Developing a gene therapy for Sjögren's syndrome

Jelle LG Vosters, Bruce J Baum†, Paul P Tak, Gabor G Illei & John A Chiorini

†Author for correspondence National Institutes of Health, Gene Therapy & Therapeutics Branch, National Institute of Dental & Craniofacial Research, DHHS, Bethesda, MD 20892, USA Tel.: +1 301 594 1193; Fax: +1 301 402 1228; bbaum@dir.nidcr.nih.gov

Sjögren's syndrome (SS) is a complex autoimmune disorder characterized by mononuclear infiltration of the exocrine glands. There is an increased interest to use immunomodulatory protein therapy to target inflammatory components thought to be important in SS. However, systemic immunomodulatory treatment has several limitations and unwanted side effects. One alternative approach is developing localized expression via gene therapy in the salivary glands. Genes encoding cytokines or cDNAs encoding soluble forms of a key cytokine receptor can be introduced directly into the salivary glands, and are likely to alter immune responses locally, but not systemically. While the etiology of SS is unclear, several gene targets are being examined in preclinical studies. The focus of this review is to summarize the approach to developing therapeutic molecules for salivary gland gene transfer that may impact the treatment of SS.

Sjögren's syndrome (SS) is a systemic autoimmune disorder of unknown etiology, characterized by mononuclear cell infiltration in exocrine glands, principally the lacrimal and salivary glands. Other organ systems are frequently involved and 5% of SS patients develop lymphoma. The inflammation of exocrine glands is most prominent, resulting in dry eyes (keratoconjunctivitis sicca) and mouth (xerostomia) [1]. SS may occur alone, termed primary SS, or in association with another defined autoimmune disease, termed secondary SS. Primary SS is one of the most common autoimmune diseases. Between half a million and 2 million individuals are affected by this disease in the USA, with women being nine-times more likely to be affected than men. In general, there are two age-peaks seen in primary SS patients, the first between 20–30 years and the second around 50–60 years.

The exact pathogenesis of SS is unknown, but is clearly multifactorial. The presence of multiple autoantibodies and chronic inflammation in the target organ indicate an autoimmune response against the exocrine glands. This so-called autoimmune exocrinopathy is supported by several findings at the histological level. Salivary and lacrimal glands in SS are characterized by large persistent mononuclear foci, consisting of both T lymphocytes (~80%) and B lymphocytes (~20%). The presence of $CD4^+$ T lymphocytes is twofold that of $CD8^+$ T lymphocytes. Variable degrees of acinar cell atrophy and progressing fibrosis can also be observed [2].

While there is effective palliative therapy for lacrimal manifestations, no effective treatment exists for the salivary gland dysfunction. The salivary gland dysfunction and associated difficulty in eating and swallowing are the major complaints of patients. Current palliative treatment for salivary gland dysfunction includes artificial saliva, frequent dental prophylaxis and/or stimulation by muscarinic agonists, such as pilocarpine and cevimeline, and the use of steroids and other immunomodulatory agents [1]. However, systemic immunosuppressive and immunomodulatory therapies, including classical immunosuppressants and tumor necrosis factor (TNF)-α inhibitors, are largely ineffective.

Inhibitors of TNF-α, such as infliximab, were studied in the treatment of SS (see below) [3,4]. However, in a double-blind, randomized pilot study of the TNF inhibitor etanercept versus placebo in 28 patients, no efficacy was observed [5]. Conclusions from this study suggest there may have been insufficient levels of the drug in the target tissues, either because the medication was unable to get to the target tissue or because higher doses of the drug are needed in order to demonstrate a therapeutic effect. Additionally, long-term exposure (1 year) may be required for the therapeutic effects to appear.

To overcome these limitations of drug delivery, gene therapy offers the possibility to engineer cells to express therapeutic proteins locally at high levels that would not lead to major side effects associated with high systemic levels. The salivary glands would be the ideal target organ for SS. In this review, we will discuss the potential use of salivary gland gene therapy in SS and potential therapeutic molecules that can be delivered via gene therapy.

