

# Sjögren's syndrome – etiopathogenesis, apoptosis and animal models

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Sjögren's syndrome (SS) is a relatively common chronic autoimmune rheumatic and lymphoproliferative disorder affecting the lachrymal and salivary glands as well as other exocrine organs throughout the body. Dryness of the mucous membranes, chronic fatigue, arthralgias and gastrointestinal symptoms are common expressions occasionally complicated by enlargement of salivary and/or lachrymal glands. Although SS is a polygenic disorder it can be characterised by mononuclear cell infiltration of exocrine tissues and in a great number of patients the presence of autoantibodies to the intracellular Ro/SSA and La/SSB antigens as well as to immunoglobulin G, i. e. rheumatoid factor. Much knowledge has during recent years been obtained about SS by studying tissue lesions *in situ* (Fig) and comparing that picture to pathophysiological events in peripheral blood.

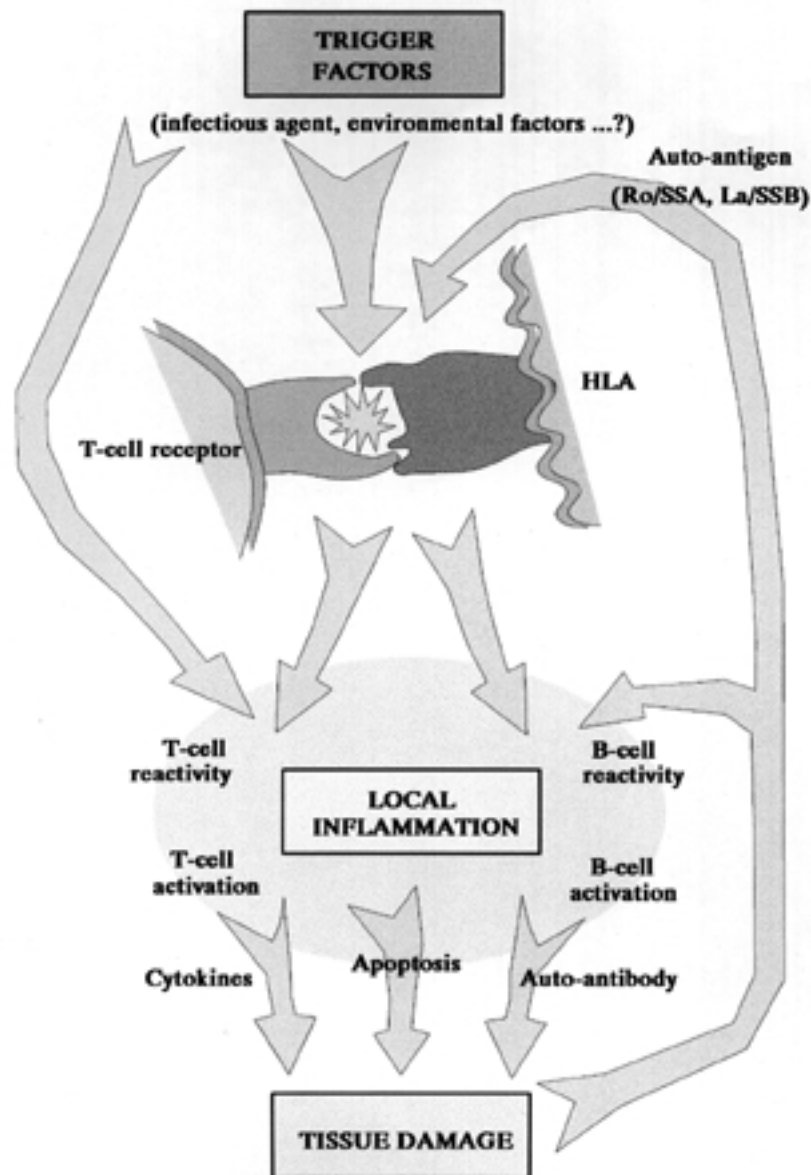
## Immunopathologic features

Immunohistologic analysis of lymphoid cell infiltration in exocrine glands in SS, where salivary glands have been mostly analysed, show a predominance of T cells with fewer B cells and macrophages (1). Adhesion molecules and activated lymphocyte function-associated antigen type 1 (LFA-1) promote homing and occasionally characteristic cell clustering similar to follicular structures of lymph nodes. Expression of the mucosal lymphocyte integrin  $\alpha 7$  and its ligand E-cadherin suggest a mucosal origin of a population of the infiltrating cells (2). There is an aberrant and differentiated expression of HLA-DR/DP/DQ molecules on acinar and ductal epithelial cells (3) presumably due to local production of IFN- $\gamma$  by activated T cells. The majority of T cells in the lymphocytic infiltrates are CD4+ T-helper cells with a CD4/CD8 ratio well over 2. Most of these T cells bear the memory phenotype CD45RO+ and express the  $\alpha$  /  $\beta$  T cell receptor and LFA-1, and may contribute significantly to B cell hyperactivity. Some studies have indicated oligoclonal expansion of certain TCR V family expressing lymphocytes.

Aberrant expression of HLA molecules by salivary gland epithelium in SS suggests that these cells may function as non-professional antigen-presenting cells interacting with CD4+ T cells. Such interaction may lead to further production of cytokines and stimulation of B cell proliferation and differentiation. For example, high levels of interleukin (IL)-1, IL-6 and tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) are produced by epithelial cells, while IL-10 and IFN- $\gamma$  are mainly produced by infiltrating T cells. IL-6 and IL-10 are also produced in increased amounts in peripheral blood (4). An identified low level of IL-2 in SS might be due to absence of T cell co-stimulatory signals resulting in the induction of anergy in the responding T cell population, but other explanations are quite plausible.

