REVIEW

Use of localised gene transfer to develop new treatment strategies for the salivary component of Sjögren's syndrome

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Effective treatment for Sjögren's syndrome (SS) might be developed locally by introducing genes encoding cytokines, which are potentially anti-inflammatory, or by introducing a cDNA encoding a soluble form of a key cytokine receptor, which can act as an antagonist and decrease the availability of certain cytokines, such as soluble tumour necrosis factor α receptors. Currently, the preferred choice of viral vector for immunomodulatory gene transfer is recombinant adeno-associated virus. The use of gene transfer to help determine the pathophysiology and to alter the course of the SS-like disease in the NOD mouse model can ultimately lead to the development of new treatments for managing the salivary component in patients with SS.

Sjögren's syndrome (SS) is an autoimmune disorder characterised by decreased lacrimal and salivary gland function, which can also affect multiple organs, including kidney and lung.¹ The principal lesion in SS is lymphocytic infiltration in target tissues.² Lacrimal and salivary gland inflammation is most prominent and associated with decreased production of tears (associated with keratoconjunctivitis sicca) and saliva (resulting in xerostomia). The reported prevalence of primary SS varies from 0.05% to 4.8% of the population and the reported incidence of cases diagnosed by a doctor is 4 per 100 000 population a year.³ The prevalence and incidence reported are influenced by subject selection and the classification criteria applied. Women are nine times more likely to be affected than men and the symptoms appear to increase with age.^{1–3}

Early events in human SS are difficult to recognise because symptoms usually manifest relatively late in the disease. This, and the inability to perform unlimited serial salivary gland biopsies in patients with SS, for practical and ethical reasons, underlines the importance of having representative animal models for the disease.

Treatment of SS currently involves the alleviation of the exocrine and systemic manifestations. In addition to local treatment using artificial tears and oral preparations, muscarinic agents, such as cevimeline (M3 muscarinic receptor-specific agonist) and pilocarpine (non-specific muscarinic agonist) are used to stimulate secretions.⁵ For systemic intervention in the disease,

hydroxychloroquine, steroids, and other immunomodulatory agents are sometimes used.^{6–7} The current array of treatments is unsatisfactory for most patients, so the development of new treatment strategies for SS is necessary. Recently, several "biological therapies", employing recombinant immunomodulatory proteins, have been shown to be useful in other autoimmune disorders.⁸ Although the potential use of these agents in SS has yet to be unequivocally demonstrated, immune modulation using recombinant proteins, such as soluble tumour necrosis factor α (TNF α) inhibitors, is inconvenient for patients to administer. Furthermore, they cannot be targeted to the active site of disease and, after injection tend to be cleared rapidly from the system.⁸ Modulation of salivary cellular functions by gene transfer into these target tissues may provide a number of therapeutic options for the management of SS.⁹ Because the exocrine manifestations of SS rather than the systemic features are prominent, we will emphasise here the role of salivary tissues as localised targets for immune modulation through gene transfer, primarily using recombinant adeno-associated viruses (AAVs). We will discuss how and where to deliver transgenes, the safety of gene transfer for patients with SS, potential candidate genes to consider for local intervention in SS, and the animal models that can be used to investigate the effects of a gene transfer intervention. We refer to other review articles for a comprehensive general review of gene transfer vectors^{10–11} or of the immunopathological mechanisms possibly operative in SS.^{12–13}

GENE TRANSFER

Gene transfer refers to a technology that delivers a segment of DNA into target cells or tissues. Gene transfer can involve the direct introduction of a gene or cDNA (that is, the transgene) into diseased cells to restore normal function. Alternatively, the target cells can be normal, but are used to produce a functional, secreted

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Abbreviations: AAV, adeno-associated virus; BlyS, B lymphocyte stimulation; ICAM, intercellular adhesion molecule; IFN γ , interferon γ ; IL, interleukin; LFA, leucocyte function associated antigen; MHC, major histocompatibility complex; NOD, non-obese diabetic; RA, rheumatoid arthritis; rAAV, recombinant adeno-associated virus; RT-PCR, reverse transcription-polymerase chain reaction; SCID, severe combined immunodeficiency; SS, Sjögren's syndrome; TNF α , tumour necrosis factor α ; VCAM, vascular cell adhesion molecule