Keywords: adeno-associated virus, gene therapy, non-obese diabetic mice, salivary glands, Sjögren's syndrome

future Medicine

Gene therapy

Gene therapy refers to an experimental procedure to deliver genes encoding proteins or inhibitory RNA to a specific target cell. This nucleic acid can act to restore normal cellular activity or to augment a cell's function. An advantage of gene therapy over conventional therapies is that expression of the therapeutic molecule can be targeted and expressed locally in a few defined cells, or expression can be directed systemically. For some therapeutic proteins, localized expression would more closely mimic their physiological pattern of expression, thus limiting side effects associated with high systemic levels.

Gene transfer is accomplished by using either viruses modified to encode the new gene or synthetic DNA–polymer complexes that enable efficient entry of the DNA into the cell [6]. Optimal vector selection depends on the tissue target and the proposed therapeutic strategy. This may require only short-term expression to overcome an acute condition or require long-term and possibly physiologically regulated expression for a chronic condition.

Long-term expression in gene therapy theoretically requires the vector to be able to integrate in the cells' genome, for example, using retrovirus- or lentivirus-based vectors [7]. A concern with this mechanism of persistence is that the integrated gene could disrupt a normal gene function, resulting in mutation or disease. This outcome has been observed in recent clinical trials with some retroviral-based vectors [8]. Alternatively, if the target cell is post-mitotic or slowly dividing, a vector system that remains episomal and transcriptionally active may prove to be efficacious.

Gene therapy for SS has focussed on salivary glands as a target tissue because loss of salivary gland function is a central aspect of SS. Delivery of the vector via the lumen of the gland is possible, via retrograde cannulation of the duct, which is routinely performed in the clinic for contrast radiography, and would be very advantageous. This route of delivery would result in both local expression of the therapeutic molecule and keep the vector at high concentration, which would enhance gene transfer. Salivary glands are a natural secretory tissue able to complete most forms of post-translational modification and importantly secrete large amounts of protein either into the saliva or depending on sorting signal sequences in the gene, the bloodstream [9,10]. Salivary epithelial cells also are a slowly dividing population, thereby making it possible to use episomal vectors for gene transfer.

While a number of viral and nonviral vectors have been tested in salivary glands, the two vector systems with the highest overall gene transfer activity are recombinant adenoviruses (rAd) and recombinant adeno-associated viruses (rAAV) [11]. Important features of recombinant adenoviral vectors include the ability to deliver DNA to a variety of cell types within the gland, and the ability to package very large genes or multiple genes in a single particle. However, the duration of gene expression from most adenoviral vectors is short, and can be accompanied by a significant immune response at high doses ($>10^{11}$ particles). rAAVs have a smaller packaging capacity, limiting the vector delivery to just a single therapeutic gene rather than multiple genes, and lower level of transgene expression compared with adenovirus vectors. However, AAV vectors are able to direct long-term expression with minimal immune response and there are currently several different types of AAV vectors that have different cell tropisms in animal models [12]. Therefore, with the AAV vectors, it might be possible to deliver one gene to ductal cells to alleviate xerostomia by creating a *de novo* saliva flow or target the expression of another gene to acinar cell to prevent apoptosis and preserve existing secretory activity.

Animal models for Sjögren's syndrome

SS is a disease of unknown etiology. Therefore, the development of animal models that mimic the disease in patients is critical not only for understanding the biology of SS, but for validating the safety and efficacy of experimental therapeutic approaches such as gene therapy. Currently, several animal models are reported to display a number of features observed in SS patients (**Table 1**). However, none of these models exactly replicates the human condition. Many models lack a decrease in salivary flow, an important clinical feature, and others have coexisting diseases, such as diabetes, not routinely observed in SS. **Table 1** is an overview of the SS animal models. The non-obese diabetic (NOD) mice are the only mice with almost all the SS features. NOD mice develop age-dependent histopathological changes in the salivary glands, which are similar to those seen in SS patients. The subset of lymphocyte infiltrations in salivary glands are predominantly $CD4^+$ T cells over $CD8^+$ T cells and B cells. Furthermore, autoantibodies against 52-kDa ribonucleoprotein SS-A/Ro, muscarinic receptor (MR) and 120-kDa α-fodrin have been detected in serum from NOD mice, although autoantibodies

Table 1. Overview of features observed in Sjögren's patients compared with animal models.

