). Still, several features differentiate chronic HCV infection and SS making an etiologic linkage less probable (Scott *et al*, 1997).

Recently, our group published data that link SS with the presence of coxsackie virus sequences in salivary gland tissues. RT-PCR for the conserved non-coding region of enteroviral RNA and subsequent sequencing, revealed 97–99% similarity with the coxsackie B4 and A13 strains (Triantafyllopoulou *et al*, 2004). These data, along with the viral ability to establish persistent noncytolytic infections *in vitro*, render coxsackie virus a probable pathogenetic factor of SS.

Summary

The target of the present paper is to point out the major role of the epithelial cell in the autoimmune lesions of SS. The exocrine manifestations of SS result mainly from the immune attack of the various organs' epithelial component. On the other hand, at the cellular/molecular level, it is clear that key pathogenetic mechanisms that lead to the initiation and perpetuation of the lesion involve the epithelial cell. We propose, therefore, the following theory that unifies most of the data, center on the role of the epithelium and justifies the name 'autoimmune epithelitis'.

In genetically liable individuals, a certain event such as a psychological and/or endocrine stress, initiates a sequel that leads to the activation of a silent offending factor (most probably an epitheliotropic virus), which results in the activation of the exocrine glands' epithelium. This leads to the recruitment, activation and expansion of lymphocytes in the lesion that in some individuals form well-organized structures resembling GCs; in patients with certain adverse prognostic factors this may progress to MALT lymphoma. Apoptosis of the epithelial cell spreads the autoimmune reaction to various antigenic determinants and as it is a normal cell-turnover mechanism, may account for the immune attack on the epithelia of other organs by sensitized lymphocytes. This insult is mostly subclinical but in a minority of patients may progress to include non-epithelial structures, and therefore becomes clinically overt. Further studies aimed at the target cell will certainly clarify these issues.

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