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T cells in primary Sjögren's syndrome: targets for early intervention

Gwenny M. Verstappen, Frans G. M. Kroese and Hendrika Bootsma

Abstract

A histologic hallmark of primary SS (pSS) is lymphocytic infiltration of the salivary and lacrimal glands, in particular by CD4⁺ T and B cells. In the early stages of the disease, infiltrates are dominated by CD4⁺ T cells, while B cell accumulation occurs at later stages. Activated T cells contribute to pathogenesis by producing pro-inflammatory cytokines and by inducing B cell activation, which results in the establishment of a positive feedback loop. In the inflamed glandular tissues, many different CD4⁺ effector subsets are present, including IFN- γ -producing Th1 cells, IL-17-producing Th17 cells and IL-21-producing T follicular helper cells. In blood from pSS patients, frequently observed abnormalities of the T cell compartment are CD4⁺ T cell lymphopenia and enrichment of circulating follicular helper T (Tfh) cells. Tfh cells are critical mediators of T cell-dependent B cell hyperactivity and these cells can be targeted by immunotherapy. Inhibition of T cell activation, preferably early in the disease process, can mitigate B cell activity and may be a promising treatment approach in this disease.

Key words: SS, lymphocytes, cytokines, T cells, immunotherapy, biologic therapies, histopathology, biomarkers

Rheumatology key messages

- CD4⁺ T cells are critically involved in pSS pathogenesis.
- Tfh cells are consistently found to be enriched in blood and likely facilitate B cell hyperactivity.
- Inhibition of T cell-B cell interaction is a promising treatment strategy for pSS.

Introduction

Primary SS (pSS) is a systemic autoimmune disease characterized by lymphocytic infiltration of the salivary and lacrimal glands. In addition to the exocrine glands, many other organs can be affected by the disease as well [1]. Hyperactivity of B cells is thought to play a central role in the pathogenesis of pSS [2]. Available evidence strongly indicates that this B cell hyperactivity is mediated by T cells [3, 4]. T cells may also be involved in a loss of self-tolerance and they secrete many pro-inflammatory cytokines associated with local inflammation in pSS, including IFN- γ , IL-17 and IL-21 [5, 6].

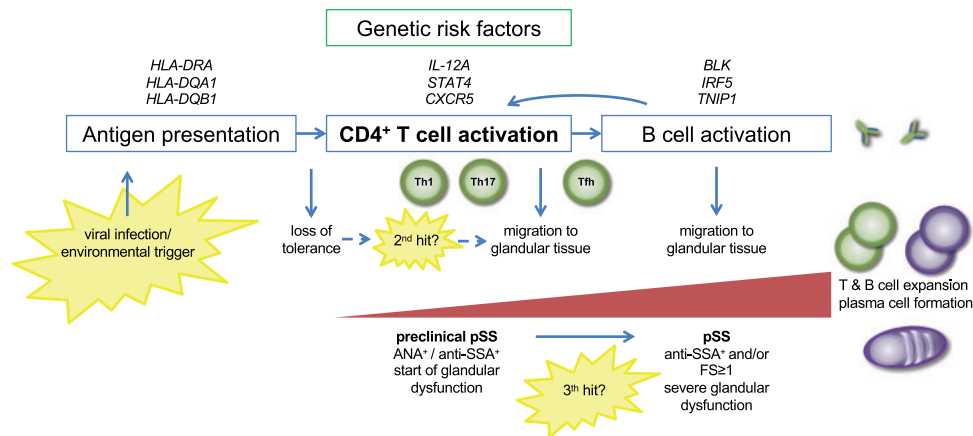
In pSS patients, T cells form a large part of the lymphocytic infiltrates observed in salivary and lacrimal gland tissues, particularly in the earlier stages of disease [7]. The infiltrated T cells are mostly CD4-expressing 'helper' T cells [8]. However, lymphocytic infiltration and loss of glandular structure are not directly related to the loss of glandular function, which suggests that (intrinsic) defects in epithelial cells contribute to the disease as well.

CD4⁺ T cells recognize antigens presented by antigen-presenting cells via class II MHC molecules. Similar to other systemic autoimmune diseases, the strongest genetic risk haplotypes for pSS were identified within the *HLA-DR* and *HLA-DQ* regions [9]. These risk haplotypes may lead to inadequate control of reactivity towards self-antigens and escape of autoreactive T cells from negative selection. HLA class II risk loci are associated with anti-SSA/SSB autoantibody presence in pSS [10]. In the majority of patients, these autoantibodies are already present years before the onset of clinical symptoms, which suggests that the induction of autoantibody-producing plasma cells by (autoreactive) T cells occurs in a preclinical stage of the disease (Fig. 1) [11]. Besides aberrant

