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Current issues in Sjögren's syndrome

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Sjögren's syndrome is a chronic autoimmune and rheumatic disorder with prominent sicca complaints from the mucous membranes because of lack of proper exocrine secretions. There is no straightforward and simple diagnostic test for Sjögren's syndrome, although several classification criteria have been designed including several oral diagnostic tests. A new set of classification criteria in a joint effort by research groups in Europe and USA has recently been presented. A large number of autoantibodies have been reported in Sjögren's syndrome where, in some cases, the antibodies are correlated with the extent and severity of disease. The finding of serum autoantibodies directed against the muscarinic M3 receptor is an important advance in understanding the pathogenesis of not only the impaired glandular function but also associated features of autonomic dysfunction in some patients. The treatment of primary Sjögren's syndrome is still mainly symptomatic.
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Historical features

Sjögren's syndrome is named after the Swedish ophthalmologist Henrik Sjögren who presented his doctoral thesis in 1933 (Sjögren, 1933). However, the disease was addressed already during the 19th century in a number of case reports describing various combinations of dry mouth, dry eyes and chronic arthritis (Jonsson, Haga and Gordon, 2001). More recently, a number of studies have presented ground-breaking reports on Sjögren's syndrome, which now can be considered as classical findings (Jonsson, 2002).

1964   Link between Sjögren's syndrome and malignant lymphoma

1965   Distinction between primary and secondary Sjögren's syndrome
1968   Histological grading assessing the infiltration of labial glands
1969   Sjögren's syndrome associated (Ro/SSA) autoantibodies
1984   Familial associations in Sjögren's syndrome
1993   Preliminary classification criteria (EU)

The salivary and lacrimal glands are the principal targets of a supposedly T cell mediated chronic inflammation, giving rise to deficient function and leading to dry eyes (keratoconjunctivitis sicca) and mouth (xerostomia). Other exocrine glands including those of the pancreas, sweat glands and mucous secreting glands of the bowel, bronchial tree and vagina, can be involved.

Epidemiology and genetics

Sjögren's syndrome is a world-wide disease and may occur in all ages. However, the peak incidence is in the fourth and fifth decades of life with a female/male ratio of 9:1. A number of studies have shown a great variation in the frequency of Sjögren's syndrome (Jonsson *et al*, 2001). The prevalence studies demonstrate that sicca symptoms and primary Sjögren's syndrome affects a considerable portion of the population, precise numbers dependent on the age group studied and on the criteria used (Silman and Rooney, 1999). Subjects identified as having primary Sjögren's syndrome in population studies often have mild to moderate complaints, and many of them have been found not to be aware of the disease. A cautious but realistic estimate from the studies presented so far will be that primary Sjögren's syndrome is a disease with a prevalence not exceeding 0.6% of the general population (six per 1000).

A prominent feature of Sjögren's syndrome is its genetic predisposition. Several families involving two or more cases of Sjögren's syndrome have been described (Reveille *et al*, 1984), and a family history with relatives having other autoimmune diseases is common (~ 30–35%) for Sjögren's syndrome patients.

The polymorphic major histocompatibility complex (MHC) genes are the best documented genetic risk

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factors for the development of autoimmune diseases overall (Nepom, 1993). With regard to Sjögren's syndrome, the most relevant MHC complex genes are the class II genes, more specifically the HLA-DR and DQ alleles (Reveille, 1992). Patients of different ethnic origin show different HLA gene associations (Kang *et al*, 1993). In Caucasians of northern and western European background, including North American Caucasians, Sjögren's syndrome is one of several autoimmune diseases associated with the haplotypes HLA-B8, DRw52, and DR3. An association with DR2 has been reported in Scandinavians (Manthorpe *et al*, 1981) and DR5 in Greeks (Papasteriades *et al*, 1988). All of the haplotypes are in strong linkage disequilibrium rendering certain difficulties to establish which of the genes contains the locus conferring the risk.

