Local immunomodulatory gene therapy for Sjögren’s syndrome
Lodde, B.M.

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Summary and Discussion

Experience with Experimental Biological Treatment and Local Gene Therapy of Sjögren’s Syndrome: Implications for Exocrine Pathogenesis and Treatment
(Continued)

Beatrijs M. Lodde¹,², Bruce J. Baum¹, Paul P. Tak², Gabor Illei¹

¹ Gene Therapy and Therapeutics Branch/NIDCR, National Institutes of Health, DHHS, Bethesda, MD, USA;
² Clinical Immunology and Rheumatology, Academic Medical Center/University of Amsterdam, Amsterdam, the Netherlands.

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Abstract

Sjögren’s syndrome (SS) is an autoimmune exocrinopathy, mainly affecting lacrimal and salivary glands and resulting in ocular and oral dryness (keratoconjunctivitis sicca and xerostomia). The etiology and pathogenesis are largely unknown and only palliative treatment is currently available. Data obtained from experimental animal and human studies employing biological agents or gene therapeutics can offer insight into the SS disease process. Here, we review the current literature on these approaches and assess the lessons learned about the SS pathogenesis.
Introduction

As of today, the exact immunopathogenesis of Sjögren’s syndrome (SS) remains unknown. However, recent advances in applications of gene therapeutics and biological agents for SS (both animal and clinical studies) could provide insight into this complex process. Herein, we explore this possibility.

Animal Studies

Several animal models of SS exist that differ in the presentation of various SS features. The non-obese diabetic (NOD) mouse, for example, is the most useful, commonly available animal model to examine the SS disease characteristics. It develops, besides type I, insulin-dependent diabetes mellitus, exocrine gland infiltrates and decreased glandular secretion, which are age and gender dependent. The male NOD mouse is used for studying dacryoadenitis, whereas sialadenitis is present in the female mouse. Lymphocytes and autoantibodies play an important role in the development of the SS-like disease in NOD mice, since NOD-scid mice and B cell-deficient NOD mice have a normal salivary gland function. We will first discuss the lessons learned from in vivo gene transfer experiments using various therapeutic genes in the NOD mouse model and other animal models of SS.

Interleukin-10

Interleukin-10 (IL-10), mainly expressed in peripheral T cells, monocytes and B cells, is a cytokine capable of inhibiting synthesis of pro-inflammatory cytokines, such as interferon-γ (IFN-γ), IL-2, IL-3, and tumor necrosis factor-α (TNF-α), and reducing the activation of monocytes/macrophages. However, IL-10 also displays immunostimulatory properties, especially on B cells and activated CD8+ T cells. Local human IL-10 (hIL-10) gene delivery to the salivary glands has proven to be successful in the female NOD mouse model for SS resulting in an increased salivary flow rate and a lower focus score (less focal infiltration in salivary glands) after both prophylactic and therapeutic administration. Increased salivary gland levels of IL-4, IL-6, IL-10, IL-12 compared to controls indicated there was no straightforward ‘repair’ of a presumed Th1-Th2 imbalance. Viral IL-10 (vIL-10) has 84% sequence homology with hIL-10 and mimics several of its immunosuppressive activities without enhancing MHC II expression on mouse B cells or costimulating mouse thymocytes or mast cell proliferation. Prophylactic in vivo transduction of the lacrimal gland with adenovirus-mediated vIL-10 (AdvIL-10) delivery partially suppressed the appearance of SS-like features such as reduced tear production, accelerated tear break-up time, ocular surface disease, and immunopathologic response. A reduced size and number
of immune infiltrates due to decreases in cells positive for CD4 and CD18 (leucocyte cell surface marker essential for leucocyte adhesion to endothelial cells and chemotaxis), reduction of MHC II expressing cells, and increase in CD8+ cells were detected in this setting.

In contrast, transgenic overexpression of endogenous IL-10 in the exocrine glands of C57BL/6 mice led to tissue destruction and the development of SS. In SS patients, salivary gland and serum IL-10 levels are variably increased, depending on disease stage and activity, and might be contributing to B cell activation and lymphoma development. Therefore, the exact role of IL-10 in SS pathogenesis still needs to be established.

