Local gene therapy and the identification of therapeutic targets in Sjögren’s syndrome

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GENE THERAPY: SJÖGREN’S SYNDROME

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ABSTRACT

Sjögren’s syndrome (SS) is characterized by inflammation and dysfunction of the secretory organs. In the majority of patients the salivary and lachrymal glands are predominantly affected, although systemic symptoms are common. The pathogenesis of the disease is not well understood and to date there is no universally effective therapy available. The development of gene therapy and in particular local gene therapy applied to the salivary glands may be effective in the disease. Animal studies have shown that treatment with immunomodulatory molecules such as interleukin 10 or vasoactive intestinal peptide can influence salivary function positively while changing the local inflammatory environment. Future research will have to show whether this approach is feasible in humans.
SJÖGREN’S SYNDROME

Sjögren’s syndrome (SS) is a systemic chronic inflammatory autoimmune disease predominantly affecting the lachrymal and salivary glands. Patients complain of dry eyes and dry mouth (sicca symptoms), the latter leading to pain, discomfort, dental caries and infection of the mouth with opportunistic pathogens such as Candida albicans. SS is often accompanied by systemic symptoms, such as Raynaud’s phenomenon, arthritis, fatigue and vasculitis. Women are nine times more likely to be affected than men and the estimated prevalence is 0.5% for the general population¹. The diagnosis is based on objective and subjective criteria of dryness of the secretory glands, inflammation of the salivary gland and the presence of auto-antibodies in the serum. It is termed primary SS (pSS) in the absence of an underlying disease, and secondary SS (sSS) when related to other autoimmune disease such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE)².

The pathogenesis and pathophysiology of SS are poorly understood. A number of human leukocyte antigen (HLA) subtypes are associated with SS³ and viral and other environmental factors together with these genetics are all thought to play a role in the initiation and progression of the disease⁴. Chronic inflammation is central to SS and this is reflected by an imbalance of pro-inflammatory cytokines over anti-inflammatory cytokines. Overexpression of numerous pro-inflammatory cytokines in the blood, salivary glands and the saliva has been shown in several studies. The most consistently upregulated cytokines are interferon \( \gamma \) (IFN\( \gamma \)), IFN\( \alpha \), interleukin (IL)-12, IL-18, IL-6, and B-Cell activating factor (BAFF). The presence of other cytokines such as IL-1\( \beta \) and IL-17 may also play a role in the pathogenesis of SS, but data on these are incomplete. Concomitantly, IL-4, an important anti-inflammatory cytokine and transforming growth factor \( \beta \) (TGF\( \beta \)) are downregulated. In contrast, the anti-inflammatory cytokine IL-10 is highly expressed in SS patients compared to healthy controls and may contribute to B cell activation and auto-antibody production (reviewed in⁵). Histologically, SS is characterized by localized infiltrates (foci) of mononuclear cells in the salivary glands. The degree of infiltration observed ranges from mild to severe and can be accompanied by glandular atrophy and fibrosis. Foci are generally comprised of T cells (80%) and B cells (20%) and in a subgroup of patients these foci are organized to resemble germinal centers⁶. SS patients are at a higher risk than the normal population of developing non-Hodgkin’s lymphoma in the exocrine glands⁷⁻⁸. A possible mechanism for this may be chronic cytokine-driven stimulation of the infiltrating B and/or T cells in these germinal centers leading to lymphomagenesis⁹.

