1

GENERAL INTRODUCTION

Partly based on:
INTRODUCTION TO PRIMARY SJÖGREN’S SYNDROME

Primary Sjögren’s syndrome (pSS) is a chronic, systemic autoimmune disease, primarily affecting the salivary and lacrimal glands [1]. Inflammation of the glands is accompanied by sicca symptoms, including a sensation of dry eyes (keratoconjunctivitis sicca) and dry mouth (xerostomia). Predominantly women are affected by the disease, and the prevalence of diagnosed pSS is estimated at 0.04% of the general population [2]. This prevalence is likely an underestimation, as misdiagnosis of patients with pSS is common due to the large diversity in initial clinical manifestations. In addition to sicca symptoms, more than 50% of patients experience extraglandular symptoms, including chronic fatigue, arthralgia and Raynaud’s phenomenon. Extraglandular manifestations may also involve the lungs, skin, kidneys and nervous systems [1]. Current treatment options for pSS are only symptomatic. In the past years, several immunomodulatory treatment approaches for pSS were evaluated, but none of them have been approved yet. Thus, there is an unmet need for treatment options that can halt or cure this disease.

Pathophysiology of pSS

The lack of efficacy of treatment modalities so far may be explained by the fact that the pathophysiology of pSS is multi-facetted and not completely understood. Both environmental and genetic factors probably contribute to disease initiation, and the few gene polymorphisms that have been associated with pSS are related to components of both innate and adaptive immune systems [3]. In particular genes that are involved in the NF-kB pathway, the interferon (IFN) signaling pathway, lymphocyte signaling, and antigen presentation have been associated with SS [4]. The strongest risk loci were found in the HLA region, with the top variants residing in the HLA-DR and HLA-DQ regions. Outside the HLA region, the strongest association was found at the Interferon Regulatory Factor 5 (IRF5) gene locus [3]. This gene is involved in IFN signaling and B cell differentiation towards plasma cells (reviewed by [4]). The involvement of IFN signaling in pSS pathogenesis is further reflected by the presence of a type I IFN signature (i.e., overexpression of type I IFN inducible genes) in 55-60% of the patients [5,6]. This signature is associated with higher systemic disease activity, higher levels of autoantibodies, and higher transcript levels of B cell activating factor (BAFF) in monocytes [5]. Also, stimulation of cultured salivary gland epithelial cells with IFN resulted in upregulation of BAFF expression by epithelial cells [7]. IFN-induced BAFF expression may link innate and adaptive immune activation in pSS.
**Histopathology**

A hallmark of pSS is focal, periductal infiltration of T lymphocytes and B lymphocytes in salivary and lacrimal gland tissues, accompanied by loss of glandular architecture and function [8]. Lymphocytes can even infiltrate the epithelium and together with proliferative metaplastic epithelial cells form characteristic lymphoepithelial lesions (LELs), which are most pronounced in the parotid gland [9]. Furthermore, periductal infiltrates can become organized in lymphoid tissue with segregated T and B cell areas and high endothelial venules. In approximately 25% of pSS patients, germinal centers (GCs) arise within this tertiary (ectopic) lymphoid tissue [10]. GCs facilitate local generation of (auto)antibody-producing plasma cells and memory B cells [11].

The periductal localization of the infiltrates illustrates the importance of the epithelium in the disease process. This epithelium is not only target of the disease but also exerts important immunological functions including cytokine production and antigen presentation [12]. In addition to periductal infiltration of the target tissue and LEL formation, a shift in the plasma cell compartment is a third histological hallmark of pSS. This shift is mostly in favor of IgG-expressing plasma cells. Increased numbers of salivary gland IgG-producing plasma cells likely contribute to the circulating levels of autoantibodies in pSS patients [13].