HLA class II allele association has been reported to differ among anti-Ro/SSA positive subjects according to the presence or absence of coexisting anti-La/SSB (Miyagawa *et al*, 1998). Distinct HLA haplotypes have been associated with a certain degree of autoantibody diversification in Sjögren's syndrome patients (Rischmueller *et al*, 1998). Of particular importance is that strong correlation has been found between anti-Ro/SSA auto antibodies and DR3/DR2 than that to the disease itself (Hamilton *et al*, 1988; Arnett, Bias and Reveille, 1989; Bolstad *et al*, 2001; Nakken *et al*, 2001). Autoantibodies to Ro/SSA and La/SSB have been found to be associated with DR3 and DQA alleles (Fei *et al*, 1991). Sjögren's syndrome patients with DQ1/DQ2 alleles have a much more severe autoimmune disease than patients with any other allelic combination at HLA-DQ (Harley *et al*, 1986). The DR3-DQ2 haplotype has been indicated as a possible marker for a more active immune response in Finnish Sjögren's patients (Kerttula *et al*, 1996).

Etiopathogenesis and immunology

Environmental factors
Among the possible etiologic and triggering factors involved in Sjögren's syndrome, the discussion about a relationship between viral infections causing development of autoimmune reactions began some decades ago. The putative role of different viruses in Sjögren's syndrome can be viewed in the light that salivary glands are a site of latent viral infections. Potential viral triggers include a number of viruses including Epstein–Barr virus (EBV), widely studied in relation to Sjögren's syndrome (James, Harley and Scofield, 2001).

Hepatitis C virus (HCV) infection has in some populations been frequently detected in patients with primary Sjögren's syndrome (Garsia-Carrasco *et al*, 1997). Analysis of the association between chronic lymphocytic sialadenitis and chronic HCV liver disease showed that histological features of Sjögren's syndrome were significantly more common in HCV-infected patients (57%) compared with controls (5%) (Haddad *et al*, 1992).

Lymphotropic viruses have the potential to trigger 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 (Haaheim *et al*, 1996). It seems reasonable that these viruses can promote autoantibody (particularly anti-La/SS-B) production through molecular mimicry or exposure of La/SS-B homologue sequences on cellular surfaces after translocation of cryptic self-determinants.

Furthermore, a possible relationship between Sjögren's syndrome and *Helicobacter pylori* infection has been suspected. In both these conditions there are increased risks of developing mucosa-associated lymphoid tissue lymphoma (Isaacson and Spencer 1987; Parsonnet *et al*, 1994). It has been suggested that infection with *H. pylori* might trigger a widespread clonal B-cell disorder in Sjögren's syndrome (De Vita *et al*, 1996). Studies on antibodies against *H. pylori* in Sjögren's syndrome though have given conflicting results as to whether seroreactivity is elevated or not (Showji *et al*, 1996; Aragona *et al*, 1999; Theander *et al*, 2001). However, improvement of sicca symptoms has been indicated after eradication treatment of *H. pylori* (Figura *et al*, 1994)

Pathology
The pathognomic histological finding in biopsies is a focal infiltration of mononuclear lymphoid cells in the salivary glands, replacing glandular epithelium (lymphoepithelial lesion), and which is progressive as demonstrated by an increase of focus score over time (Jonsson *et al*, 1993). Another pattern of inflammation in labial salivary gland biopsy is chronic sialadenitis characterized by scattered mononuclear cell infiltration without focal aggregates and accompanied by degenerative changes (acinar atrophy, ductal hyperplasia, fibrosis and/or fatty infiltration). This pattern is not considered to be typical for primary Sjögren's syndrome, and often leads to glandular atrophy and xerostomia.

Immunopathology and autoimmunity
Immunohistologic analysis of lymphoid cell infiltration in exocrine glands in Sjögren's syndrome shows a predominance of T cells with fewer B cells and macrophages (Jonsson *et al*, 2001). 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 $\alpha^E\beta_7$ and its ligand E-cadherin suggest a mucosal origin of a subpopulation of the infiltrating cells (Kroneld *et al*, 1998). There is an increased expression of HLA-DR/DP/DQ molecules on acinar and ductal epithelial cells (Jonsson *et al*, 1987) presumably because of local production of IFN-γ by activated T cells. The majority of T cells in the lympocytic infiltrates are CD4^{+} T-helper cells with a CD4/CD8 ratio well over two. Most of these T cells bear the memory phenotype CD45RO^{+} and express the α/β T cell receptor and LFA-1, and may contribute significantly to B cell hyperactivity. There is indication of oligoclonal expansion of certain TCR V$_\beta$

family expressing lymphocytes (Sumida et al, 1992). The findings in peripheral blood in Sjögren's syndrome have yielded findings similar to those in salivary glands, although a difference in magnitude of immune activation is often evident.