Human	Id3 -/-	NOD	SS-B immune	NFS/sld	IQI/Jic	MRL/lpr	NZB/W	Aly/Aly
Xerostomia	Yes	Yes	Yes	Yes				
Keratoconjuctivitis sicca	Yes	Yes		Yes			Yes	
Autoantibodies (anti-SSA/SSB/MR, ANA)	Yes	Yes	Yes	Yes	Yes	Yes		
Histology: $CD4^+ > CD8^+ >$ B cells	Yes	Yes	Yes			Yes		
Age-dependent onset	Yes	Yes	Yes		Yes		Yes	Yes
Female > male	Yes	Yes		Yes	Yes	Yes		
Genetically defined phenotype	Yes							

ANA: Antinuclear antibody; anti-MR: Anti-muscarinic receptor; NOD: Non-obese diabetic.

to 60-kDa SS-A/Ro or SS-B/La are not detected. Importantly, the development of sialadenitis in NOD mice is accompanied by loss of salivary gland function. However, these mice also develop insulin-dependent diabetes mellitus (IDDM), a condition that is not routinely observed in patients. Furthermore, the SS phenotype in this mouse line is highly unstable and genetically poorly defined, which renders it difficult to work with or compare results when the mice are obtained from different suppliers [13]. Recently, a NOD strain (C57BL/6.NODc3.NODc1t) was developed that displays autoimmune exocrinopathy, including salivary gland dysfunction and lymphocyte infiltrations as seen in SS, without exhibiting autoimmune diabetes [14]. Despite their limitations, NOD mice are useful in evaluating localized gene transfer strategies for SS.

Experience to date

Despite the lack of success with systemic administration of immunomodulatory proteins such as etanercept or infliximab in clinical trials, the use of gene transfer to salivary glands in mice has demonstrated that local expression of immunomodulatory proteins can preserve salivary flow and decrease gland infiltrates [15,16]. The first report of successful rAAV-immunomodulatory salivary gland gene delivery in NOD mice resulted from the retroductal delivery of human interleukin (IL)-10. In that study, we demonstrated *in vitro* and *in vivo* expression of biologically active human IL-10 and clinical benefits [15]. Recently, a second successful application of salivary gland gene transfer in NOD mice was reported. Vasoactive intestinal peptide (VIP), which is an immunomodulatory hormone, was delivered to salivary glands using an AAV-vector. This local delivery of VIP resulted in disease-modifying and immunosuppressive effects in submandibular salivary glands of NOD mice [16]. These two reports show that salivary gland gene transfer can provide local beneficial effects. The difficulty using this strategy in SS patients is the multifactorial, and currently unclear, pathogenesis of the disease. Choosing the right transgene or transgenes is not easy.

What genes to use?

In attempting to develop a gene therapy for salivary glands in SS, a critical question is what therapeutic molecule should be delivered? The previously mentioned studies in NOD mice demonstrate that local expression of immunomodulatory proteins can be effective in the treatment of SS. Owing to the multifactorial nature of SS, in addition to immunomodulatory proteins, other candidate genes could act to prevent disease progression, blunt the immune response, or restore salivary flow (**Tables 2 & 3**).

Immunological therapy

One of the early inflammatory events in autoimmune exocrinopathy is the recruitment of lymphocytes into the exocrine glands. Intercellular adhesion molecule (ICAM)-1, vascular cell

Table 2. Protein therapeutics in clinical trials of autoimmune diseases.

Target	Agent	Tested disease
ICAM-1	Antisense inhibitor	Crohn, UC
VCAM-1	Probucol derivate	RA
TNF-α	mAb TNF-α	RA,SS
	TNF-α receptor/IgG1	RA,SS
IFN-γ	mAb IFN-γ	RA
IFN-receptor	rIFN-α	SS
IL-1	IL-1RA	RA
Blys	mAb Blys	SLE
	Blys-receptor/IgG	SLE
CD20	mAb CD20	SS

Blys: B-lymphocyte stimulator; ICAM: Intercellular adhesion molecule; IFN: Interferon; Ig: Immunoglobulin; IL1-(RA): Interleukin 1-(receptor antagonist); mAb: Monoclonal antibody; RA: Rheumatoid arthritis; (r)IFN: (Recombinant) interferon; SLE: Systemic lupus erythematosus; SS: Sjögren's syndrome; TNF: Tumor necrosis factor; UC: Ulcerative colitis; VCAM: Vascular cell adhesion molecule.

adhesion molecule (VCAM)-1 and integrins play a crucial role in this tissue-specific homing [17]. Agents that could address this pathway include antisense inhibitors of ICAM-1 and a probucol derivative inhibitor of VCAM-1, which are designed to inhibit lymphocyte adhesion and penetration of endothelial cell surfaces [18].