THE SALIVARY GLAND IN SS

The salivary gland is primarily composed of two cell types: Epithelial (ductal) cells and acinar (secretory) cells. Acinar cells are the only cells in the gland that produce saliva, they also produce more than 80% of the proteins found in saliva. The fluid secreted by acinar cells is modified by the ducts during its passage to the mouth. NaCl is reabsorbed from the secreted fluid whereas K⁺ and HCO3⁻ are added by the ductal cells in addition to the secretion of proteins such as growth factors and
immunoglobulin (Ig) A (reviewed in\textsuperscript{19}). In SS both acinar and ductal cells are thought to be involved in the pathogenesis of the disease. Acinar cells lose their ability to secrete fluid, due to destruction by inflammation, altered cell signaling or aberrations in the neurological signal that drives secretion. The ductal epithelial cells also play an important role in SS. Ductal cells can function as antigen presenting cells in the gland\textsuperscript{11} and express co-stimulatory molecules such as CD40\textsuperscript{12}, CD80 and CD86\textsuperscript{13} indicating an active role in inflammation. Moreover, histologically most infiltrates are found around the ducts, thus SS is also known as autoimmune epithelitis\textsuperscript{14}. Figure 1 shows a simplified image of the major processes involved in SS. The cause of sicca symptoms experienced by SS patients can be directly due to the destruction or dysfunction of parenchymal tissue of the secretory organs by inflammation in some patients. Recently recognized dysfunction without overt infiltration in a significant number of patients has lead to the hypothesis that the dryness of the gland may be caused by a mechanism independent of, or in addition to destruction\textsuperscript{15-16}. The type 3 muscarinic receptor (M3R), which is part of the parasympathetic nervous system, is the main neurological receptor involved in saliva secretion in murine models as shown by knockout studies\textsuperscript{17}. It is also thought to be the main receptor involved in human saliva secretion. There is cumulating evidence that serum of a subgroup of patients interferes with the function of the M3R \textit{in vitro}\textsuperscript{18-19}. The details of this interaction are still unclear. One mechanism could be that autoantibodies against the M3R are involved, but data on the detection of these antibodies are conflicting\textsuperscript{20-22}.

\section*{CURRENT TREATMENT FOR SS}

To date there is no universally effective therapy for SS. Therapy is based on symptom relief and the prevention and treatment of complications. Patients are advised to take extra oral care and undergo regular dental check ups. For the sicca symptoms, artificial tears, punctal plugs and artificial saliva can be applied. Some patients receive symptomatic relief from secretagogues such as cimeveline and pilocarpine\textsuperscript{23}. The use of immunosuppressants has been unsatisfactory; these drugs can improve systemic conditions associated with the disease but the drugs have little effect on the sicca symptoms\textsuperscript{24-27}. Most cytokine-directed therapies have also proved ineffective in SS, though these therapies have been applied successfully in the treatment of other rheumatoid diseases. For instance, blocking the pro-inflammatory cytokine tumor necrosis factor $\alpha$ (TNF$\alpha$) in RA, an autoimmune disease with many similarities to SS, has lead to greatly improved disease control with an acceptable safety profile\textsuperscript{28}. However, unlike RA, the use of TNF$\alpha$ blockers did not lead to improved saliva or tear production in clinical trials with SS patients\textsuperscript{29}. Another recently investigated therapy targets B cells. Rituximab (RTX), an anti-CD20 antibody that depletes CD20$^+$ B cells, was first approved for treatment of patients with relapsed or refractory lymphoma\textsuperscript{30} and has quickly revolutionized the treatment of B cell lymphomas. To date RTX has been used not only in malignancies but also has been successfully used in the treatment of autoimmune diseases like RA\textsuperscript{31} and in small pilot studies for the treatment of SLE\textsuperscript{32}. RTX in patients with SS resulted in depletion of peripheral B cell without having an effect.
GENE THERAPY FOR SS

In summary, several treatments that are effective in other autoimmune inflammatory diseases failed in SS. There are many reasons for this failure: First, it is possible that these drugs are aimed at the wrong target. The inhibition of TNFα in SS patients for instance was shown to lead to paradoxical upregulation of TNFα, and also IFNα and BAFF were shown to be upregulated after treatment36. Second, it is possible that these drugs reached suboptimal dose levels in the affected organs due to poor penetration. Third, it is possible that the drug was not given for a sufficient period of time or too late in the course of the disease to elicit a response. SS patients often suffer from symptoms long before they are diagnosed1. A therapy therefore may need to be applied long term to reverse damage that has accumulated over several years. As a therapeutic approach, gene therapy may offer the possibility to address some of these concerns.