**Extraglandular manifestations**

Extraglandular manifestations of pSS can be differentiated in peri-epithelial or immune complex-mediated manifestations. Examples of peri-epithelial manifestations are interstitial nephritis and obstructive bronchiolitis. Cutaneous vasculitis, peripheral neuropathy, and glomerulonephritis are examples of immune complex-mediated manifestations [14,15]. Patients with peri-epithelial manifestations usually have a more stable disease than patients with immune complex mediated-manifestations. In addition, hematologic abnormalities, such as leucopenia (including lymphopenia), anemia and thrombocytopenia, are common in pSS patients [16,17]. Patients with pSS also have a 5- to 16-fold increased risk for the development of malignant B cell lymphoma (reviewed by [18]). Eventually, 5-10% of patients develop a lymphoma in the salivary glands, particularly of the mucosa-associated lymphoid tissue (MALT) type [19,20]. These lymphomas may well reflect the characteristic B cell hyperactivity seen in these patients (see below).

The systemic activity of pSS is strongly associated with several serologic abnormalities, including low C4 levels, hypergammaglobulinemia, cryoglobulinemia and higher levels of rheumatoid factor and anti-SS-A(Ro)/SS-B(La) autoantibodies [17,21]. These serologic signs may therefore predict the evolution of extraglandular symptoms and identify patients in need for systemic treatment.
**T cell-dependent B cell hyperactivity**

Increased levels of autoantibodies, together with the presence of hypergammaglobulinemia and cryoglobulins, reflect the ongoing T cell-dependent B cell hyperactivity in pSS patients. An important cytokine axis that appears to be involved in this T cell-dependent B cell hyperactivity is the IL-6/IL-21 axis. IL-6 is overexpressed in saliva, tears and minor salivary glands of pSS patients (reviewed by [22]). One of the many effector functions of IL-6 is direct and/or indirect stimulation of B cell proliferation and differentiation into plasma cells (reviewed by [23]). Indirect stimulation of B cells by IL-6 is mediated via differentiation of naïve CD4+ T cells into T follicular helper (Tfh) cells and induction of IL-21 production by these cells in response to IL-6 [24]. IL-21 is a potent inducer of plasma cell formation and is involved in GC B cell selection [25,26]. Therefore, the IL-6/IL-21 axis is thought to play a pivotal role in B cell activation in pSS patients. Furthermore, together with TGF-β, IL-6 may contribute to immunopathology by the induction of Th17 cell differentiation [27].

Despite the notion that B cell hyperactivity is a central event in the disease process, their specific pathogenic role remains controversial. Current evidence indicates that this role goes beyond autoantibody production [28], as antigen presentation and cytokine production by B cells might be significantly involved in pathogenesis of pSS. Activity of the IL-6/IL-21 cytokine axis and subsequent stimulation of both antibody-dependent and antibody-independent B cell functions may result in a pro-inflammatory amplification loop, which enhances infiltration of lymphocytes and non-lymphoid mononuclear cells to the target tissues of pSS patients. Together with the autoreactivity, apoptosis and possibly also intrinsic defects of the glandular epithelium, the inflammation contributes to dysfunction or even destruction of the exocrine glands and other tissues and finally in worsening of the clinical symptomatology. Expanding knowledge of the various cell types and mediators involved in immunopathology of pSS has opened new ways for the development of selective treatment modalities in pSS. Vice versa, application of the newly developed treatment modalities may help to understand the pathogenesis of the disease.

**Treatment of pSS**

*Conventional synthetic immunomodulatory drugs in pSS*

The use of conventional immunomodulatory drugs in pSS is largely extrapolated from its effectiveness in other autoimmune diseases, such as rheumatoid arthritis (RA) or systemic lupus erythematosus (SLE). Examples of frequently prescribed ‘off-label’ drugs in pSS are prednisone, hydroxychloroquine, and azathioprine. Prednisone is a synthetic corticosteroid with broad immunosuppressive effects. In pSS patients, low-dose prednisone is used for the treatment of arthritis and cutaneous symptoms,
based on clinical experience. High-dose prednisone is used to treat severe systemic manifestations of pSS. Evidence regarding the use of prednisone for the treatment of pSS is limited, as trials were small and specifically designed to assess the effect on sicca features. Prednisone seems to improve hypergammaglobulinemia and lymphopenia [29], which are common biological abnormalities in pSS patients, associated with systemic disease activity [17,21].

