T cell-dependent B cell hyperactivity in primary Sjögren's syndrome

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TH17 CELLS IN PRIMARY SJÖGREN'S SYNDROME: PATHOGENICITY AND PLASTICITY

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ABSTRACT

Th17 cells play an important physiological role at mucosal barriers, and are involved in inflammatory responses to pathogens. Th17 cells and their signature cytokine IL-17 are also present in salivary gland lesions of primary Sjögren's syndrome (pSS) patients and can be elevated in their peripheral blood. In pSS patients, clear correlations between increased Th17 cell activity and symptoms of the disease have not been found, but Th17 cells may contribute to disease progression, for example by supporting autoreactive B cell responses. In mouse models of pSS, Th17 cells play an important role in pathogenesis, particularly at disease onset, when there is a disturbed balance between T effector and T regulatory cells. Studying the pathogenicity of Th17 cells in humans is complicated due to the plasticity of this cell subset, allowing them to obtain different effector functions depending on the local environment. Th17 cells can develop towards Th17.1 cells, producing both IL-17 and IFN-γ, or even towards Th1-like cells producing IFN-γ in the absence of IL-17. These effector subsets may be more pathogenic than bona fide Th17 cells. Co-expression of IFN-γ by Th17 cells has been shown to promote chronic inflammation in several autoimmune diseases and may also contribute to pSS pathogenesis. In line with the noticeable role of IL-17 in pSS mouse models, interference with Th17 cell generation, recruitment or effector functions (e.g. IL-17 inhibition) can prevent or ameliorate disease in these models. Therapies targeting Th17 cells or IL-17 have not been tested so far in pSS patients, although treatment with rituximab seems to lower local and systemic IL-17 protein levels, and to a lesser extent also chemokine receptor-defined Th17 cells. In this review we discuss current knowledge of pathogenicity and plasticity of Th17 cells in human pSS and murine models of pSS. We postulate that plasticity towards Th17.1 cells in pSS may enhance pathogenicity of Th17 cells at the main target sites of the disease, i.e. salivary and lacrimal glands.

INTRODUCTION

Primary Sjögren's syndrome (pSS) is a systemic autoimmune disease, primarily affecting the salivary and lacrimal glands. Oral and ocular dryness, fatigue and pain are predominant symptoms of pSS. The disease is clinically heterogeneous and many extraglandular organs can be involved during the course of the disease [1]. The pathophysiology of pSS is multi-facetted and not completely understood. Both environmental and genetic factors are likely involved in disease initiation, and the few gene polymorphisms that are associated with pSS are related to components of both innate and adaptive immune systems [2]. No polymorphisms in genes encoding salivary or lacrimal components have been identified. Involvement of the adaptive immune system is evident in the affected exocrine glands of pSS patients, where main histopathological findings include periductal focal infiltration of mononuclear cells, largely consisting of $CD4^+$ T cells and B cells [3]. These periductal infiltrates can be organized into ectopic lymphoid structures with segregated T and B cell areas. In approximately 25% of the patients, these structures contain germinal centers, which promote local expansion of (auto)antigen-specific (memory) B cells [4,5]. The occurrence of ectopic germinal centers, together with hypergammaglobulinemia and presence of autoantibodies underlines the important role of B cell hyperactivity in pSS pathogenesis [6]. It is, however, important to note that $CD4^+$ T cells predominate the periductal infiltrates in patients with mild lesions [3]. Growing evidence suggests that the crosstalk between $CD4^+$ T cells and B cells forms a crucial step in pSS pathogenesis and a suitable target for treatment [7,8].

Different $CD4^+$ T cell subsets seem to contribute to pSS pathogenesis, including T helper 1 (Th1) cells, follicular T helper (Tfh) cells and T helper 17 (Th17) cells, although the relative importance of each subset remains a matter of debate. After the first discoveries of a link between Th17 cells and autoimmunity, several human and murine studies investigated the role of Th17 cells in pSS pathogenesis, as summarized in Table 1. In 2008, the first studies showed that IL-17, the signature cytokine of Th17 cells, is present within lymphocytic infiltrates of minor salivary gland tissue from pSS patients [9,10]. Presence of IL-17 was predominantly observed in CD4+ T cell-rich areas of the periductal infiltrates [10]. Also IL-17 mRNA levels were elevated in minor salivary glands of pSS patients, compared with non-SS sicca patients [11]. Subsequent studies focused on the presence of Th17 cells within the glands. However, there is not a single marker that identifies Th17 cells exclusively. In current literature, Th17 cells have been identified either by expression profiles of their signature cytokines IL-17 and IL-22, by the expression of chemokine receptors (CCR6, CCR4, CD161, podoplanin) and/or by means of transcription factors (RORγ, STAT3). To complicate matters further, Th17 cells can acquire functional characteristics of regulatory T (Treg) cells, Th1 cells and Tfh cells and even can downregulate IL-17 production, illustrating the plasticity of this cell subset [12].

TABLE 1 | Evidence for the involvement of Th17 cells in pSS pathogenesis.