B cell activation is a very consistent immunoregulatory abnormality in SS. The B cells make up roughly 20 % of the infiltrating cell population in SS glands. The B cells produce increased amounts of immunoglobulins with autoantibody activity for IgG (rheumatoid factor), Ro/SSA and La/SSB (5). A substantial number of the B cells are CD5+ (B-1 cells). Out of the isotypes, IgG predominates in contrast to IgA, which dominates in normal salivary glands.

Among the infiltrating T cells some express activation markers such as CD25, proto-oncogene products and HLA-DR, but few T cells proliferate as determined by cell cycle studies and autoradiography. Also it seems difficult to stimulate the T lymphocytes in SS with the autoantigens Ro/SSA and La/SSB. This can suggest that memory T cells in the infiltrates may be semiactivated and engaged in low-grade responses to persisting antigen(s). By avoiding hyperstimulation, the T cells may circumvent the signals that lead to apoptosis.



**Figure.** A schematic presentation of potential immune components in the pathogenesis of Sjögren's syndrome.

### Live or let die? Infiltrating cells have an opinion on the destiny of glandular cells in SS

Even though the actual mechanism(s) behind the characteristic glandular destruction of SS salivary glands remains obscure, immunopathological findings demonstrate that infiltrating cytotoxic T cells (CTL) could play a major role in this event. Upon recognition of a proper MHC-antigen complex presented by a target cell, CTLs induce cell death through one of its two main and independent pathways, the perforin-mediated or the Fas-mediated pathway. In the Fas-mediated pathway apoptotic death signals are transmitted to the target cell upon crosslinkage of two cell surface molecules, Fas ligand (FasL) on the CTL and Fas on the target cell. Fas (APO1 antigen, CD95) is a member of the F- receptor family and is expressed constitutively or induced after activation on a variety of cell types, including epithelial cells (6). Fas L, a type II transmembrane protein, is expressed in non-lymphoid sites such as the cornea and testis, and on activated T lymphocytes (7).

Interestingly, expression of Fas has also been detected among infiltrating mononuclear cells in salivary glands of MRL/*lpr* mice, a murine model featuring similar pathogenesis as systemic lupus erythematosus (SLE) and SS (8).

The Fas-mediated pathway plays an important role in regulating lymphocyte apoptosis and thereby maintaining lymphocyte homeostasis by deletion of mature T cells and clonal deletion of autoreactive T and B cells. Apoptosis, or programmed cell death, is also relevant for the down-regulation of immune responses after activation and proliferation of T and B cells (9). Defective expression of the *fas*/*fasL* and other pro- and antiapoptotic genes such as *bcl-2*, *bcl-x*, *bax*, and *c-myc* would consequently result in marked accumulation of mature lymphocytes and could contribute to the development of autoimmune disorders. A defective apoptotic pathway via Fas/FasL was also demonstrated to induce lymphoproliferative and autoimmune disease in *lpr* and *gld* strains of mice, which are models for SLE and SS.

