Local gene therapy and the identification of therapeutic targets in Sjögren’s syndrome
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CYTOKINES IN SJÖGREN’S SYNDROME: POTENTIAL THERAPEUTIC TARGETS

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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. The pathogenesis of the disease is 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. The chronic inflammation in SS patients is further 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.

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 γ (IFNγ). A Th1 response clears intracellular organisms, but is also involved in many autoimmune diseases. The Th2 response is characterized by a humoral immune response and results in the activation of B cells and the production of antibodies. The main cytokine involved in Th2 responses is interleukin 4 (IL-4). An imbalanced Th2 response results in allergies but is also linked to autoimmunity (reviewed in 4). SS is thought to be a Th1 dominated disease primarily because IFNγ 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 IFNγ and IL-2 ex vivo, but no or low levels of the Th2 cytokine IL-4. However, the significant hypergammaglobulinaemia 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 SJÖGREN’S SYNDROME

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 excretory glands. The imbalance of pro- over anti-inflammatory cytokines results in cumulative damage in the glands leading to decreased secretory function. However, although infiltration of the gland by lymphocytes is a hallmark of SS, some patients suffer from significant secretory dysfunction without major glandular destruction. Since 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. Moreover, SS patients are at a higher risk than the normal population for developing non-Hodgkin’s lymphoma in the exocrine glands. The mechanisms responsible for lymphomagenesis include cytokine-driven chronic stimulation of B and/or T cells and formation of ectopic germinal centers. In addition, since cytokines are key molecules in systemic inflammation, they may contribute to systemic complications of SS as well. Many cytokines that are found to be highly expressed in the glands and serum have been related to episodes of vasculitis or other systemic features.

MAJOR PRO-INFLAMMATORY CYTOKINES IN SJÖGREN’S SYNDROME

The major pro-inflammatory cytokines found to be important in SS are the interferons, IL-12, IL-18, tumor necrosis factor α (TNFα), 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 play an important role in SS is still unknown.

Interferons

Interferons were the first cytokines discovered. They were found to play a crucial role in the innate immune response against viruses, over the past decades their function has turned out to be much broader. Interferons activate T cells and macrophages, affect class switching of B cells, enhance antigen presentation and upregulation of inter-cellular adhesion molecules, all leading to an activated immune state ready to fight off pathogens. IFNs also play a crucial role in autoimmunity. There are two groups of interferons; Type I interferon (IFNα and IFNβ) is secreted by virus-infected cells and plasmacytoid dendritic cells (pDC) while type II, or IFNγ is mainly secreted by T cells, natural killer (NK) cells and macrophages.

IFNs play a central role in the pathogenesis of SS. They are aberrantly expressed in patients and most cytokines and transcription factors that are overexpressed in patients are IFN inducible. Moreover, activated pathways that are related to IFN signalling were significantly different between SS patients and healthy volunteers. This profile has been referred to as ‘the interferon signature’.
IFNα
Low levels of IFNα are constitutively present in a variety of cells and are found circulating in the blood, whereas high titres are rapidly produced in the presence of danger signals such as a viral infection. IFNα is also upregulated in autoimmune diseases like systemic lupus erythematosus (SLE).

In salivary gland biopsies from SS patients IFNα is detected at higher levels in acini and endothelial cells compared to controls. Moreover, IFNα is mainly secreted by pDC, these cells are found in the salivary glands of SS patients but not in healthy controls. Serum levels of IFNα were found to be high in SS patients compared to healthy individuals by some groups, but not by others. On the contrary, some groups observed low circulating levels of IFNα and argued that this could lead to reduced beneficial NK cell activity and reduced anti-viral defense mechanisms in patients.