Reference	Publication	Study population	Key observations related to Th17 cells
Human			
[8]	Verstappen et al., 2017	pSS patients before and after abatacept treatment	Patients with pSS have elevated frequencies of circulating Th17 cells (CCR6+CCR4+), compared with controls. These cells are not affected by abatacept treatment.
[9]	Nguyen et al., 2008	pSS patients	Protein expression of IL-17 and IL-23 in lymphocytic foci in minor salivary glands of pSS patients. IL-17 levels in serum and saliva of pSS patients comparable to non-SS sicca patients.
[10]	Sakai et al., 2008	pSS patients	Protein expression of IL-17 in minor salivary glands was predominantly found in CD4+ T cell areas, but also co-localized to some extent with CD8+ T cells and ductal epithelial cells.
[11]	Katsifis et al., 2009	pSS patients	Local IL-17 protein and mRNA levels, together with IL-6 and IL-23 mRNA, increase with progression of lesion severity in minor salivary glands of pSS patients. Plasma IL-17 levels were significantly higher in pSS patients, compared with controls.
[13]	Ciccia et al., 2014	pSS patients before and after RTX treatment	Salivary gland expression of IL-17, but not of IL-23p19 and p-STAT3, decreased by rituximab treatment.
[14]	Liu et al., 2017	pSS patients	IL-17A conjunctival mRNA and protein expression in tears higher in pSS, compared with non-SS group with dry eye disease.
[15]	Ciccia et al., 2012	pSS patients	IL-22 is present in minor salivary gland tissue of pSS patients and Th17 cells are a major source of this cytokine.
[16]	Blokland et al., 2017	pSS patients	Percentages of peripheral IL-17-producing CD4+ T cells were similar between pSS patients and controls. CCR9+ Th-cells produced IL-17 upon antigen and IL-7 stimulation.
[17]	Verstappen et al., 2017	pSS patients before and after RTX treatment	Frequency of IL-17-producing CD4+ T cells in PBMCs from pSS patients at baseline was similar to controls, but these cells significantly decreased by rituximab treatment, together with serum levels of IL-17.
[18]	Bikker et al., 2012	pSS patients	Ex vivo and IL-7-induced IL-17A production is similar in pSS patients and controls
[19]	Kwok et al., 2012	pSS patients	Higher frequency of IL-17-producing CD4+ T cells in PBMCs from pSS patients, compared with controls.
[20]	Pollard et al., 2013	pSS patients	Several Th17-related cytokines (IL-17, GM-CSF, IL-1β) were significantly elevated in pSS patients, compared with controls.
[21]	Reksten et al., 2009	pSS patients	Higher levels of Th17-associated cytokines in pSS patients with germinal center (GC) formation in their salivary glands, compared with GC-negative patients.
[22]	Alunno et al., 2013	pSS patients	IL-17-producing CD4-CD8- T cells are expanded in PBMCs from pSS patients, are also present in minor salivary glands and are resistant to in vitro dexamethasone suppression.
[23]	Fei et al., 2014	pSS patients	Glandular IL-17 protein expression increased with progression of lesion severity. CD4+IL-17+ cells in peripheral blood of pSS patients and serum IL-17 were significantly increased, compared with controls.

TABLE 1 | Continued

Reference	Publication	Study population	Key observations related to Th17 cells
Mouse			
[9]	Nguyen et. al., 2008	C57BL/6.NOD-Aec1Aec2 mice	IL-17A, IL-17R and IL-23 expression in salivary glands when infiltrates occur, Tbet is increased in the pre-disease phase.
[24]	Voigt et. al., 2016	C57BL/6.NOD-Aec1Aec2 x IL-17 KO mice	IL-17 deficient C57BL/6.NOD-Aec1Aec2 mice are protected against disease development.
[25]	Wanchoo et. al., 2017	C57BL/6.NOD-Aec1Aec2 mice	TCR repertoires of Th1 and Th17 cells in salivary gland infiltrates are restricted.
[26]	Lin et. al., 2015	C57BL/6J and IL-17 KO mice with ESS	Th17 cells are increased in salivary gland peptide induced disease. IL-17 deficient mice are protected, and transfer of Th17 cells in IL-17 deficient mice restores disease phenotype.
[27]	Iizuka et. al., 2015	RORγt Tg mice and RAG KO mice	RAG deficient mice develop pSS phenotype upon transfer of RORγt overexpressing CD4+ T cells, but not when these cells are IL-17 deficient.
[28]	Lee et. al., 2012	C57BL/6.NOD-Aec1Aec2 with IL27 expression in salivary glands	IL-27 expression through rAAV2-*IL27 vector injection*, which induces Th1 and inhibits Th17 cells is most effective after onset of glandular disease
[29]	Contreraz-Ruiz et. al., 2017	TSP1 KO mice with TSP1 peptide treatment	Treatment of TSP1 KO mice with TSP1 derived peptide increases Treg cells and reduces Th17 cells, and attenuates disease symptoms.
[30]	Coursey et. al., 2017	NOD.B10.H2^{b}	Treg cell function is hampered and Treg cells cells start to produce IL-17 and IFNγ.
[31]	Iizuka et. al., 2010	M3R KO and RAG KO mice	Transfer of M3R deficient splenocytes in RAG deficient mice leads to Th17.1 infiltration in salivary glands and pSS like symptoms.
[32]	Tahara et. al., 2017	M3R KO and RAG KO mice with anti-RORγt treatment	RORγt antagonist treatment after transfer of M3R deficient splenocytes into RAG deficient mice reduces both IL-17 and IFNγ in spleen and LN.
[33]	Nguyen et. al., 2010	C57BL/6J with IL-17A expression in salivary glands	IL-17A expression through Adenovirus 5 cannulation in salivary glands leads to pSS-like phenotype

In this review we will discuss current knowledge of Th17 cells in pSS pathogenesis and mouse models of pSS, including their phenotype, localization, function and correlation with clinical features of the disease. We will focus on the relation between pathogenicity and plasticity of Th17 cells and postulate that plasticity towards Th1-like cells in pSS may enhance pathogenicity of Th17 cells at the main target sites of the disease, i.e. salivary and lacrimal glands.