protein to correct malfunctions in other cells and tissues. Generally, there are two ways to transfer genes, using viruses or using non-viral methods.^{9–10} For clinically successful gene transfer it is essential to have a good understanding of the normal physiology of the target cell, and the clinical features, immunological, molecular and cellular pathobiology of the disease. For salivary glands, as well as other tissues, an effective gene delivery vector requires several components. The vector must appropriately package the gene providing protection from the extracellular environment. It must allow binding and uptake by the target cells and should facilitate delivery of the gene to the nucleus. Ideally, for a rheumatic disease, the vector carrying the gene of interest should provide stable expression—for example, by integrating the transgene into the host genome. Also it is desirable to provide tissue-specific transcriptional control—for example, using cell-specific promoters and/or other regulatory elements, to prevent unwanted transgene expression elsewhere.¹⁴ For persistence of gene expression, the vector and gene product should not be immunogenic to avoid a potent immune response and destruction of the transduced cell.¹⁵

Methods of gene delivery in salivary glands

Non-viral vectors

Naked DNA plasmids, either alone or complexed with various cationic molecules, can be used to deliver genes. In vitro, generally good transfection, with many cell types can be achieved. Complexing plasmid DNA with cationic formulations enhances cellular entry, the transfer of a transgene by a non-viral vector, and expression of that transgene in the target cell. Non-viral vectors, in general, are significantly less immunogenic than viral vectors,¹⁶ but in vivo, the efficiency of gene transfer of non-viral vectors is low, making them less favourable for in vivo gene transfer at present (table 1).

Viral vectors

Viruses have evolved efficient ways to enter into host cells and replicate. This ability makes them useful as gene transfer vectors. Currently, viral vectors provide the most efficient method of performing clinically useful gene transfer. The vast majority of gene transfer studies in salivary glands have used adenoviruses, and more recently recombinant adeno-associated viruses (rAAVs). Occasionally other viral vectors have been employed in salivary glands. For example, there are single reports of recombinant vaccinia, herpes, and lentiviruses being used.¹⁷ Such infrequently used vectors will not be discussed further here.

“Viruses are efficient gene transfer vectors”

Ideally, all recombinant viral vectors should be constructed as being non-competent for replication, and deleted of viral

structural genes, which may be cytotoxic or immunogenic. Viral vectors should also retain full infectious ability and mediate the required level of transgene expression in the target cells. Retroviral vectors can integrate into the host chromosome. However, transduction by most retroviral vectors (not lentiviral vectors, however) requires cell division, and at present they also cannot be grown in high titres. Consequently, most retroviral vectors can only be used ex vivo,¹⁸ an impractical feature for use with salivary glands.

Adenoviral vectors can efficiently infect dividing and non-dividing cells, including highly differentiated tissues such as salivary glands. They can be easily grown in large amounts and have efficient transduction rates in vivo. For these reasons, adenoviral vectors have commonly been used in salivary gland gene transfer studies. However, these vectors do not mediate transgene integration into the host cell genome. The absence of genomic integration results in transient expression of the transgene. In addition, adenoviral vectors elicit a potent host immune response in the salivary gland, making them for many clinical applications less than ideal vectors.^{15–19}

Recently, many laboratories have explored the possible use of rAAVs (serotype 2) for in vivo gene transfer. AAV is a small single stranded DNA parvovirus. Wild-type AAV has the ability to integrate into a specific non-mutagenic site on chromosome 19. However, although rAAVs do not retain this ability for site-specific integration, they do appear to provide stable expression through a long term extrachromosomal presence. rAAVs have the potential for transducing a wide range of host cells and are considered to be non-pathogenic.²⁰ Importantly, rAAV serotype 2 transduces the ductal cells in salivary glands.^{21–22}

Systemic versus local salivary gland delivery

Gene delivery can be performed systemically or locally. When a transgene is delivered in patients with SS by a route that is remote from the active disease site, such as into muscle or intravenously, more systemic sequelae, both related to the vector and to the transgene product, can be expected. Conversely, local delivery of a transgene at the site of disease leads to highly localised therapeutic effects, while minimising the risk of undesirable systemic dissemination of vector and transgene. Additionally, it is possible to regulate the secretory characteristics of transgene products.²³ This could lead to controlled levels of therapeutic secretory proteins systemically in lower quantities, which may be advantageous in treating SS.