IFNγ
IFNγ is the major cytokine involved in a Th1 response, a response designed to clear intracellular pathogens. However, overexpression of IFNγ and an exaggerated Th1 response are also involved in many autoimmune diseases, such as RA and multiple sclerosis (MS). The production of IFNγ is directly stimulated, amongst other cytokines, by IFNα and IFNβ. The presence of IFNγ creates a pro-inflammatory environment in the salivary gland as illustrated by the observation that treatment of human salivary gland (HSG) cells with IFNγ, in the presence or absence of TNFα, results in increased levels of adhesion molecules and upregulation of antigen presenting molecules on the cell surface.

IFNγ mRNA is overexpressed by infiltrating cells in the salivary gland of patients with primary SS, but has normal systemic levels in the same patients. Contradicting results have been published on whether healthy individuals express IFNγ in the salivary gland at constitutive levels. IFNγ inducible proteins, however, were clearly found in salivary glands of SS patients but not in healthy controls, supporting the notion of increased IFNγ expression in SS.

Interestingly, IFNγ is also highly expressed in individuals with sicca symptoms who do not have any histological signs of inflammation in the gland. It is possible that, in addition to maintaining an inflammatory response with recruitment of T and B cells, IFNγ also has a direct effect on the secretory function of the gland. In vitro data support this: prolonged treatment of HSG with IFNγ in the presence or absence of TNFα leads to a persistent depletion of intracellular Ca2+ stores and thus to an exhausted response system. Moreover, IFNγ reduces the growth of HSG in a concentration dependent way, suggesting that IFNγ may impair damage repair in the salivary gland.

IL-12 and IL-18
IL-12 and IL-18 are pro-inflammatory cytokines, closely related to IFNγ, that work synergistically to drive a Th1 response. They are both predominantly secreted by monocytes and macrophages and promote IFNγ secretion. IL-12 and IL-18 were found to be overexpressed in SS. Expression of IL-12 was primarily observed in the infiltrating cells. 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-versus-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\textsuperscript{37,40-41}. IL-18 may be involved in the lymphomagenesis of SS patients, since 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\textsuperscript{37,41}.

**TNFα**

TNFα is produced by monocytes, CD4\(^+\) T cells and epithelial cells. It upregulates the apoptotic receptor Fas on many cells including HSG\textsuperscript{42} and in combination with IFNγ sensitizes cells to apoptosis\textsuperscript{43-44}. Moreover, it also plays a role in the presentation of autoantigens since 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 TNFα\textsuperscript{45}.

High levels of TNFα and TNFα secreting cells have been found in peripheral blood and in lymphocytic infiltrates in salivary gland biopsies from patients with SS\textsuperscript{22,46-47}. TNFα, 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\textsuperscript{47}. The expression level of TNFα did not correlate with the focus score\textsuperscript{48}, but serum levels are especially high in patients positive for rheumatoid factor (RF)\textsuperscript{49} suggesting a correlation of TNFα expression and severity of systemic involvement.

**IL-1β**

IL-1β activates vascular endothelium and lymphocytes. Together with TNFα 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\textsuperscript{46,50}. Immunohistochemical staining of salivary glands of SS patients showed expression of IL-1β whereas biopsies from controls did not\textsuperscript{22}.

**IL-6**

IL-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\textsuperscript{51}. IL-6 has an active role in T cell stimulation and recruitment since it promotes the transition of naïve T cells to cytotoxic T cells. It also upregulates intercellular adhesion molecule 1 (ICAM-1) which functions as a receptor for activated T cells and a co-stimulatory molecule for B cells, on many cells\textsuperscript{52}.

IL-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\textsuperscript{49,53-57}. IL-6 in saliva of SS patients was found to be consistently high. Moreover, it was found in labial gland biopsies of SS, but was not detected or was
detected at lower levels in healthy controls\textsuperscript{52,55,57-59}. IL-6 together with TNF\(\alpha\) seems to be directly associated with inflammation of the gland since these two cytokines are overexpressed in saliva of SS patients but not in patients with drug-induced xerostomia\textsuperscript{56}.

