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Ann Rheum Dis 2006;65;1406-1413; originally published online 31 Jul 2006;
doi:10.1136/ard.2006.052761

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REVIEW

Experience with experimental biological treatment and local gene therapy in Sjögren's syndrome: implications for exocrine pathogenesis and treatment

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Sjögren's syndrome is an autoimmune exocrinopathy, mainly affecting the lacrimal and salivary glands, and resulting in ocular and oral dryness (keratoconjunctivitis sicca and xerostomia). The aetiology and pathogenesis are largely unknown, and only palliative treatment is currently available. Data obtained from experimental animal and human studies using biological agents or gene therapeutics can offer insight into the disease process of Sjögren's syndrome. This article reviews the current literature on these approaches and assesses the lessons learnt about the pathogenesis of Sjögren's syndrome.

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Accepted 22 July 2006
Published Online First 31 July 2006

Sjögren's syndrome is an autoimmune exocrinopathy of unknown aetiology, mainly affecting the lacrimal and salivary glands. Lymphoid infiltrates, consisting primarily of T cells and to a lesser extent B cells and macrophages, cause destruction, dysfunction or atrophy of the secretory glands, resulting in ocular and oral dryness (keratoconjunctivitis sicca and xerostomia).[1 2] Sjögren's syndrome occurs predominantly in perimenopausal and postmenopausal women. The distinction between primary and secondary Sjögren's syndrome is that secondary Sjögren's syndrome develops in the presence of other connective-tissue diseases, such as systemic lupus erythematosus and rheumatoid arthritis.[3] Currently, although several approaches focusing on immunomodulatory drugs have been used, only palliative treatment is available for the exocrine dysfunction.

The autoimmune excrinopathy is thought to develop in two separate phases in genetically predisposed people. An unknown environmental stimulus (eg, viral infection) initiates the lymphocyte-independent phase in which inappropriate apoptosis of epithelial cells gives rise to apoptotic autoantigens. These autoantigens may attract lymphocytes in the second, lymphocyte-dependent phase characterised by the production of cytokines and autoantibodies. This subsequent specific immune attack can be exacerbated by a reduced rate of apoptosis among lymphocytes, resulting in death of epithelial cells and a loss of secretory function.[2 4 5] In addition, dysfunction of residual glandular epithelial cells can occur indirectly, possibly owing to the effects of cytokines, autoantibodies (eg, anti-muscarinic receptor antibodies) or parasympathetic nervous system dysfunction in patients with Sjögren's syndrome.[6 7]

Although the exact immunopathogenesis has as yet not been elucidated, Sjögren's syndrome is characterised by an imbalance in cytokine production, locally as well as systemically, depending on disease stage and severity. Only recently has an international group agreed on standard primary and secondary outcome measures for clinical studies on patients with Sjögren's syndrome.[8] Additionally, the pattern of cytokine abnormalities is variable in different studies and is not sufficient to distinguish patients with Sjögren's syndrome from controls.[2 9–12] For example, Pertovaara *et al*[11] found a T helper (Th)2 cytokine profile to be associated with signs of a milder form of primary Sjögren's syndrome, and in line with this observation Mitsias *et al*[12] detected Th2 cytokines in human labial salivary glands of patients with Sjögren's syndrome when there was only low-grade infiltration. A Th1 cytokine pattern was associated with a later disease stage (definite Sjögren's syndrome) and advanced lymphocyctic infiltration.[12] However, the cytokine profile in biopsy specimens of the submandibular gland (SMG), with simultaneous expression of interferon (IFN)γ, interleukin (IL)2, IL4 and IL13, provides an argument against a simple Th1 or Th2 predominance in patients with Sjögren's syndrome.[2] It is important to recognise that the division of Th lymphocytes into Th1 and Th2 subgroups is eminent in animals, but is not well defined in humans.[9 12]

Interestingly, recent studies showing an increased expression of IFN and associated genes in minor salivary glands of patients with primary Sjögren's syndrome support the possible interaction between the innate and adaptive immune system in pathogenesis of Sjögren's syndrome.[13 14] Also, an antigen-driven, germinal centre-type B cell response has been suggested to take place in the salivary glands of patients with Sjögren's syndrome.[15] These B cells represent a unique, highly selected and differentiated