Role of Th17 cells in pSS

Th17 cells play an important physiological role at mucosal sites of healthy individuals. The main effector cytokines of Th17 cells are IL-17 an IL-22. These cytokines support the epithelial barrier integrity by stimulation of tight junction protein formation [34], and

IL-22 has an important role in epithelial cell survival and proliferation [35]. Th17 cells also act as first defense against microbes by stimulating the production of antimicrobial peptides and chemokines to attract leukocytes when the epithelial barrier is breached [36]. Initially, activation and polarization of Th17 cells may be initiated by dendritic cells in lymph nodes draining the salivary and lacrimal glands, whereas in later phases of the disease this may also happen locally in the inflamed glandular tissue. These dendritic cells secrete Th17 cell polarizing cytokines, including TGF-β and IL-23 (Figure 1). Ductal epithelial cells of the glands may also produce cytokines important for Th17 polarization, such as IL-1β [37]. Activated Th17 cells promote inflammation by stimulating release of pro-inflammatory cytokines in the inflamed exocrine glands, including IL-6 and TNF, by virtue of IL-17 and IL-22 secretion and its binding to their receptors expressed on stromal and epithelial cells [38] (Figure 1). Expression of IL-17R was observed in a neoplastic parotid gland cell line [10], and is likely also expressed by ductal epithelial cells in pSS patients. IL-17 was also shown to induce matrix metalloproteinase 1 (MMP-1) and MMP-3 release from synovial fibroblasts in rheumatoid arthritis, which may cause tissue destruction [39]. In salivary gland tissue of pSS patients, particularly MMP-9 expression is increased and is associated with acinar damage [40]. Interleukin-17 also promotes MMP-9 production by epithelial cells [41].

In addition to their role in tissue inflammation, Th17 cells also may contribute more specifically to autoimmune processes by the following mechanisms (i) supporting isotype class switching upon B cell receptor stimulation, both via IL-17 and IL-21 production [42,43], (ii) regulating glycosylation of autoreactive antibodies [44], (iii) affecting trafficking of B cells within the GC resulting in disturbed selection of B cells and formation of autoantibodies [45] and (iv) supporting formation of ectopic lymphoid tissue and ectopic germinal centers (GCs) [45–47] (Figure 1). Whether these functions of Th17 cells are involved in pSS pathogenesis is currently unknown. There is some support for a role of IL-22 in ectopic lymphoid tissue formation in pSS. Administration of luciferase-encoding replication-defective adenovirus (Ad5) through intraductal cannulation into the salivary glands of C57BL/6 mice leads to lymphocytic infiltration of these glands, and ectopic lymphoid tissue formation. Knockout or blockade of IL-22 in this model impaired ectopic lymphoid tissue formation [48]. This was probably caused by reduced IL-22-mediated CXCL12 and CXCL13 production by stromal cells in these IL-22 deficient animals. In summary, numerous potential effector functions of Th17 cells may contribute to pathogenesis in autoimmune conditions in general, and pSS in particular [49].

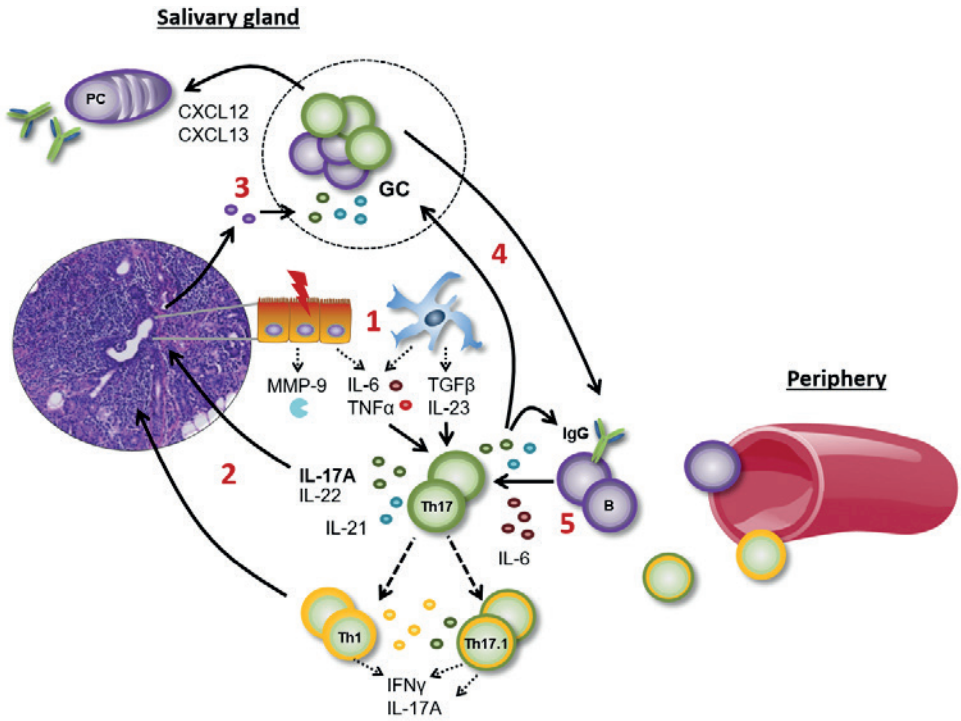

FIGURE 1 | Role of Th17 cells in primary Sjögren's syndrome (pSS) patients. 1) Environmental factors activate epithelial cells and dendritic cells (in blue). These cells secrete pro-inflammatory cytokines and present antigens, resulting in activation of Th17 cells. 2) Th17 cells infiltrate the salivary gland and may differentiate towards Th17.1 cells or Th1 cells. Pro-inflammatory cytokines (IL-17A, IL-22, IFNγ) are secreted by these cells and bind to their receptors expressed on stromal and epithelial cells. Tissue inflammation is exacerbated and more pro-inflammatory factors are secreted by epithelial cells. 3) CXCL12 and CXCL13 are expressed by stromal and epithelial cells and, together with antigen presentation by follicular dendritic cells, can induce germinal center (GC) formation in salivary glands. 4) The GC generates plasma cells producing autoantibodies and memory B cells switched to IgG, which is stimulated by IL-17 and IL-21. 5) B cells in secondary lymphoid organs and salivary glands produce IL-6, further stimulating formation of Th17 cells.