Immune cells use cyto- and chemokines as signals for directing and generating immune responses. In 1994, Fox and colleagues proposed a model for the pathogenesis of SS, in which TNF-α and interferon (IFN)-γ have important roles as regulatory proteins that induce autoimmune sialadenitis. These cytokines are still considered to be prime targets in clinical trials of autoimmune disorders.

TNF-α plays a distinct role in immune system homeostasis and function. In many inflammatory and autoimmune diseases, increased TNF-α production is a pathological trigger, and agents have been developed to inhibit its overproduction. For example, infliximab is a chimeric monoclonal antibody that binds both soluble and transmembrane TNF-α, and etanercept, a fusion molecule of TNF-α receptor p75 (TNFR2) and imunoglobulin (Ig)G1, which binds soluble TNF-α; both have been beneficial in the treatment of other autoimmune diseases. Side effects of systemic anti-TNF-α antibody therapy include lymphoma and infections [19]. Salivary gland gene transfer with these inhibitors might be an excellent alternative to overcome, at least in theory, the side effects and negative results of systemic anti-TNF-α therapy in SS patients. Salivary gland gene transfer with these inhibitors may also enable long-term localized expression. A gene therapy clinical trial involving local expression of soluble TNFR2–IgG fusion protein (e.g., etanercept) in the affected joints of RA patients has recently begun. If this study is beneficial, it would reinforce the use of a localized gene-transfer approach for the treatment of SS.

IFN-γ is another key immunoregulator, and its altered expression can cause pathological conditions [20,21]. Clinically, inhibition of IFN-γ is possible with inhibitory IFN-γ antibodies, and studies in autoimmune disorders, for example, RA, demonstrate efficacy after intramuscular injection without serious side effects [21]. To date, anti-IFN-γ antibodies have not been tested in SS. Intramuscular injection of plasmids encoding a recombinant IFN-γ receptor–IgG fusion protein have been effective in treating both Type 1 diabetes and systemic lupus erythmous (SLE) in mouse models, and no significant side effects are reported [22].

Type I interferons link innate and adaptive immunity. Recently, increased expression of IFN-α-regulated genes was described in the salivary gland of SS patients, raising the possibility of IFN-α blockade as a potential therapy [23]. However, clinical trials of IFN-α in SS patients (150 international units [IU] of IFN-α three-times per day by oral lozenges) resulted in a significant increase in unstimulated salivary flow, without causing significant side effects [24,25]. However, the authors did not observe any difference in stimulated flow or change in oral dryness.

Table 3. Candidate transgenes for gene therapy in Sjögren's syndrome.

Product	Goal
IFN-α	Antagonize IFN-γ; anti-inflammatory
sIFNR	Antagonize IFN-γ
sTNFR	Antagonize TNF-α
IL-10	Anti-inflammatory
sICAM-1	Reduce lymphocyte adhesion
sVCAM-1	Reduce lymphocyte adhesion
sCTLA-4, sCD40	Inhibit lymphocyte costimulation
anti-Blys	Decrease auto-reactive B-cells
sCD20	B-cell depletion
AQP-1	Restore fluid secretion
AQP-5	Restore fluid secretion

AQP: Aquaporin; Blys: B-lymphocyte stimulation; IFN: Interferon; IL: Interleukin; sCTLA: Soluble cytotoxic lymphocyte antigen; sICAM: Soluble intercellular adhesion molecule; sIFNR: Soluble interferon-γ receptor; sTNFR: Soluble tumor necrosis factor receptor; sVCAM: Soluble vascular cell adhesion molecule.

