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INVITED MEDICAL REVIEW

Cytokines in Sjögren's syndrome

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Cytokines play a central role in the regulation of immunity and are often found to be deregulated in autoimmune diseases. Sjögren's syndrome is a chronic autoimmune disease characterized by inflammation and loss of secretory function of the salivary and lachrymal glands. This review highlights the current knowledge of the expression and the function of pro- and anti-inflammatory cytokines both locally and systemically in Sjögren's syndrome patients. In the salivary glands, saliva and serum of these patients, many pro-inflammatory cytokines are upregulated. Concomitantly, most anti-inflammatory cytokines are not detectable or are expressed at low levels. Besides a role in inflammation, cytokines are also thought to be involved in salivary gland dysfunction by directly interfering with the epithelial cells in the glands. Future research on the role of novel cytokines in Sjögren's syndrome in combination with a better understanding of the effect of cytokines on exocrine dysfunction will aide the identification of the best therapeutic targets for Sjögren's syndrome.
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Sjögren's syndrome

Sjögren's syndrome (SS) is a systemic chronic inflammatory autoimmune disorder that affects secretory organs such as the salivary and lachrymal glands. Patients complain of dry eyes and dry mouth (sicca symptoms) and often have systemic manifestations, such as Raynaud's phenomenon, arthritis, fatigue and vasculitis. Its estimated prevalence is 0.5% with a female to male ratio of 9:1 (Fox, 2005). The pathogenesis of the disease remains largely unknown and to date no universally effective therapy is available. The histological hallmark of SS is the presence of focal infiltration of T and, to a lesser degree, B lymphocytes in the salivary glands indicating a chronic inflammatory process (Daniels and Fox, 1992; Vitali *et al*, 2002). This chronic inflammation is reflected by an imbalance in cytokines both locally in the glands and systemically in the blood. The scope of this review is to summarize the current data on cytokine abnormalities described in patients with SS.

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Cytokines

Cytokines are powerful regulators of the innate and adaptive immune system. They play a central role in controlling the direction, amplitude and duration of the inflammatory response. Aberrations in their expression may lead to immune deficiency, allergy or autoimmunity. Cytokines are pleiotropic and can be secreted by hematopoietic cells and numerous other cell types. Most cytokines have a predominantly pro- or anti-inflammatory effect, but many can exert both functions depending on their environment.

Immune responses have been traditionally divided into two groups, Th1 and Th2, although it has recently become clear that many immune responses have features of both. A Th1 response is characterized by activation of effector T cells and the production of interferon (IFN)-γ. A Th1 response clears intracellular organisms, but is also involved in many autoimmune diseases. The main feature of Th2 responses is a humoral immune response through the activation of B cells and the production of antibodies. The main cytokine involved in Th2 responses is interleukin (IL)-4. An imbalanced Th2 response results in allergies but is also linked to autoimmunity [reviewed in Crane and Forrester (2005)]. SS is thought to be a Th1 dominated disease primarily because IFN-γ and its related cytokines are consistently found to be highly expressed in SS patients. Moreover, salivary gland-derived T cells from patients produce the Th1 cytokines IFN-γ and IL-2 *ex vivo*, but no or low levels of the Th2 cytokine IL-4 (Brookes *et al*, 1996). However, the significant hypergammaglobulinemia and high levels of autoantibodies together with high expression of IL-10, another Th2 cytokine, demonstrate a simultaneous activation of the Th2 response.

These data reflect that although the Th1/Th2 concept is useful in understanding cytokine networks and responses, its direct applicability in systemic human autoimmune diseases, such as SS, is limited.