Abbreviations: AAV, adeno-associated virus; BAFF, B cell-activating factor; FasL, Fas ligand; IFN, interferon; MALT, mucosa-associated lymphoid tissue; NFκB, nuclear factor κB; NOD, non-obese diabetic; rAAV2, recombinant serotype 2 AAV vector; RANTES, regulated on activation, normal T cell expressed and secreted; SMG, submandibular gland; TNF, tumour necrosis factor; TNFRp55-Ig, human 55-kDa TNF receptor extracellular domain linked to a mouse IgG heavy chain; VIP, vasoactive intestinal peptide

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B cell population, suggesting a key role of the target organ in recruitment of inflammatory cells, lymphoid neogenesis and propagation of the disease process.[9] The B-cell-activating factor (BAFF), or B lymphocyte stimulator, induced by IFN and a member of the tumour necrosis factor (TNF)α superfamily, likely has an important role. Dysregulated BAFF expression has been proposed to lead to disease progression, perpetuation of humoral autoimmunity[16–18] and the formation of germinal centres in patients with Sjögren's syndrome.[19] Moreover, BAFF has been implicated in the development of B cell malignancies.[18]

Recent advances in applications of gene therapeutics and biological agents in the treatment of patients with Sjögren's syndrome (both animal and clinical studies) should provide insight into the complex immunopathogenesis of Sjögren's syndrome. Herein, we explore this possibility.

Gene transfer methods

Treating a chronic autoimmune disease such as Sjögren's syndrome requires long-term therapeutic approaches. Thus, when using available biological agents such as recombinant proteins that have relatively short half-lives, patients or animals must receive frequent injections. This is inconvenient, uncomfortable and kinetically less than ideal. Conversely, gene transfer offers the potential for stable and regulated expression of the therapeutic protein,[19] although existing gene transfer vectors are imperfect. As yet, no clinical gene transfer studies have been conducted on patients with Sjögren's syndrome. All studies described herein are from animal models of Sjögren's syndrome.

Several gene delivery systems are currently available. Recombinant viral vectors are now particularly useful for their efficiency in mediating gene transfer to targeted cells.[19] However, viral vectors elicit host immune responses, may have a limited packing capacity and, for some, random integration into the host genome is a major concern.[20] For example, the non-enveloped adenovirus conveys robust transgene expression, but this is short lived (few weeks) because of a potent immune response.[21] The single-stranded RNA retrovirus integrates in the host genome and is thus capable of mediating extremely long-term transgene expression. As it is currently possible to generate only low titres of retroviral vectors, their use is typically limited to ex vivo applications.[22] Furthermore, retroviral vectors pose a major safety concern—namely, insertional mutagenesis—as has recently been reported in a severe combined immunodeficiency trial.[23] Non-viral methods, such as plasmid–cationic liposome mixtures, DNA–protein conjugates and naked DNA,[20] generally pose less of a safety risk than viral vectors, but have low transduction efficiency and very short periods (days) of transgenic protein expression.[19] Alternatively, adeno-associated virus (AAV)—a small, single-stranded DNA, non-pathogenic virus—has shown considerable promise as a viral vector for gene therapy. The widely used recombinant serotype 2 AAV vectors (rAAV2) are capable of infecting several mammalian cells, dividing as well as non-dividing, and eliciting a minimal immune response.[21 24] Consequently, transgene expression from rAAV2 vectors, although often more modest than that seen with adenoviral vectors, is stable and lasts for years, and rAAV2 vectors have proved to be useful in animal models of Sjögren's syndrome.

ANIMAL STUDIES

Several animal models of Sjögren's syndrome exist, differing in the presentation of various features of Sjögren's syndrome.[19 25] The non-obese diabetic (NOD) mouse, for example, is the most useful, commonly available animal model to examine the disease characteristics. It develops, besides type 1 diabetes mellitus, exocrine gland infiltrates and decreased glandular secretion, which are age and sex dependent.[1 4 26] The male NOD mouse is used for studying dacryoadenitis, whereas sialadenitis is studied in the female mouse. Lymphocytes and autoantibodies play an important part in the development of Sjögren's syndrome-like disease in NOD mice, as NOD-*scid* mice and B cell-deficient NOD mice have a normal salivary gland function.[27] We will first discuss the lessons learnt from in vivo gene transfer experiments using various therapeutic genes in the NOD mouse model and other animal models of Sjögren's syndrome.