The communication between immune cells offers another point of intervention in the treatment of SS. The regulation of the adaptive immune response against foreign antigens is initiated by the activation of T lymphocytes, which promote B cells, monocytes and dendritic cells. One strategy to decrease the function of T lymphocytes is to block activated or cytotoxic T cells. In localized gene therapy, expression of soluble cytotoxic lymphocyte antigen (CTLA)-4, which binds B7 (an important costimulatory molecule) and subsequently inhibits costimulation of T and B lymphocytes, may be useful. Another potential therapeutic molecule is soluble CD40, which can disrupt the CD40–CD40 ligand (CD40–CD154) interaction and also inhibit T and B lymphocytes costimulation [26].

Several studies have documented a pivotal role of B cells in the pathogenesis of autoimmune disease, specifically, production of autoantibodies. B-lymphocyte stimulator (BLys) is responsible for the differentiation, maturation and survival of B cells and has been detected in the serum of patients with SS, rheumatoid arthritis (RA) and SLE [27]. In SLE patients, clinical trials have been initiated to investigate the effect of anti-BLyS monoclonal antibodies and BLys receptor–IgG fusion protein. Results from a Phase I study in SLE have demonstrated that the antagonist is biologically active and safe [28]. No clinical trials using BLyS antagonists in SS patients have been initiated. Another target is B-cell surface antigen CD20, which can be inhibited by its antibody, resulting in B-cell depletion. Clinical trials have been performed that infused anti-CD20 antibodies into patients with either early primary SS or primary SS with mucosa-associated lymphoid tissue (MALT)-type lymphoma. Results have been beneficial for MALT lymphoma, but for patients with early primary SS, human antichimeric antibody formation was reported [29,30].

Nonimmunological therapy

Dysregulation of apoptosis may play a crucial role in the pathogenesis of SS in two ways: the resistance to apoptosis of infiltrating mononuclear cells and an increase in apoptosis of the exocrine gland epithelial cells. Apoptosis involves a complex cascade that could be influenced at many sites; for example, surface receptors, initiators or intracellular targets [31–33]. Several molecules might be useful to target apoptosis, such as Bcl-2, Bax, FasL, caspase, perforin and granzyme B. Indeed, transplantation and cancer studies delivering viral vectors expressing anti-apoptotic and proapoptotic molecules have been beneficial [34,35]. Similarly, such molecules may be useful for gene therapy targeting apoptotic protecting molecules; for example, Bcl-2, expressed by residing lymphocytes in the salivary glands of SS patients, by monoclonal antibodies or soluble receptors.

Acetylcholine mediates parasympathetic neurotransmission to salivary and lacrimal glands through a family of muscarinic receptor subtypes. Many studies have demonstrated an association between inhibition of neurotransmission through muscarinic receptor 3 (M_3R) and anti-M_3R antibodies in SS patients. However, *in vivo* anti-M_3-R antibodies have not currently been shown to directly affect salivary gland function [36,37]. The importance of developing therapies to block anti-M_3-R antibodies requires a better understanding of their role in this disease.

Aquaporins (AQPs) are membrane proteins that function as water channels and enable transcellular movement of water in response to osmotic gradients. A deficiency or alteration in these molecules could lead to sicca symptoms within SS patients. In 2000, Beroukas and colleagues reported a down-regulation of AQP-1 expression in myoepithelial cells in SS patients, using high-resolution confocal microscopy [38]. Although the role of aquaporins in SS pathology is unclear, they may be potentially useful therapeutic molecules. For example, fretrograde ductal administration of a recombinant adenovirus encoding human AQP-1 within irradiated minipig parotid glands led to significant recovery of secretory function [39].

At present, it is difficult to determine the best single-target gene and it is possible that multiple genes must be administered for maximal effect.

Conclusion

At present, the primary treatment of autoimmune disorders is immune modulation. The production of pro-inflammatory cytokines by epithelial cells, as well as lymphocytes, in SS is a hallmark of this disease. However, the etiology of SS is clearly multifactorial and suggests that numerous targets for therapeutic intervention are possible, as indicated herein. Using gene therapy directed at the salivary gland would create a local expression of the therapeutic molecule that would more closely mimic their natural expression and limit side effects associated with systemic expression. Furthermore, the technology to delivery genes to the salivary

gland already exists, and positive results have been reported in proof-of-principle studies in animal models of SS.