The B cells make up roughly 20% of the infiltrating cell population in affected glands. The B cells produce immunoglobulins with autoantibody activity for IgG (rheumatoid factor), Ro/SSA and La/SSB (Halse et al, 1999a). A substantial number of the B cells are of CD5^{+} phenotype (B-1 cells) (Dauphinee, Tovar and Talal, 1988). Production of IgG predominates in Sjögren's syndrome whereas synthesis of IgA is more abundant in normal salivary glands.

A large number of autoantibodies have been reported in both primary and secondary Sjögren's syndrome, reflecting both B cell activation and a loss of immune tolerance in the B cell compartment (MacSween, Govidie and Anderson, 1967; Feltkamp and Van Rossum, 1968; Manthorpe, Permin and Tage-Jensen, 1979; Ben-Chetrit et al, 1988; Atkinson et al, 1989; Inagak et al, 1991; Hauschild et al, 1993; Markusse, Otten and Vroom, 1993; Kausman et al, 1994; Tzioufas and Moutsopoulus, 1994; Boire et al, 1995; Bacman et al, 1996, 1998; Kino-Ohsaki et al, 1996; Haneji et al, 1997; Font et al, 1998; Elagib et al, 1999; Freist et al, 1999; Ono et al, 1999; Watanabe et al, 1999). In some cases, the presence of these antibodies is related to the extent and severity of disease in Sjögren's syndrome. The non-organ-specific autoantibodies anti-Ro/SSA and anti-La/SSB are the diagnostically most important and the best characterized autoantibodies in primary Sjögren's syndrome (Jonsson et al, 2001). The majority of anti-Ro-positive sera also react with the denatured form of a 52-kDa protein termed Ro52, which is structurally distinct from Ro60 and probably does not directly associate with the Ro ribonucleoprotein particle (Ben-Chetrit et al, 1988; Boire et al, 1995). However, the two Ro proteins colocalize to surface membrane blebs on apoptotic cells where they may become targets of an autoimmune response (Ohlsson et al, 2002).

Anti-thyroid microsomal and antigastric parietal cell antibodies occur in about one-third of patients with both primary and secondary Sjögren's syndrome but other organ-specific antibodies are infrequent (Morrow et al, 1999). Antibodies to salivary duct antigens were described over 30 years ago but they have remained poorly characterized and their clinical significance is uncertain (MacSween et al, 1967; Feltkamp and Van Rossum, 1968). However, a recent study has demonstrated that this reaction is because of cross-reactivity with blood group antigens (Goldblatt et al, 2000).

Several other autoantibodies have been reported to be frequently present in the sera of patients with primary Sjögren's syndrome including antibodies directed against carbonic anhydrase (Inagak et al, 1991; Kino-Ohsaki et al, 1996), proteasomal subunits (Freist et al, 1999) and α-fodrin (Haneji et al, 1997). These findings are intriguing but await independent confirmation in larger cohorts of Sjögren's syndrome patients. The finding of serum autoantibodies directed against the

muscarinic M3 receptor (expressed in salivary and lacrimal glands) in the majority of patients is an important advance in understanding the pathogenesis of impaired glandular function in Sjögren's syndrome (Bacman et al, 1996, 1998). This is of high interest as a recent study has shown that M3-muscarinic receptors are up-regulated in glandular acini (Beroukas et al, 2002).

Studies in the non-obese diabetic (NOD) mouse have indicated that muscarinic receptor autoantibodies are directed against the agonist-binding site of the molecule on the cell surface and interfere with secretory function of exocrine tissues in Sjögren's syndrome (Robinson et al, 1998a). Inhibitory effects of these autoantibodies on parasympathetic neurotransmission in Sjögren's syndrome have recently been experimentally shown (Waterman, Gordon and Rischmueller, 2000). However, the clinical significance of these antibodies in Sjögren's syndrome remains to be elucidated (Humphreys-Beher et al, 1999).

