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

Sjögren’s syndrome

Sjögren’s syndrome (SS) is a common autoimmune disease. It affects many organs in the body, including and foremost the secreting glands such as the lachrymal and salivary glands. The diagnosis is based on subjective symptoms and objective measurements of the function of the lachrymal and salivary glands and the presence of signs of inflammation. The disease is nine times more common in women than in men and manifests itself mainly in the menopausal age. Patients suffer from (often severe) dry eyes and dry mouth leading to increased risk of infections, dyscomfort and pain in these areas. Furthermore, patients may also have signs of vascular inflammation (vasculitis), joint pain and can experience extreme fatigue. The cause of SS, as in many autoimmune diseases, remains unknown. There is currently no universally effective therapy and patients have to rely on symptomatic treatment. One of the main reasons for the lack of a good remedy is the limited existing knowledge on the pathogenesis and disease progression.

Inflammation in SS; a disordered system

One of the hallmarks of SS is inflammation of the salivary gland characterized by the influx of immune cells and their clustered organization. The recruitment into the salivary gland and the development, survival and function of these immune cells is in part controlled and influenced by cytokines, protein messengers that can inhibit, stimulate and induce other cytokines and that can pass signals to other cells. In the salivary gland, blood and saliva of patients with SS, differences in the presence of these cytokines can be detected when compared with healthy volunteers. Moreover, specific differences can be found with people who have a dry mouth and eyes, but not SS (sicca patients). A number of therapies based on normalizing the cytokine balance—such as anti-TNF therapy, of which some work well in other related autoimmune diseases such as rheumatoid arthritis—were not effective in the treatment of SS. Chapter 2 in this thesis describes the dysregulated cytokine network and the cytokine-based therapeutic studies in humans that mostly failed.

Gene therapy in SS

The reason why the described cytokine targeted therapies do not work is only partially understood. It is possible that the drug did not penetrate the most affected organs and/or prolonged therapy may be needed to elicit a substantial beneficial effect. Gene therapy, in particular local gene therapy in the salivary gland, could overcome these problems. Through the introduction of certain genes in the salivary gland, proteins can be produced and secreted by the salivary gland cells which can then influence the local inflammatory environment. So-called adeno-associated viral viruses (AAV’s) can infect epithelial cells of the salivary gland; recombinant AAVs can not multiply and are not pathogenic. These viruses can be made to carry genes that encode for proteins that can affect the inflammatory milieu. When introduced into the salivary gland, local, long-term and stable production of these therapeutic proteins can be achieved.
Chapter 3 discusses in detail the possibilities and limitations of gene therapy in SS and proposes a number of candidate targets that can be addressed. These targets include cytokines, adhesion molecules, and the co-stimulatory pathway.

Identifying targets for the treatment of SS; studies in mice
Some of the targets proposed in chapter 3 were studied in the non-obese diabetic (NOD) mouse. The NOD mouse spontaneously develops a large number of immune-mediated disorders in life, of which diabetes is most well known. Besides diabetes it also develops a syndrome that resembles human SS; with age these mice develop inflammation of the salivary and lacrimal glands. In addition, salivary flow may decrease with age, predominantly in female mice. Chapter 4 describes a number of observations in this spontaneous course of the disease in the NOD. We investigated in detail the inflammation of the salivary gland and found that macrophages are infiltrated in the gland before other immune cells enter and possibly, in cooperation with the epithelial cells, produce cytokines that attract other inflammatory cells. In addition, we also found that the adhesion molecule intercellular adhesion molecule type 1 (ICAM-1) is strongly present before infiltration of cells. ICAM-1 aids immune cells to exit the bloodstream and is involved in co-stimulatory pathways, crucial for reciprocal activation of B and T cells. CD40, also involved in co-stimulatory pathways was found to be upregulated in focal infiltrates (foci) but was not detected prior to the formation of foci. The growth factor B cell activating factor, BAFF, which is important for the development and survival of subsets of B lymphocytes, is strikingly present in the epithelial cells of the ductal structures of the salivary gland. In late stage disease, BAFF production significantly increases in these epithelial cells suggesting an important role for this growth factor at the more advanced phase of inflammation. These data show that autoimmune inflammation in the NOD mice is a multi-step process directed by many different players at different stages of the disease. Understanding these stages and the individual key players important in each of these stages is crucial for the discovery of an effective therapy in mice and may guide us in the development of a future therapy in humans.