Future perspective

Gene therapy is still very much an experimental therapy. However, advances in vector design have made gene therapy a viable approach for the treatment of a variety of diseases. In order to rigorously test the feasibility of salivary gland gene therapy for SS, it is important to have stable animal models, both to assess potential transgene efficacy and increase understanding of the fundamental mechanisms underlying disease pathogenesis. SS is a complex disease, and it may require a combinatorial therapy that targets different aspects of the disease. In this context, gene therapy may be especially promising due to its ability to deliver both secreted and intracellular proteins to defined cell types.

Executive summary

Sjögren's syndrome & current treatment

- Sjögren's syndrome (SS) is a multifactorial autoimmune disorder characterized by mononuclear cell infiltration in the exocrine glands.
- SS occurs alone (primary) or in association with another autoimmune disorder (secondary).
- Current treatments are saliva substitutes, artificial tears and hydroxochloroquine.

Systemic clinical trials in autoimmune disorders

- Current clinical trials indicate that immunomodulatory protein therapy can benefit patients with certain autoimmune diseases.
- Protein therapies suffer from ineffective administration routes, which require frequent delivery and lead to frequent systemic side effects.

Gene therapy

- The process of delivering DNA-encoding therapeutic proteins to target cells or tissue.
- Targeted delivery can result in higher local protein concentrations,but low systemic levels.
- The salivary glands in SS patients are ideal depot organs for expression of therapeutic proteins.
- Recombinant adenovirus (rAd) and adeno-associated virus (rAAV) are effective vectors in the salivary gland.

Therapeutic molecules for gene therapy

- Potential target molecules for gene therapy fall into several categories effectors of inflammation, apoptosis, parasymphathetic cell signaling and water channel proteins.

Animal models for Sjögren's syndrome

- Gene therapy has been tested in nonobese diabetic (NOD) mice.
- NOD mice are a widely used model for SS, but lack stability and develop diabetes.
- New models may overcome the limitations of the NOD model.

Conclusion

- Gene therapy is a new approach in SS treatment, which may benefit patients by delivering localized expression of therapeutic molecules that limit systemic side effects.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Fox RI: Sjogren's syndrome. *Lancet* 366(9482), 321–331 (2005).

• **Clinical overview of Sjögren's syndrome (SS).**

2. Hansen A: Immunopathogenesis of primary Sjögren's syndrome: implications for disease management and therapy. *Curr. Opin. Rheumatol.* 17 (5), 558–565 (2005).
3. Steinfeld SD, Demols P, Appelboom T: Infliximab in primary Sjögren's syndrome: one-year followup. *Arthritis Rheum.* 46(12), 3301–3303 (2002).

• **Two studies demonstrating conflicting results of infliximab in primary SS.**

4. Mariette X, Ravaud P, Steinfeld S *et al.*: Inefficacy of infliximab in primary Sjögren's syndrome: results of the randomized, controlled trial of remicade in primary Sjögren's syndrome (TRIPSS). *Arthritis Rheum.* 50(4), 1270–1276 (2004).

• **Two studies demonstrating conflicting results of infliximab in primary SS.**

5. Sankar V, Brennan MT, Kok MR *et al.*: Etanercept in Sjögren's syndrome: a twelve-week randomized, double-blind, placebo-controlled pilot clinical trial. *Arthritis Rheum.* 50(7), 2240–2245 (2004).

• **Interesting study showing no beneficial effect of etanercept in SS.**

6. Ratko TA, Cummings JP, Blebea J, Matuszewski KA: Clinical gene therapy for nonmalignant disease. *Am. J. Med.* 115(7), 560–569 (2003).
7. Shai E, Falk H, Honigman A, Panet A, Palmon A: Gene transfer mediated by different viral vectors following direct cannulation of mouse submandibular salivary glands. *Eur. J. Oral Sci.* 110(3), 254–260 (2002).
8. Vanden-Driessche T, Collen D, Chuah MK: Biosafety of onco-retroviral vectors. *Curr. Gene Ther.* 3(6), 501–515 (2003).
9. Wang J, Voutetakis A, Zheng C, Baum BJ: Rapamycin control of exocrine protein levels in saliva after adenoviral vector-mediated gene transfer. *Gene Ther.* 11(8), 729–733 (2004).