Hydroxychloroquine (HCQ) is a disease-modifying antirheumatic drug (DMARD) that suppresses endosomal activation of Toll-like receptor (TLR)7 and TLR9 [30,31]. Consequently, HCQ impairs innate immune responses, including pro-inflammatory cytokine (e.g. interferon) production. In pSS patients, HCQ is used for the treatment of articular and skin involvement based on the efficacy observed in SLE and RA. However, two placebo-controlled trials did not show a clinical benefit of HCQ in pSS patients [32,33]. The suggested application of HCQ for treatment of articular involvement in pSS was not confirmed by these trials. Too few patients with skin involvement were included to draw any conclusion concerning this manifestation. No clear benefit has been demonstrated of other conventional immunomodulatory drugs that were evaluated in pSS patients (reviewed by [34]). These drugs include azathioprine, methotrexate and mycophenolic acid. Treatment with leflunomide showed only modest clinical efficacy in a phase II open-label study, but did ameliorate leucocytoclastic vasculitis in three pSS patients [35]. The high rate of adverse events reported for many of these conventional immunomodulatory drugs raises concerns about whether they should be prescribed off-label in pSS.

**Biologic immunomodulatory agents in pSS**

Anti-TNF agents were the first biologic drugs evaluated in pSS patients. Unexpectedly, at that time, these biologicals did not show efficacy [36,37]. In 2005, rituximab, a chimeric anti-CD20 monoclonal antibody, was introduced in pSS for the treatment of pSS patients with MALT lymphoma. Thereafter, several open-label and placebo-controlled trials followed to evaluate the efficacy of rituximab in the treatment of pSS, which has been a hotly debated issue ever since [38]. In addition to B cell depletion therapy with rituximab, other potential drugs for pSS that target B cells directly are anti-CD22 antibodies (e.g. epratuzumab), anti-CD40 antibodies (e.g. CFZ533), and antibodies that bind to the BAFF receptor (e.g. VAY736). BAFF signaling is involved in survival, activation and differentiation of B cells (reviewed by [39]). Next to B cells, additional targets for treatment have emerged as a result of advanced understanding of pSS pathogenesis. None of these biologicals have yet been approved for the treatment of pSS.

Given the recognized role for T cells in pSS pathogenesis, targeting of these cells is also considered to be a rational therapeutic option in pSS. Abatacept is a fully human
fusion molecule of IgG-Fc and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4). Abatacept prevents the co-stimulatory interaction between antigen-presenting cells, including B cells, and T cells. Selective co-stimulation modulation by abatacept is presumed to inhibit full T cell activation and T cell-dependent B cell activation. Additionally, other strategies that block co-stimulatory pathways (e.g. anti-CD40) or cytokine binding (e.g. anti-IL-6R) are currently being evaluated in pSS. Targeted synthetic DMARDs that inhibit intracellular BCR- or cytokine receptor signaling, such as Bruton’s tyrosine kinase (BTK) inhibitors, phosphoinositide 3-kinase (PI3K) inhibitors and Janus kinase (JAK) inhibitors, have become available as well (illustrated in Figure 1). In summary, many possible treatment options for pSS are underway and have the potential to halt disease progression and ameliorate symptoms. However, to realize patient-tailored treatment we need additional biomarkers that can predict (progression of) systemic disease activity and treatment response.

