Local immunomodulatory gene therapy for Sjögren’s syndrome
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CHAPTER 1

General Introduction

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

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Sjögren’s syndrome

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

The autoimmune exocrinopathy is thought to develop in two separate phases in genetically predisposed individuals. An unknown environmental stimulus (e.g., a 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 characterized 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 further epithelial cell death and loss of secretory function.\(^2\,^4\,^5\) In addition, dysfunction of residual glandular epithelial cells can occur indirectly possibly due to the effects of cytokines, autoantibodies (e.g., anti-muscarinic receptor antibodies) or parasympathetic nervous system dysfunction in SS patients.\(^6\,^7\)

Although the exact immunopathogenesis has as yet not been elucidated, SS is characterized 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 in SS patients.\(^8\) Additionally, the pattern of cytokine abnormalities is variable in different studies and is not sufficient to distinguish SS patients from controls.\(^2\,^9\,^10\) For example, Pertovára et al. found a T helper (Th) 2 cytokine profile to be associated with signs of a milder form of primary SS\(^11\), and in line with this observation Mitsias et al. detected Th2 cytokines in human labial salivary glands of SS patients when there was only low-grade infiltration. A Th1 cytokine pattern was associated with a later disease stage (definite SS) and advanced lymphocytic infiltration.\(^12\) However, the cytokine profile in submandibular gland biopsies with simultaneous expression of interferon-γ (IFN-γ), interleukin-2 (IL-2), IL-4 and IL-13 provides an argument against a simple Th1 or Th2 predominance in SS patients.\(^9\) It is important to recognize that the division of T-helper lymphocytes into Th1 and Th2 subgroups is eminent in animals, but in humans
Interestingly, recent studies showing an increased expression of interferon and associated genes in minor salivary glands of primary SS patients support the possible interaction between the innate and adaptive immune system in SS pathogenesis.\textsuperscript{13,14} In addition, an antigen-driven, germinal center-type B cell response has been suggested to take place in the salivary glands of SS patients.\textsuperscript{15} These B cells represent a unique, highly selected and differentiated B cell population, suggesting a key role of the target organ in recruitment of inflammatory cells, lymphoid neogenesis and propagation of the disease process.\textsuperscript{9} The B-cell-activating factor (BAFF), or B lymphocyte stimulator (Blys), induced by IFN and a member of the tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) superfamily, likely plays an important role. Dysregulated BAFF expression has been proposed to lead to disease progression, perpetuation of humoral autoimmunity,\textsuperscript{16-18} and the formation of germinal centers in SS.\textsuperscript{18} Moreover, BAFF has been implicated in the development of B cell malignancies.\textsuperscript{18}

Gene transfer methods
Treating a chronic autoimmune disease such as SS 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,\textsuperscript{19} although existing gene transfer vectors are imperfect. As yet, no clinical gene transfer studies have been conducted for SS. All studies described herein are from SS animal models.

Several gene delivery systems are currently available. Recombinant viral vectors are now particularly useful for their efficiency in mediating gene transfer to targeted cells.\textsuperscript{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.\textsuperscript{20} For example, the non-enveloped adenovirus (Ad) conveys robust transgene expression, but this is short-lived (few weeks) due to a potent immune response.\textsuperscript{21} The single-stranded RNA retrovirus integrates in the host genome and is thus capable of mediating extremely long-term transgene expression. However, because it is currently only possible to generate low titers of retroviral vectors their use is typically limited to \textit{ex vivo} applications.\textsuperscript{22} Furthermore, retroviral vectors pose a major safety concern: insertional mutagenesis, as has recently been reported in a severe combined immunodeficiency trial.\textsuperscript{23} Non-viral methods, such as plasmid-cationic liposome mixtures, DNA-protein conjugates, and naked DNA,\textsuperscript{20} generally pose less of a safety risk than viral vectors, but suffer from low transduction efficiency and very short periods
(days) of transgenic protein expression. 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 numerous mammalian cells, dividing as well as non-dividing, and elicit a minimal immune response. Consequently, transgene expression from rAAV2 vectors, while often more modest than that seen with Ad vectors, is very stable and lasts years, and rAAV2 vectors have proven to be useful in animal model studies of SS.

Content of this Thesis

Part I. Gene Therapy Vectors

Chapter 2 describes the advances made in vector-mediated gene transfer, thereby focusing on salivary glands and SS. Ads offer a strong, but rather short-lived protein expression, as described above. They have merely a place in proof-of-concept experiments. In Chapter 3 the development of such an Ad vector is outlined. It is the first time a viral vector encoding vasoactive intestinal peptide (VIP) has been successfully produced and tested.

Part II. Gene Therapy in an Animal Model of Sjögren's Syndrome

SS patients could potentially benefit from immunomodulatory gene therapy. In addition, this form of treatment could offer more insight into the complex and largely unknown pathogenesis of SS. The non-obese diabetic (NOD) mouse is the most useful, commonly available animal model to examine the SS disease characteristics. It develops, besides type I, insulin-dependent diabetes mellitus (IDDM), exocrine gland infiltrates and decreased glandular secretion, which are age and gender dependent. An rAAV2 encoding human IL-10 (hIL-10) was tested in the female NOD mouse, as described in Chapter 4. Mice were administered rAAV2hIL-10 or a control virus expressing β-galactosidase (rAAV2LacZ) before or after disease onset. SS, as well as IDDM parameters, were monitored and compared. The next two chapters illustrate the testing of VIP (Chapter 5) and an NF-κB inhibitor, IκBα(sr) (Chapter 6), in a comparable setting, only with administration before onset of disease.

The most commonly used animal model to study SS uses the NOD mouse. It has been known that the IDDM phenotype is not stably expressed and in Chapter 7 several experimental studies are compared to evaluate the expression of the SS phenotype in this mouse.
In the concluding **Chapter 8** a summary of several of the preceding chapters is depicted. Additionally, the lessons learned from the use of gene therapeutics and biological agents for the exocrine pathogenesis of SS are summarized and, based on this information, a potentially optimal treatment for SS is given.

**Questions in this thesis**

1. Can the course of events in SS be altered by gene therapy with immunomodulatory genes?
2. Is the administration of rAAV2hIL-10 after the onset of disease as effective as administration before onset?
3. Are the purported roles of VIP (immunomodulatory, secretory, trophic stimulatory) useful in the management of SS?
4. Does rAAV2IkBa(sr) alter disease markers in a mouse model of SS?
5. How stable is the SS phenotype in the NOD mouse model?
6. What lessons can be learned about the exocrine pathogenesis of SS from gene and biological therapeutics?

**References**