**BAFF**

One of the newest growth factors implicated in SS is BAFF which belongs to the superfamily of TNF related cytokines and promotes B cell survival\textsuperscript{60}. BAFF exists in a membrane bound and a secreted form. BAFF induces major lymphoproliferative disorders in transgenic mice with B cell hyperplasia and hyperglobulinaemia resembling the autoimmune phenotype of SLE\textsuperscript{61}. At a later age these mice develop a SS like disease with infiltrates in the salivary gland and a reduced salivary flow\textsuperscript{62}.

BAFF 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\textsuperscript{63}. SS patients also have high serum and salivary levels compared to healthy individuals whereas the expression levels of membrane bound BAFF does not differ between patients’ and healthy controls’ epithelial cells\textsuperscript{64,65}. 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\textsuperscript{66-68}. 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 high levels of gammaglobulin\textsuperscript{69}. This may indicate that BAFF is important in germinal center formation and may contribute to lymphomagenesis, but data on this are still inconclusive\textsuperscript{66-68}.

**IL-17 and IL-23**

A recently discovered subset of T lymphocytes involved in inflammation and autoimmunity, Th17 cells, was originally discovered in mice and is characterized by the secretion of the powerful pro-inflammatory cytokines IL-17 and IL-23\textsuperscript{70,71} and IFN\(\gamma\) when cells are stimulated with IL-12\textsuperscript{72}. In humans, Th17 cells are derived from memory T cells under the influence of IL-1\(\beta\), IL-6 and/or IL-23. IL-4 inhibits the development of Th17\textsuperscript{73-76}. 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 intercellular adhesion molecules in a variety of cells\textsuperscript{77}.

IL-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 fifty percent 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 IL17 but this was confined to ductal epithelium only\textsuperscript{39,57}. 
CHAPTER 2

ANTI-INFLAMMATORY CYTOKINES

Many pro-inflammatory cytokines are overexpressed in SS. Concomitantly, some, but not all of the anti-inflammatory cytokines are lacking or are expressed at relatively low levels.

**TGFβ**

Transforming growth factor β (TGFβ) is a bipolar cytokine. TGFβ is crucial to development of immunity. It is often associated with exaggerated immune excitability and overexpression is associated with increased fibrosis. Conversely, TGFβ is key in limiting innate and adaptive immune responses, particularly self-reactive T cells, to restore immune homeostasis and to prevent autoimmunity (reviewed in78-79).

TGFβ mRNA is found in normal and SS salivary glands40, but is reduced in SS patients with a high focus score80. TGFβ 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 gland81.

**IL-4**

IL-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 patients40. Moreover, the ratio of the pro-inflammatory cytokine IFNγ to IL-4 is higher in the salivary gland and lower in the peripheral blood of patients29 reflecting a skewed immune pattern towards a Th1 response locally in the gland.

**IL-10**

IL-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 IFNγ it can exert a pro-inflammatory effect82. IL-10 is an important B cell activating factor and prolonged stimulation of naïve B cells with IL-10 leads to plasma cell formation83-84.

High plasma levels of IL-10 correlate with a higher susceptibility for SS24,85. High serum levels of IL-10 in SS patients are associated with higher titters of IgA rheumatoid factor, 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 vasculitis24. T cells isolated from salivary glands from SS patients produce significantly higher levels of IL-10 in contrast to the circulating T cells of the same patients5. 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 rate86. 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. Since 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.

In table 1 the cytokines we previously discussed and their role and expression levels are summarized. In short, the cytokine imbalance in SS is characterized by the
overexpression of pro-inflammatory cytokines, such as IFNγ, 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. The presence of other cytokines like IL-1β and IL-17 may also play a role in the pathogenesis of SS, but data on these are incomplete. Concomitantly, IL-4 and TGFβ, two important anti-inflammatory cytokines are downregulated. In contrast, the anti-inflammatory Th2 cytokine IL-10 is highly expressed in SS patients compared to healthy controls and may contribute to B cell activation and autoantibody production. Figure 1 depicts (in a simplified way) the cytokines, the processes they are involved in and their relationship to each other.