Interleukin 10

IL10, mainly expressed in peripheral T cells, monocytes and B cells, is a cytokine capable of inhibiting synthesis of pro-inflammatory cytokines such as IFNγ, IL2, IL3 and TNFα, and reducing the activation of monocytes or macrophages. However, IL10 also shows immunostimulatory properties, especially on B cells and activated CD8+ T cells.[28] Local human IL10 gene delivery to the SMGs has proved to be successful in the female NOD mouse model for Sjögren's syndrome, resulting in an increased salivary flow rate and a lower focus score (less focal infiltration in the SMGs) after both prophylactic and therapeutic administration. Increased SMG levels of IL4, IL6, IL10 and IL12 in the NOD mice compared with those in controls indicated that there was no straightforward ''repair'' of a presumed Th1–Th2 imbalance.[26] Viral IL10 has 84% sequence homology with human IL10 and mimics several of its immunosuppressive activities, without increasing 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 viral IL10 delivery partially suppressed the appearance of Sjögren's syndrome-like features, such as reduced tear production, accelerated tear break-up time, ocular surface disease and immunopathological response.[29] A reduced size and number of immune infiltrates due to decrease in cells positive for CD4 and CD18 (leucocyte cell surface marker essential for leucocyte adhesion to endothelial cells and chemotaxis), reduction of cells expressing MHC II and increase in CD8+ cells were detected in this setting.

In contrast, transgenic overexpression of endogenous IL10 in the exocrine glands of C57BL/6 mice led to tissue destruction and the development of Sjögren's syndrome.[30] In patients with Sjögren's syndrome, salivary gland and serum IL10 levels are variably increased, depending on disease stage and activity, and might be contributing to B cell activation and lymphoma development.[12 31–33] Therefore, the exact role of IL10 in the pathogenesis of Sjögren's syndrome still needs to be established.

TNFα inhibition

TNFα is a dominant pro-inflammatory cytokine that is increased in the glands of patients with Sjögren's syndrome: TNFα and its cognate receptors have been found on infiltrating mononuclear inflammatory cells, vascular endothelial cells, ductal epithelial cells and fibroblasts.[34 35] However, the exact role of TNFα in autoimmune pathology is yet to be determined.[4] An adenovirus encoding the human 55-kDa TNF receptor extracellular domain linked to a mouse immunoglobulin (Ig)G heavy chain (TNFRp55-Ig) was used in a dacryoadenitis rabbit model. Prophylactic administration to the lacrimal glands concurrently with the induction of dacryoadenitis led to a partial suppression of Sjögren's syndrome-like features. Tear production decreased in the control group, but was unchanged in the treated group, whereas the tear break-up time and Rose Bengal staining (an indicator of corneal surface defects) were similar for both groups. This is a reflection of immunoregulation in the gland, but not in the conjunctiva.[36] The same research group also observed a therapeutic effect of vector delivery—that is, after

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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).[37] Thus, local TNFα inhibition shows promising results for the ophthalmological component of Sjögren's syndrome-like disease.

In preliminary experiments, we administered an rAAV2 vector encoding TNFRp55-Ig to submandibular glands of the NOD mice before the onset of Sjögren's syndrome-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.[38]

Vasoactive intestinal peptide

Vasoactive intestinal peptide (VIP) is a small neuropeptide with pleiotropic functions in the neuroimmunoendocrine network.[39] Being an immunomodulator, secretory and trophic stimulus VIP could be an interesting therapeutic candidate for Sjögren's syndrome,[40] although a short half life limits its use. Delivery of rAAV2hVIP to the SMGs of NOD mice in a prophylactic experimental design resulted in an increased salivary flow rate and a reduction in SMG levels of the cytokines IL2, IL10, IL12 (p70) and TNFα, and in serum levels of the chemokine RANTES (regulated on activation normal T cell expressed and secreted).[40] It was hypothesised that secreted hVIP directly increased salivary secretion of acinar cells and/or influenced the immune milieu. Delgado *et al* postulated VIP to be a Th2 cytokine with a key role in neuroimmunology[41 42]—that is, VIP production by Th2 cells, as well as VIP stimulation of Th2 and inhibition of Th1 functions. On 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 a strict Th2 cytokine in this model of Sjögren's syndrome.