Delivery to salivary glands

The anatomical configuration of major salivary glands favours localised tissue modulation by gene delivery. When a vector is introduced into major salivary glands in vivo through a cannulated duct, it can reach the luminal surface

Table 1 Vectors commonly used for salivary gland gene transfer

Vector	Advantage	Disadvantage
Adenovirus	Highly efficient transduction	Short term expression of the transgene (weeks) Potent immune response possible
AAV	Relatively simple production Transduces non-dividing cells Large gene packaging capacity Efficient transduction Transduces non-dividing cells Long term expression (months/years) Modest immune response	Limited packaging capacity (max 4.6 kb) Difficult to produce
Non-viral	Simple to produce Low immune response Inexpensive	Low transduction efficiency Relatively short term expression (days)

AAV, adeno-associated virus.

Figure 1 Ductal cannulation of submandibular glands of a rat by using custom made cannulas. A maximal volume of 50 μ l (mouse) or 200 μ l (rat) of gene transfer vector can be delivered in a retrograde fashion using 3/10 ml insulin syringes connected to the cannulas. Arrows indicate the cannulas (see Baum *et al*²⁴). Photo courtesy of Dr L Baccaglini.

of virtually all epithelial cells (fig 1).²⁴ Ductal cannulation of major salivary glands is a routine clinical procedure used for sialography, and clinically no anaesthesia is required. The major salivary glands are also well encapsulated, which minimises the risk of dissemination of gene transfer vectors beyond the gland.²⁴ Many reports of successful salivary gland gene transfer have been published (for a recent review see Baum *et al*²⁴). These features probably ensure effective local gene transfer, particularly useful in a disease such as SS.

Safety of gene delivery

In any medical intervention there are risks and benefits. At present, gene transfer is a developing therapeutic strategy that has resulted in significant clinical problems without any unqualified clinical success.²⁶ None the less, gene transfer strategies are based on sound science, albeit incompletely understood at present.²⁷ Currently, with gene transfer, the use of viral vectors presents the most risk to patients. Although viral vectors are modified to minimise their risk of replication, because our knowledge of vector biology is not complete problems can occur. For example, recombination events might take place creating replication competent viruses. As we have recently learnt from the studies in Paris using retroviral vectors to treat SCID-XI patients, unique vector integration events with severe consequences are possible.²⁸ Also vector immune response is a major concern, and some vectors, especially adenoviral vectors, can elicit potent immune responses.²⁶

“Vector safety issue has to be solved before gene transfer is widely used in daily clinical practice”

These potential risks must be considered in the light of patient needs and benefits. It is still highly probable that gene transfer strategies will provide opportunities for new treatments for many patients with diseases that are both fatal and associated with excess morbidity. Although most patients with SS have a normal lifespan, patients with more severe manifestations have greater morbidity, including vasculitis and lymphoma, as well as an increased risk of mortality.²⁹ Importantly, patients with SS have considerable difficulties with chewing, swallowing, speech, Candidal infections, and other oral problems that have a deleterious impact on their quality of life.³⁰ Current treatments for SS are inadequate. At present, we consider that localised delivery of targeted genes

using rAAV serotype 2 vectors is a promising application of gene transfer for use in SS.²¹ rAAV vectors provide a high level of “relative safety”. These vectors allow long lived transgene expression in the absence of chromosomal integration, they are naturally replication incompetent, and they elicit a minimal to modest immune response. Currently, rAAV vectors in our view are the most reasonable viral vectors to use in the treatment of a chronic autoimmune disease such as SS. Additionally, human salivary glands are well encapsulated, a circumstance that should prevent the undesirable spread of vector beyond the gland. Furthermore, the use of specific salivary cell promoters in recombinant vectors should limit transgene expression to glandular parenchyma.¹⁴ Finally, salivary glands are not vital organs, necessary for survival. If a severe adverse effect occurs a salivary gland can be removed surgically. The history of biomedical research has shown that there are risks in the development of new medical treatments. However, failure to carefully explore reasonable new treatment options for patients, in the absence of suitable conventional treatments, is not appropriate.

PATHOPHYSIOLOGY AND IMMUNOLOGICAL TARGETS FOR GENE TREATMENT

The pathogenesis of SS is not fully understood at present. Thus, there are no clearly defined molecular targets for gene transfer or conventional treatments. Although the exact pathophysiology is unknown, several possible immunological mechanisms, probably operative in the tissue destruction of salivary glands, have been identified. These may provide general targets for localised immunomodulatory gene treatment in salivary glands. Table 2 shows examples of potential candidate genes.