**Figure 1.** The effect of key cytokines on the different aspects of Sjögren’s syndrome (SS). An imbalance in the local expression of pro-inflammatory and anti-inflammatory cytokines leads to chronic inflammation and salivary gland dysfunction. Pro-inflammatory cytokines are shown in dark gray boxes, anti-inflammatory in green. IL-10, a bipolar cytokine with known pro- and anti-inflammatory characteristics, is shown in green and gray. The effect of cytokines on the most important pathological processes (white ovals) in SS are shown by blue arrows. The effect on cytokines on each other is shown in orange arrows. IL-4 and TGFβ are expressed at low levels or not detectable in SS. IL-17 and IL-23 (in orange) may play a role in SS (dotted lines) but conclusive data on this is not yet available. Cytokines in the red framed boxes depict cytokines which may provide a good target for therapy. DC, dendritic cells, IL, interleukin, TGF, transforming growth factor, BAFF, B cell activating factor, TNF, tumor necrosis factor, IFN, interferon.
Table 1. Cytokines involved in patients with Sjögren’s syndrome (SS), their function, their relative expression levels in salivary gland (SG) and serum compared to healthy controls and their proposed effect in SS. Natural killer, NK; major histocompatibility complex, MHC; human salivary gland cells, HSG; germinal centers, GC.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>General effect</th>
<th>Expression SG</th>
<th>Serum levels</th>
<th>Proposed effect in SS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNα</td>
<td>Anti viral response, activation of NK cells, induction of MHC I and cellular adhesion molecules, induces IFNγ</td>
<td>↑18,22</td>
<td>↑23-24, =14, ↓20</td>
<td>Upregulation of numerous pro-inflammatory pathways(^\text{18}), reduced NK cell activity/ reduced anti viral response(^\text{25})?</td>
</tr>
<tr>
<td>IFNγ</td>
<td>Central Th1 cytokine, antiviral and anti bacterial response. Induction of numerous pro-inflammatory cytokines, up-regulation of class I and II MHC antigens and leukocyte adhesion molecules, modulation of macrophage effector functions</td>
<td>↑21-22,29,31,33</td>
<td>=21-22,29</td>
<td>Depletion of Ca(^+) stores in HSG(^\text{34}), reduced growth of HSG(^\text{28,35}), induction of adhesion molecules and antigen presenting molecules on HSG(^\text{28})</td>
</tr>
<tr>
<td>IL-12/IL-18</td>
<td>Induction of IFNγ secretion, CD4(^+) T cell differentiation to Th1 cells</td>
<td>↑37-41</td>
<td>↑37-38</td>
<td>GC formation and lymphomagenesis/ auto-antibody antibody formation(^\text{37,40-41})</td>
</tr>
<tr>
<td>TNFα</td>
<td>Induction of apoptosis, activation of vascular endothelial cells and macrophages, clearing of pathogens</td>
<td>↑22,47</td>
<td>↑46,49,87</td>
<td>Auto-antigen presentation of Ro and La(^\text{45}), correlates with number of systemic complications(^\text{49})</td>
</tr>
<tr>
<td>IL-1β</td>
<td>Activation of vascular endothelium, activation of lymphocytes and NK cells, major component of acute inflammatory response</td>
<td>↑22</td>
<td>↑46,50</td>
<td>?</td>
</tr>
<tr>
<td>IL-6</td>
<td>B cell proliferation and terminal differentiation into plasma cells, T cell stimulation and recruitment, acute phase responses</td>
<td>↑(↑ in saliva)(^\text{22,55,57-59})</td>
<td>↑49,53-57</td>
<td>Correlates with degree of inflammation and systemic features(^\text{49,53-57})</td>
</tr>
<tr>
<td>BAFF</td>
<td>B cell development, maturation and survival</td>
<td>=/↑ in infiltrating T cells(^\text{53-65})</td>
<td>↑64-68</td>
<td>Resistance to apoptosis in B cells(^\text{54}), autoantibody formation/ GC formation and lymphoma genesis(^\text{66-68})</td>
</tr>
<tr>
<td>IL-17/IL-23</td>
<td>Clearing of extracellular pathogens, essential for many auto immune diseases in animal models, induction of many pro-inflammatory cytokines</td>
<td>↑ in infiltrating cells(^\text{39,55}), = in saliva(^\text{37})</td>
<td>=57</td>
<td>?</td>
</tr>
<tr>
<td>TGFβ</td>
<td>Anti-inflammatory effect, overexpression is related with fibrosis</td>
<td>=(^\text{40/ high focus score})(^\text{80,81})</td>
<td>?</td>
<td>Reduced protection from autoimmunity, inflammation?</td>
</tr>
</tbody>
</table>
CURRENT EXPERIENCE WITH CYTOKINE DIRECTED THERAPIES