THE USE OF LOCAL GENE THERAPY

Since the salivary gland is heavily involved in the pathogenesis of SS, locally applied therapy to the salivary gland is very attractive and has a number of advantages over systemic therapy. First, the salivary glands can be easily reached by retrograde cannulation of the orifices of the salivary ducts in the mouth (as is routinely performed in scintigraphy of the salivary glands). This technique can be used not only to introduce anti-inflammatory small molecules directly to the gland in a non-invasive manner but can also be used to introduce vectors able to direct the expression of immunomodulatory proteins or the gland physiology. Second, the entire salivary gland can be targeted since the luminal membrane of both the acinar and ductal cells within the gland is exposed. Third, because of its natural secretory activity, the salivary gland is an excellent organ to locally achieve a high level of the drugs compared to systemic administration. Fourth, long-term expression is possible from the salivary gland due to the relatively low turnover of the epithelial cells in the gland. Fifth, systemic exposure to the drugs can be limited by treating the salivary glands only, possibly leading to fewer side effects.

EXPERIENCE WITH LOCAL GENE THERAPY

Several studies have recently been conducted to address the utility of gene therapy in treating salivary gland dysfunction and inflammation in animal models of SS (Table
1). The non-obese diabetic (NOD) mouse model is the most widely used model to study the spontaneous development and treatment of SS. Classically, this mouse is studied for diabetes. From the age of 10 weeks these mice spontaneously develop insulin-dependent diabetes preceded by autoimmune insulitis of the pancreas. Independently of this disease and a few weeks later than the onset of diabetes the mice can also spontaneously develop autoimmune exocrinopathy of the salivary glands with gender-dependent loss in gland activity and lymphocytic infiltration of the salivary and lachrymal glands. With age, NOD mice also may develop auto-antibodies against the nuclear antigens Ro and La. However this SS-like phenotype is unstable and the factors that contribute to the disease are still largely unknown. One of the first studies to test the local application of gene therapy to the salivary gland used a viral vector encoding the anti-inflammatory cytokine IL-10. IL-10 was shown to be

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**Figure 1. Simplified schematic of fluid secretion in the salivary gland and the processes involved in Sjögren's syndrome.** Upon stimulation of the Muscarinic type 3 receptor (M3R) and via Ca²⁺ signaling, fluid is secreted by the acinar cells through the aquaporins (AQP). The fluid (blue arrows) passes through the ducts into the oral cavity while epithelial cells modify its content. In SS reduced saliva can be the result of many processes interfering with the secretory capacity of the gland (thick black arrows). The infiltrating B and T cells interacting with each other through their respective receptors in interaction with the MHC-peptide complex and the epithelial cells and dendritic cells (DC) are shown in the enlarged area (the co-stimulatory molecules are depicted as black T's). Each of the processes involved in SS can potentially be targeted with gene therapy. (IgA, immunoglobulin type A; B, B cell; T, T cell).
beneficial in a number of preclinical models of autoimmune diseases. IL-10 was administered locally to the salivary glands of NOD mice by retrograde cannulation of the salivary gland ducts, or intramuscular injection in the quadriceps muscle using a recombinant adeno associated viral type 2 (AAV2) vector. The vector was given at 8 weeks, before onset of the disease, and at 16 weeks, at a more progressive stage of the disease. At 20 weeks, the mice treated with IL-10 directly into the salivary gland showed significantly higher salivary flow in the early and late treatment group compared with the intramuscular or control vector treatment groups.

Similarly, positive results have also been observed using an AAV vector encoding the neuropeptide vasoactive intestinal peptide (VIP). This peptide has a broad range of functions. It is a neurotransmitter causing vasodilatation, but is also known to be involved in immune tolerance and is a potent suppressor of a variety of pro-inflammatory cytokines. NOD mice were treated with a single dose of $1 \times 10^{11}$ viral particles containing the VIP gene per salivary gland. At the age of 16 weeks, 8 weeks after the treatment, VIP treated mice showed no loss of salivary flow compared to the control group. There was no difference in focus score or apoptotic index between the different groups, but there was significantly less IL-2, IL-12, TNFα, and IL-10 present in glandular extracts, in contrast to serum levels, where no difference was observed.