Cytokines in relation to the microenvironment

Immune cells produce a variety of cytokines that allow them to communicate with each other and produce an extensive, but generally self limited response. Cytokines bind to cell surface receptors present on many cell types. Epithelial cells can also produce different cytokines, and thus participate in immune events (fig 2). Also, T cell subsets have been categorised by the cytokines that they produce. For example, in mice, clones of CD4⁺ T cells show divergence into T helper cell type 1 (Th1) and T helper cell type 2 (Th2) subpopulations.³¹ The Th1 cells produce cytokines such as interleukin (IL)2, interferon gamma (IFN γ), and lymphotoxin that are associated with cell mediated immunity.³² Such activities are effective in combating viruses or intercellular organisms.³³ Th2 cells produce IL4, IL5, IL6, IL10, and IL13, which stimulate humoral responses.³⁴ In humans, the divergence of CD4⁺ T cells into Th1 and Th2 subsets is less clear.^{35 36}

Several studies, using reverse transcription-polymerase chain reaction (RT-PCR) indicate that there is an increased level of classic Th1 like cytokines—notably, IL2, IFN γ , the

Table 2 Candidate targets for gene transfer

Product	Remark	Ref
Anti-CD20	Depletion of B cells	64
sTNFR	Antagonise TNF α	44, 45
IL10/vIL10	Inhibit Th1 activity, anti-inflammatory	21, 48
sCTLA-4, CD40	Inhibit costimulation lymphocytes	60, 61
Anti-BlyS	Decrease of autoreactive B cells	63

sTNFR, soluble tumour necrosis factor receptor; vIL10, viral IL10; sCTLA-4, soluble cytotoxic lymphocyte antigen-4; anti-BlyS, anti-B lymphocyte stimulation.

Figure 2 Schematic drawing of a salivary gland with a high endothelial venule, representing the lymphocyte traffic, the role of intercellular adhesion molecule-1 (ICAM-1)/leucocyte function associated antigen-1 (LFA-1), the formation of Th1- and Th2-like lymphocytes, and the key regulatory cytokines and their interactions in SS. The imbalance in Th1-like cells, producing proinflammatory cytokines, and Th2-like cells, results in local inflammation. The T-B cell interaction represents a pathway leading to autoantibody formation. This inflammatory microenvironment might result in tissue damage and disruption of secretory function. Also, note that the salivary gland epithelial cell can function as an antigen presenting cell (APC). Th1, T helper 1-like lymphocytes; Th2, T helper 2-like lymphocytes; Lymph, lymphocytes.

proinflammatory cytokines IL1 β , and TNF α , as well as an increase in IL10, in salivary tissue of patients with SS.^{4, 37–41} For example, Fox *et al* found that salivary gland epithelial cells produced over 40-fold more IL1 α , IL6, and TNF α mRNA than epithelial cells from subjects with histologically normal salivary glands.³⁹ Furthermore, these investigators found increased levels of IL1 β , IL6, IL10, TNF α , and IFN γ in the parotid saliva of patients with SS using an enzyme linked immunosorbent assay (ELISA). This suggests that the raised mRNA levels detected in minor gland tissue by RT-PCR are consistent with local cytokine protein synthesis and release. However, an important caveat to these results is that the mRNAs for all of the above cytokines, with the exception of IFN γ , have been detected in salivary tissues of healthy volunteers.^{39, 42} Interestingly, Mitsias *et al* found a positive correlation between IFN γ mRNA levels in cultured labial salivary glands and the intensity of the lymphocytic infiltration in the glands of patients with SS.⁴³ None the less, it has been suggested that IL1 α and TNF α have important roles as regulatory proteins inducing autoimmune related tissue damage (fig 2).³⁸ Indeed, patients with rheumatoid arthritis (RA) have been successfully treated by blocking TNF α , for instance with soluble TNF α receptor (etanercept).⁴⁴ Recently, an uncontrolled trial with an anti-TNF α antibody (infliximab) suggested that this was beneficial in the treatment of SS.⁴⁵

Although the roles of individual cytokines in the pathogenesis of SS still have not been clearly established, the proinflammatory cytokines probably stimulate cytotoxic T cell processes within the gland. IL10 is mainly produced by Th2 like cells,⁴⁶ and IL10 is associated with a tissue protective role because it prevents an unchecked immune response to pathogens which might cause undue tissue destruction. Likewise, it has been suggested that IL10 plays a part in peripheral tolerance and in protection against autoimmunity.⁴⁶ For example, IL10 can reverse the cartilage degradation induced by antigen stimulated mononuclear cells and inhibit proinflammatory cytokine production.⁴⁷ Furthermore, there is

evidence suggesting that IL10 has a protective role in Fas related apoptosis.⁴⁶

"IL10 may be useful locally in the management of SS"