Cytokines and salivary gland dysfunction in SS

Cytokines may contribute to the pathogenesis of SS in various ways. They play a central role in the initiation and perpetuation of inflammation in the secretory glands. The imbalance of pro- over anti-inflammatory cytokines results in cumulative damage in the glands leading to decreased secretory function. Although infiltration of the gland by lymphocytes is a hallmark of SS, some patients suffer from significant secretory dysfunction without major glandular destruction (Fox and Speight, 1996; Humphreys-Beher *et al*, 1999). As some cytokines are upregulated even in the absence of lymphocytic infiltrates, they may have a direct effect on epithelial cells independent from damage caused by inflammation. As cytokines are key molecules in systemic inflammation, they may contribute to systemic complications of SS as well. Many cytokines that are overexpressed in the glands and serum have been related to episodes of vasculitis or other systemic features. SS patients are also at a higher risk than the normal population for developing non-Hodgkin's lymphoma in the exocrine glands (Voulgarelis *et al*, 1999; Tzioufas and Voulgarelis, 2007). Although the mechanisms responsible for lymphomagenesis is not well understood, it most likely includes cytokine-driven processes, such as chronic stimulation of B and/or T cells and the formation of ectopic germinal centers (Mariette, 1999) (see Table 1).

Major pro-inflammatory cytokines in SS

The major pro-inflammatory cytokines found to be important in SS are the IFNs, IL-12, IL-18, tumor necrosis factor (TNF)-α, IL-1β, IL-6 and B-cell activating factor (BAFF). Whether the recently discovered IL-17, IL-23 and the related T-cell subset (Th17 cells) play an important role in SS remains unknown.

Interferons

Interferons were the first cytokines discovered. They play a crucial role in the innate immune response against viruses (Isaacs and Lindenmann, 1957), but over the past decades, it was shown that they have a much broader spectrum of function. They activate T cells and macrophages, affect class switching of B cells, and enhance antigen presentation and upregulation of intercellular adhesion molecules (ICAM), all leading to an activated immune state ready to fight off pathogens. IFNs also play a crucial role in auto-immunity (Gota and Calabrese, 2003; Baccala *et al*, 2005; Bave *et al*, 2005) (Tak, 2004). There are two groups of IFNs; Type I (IFN-α and -β) is secreted by virus-infected cells and plasmacytoid dendritic cells (pDC), while Type II or IFN-γ is mainly secreted by T cells, natural killer (NK) cells and macrophages (Le Page *et al*, 2000).

Interferons play a central role in the pathogenesis of SS. They are aberrantly expressed in patients and many cytokines and transcription factors that are overexpressed in patients are IFN inducible (Bave *et al*, 2005; Hjelmervik *et al*, 2005; Gottenberg *et al*, 2006; Hu *et al*, 2007; Wildenberg *et al*, 2008). Moreover, pathways that are related to IFN signaling are significantly upregulated in SS patients (Gottenberg *et al*, 2006). This profile has been referred to as 'the interferon signature' (Bave *et al*, 2005).

IFN-α

Low levels of IFN-α are constitutively present in a variety of cells and are found circulating in the blood, whereas high titers are rapidly produced in the presence of danger signals such as a viral infection (Taniguchi and Takaoka, 2001). IFN-α is also upregulated in autoimmune diseases such as systemic lupus erythematosus (SLE) (Ronnblom and Alm, 2001).

In salivary gland biopsies from SS patients, IFN-α is detected at higher levels in acini and endothelial cells compared with controls (Oxholm *et al*, 1992), but IFN-α is mainly secreted by pDCs, which are found in the salivary glands of SS patients but not in healthy controls (Gottenberg *et al*, 2006). Serum levels of IFN-α were found to be high in SS patients compared with normal individuals by some groups (Anaya *et al*, 2005; Szodoray *et al*, 2005), but not by others (Bave *et al*, 2005). On the contrary, some groups observed low circulating levels of IFN-α and argued that this could lead to reduced beneficial NK cell activity and reduced anti-viral defense mechanisms in patients (Shiozawa *et al*, 1990).