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Fig. 1 Proposed role of CD4⁺ T cells in primary Sjögren's syndrome (pSS) pathogenesis

Antigen is presented to CD4⁺ T cells via MHC class II (HLA) molecules, resulting in CD4⁺ T cell activation. Risk loci in HLA-DR and HLA-DQ regions, associated with pSS, may be involved in a loss of tolerance to self-antigens. Depending on the type of antigen and additional environmental cues, differentiation of naïve cells into Th1 cells, Th17 cells and Tfh cells is induced. IL-12A and STAT4 risk variants may contribute to enhanced Th1 cell differentiation. A second, local hit may induce migration of effector CD4⁺ T cells to salivary and/or lacrimal gland tissues. This stage is clinically reflected in features suggestive of pSS, without the presence of focal periductal infiltrates and without evident signs of B cell hyperactivity. A third hit is probably required to establish a positive feedback loop between T cells and B cells, resulting in T cell-dependent B cell hyperactivity. In this stage, typical features associated with pSS become evident.

thymic T cell selection, alternative explanations for the observed autoreactivity are cross-reactivity between foreign and self-antigens or mimicry with microbial antigens [12, 13].

CD4⁺ T cells in pSS patients have been extensively studied over the years, particularly in blood. Th1 cells were the first CD4⁺ effector subset to be recognized and associated with autoimmunity. Initial studies observed that in inflamed glandular tissue of pSS patients, the majority of CD4⁺ T cells expressed IFN- γ [8, 9], consistent with a Th1 cell phenotype. Additionally, the role of more recently identified subsets, including Th17 cells and follicular helper T (Tfh) cells, has been assessed in numerous autoimmune diseases, including pSS (reviewed by Patel and Kuchroo [14] and Vinuesa and Linterman [15]). In this review we summarize our current knowledge of the contribution of CD4⁺ T cells in pSS pathogenesis and briefly appraise the role of CD8⁺ T cells in the disease. Furthermore, T cell-targeting therapies for pSS will be discussed.

CD4⁺ T cell lymphopenia

A typical laboratory finding in pSS is a decrease in CD4⁺ T cell numbers in the blood, which can even lead to CD4⁺ T cell lymphopenia [16]. Although its origin and implications remain largely unclear, CD4⁺ T cell lymphopenia is a predictive factor of lymphoma development in pSS (reviewed by Nocturne and Mariette [17]). A recent study showed that lower CD4⁺ T cell counts were significantly correlated with higher systemic disease activity scores [18], as measured by the EULAR SS disease activity index (ESSDAI) [19].

The same study also showed that decreased CD4⁺ T cell numbers in blood were associated with increased numbers of lymphocytes, including CD4⁺ T cells, in minor salivary glands (MSGs), favouring the hypothesis that this lymphopenia in blood is a result of CD4⁺ T cell migration to inflamed tissues [18]. However, direct evidence for this hypothesis is lacking. Another possible explanation for CD4⁺ T cell lymphopenia in pSS is the increased differentiation rate of naïve T cells into effector T cells, which have a more rapid turnover than the usually long-lived naïve cells [20]. Because of CD4⁺ T cell lymphopenia, comparisons between the numbers of circulating CD4⁺ T cell subsets in pSS patients and healthy controls (HCs) are difficult to interpret. For this reason, many studies have assessed the frequencies of these subsets to identify changes in the CD4⁺ T cell compartment in pSS.

Effector CD4⁺ T cell subsets in blood and salivary gland tissue of pSS patients

Various CD4⁺ T cell subsets can be discriminated by surface molecule expression, such as CD45RA/CD45RO for differentiation between naïve and memory cells and expression of chemokine receptors for recognition of different effector subsets. Also, *in vitro* cytokine production can be used for phenotyping. However, the use of different definitions makes it difficult to compare various studies.

Th1/Th2 cells

One of the first studies that investigated Th1/Th2 balance in matched blood and MSG tissue samples showed that

serum levels of IFN- γ were decreased while the number of IFN- γ ⁺ cells in MSG tissue was increased in pSS compared with non-SS sicca patients [21]. No differences in Th2 cell activity, assessed by IL-4 protein levels in serum and the number of IL-4⁺ cells in the glands, were observed. Subsequent studies showed that neither numbers nor frequencies of Th1 and Th2 cells in the blood from pSS patients were aberrant [22, 23]. However, support for local involvement of Th1 cells has been substantiated by a more recent study showing that local IFN- γ (type II IFN) activity, assessed by the detection of IFN-inducible guanylate binding protein 1, was associated with the degree of CD45⁺ infiltration in the MSGs of pSS patients [24]. Th1 cells are likely attracted to the salivary glands via secretion of the pro-inflammatory chemokines CXCL9 and CXCL10 by ductal epithelial cells. These chemokines are the ligands for the CXCR3 receptor on Th1 cells [25]. While Th1 cell-related mRNA transcripts (e.g. IFN- γ) were detected in glandular tissue of the vast majority of pSS patients, Th2 cell-related transcripts seem to be present only in patients with strong B cell accumulation [26]. Furthermore, Th1 cell-related mRNA transcripts (e.g. IFN- γ , T-bet) were more abundant in the MSG tissue of pSS patients without germinal centres (GCs), while Th2 cell-related mRNA transcripts (GATA3 and IL-4) were almost exclusively detected in GC-positive pSS patients [27]. Although IL-4 and GATA3 are Th2 cell-associated molecules, IL-4-producing T cells within B cell follicles have phenotypic characteristics of Tfh cells (see below) [28]. Therefore it is tempting to speculate that the cells responsible for higher IL-4 levels in GC-positive patients are in fact Tfh cells.