Nuclear factor κB inhibitor α

The role of individual cytokines in the pathogenesis of Sjögren's syndrome is unclear. Although overexpressing or blocking these molecules can result in some clinical improvement, studies thus far suggest that there is no noticeable Th1–Th2 shift in the cytokine profile. Therefore, it might be preferable to induce a more general, but cell-type-localised, blockade of downstream immunoregulatory events. Nuclear factor (NF)κB, a group of inducible dimeric transcription factors, is expressed in all cell types. After stimulation and degradation of its inhibitory protein, IκB, cytoplasmic NFκB is translocated to the nucleus, where it has an important regulatory role in the cellular response to inflammatory processes.[43–45] An IκBα mutant that renders the inhibitor a super repressor (IκBα (sr)) is resistant to immediate degradation on stimulation, thereby preventing NFκB activation.[44] To test whether this agent would be useful in treatment of patients with Sjögren's syndrome, rAAV2IκBα (sr) was administered to the SMGs of female NOD mice. Inhibition of the NFκB pathway resulted in the reduction of several cytokines in the SMG (IL2, IL10, IL12 (p70), TNFα and RANTES) and also considerably improved salivary flow rate (Lodde *et al*, unpublished observations). Again, as above, a reduction in clinical parameters of Sjögren's syndrome was observed, but there was no shift from Th1 to Th2.

Anti-CD4 antibody

The molecular marker CD4 is expressed by activated Th cells–the most predominant cell type in glandular tissue of patients with Sjögren's syndrome.[2] Studies by Thompson *et al*[27] and Arakaki *et al*[46] have clearly shown that CD4+ T cells have a key role in the development and maintenance of Sjögren's syndrome. For example, Thompson *et al*[27] reported a 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. The anti-CD4 antibody led to tolerance induction, possibly by the generation of regulatory T cells. Arakaki *et al*[46] showed that adoptive transfer of autoreactive, CD4+ T cells into normal syngeneic recipients induced autoimmune lesions similar to those in patients with Sjögren's syndrome. The autoreactive, CD4+ T cell lines recognised synthetic α-fodrin; a membrane skeleton protein. Of interest, autoantibodies to α-fodrin have been detected in human Sjögren's syndrome[47 48] and in NOD mice.[51] Similarly, topical application of an anti-CD4 monoclonal antibody to the eye also suppressed the local activation of CD4+ T cells rather than deleting them, which reduced the expansion of pathological CD4+ T cells against α-fodrin.[50]

Ciclosporin A

Although ciclosporin 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 the transcription factor NF-AT (nuclear factor of activated T cells). This leads to reduced transcription of several cytokine genes, including IL2, IL3 and IL4, and TNFα. It acts primarily on T cells, inhibiting their activation.[51] Topical ciclosporin A seems to improve tear secretion in mouse models of Sjögren's syndrome, 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 expression of Fas-ligand (FasL) expression on infiltrating lymphocytes (NOD mice).[52] The key mechanism for the therapeutic effect of topical ciclosporin A for keratoconjunctivitis sicca seems to be inhibition of apoptosis.[53] In addition, Strong *et al*[53] hypothesised that this occurs through either reduction of proapoptotic cytokines on the ocular surface or inhibition of the caspase cascade.

Apoptosis

Dysregulation of apoptosis may have a crucial role in the pathogenesis of Sjögren's syndrome. Epithelial cells seem 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 IFNγ and TNFα.[5] Fas–FasL is discussed in the next paragraph, data on TNFα inhibition have been presented earlier, and no information on treatments targeting the other molecules is currently available.

Fas–FasL

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.[5] Increased expression of the antiapoptotic protein Bcl-2 associated with a decreased sensitivity to Fas-mediated apoptosis has been described in infiltrating lymphocytes of patients with Sjögren's syndrome.[54 55] FasL is thought to be increased on infiltrating mononuclear cells, contributing to gland destruction, but other evidence indicates that soluble FasL is produced by the salivary gland itself.[56] On the basis of studies on salivary glands of Fas-deficient B6-*lpr/lpr* mice and FasL-deficient B6-*gld/gld* mice, Fleck *et al*[56 57] concluded that a defect in Fas-mediated apoptosis of immune cells leads to an up regulation of the immune response. After murine