Rheumatoid factor is detected in the serum and saliva of 60–80% of primary Sjögren's syndrome patients (Atkinson et al, 1989; Markusse et al, 1993). There appears to be little role for somatic hypermutation in their generation in contrast to rheumatoid factor in rheumatoid arthritis (Elagib et al, 1999). A significant number of patients with primary Sjögren's syndrome have mixed oligoclonal cryoglobulins, many of them having IgM rheumatoid factor activity (Tzioufas et al, 1986). The latter frequently possess cross-reactive idiotypes notably the 17.109 (V kappa III b related) and G-6 (VH1 related) idiotypes that may serve as markers for lymphoma development in primary Sjögren's syndrome (Fox et al, 1986a; Tzioufas et al, 1996).

Oligoclonal or monoclonal B cell expansion, arising mainly from salivary glands but also from visceral organs and lymph nodes, has been reported to occur in 14–100% of Sjögren's syndrome patients (Anaya et al, 1996). In this respect, Sjögren's syndrome appears to be a crossroad between autoimmunity and malignancy and it is suggested that patients with evidence of clonal expansions of B cells in their salivary glands are at high risk of developing malignant lymphoma (Hyjek, Smith and Isaacson, 1988; Diss et al, 1995; Jordan et al, 1995). Various studies have reported that between 25 and 80% of salivary lymphoid infiltrates have morphologic and/or immunophenotypic evidence of low-grade lymphomas (Harris, 1999). However, there is no absolute correlation between clonality and the development of lymphoma. Although a high proportion of lymphoid cells may show evidence of immunoglobulin gene rearrangements, clonality does not necessarily predict progression to clinically overt lymphoma. The clinical benefit of immunogenotypic analysis in the diagnosis of salivary gland lymphoma in Sjögren's syndrome remains to be defined (His et al, 1996; Quintana, Kapadia and Bahler, 1997). A recent study reported that a history of swollen salivary glands, lymphadenopathy and leg ulcers predicted lymphoma development in patients with primary Sjögren's syndrome (Sutcliffe et al, 1998).

Immune-mediated tissue destruction

Highly up-regulated expression of HLA molecules, and the more recently demonstrated B-7 co-stimulatory molecules (Manoussakis *et al*, 1999), by salivary gland epithelium in Sjögren's syndrome is a potentially effective local antigen-presenting mechanism whereby HLA antigens could be involved in exocrine glandular destruction mediated directly or indirectly by CD4$^+$ T cells. Such interaction may lead to further production of cytokines and stimulation of B cell proliferation and differentiation. Indeed, high levels of three tissue destructive cytokines, interleukin (IL)-1βT, IL-6 and tumour necrosis factor-α (TNF-α), are produced by epithelial cells. Infiltrating T cells mainly produce IL-10 and IFN-γ, while IL-6 and IL-10 are also elevated in peripheral blood (Halse *et al*, 1999b). A low capacity to produce IL-2 in Sjögren's syndrome might be because of absence of the T cell costimulatory signals resulting in the induction of anergy in the responding T cell population, but other explanations are also possible.

Recently conducted studies on chemokine patterns have pointed further to the role of epithelial cells in the pathogenesis of Sjögren's syndrome (Amft and Bowman 2001; Xanthou *et al*, 2001; Salomonsson *et al*, 2002). This offers new insight into the mechanisms of leukocyte attraction and formation of secondary lymphoid tissue structures.

Even though the mechanism(s) behind the characteristic destruction of salivary glands in Sjögren's syndrome remain obscure, immunopathological findings demonstrate that infiltrating cytotoxic T cells (CTL) could play a 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. Interestingly, expression of Fas has also been detected among infiltrating mononuclear cells in salivary glands of MRL/*lpr* mice, a murine model displaying similar features as human systemic lupus erythematosus and Sjögren's syndrome (Skarstein *et al*, 1997).

Expression of Fas, Fas-L, Bcl-2 and other apoptosis associated genes/proteins has been detected by RT-PCR and immunohistochemical staining of minor salivary glands in patients with Sjögren's syndrome (Kong *et al*, 1997; Nakamura *et al*, 1998). In particular, ductal and acinar epithelial cells but to some degree also infiltrating mononuclear cells express abnormal levels of Fas and FasL, especially in cases with heavy mononuclear cell infiltration. Ductal epithelial cells expressing Fas were usually situated inside or adjacent to a dense focus (Ohlsson *et al*, 2002). Most *in situ* studies have clearly shown a low grade or even absence of apoptosis among infiltrating mononuclear cells (Kong *et al*, 1997; Nakamura *et al*, 1998). The presence of granzyme A in Sjögren's glands (Alpert *et al*, 1994) suggests that rather than apoptosis the perforin pathway of CTL mediated killing may be involved in destruction of salivary glands.