**Tumor necrosis factor-α inhibition**

TNF-α is a dominant pro-inflammatory cytokine that is increased in SS glands: TNF-α and its cognate receptors have been found on infiltrating mononuclear inflammatory cells, vascular endothelial cells, ductal epithelial cells, and fibroblasts. However, the exact role of TNF-α in autoimmune pathology has yet to be determined. An adenovirus encoding the human 55-kDa TNF receptor extracellular domain linked to a mouse immunoglobulin G (IgG) heavy chain (AdTNFRp55-Ig) was utilized in a dacryoadenitis rabbit model. Prophylactic administration to the lacrimal glands concurrently with induction of dacryoadenitis led to a partial suppression of SS-like features. The tear production decreased in the control group, but was unchanged in the treated group, while the tear break-up time and Rose Bengal staining (an indicator of corneal surface defects) properties were similar for both groups. This is a reflection of immunoregulation in the gland, but not the conjunctiva. The same research group also observed a therapeutic effect of vector delivery, i.e., after disease had been induced, tear production returned to normal levels, tear break-up time and Rose Bengal score improved, and immunopathology diminished (lower CD4:CD8 ratio and reduced infiltration of T cells and leucocytes). Thus, local TNF-α inhibition shows promising results for the ophthalmological component of the SS-like disease.

In preliminary experiments, we administered an rAAV2 vector encoding TNFRp55-Ig to NOD mouse submandibular glands prior to the onset of SS-like pathology. Eight weeks after vector delivery, animals were evaluated for salivary flow and glandular inflammatory infiltrates (focus scores). At a dose of $10^9$ vector particles/gland, rAAV2TNFRp55-Ig led to a considerable increase in salivary flow, as well as a reduction in focus scores, compared with mice receiving a control vector.

**Vasoactive intestinal peptide**

Vasoactive intestinal peptide (VIP) is a small neuropeptide with pleiotropic func-
tions in the neuro-immuno-endocrine network.\textsuperscript{19} Being an immunomodulator, and secretory and trophic stimulus VIP could be an interesting therapeutic candidate for SS,\textsuperscript{20} although a short half-life limits its usage. Delivery of rAAV2hVIP to the salivary glands of NOD mice in a prophylactic experimental design resulted in an increased salivary flow rate and a reduction of salivary gland levels of the cytokines IL-2, IL-10, IL-12(p70) and TNF-\(\alpha\), and serum levels of the chemokine RANTES.\textsuperscript{20} It was hypothesized that secreted hVIP directly enhanced saliva secretion by acinar cells and/or influenced the immune milieu. Delgado \textit{et al.} postulated VIP to be a Th2 cytokine with a key role in neuroimmunology,\textsuperscript{21,22} i.e., VIP production by Th2 cells, as well as VIP stimulation of Th2 and inhibition of Th1 functions. Upon gene transfer of hVIP, no marked shift from Th1 to Th2 cytokine production was observed, but rather a down-modulation of several Th1 and Th2 cytokines was seen. This indicated that VIP acted as a more overall immunosuppressant than strict Th2 cytokine in this SS model.

\textit{Nuclear factor \(\kappa\)B inhibitor \(\alpha\)}

As noted above, the role of individual cytokines in SS pathogenesis is unclear. Although overexpressing or blocking these molecules can result in some clinical improvement, studies thus far suggest there is no significant Th1-Th2 shift in cytokine profile. Therefore, it might be preferable to induce a more general, but cell-type-localized, blockade of downstream immunoregulatory events. Nuclear factor \(\kappa\)B (NF-\(\kappa\)B), a group of inducible dimeric transcription factors, is expressed in all cell types. Following stimulation and degradation of its inhibitory protein, I\(\kappa\)B, cytoplasmic NF-\(\kappa\)B is translocated to the nucleus, where it plays an important regulatory role in the cellular response to inflammatory processes.\textsuperscript{23-25} An I\(\kappa\)B\(\alpha\) mutant that renders the inhibitor a super repressor (I\(\kappa\)B\(\alpha\)(sr)) is resistant to immediate degradation upon stimulation, thereby preventing NF-\(\kappa\)B activation.\textsuperscript{25} To test if this agent would be useful in SS treatment, rAAV2I\(\kappa\)B\(\alpha\)(sr) was administered to salivary glands of female NOD mice. Inhibition of the NF-\(\kappa\)B pathway resulted not only in the reduction of several cytokines in the salivary gland (IL-2, IL-10, IL-12(p70), TNF-\(\alpha\), and RANTES), but also considerably improved salivary flow rate (Chapter 6). Again, as above, a reduction in clinical SS parameters was observed, but there was no shift from Th1 to Th2.