Accordingly we have suggested that IL10 may be a general immunomodulatory factor useful locally in the management of SS.²¹ Currently we are using an rAAV vector, rAAV hIL10, for local delivery of the human hIL10 gene into the salivary glands.^{21, 48} This vector, when injected into IL10 knockout mice, prevented development of endotoxic shock subsequently induced by lipopolysaccharide.⁴⁹ This dramatic biological protection occurred although only modest levels of hIL10 were detectable in serum.⁴⁹ The expression of hIL10 in mice after salivary gland rAAV hIL10 delivery is stable for more than eight weeks, the longest time studied and improves salivary secretion.^{21, 48} Although use of this vector thus far seems promising for SS, it is tempered by remembering that IL10 can act as a potent stimulator of B cell responses.^{50, 51} IL10 induces differentiation and activation of human B cells, which subsequently secrete larger amounts of IgA, IgG, and IgM. Therefore, treatment with IL10 theoretically could drive an increase in immunoglobulin production within infiltrated salivary glands (fig 2). This is of particular concern in an autoimmune disease such as SS, which is associated with an increased risk of B cell lymphoid malignancy.⁵²

In summary, the epithelial cells in the salivary glands are active participants in the autoimmune mediated process of primary SS, as shown by their ability to express a high frequency and wide variety of cytokines. The presence of an infiltrating lymphoid focus within the gland seems to be associated with a dysregulation of normal cytokine gene expression by salivary epithelial cells.⁴² Local microenvironmental modulation of dysfunctional salivary epithelial cells with genes for specific cytokines, such as IL10, may result in therapeutic benefit.

Adhesion molecules and major histocompatibility complex (MHC) class II

Adhesion molecules expressed on the surface of immune cells transduce a variety of signals and mediate important cellular interactions by binding to specific receptors expressed on target cells or by attaching to extracellular matrix components in a site-specific manner. Aberrant expression of certain adhesion molecules (mRNA and protein level) in vivo has been described in patients with autoimmune connective tissue diseases.⁵³ For example, immunohistochemical analyses of salivary gland biopsy specimens from patients with SS show a marked expression of vascular cell adhesion molecule-1 (VCAM-1) and ICAM-1 in venules surrounded by infiltrated CD4⁺ CD45RO⁺ T cells.⁵⁴ E-selectin was expressed on vascular endothelium with weak intensity.⁵⁴ Cytokine mediated up regulation of VCAM-1 and ICAM-1, which facilitates recruitment of VLA-4 and LFA-1 expressing T cells, might contribute to lymphoid cell infiltration in the salivary and lachrymal glands in SS.⁵⁴ Interference with this process by blocking the function of adhesion molecules may thus provide a useful therapeutic approach in SS. However, there is no conclusive evidence for tissue-specific T cell entry mediated by adhesion molecules in SS.⁵⁵

T cells

Immunohistological studies show that 75% of the cells infiltrating salivary glands in SS are T lymphocytes, mainly of the helper/inducer (CD4) subset.² The presence of macrophages and B cells is less prominent.¹³ The T cells bear mostly the memory phenotype CD45RO⁺ expressing the α/β T cell receptor and the adhesion molecule LFA-1,^{13, 56} which promotes homing in the exocrine tissue. T cells within the gland also show an up regulation of bcl-2 and bcl-x_L, which would probably result in a resistance to apoptosis.^{57, 58} The expression of HLA-DR in the salivary and lachrymal gland epithelial cells allows the cells to present antigens to T helper cells. Correspondingly, activated CD4⁺ T cells can induce the production of proinflammatory factors by epithelial cells as well as by other lymphocytes.^{4, 59} Therapeutic strategies directed at T cells could include the blocking of the activated or cytotoxic T cells. This could employ, for example, soluble cytotoxic lymphocyte antigen-4 (CTLA-4), which will disrupt B7-CD28 binding, or soluble CD40, which will disrupt CD40-CD40 ligand interaction, thus inhibiting the costimulation of T cells.^{60, 61}

B cells

B cells form about 20% of the infiltrating lymphocytes in the salivary glands of patients with SS, and they are responsible for the production of autoantibody immunoglobulins, such as anti-Ro and La.¹³ Increased levels of B lymphocyte stimulator (BLyS) have been detected in the serum of patients with SS. The level of BLyS correlates with the level of autoantibodies, suggesting that BLyS may play a part in activating autoreactive B cells.⁶² Also, mice transgenic for BAFF develop a disease with SS characteristics, which is manifested by severe sialadenitis, decreased saliva production, and destruction of submaxillary glands, providing a potential model for studying this aspect of the role of autoreactive B cells in SS.⁶³ Additionally, Edwards and Cambridge described an improvement in RA after treatment with rituximab, an anti-CD20 antibody, effectively causing subtotal depletion of B cells.⁶⁴ Both anti-BLyS and anti-CD20 antibody could be used as therapeutic agents in SS (table 2).