IFNα was the first cytokine used in a therapy for SS patients based on the observation that SS patients have low levels of circulating IFNα (a view which has since been challenged) and reduced sensitivity of NK cells indicating a potentially reduced antiviral response. Early small studies used high dose parenteral IFNα with overall positive results on salivary gland function and focus score. At the same time, several groups showed that oromucosal administration of low dose IFNα was biologically and clinically effective in animal models of autoimmune diseases. These observations and the concern about potential toxicities associated with high dose IFNα led to studies evaluating low dose oral IFNα. Initial studies showed improvement in some but not the same outcome measure of salivary gland function and focus score. A Phase III study of 497 patients treated with placebo or IFNα lozenges chose stimulated salivary flow and subjective oral dryness as the co-primary outcomes. This study was negative for these primary endpoints because both the placebo and the interferon treated groups showed significant but similar improvement in stimulated saliva. Interestingly, compared to placebo recipients those treated with IFNα had a significantly higher improvement in unstimulated salivary flow, and showed improvement in several other subjective secondary endpoints.

Preliminary studies with TNF-blocking agents were also encouraging with positive outcome in both objective and subjective parameters after infliximab treatment. However, a larger randomized, double-blind, placebo-controlled study of infliximab with 103 SS patients showed no difference in response between the placebo versus the infliximab treated. Similarly, etanercept was also not more effective than placebo in a 12 week placebo-controlled.

A major limitation of these studies is that they do not provide an explanation for the disappointing results. The negative results could be due to suboptimal study design, ineffective dosing, insensitive outcome measures, biologic inefficacy or the combination of these factors. Patient selection is important and should be tailored to the goal of the therapy. If improving salivary flow is the goal, including only patients

<table>
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>IL-4</td>
<td>CD4+ T cell differentiation to Th2 response, inhibition of Th17 differentiation</td>
<td>↓60</td>
<td>↓(related to IFN levels)</td>
<td>Reduced protection from autoimmunity, inflammation?</td>
</tr>
<tr>
<td>IL-10</td>
<td>Anti-inflammatory, stimulation of immunoglobulin secretion from B cells, pro-inflammatory in presence of IFNγ</td>
<td>↑↑24,85</td>
<td>↑↑24,85</td>
<td>Higher susceptibility in individuals with high serum levels, systemic complications, direct relation with dryness?</td>
</tr>
</tbody>
</table>

Table 1. Continued.
who have some salivary function will improve the chance to show an effect, since patients who may not have any functional tissue left due to fibrosis or atrophy will be excluded. The importance of this consideration was already shown in a study with rituximab\textsuperscript{98}. Alternatives to traditional outcome measures of saliva and tear flow are much needed, since our current measures are both insensitive and non specific and do not distinguish between disease activity and damage. Most importantly, early clinical studies should address the biologic effects of the treatments. For example, it is not known whether the lack of efficacy of TNF-inhibitors was due to suboptimal doses, which were insufficient to suppress TNF in the target organs or if effective suppression of TNF was achieved but other mechanisms of the underlying pathophysiology negated the benefit of TNF-blockade. This latter mechanism is supported by recent observations showing paradoxical elevation of TNF\textsubscript{α}\textsuperscript{87}, IFN\textsubscript{α} and BAFF\textsuperscript{99} after etanercept treatment.