IFN-γ

Interferon-γ is the major cytokine involved in a Th1 response, a response designed to clear intracellular pathogens. However, overexpression of IFN-γ and an exaggerated Th1 response are also involved in many autoimmune diseases such as rheumatoid arthritis (RA) and multiple sclerosis (Schulze-Koops and Kalden, 2001; Hemmer *et al*, 2006). The production of IFN-γ is directly stimulated amongst other cytokines by IFN-α and IFN-β (Baccala *et al*, 2005). The presence of IFN-γ creates a pro-inflammatory environment in the salivary gland as illustrated by the observation that treatment of human salivary gland (HSG) cells with IFN-γ, in the presence or absence of TNF-α, results in increased levels of adhesion molecules and upregulation of antigen presenting molecules on the cell surface (Wu *et al*, 1994).

Table 1 Effects of cytokines in Sjögren's syndrome (SS)

Cytokines in SS are thought to play a role in
Inflammation; initiation and progression of inflammatory damage in the secretory organs
Direct effect on cells in the secretory glands leading to *impaired fluid secretion*
Chronic stimulation of B and T cells leading to *lymphomagenesis*
Systemic complications

Interferon-γ mRNA is overexpressed by infiltrating cells in the salivary gland of patients with primary SS, but has normal systemic levels in the same patients (Oxholm *et al*, 1992; van Woerkom *et al*, 2005). Contradicting results have been published on whether healthy individuals express IFN-γ in the salivary gland at constitutive levels (Oxholm *et al*, 1992; Fox *et al*, 1994; Konttinen *et al*, 1999; Wakamatsu *et al*, 2007). IFN-γ-inducible proteins, however, were clearly found in salivary gland of SS patients but not in healthy controls (Wakamatsu *et al*, 2006, 2007), supporting the notion of increased IFN-γ expression in SS.

Interestingly, IFN-γ is also highly expressed in individuals with sicca symptoms who do not have any histological signs of inflammation in the gland (van Woerkom *et al*, 2005). It is possible that, in addition to maintaining an inflammatory response with recruitment of T and B cells, IFN-γ also has a direct effect on the secretory function of the gland. *In vitro* data support this: prolonged treatment of HSG with IFN-γ in the presence or absence of TNF-α leads to a persistent depletion of intracellular Ca^{2+} stores and thus to an exhausted response system (Wu *et al*, 1996). Moreover, IFN-γ reduces the growth of HSG in a concentration-dependent way (Wu *et al*, 1994; Daniels *et al*, 2000), suggesting that IFN-γ may impair damage repair in the salivary gland.

IL-12 and IL-18

Interleukin-12 and IL-18 are pro-inflammatory cytokines, closely related to IFN-γ, which work synergistically to drive a Th1 response. They are both predominantly secreted by monocytes and macrophages and promote IFN-γ secretion (Dinarello, 2007).

Interleukin-12 and IL-18 were found to be overexpressed in SS. Expression of IL-12 was primarily observed in the infiltrating cells (Bombardieri *et al*, 2004; Eriksson *et al*, 2004; Sakai *et al*, 2008), whereas IL-18 was detected in acinar cells, ductal cells, and macrophages in salivary glands of SS patients, but not in healthy subjects, patients with chronic graft-*vs*-host disease or chronic non-SS sialoadenitis. Some groups, but not all, found that IL-18 is particularly high in those patients with anti-Ro and anti-La autoantibodies (Kolkowski *et al*, 1999; Bombardieri *et al*, 2004; Manoussakis *et al*, 2007). IL-18 may be involved in the lymphomagenesis of SS patients, because high levels of IL-18 correlated with low levels of circulating complement, salivary gland enlargement and germinal center formation, all of which are thought to be risk factors for lymphoma development (Bombardieri *et al*, 2004; Manoussakis *et al*, 2007).

TNF-α

Tumor necrosis factor-α is produced by monocytes, CD4+ T cells and epithelial cells. It upregulates the apoptotic receptor Fas on many cells including HSG (Matsumura *et al*, 2002) and in combination with IFN-γ sensitizes cells to apoptosis (Kamachi *et al*, 2002; Kulkarni *et al*, 2006). Moreover, it also plays a role in the presentation of autoantigens as the nuclear antigens Ro, La and alpha fodrin, recognized by autoantibodies in many SS patients, are only transported to the membrane surface of salivary gland cells, which undergo apoptosis in the presence of TNF-α (McArthur *et al*, 2002).