M3 isoform of the muscarinic receptor

The M3 isoform of the muscarinic receptor is an important neurotransmitter receptor involved in salivary fluid secretion.⁶⁵⁻⁶⁷ There is a reported 100% prevalence of antimuscarinic

M3 receptor antibody (M3R) in patients with primary SS, which is also detected in patients with secondary SS.⁶⁸ The presence of these antibodies in secondary SS is not necessarily expected and may provide evidence for a common pathogenetic link between primary and secondary SS. These autoantibodies may contribute to sicca symptoms (that is, by blocking of M3 receptors on acinar cells, thus preventing activation of fluid secretion) and may explain associated features of autonomic dysfunction in some patients.⁶⁸ Direct M3 isoform receptor autoantibody mediated tissue damage might occur through nitric oxide generation and accumulation.⁶⁵⁻⁶⁷ Muscarinic receptor stimulation with pharmacological agents improves secretory function in patients with SS.⁶⁷

Apoptotic processes

Apoptotic processes could have an important role in the pathogenesis of SS. Defective apoptosis in lymphocytes could result in the accumulation of lymphoid cells in the affected tissue. Moreover, an increase in apoptosis of the exocrine gland epithelial cells might explain the loss of secretory function and the resulting exposure of intracellular autoantigens, which may evoke or enhance autoantibody production.⁶⁹ The apoptotic process includes many potential therapeutic targets, including cell surface molecules such as Fas, intracellular molecules (for example, p53, Bcl-2 family members) and enzymatic pathways involving perforins and granzyme B.^{70, 71}

There are a number of conflicting reports about the level of apoptosis in the lymphocytes of patients with SS. For example, T cells from patients with SS show accelerated apoptosis in vitro.^{57, 58} On the other hand, in situ DNA nick end labelling (TUNEL) studies have shown a low rate of apoptosis among infiltrating mononuclear cells.⁷² As noted above, increased apoptosis of ductal and acinar cells could provide an explanation for the loss of secretory function and the formation of autoantigens. However, Kong *et al* described an increase in Fas-Fas ligand expression in salivary glands of patients with SS,⁷³ while Ohlsson *et al* found only a low level of cell death among epithelial cells in patients with secondary SS.⁷² Recently Mariette *et al* showed a colocalisation of p53 and its transcription factor p21 in salivary ductal cells surrounding lymphoid foci in the minor gland biopsy specimens from 8/10 patients with SS.⁷⁴ This could indicate that ductal cells have time to repair DNA damage and thus prevent apoptosis.⁷⁴ Nagaraju *et al* reported that several generally expressed autoantigens (for example, α -fodrin, La, and nuclear mitotic apparatus protein), and tissue restricted autoantigens (for example, M3R) targeted in SS, are specifically cleaved by granzyme B, generating unique fragments and thus creating neoantigens.⁷¹ They argued that granzyme B cleavage of target molecules during apoptosis may be critical for autoantibody generation in SS.⁷¹

Overall, the status of apoptotic processes in the salivary glands of patients with SS is not clear; glandular hypofunction does not appear to result solely from glandular destruction through apoptosis. Moreover, normal acinar cells may be present in patients with SS with hyposalivation,⁷⁵ suggesting that other immunopathological mechanisms are involved.⁷⁶

Although the pathophysiological systems in SS are complex, and a precise disease mechanism is far from understood, we are employing localised gene transfer to salivary glands for two purposes: both for testing potential therapeutic efficacy and as a tool to facilitate an understanding of pathological mechanisms. With this approach it is possible to test many individual molecules, in defined models, and thus provide useful information on the possible role of these molecules in SS, even if they are not useful for treatment (table 2).