\textit{Anti-CD4 antibody}

The molecular marker CD4 is expressed by activated Th cells, the most predominant cell type in SS glandular tissue.\textsuperscript{26} Studies by Thompson \textit{et al.}\textsuperscript{6} and Arakaki \textit{et al.}\textsuperscript{27} have clearly shown that CD4\(^+\) T cells play a key role in the development and maintenance of SS. For example, Thompson \textit{et al.} reported prevention of lymphocytic infiltration and resolution of established pathology of the salivary glands in NOD mice treated with a non-depleting anti-CD4 antibody; salivary function was not assessed.\textsuperscript{6} The anti-CD4 antibody led to tolerance induction,
possibly by the generation of regulatory T cells. Arakaki et al. showed that adoptive transfer of autoreactive CD4+ T cells into normal syngeneic recipients induced autoimmune lesions similar to those of SS.27 The autoreactive CD4+ T cell lines recognized synthetic α-fodrin, a membrane skeleton protein. Of interest, autoantibodies against α-fodrin have been detected in human SS28,29 and NOD mice.30 Similarly, topical eye administration of an anti-CD4 monoclonal antibody also suppressed the local activation of CD4+ T cells rather than deleting them, which reduced the expansion of pathologic CD4+ T cells against α-fodrin.31

**Cyclosporine A**

Although cyclosporine is not strictly considered to be a biological agent, we have included it here, because this small fungal peptide acts by inhibiting nuclear translocation of transcription factor NF-AT (nuclear factor of activated T cells). This leads to reduced transcription of several cytokine genes, including IL-2, IL-3, and IL-4, and TNF-α. It acts primarily on T cells, inhibiting their activation.32 Topical cyclosporine A appears to improve tear secretion in SS mouse models by preventing lymphocyte-induced apoptosis of acinar cells. In one model this was achieved by preventing lymphocyte infiltration (NFS/sld mice) and in the other by reducing Fas-ligand (FasL) expression on infiltrating lymphocytes (NOD mice).33 The key mechanism for the therapeutic effect of topical cyclosporine A for keratoconjunctivitis sicca appears to be inhibition of apoptosis.34 In addition, Strong et al. hypothesized this occurs through either reduction of pro-apoptotic cytokines on the ocular surface or inhibition of the caspase cascade.34

**Apoptosis**

Dysregulation of apoptosis may play a crucial role in SS pathogenesis. Epithelial cells appear to undergo increased apoptosis, whereas infiltrating mononuclear cells show reduced apoptotic rates. T cells can induce apoptotic cell death by three different mechanisms: 1) Fas-FasL interaction; 2) release of proteases, such as perforin and granzyme B; and 3) production of cytokines, such as IFN-γ and TNF-α.35 Fas-FasL is discussed in the next paragraph, data on TNF-α inhibition have been presented above and no information on treatments targeting the other molecules is currently available.

**Fas/Fas-ligand**

Fas (Apo-1/CD95) is ubiquitously expressed on cells; FasL (CD95L) has a more restricted expression and is present on activated T lymphocytes. Binding of FasL to the Fas antigen activates the caspase cascade, ultimately leading to nuclear DNA fragmentation and apoptosis in susceptible cells.35 Increased expression of the anti-apoptotic protein Bcl-2 associated with a decreased sensitivity to Fas-mediated apoptosis has been described in infiltrating lymphocytes of SS patients.36,37 FasL is thought to be increased on infiltrating mononuclear cells, con-
tributing to gland destruction, but other evidence indicates that soluble FasL is produced by the salivary gland itself. Fleck et al. concluded, based on studies in salivary glands of Fas-deficient B6-lpr/lpr mice and FasL-deficient B6-gld/gld mice that a defect in Fas-mediated apoptosis of immune cells leads to an up-regulation of the immune response. Following murine cytomegalovirus infection these mutant mice develop a severe, acute and chronic sialadenitis, featured by multiple focal infiltrates. The chronic sialadenitis is due to defective Fas-mediated apoptosis, which allows activated T cells to persist in the salivary glands despite the absence of detectable virus. Interestingly, the SS-like disease does not develop without viral induction. Fas-mediated apoptosis is subsequently required for down-modulation of the inflammatory response to prevent this postviral, chronic disease and was achieved by FasL gene transfer to B6-gld/gld mice. Acinar and ductal cells were not sensitive to FasL-mediated apoptosis and a role of FasL-mediated epithelial cell apoptosis in SS is unlikely, despite documented increase in Fas expression. Instead, abnormal expression of the pro-apoptotic protein Bax by acinar cells or the presence of IFN-γ may represent a pathological feature.