10. Wang J, Voutetakis A, Rivera VM *et al.*: Rapamycin control of transgene expression from a single AAV vector in mouse salivary glands. *Gene Ther.* 13(2), 187–190 (2006).
11. Baum BJ, Voutetakis A, Wang J: Salivary glands: novel target sites for gene therapeutics. *Trends Mol. Med.* 10(12), 585–590 (2004).
12. Katano H, Kok MR, Cotrim AP *et al.*: Enhanced transduction of mouse salivary glands with AAV5-based vectors. *Gene Ther.* 13(7), 594–601 (2006).
13. Lodde BM, Mineshiba F, Kok MR *et al.*: NOD mouse model for Sjögren's syndrome: lack of longitudinal stability. *Oral Dis.* (In Press).

• **Study demosntrating the lack of longitudinal stability in non-obese diabetic (NOD) mice.**

14. Cha S, Nagashima H, Brown VB, Peck AB, Humphreys-Beher MG: Two NOD Idd-associated intervals contribute synergistically to the development of autoimmune exocrinopathy (Sjögren's syndrome) on a healthy murine background. *Arthritis Rheum.* 46(5), 1390–1398 (2002).
15. Kok MR, Yamano S, Lodde BM *et al.*: Local adeno-associated virus-mediated interleukin 10 gene transfer has disease-modifying effects in a murine model of Sjögren's syndrome. *Hum. Gene Ther.* 14(17), 1605–1618 (2003).

•• **First successful gene therapy of NOD mice.**

16. Lodde BM, Mineshiba F, Wang J *et al.*: Effect of human vasoactive intestinal peptide gene transfer in a murine model of Sjogren's syndrome. *Ann. Rheum. Dis.* 65(2), 195–200 (2006).
17. Turkcapar N, Sak SD, Saatci M, Duman M, Olmez U: Vasculitis and expression of vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and E-selectin in salivary glands of patients with Sjögren's syndrome. *J. Rheumatol.* 32(6), 1063–1070 (2005).
18. Delaleu N, Jonsson MV, Jonsson R: Disease mechanisms of Sjögren's syndrome. *Dis. Mech. Drug Discovery Today.* 1(3), 329–336 (2004).
19. Scheinfeld N: A comprehensive review and evaluation of the side effects of the tumor necrosis factor-α blockers etanercept, infliximab and adalimumab. *Dermatol. Treat.* 15 (5), 280–294 (2004).
20. Skurkovich B, Skurkovich S: Anti-interferon-γ antibodies in the treatment of autoimmune diseases. *Curr. Opin. Mol. Ther.* 5(1), 52–57 (2003).
21. Sigidin YA, Loukina GV, Skurkovich B, Skurkovich S: Randomized, double-blind trial of anti-interferon-γ antibodies in rheumatoid arthritis. *Scand. J. Rheumatol.* 30(4), 203–207 (2001).
22. Prud'homme GJ, Lawson BR, Theofilopoulos AN: Anticytokine gene therapy of autoimmune diseases. *Expert Opin. Biol. Ther.* 1(3), 359–373 (2001).
23. Gottenberg JE, Cagnard N, Lucchesi C *et al.*: Activation of IFN pathways and plasmacytoid dendritic cell recruitment in target organs of primary Sjogren's syndrome. *Proc. Natl Acad. Sci.* 103(8), 2770–2775 (2006).
24. Cummins MJ, Papas A, Kammer GM, Fox PC: Treatment of primary Sjögren's syndrome with low-dose human interferon α administered by the oromucosal route: combined Phase III results. *Arthritis Rheum.* 49(4), 585–593 (2003).
25. Yamada S, Mori K, Matsuo K, Inukai A, Kawagashira Y, Sobue G: Interferon-α treatment for Sjögren's syndrome associated neuropathy. *J. Neurol. Neurosurg. Psychiatry* 76(4), 576–578 (2005).
26. Howard LM, Miller SD: Immunotherapy targeting the CD40/CD154 costimulatory pathway for treatment of autoimmune disease. *Autoimmunity* 37(5), 411–418 (2004).
27. Mariette X, Roux S, Zhang J *et al.*: The level of BLyS (BAFF) correlates with the titre of autoantibodies in human Sjögren's syndrome. *Ann. Rheum. Dis.* 62(2), 168–171 (2003).
28. Stohl W: Targeting B lymphocyte stimulator in systemic lupus erythematosus and other autoimmune rheumatic disorders. *Expert Opin. Ther. Targets* 8(3), 177–189 (2004).
29. Pijpe J, van Imhoff GW, Spijkervet FK *et al.*: Rituximab treatment in patients with primary Sjögren's syndrome: an open-label Phase II study. *Arthritis Rheum.* 52(9), 2740–2750 (2005).
30. Eisenberg R, Looney RJ: The therapeutic potential of anti-CD20: What do B-cells do? *Clin. Immunol.* 117(3), 207–213 (2005).
31. Patel YI, McHugh NJ: Apoptosis – new clues to the pathogenesis of Sjogren's syndrome? *Rheumatology* 39(2), 119–121 (2000).
32. Manganelli P, Fietta P: Apoptosis and Sjögren syndrome. *Semin. Arthritis Rheum.* 33(1), 49–65 (2003).
33. Ramos-Casals M, Font J: Primary Sjögren's syndrome: current and emergent aetiopathogenic concepts. *Rheumatology* 44(11), 1354–1367 (2005).