While the presence of IFN- γ -producing Th1 cells within lymphocytic infiltrates in pSS is evident, little is known about their contribution to hyposalivation and/or destruction of the acinar and ductal epithelium *in vivo*. Evidence from *in vitro* studies with cultured intestinal epithelial cells suggests that IFN- γ can alter tight junction function and increase permeability across the epithelium (reviewed by Walsh *et al.* [29]). Alterations in tight junction components were also observed in MSGs of pSS patients and *in vitro* exposure of acinar cells to IFN- γ could mimic these alterations [30]. In addition to an effect on tight junctions, IFN- γ could also induce Fas-mediated apoptosis in salivary gland epithelial cell (SGEC) line cultures [31]. Together, these results suggest that accumulation of IFN- γ -producing Th1 cells in the exocrine glands may contribute to epithelial cell damage and, consequently, diminished saliva secretion.

IL-17-producing cells

The greatest evidence for a pathogenic role of Th17/IL-17-producing cells comes from mouse models of pSS (reviewed by Verstappen *et al.* [32]). In different models, IL-17 knock-out mice were protected from disease development [33, 34]. In human pSS, IL-17 protein and mRNA is increased in MSG tissue of pSS compared with non-SS sicca patients [35–37]. In peripheral blood, frequencies of Th17 cells (defined as CD4⁺CD45RA⁻FoxP3⁻CXCR5⁺CXCR3⁻CCR4⁺CCR6⁺ cells)

were increased at least in pSS patients with moderate to high disease activity [3, 23]. In addition, mRNA levels of the Th17 cell-associated transcription factor RAR-related orphan receptor (ROR)- γ t and its co-activator Transcriptional coactivator with PDZ-binding motif (TAZ) were higher in circulating memory CD4⁺ T cells from pSS patients compared with HCs [38]. On the other hand, when Th17 cells were defined by *in vitro* IL-17 production, most studies did not find aberrant numbers and/or frequencies of these cells in the blood of pSS patients [22, 23, 39].

In addition to a typical pattern of chemokine receptor expression by Th17 cells, all IL-17-producing T cells express the C-type lectin CD161 [40, 41]. However, not all CD161⁺ T cells produce IL-17. In blood from pSS patients, the percentages of both CD161⁺ROR γ t⁺ (Th17-like) cells and CD161⁺ROR γ t⁻ cells were increased compared with HCs [42]. The percentages of CD161⁺ROR γ t⁺ cells correlated with the presence of anti-SSA/-SSB autoantibodies and IgG levels in serum, but not with ESSDAI scores [42]. CD161 functions as a homing factor to mucosal tissues and as a costimulatory receptor in the context of TCR stimulation [43]. CD161⁺ T cells were present in MSG tissue of pSS patients with a focus score ≥ 1 and a considerable part of these cells (~40%) co-expressed HLA-DR, indicating an activated phenotype [42]. Whether these local CD161⁺ T cells produce IL-17 and/or IFN- γ is not known. Another subset of Th17-like cells, i.e. IL-17-producing CD4⁺CD8⁻ 'double negative' T cells, was also expanded in peripheral blood and MSG tissue of pSS patients [44]. These cells were mostly unconventional TCR γ δ ⁺ T cells, which suggests that activation occurs in an MHC-independent manner. The presence of various types of IL-17-producing cells in the glandular tissue may contribute to local inflammation, likely via the pro-inflammatory effects of IL-17 on epithelial cells (e.g. induction of MMP secretion, dysregulation of tight junction proteins) and support of ectopic lymphoid tissue formation (reviewed by Verstappen *et al.* [32]). However, studying the contribution of Th17 cells to pSS pathogenesis in humans is complicated by their plasticity. Th17 cells may readily develop into various subsets, including Th1 cells and peripherally induced Treg cells. We have previously suggested that plasticity towards Th17.1 cells, co-expressing IL-17/IFN- γ (and CCR6/CXCR3), may enhance their pathogenicity [32]. In conclusion, the pathogenic role of IL-17-producing cells observed in mouse models of SS has only been partly confirmed in human pSS and needs further investigation.