Th17 cells and glandular inflammation in pSS

Interleukin-17 protein and mRNA, as well as cells expressing the Th17-associated transcription factor RORγ, are present in minor salivary gland tissue of pSS patients, mainly in CD4+ T cell-rich areas [9–11,13,14]. IL-17 is also present in saliva and tears from pSS patients, and in tears, levels are higher compared with non-SS sicca controls [14,50]. Although it is likely that Th17 cells are the main source of IL-17 in the inflamed exocrine glands, γδ T cells, NK cells, innate lymphoid cells (ILCs) including lymphoid tissue inducer cells, and $CD8^+$ T cells are also potent sources of IL-17 [49]. Double negative (CD4-CD8-) T cells and CD8+ T cells that are positive for IL-17 are actually present in minor salivary glands of pSS patients, albeit in low numbers. Immunohistochemical analysis initially

suggested that also mast cells were a source of IL-17 in inflamed salivary glands [13]. Recent findings show, however, that mast cells do not produce IL-17 themselves, but actively capture IL-17 by endocytosis [51].

The number of IL-17-positive cells and IL-17 mRNA levels in minor salivary gland biopsies correlate with focus score, a measure of glandular inflammation [11]. Another Th17 cell-associated cytokine that is present in salivary gland tissue of pSS patients is IL-22, which seems to co-localize with mononuclear cells and ductal epithelial cells [15]. The same study showed that, after in vitro stimulation, IL-22 is mainly co-expressed by IFN-γ- or IL-17-producing CD4+ T cells isolated from minor salivary glands and only a small proportion of CD4+ T cells expressed IL-22 alone [15]. The IL-22 receptor (IL-22R) is usually expressed by nonhematopoietic cells at barrier surfaces [52]. However, only few ductal and acinar epithelial cells in the salivary glands of pSS and non-SS sicca patients seem to express IL-22R, and aberrant protein expression of IL-22R was observed among infiltrating mononuclear cells in pSS patients [53]. The nature and function of this IL-22R expression on mononuclear cells is, however, unclear.

The developmental origin of IL-17- and IL-22-expressing T cells in salivary glands is not exactly known, and both local differentiation from naïve CD4+ T cells as well as recruitment of Th17(-like) effector cells from the peripheral blood may contribute to the local pool of IL-17- and IL-22-expressing cells (Table 2). Naïve T cells can differentiate locally into Th17 cells in the presence of antigen presenting cells (APCs) and the essential cytokines IL-6 and TGF-β [54]. IL-6 is present in salivary gland tissue and saliva of pSS patients and local IL-6 expression increases with a higher focus score [55]. TGF-β is also produced in salivary gland tissue of both healthy individuals and pSS patients [11]. Th17 cell differentiation is further amplified by IL-21 and this cytokine is abundantly expressed in the glandular infiltrate of pSS patients [56,57]. In addition to IL-6 and TGF-β, also the pro-inflammatory chemokines CXCL9 and CXCL10 may play a role in local polarization of Th17 cells. Activated $CD4^+$ T cells may express CXCR3 and ligation of CXCR3 not only leads to upregulation of Tbet, the transcription factor driving Th1 cell differentiation, but also to RORγ expression and Th17 cell formation [58]. In this context it is relevant to mention that CXCL9 and in particular CXCL10 are secreted in high quantities by ductal epithelial cells from pSS patients in response to IFN-γ [59] and likely also to IFN-α [60].

Besides local differentiation of naïve cells and polarization of Th1 cells, Th17 cells can also be recruited from the circulating pool of Th17 cells by chemokines that are secreted in the salivary glands. An important pathway for direct recruitment of Th17 cells to the inflamed tissue is via the CCL20/CCR6 signaling axis [16,61]. CCL20 is not only important for recruitment of Th17 cells, but also for activation of these cells, as binding of CCL20 to CCR6 induces calcium influx in Th17 cells [62]. CCL20-mRNA transcripts were, however, only detected at low levels and in few pSS patients as revealed by qPCR [63,64]. Thus, the role for CCL20 in the recruitment of Th17 cells to the salivary glands seems limited.

Th17(-like) cells may, however, also be attracted by other chemokines, such as CCL25/CCR9. Recently it was shown that IL-17-producing CCR9$^+$ T cells home in small numbers to the inflamed salivary gland under the influence of CCL25 [16]. Another signaling axis that may contribute to recruitment of both naïve and central memory (Th17) cells consists of CCR7 and its ligands CCL19 and CCL21, all of which are highly expressed in salivary gland tissue of pSS patients [65,66].

TABLE 2 | Ligands and receptors that promote Th17 cell polarization, recruitment and maintenance in (inflamed) human salivary glands.

Expressed by naïve / activated T cell	Ligand	Expressed by	Effect	Reference
Local polarization of naïve CD4$^+$ T cells into Th17 phenotype				
IL-6 receptor	IL-6	APC, ductal epithelial cells	IL-6 and TGFβ together promote Th17 differentiation by upregulating RORγt and IL-23R expression on Th17 cells	[11,54,55]
TGFβ receptor	TGFβ	APC		
IL-21 receptor	IL-21	Tfh cells/Th17 cells	amplification of Th17 differentiation	[56,57]
CXCR3	CXCL9 / CXCL10	ductal epithelial cells (among others)	upregulation of Tbet and RORγt on T cells	[58–60]
Expressed by Th17 cell	**Ligand**	**Expressed by**	**Effect**	**Reference**
Recruitment of Th17 cells to the salivary glands				
CCR6	CCL20	salivary gland epithelial cells (low expression)	homing of Th17 cells to salivary glands and activation of these cells	[61,62,64]
CCR7	CCL19 / CCL21	salivary gland stromal cells (high expression)	homing of naïve T cells and central memory Th17 cells to salivary glands	[65,66]
CCR9	CCL25	inflamed salivary gland tissue (epithelial cells)	homing of CCR9+IL-17+ T cells to salivary glands	[16]
Maintenance of Th17 cells in salivary glands				
IL-23 receptor	IL-23	APC	expansion and maintenance of Th17 cells and production of cytokines	[9,11,49]
IL-7 receptor	IL-7	salivary gland stromal cells	maintenance of pathogenic Th17 cells	[68,69]
IL-15 receptor	IL-15	salivary gland epithelial cells	maintenance of pathogenic Th17 cells	[68,70]