Considerations regarding animal models
Although the NOD mouse is a commonly used model for insulin-dependent diabetes mellitus, it is not perfect. Several immunomodulatory treatments were successful in animals, but this strategy failed to translate to clinical trials in patients with diabetes mellitus. It was hypothesized that the NOD mouse represents only one pathogenic mechanism, whereas in humans different processes might lead to a final common pathway. The same could apply to the SS-like disease in NOD mice and human SS. Importantly, until now, there have been no clinical trials using gene therapy in SS. From the mouse studies described above, although localized salivary gland gene transfer is clearly beneficial in SS murine models, it remains to be seen whether these findings are relevant to the presentation of the disease in humans.

Clinical Studies

TNF-α inhibition
Based on their successful use in rheumatoid arthritis, TNF-α blockers have also been tested in SS. A pilot study and one-year follow-up open trial with infliximab, a chimeric monoclonal antibody against TNF-α, showed an improvement in all objective and subjective SS parameters tested. In the pilot study, statistically significant improvement was seen in global (patient’s global assessment, patient’s assessment of pain, physician’s global assessment), peripheral (tender joint/count), fatigue (patient’s fatigue assessment), laboratory (erythrocyte
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The sedimentation rate, numbers of peripheral CD4+ and CD8+ cells) and local (patient’s dry mouth and dry eyes assessment, unstimulated salivary flow rate) assessments during the six-week treatment and two-month follow-up period. Global, peripheral, fatigue and local assessments were also significantly improved in the subsequent one-year follow-up study. However, these results could not be confirmed in subsequent studies. Randomized, double-blind, placebo-controlled trials with infliximab48 and etanercept, a soluble human TNF-α-p75-receptor fusion protein,49 demonstrated no beneficial effect. A pilot, open-label study with etanercept only reported less fatigue and a decreased erythrocyte sedimentation rate in a small subgroup.50 These observations could be due to a number of various factors, such as patient characteristics, suboptimal outcome measures, and lack of biological efficacy. Moreover, both of these recombinant proteins, with a limited half-life, were administered systemically and may not have achieved therapeutic levels locally in the target glands. Unfortunately, none of the studies evaluated tissue levels of the drug or local TNF-α activity before or after treatment. Local gene transfer of a TNF-α blocker could perhaps change the outcomes. In addition, it is possible TNF-α does not play such an important role as previously thought in SS.59

Thalidomide

Thalidomide, which can function as a TNF-α inhibitor, was tested in a 12-week randomized, double-blind, placebo-controlled pilot clinical trial. Unfortunately, thalidomide treatment was associated with unacceptable adverse effects and too few patients completed the study to address potential efficacy. Despite the prominent adverse effects, the possibility that thalidomide may be beneficial and safe in SS at much lower doses could not be ruled out.51 In a rheumatoid arthritis trial the frequency of adverse effects of thalidomide treatment was also high.52,53

Interferon-α

There are three major interferon classes with type I interferon consisting of 14 IFN-α isoforms among others. IFN-α has been shown to enhance phagocytic antigen processing and immune regulatory activity of macrophages, specific cytotoxicity of lymphocytes for target cells, and natural killer cell activity. It can be administered in a high-dose injection or low-dose lozenge. To date, one phase III trial has been completed, where low-dose IFN-α was given by the oromucosal route.54 A significant increase in unstimulated, whole salivary flow rate was seen in patients with primary SS, without causing significant adverse events. The coprimary endpoints of stimulated whole salivary flow and oral dryness were not significantly improved. However, a recent report seems to contradict the positive finding of the phase III trial: Båve et al. reported that patients with primary SS have an activated type I IFN.55 A viral infection may initiate the production of IFN, but the continued IFN-α synthesis is caused by RNA-
containing immune complexes that activate plasmacytoid dendritic cells to pro-
long IFN-α production at the tissue level. This IFN-α promotes the autoimmune
process by a vicious circle-like mechanism with increased autoantibody produc-
tion and formation of more endogenous IFN-α inducers. The authors hypothe-
sized that oral IFN-α treatment may possibly act by increasing saliva secretion
via upregulation of transcription of aquaporin 5, a membrane water channel,56
without influencing the underlying autoimmune process that could still be main-
tained by IFN-α.