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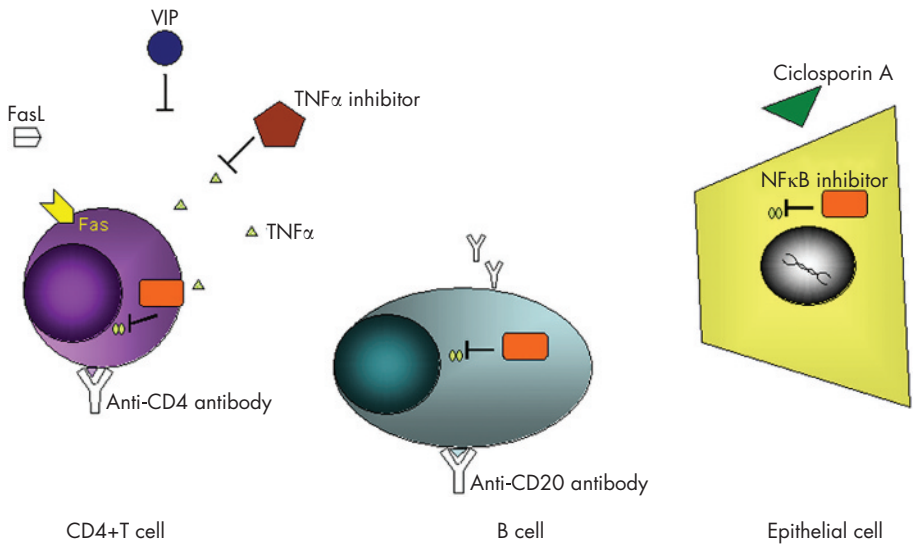

Figure 1 Target sites of therapeutic molecules. Schematic view of the target site of different therapeutic molecules. T cells and their cytokines can be blocked by an anti-CD4 antibody, vasoactive intestinal peptide (VIP) or a tumour necrosis factor (TNF)α inhibitor, whereas an anti-CD20 antibody inhibits B cells. Ciclosporin A can inhibit apoptosis of epithelial cells, whereas Fas ligand (FasL) stimulates apoptosis of overaccumulating CD4+ T cells. A nuclear factor (NF)κB inhibitor could exert its effects in epithelial cells, and T and B lymphocytes.

cytomegalovirus infection, these mutant mice develop a severe, acute and chronic sialadenitis, featured by multiple focal infiltrates. The chronic sialadenitis is caused by defective Fas-mediated apoptosis, which permits activated T cells to persist in the salivary glands despite the absence of detectable virus.[56] Interestingly, Sjögren's syndrome-like disease does not develop without viral induction. Fas-mediated apoptosis is subsequently required for the down-modulation of the inflammatory response to prevent this postviral, chronic disease,[56 57] which was achieved by FasL gene transfer to B6-*gld/gld* mice.[56] Acinar and ductal cells were not sensitive to FasL-mediated apoptosis and, despite documented increase in Fas expression,[29 54 56] a role of FasL-mediated epithelial cell apoptosis in Sjögren's syndrome is unlikely. Instead, abnormal expression of the proapoptotic protein Bax by acinar cells[55] or the presence of IFNγ[58] may represent a pathological feature.

Considerations about animal models

Although the NOD mouse is a commonly used model for type 1 diabetes mellitus, it is not perfect.[59–62] Several immunomodulatory treatments were successful in animals, but this strategy failed to translate to clinical trials in patients with diabetes mellitus. It was hypothesised that the NOD mouse represents only one pathogenic mechanism, whereas in humans different processes might lead to a final common pathway.[59] The same could apply to the Sjögren's syndrome-like disease in NOD mice and Sjögren's syndrome in human.[63] Importantly, until now, there have been no clinical trials using gene therapy in Sjögren's syndrome. From the mouse studies described above, although localised salivary gland gene transfer is clearly beneficial in murine models of Sjögren's syndrome, it remains to be seen whether these findings are relevant to the presentation of the disease in humans.