Treg cells

Conflicting data exist about the involvement of Treg cells in pSS [39, 45–47]. Because the developmental pathways of Th17 and Treg cells are reciprocal, increased frequencies of Th17 cells are often accompanied by reduced frequencies of Treg cells. This Th17/Treg balance seems to be disturbed in several autoimmune conditions [48]. However, such an imbalance is not evident in pSS patients. The discrepancy between various studies on the numbers and frequencies of Treg cells may be partly

explained by their definition. Not all studies discriminate between CD45RA⁺naïve (thymus-derived) Treg cells and CD45RA⁻ memory Treg cells, which comprise mostly peripherally derived Treg cells. Treg cells can be adequately identified by high expression levels of the IL-2 receptor alpha chain (CD25) and the transcription factor FoxP3 [49]. FoxP3 expression is highly associated with suppressor activity [50]. CD25 expression alone has been used in many studies to identify Treg cells in pSS patients, but CD25 can be upregulated by all CD4⁺ T cells upon activation. In addition to CD25 and FoxP3, Treg cells can express chemokine receptors that may overlap with effector subsets. CXCR5-expressing Treg cells, for example, are considered as regulatory counterparts of Tfh cells (both subsets will be discussed further below).

Despite the existence of conflicting data, a recent study showed that in various systemic autoimmune diseases, including pSS, frequencies of activated memory Treg cells were increased while frequencies of naïve Treg cells were unchanged [51]. We also found that the frequencies of memory Treg cells were increased in pSS patients compared with HCs [3]. In this cohort, most pSS patients had moderate–high systemic disease activity (ESSDAI > 5). In contrast, in our inception cohort, with shorter disease duration and on average lower disease activity scores, we did not find a significant change in memory Treg cell frequencies in pSS compared with non-SS sicca patients (unpublished data). These data suggest that memory Treg cell frequencies are related to disease activity, possibly as a consequence of excessive T cell activation in patients with more severe disease. Correspondingly, higher frequencies of activated memory Treg cells were present in IFN-positive pSS patients compared with IFN-negative patients and HCs and these IFN-positive patients exhibited significantly higher ESSDAI scores than IFN-negative patients [52].

FoxP3-expressing cells were also studied in MSG tissue of pSS patients by immunohistochemistry. The frequency of FoxP3⁺ cells correlated positively with the biopsy focus score [37, 53]. Whether Treg cells in salivary glands of pSS patients exhibit full suppressive capacity is unknown. Two studies investigated the suppressive capacity of CD4⁺CD25^{high} T cells in blood from pSS patients, but with conflicting results [45, 54]. In conclusion, the frequencies of (memory) Treg cells are increased in the blood and tissue of pSS patients, in particular in patients with high disease activity, but the functional capacity of these cells in pSS remains ambiguous.

Tfh cells and follicular regulatory T (Tfr) cells

Although the necessity of T cell help for antibody responses was described decades ago, the recognition of a dedicated subset of B cell helper T cells (Tfh cells) followed much later. First, the chemokine receptor CXCR5, promoting migration to B cell follicles, was linked to Tfh cells [55]. Subsequently it was revealed that Tfh cell differentiation is driven by the transcription factor Bcl-6 and that activated Tfh cells express high levels of Inducible T-cell COStimulator (ICOS) and PD-1 [15]. Tfh cells facilitate