Cyclosporine A
The most important advance in the treatment of ocular manifestations of SS is
the introduction of topical anti-inflammatory agents such as cyclosporine A,
which increases tear production and decreases symptoms without any signifi-
cant side effects.32 A phase III study showed that 0.05% cyclosporine A im-
proved subjective measures of dry eye57 and the FDA has approved marketing
of this emulsion for topical dry eye treatment. Immune- and apoptosis-related
markers were reduced in the conjunctival epithelium after six months of treat-
ment, consistent with topical cyclosporine A acting through inhibition of the
apoptosis mechanism.58 Systemic administration of cyclosporine A, however,
leads to increased lymphocytic infiltration and has been unsuccessful in hu-
mans.32

Depletion or modulation of B cells
SS is specifically characterized by B cell hyperactivity59 and patients have a 44
times increased risk of developing B cell non-Hodgkin’s lymphoma.60 Rituximab
is a chimeric monoclonal anti-CD20 antibody that depletes CD20+ B cells from
the circulation. Findings of a phase II study suggest that rituximab is effective in
the treatment of primary SS, showing significant improvements in the Rose Ben-
gal score in the group with early primary SS and the group with primary SS and
mucosa-associated lymphoid tissue (MALT)-type lymphoma (MALT/primary SS),
and in tear break-up time in the group with early primary SS. Stimulated sub-
mandibular/sublingual saliva secretion increased significantly in patients whose
stimulated salivary flow was >0.10 mL/minute at baseline (all patients with early
primary SS and two with MALT/primary SS). B cell depletion was accompanied
by a significant decrease in IgM rheumatoid factor levels at week 5 in patients
with MALT/primary SS. Additionally, three of seven patients with MALT-type
lymphoma had a complete remission, but the high incidence of human anti-
chimeric antibodies and associated side effects, such as serum sickness, ob-
served in this study needs further evaluation.61 In a retrospective study six pri-
mary SS patients who had been given rituximab were evaluated. Five showed
improvement of several manifestations, notably swelling of the parotid gland,
arthralgias, and cryoglobulinemia related vasculitis, but no conclusions could be
drawn about the effect of rituximab on dryness.\textsuperscript{62} In an open-label phase I/II trial studying fifteen primary SS patients treated with epratuzumab, a humanized monoclonal anti-CD22 antibody, improved objective and subjective measures were seen in several patients: 10 of 14 patients showed lacrimal flow and 5 showed salivary flow increases. Symptomatic patients at study entry reported clinical improvement of dry eyes (58%), dry mouth (36%), fatigue (65%), tender point (67%) or tender joint (100%) counts.\textsuperscript{63} The modulating antibody led to B cell levels decreasing by circa 60%, but T cell levels, immunoglobulins, and routine safety laboratory parameters remained unchanged. A complete depletion of circulating B cells appears therefore not essential for clinical efficacy.

\textit{Prednisone}

Glucocorticoids are potent inhibitors of NF-κB activation,\textsuperscript{64} most likely through protein-protein interactions between the glucocorticoid receptor and NF-κB subunits, possibly in the nucleus.\textsuperscript{65} They are mostly used for treating extraglandular manifestations of SS. A six-month trial with oral prednisone (30 mg, alternate days) failed to improve functional and histological parameters in primary SS patients.\textsuperscript{66} However, salivary flow and subjective measures were enhanced in selected patients. In addition, a decrease of serum total protein, IgG, IgA and erythrocyte sedimentation rate, and an increase in white cell count were observed. Although local corticosteroid irrigation therapy of the parotid gland relieved xerostomia in SS patients,\textsuperscript{67} the adverse effects of topical use of corticosteroids in the eye outweigh the possible benefits and is therefore not recommended.\textsuperscript{32}