Among the salivary gland 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. Also, it seems difficult to stimulate the T lymphocytes in Sjögren's syndrome with the autoantigens Ro/SSA and La/SSB (Halse *et al*, 1996) although a recent study has indicated no T cell responses, at least to La/SSB (Davies *et al*, 2002). These findings suggest that many cells are of memory T cell phenotype; either few of them are autoantigen specific or alternatively many of them are in a state of anergy. In both cases lack of stimulation of T cells will also hamper the apoptotic signals.

Animal models

As already alluded to there are genetic associations which may predispose individuals to Sjögren's syndrome, in particular the genes encoding products of the MHC and immune receptors, but also other genes. It is thus natural to seek more knowledge using genetically well-characterized, inbred animal models that are available for study (Jonsson and Skarstein 2001). In particular, the current challenge is to find links between a particular genetic background and phenotypic expression(s) of this disease.

Any proposed animal model should fulfill certain criteria and features found in the human disease. Moreover, the clinical symptoms of Sjögren's syndrome 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 (Jonsson and Skarstein 2001).

The earlier attempts to induce Sjögren's syndrome in animals by injection with salivary gland extracts with or without adjuvants and/or other supplements would give rise to 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 of Sjögren's syndrome are the mice with spontaneous autoimmune disease with long-lasting and progressive exocrinopathy, but even in these cases the disorder has at best represented only secondary Sjögren's syndrome (Jonsson and Skarstein 2001). Both anti-Ro (Wahren *et al*, 1994) and anti-La (St. Clair *et al*, 1991) have been detected in murine models of spontaneous Sjögren's syndrome. As these autoantibodies are the dominant serological marker in patients with primary Sjögren's syndrome, this finding is an important starting point for future work.

NOD.B10.H2b mice have been found to exhibit exocrine gland lymphocytic infiltration typical of Sjögren's syndrome-like disease and dysfunction observed in NOD mice, but without the insulitis and diabetes (Robinson *et al*, 1998b). 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 Sjögren's syndrome.

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More recently, gene segregation experiments on a (NOD.QxB10.Q) F2 cross and genetic mapping have revealed one locus associated with sialadenitis on chromosome 4 (LOD score 4.7) (Johansson *et al*, 2002). In this study it was shown that the genetic control of sialadenitis seemed to be unique in comparison with diabetes and also arthritis, as no loci associated with these diseases have been identified at the same location.

Clinical presentation

Signs and symptoms

Sjögren's syndrome presents with a wide variety of clinical features (Jonsson *et al*, 2001). Onset of the disease is insidious and patients have difficulty in determining when the disease actually started. Keratoconjunctivitis sicca and xerostomia (so-called sicca complex) are the main clinical presentations in adults, whereas bilateral parotid swelling can be an obvious sign at juvenile disease onset. More than half of the patients may develop an extraglandular manifestation during the evolution of the disease. Occasionally, systemic features may lead to diagnosis.

The spectrum of the disease extends from an organ-specific autoimmune disorder to a range of systemic manifestations (musculoskeletal, pulmonary, gastric, hematologic, dermatologic, renal, and nervous system involvement). Sjögren's syndrome may develop alone (primary) or in association with almost any of the autoimmune rheumatic diseases (secondary), the most frequent being rheumatoid arthritis and systemic lupus erythematosus.

Arthritis, Raynaud's phenomenon and leukocytoclastic vasculitis, in addition to focal myositis and lymphadenopathy are the most common extraglandular manifestations of primary Sjögren's syndrome. With regard to pulmonary involvement, diffuse interstitial pneumonitis has been documented. A wide range of neurological disorders has been reported, the peripheral nervous system being most frequently affected (neuropathy) but occasionally also CNS. Interstitial nephritis in a subclinical form, and as a cause of renal tubular acidosis or nephritogenic diabetes insipidus, occurs in about 30% of the patients.

Lymphomas, almost exclusively of B cell lineage, are a characteristic but unusual feature of Sjögren's syndrome, occurring in about 5% of the patients (Voulgarelis *et al*, 1999). This complication of Sjögren's syndrome is particularly found in patients with high levels of immunoglobulins, autoantibodies and cryoglobulins. When the lymphoma develops, the immunoglobulin levels often drop and the autoantibodies disappear.