Table 3 Comparison of reported features in animal models of SS with the human disease

Strain	Clinical feature		Histological*		Salivary	Associated features	Age of detection	Ratios female: male	Reference
	KCS	Xerostomia	Serological markers	Lachrymal					
Human	+	+	Hypergamma-globulinaemia, ANA, SSA/SSB, RF	+	+	Secondary: SLE, RA, scleroderma, glomerulonephritis	>50 Years	9:1	1, 13
NOD	+	+	Anti-M3, SSA/SSB	+	+	IDDM	8 Weeks	F>M	89, 90
NOD.B10.H2b	-	-	ND	+	+	SCID, IDDM, glomerulonephritis	8-12 Weeks	ND	93
NOD-SCID	-	-	ND	-	-	Arthritis, vasculitis, lymphadenopathy as above, etc	8-10 Weeks	ND	94
MRL/lpr	-	-	SSA/SSB	+	+	Fulminant, chronic	?	F>M	79, 84
MRL+/-lpr	-	-	SSA/SSB	ND	+	Glomerulonephritis, lupus less fulminant	?	F>M	81
MRL/+	-	-	SSA/SSB	+	+	Glomerulonephritis, lupus	?	F>M	81
NZB	+	-	-	+	+	Glomerulonephritis, more severe	16 Weeks	1:1	77
NZB/NZW	+	-	-	+	+	ND	16 Weeks	1:1	77
NFS/sld	-	-	Ab against ssDNA, anti-salivary duct Ab	+	+		4-8 Weeks	Lesions F>M	86, 87

NOD, non-obese diabetic; NOD.B10.H2b, substrain of NOD which lacks insulitis; MRL/lpr, MRL+/-lpr, different mouse substrains developed from crosses involving a number of standard inbred strains (AKR/J, C57BL/6J, C3H/Di, and LG/J); a mutation; lpr (lymphoproliferation) was found in the 12th generation of brother-sister matings. NFS/sld, NSF mice carrying mutant sld gene, thymectomised three days after birth; IDDM, insulin dependent diabetes mellitus; SLE, systemic lupus erythematosus; RA, rheumatoid arthritis; SCID, severe combined immunodeficiency; ND, not done; Ab, antibody; KCS, keratoconjunctivitis sicca.
*Cellular lymphocytic infiltrate and tissue damage.

ANIMAL MODELS FOR SJÖGREN'S SYNDROME

To develop any new treatment for a disease a valid preclinical animal model is critical. The ideal animal model for SS would fulfil all of the known features of human SS, including clinical characteristics of dry eyes and dry mouth, lymphocytic cell infiltration of lachrymal and salivary glands with preferential destruction of acinar epithelium, and characteristic serological markers. Moreover, a useful animal model creates the opportunity to study early stages of the disease. A variety of animal models for SS have been used. However, none of the current models has all desired characteristics of SS. Table 3 provides a brief comparison of the most commonly used animal models of SS and their features in relation to human SS. These models are discussed in more detail below.

NZB and NZB/NZW mice

Kessler first described spontaneous histopathological changes in the NZB and NZB/NZW mouse strains, which were similar to SS.⁷⁷ Mononuclear cell infiltrates were noted in the lachrymal and to a lesser extent in the salivary glands. Sialochemical and modified Schirmer tests on these animals showed, however, that salivary gland pathological sequelae were present in only a small number of animals.⁷⁸ Consequently, there has been little additional immunological characterisation of the exocrine tissues in these mice.

MRL/lpr mice

Use of the MRL mouse and substrains as a model for SS has been described by Jonsson.⁷⁹ Hayashi *et al* have also analysed local cytokine expression production in MRL/lpr mice.⁸⁰ These mice exhibit an overrepresentation of T cells bearing CD4 and V (β) 8 molecules, which suggests chronic antigenic stimulation in their salivary glands.⁸¹ In addition, there is a local expression of the proinflammatory cytokine genes, IFNγ and IL12 (p40). This suggests that there may be a pathogenic trigger in the epithelial cells. In particular, a high level of local expression of IL12 mRNA was detected earlier in the proinflammatory stage of autoimmune lesions.⁸¹ In MRL/lpr mice, overexpression of IL1β and TNFα was detected before the onset of inflammatory lesions in the salivary gland, and the up regulation of IL6 mRNA was also found in the autoimmune sialadenitis occurring in MRL/lpr mice.⁸²

NZB, NZB/NZW and MRL/lpr mice, of which there are different congenic strains, were originally bred to study autoimmunity in general, and have also been employed as models for systemic lupus erythematosus,⁸³ RA,⁸⁴ and scleroderma.⁸⁵ Although they share a number of common immunological and histopathological features, all of these mice lack the critical feature of the loss of secretory function by the exocrine glands (table 3).