\textbf{Concluding remarks}

Several important lessons can be learned from these studies in animals and humans using gene transfer and biologicals (Table 1). Firstly, Th2 cytokines may have a therapeutic effect, but there is no ‘correction’ of the proposed Th1-Th2 imbalance. In fact, it seems the abnormalities in SS cannot be ascribed to a simple imbalance. Raz \textit{et al.} previously addressed the issue with respect to type I diabetes mellitus whether a cytokine shift has a primary role in suppressing the disease or it is only a biomarker.\textsuperscript{44} They concluded, based on knock-out and vaccination models, it is possible the cytokine shift is only a marker of the cellular change from a Th1 population to Th2 cells rather than the primary mechanism of protection. Cytokines, such as IL-4, IL-10, and IFN-γ, probably have some role, but it is unlikely they solely regulate the phenomenon.

Secondly, it may be more therapeutically beneficial to administer a transgene locally to glands via a viral vector rather than to deliver the encoded protein as a
<table>
<thead>
<tr>
<th>Target</th>
<th>Animal</th>
<th>Human</th>
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<tr>
<td>IL-10</td>
<td>Overexpression is causal factor, but can have therapeutic value without increased risk of lymphoma (vIL-10)</td>
<td>-</td>
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<tr>
<td>TNF-α inhibitor</td>
<td>Efficacy in prophylactic and treatment setting</td>
<td>Not important or local levels too low</td>
</tr>
<tr>
<td>VIP</td>
<td>Immunomodulator (Th2 cytokine), secretory and trophic stimulus</td>
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<tr>
<td>Anti-CD4 Ab</td>
<td>CD4⁺ T cells play key role</td>
<td>B cell modulation sufficient for clinical efficacy</td>
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<td>B cell depletion/ modulation</td>
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<tr>
<td>NF-κB inhibitor</td>
<td>NF-κB pathway important</td>
<td>-</td>
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<tr>
<td>Cyclosporine A (topical)</td>
<td>Prevention of lymphocyte-induced acinar apoptosis</td>
<td>Inhibition of epithelial cell apoptosis</td>
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<tr>
<td>Fas/FasL</td>
<td>Defect Fas-mediated apoptosis in infiltrating lymphocytes, role Fas in epithelial cell apoptosis unlikely</td>
<td>-</td>
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<tr>
<td>IFN-α</td>
<td>-</td>
<td>Phase III trial: increased unstimulated SFR, but increased endogenous IFN-α in SS (viral/autoantigen?)</td>
</tr>
<tr>
<td>Prednisone</td>
<td>-</td>
<td>Increased SFR and subjective measures in selected patients</td>
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</tbody>
</table>

Table 1. Conclusions drawn from animal and human SS studies using different targeted biologicals and transgenes

Ab – antibody; Fas/L – Fas ligand; IFN – interferon; IL – interleukin; NF-κB – nuclear factor κB; SFR – salivary flow rate; TNF-α – tumor necrosis factor-α; vIL-10 – viral interleukin-10; VIP – vasoactive intestinal peptide
systemic biological. Initial gene transfer studies in the lacrimal and salivary glands, using vectors encoding TNFRp55-Ig (similar to etanercept), appear to have some efficacy. Conversely, systemically delivered etanercept is not beneficial for SS patients. The paradigm of local vector delivery may be important and useful for testing other transgenes encoding therapeutic proteins with little to no benefit when given by the recommended systemic route.

Thirdly, there also appears to be therapeutic potential for strategies targeting the NF-κB pathway, which is important in the inflammatory process in SS by mediating the effects of various cytokines. Although systemic treatment with prednisone, an NF-κB inhibitor, did not show improvement in salivary nor lacrimal function, local irrigation of parotid glands with corticosteroids improved saliva production. This is supported by preliminary data from the NOD mouse suggesting that NF-κB blockade in the gland results in improved salivary function. Furthermore, both T and B cells are clearly important to the SS pathogenesis and deleting or blocking them may have therapeutic value. For example, blocking IFN pathways or inhibiting the consequences of IFN production, such as B-cell-activating factor (BAFF/Blys) secretion, have been proposed and may be useful.68

Finally, apoptotic pathways appear to be important to the SS pathogenesis. Although infiltrating lymphocytes demonstrate a defective Fas-mediated apoptosis, the role of Fas in the apoptosis of epithelial cells is unclear. Importantly, the beneficial effect of topical cyclosporine A, which inhibits epithelial cell apoptosis and decreases subjective dry eye measures, suggests that targeting molecules essential to apoptosis may be therapeutically beneficial (Table 1).