High levels of TNF-α and TNF-α secreting cells have been found in peripheral blood and in lymphocytic infiltrates in salivary gland biopsies from patients with SS (Oxholm *et al*, 1992; Koski *et al*, 2001; Willeke *et al*, 2003). TNF-α and its two receptors, TNFR-p55 and TNFR-p75, are present in biopsies of healthy controls but are expressed at higher levels in SS patients (Koski *et al*, 2001). The expression level of TNF-α did not correlate with the focus score (Moutsopoulos *et al*, 2008), but serum levels are especially high in patients positive for rheumatoid factor (RF) (Garcic-Carrasco *et al*, 2001) suggesting a correlation of TNF-α expression and severity of systemic involvement.

IL-1β

Interleukin-1β activates vascular endothelium and lymphocytes. Together with TNF-α, it is considered to be the key inflammatory cytokine in chronic inflammation; however, surprisingly little is known about its role in SS. IL-1β secreting circulating lymphocytes are significantly upregulated in SS patients compared with healthy controls and non-SS sicca patients, and its level correlates with disease duration and RF levels (Willeke *et al*, 2003; Ek *et al*, 2006). Immunohistochemical staining of salivary glands of SS patients showed expression of IL-1β, whereas biopsies from controls did not (Oxholm *et al*, 1992).

IL-6

Interleukin-6 is important for B cell growth and differentiation. It is thought to induce the production of autoreactive antibodies by infiltrating B cells via upregulation of specific cytokines and through its effect on the terminal differentiation of the immunoglobulin producing plasma B cell (Ishihara and Hirano, 2002). IL-6 has a role in T-cell stimulation and recruitment as it promotes the transition of naïve T cells to cytotoxic T cells. It also upregulates ICAM-1, which functions as a receptor for activated T cells and on many cells as a co-stimulatory molecule for B cells (Chen *et al*, 2006).

Interleukin-6 was found highly expressed in serum and in peripheral circulating lymphocytes of SS patients, and was absent in most of the healthy controls. High levels of IL-6 correlated with the degree of infiltration in the gland and the number of extraglandular symptoms (Garcic-Carrasco *et al*, 2001; Hulkkonen *et al*, 2001b; Boras *et al*, 2004; Szodoray *et al*, 2004a; Vucicevic Boras *et al*, 2006; Nguyen *et al*, 2008). IL-6 was found consistently at high levels in saliva of SS patients. Moreover, it was found in labial gland biopsies of SS, but was not detected or was detected at lower levels in

healthy controls (Oxholm *et al*, 1992; Grisius *et al*, 1997; Tishler *et al*, 1999; Boras *et al*, 2004; Nguyen *et al*, 2008). IL-6 together with TNF-α seems to be directly associated with inflammation of the gland as these two cytokines are overexpressed in saliva of SS patients but not in patients with drug-induced xerostomia (Vucicevic Boras *et al*, 2006).

BAFF

One of the most recently described cytokines implicated in SS is BAFF, which belongs to the superfamily of TNF-related cytokines and promotes B-cell survival (Mackay and Browning, 2002). BAFF exists in a membrane bound and a secreted form. BAFF induces major lymphoproliferative disorders in transgenic mice with B-cell hyperplasia and hyperglobulinemia resembling the autoimmune phenotype of SLE (Gross *et al*, 2000). At a later age, these mice develop an SS-like disease with infiltrates in the salivary gland and a reduced salivary flow (Mackay *et al*, 1999).