CLINICAL STUDIES

TNFα inhibition

On the basis of their successful use in treatment of patients with rheumatoid arthritis, TNFα blockers have also been tested in patients with Sjögren's syndrome. A pilot study and a 1-year follow-up open trial with infliximab, a chimeric monoclonal antibody to TNFα, showed an improvement in all objective and subjective parameters of Sjögren's syndrome tested.[64 65] In the pilot study, statistically significant improvement was seen in global (patient's global assessment, patient's assessment of pain and doctor's global assessment), peripheral (tender joint or point count), fatigue (patient's fatigue assessment), laboratory (erythrocyte sedimentation rate, numbers of peripheral CD4+ and CD8+ cells) and local (patient's dry mouth and dry eyes assessment and unstimulated salivary flow rate) assessments during the 6-week treatment and 2-month follow-up period. Global, peripheral, fatigue and local assessments were also markedly improved in the subsequent 1-year follow-up study. However, these results could not be confirmed in later studies. Randomised, double-blind, placebo-controlled trials with infliximab[66] and etanercept, a soluble human TNFα–p75 receptor fusion protein,[67] showed no beneficial effect. A pilot, open-label study with etanercept only reported less fatigue and a decreased erythrocyte sedimentation rate in a small subgroup.[68] These observations could be owing to several factors, such as patient characteristics, suboptimal outcome measures and a lack of biological efficacy. Moreover, both of these recombinant proteins, with a limited half life, were given 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 TNFα activity before or after treatment. Local gene transfer of a TNFα blocker could perhaps change the outcomes. In addition, it is possible that TNFα does not have such an important role as previously thought in Sjögren's syndrome.[67]

Thalidomide

Thalidomide, which can function as a TNFα inhibitor, was tested in a 12-week randomised, double-blind, placebo-controlled pilot clinical trial. Unfortunately, thalidomide treatment was associated with unacceptable adverse effects, and too few patients completed the study to consider potential efficacy. Despite the prominent adverse effects, the possibility that thalidomide may be beneficial and safe at much lower doses in patients with Sjögren's syndrome could not be ruled out.[69] In a trial on patients with rheumatoid arthritis, the frequency of adverse effects of thalidomide treatment was also high.[70 71]

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Table 1 Conclusions drawn from animal and human studies on Sjögren's syndrome, using different targeted biologicals and transgenes[27 28 30 31 37 38 41 47 51 53 54 57 58 64–69 72 73 75 76 79 84]

Target	Animals	Humans
IL10	Overexpression is causal factor, but can have therapeutic value without increased risk of lymphoma (vIL10)	
TNFα inhibitor	Efficacy in prophylactic and treatment setting	Not important or local levels too low
VIP	Immunomodulator (Th2 cytokine), secretory and trophic stimulus	
Anti-CD4 Ab	CD4+ T cells have a key role	
B cell depletion or modulation		B cell modulation sufficient for clinical efficacy
NFκB inhibitor	NFκB pathway important	
Ciclosporin A (topical)	Prevention lymphocyte-induced acinar apoptosis	Prevention inhibition apoptosis epithelial cells
Fas–FasL	Defect Fas-mediated apoptosis infiltrating lymphocytes, role of Fas in epithelial cell apoptosis unlikely	
IFNα		Phase III trial: increased unstimulated SFR, but increased endogenous IFNα in SS (viral/autoantigen?)
Prednisone		Increased SFR and subjective measures in selected patients

Ab, antibody; FasL, Fas ligand; IFN, interferon; IL, interleukin; NF, nuclear factor; SFR, salivary flow rate; SS, Sjögren's syndrome; TNFα, tumour necrosis factor α; vIL10, viral interleukin 10; VIP, vasoactive intestinal peptide.

Interferon α

IFN has three major IFN classes, with type I IFN consisting of 14 IFNα isoforms among others. IFNα improves phagocytic antigen processing and immune regulatory activity of macrophages, specific cytotoxicity of lymphocytes for target cells and natural killer cell activity. It can be given in a high-dose injection or low-dose lozenge. To date, one phase III trial has been completed, where low-dose IFNα was given by the oromucosal route.[72] A large increase in unstimulated whole salivary flow rate was seen in patients with primary Sjögren's syndrome, without causing major adverse events. The coprimary end points of stimulated whole salivary flow and oral dryness were not markedly improved. However, a recent report seems to contradict the positive finding of the phase III trial: Båve *et al*[73] reported that patients with primary Sjögren's syndrome have an activated type I IFN. Viral infection may initiate the production of IFN, but the continued IFNα synthesis is caused by RNA-containing immune complexes that activate plasmocytoid dendritic cells to prolong IFNα production at the tissue level. This IFNα promotes the autoimmune process by a vicious circle-like mechanism, with increased autoantibody production and formation of more endogenous IFNα inducers. The authors hypothesised that oral IFNα treatment may possibly act by increasing saliva secretion via up regulation of transcription of aquaporin 5, a membrane water channel,[74] without influencing the underlying autoimmune process that could still be maintained by IFNα.