Based on the immunological aberrations found in the salivary glands of NOD mice, gene therapeutical approaches aimed at these abnormalities were tested and described in chapter 5, 6 and 7. AAVs were used to deliver specific inhibitors/modulators to the salivary glands and their effect on inflammation and salivary gland function was determined. In chapter 5, an AAV was constructed encoding soluble ICAM-1 (sICAM-1). Expression of the soluble molecule competes with endogenous cell bound ICAM-1 for binding with its ligands and should therefore inhibit cell-adhesion and co-stimulation. When sICAM-1 was administered before focal inflammation was present in the salivary gland, less inflammation was observed 12 weeks later. However, if the AAV vector was given when focal inflammation was present, sICAM-1 did not positively affect the disease and the number of T cells in the salivary gland was increased. This indicates that early intervention with the ICAM-1-ligand binding can positively affect disease outcome, but late treatment may worsen the disease and increase inflammatory T cells in the glands. In chapter 6, CD40, also involved in the co-stimulatory pathway,
was expressed in a soluble form at different disease stages. Although the treatment resulted in changes in mRNA profiles related to inflammation, no effect was seen on focal inflammation or on salivary gland function. This study suggests that CD40 is not a good target in the treatment of SS. In chapter 7 gene therapy with an AAV-vector encoding for the transmembrane and CAML interactor receptor (TACI) of BAFF and a proliferation inducing ligand (APRIL), two cytokines involved in B cell stimulation and survival, is described. The soluble form of this receptor should scavenge these cytokines and prevent it from binding to the endogenous receptor on cells. This therapy reduced inflammation in the salivary gland based on B cell and immunoglobulin (type IgG) reduction. This data suggests that the use of soluble TACI may be effective in the treatment for SS. Taken together, these three studies indicate that timing and choosing the correct target are of considerable importance in the treatment of SS-like disease in NOD mice. This will likely also be the case in humans.

Novel therapeutic targets in patients with SS

In chapter 8 we made the transition from mice to humans. The salivary glands and peripheral blood of patients with SS, those with complaints of dry mouth and eyes but without SS (sicca patients) and healthy individuals were studied. We showed how they differ in the presence and distribution of two key inflammatory related cytokines, BAFF, previously also described in the NOD mouse, and APRIL. We found that APRIL is expressed at lower levels in the salivary glands of SS patients compared with healthy volunteers and sicca patients. At the same time some patients had strongly increased APRIL levels in the peripheral blood. BAFF was detected in the ductal cells and in infiltrating cells which confirms previous observations by other researchers. The receptors to which these two cytokines bind were also present in varying degrees when compared between patients and healthy people. These abnormalities indicate that a therapy inhibiting BAFF in the salivary gland may be beneficial, but local inhibition of APRIL may not be effective in the treatment of SS.

Future directions and considerations

Our proof-of-principle studies show that gene therapy can be effective for the treatment of the inflammatory component of SS in NOD mice. However, none of the gene therapy studies in NOD mice increased salivary flow. This is a major concern, since dryness is an important and very debilitating component of SS. Therefore, the quest for an ideal target in the treatment of SS has not ended with this thesis. It is possible that a combination of therapies could lead to better results including improved salivary gland function. In addition, other approaches besides the use of a soluble receptor needs to be explored. The silencing of genes in the cell resulting in decreased production of proteins such as receptors, cytokines and chemokines should also be further investigated. In addition the overexpression of cytokines that are naturally low in SS and have anti-inflammatory properties should also be explored in the future. The application of AAV-vectors is not limited to the expression of soluble receptors (as in this study) and could be used for these purposes. In parallel, more research is needed in patients with SS aimed at the identification of novel targets, and to provide more insight into the natural progression
of the disease. Attempts should be made to identify different natural disease stages, since certain therapies may be beneficial at an early phase but could be detrimental at a more progressed disease stage.

Conclusion
The research presented herein summarizes the current knowledge on cytokines and their presence in SS, it identifies immunological targets in mice with an SS-like disease and shows that local gene therapy can be successful for the treatment of the inflammatory salivary gland component of a SS-like disorder in mice. The chance for success of this approach depends on proper timing and should be aimed at the right target.