• **Clear overview of the aetiopathogenic concepts in SS.**

34. Huh WK, Gomez-Navarro J, Arafat WO *et al.*: Bax-induced apoptosis as a novel gene therapy approach for carcinoma of the cervix. *Gyn. Onc.* 83(2), 370–377 (2001).
35. Ritter T, Kupiec-Weglinski JW: Gene therapy for the prevention of ischemia/reperfusion injury in organ transplantation. *Curr. Gene Ther.* 5(1), 101–109 (2005).
36. Dawson LJ, Allison HE, Stanbury J, Fitzgerald D, Smith PM: Putative anti-muscarinic antibodies cannot be detected in patients with primary Sjögren's syndrome using conventional immunological approaches. *Rheumatology* 43(12), 1488–1495 (2004).
37. Kovács L: Clinical associations of autoantibodies to human muscarinic acetylcholine receptor 3213–228 in primary Sjögren's syndrome. *Rheumatology* 44(8), 1021–1025 (2005).
38. Beroukas D, Hiscock J, Gannon BJ, Jonsson R, Gordon TP, Waterman SA: Selective down-regulation of aquaporin-1 in salivary glands in primary Sjögren's syndrome. *Lab. Invest.* 82(11), 1547–1552 (2002).
39. Shan Z, Li J, Zheng C *et al.*: Increased fluid secretion after adenoviral-mediated transfer of the human aquaporin-1 cDNA to irradiated miniature pig parotid glands. *Mol. Ther.* 11(3), 444–451 (2005).

Affiliations

• *Jelle LG Vosters*
National Institutes of Health, Gene Therapy & Therapeutics Branch, National Institute of Dental & Craniofacial Research, DHHS, Bethesda, MD 20892, USA
and,
Division of Clinical Immunology & Rheumatology, Academic Medical Center/University of Amsterdam, 1100 DE Amsterdam, The Netherlands
Tel.: +1 301 451 9801;
Fax: +1 301 402 1228;
vostersj@mail.nih.gov

• *Bruce J Baum*
National Institutes of Health, Gene Therapy & Therapeutics Branch, National Institute of Dental & Craniofacial Research, DHHS, Bethesda, MD 20892, USA
Tel.: +1 301 594 1193;
Fax: +1 301 402 1228;
bbaum@dir.nidcr.nih.gov

• *Paul P Tak*
Division of Clinical Immunology & Rheumatology, Academic Medical Center/University of Amsterdam, 1100 DE Amsterdam, The Netherlands
Tel.: +31 205 662 171;
Fax: +31 206 919 658;
p.p.tak@amc.uva.nl

- *Gabor G Illei*
  National Institutes of Health, Gene Therapy & Therapeutics Branch, National Institute of Dental & Craniofacial Research, DHHS, Bethesda, MD 20892, USA
  Tel.: +1 301 496 4072;
  Fax: +1 301 402 1228;
  illeig@mail.nih.gov
- *John A Chiorini*
  National Institutes of Health, Gene Therapy & Therapeutics Branch, National Institute of Dental & Craniofacial Research, DHHS, Bethesda, MD 20892, USA
  Tel.: +1 301 496 4279;
  Fax: +1 301 402 1228;
  jchiorini@dir.nidcr.nih.gov