Treatment of NOD mice with both the IL-10 and the VIP constructs did not lead to noticeable side effects; the mice appeared to be healthy during treatment. In contrast, local application of TNF inhibitors did not improve inflammation and dysfunction of the salivary glands in NOD mice. This supports the lack of efficacy seen in previous studies in humans when systemic treatment was studied. Salivary gland-directed gene therapy with a soluble TNF receptor (sTNFR1:IgG) in NOD mice decreased salivary gland activity. Treatment decreased some pro-inflammatory cytokines locally in the gland, while TGFβ was upregulated. In plasma however, the opposite was observed, with upregulated pro-inflammatory cytokines and downregulated TGFβ levels. The reason for the failure of this treatment in mice is not completely understood, but could be due to circulating receptor-complex formation or underlying mutations in the TNF pathway as have been described in humans and mice. This observation further emphasizes the negative effect of this class of drug on salivary gland function and suggests that other molecules should be explored as targets for therapeutic intervention in SS.

**FUTURE THERAPEUTIC TARGETS**

Although the studies with IL-10 and VIP are promising, other potential therapeutics should be explored not only as possible future drugs but also to better understand the underlying pathophysiology associated with SS. Novel therapeutics in SS can be divided in an immunological and a non-immunological group. Examples of immunological targets include molecules that can interfere with the proliferation and activation of B and T cells or affect chemotaxis of lymphocytes, macrophages and dendritic cells. Non-immunological therapies comprise of molecules that enhance fluid secretion, for instance the introduction of water channels such as aquaporins.
Similarly, therapies that enhance the sensitivity of salivary and lachrymal glands to neuro-stimulatory signals could be explored as a therapeutic approach for the treatment of SS.

**IMMUNOLOGICAL TARGETS**

**Cytokines: IFNα, IFNγ and IL-12**

Many pro-inflammatory cytokines are upregulated in SS and blocking one or more of these cytokines may result in reduced inflammation. Some of these cytokines are also involved in secretory dysfunction making them even more interesting candidates for treatment. IFNα and IFNγ together with IL-12, are all closely related in function and are all overexpressed in SS patients.

In addition, the majority of cytokines and transcription factors that are overexpressed in patients are IFN inducible, a profile that has been referred to as ‘the interferon signature’. IFNγ is also highly expressed in individuals with sicca symptoms who do not have any histological inflammatory markers in the salivary gland. Moreover, prolonged treatment of the human salivary gland cell line (HSG) with IFNγ in the presence or absence of TNFα leads to a persistent depletion of intracellular Ca2+ stores (important for signal transduction leading to fluid secretion), and thus to an exhausted response system. These two observations suggest that IFNs can affect the secretory capacity of the glands. Since IFNγ also reduces the growth of HSG in a concentration dependent way in vitro interfering with the interferon system, for instance by introducing a soluble receptor, may reduce sicca symptoms, local damage and inflammation in the salivary glands of SS patients.

**Chemokines**

Chemokines are chemotactic cytokines that are very important in orchestrating mobilizing and, to a lesser degree, regulating homeostasis of a wide range of hematopoietic cells. The role of chemokines in many autoimmune disease such as RA and autoimmune thyroiditis is well established and there is evidence they play an important role in SS as well. Chemokines are important for the homing of T and B cells to the gland and for the survival of malignant B cells. The chemokines CXCL9

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**Table 1. Salivary gland gene therapy in NOD mice.**

<table>
<thead>
<tr>
<th>Gene and vector</th>
<th>Results:</th>
</tr>
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<tbody>
<tr>
<td>IL10 AAV2</td>
<td>Prevented loss of salivary flow, reduced number of infiltrates in the salivary gland</td>
</tr>
<tr>
<td>VIP AAV2</td>
<td>Prevented loss of salivary flow, no effect on infiltrates in the salivary gland</td>
</tr>
<tr>
<td>sTNFR1:IgG1 AAV2</td>
<td>Reduced salivary flow over time, reduction in pro-inflammatory cytokines, upregulation of systemic pro-inflammatory cytokines</td>
</tr>
</tbody>
</table>

NOD, non-obese diabetic mouse; IL10, interleukin 10; AAV, adeno-associated virus; VIP, vasoactive intestinal peptide; sTNFR1:IgG1, soluble tumor necrosis factor receptor type 1.
and CXCL10 for instance, were found to be significantly upregulated in SS salivary glands, but not in normal salivary glands. These chemokines were predominantly expressed in ductal epithelium in close proximity to lymphoid infiltrates. Moreover high titers of CXCL9 and CXCL10 were produced after patient-derived SG cells were treated with IFNg in vitro. Upregulation of these chemokines is known to attract the IFNγ producing plasmacytoid DC and B cells. Interfering with such a system may be useful in reducing inflammation in the gland by blocking chemotaxis of lymphocytes and dendritic cells, and may also reduce the risk of developing lymphoma.