Based on these observations, we can categorize different treatment options and the ways of delivery, such as mode, route and time of administration. Gene transfer has shown to be promising in several animal models and has the advantage over repetitive administration of recombinant proteins, that theoretically only one injection would be needed for long-term protein expression. The administration route is of importance for several reasons. For example, local therapy of a TNF-α inhibitor transgene in a rabbit model proved to be successful, whereas systemic delivery of the recombinant protein to humans failed to improve the SS disease features, suggesting that with long-term local expression we may achieve higher concentrations of therapeutic molecules. Moreover, as the systemic effects with local expression of these molecules are expected to be much lower, an increased concentration at the site of inflammation could be achieved with likely much less systemic side effects leading to a more desirable therapeutic effect and safety margin. Finally, results obtained in a prophylactic setting are hopeful, but several potentially therapeutic molecules still require testing as a therapeutic treatment, resembling the clinical situation.
SS has a complex etiology, where environment, genetics, and disease stage all play a role; there is not one critical factor. Table 2 and the Figure give a summary of the three most important cell types involved and the influences of the different treatments presented herein. T cells and their cytokines can be blocked by an anti-CD4 antibody, VIP, or a TNF-\(\alpha\) inhibitor, while an anti-CD20 or anti-Blys antibody inhibits the B cell site of the equation. Cyclosporine A can inhibit apoptosis of epithelial cells, whereas FasL stimulates CD4\(^+\) T cell apoptosis. An NF-\(\kappa\)B inhibitor could exert its effects on epithelial cells, T and B lymphocytes.

**Figure. Target sites of therapeutic molecules**

Schematic view of the target site of different therapeutic molecules. T cells and their cytokines can be blocked by an anti-CD4 antibody, vasoactive intestinal peptide (VIP), or a tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) inhibitor, while an anti-CD20 antibody inhibits B cells. Cyclosporine A can inhibit apoptosis of epithelial cells, while Fas-ligand (FasL) stimulates apoptosis of overaccumulating CD4\(^+\) T cells. A nuclear factor \(\kappa\)B (NF-\(\kappa\)B) inhibitor could exert its effects in epithelial cells, T and B lymphocytes.

Local (gene) therapy of the exocrine component of SS appears to be kinetically optimal and clinically most appropriate. We propose that local delivery of an immunomodulatory and anti-apoptotic transgene could reduce the gland inflammation by affecting multiple downstream targets. Furthermore, a combination of transgenes—e.g., an NF-\(\kappa\)B inhibitor can be combined with an anti-CD4 antibody, FasL and/or VIP—may be particularly useful.

Clearly, our present understanding of the SS pathogenesis is inadequate to precisely define molecular targets for treatment. Nonetheless, as we have tried to demonstrate herein, several reasonable potential therapeutic targets can be identified in SS. Rigorous testing of these will help both to understand SS pathogenesis and to develop novel therapeutics.
Table 2. Importance of epithelial, T and B cells in the pathogenesis of Sjögren’s syndrome

Evidence for the importance of the three different cell types at the molecular level, as learned from the disease process, is shown. The possible therapeutic intervention (bold) in this process is also displayed.

<table>
<thead>
<tr>
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<th>Epithelial cells</th>
<th>T cells</th>
<th>B cells</th>
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<tr>
<td>Disease stage</td>
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<td>• continuous</td>
<td>• onset?</td>
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<tr>
<td>Evidence</td>
<td>• autoantibodies</td>
<td>• cytokines (anti-TNF-α, VIP)</td>
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<td></td>
<td>• cytokines</td>
<td>• cytokine shift?</td>
<td>• lymphoma (late)</td>
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<tr>
<td></td>
<td>• apoptosis</td>
<td>• antibodies (anti-CD4)</td>
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<td></td>
<td>• neo-antigens (cyclosporine A)</td>
<td>• decreased apoptosis (FasL)</td>
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</tbody>
</table>


Chapter 8

Summary and Discussion