Not only pro-inflammatory cytokines that induce or amplify Th17 cell differentiation, but also cytokines that are important for homeostasis of Th17 cells may contribute to Th17-mediated pathology in inflamed tissue. IL-23 is important for expansion and maintenance of Th17 cells by STAT3 activation and is present in glandular infiltrates [9,11,49]. Production of IL-23 by macrophages is at least in part mediated by the activation of interferon regulatory factor 5 (IRF5) [67]. Interestingly, polymorphisms of the IRF5 gene locus are associated with pSS and may enhance IL-23 production

[2]. IL-7 and IL-15 can also sustain pathogenic Th17 cells, which is mediated by STAT5/Akt signaling [68]. Elevated levels of IL-7 are observed in minor salivary glands of pSS patients, compared with non-SS sicca patients, and IL-7 is largely produced by stromal cells in the glands [69]. IL-15 can be produced by salivary gland epithelial cells of pSS patients in response to TLR2 stimulation in vitro [70].

Taken together, the inflamed exocrine glands in pSS constitute a microenvironment that enables local polarization and recruitment of (precursor) Th17 cells. Although their contribution to the disease is not clear yet, local Th17 cells can acquire several effector functions that are potentially pathogenic.

Th17 cells and systemic inflammation in pSS

In addition to glandular Th17 cell activity, also circulating Th17 cells and serum levels of IL-17 have been studied in the past decade in pSS, but with conflicting results [9,11,17–21]. Some studies report an increase in circulating Th17 cells and/or serum levels of IL-17, whereas others do not find a difference between pSS patients and healthy controls. It should be noted that different definitions of Th17 cells were used in these studies.

Recently, we found in two independent study cohorts that proportions of circulating Th17 cells, as defined by their chemokine receptor expression profile (CD4+CD45RA-FoxP3-CXCR5-CXCR3-CCR4+CCR6+), were increased in pSS patients compared to healthy controls [8,17]. Both studies included patients with moderate systemic disease activity, as measured by ESSDAI, the EULAR Sjögren's Syndrome Disease Activity Index (median ESSDAI scores in these study cohorts: 11 and 8, respectively). Despite this increase in chemokine-receptor defined Th17 cells in these patients, proportions of circulating CD4+IL-17+ T cells were not elevated [17], consistent with a previous report [18]. The relative increase in Th17 cells, as defined by chemokine receptor expression, was not observed when comparing pSS patients with non-SS sicca patients in a diagnostic cohort that included patients clinically suspected with pSS (Verstappen & Kroese, unpublished data). In this cohort, systemic disease activity in pSS patients was low (median ESSDAI score = 4). These findings indicate that elevated levels of Th17 cells are possibly only seen in pSS patients with moderate to high systemic disease activity.

Alternative definitions of Th17 cells have been adopted to study the prevalence of Th17 cells in peripheral blood of pSS patients. For example, expression of the C-type lectin CD161, in combination with RORγ, the master transcription factor required for generation of Th17 cells and IL-17 production, has been used [71]. In pSS patients, CD4+CD161+RORγ+ T cells were increased and this increase correlated positively with anti-SSA/SSB autoantibody status and serum IgG level, but not with systemic disease activity, as measured by ESSDAI [72]. Recent findings show that, in addition to typical CCR4+CCR6+ Th17 cells, also circulating 'Tfh-like' CCR9+CD4+ and CXCR5+CD4+ T

cells from pSS patients are capable of producing IL-17 [16]. Regarding the latter Tfh-like subset, a fraction of these cells appears to co-express CCR6, and thus may also be considered as a Th17 cell subset. These CD4+CXCR5+CCR6+ T cells were elevated in peripheral blood of pSS patients [73]. Lastly, circulating double negative (CD4-CD8-) T cells, which consist largely of γδ+ T cells, are a potential source of IL-17 in pSS patients [22]. Also these double negative T cells that produce IL-17 are expanded in peripheral blood of pSS patients [22]. The chemokine receptor profile of double negative T cells still needs to be defined.

Even though definitions of Th17 cells vary, these cells thus seem to be increased in peripheral blood of pSS patients. Likely, both circulating and local Th17 cells contribute to serum levels of IL-17, although, as mentioned before, also other cell types are able to produce this pivotal Th17 cell cytokine. Nearly all studies showed increased IL-17 (i.e. IL-17A) levels in serum of pSS patients. However, a correlation between serum IL-17 levels and disease activity has not been reported [9,11,20,21,23,74]. Reksten et al. showed that serum levels of IL-17 were higher in pSS patients with GCs in their minor salivary gland biopsies compared to GC-negative patients [21]. Subsequently they observed that serum IL-17 levels correlated positively with levels of anti-Ro/SSA and anti-La/SSB autoantibodies, but not with clinical features of the disease [74]. These findings, together with our observations that circulating Th17 cells are increased only in patient cohorts with moderate-to-high systemic disease activity, but not in patients with low systemic disease activity, indicate that numbers of circulating Th17 cell and levels of serum IL-17 are associated with disease severity and/or with certain stages of the disease. In line with this notion, a positive correlation between disease duration and levels of circulating Th17 cells and serum IL-17 was observed in mouse models of pSS [75].