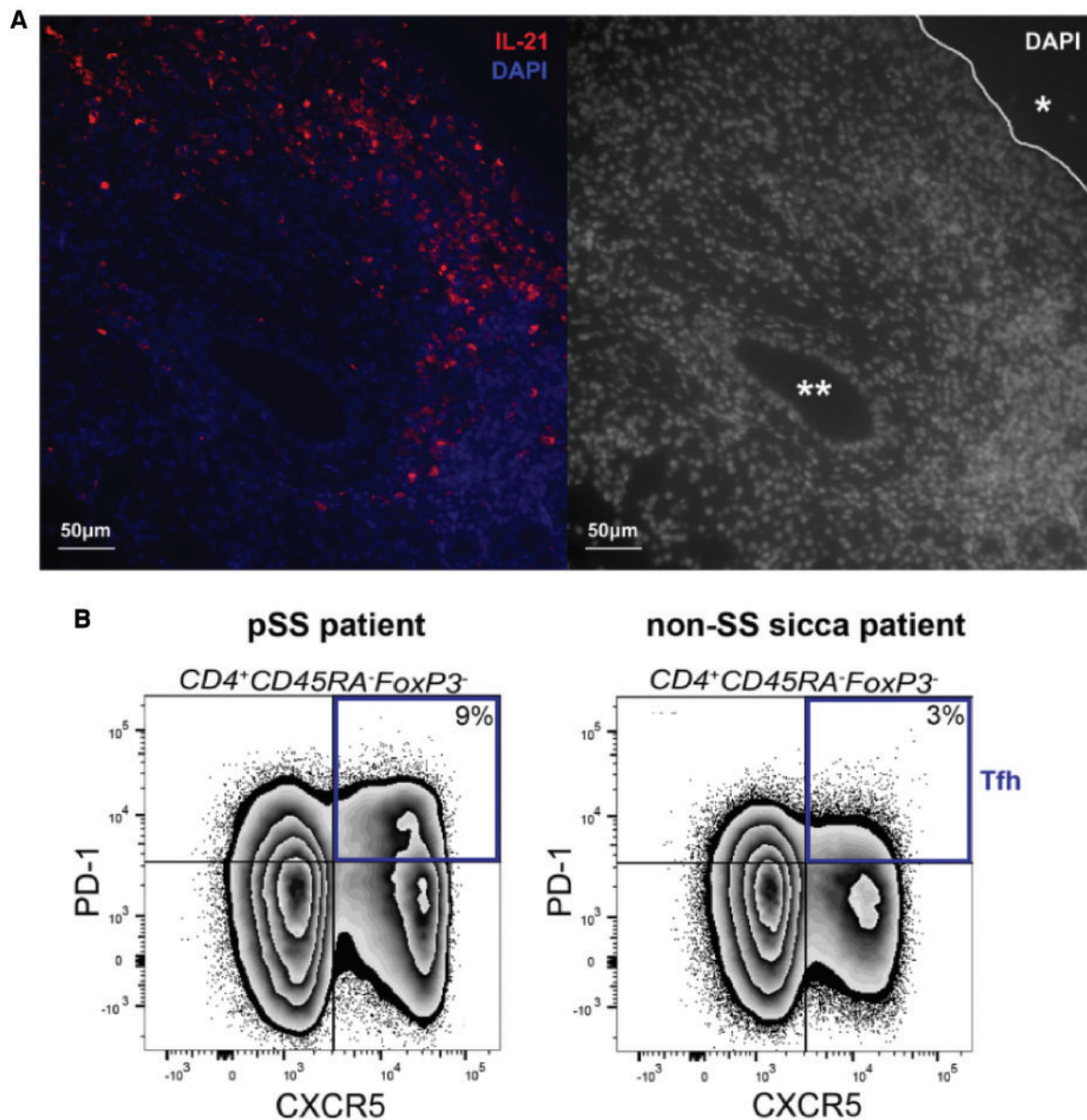
T cell-dependent B cell responses, mainly by secretion of IL-21. This cytokine is a key driver of B cell activation and differentiation towards plasma cells [56]. Increased frequencies of Tfh cells have been associated with several B cell-mediated autoimmune diseases [57]. Also in pSS, frequencies of Tfh cells are increased in blood and glandular tissue [58–61]. We found in separate cohorts that the frequencies of circulating Tfh (cTfh) cells, defined as CD4⁺CD45RA⁻CXCR5⁺PD-1⁺ cells (Fig. 2), were increased in pSS patients compared with HCs [3, 23]. This increase was already present at the time of diagnosis and the frequencies of activated Tfh cells correlated positively with ESSDAI scores [62, 63].

Identification of Tfh cells within glandular tissue is more complicated. Detection by immunohistochemistry using CXCR5 expression is impeded because of the abundance of B cells that also express this receptor. Quantification of these cells by flow cytometry is hampered by the fact that when biopsies are processed into cell suspensions using enzymatic digestion, CXCR5 expression is lost. Also Bcl-6, although essential for Tfh cell induction, is not suitable for Tfh cell identification in lymphoid tissues [64], because Bcl-6 is probably downregulated in human Tfh cells after antigen exposure [65]. A recent study that analysed MSG cell suspensions by flow cytometry therefore defined Tfh cells as PD-1⁺ICOS⁺ cells. Their results indicate that this phenotype represents ~9% of the total CD4⁺ T cells [63]. The presence of glandular Tfh cells is further supported by the significant amount of IL-21 protein and mRNA in the salivary glands of pSS patients (Fig. 1) (reviewed by Kwok *et al.* [6]), although this cytokine can also be produced by other T cell subsets, such as Th17 cells and peripheral helper T cells. Peripheral helper T cells (CXCR5⁻PD-1^{high}) express Tfh cell-related factors, including IL-21, CXCL13 and ICOS, but lack CXCR5 expression. The frequencies of peripheral helper T cells were increased in blood from pSS patients compared with HCs [3, 66, 67].