Concerning secondary Sjögren's syndrome, rheumatoid arthritis patients with sicca complex tend to have more severe disease, with frequent extra-articular manifestations including vasculitis presented as digital infarcts and/or cutaneous ulcers. In systemic lupus erythematosus, patients with concomitant Sjögren's syndrome have a lower frequency of glomerulonephritis and a relatively good prognosis. Primary biliary cirrhosis and scleroderma, although rare in general, are frequently complicated by Sjögren's syndrome. Other autoimmune diseases, which have been described in association with Sjögren's syndrome, include polymyositis, mixed connective tissue disease, chronic active hepatitis and Hashimoto's thyroiditis (Morrow *et al*, 1999).

Oral diagnostic tests

For the diagnosis of Sjögren's syndrome the most practical criteria to use is the recently modified European criteria that include a list of exclusions (Vitali *et al*, 2002). In addition to the subjective symptoms of dry eyes and dry mouth the following objective items should be fulfilled: (i) ocular signs by Schirmer's I test and/or Rose Bengal score; (ii) focal sialadenitis by histopathology; (iii) salivary gland involvement by either salivary scintigraphy, parotid sialography or unstimulated salivary flow; (iv) autoantibodies of Ro/SSA and/or La/SSB specificity.

Xerostomia is of common occurrence in Sjögren's syndrome, and the easy accessibility of saliva also supports the use of sialometry and sialochemistry in the diagnosis of the disease (Kalk *et al*, 2001). Despite the fact that it is rather inaccurate and impure, the collection and analysis of whole saliva is the most used technique for sialometry. Saliva, which is produced by the three major and numerous minor submucosal salivary glands, exhibits great flow variations among healthy individuals, and in the same individual under diverse conditions (Dawes 1987; Tishler *et al*, 1997). The test should therefore be standardized; the unstimulated whole saliva collection test is performed for 15 min, and the test is considered positive when ≤ 1.5 ml whole saliva is collected.

Using sialochemical analysis, a spectrum of salivary components (sodium, chloride, calcium, phosphate, urea, amylase, lactoferrin, mucin and total protein) can be assessed. Kalk *et al* (2002), found that patients with Sjögren's syndrome clearly differed from those who tested negatively for Sjögren's syndrome according to the European classification criteria, showing lower sublingual/submandibular flow rates and appreciably changed salivary composition. They reached the conclusion that glandular sialometry and sialochemistry are not only useful tools for differentiating Sjögren's syndrome from other salivary gland diseases in clinical practice, but these investigations also have potential as diagnostic criteria for Sjögrens syndrome, showing distinct sialometric and sialochemical changes as well as profiles. Results from further studies (Kalk *et al*, 2002) have shown that the most accurate test for diagnosing Sjögren's syndrome, combines the stimulated sublingual/submandibular flow rate and parotid sodium and chloride concentration as salivary variables, with a high sensitivity and specificity. Because these non-invasive diagnostic tests can be easily applied, do not need a laboratory other than for routine blood testing, and are very accurate, gland-specific sialometry and sialochemistry may eventually replace other, more invasive, diagnostic techniques for diagnosing Sjögrens syndrome.

Other tests used to evaluate salivary gland involvement include parotid sialography, salivary gland scintigraphy and ultrasound investigation. The sialography typically shows sialectasias in contrast to the fine arborization seen in normal parotid ductules. In the scintigraphic test, ^{99m}Tc-pertechnate is given intravenously, and in Sjögren's syndrome patients the typical finding is decreased uptake in response to stimulation of the parotid and submandibular salivary glands. This test is a sensitive and valid method to measure abnormalities in salivary gland function in the hands of skilled personal (Håkansson et al, 1994). The value of further non-invasive techniques in Sjögren's syndrome is exemplified with the development of magnetic resonance imaging including also sialography, giving complementary information on the progressive pathologic changes of glandular parenchyma (Niemelä et al, 2001).