Expression of *fas*, *fasL*, *bcl-2* and other apoptosis associated genes/proteins has also been detected by RT-PCR and immunohistochemical staining of minor salivary glands from patients with SS (10,11). In particular, ductal and acinar epithelial cells but to some degree also infiltrating mononuclear cells express abnormal levels of Fas and FasL in SS, especially in cases with severe mononuclear cell infiltration. Ductal epithelial cells expressing Fas were usually situated inside or close to a dense focus. Together with the finding that expression of FasL was seen in some of the infiltrating mononuclear cells scattered around the acini and ductal regions, these findings confirm the hypothesis that infiltrating CTLs expressing FasL crosslink to Fas on glandular cells and thereby induce apoptosis in these cells. Upregulation of different apoptosis-inducing molecules such as Fas and impaired expression of Bcl-2, an inhibitor of apoptosis, in ductal and acinar cells suggest that these cells are directed to commit apoptosis. *In situ* DNA nick-end-labelling (TUNEL) also confirms, as suggested in some studies, that these cells die through apoptosis in a higher frequency in SS than in healthy individuals.

Most *in situ* studies have clearly shown a low or even absence of apoptosis among infiltrating mononuclear cells (10,11). An upregulated Bcl-2 expression has been detected which may contribute to the inhibition of apoptosis in these cells. Other extrinsic factors that could block apoptosis in T lymphocytes include cytokines (IL-2, IL-10 and TNF- ) and adhesion molecules.

The presence of granzyme A in SS salivary glands (12) suggests that also the perforin pathway of CTL killing may be involved in glandular destruction of SS salivary glands. There has been some contradictions concerning expression studies of apoptotic associated genes, especially in acinar cells. In addition, the actual mechanism behind CTL glandular destruction in SS still remains unclear. Therefore, it is of importance to look further into the obviously complex pictures of apoptosis in SS.

### **A possible viral etiology**

Among the possible etiologic and triggering factors involved in SS, the discussion about a relationship between viruses and autoimmunity began some decades ago. Of importance, the epidemiology of relevant viruses must be taken into consideration when interpreting the association between a virus and disease. Although there is no direct proof that any virus is the etiologic agent of SS, they might be involved as etiologic co-factors.

The possible etiologic role of different viruses in SS can be explained by the fact that salivary glands are a site of latent infection by viruses. Potential viral triggers include a number of viruses. Among these, Epstein-Barr virus (EBV) has been widely studied in relation to SS. These investigations suggest that SS results in part from an abnormal immune response to an ubiquitous virus, such as EBV (13). A higher prevalence of human herpesvirus-6 (HHV-6) antibodies has also been detected in patients with SS than in normal individuals (36% versus 10%) (14). By contrast, some

investigators found normal antibody prevalence to HHV-6 (15). Difficulties in analysing the possible viral role are related to the high prevalence of both herpesviruses (EBV and HHV-6) in the normal population.

Retroviruses are known to infect cells of the immune system and cause abnormalities in immune regulation. High serum titres of anti-human T lymphotropic virus type I (HTLV-I) antibodies and a high prevalence of salivary IgA-class anti-HTLV-I antibodies in patients with SS were reported (16). One study showed that 33% of sera obtained from patients with primary SS contained antibodies that reacted on immunoblot with p24 of the human immunodeficiency virus (HIV) gag protein (17).

Hepatitis C virus (HCV) infection has also, in some populations, been frequently detected (14%) in patients with primary SS, and liver involvement was found to be present in all these patients (18). Analysis of the association between chronic lymphocytic sialadenitis and chronic HCV liver disease showed that histological features of SS were significantly more common in HCV-infected patients (57%) compared with controls (5%) (19).

Lymphotropic viruses have the potential to affect the autoimmune process. Some of the immunoreactive regions within the La/SS-B protein have been found to have sequence similarities with proteins of EBV, HHV-6 and HIV-I (20). It seems possible that these viruses can promote autoantibody (particularly anti-La/SS-B) production through molecular mimicry or exposure of La/SS-B on cellular surface after translocation of cryptic self-determinants.