Whether glandular Tfh cells are formed locally or whether these cells differentiate in secondary lymphoid tissues and subsequently migrate to inflamed glandular tissues is unknown, but most likely both routes are active. An essential cytokine for Tfh cell differentiation is IL-6, which is elevated in the blood and salivary gland tissue of pSS patients [26, 68, 69]. Importantly, *in vitro* experiments with SGEC lines derived from pSS patients showed that epithelial cells can promote differentiation of CD4⁺naïve T cells into Tfh cells via upregulation of ICOS-L and IL-6 [70], supporting the possibility of local formation in the salivary glands. At the same time, a relatively large fraction of the circulating Tfh cells in pSS patients expresses CXCR3 (unpublished observations), which enables migration to the inflamed salivary glands where CXCL10 is produced [25].

In addition to Tfh cells, their regulatory counterparts, i.e. Tfr cells, have been identified on the basis of their simultaneous expression of FoxP3 and CXCR5 [71]. These cells are able to control Tfh cell proliferation and B cell activation in secondary (and probably also tertiary) lymphoid tissues (reviewed by Sage and Sharpe [72]). Although Tfr

Fig. 2 Characteristic features of pSS in the tissue and blood involve Tfh cells and IL-21



(A) Immunofluorescent staining of IL-21 protein in inflamed parotid gland tissue of a pSS patient. 4',6-diamidino-2-phenylindole was used to image the nuclei. *Excretory duct. **Striated duct. **(B)** A representative example of a flow cytometric analysis of circulating T cells illustrates the increase in Tfh cells, defined as $CD4^+CD45RA^-FoxP3^-CXCR5^+PD-1^+$ cells, in pSS patients compared with non-SS sicca patients.

cells, similar to Tfh cells, mainly exert their functions within lymphoid tissue, low numbers of circulating Tfr (cTfr) cells can also be found in blood. Two studies have shown that not only cTfh cells but also cTfr cells are enriched in blood from pSS patients [62, 63]. These cTfr cells were even more increased than cTfh cells, resulting in a significantly higher cTfr:cTfh ratio. Tfr cells were also present within the MSG tissue in majority of the pSS patients [63]. In human lymph nodes, Tfr cells are mainly located

at the border between the T cell zone and the B cell follicle and are rarely found within the GC [64]. A similar exclusion of Tfr cells was seen in ectopic GCs in the salivary gland tissue of pSS patients [73]. By their positioning at the T cell/B cell border, Tfr cells can control the input and/or output of the GC reaction by interacting with B cells and Tfh cells trafficking into and out of the GC. Fonseca *et al.* [74] showed that most cTfr cells in the peripheral blood of HCs have a naïve-like phenotype and

lack B cell suppressive capacity. However, cTfr cells were absent from the thymus and generated in peripheral lymphoid tissues. Their increased frequency in pSS patients may reflect ongoing T cell differentiation in secondary lymphoid organs.

A different 'Tfh-like' subset, defined by CCR9 expression, was also increased in blood from pSS patients, and small numbers of these cells were found in the MSG tissue of these patients [75, 76]. CCR9⁺ T cells share phenotypic and functional features with Tfh cells, and in HCs they typically exert their function at mucosal sites [76]. CCR9⁺ T cells have heterogeneous effector functions *in vitro*, and both cells from pSS patients and HCs are able to secrete various cytokines, including IL-21, and induce IgG production by B cells [75]. CCR9⁺ T cells can migrate towards the chemokine CCL25, which is produced in inflamed salivary gland tissue of pSS patients. CCL25 levels increased with disease severity and an influx of CCR9⁺ T cells may contribute to local B cell activation [75]. Although Tfh cells and CCR9⁺ T cells share the capacity to produce IL-21, the numbers of CCR9⁺ T cells in the blood and glandular tissue of pSS patients are essentially lower than Tfh cells and their relative contribution to humoral immune activation in addition to Tfh cells remains to be established.

Together, the available evidence shows that different CD4⁺ T cell subsets with B helper capacity are enriched in the blood and salivary gland tissue of pSS patients, supporting B cell hyperactivity. This B cell hyperactivity may contribute to the disease process and disease activity by autoantibody formation and pro-inflammatory cytokine production.