NFS/sld mice

The NFS/sld mice exhibit a sublingual gland differentiation arrest and show significant inflammatory (T cell infiltrates) changes, which after neonatal thymectomy develop in both the salivary and lachrymal glands.⁸⁶ These T cell infiltrates are reactive to α-fodrin (120 kDa), a product of fodrin cleavage by caspase. Fodrin is a 250 kDa membrane associated cytoskeletal protein, found in many tissues. Sera from patients with SS react positively with purified α-fodrin (120 kDa) and recombinant human α-fodrin protein.^{86, 87} It remains unclear whether production of antibodies against α-fodrin is a primary event in SS, as these antibodies are also found in other autoimmune diseases.⁸⁸ These mice have not been widely studied, and are not readily available or easily used. They also do not appear to exhibit salivary hypofunction.

Non-obese diabetic (NOD) mice

With increasing age, NOD mice develop, in addition to insulin dependent diabetes mellitus, sex (female) specific histopathological changes in the salivary glands similar to those of human patients with SS.⁸⁹⁻⁹⁰ Perhaps more importantly, they exhibit the critical clinical manifestation of declining exocrine tissue secretory function, that is, salivary hypofunction (table 3). Similar to findings in patients with SS, mRNA transcripts for IL1 β , IL2, IFN γ , TNF α , IL10, as well as minimal expression of IL4, occur in NOD mice.⁹¹ Within the lymphocytic infiltrates the most intense immunohistochemical staining is for TNF α , IL2, and IFN γ .⁹¹ This suggests that these cytokines may be involved in parenchymal damage, as is suspected in patients with SS.

In NOD mice, the unique expression of MHC I-A(g7) is crucial for the development of diabetes.⁹² Robinson *et al* developed a strain termed NOD.B10.H2b, which has an MHC congenic to NOD (table 3).⁹³ These mice exhibit lymphocytic infiltrates in the exocrine tissues, typical of SS-like disease as is seen in NOD mice, but they lack the insulinitis and diabetes.⁹² This implies that the insulinitis and sialadenitis are not genetically linked in NOD mice.

Studies that have focused on the genetic profile of the NOD mouse, have suggested that both "immune" and "non-immune" related genes are involved in the pathogenesis. The immunodeficient NOD mouse—that is, carrying the severe combined immunodeficiency (SCID) genetic profile, lacks lymphocytes almost completely. NOD-SCID mice provide the strongest evidence for the role of "non-immunogenic" genes in disease pathogenesis in this model.⁹⁴ The origin of this abnormality probably resides in the salivary glands, although the mechanism is unclear. Recently, Van Blokland *et al* have argued that apoptosis was not a major factor in the onset of the sialadenitis (table 3).⁹⁵

Overall, of the common animal models available, the NOD mouse seems to be the most useful for studying the pathogenesis of SS.⁸⁹⁻⁹¹ Although not ideal, it features certain key immunological and clinical characteristics of SS.

CONCLUDING REMARKS

Immunomodulation is the first line of treatment for many autoimmune diseases. Because we do not know the exact cause of SS, the use of immunomodulatory treatments (genetic or conventional) must proceed cautiously. Ideally, immunological treatment is based on evidence that the chosen target plays a key part in the disease activity. In SS there is as yet no clear cut target for gene based or conventional treatment. Furthermore, in SS, the exocrine inflammation often occurs without prominent systemic manifestations, implying that local administration of immunomodulatory agents would be useful and possibly desirable. Although the clinical symptoms of SS can be highly debilitating, the systemic morbidity is generally mild, and mortality may not be significantly increased in patients with primary SS, except in a subset of these patients.⁹⁶

As noted above, many factors, including cytokines, and MHC class II expression and adhesion molecules can serve as potential immunoregulatory targets. The production of proinflammatory cytokines by epithelial cells as well as lymphocytes is one of the most frequently described features in autoimmune diseases. Thus, effective treatment might be developed for SS by locally introducing genes encoding cytokines, which are potentially anti-inflammatory, such as IL10, or by introducing a cDNA encoding a soluble form of a key cytokine receptor, which has the ability to function as an antagonist and decrease the availability of certain cytokines, such as soluble TNF α receptors.

We currently think that rAAV is the preferred viral vector for immunomodulatory gene transfer in salivary glands

(table 1) as it results in stable levels of transduction, and evokes a relatively low immune response. In the absence of clear targets we also propose to use gene transfer both to help elucidate the pathophysiology and to alter the course of the SS-like disease in the NOD mouse model. We are optimistic that this approach may ultimately lead to the development of new treatments for managing the exocrine pathology in patients with SS.

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