Serologic tests

Anti-La/SSB antibodies were first defined by immunodiffusion technique in association with anti-Ro60 precipitins (Clark et al, 1969). Recent studies have shown that up to 40% of anti-La-positive sera are negative on immunodiffusion and detectable only by immunoblot or enzyme-linked immunosorbant assay (ELISA). These are termed non-precipitating anti-La antibodies (Alspaugh and Tan 1975; Gordon, Mavrangelos and McCluskey, 1992). Anti-La is invariably accompanied by anti-Ro reflecting the physical association of these molecules in Ro/La ribonucleoprotein particles. In contrast, anti-Ro antibodies frequently occur in the absence of anti-La reactivity.

The reported frequencies of anti-Ro and anti-La depend on the methods of detection and referral bias of the center performing the study. Overall, anti-Ro precipitins occur in approximately 60–75% of primary Sjögren's syndrome and are also observed in cases of secondary Sjögren's syndrome irrespective of the association with systemic lupus erythematsosus, progressive systemic sclerosis, rheumatoid arthritis or primary biliary cirrhosis (Reichlin and Scofield 1996). Anti-La was initially reported to occur in up to 40% of patients with primary Sjögren's syndrome. Even higher frequencies were reported when ELISA or immunoblotting was used to analyze anti-La (Keech et al, 1996). Further studies have shown that combined detection of anti-La and anti-Ro antibodies have a higher diagnostic specificity for primary Sjögren's syndrome than anti-Ro alone (Venables et al, 1989).

Although the pathogenetic role of anti-Ro and anti-La in Sjögren's syndrome is not established, positive serology is associated with a high frequency of palpable purpura, leukopenia, lymphopenia and hypergammaglobulinaemia, and with more severe glandular disease (Harley 1989; Atkinson et al, 1992; Gerli et al, 1997). Recent studies have also found salivary enrichment of anti-Ro and anti-La in patients with Sjögren's syndrome suggesting local autoantibody production in salivary glands (Halse et al, 2000; Horsfall and Rose, Maini, 1989), as well as the presence of Ro52, Ro60 and La autoantibody-producing cells in salivary gland biopsy samples from patients with Sjögren's syndrome (Tengnér et al, 1998; Halse et al, 1999a).

Labial salivary gland biopsy

The labial salivary gland biopsy has an important role in establishing the diagnosis of Sjögren's syndrome. It is performed preferentially according to the procedure described by Daniels (Daniels 1984, 1986). After local anesthesia, a 1.5–2 cm incision is made parallel to the vermilion border in the middle of the lower lip, between the midline and the corner of the mouth. At least five lobes of labial glands are then obtained by blunt dissection. After routine histological fixation and preparation the biopsy is evaluated according to a procedure where an inflammatory focus is defined as an accumulation of at least 50 mononuclear leukocytes per 4 mm^2 (Daniels 1986b). According to the European criteria (Vitali et al, 2002, 1993) a biopsy is positive at focus score $\geq$ 1 per 4 mm^2, while the California criteria (Fox et al, 1986b) defines positive biopsy as >1 focus. Occasionally so-called epi-myoepithelial islands are seen in labial gland biopsies but these are thought to be more common in the major glands. The designation epi-myoepithelial is to be avoided as the lesions have been found to be devoid of myoepithelial cells (Ihrler et al, 1999). The more correct term would be to use lympho-epithelial lesion.

One differential diagnostic feature to focal sialadenitis is the granulomatous inflammation as seen in connection with, for example, sarcoidosis. The specificity of a positive labial salivary gland biopsy is 86.2% and the sensitivity is 82.4% in patients with primary Sjögren's syndrome diagnosed according to the European criteria (Vitali et al, 1994). The focus score is connected to the presence of keratoconjunctivitis sicca and autoantibodies (Atkinson et al, 1992; Daniels and Whitcher, 1994), while the correlation with xerostomia is less evident (Jonsson et al, 1993).

Treatment and prognosis

At present, treatment for most patients is essentially symptomatic. The patient should regularly visit a rheumatologist as well as an ophthalmologist and dentist in order to prevent and treat the consequences of mucosal dryness, in addition to extraglandular manifestations and other associated complications.

Artificial tears, ointments and soft contact lenses often alleviate the patient's ocular complaints, and are of importance in preventing corneal damage and conjunctivitis (Oxholm et al, 1992). Another treatment option for dry eye is 'punctal occlusion' by using a variety of 'plugs' to occlude the punctal openings at the inner aspects of the eyelids (Fox 1992). Using this procedure, the instilled artificial tears will remain in the eye for longer time.