**FIGURE 1 | Targets for systemic treatment with biologic agents in primary Sjögren’s syndrome (pSS).** Different aspects of the inflammatory response in target tissues of pSS are shown. The pathogenic role of epithelial cells in the disease process is reflected by infiltration of mononuclear cells in epithelial tissues. Furthermore, epithelial cells secrete inflammatory proteins, such as type I IFNs, BAFF, IL-6 and chemokines. There is a subsequent migration of various cell types to the tissue and then all elements to carry out (auto-) immune responses are in place. Key targets for treatment and biologic agents that have been investigated are illustrated. APRIL: A proliferation-inducing ligand; BAFF: B cell activating factor; DC: Dendritic cell; PC: Plasma cell; pDC: Plasmacytoid dendritic cell; Th-cell: T-helper cell; TLR: Toll-like receptor.
AIM OF THIS THESIS

The aim of this thesis was to assess the role of T cell-dependent B cell hyperactivity in pSS, both as a biomarker of disease (activity) and as a target for treatment. Biomarkers in blood and tissue were studied in a variety of patient cohorts, and effects of immunomodulatory therapies on the immune system of treated patients were evaluated.

In the studies described in part one of this thesis, we investigated the relevance of several T cell and B cell-related biomarkers of pSS, and discussed their role in disease initiation, clinical manifestation, and/or disease progression. In chapter 2 we reviewed the role of Th17 cells in pSS pathogenesis, also in relation to their plasticity, i.e. ability to adapt different effector functions. In the study described in chapter 3a we show that in addition to the elevated frequencies of Tfh cells, the ratio between Tfh cells and T follicular regulatory (Tfr) cells is altered in pSS patients, already at the time of diagnosis. Furthermore, we show that Tfr cells from pSS patients express lower levels of CTLA-4, a receptor involved in immune suppression. These alterations may have important implications for establishment of B cell hyperactivity. In the study described in chapter 3b, we evaluated the potential role of the Tfr/Tfh ratio and frequency of activated Tfh cells in blood as biomarkers of pSS. In chapter 4 we moved to the B cell side and revealed the phenotype and gene expression profile of FcRL4+ B cells, a subtype of mucosa-associated B cells. These cells were found in close association with the ductal epithelium in the inflamed salivary glands, and are thought to be the cell type from which MALT lymphomas arise. Our findings help to elucidate their role in pSS pathogenesis. In the study described in chapter 5 the pathological role of B cells was assessed from a more clinical perspective. Possible applications of serum immunoglobulin free light chains (FLC) as biomarkers of MALT lymphoma and systemic disease activity in pSS are shown. We also provide evidence for a role of FLCs as biomarkers of treatment response, making the study described in this chapter a stepping stone to the second part of this thesis.

In part two of this thesis we focused on the effect of immunomodulatory drugs on the immune system of treated pSS patients. We went back from bedside to bench to study which cell types and cytokines were affected by treatment and were important for treatment response. In the study described in chapter 6 we show that the effects of B cell depletion therapy with rituximab are not restricted to B cells, but that this treatment also significantly affects the T cell compartment, in particular Tfh cells. Chapter 7 provides an overview of available data on the efficacy of rituximab in pSS, including clinical and biological effects, and underlines the value of this treatment for a subgroup of pSS patients. The study described in chapter 8 illustrates that blockade of CD28-mediated T cell co-stimulation by abatacept has significant effects on T cell-dependent B cell hyperactivity. The research reported in chapter 9 focused on the
expression of the BCR signaling molecule Bruton’s tyrosine kinase (BTK) by B cells. We show that a subgroup of pSS patients has increased BTK levels across different B cells subsets, indicating a lower threshold for activation. In addition, we were able to show that abatacept treatment reduced BTK levels, illustrating the positive feedback loop between T cell and B cell activation. Finally, the results presented in this thesis are summarized and discussed in chapter 10. Based on our findings we consider ways to accelerate establishment of effective treatment modalities for pSS.
REFERENCES


PART I

T CELL-DEPENDENT B CELL HYPERACTIVITY: BIOMARKER OF DISEASE?