B-cell activating factor is equally expressed in *ex vivo* cultured epithelial cells of the salivary gland of healthy individuals and SS patients; however, patients also express BAFF at low levels in infiltrating T cells in the salivary gland, whereas healthy people do not (Ittah *et al*, 2006). SS patients also have high serum and salivary levels compared with healthy individuals, whereas the expression levels of membrane bound BAFF does not differ between patients' and healthy controls' epithelial cells (Daridon *et al*, 2007; Pers *et al*, 2007). Plasma and salivary secreted BAFF levels are especially higher in patients with hypergammaglobulinaemia, higher focus scores and in patients positive for anti-Ro and anti-La antibodies (Mariette *et al*, 2003; Gottenberg *et al*, 2005; Jonsson *et al*, 2005). BAFF induces a significant anti-apoptotic effect in peripheral B cells of SS patients; this effect is even more evident in B cells from SS patients with hypergammaglobulinemia (Szodoray *et al*, 2004b). This may indicate that BAFF is important in germinal center formation and may contribute to lymphomagenesis, but data on this are still inconclusive (Mariette *et al*, 2003; Gottenberg *et al*, 2005; Jonsson *et al*, 2005).

IL-17 and IL-23

A recently discovered subset of T lymphocytes involved in inflammation and autoimmunity, the so called Th17 cells, was originally discovered in mice and is characterized by the secretion of the powerful pro-inflammatory cytokines IL-17 and IL-23 (Cua *et al*, 2003; Park *et al*, 2005) and IFN-γ when cells are stimulated with IL-12 (Annunziato *et al*, 2007). In humans, Th17 cells are derived from memory T cells under the influence of IL-1β, IL-6, and/or IL-23. IL-4 inhibits the development of Th17 cells (Acosta-Rodriguez *et al*, 2007; van Beelen *et al*, 2007; Chen *et al*, 2007; Wilson *et al*, 2007). Th17 cells are very effective in clearing extracellular pathogens. They are also believed to have a pivotal role in the initiation and perpetuation of autoimmunity. IL-17 induces the expression of a variety of pro-inflammatory cytokines such as IL-6, TNF and ICAM in a variety of cells (Steinman, 2007).

Interleukin-17 may be an important player in the pathogenesis of SS, but data are lacking to support this to date. IL-17 could be detected in serum and saliva of about 50% of a small group of SS patients, but also in a similar percentage in healthy control subjects. In biopsies of patients, the lymphocytic foci stained positive for both IL-17 and IL-23, especially in the CD4+ T cells, and showed diffuse staining on epithelial cells. Healthy individuals and sicca patients also showed low expression of IL-17 but this was confined to ductal epithelium (Nguyen *et al*, 2008; Sakai *et al*, 2008).

Anti-inflammatory cytokines

In contrast to the overexpression of pro-inflammatory cytokines, anti-inflammatory cytokines are undetectable or are expressed at relatively low levels in SS. Interestingly, IL-10, which is considered an anti-inflammatory cytokine, is overexpressed in SS.

Transforming growth factor-β (TGF-β)

Transforming growth factor-β is a bipolar cytokine crucial to the development of immunity. It is often associated with exaggerated immune excitability and overexpression is associated with increased fibrosis. Conversely, TGF-β is key in limiting innate and adaptive immune responses, particularly self-reactive T cells, to restore immune homeostasis and to prevent autoimmunity [reviewed in (Prud'homme and Piccirillo, 2000; Wahl, 2007)].

Transforming growth factor-β mRNA is found in normal and SS salivary glands (Kolkowski *et al*, 1999), but is reduced in SS patients with a high focus score (Ogawa *et al*, 1995). TGF-β is immunohistochemically detected in the ductal epithelial cells of normal and inflamed salivary gland tissues but is absent in ductal epithelial cells surrounded by infiltrated activated T cells in the diseased gland (Kizu *et al*, 1996).

IL-4

Interleukin-4 is another classical anti-inflammatory cytokine and the main cytokine in a Th2 type immune response. This cytokine is absent or low in mucosal biopsies of SS patients (Kolkowski *et al*, 1999). Moreover, the ratio of the pro-inflammatory cytokine IFN-γ to IL-4 is higher in the salivary gland and lower in the peripheral blood of patients (van Woerkom *et al*, 2005) reflecting a skewed immune pattern towards a Th1 response locally in the gland.