Ciclosporin A

The most important advancement in the treatment of ocular manifestations of Sjögren's syndrome is the introduction of topical anti-inflammatory agents such as ciclosporin A, which increases tear production and decreases symptoms without any major side effects.[52] A phase III study showed that 0.05% ciclosporin A improved subjective measures of dry eye,[75] and the Food and Drug Association, Rockville, Maryland, USA, has approved marketing of this emulsion for topical application for dry eyes. Immune-related and apoptosis-related markers were reduced in the conjunctival epithelium after 6 months of treatment, consistent with topical ciclosporin A acting through inhibition of the apoptosis mechanism.[76] Systemic treatment with ciclosporin A, however, leads to increased lymphocytic infiltration and has been unsuccessful in humans.[52]

Depletion or modulation of B cells

Sjögren's syndrome is specifically characterised by B cell hyperactivity,[77] and patients have a 44 times increased risk of developing B cell non-Hodgkin's lymphoma.[78] 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 patients with primary Sjögren's syndrome, showing marked improvements in the Rose Bengal score in the group with early primary Sjögren's syndrome and in the group with mucosa-associated lymphoid tissue (MALT) or primary Sjögren's syndrome, and in tear break-up time in the group with early primary Sjögren's syndrome. Stimulated submandibular or sublingual salivary secretion increased considerably in patients whose stimulated salivary flow was >0.10 ml/min at baseline (all patients with early primary Sjögren's syndrome and two patients with MALT or primary Sjögren's syndrome). B cell depletion was accompanied with a marked decrease in IgM rheumatoid factor levels at week 5 in patients with MALT or primary Sjögren's syndrome. Additionally, three of seven patients with MALT-type lymphoma had a complete remission, but the high incidence of human antichimeric antibodies and associated side effects, such as serum sickness, observed in this study needs further evaluation.[79] In a retrospective study, six patients with primary Sjögren's syndrome who had been given rituximab were evaluated. Five showed improvement of several manifestations, notably swelling of the parotid gland, arthralgias and cryoglobulinaemia-related vasculitis, but no conclusions could be drawn about the effect of rituximab on dryness.[80] In an open-label phase I–II trial on 15 patients with primary Sjögren's syndrome treated with epratuzumab, a humanised monoclonal anti-CD22 antibody, improved objective and subjective measures were seen in several patients: 10 of 14 patients showed lacrimal flow and 5 showed increased salivary flow. Patients with symptoms at study entry reported clinical improvement of dry eyes (58%), dry mouth (36%), fatigue (65%), tender point (67%) or tender joint (100%) counts.[81] The modulating antibody led to B cell levels decreasing by about 60%, but T cell levels, immunoglobulins and routine safety laboratory parameters remained unchanged. A complete depletion of circulating B cells therefore does not seem essential for clinical efficacy.

Prednisone

Glucocorticoids are potent inhibitors of NFκB activation,[82] most likely through protein–protein interactions between the glucocorticoid receptor and NFκB subunits, possibly in the nucleus.[83] They are mostly used for treating extraglandular manifestations of Sjögren's syndrome. A 6-month trial with oral prednisone (30 mg on alternate days) failed to improve functional and histological parameters in patients with

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Table 2 Importance of epithelial, T and B cells in the pathogenesis of Sjögren's syndrome

	Effects of NFκB inhibitors on		
	Epithelial cells	**T cells**	**B cells**
Importance	+	+	+
Disease stage	early?	continuous	onset?, chronic
Evidence	cytokines, apoptosis, neo-antigens **(ciclosporin A)**	cytokines **(anti-TNFα, VIP)**, cytokine shift?, antibodies **(anti-CD4)**, decreased apoptosis **(FasL)**	autoantibodies, lymphoma (late), antibodies **(rituximab, anti-Blys)**
	↖	↑ **NF-κB inhibitor**	↗

Blys, B lymphocyte stimulator; NF, nuclear factor; TNFα, tumour necrosis factor α; VIP, vasoactive intestinal peptide.
Evidence is shown for the importance of the three different cell types at the molecular level, as learnt from the disease process. The possible therapeutic intervention in this process is also shown in bold.