Th17 cells in mouse models of pSS

Mouse models of pSS are very useful to study aspects of the disease that otherwise cannot be addressed. Although these models often only mimic part of the pathology found in pSS patients, they do give important insights in the role of individual cells or cytokines, and provide the opportunity to study disease kinetics.

The most extensively used animal model to study pSS is the C57BL/6.NOD-*Aec1Aec2* mouse. These mice harbor two susceptibility loci that promote a spontaneous pSS-like autoimmune phenotype, featuring salivary and lacrimal gland dysfunction leading to decreased saliva production and ocular inflammation [76]. In these mice, RORγt, IL-17 and IL-17R mRNA expression were found in the salivary (submandibular) glands [9]. Elevated IL-17 and IL-17R expression was also seen at the ocular surface [77]. Correspondingly, Th17 cells were present in the immune infiltrates in salivary and lacrimal glands of

affected mice [24,77]. However, only low levels of IL-17 were found in serum of these mice [9]. Despite these low serum IL-17 levels, IL-17 seems to play an important role in pSS-like disease in this model. This is illustrated by the observation that IL-17-deficiency in C57BL/6.NOD-*Aec1Aec2* mice significantly reduces the pro-inflammatory response in the salivary glands and restores normal secretory function, particularly in female animals [24]. In addition, these mice exhibit an altered specificity of auto-antibodies compared to IL-17-sufficient C57BL/6.NOD-*Aec1Aec2* mice, illustrating the role of IL-17 in promoting autoreactive B cells responses. This effect is probably mediated by affecting the numbers of both GC B cells and plasma cells [24]. These data suggest that IL-17 is particularly pathogenic at the site of inflammation. This is further supported by a model in which SS-non-susceptible C57BL/6J mice received local IL-17A gene transfer in the salivary glands, resulting in glandular inflammation, autoantibody production and decreased saliva production [69]. In addition to pro-inflammatory roles of IL-17 in C57BL/6.NOD-*Aec1Aec2* mice, a recent study also shows that T cell receptor repertoires of Th1 and Th17 cells in the salivary glands are limited compared to wild type controls, particularly in female animals [25], suggesting they may be skewed towards recognition of autoantigens.

In a second mouse model of pSS, disease is induced by immunization with autoantigenic peptides derived from salivary glands [78]. Also in these mice, Th17 cells are abundantly present in the salivary gland infiltrates and draining lymph nodes, and are the main IL-17 producing T cell subset [78]. In parallel, these mice have high serum levels of IL-6 and TGFβ, which are essential cytokines for Th17 differentiation. Importantly, IL-17-deficient mice immunized with salivary gland peptides are completely protected from disease development and adoptive transfer of Th17 cells (polarized in culture) to these mice restores the autoimmune phenotype [26]. Also a third mouse model, in which RORγt is overexpressed, illustrates the importance of Th17 cells in development of pSS-like disease [27]. These mice exhibit increased IL-17 production by T cells and concomitantly pSS-like features including salivary and lacrimal gland inflammation and autoantibody production [27]. Increased expression of CCR6 was found on splenic $CD4^+$ T cells in these mice, and the ligand for CCR6 (i.e. CCL20) was abundantly expressed in the salivary glands, enabling homing of circulating Th17 cells to these glands [27].

These models not only reveal that Th17 cells are crucial cells for development of pSS-like disease, but also give important clues about their relevance at different time points of disease onset and progression. In the C57BL/6.NOD-*Aec1Aec2* mice, IL-17, IL-23 and RORγt expression increase when the infiltrates arise in the salivary glands, whereas they drop again after development of full-blown disease [9]. These findings suggest that Th17 cells may play a local temporal role at early stages of the disease. However, before the function of Th17 cells becomes apparent, Th1 cells appear to be involved. Even before infiltrates are formed in the salivary glands, levels of Tbet, the transcription

factor driving Th1 cell differentiation, are increased in submandibular glands, in line with the crucial role for IFNγ in the pre-clinical onset of disease in NOD mice [9,79]. This temporal balance between Th1 and Th17 cells in the glandular tissue may determine the development of the autoimmune phenotype. This is further illustrated by gene therapy of these C57BL/6.NOD-*Aec1Aec2* mice with IL-27, a cytokine that promotes Th1 and inhibits Th17 development. Initiation of treatment after disease onset, i.e. at a time point when Th17 cells are thought to play a role, is more effective than treatment before disease onset, i.e. when Th1 cells are involved [28]. Also in the salivary gland peptide-immunized model, first Th1 cells are increased in the salivary glands, and later on Th17 cells predominate [26].

Taken together, there is strong evidence in mice that Th17 cells are a driving force in the pathogenesis of pSS(-like) disease. The pSS mouse models further indicate that Th17 cells and IL-17, are particularly involved in the early phase of disease, a finding that may be more challenging to confirm in pre-clinical disease in humans.

Th17/Treg imbalance in pSS

Autoimmune diseases are frequently linked to an altered Th17/Treg ratio and commitment to one of these lineages is tightly regulated by distinct signaling molecules [80]. Available evidence indicates that there is, however, no imbalance in proportions of effector Th17 cells and Treg cells in pSS patients, as both subsets are equally increased in the periphery of pSS patients with moderate systemic disease activity [8]. Furthermore, the numbers of both Th17 cells and FoxP3+ cells in minor salivary gland tissue correlate positively with focus score/grade of inflammation [11,81]. It is not known though whether the population of FoxP3+CD4+ T cells in pSS patients is functionally normal and is able to suppress effector T cells.