**FUTURE TARGETS**

Despite the disappointing clinical results to date cytokines remain attractive albeit challenging therapeutic targets. The pharmaceutical industry shows little interest to develop biologic therapies primarily for SS; therefore we will focus on cytokines which could be targeted with biologics that are available or are in clinical testing for other indications (Table 2).

IFN-regulated genes are overexpressed in both the exocrine glands and peripheral blood in SS suggesting an exaggerated interferon response\textsuperscript{100}. Suppressing IFN\textsubscript{α} represents an appealing therapeutic target but the clinical significance of the IFN signature and the role of IFN\textsubscript{α} in SS, in general is not yet understood. First, the IFN signature showed no association with any clinical manifestations other than the presence of anti-SS\textsubscript{a} and SS\textsubscript{b} antibodies in SS\textsuperscript{100} and, as discussed above, therapeutic trials with IFN\textsubscript{α} showed no harmful effects and may have had some benefit. So, on the one hand, although conventional wisdom overwhelmingly supports the blockade of IFN\textsubscript{α} as a treatment option in SS, based on the available clinical data of IFN\textsubscript{α} treatment of patients with established SS, it should not be dismissed as a potential therapy. On the other hand, the majority of experts support the idea of blocking IFN\textsubscript{α} to treat SS. Several IFN blocking agents are under development. Single administration of an anti-IFN\textsubscript{α} monoclonal antibody in SLE successfully suppressed the IFN signature\textsuperscript{101}. Further studies are required to test whether this translates into clinical benefit. Multiple dosing studies are underway and if they show a reasonable safety profile interferon blocking agents should be tested in SS.

A more recently described cytokine implicated in SS is BAFF a major promoter of B cell survival. In patients with SS BAFF levels are elevated in the serum, saliva and exocrine glands\textsuperscript{65}. BAFF is upregulated in salivary gland epithelial cells in vitro after viral infection and after treatment with IFN\textsubscript{α}\textsuperscript{102} suggesting that it may represent a link between innate and adaptive immunity. Moreover, BAFF seems to be involved in the formation of ectopic germinal centers in the salivary glands which may be an important step in lymphomagenesis. Several anti-BAFF agents are currently tested
in autoimmune diseases. In SS, studies with belimumab, an anti-BAFF monoclonal antibody, which showed efficacy in SLE\textsuperscript{103}, are planned.

IL-6, a potent pro-inflammatory cytokine, is involved in acute phase reactions and both B and T cell responses. It was found to be consistently high in saliva and serum, and is highly expressed in the salivary glands of SS patients but not in subjects with xerostomia only\textsuperscript{22,55,57-59}. A monoclonal antibody against the IL-6 receptor exhibited efficacy and a good safety profile in rheumatoid arthritis (RA)\textsuperscript{104}. The same antibody led to normalization of the abnormal peripheral B lymphocyte repertoire in a pilot study in patients with SLE\textsuperscript{105}. B cell abnormalities are similar between SS and SLE, and are characterized by a shift to increased plasma cell and memory B cell populations. Therefore, blocking IL-6 or its receptor may have a beneficial effect on both the local inflammatory process and systemic autoimmunity in SS.