IL-10

Interleukin-10 is an anti-inflammatory cytokine involved in Th2 type responses. IL-10 produced by regulatory T cells suppresses the effector immune responses. However, in the presence of IFN-γ, it can exert a pro-inflammatory effect (Sharif *et al*, 2004).

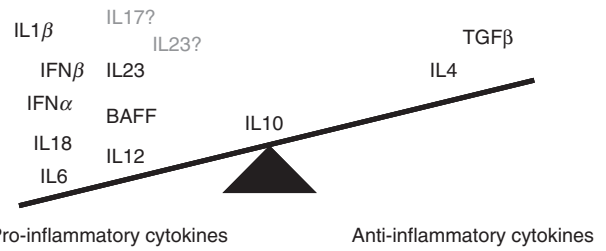

Figure 1 The cytokine profile found in Sjögren's syndrome (SS) is imbalanced with the overexpression of numerous pro-inflammatory cytokines (on the left) *vs* low or undetectable levels of anti-inflammatory cytokines (on the right). The bipolar interleukin (IL)-10 is highly expressed and may exert both anti- and pro-inflammatory effects in SS. The expression levels of the pro-inflammatory cytokines IL17 and IL23 remain unknown. TGF, transforming growth factor; IFN, interferon; BAFF, B-cell activating factor

IL-10 is important for B cell activation and prolonged stimulation of naïve B cells with IL-10 leads to plasma cell formation (Rousset *et al*, 1992, 1995).

High plasma levels of IL-10 correlate with a higher susceptibility for SS (Hulkkonen *et al*, 2001a; Anaya *et al*, 2005). High serum levels of IL-10 in SS patients are associated with higher titers of IgA RF, anti-Ro, and anti-La antibodies, and with the severity of lymphocytic infiltration in the salivary gland. Moreover, patients who have high levels of IL-10 had significantly more episodes of cutaneous vasculitis (Anaya *et al*, 2005). T cells isolated from the salivary gland from SS patients produce significantly higher levels of IL-10 in contrast to the circulating T cells of the same patients (Brookes *et al*, 1996). A significant elevation of IL-10 was found in saliva of SS patients compared with healthy controls. In patients, these elevated IL-10 levels significantly correlated with the severity of dryness of the mouth and eyes and with the erythrocyte sedimentation rate (Bertorello *et al*, 2004). These data indicate that higher levels of circulating IL-10 are associated with more systemic involvement and also play a role in the local inflammatory process. As high IL-10 levels are related to more severe disease, it is possible that the increased secretion of IL-10 represents an anti-inflammatory control mechanism, while it contributes at the same time to B-cell activation.

Summary

Cytokines play a central role in the regulation of immunity and dysregulation of the cytokine network contributes to both systemic and exocrine manifestations of SS. The cytokine imbalance in SS is characterized by the overexpression of pro-inflammatory cytokines such as IFN-γ, IL-12 and IL-18. Two other cytokines, IL-6 and BAFF, which are important in T- and B-cell activation and autoantibody production, are also upregulated. Concomitantly, IL-4 and TGF-β, two important anti-inflammatory cytokines are downregulated, possibly indicating loss of protection from autoimmunity. In contrast, the anti-inflammatory Th2 cytokine IL-10 is highly expressed in SS patients and albeit ineffective in controlling inflammation, it may contribute to B-cell activation and autoantibody production (see Figure 1). Although the expression levels of many cytokines are known in SS, less remains known about the mechanisms by which they contribute to exocrine gland dysfunction. Future research will need to be expanded to other cytokines important in chronic inflammation, such as IL-1β, IL-17 and IL-23 as well as the effects of these cytokines on both local and systemic inflammation and secretory function. A better understanding of the abnormalities in the cytokine network and their role in the pathogenesis of SS will likely lead to the identification of the best therapeutic targets for this disease.

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Author contributions

All authors contributed to this manuscript.

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