Although these observations strongly argue that there is no Th17/Treg imbalance in human pSS, several mouse models suggest that an imbalance between Th17 cells and Treg cells could underlie the development of this disease (Figure 2). This imbalance may be a result of increased IL-6 in the inflammatory environment. TGFβ in the absence of IL-6 induces Treg differentiation, but TGFβ and IL-6 together promote Th17 differentiation [82]. In C57BL/6.NOD.*Aec1Aec2* mice, Treg cells are decreased compared to wild-type control mice in the lacrimal gland already at an early pre-clinical disease age, when Th17 cell numbers and IL-17A expression are increased [77]. Consistent with these findings, transient depletion of Treg cells in NOD mice led to increased salivary gland infiltrates [83]. A role for Th17/Treg imbalance in disease induction is further illustrated in mice lacking thrombospondin-1 (TSP1), an important activator of latent TGFβ in vivo [84]. These mice spontaneously develop ocular inflammation accompanied by dry eye symptoms and anti-SSA and anti-SSB antibodies [85]. Increased splenic Th17 cells and lacrimal IL-17 protein levels in these mice were accompanied by a decrease in splenic

Treg cells [85]. In vivo administration of TSP1-peptide to TSP1 knock-out mice induced formation of FoxP3+ Treg cells, and decreased Th17 cells, attenuating symptoms of disease [29].

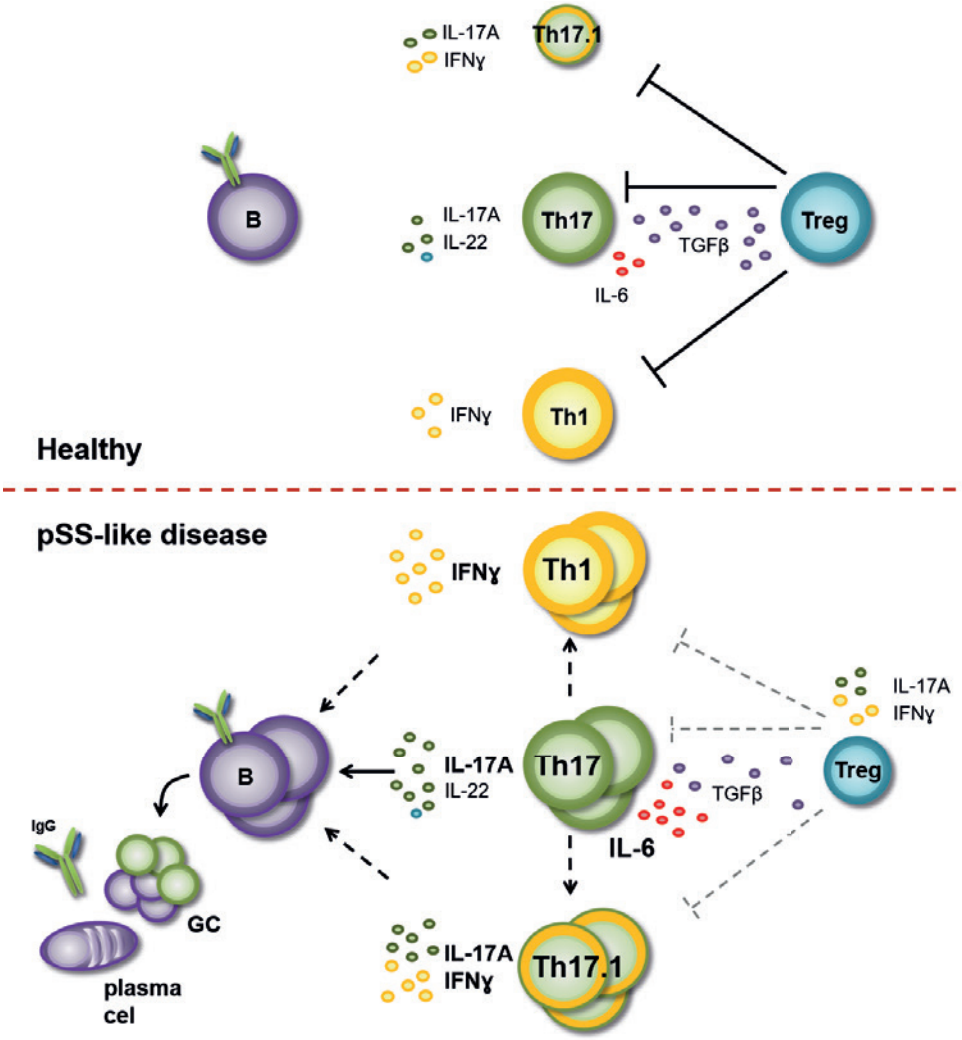

FIGURE 2 | Insights on Th17 cell plasticity from pSS mouse models. In a healthy situation, there is no inflammation in the salivary glands and at the ocular surface. Treg cells control Th1 cells, Th17 cells and the small number of Th17.1 cells present in the body. However, in mice with pSS-like disease, IL-6 levels increase, shifting the balance between Treg cells and Th17 cells. Treg cells are reduced in number, lose their regulatory capacity and sometimes start producing IL-17 and IFNγ. Simultaneously, the number of Th17 cells increases, and these cells can convert to IL-17 and IFNγ producing Th17.1 cells, or to IFNγ single producing Th1-like cells. Together, these cells can promote germinal center formation, and support differentiation of B cells into class-switched plasma and memory cells.

In a different NOD model bearing an altered MHC region (NOD.B10.H2^{b} mice), animals spontaneously develop ocular surface disease upon aging. In these aged mice, FoxP3+ Treg cells aberrantly co-express Tbet and RORγt and produce IFN-γ and IL-17. At the same time, aged Treg cells in NOD.B10.H2^{b} mice exhibit lower suppressive capacity compared to Treg cells from young mice. Transfer of CD4^{+}CD25^{+} Treg cells from these aged mice into T and B cell-deficient (RAG1-deficient) animals induced a similar phenotype of periductal inflammation in the lacrimal glands as transfer of CD4^{+}CD25^{-} T helper cells [30]. These results confirm that Treg cells can acquire pro-inflammatory features associated with Th1 and Th17 cells.