Overexpression of IL-12 and IL-18 is associated with inflammation and decreased function in the gland as well as lymphomagenesis. Limited, preliminary studies with an IL-18 binding protein were performed in rheumatoid arthritis and psoriasis\textsuperscript{106}. A monoclonal antibody against the p40 subunit of IL-12 showed beneficial effects in Crohn’s disease and psoriasis. Since the p40 subunit is shared with the recently discovered IL-23\textsuperscript{107}, it is likely that, at least some of, the beneficial effects are due to blocking IL-23\textsuperscript{108}. IL-23 was found at higher levels in the salivary gland in SS\textsuperscript{57,109} and if its role in chronic inflammation were confirmed, blocking the shared p40 subunit of IL-12 and IL-23 would be appealing. IL-17 secreting CD4\textsuperscript{+} T cells have recently been identified as a specific subset with an important role in inflammation and autoimmunity.

<table>
<thead>
<tr>
<th>Target</th>
<th>Rationale for blocking in SS</th>
<th>Stage of drug development</th>
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<tbody>
<tr>
<td>IFN\textalpha</td>
<td>decrease inflammation, reverse Th1 type immune response</td>
<td>anti-IFN\textalpha monoclonal antibody: single dose suppressed interferon signature in SLE, multiple dose Phase I study completed</td>
</tr>
<tr>
<td></td>
<td>administration: enhancing NK cell activity? multiple parenteral formulations approved, oral lozenge in clinical studies</td>
<td></td>
</tr>
<tr>
<td>BAFF</td>
<td>decrease B cell activation, prevention of GC formation and lymphoma-genesis, reduction of autoantibodies</td>
<td>phase III studies have shown efficacy in SLE</td>
</tr>
<tr>
<td>IL-6</td>
<td>decrease systemic and local inflammation, decreasing B cell activation, decrease plasma cell formation, reduction of autoantibodies</td>
<td>phase III studies in RA successfully completed, pilot study in SLE showed normalization of B cell repertoire</td>
</tr>
<tr>
<td>IL-12/IL-23</td>
<td>decrease inflammation, reduction of GC formation and lymphoma-genesis</td>
<td>phase III clinical trial in psoriasis successfully completed, phase II for Crohn’s disease currently undertaken</td>
</tr>
<tr>
<td>IL17</td>
<td>reverse autoimmunity, reducing inflammation</td>
<td>phase II clinical trial for rheumatoid arthritis, Crohn’s disease and psoriasis currently undertaken</td>
</tr>
</tbody>
</table>
SS patients have increased expression of IL-17 in the salivary glands\textsuperscript{39,57,109} and higher levels in the serum\textsuperscript{109}. Further studies are needed to establish the role of IL-17 in humans but it may represent an exciting future target.

Successful cytokine-based therapies must have a reasonable safety profile, should reduce inflammation systemically and locally and should restore the secretory function. Because of the redundancy of the cytokine network targeting a single candidate may not achieve all criteria. Therefore, for an effective therapeutic response it may be necessary to use a combination of cytokine targets concomitantly or sequentially or target downstream effector molecules shared by several cytokines. A major limitation of these approaches is the increased risk of potentially severe side effects, which is not justified for many SS patients. Because the salivary glands are relatively easily accessible, an alternative to systemic treatment, which would greatly improve the risk benefit ratio of cytokine based therapy for SS patients, would be the local delivery of cytokines or their inhibitors, for example by gene therapy, successfully applied to animal models of SS\textsuperscript{110-111}. The increasing availability of biologics and the potential of gene therapy are exciting, but identifying the right target remains a challenge that can only be overcome by a better understanding of the pathogenesis of SS. Well designed proof of concept studies addressing the biologic effects of cytokine directed therapies will facilitate the identification of targets which can be tested for clinical efficacy. Since SLE and SS share many pathophysiological similarities and a large proportion of SLE patients have coexisting SS, SLE patients enrolled in studies with biologics that may work in SS should be evaluated for SS, as well. This could be done relatively easily and would provide significant information about the potential value of various cytokines as potential targets in SS.

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CHAPTER 2