TCR specificities in pSS patients

To date, it is not known whether infiltrated (effector) T cells recognize autoantigens, salivary gland-specific proteins, microbial peptides or even other targets within the inflamed glandular tissue. T cells can be activated locally by professional antigen-presenting cells, but also by SGECs. In the inflamed glandular lesions of pSS patients, SGECs aberrantly express HLA-DR and B7 (CD80/CD86) costimulatory molecules, particularly in response to IFN- γ [77, 78]. Single-cell analysis of glandular T cells showed that TCR sequence diversity in the salivary gland was reduced and that there were more clonal expansions in the salivary glands of pSS patients compared with blood [79]. A more restricted local TCR repertoire in pSS was also observed by single-cell analysis of Th1 and Th17 cells isolated from salivary gland tissues of pSS patients compared with non-SS sicca patients [80]. In addition, Joachims *et al.* [79] showed that expanded clones of memory CD4⁺ T cells in the salivary glands displayed sequence similarity both within expanded clones of the same individual and among individual patients, indicating local shared antigen recognition. They also observed that an increased frequency of clonal expansions within the glands was correlated with decreased unstimulated salivary flow and increased salivary gland fibrosis. Based on these findings the authors hypothesized that damage to the salivary glands may depend on the expansion of self-

reactive T cells that recognize exocrine gland-specific antigens. This damage is likely mediated by cytokine production, e.g. IFN- γ . This hypothesis is supported by an experimental mouse model in which mice were immunized with M3 muscarinic acetylcholine receptor (M3R) peptides to induce SS. In this model, M3R-specific T cells produce large amounts of IFN- γ and IL-17 [81]. When M3R-immunized mice were treated with an antagonistic altered M3R peptide ligand (that harbours an amino acid substitution at the TCR contact site), anergy of CD4⁺ M3R-reactive T cells was induced and sialoadenitis was suppressed [82]. Thus, at least in an experimental model, recognition of local antigen by CD4⁺ T cells may result in T cell expansion, pro-inflammatory cytokine production and consequently gland dysfunction. Together, the available evidence suggests that at least a proportion of CD4⁺ T cells expand locally after antigen recognition in the salivary glands and these antigens may be shared between individuals. The dominant antigens that are recognized remain to be elucidated.

Involvement of CD8⁺ T cells in the pathogenesis of pSS

Although the majority of T cells within the glandular infiltrates of pSS patients are CD4⁺ cells, CD8⁺ T cells are also present. Part of these CD8⁺ T cells show an activated phenotype, as reflected in higher expression levels of HLA-DR. Increased proportions of HLA-DR⁺ T cells were associated with higher disease severity [18]. Also in the blood of anti-SSA⁺ pSS patients, increased HLA-DR expression by both CD4⁺ and CD8⁺ T cells was observed and the frequencies of HLA-DR-expressing activated CD4⁺ and CD8⁺ T cells in blood correlated with ESSDAI scores [18]. Furthermore, the proportion of activated CD8⁺ T cells in blood was associated with a multi-omic-based disease signature of pSS, which was based on whole blood transcriptomes, serum proteomes and peripheral immunophenotyping [83]. The expression of CXCR3 by activated CD8⁺ T cells in pSS patients may be important for their migration to the inflamed salivary glands. Indeed, in mice it was shown that after viral infection, recruitment of activated CD8⁺ T cells to salivary gland tissue was dependent on CXCR3 [84]. We speculate that chronic antigen stimulation and systemic inflammation, reflected as higher ESSDAI scores, results in the activation of CD8⁺ T cells in secondary lymphoid organs, CXCR3 upregulation and consequent migration to the salivary glands. Whether CD8⁺ T cells, in turn, contribute to glandular dysfunction or systemic disease activity is unknown.

T cell-targeting treatment of pSS patients

As indicated previously, CD4⁺ T cell activation is needed for the establishment of B cell (hyper)activation in pSS. Restriction of T cell-dependent B cell hyperactivity might therefore be an important target for the treatment of pSS patients. Abatacept is a biologic DMARD that binds to CD80/86 on antigen-presenting cells (including

B cells). Consequently, it impairs CD28-mediated T cell activation. The first open-label study on the effects of abatacept in pSS showed that blood CD4⁺ T cell numbers, adjusted for disease duration, increased following treatment. This partial recovery of lymphopenia may be clinically beneficial, as CD4⁺ T cell lymphopenia is associated with systemic disease activity and lymphomagenesis [17, 18, 85]. Abatacept treatment also reduced Treg cell numbers in MSG tissue, along with an increase in stimulated saliva production, adjusted for disease duration [86]. A second open-label study showed that saliva production rates stabilized over the treatment period (24 weeks). This second study also found that abatacept treatment significantly improved systemic disease activity scores, as measured by ESSDAI [87]. Additionally, we showed that abatacept selectively reduced the percentages and numbers of cTfh cells and memory Treg cells to levels seen in HCs [3]. Furthermore, abatacept treatment resulted in decreased ICOS expression by the remaining cTfh cells, which correlated significantly with the reduction in ESSDAI scores [3]. In RA patients, the frequency of cTfh cells at baseline was an independent predictor of response to abatacept [88]. In pSS patients, treatment with abatacept had not only significant effects on cTfh cells, but also on B cell activity, reflected in decreased serum autoantibody levels, frequencies of circulating plasmablasts and protein levels of Bruton's tyrosine kinase in

B cells [3, 89]. The effects of abatacept on B cell activity in pSS provide strong evidence that T cells and B cells act in a positive feedback loop. Consistent with this notion, B cell depletion therapy with rituximab had significant effects on the CD4⁺ T cell compartment in pSS patients [23, 90]. In particular, levels of cTfh cells and Th17 cells were reduced by rituximab, and this reduction in cTfh cells was associated with the decrease in ESSDAI scores over time [23].