The co-stimulatory pathway

The co-stimulatory pathway has been shown to play a role in many autoimmune diseases. In studies on RA and psoriasis, the principle of blocking co-stimulatory pathways as a treatment has been shown to be safe and effective. In short, to activate B and T cells, simultaneous binding of co-stimulatory molecules is required in addition to the binding of the T or B cell receptor with the peptide-major histocompatibility complex (MHC). In the absence of the co-stimulatory signal, binding of the T or B cell receptor to the peptide-MHC can lead to tolerance and anergy. Co-stimulatory signals are bidirectional and can lead to activation of B cells, upregulation of adhesion molecules, class switching and enhancement of CD4+ and CD8+ effector functions as well as many other pro-immune activities. One of the co-stimulatory pathways known to be involved in SS is the CD40 interaction with CD40 ligand (CD40L/CD154). In NOD mice, blocking the CD40–CD40L interaction around 4 weeks of age prevents the onset of insulitis and diabetes by inhibiting the development and chemotaxis of pathogenic Th1 cells to the islets of Langerhans in the pancreas, the effect on the SS phenotype has not been studied yet. Blocking these pathways in SS may promote tolerance over autoimmunity by reducing auto-reactive T cells, moreover it may affect the activation of B cells and the differentiation of B cells into plasma cells.

NON-IMMUNOLOGICAL TARGETS

Aquaporins

In order to treat the main symptoms of SS, dry mouth and dry eyes, gene therapy could be used to enhance fluid secretion by introducing water channels (AQP) into the ducts of the salivary glands. This re-engineering of the salivary gland has been successfully performed in rats, mini pigs and non-human primates for the treatment of radiation-induced xerostomia. In these studies, vectors encoding water channels were used weeks to months after radiation induced loss of salivary flow. The treatment restored salivary gland activity without any significant side effects. Currently a Phase I trial is ongoing to evaluate the safety of this approach (clinicaltrials.gov, NCT00372320). In this trial, patients with a history of damage of the salivary glands due to head and neck radiation and objective and subjective symptoms of dry mouth receive an adenoviral vector encoding AQP type 1 locally in the parotid gland. The primary outcome of this study is to determine safety of this novel treatment. The secondary outcome will be to measure the effectiveness of gene transfer of AQP1
to increase parotid gland salivary output and improve symptoms associated with irradiation-induced parotid hypofunction. If this proves to be safe, further studies will provide us with more information on the efficacy of the therapy and may lead to studies on its applicability in SS.

Neuro-stimulatory pathway
Evidence is accumulating that the function of the muscarinic type 3 receptor (M3R) is impaired in SS. It is thought that antibodies blocking the M3R are responsible for the dysfunction of the receptor, leading to dryness of the secretory organs, but it has been challenging to prove this in a direct manner. Enhancing the sensitivity of the M3R by using soluble receptors as decoy for possible autoantibodies or introducing properly functioning receptors in excess to the gland may overcome the secretory dysfunction. However, at this moment the mechanism responsible for the malfunction of the receptor is not fully understood and further research will be necessary to identify its exact nature.

FUTURE DIRECTIONS AND LIMITATIONS
The most difficult challenge facing the treatment of SS to date is the identification of the proper target(s). Since we understand little of the pathogenesis of SS and we do not know the autoantigen that is recognized, choosing the correct target is largely empirical. However, by using local delivery of key immunomodulatory proteins or other potential therapeutic molecules directly to the gland in animal models we can begin to identify the critical pathways involved in the inflammatory process and the loss of secretory function.

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REFERENCES


47. Nguyen, C.Q., et al. Identification of possible candidate genes regulating