primary Sjögren's syndrome.[84] However, salivary flow and subjective measures were improved in selected patients. In addition, a decrease in serum total protein, IgG, IgA and erythrocyte sedimentation rate, and an increase in white cell count were observed. Although local corticosteroid irrigation treatment of the parotid gland relieved xerostomia in patients with Sjögren's syndrome,[85] the adverse effects of topical use of corticosteroids in the eye outweigh the possible benefits and is therefore not recommended.[52]

CONCLUDING REMARKS

Several important lessons can be learnt (table 1) from these studies on animals and humans using gene transfer and biologicals. Firstly, Th2 cytokines may have a therapeutic effect, but there is no "correction" of the proposed Th1–Th2 imbalance. In fact, it seems that the abnormalities in Sjögren's syndrome cannot be ascribed to a simple imbalance. Raz *et al*[60] previously considered the issue whether a cytokine shift has a primary role in suppressing the disease or it is only a biomarker with respect to type I diabetes mellitus. They concluded, on the basis of knockout and vaccination models, that it is possible that 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 IL4, IL10 and IFNγ probably have some role, but they may not 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 systemic biological. Initial gene transfer studies on the lacrimal and salivary glands, using vectors encoding TNFRp55-Ig (similar to etanercept), seemed to show some efficacy. Conversely, systemically delivered etanercept is not beneficial for patients with Sjögren's syndrome. 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 seems to be a therapeutic potential for strategies targeting the NFκB pathway, which is important in the inflammatory process in Sjögren's syndrome, by mediating the effects of various cytokines. Although systemic treatment with prednisone, an NFκB inhibitor, did not show improvement in salivary or lacrimal function, local irrigation of parotid glands with corticosteroids improved saliva production. This is supported by preliminary data from the NOD mouse, suggesting that NFκB blockade in the gland results in improved salivary function. Furthermore, both T and B cells are clearly important to the pathogenesis of Sjögren's syndrome, and deleting or blocking them may have therapeutic value—for example, methods such as blocking IFN pathways or inhibiting the consequences of IFN production, such as BAFF secretion, have been proposed and may be useful.[15]

Finally, apoptotic pathways seem to be important to the pathogenesis of Sjögren's syndrome. Although infiltrating lymphocytes show a defective Fas-mediated apoptosis, the role of Fas in the apoptosis of epithelial cells is unclear. Importantly, the beneficial effect of topical ciclosporin 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).

On the basis of these observations, we can categorise 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, in that theoretically, only one injection would be needed for long-term protein expression. The delivery route is important for several reasons. For example, local therapy of a TNFα inhibitor transgene in a rabbit model proved to be successful, whereas systemic delivery of the recombinant protein in humans failed to improve the disease features of Sjögren's syndrome, 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 probably 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.

Sjögren's syndrome has a complex aetiology, where environment, genetics and disease stage all have a role; there is not one critical factor. Table 2 and fig 1 give a summary of the three most important associated cell types 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 TNFα inhibitor, whereas an anti-CD20 or anti-B lymphocyte stimulator antibody inhibits the B cell site of the equation. Ciclosporin A can inhibit apoptosis of epithelial cells, whereas FasL stimulates CD4+ T cell apoptosis. An NFκB inhibitor could exert its effects on epithelial cells, and on T and B lymphocytes.

Local (gene) therapy of the exocrine component of Sjögren's syndrome seems to be kinetically optimal and clinically most appropriate. We propose that the local delivery of an immunomodulatory and antiapoptotic transgene could reduce the gland inflammation by affecting multiple downstream targets. Furthermore, a combination of transgenes—for example, an NFκB inhibitor combined with an anti-CD4 antibody, FasL or VIP—may be particularly useful.

Clearly, our present understanding of the pathogenesis of Sjögren's syndrome is inadequate to precisely define

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molecular targets for treatment. Nonetheless, as we have tried to show in this article, several reasonable potential therapeutic targets can be identified in patients with Sjögren's syndrome. Rigorous testing of these will help both to understand the pathogenesis of Sjögren's syndrome and to develop novel therapeutics.

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Funding: This research was supported by the Intramural Research Program of the NIDCR/NIH.

Competing interests: None.

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