In contrast to the targeted biologic DMARDs, conventional DMARDs (cDMARDs) often have broad immunosuppressive effects. Several cDMARDs, in particular CSA and LEF, exert inhibitory effects on T cell activation and proliferation (reviewed by van der Heijden *et al.* [91]). Although topical ophthalmic use of CSA for dry eye disease, associated with pSS, is supported by the literature (reviewed by Ramos-Casals *et al.* [92]), evidence of the efficacy of systemic CSA in pSS patients is lacking. There is evidence that LEF may be effective in pSS [93], and the combined efficacy of LEF and HCQ is currently under investigation.

In addition to abatacept and LEF, several other immunomodulatory treatments that target T cells directly or indirectly are now under investigation in pSS. Recently a clinical trial with low-dose IL-2 therapy in 190 pSS patients was completed. The rationale for such an approach is to restore the balance between effector T cells and Treg cells.

TABLE 1 Key findings describing changes in the CD4⁺ T cell compartment of patients with pSS

Finding	Reference
Blood	
CD4 ⁺ T cell lymphopenia is a predictive factor of lymphoma development and is associated with higher systemic disease activity and with increased numbers of lymphocytes in MSGs of pSS patients.	[17, 18, 85]
Different subtypes of IL-17-producing CD4 ⁺ T cells are enriched in peripheral blood from at least a subgroup of pSS patients.	[3, 23, 38, 42]
Frequencies of memory Treg cells are increased in peripheral blood from pSS patients, at least in patients with moderate-high disease activity.	[3, 51, 52]
Frequencies of circulating Tfh cells are increased in pSS patients compared with non-SS sicca controls and healthy individuals. Frequencies of activated cTfh cells (CD4 ⁺ CD45RA ⁻ CXCR5 ⁺ PD-1 ⁺ ICOS ⁺) correlate with systemic disease activity.	[3, 23, 62, 63]
Circulating Tfr cells are enriched in peripheral blood from pSS patients, resulting in a higher cTfr:cTfh ratio in pSS patients compared with healthy individuals.	[62, 63]
The proportion of CCR9 ⁺ 'Tfh-like' cells is increased in peripheral blood from pSS patients compared with healthy individuals.	[75]
Tissue	
IFN- γ -producing CD4 ⁺ T cells (Th1 cells) are present within lymphocytic infiltrates and IFN- γ (type II IFN) activity is associated with the degree of CD45 ⁺ infiltration in MSGs of pSS patients.	[21, 24]
IL-17 protein and mRNA is increased in MSG tissue of pSS patients compared with non-SS sicca controls.	[35-37]
The frequency of FoxP3 ⁺ cells in MSGs correlates positively with the biopsy focus score.	[37, 53]
Tfh-like cells (CD4 ⁺ PD-1 ⁺ ICOS ⁺) make up a significant part of the T cell infiltrate in MSGs of pSS patients and likely form a major source of IL-21.	[63]
The TCR repertoire of glandular CD4 ⁺ T cells indicates local antigen recognition (and expansion) by these cells.	[79]

Indeed, the number of Treg cells in the blood increased significantly after treatment, but clinical efficacy was lacking [39]. An important limitation of this study is that 89% of patients were concomitantly treated with immunosuppressants. Better insight into the role of Treg cells in pSS is necessary to support the use of Treg-targeted treatment in this disease. Other biologic/synthetic DMARDs that are currently under investigation and may affect T cell activation include anti-IL-6R treatment with tocilizumab, anti-CD40 treatment with CFZ533, anti-ICOSL treatment with AMG557 and JAK1 inhibition with filgotinib. The clinical efficacy as well as the effects of these treatments on T (and B) cells in pSS patients are eagerly awaited.

Conclusion

Current evidence suggests that CD4⁺ T cells, and perhaps also CD8⁺ T cells, can contribute significantly to local and systemic inflammation in pSS (Table 1). In particular, T cell subsets that support B cell function, e.g. Tfh and Tfh-like cells, appear to play a major role in pSS, either in the glandular tissue itself, at distinct inflamed sites or in secondary lymphoid organs. We presume that interruption of T cell–B cell interaction is crucial for successful treatment of this disease. If T cell activation can be impaired by treatment, preferably early in the course of disease, excessive B cell activation and damage to glandular tissue by B and T cell–derived pro-inflammatory cytokines may be attenuated.

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