### **Animal models in the study of SS**

As already alluded to there are genetic associations which may predispose to this disease, in particular the genes encoding products of the MHC, T and B cell receptors, but also other candidate genes are relevant. It is thus natural to seek more knowledge in genetically well characterised, inbred and controlled animal models which are available (21). In particular, the current challenge will be to find links between a particular genetic set up and phenotypic expression(s).

However, certain criteria and features of human SS should be fulfilled in any proposed animal model. Moreover, the clinical symptoms of SS in humans usually appear relatively late in life thus making examination of early events difficult. An animal model of the disease would make it possible to study earlier events and to identify potentially important immune reactions in the pathogenesis of this disease. Finally, both immune manipulation and the effects of drug therapy can be studied in animals (21).

The earlier reports on attempts to induce SS in animals by injection with salivary gland extracts with or without adjuvants and/or other supplements largely produced a transient inflammation which was self limiting and did not mirror the human disease in either the temporal course of events or in the serological profile. The better models are the mice with spontaneous autoimmune disease with long-lasting and progressive exocrinopathy, but even in these examples the disorder at best represents only secondary SS (21).

Of high relevance, both anti-Ro/SSA (22) and anti-La/SSB have been more recently described in spontaneous models. These findings of a similar serologic profile of specific antibodies to La/SSB and/or Ro/SSA which characterise the majority of patients with SS are of fundamental importance for future work.

Interesting observations have been made in the MRL/*lpr* mouse where the *lpr* genotype has been identified as a mutation in the gene encoding Fas which is a cell surface protein that mediates apoptosis. Apoptotic cells were absent or appeared at very low frequency among the infiltrating mononuclear cells in salivary glands. Based on the analysis of the apoptotic activity, the T cells seemed to be rescued from apoptosis due to a failure in signalling (8).

To analyse Fas and TNF receptor I (TNFRI) apoptosis pathways in salivary gland inflammatory disease induced by murine cytomegalovirus (MCMV) infection, four different strains of mice were infected (23). Although acute salivary gland inflammation developed in all MCMV-infected mice only the B6-*lpr/lpr* showed chronic inflammation. Apoptotic cells were detected during the acute, but not the chronic, phase of inflammation. Both Fas- and TNFRI-mediated apoptosis was found to contribute to the clearance of MCMV-infected cells in salivary glands. However, because Fas-mediated apoptosis is necessary for the down-modulation of the immune response, a defect in this process can lead to a postinfection, chronic inflammatory response that resembles SS.

In order to better understand the role of IL-10 in SS, transgenic mice were constructed (24). The transgenic expression of IL-10 induced apoptosis of glandular tissue and infiltration of lymphocytes consisting of primarily FasL+, CD4+ T cells as well as *in vitro* upregulation of FasL expression on T cells. Altogether, this suggested that glandular overexpression of IL-10 and the subsequent Fas/FasL-mediated bystander tissue destruction is a causal factor in the development of SS.

The appearance of autoimmune diabetes prior to autoimmune exocrinopathy in the NOD mouse suggests that it is a model of secondary but not primary, autoimmune sicca complications. Since the unique MHC I-A(g7) expression in NOD mice is essential for the development of insulinitis and diabetes in these animals, the exocrine gland function in NOD.B10.H2b mice, which have an MHC congenic to NOD, was investigated as a potential model for primary SS (25). NOD.B10.H2b mice exhibited the exocrine gland lymphocytic infiltration typical of SS-like disease and dysfunction observed in NOD mice, but without the insulinitis and diabetes. This suggests that the unique MHC I-A(g7) is not essential for exocrine tissue autoimmunity. Furthermore, these findings indicate that murine sicca syndrome occurs independently of autoimmune diabetes and that the congenic NOD.B10.H2b mouse represents a novel murine model of primary SS.

Thus it appears that there are currently some good experimental models, which fulfil at least most of the criteria of SS. Moreover, elucidating the molecular aspects of human autoimmune exocrinopathy would certainly benefit largely from exploring further relevant transgenic and knock-out mice.

## Conclusion

The etiology of SS is still a matter of conjecture although several hypotheses prevail. Nevertheless, there is considerable evidence that an – as yet unknown – initiating factor set against the appropriate genetic background may invoke immunologically mediated inflammatory mechanisms which result in the chronic exocrine lesions. T cell mediated autoimmune responses in the glandular tissue as well as apoptotic events may be of central importance in the pathogenesis of SS.

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