The management of dry mouth aims to prevent and treat infections, periodontal disease and dental caries. To reduce the risk of caries, it is necessary to maintain a

good oral hygiene and use sugarless sweets and chewing gums to stimulate residual salivary flow. Artificial saliva products and special toothpaste may also be of benefit for certain patients, and fluoride supplementation is advocated. Eradication of oral candidiasis usually provides significant improvement of oral symptoms.

Oral pilocarpine has been shown to be a safe treatment and provide significant subjective and objective benefits for patients with Sjögren's syndrome, suffering from symptoms associated with xerostomia (Nusair and Rubinow 1999). Another potential therapy includes systemic use of interferon-alpha, which may be of benefit for the symptoms associated with xerostomia (Shiozawa *et al*, 1993; Ship *et al*, 1999). Cemiveline, a novel quinuclidine derivative of acetylcholine exhibiting high affinity for the muscarinic M3 receptor, has long lasting sialogogic action and few side-effects (Ninomiya *et al*, 1998).

Hydroxychloroquine, azathioprine, cyclosporin-A and cyclophosphamide may be useful as immunomodulating agents reducing immune activation and lymphoproliferation and sometimes are employed in patients with Sjögren's syndrome with extraglandular symptoms (Kruize *et al*, 1993; Fox, Guarrasi and Krubel, 1996). Administration of NSAIDs and systemic steroids have been suggested to improve the signs and symptoms of Sjögren's syndrome but are mainly used for treatment of severe extraglandular complications such as arthritis, vasculitis, pulmonary and renal involvement (Fox *et al*, 1993).

There are few studies on the natural course of primary Sjögren's syndrome, but it has been described as a stepwise, gradual progression from a disorder mainly in exocrine glands, to systemic extraglandular features and finally to lymphoid neoplasia development (Moutsopoulos and Manoussakis, 1989). However, in general primary Sjögren's syndrome is characterized by a stable and rather mild course of glandular and extraglandular manifestations, in contrast to the increased risk for development of malignant lymphoma (Voulgarelis *et al*, 1999).

Serology can be useful in predicting the subsequent outcome and complications in patients with primary Sjögren's syndrome. The presence of anti-SSA antibodies may identify patients with systemic disease (Kelly *et al*, 1991), and in anti-SSA/anti-SSB positive patients the relative risk of developing non-Hodgkin lymphoma has been reported as high as 49.7 after 10 years follow-up (Davidson *et al*, 1999). The development of extraglandular manifestations seems to be influenced by a number of factors including the MHC HLA B8 and DR3 expression (Pease *et al*, 1993).

Spontaneous symptomatic improvement has been described in 12% of patients with primary Sjögren's syndrome, especially in elderly patients with some clinical overlap with systemic lupus erythematosus (Kelly *et al*, 1991). In a recent study on survivorship in a population-based cohort followed from 1976 to 1992, the authors did not demonstrate increased mortality of patients with primary Sjögren's syndrome (Martens *et al*, 1999).

Conclusion

The etiology and pathogenesis of Sjögren's syndrome is still a matter of speculation although several hypotheses prevail. Nevertheless, there is considerable evidence that some – as yet unknown – initiating factor(s) set against the appropriate genetic background may evoke immunologically mediated inflammatory mechanisms that result in the chronic exocrine gland lesions. T cell mediated autoimmune responses in the glandular tissue as well as dysregulated apoptosis are currently considered to be of central importance in the pathogenesis. A plethora of autoantibodies have been linked to this autoimmune exocrinopathy although their role is not always well defined. Accordingly, B cell activation is a very consistent immunoregulatory abnormality in Sjögren's syndrome.

The search for susceptibility genes in families with Sjögren's syndrome is ongoing with the same approach employed as in the other chronic autoimmune diseases. Two major strategies are utilized; the position independent candidate gene approach with mutation screening of suspected disease related genes, and full genome scanning (microsatellite analysis) in humans as well as in animal models to determine susceptibility chromosomal regions which later will be used in a positional candidate gene strategy.

A challenge in Sjögren's syndrome will be to stratify the disease process including genetic and environmental triggers. Identification of new genetic markers and better characterization of novel autoantibodies (e.g. those directed against muscarinic receptors in exocrine glands) may lead to the development of better diagnostic and prognostic tests in Sjögren's syndrome including its systemic complications.

Acknowledgements

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