Together, these murine models illustrate that not only the enhanced pro-inflammatory features of Th17 cells can promote disease, but that changes in Treg cells, both in number or function, may contribute to disease progression. Functional assays with human Treg cells from pSS patients could clarify whether decreased suppressive capacity or even pro-inflammatory capacity of Treg cells also plays a role in the development of disease in patients.

Plasticity of Th17 cells in pSS

Both in humans and mice, Th17 cells are not a "fixed" subset, but can acquire features from, or differentiate towards, other effector types, i.e. Th1 and Treg cells [86]. The transformation of typical Th17 cells towards Th17.1 cells is most intensively studied, especially in the context of autoimmunity [87] (Figure 2). These Th17.1 cells co-express CXCR3 and CCR6 and produce both IL-17 and IFN-γ.

Plasticity of Th17 cells in humans is, however, a relatively unexplored field. In patients with Crohn's disease, Th17.1 cells are pathogenic and promote chronic inflammation [88]. Furthermore, in patients with multiple sclerosis Th17.1 cells reacted strongly against self-antigens[89]. The factors that drive this plasticity in humans are not fully understood, but some indications may come from a murine model of experimental autoimmune encephalomyelitis. In these mice, transformation of Th17 cells to both IFN-γ-single producing Th1 cells and IFNγ/IL-17 double producing Th17.1 cells was driven by high IL-7 expression [90]. Interestingly, in salivary gland tissue of pSS patients, IL-7 is abundantly present [69], and may drive the plasticity of Th17 cells to IFN-γ single or double producing Th17.1 cells. Besides plasticity of Th17 cells, plasticity of other effector T cell subsets may also contribute to the pathology seen in pSS patients. For example, it has been shown in mice with experimental autoimmune encephalomyelitis (EAE) that Tfh cells can aberrantly express IL-17, and these IL-17-producing Tfh cells could augment the formation of autoreactive B cells by stimulating ectopic germinal center formation and impairing chemotactic migration of B cells out of the germinal center [47]. Aberrant expression of IL-17 by Tfh cells may also play a role in later phases of pSS pathogenesis when germinal center containing ectopic lymphoid tissue is present.

The possible contribution of Th17 cell plasticity to pathogenicity in pSS is further illustrated by a Sjögren mouse model driven by an immune response against the M3 muscarinic acetylcholine receptor (M3R) [91]. Under physiological conditions, cholinergic stimulation of these receptors leads to an increase of saliva secretion. Immunization of M3R-deficient mice with M3R peptides induces a strong immune response that results in formation of autoantibodies directed against M3R, that block the cholinergic stimulation and lead to reduced saliva production. Such blocking autoantibodies against these receptors have also been described in human pSS patients [91,92]. Besides autoantibody formation, the M3R immunized mice exhibit an increase in IL-17A and IFNγ producing Th17.1 cells in the spleen [31]. Adoptive transfer of splenocytes from these mice into T- and B-lymphocyte deficient animals induced severe pSS-like disease with anti-M3R autoantibody formation and Th17.1 cells infiltrating the salivary glands associated with decreased saliva production [31]. Treatment of these mice with a RORγt antagonist after the transfer of splenocytes, reduced both IL-17 and IFNγ *in vivo*, and partially abrogated disease [32]. These data suggest that Th17 cells could co-produce IL-17 and IFNγ, or that Th17 cells might convert to Th1 cells post-transfer. Although this model is not completely equivalent to pSS pathogenesis, it does show many similarities with human disease, including inflammation specifically of the salivary and lacrimal glands, but not of the intestines or liver, and a similar cellular composition of mononuclear infiltrates in the glands.

In summary, although the data are scarce, they indicate that plasticity of Th17 cells towards more pathogenic Th17.1 cells or Th1 cells may contribute to disease progression in pSS.

Effect of treatment on Th17 cells/IL-17 in pSS

Immunomodulatory treatment of pSS patients may provide important insights into the role of various cell types in pathogenesis. One of the first biological DMARDs that was clinically tested in pSS patients was the TNF-alpha inhibitor etanercept. Markers of activation on B cells and CD4+ T cells were not significantly altered by etanercept treatment, in line with a lack of clinical benefit [93]. Plasma IL-17 levels were also unaffected [11]. Subsequently, several studies assessed the efficacy of B cell depletion therapy with rituximab. Although the clinical benefits are a matter of debate [94], many biological parameters are affected, including Th17 cell-related biomarkers [95]. Rituximab treatment resulted in decreased IL-17 protein expression in minor salivary gland tissue of pSS patients, despite the finding that factors that are important for maintenance of Th17 cell , viz. pSTAT3 and IL-23, were not altered [13]. Dendritic cells and macrophages are major sources of IL-23 and these cells are likely not affected by B cell depletion therapy [57]. In addition to reduced IL-17 expression in the salivary glands, we found decreased frequencies of circulating IL-17+CD4+ T cells and to a smaller

extent also chemokine receptor-defined Th17 cells after rituximab treatment [17]. The decrease of IL-17+CD4+T cells over time correlated with decreasing levels of IgG and autoantibodies, suggesting that IL-17 and autoantibody formation are somehow related. Also serum levels of IL-17 decreased in this study [17]. In a previous study, we found that serum IL-6 levels were also significantly reduced by rituximab treatment [20]. We therefore postulated that the effect of B cell depletion therapy on Th17 cells and IL-17 production is mediated by depletion of IL-6-producing B cells [17]. As mentioned before, IL-6 supports Th17 cell differentiation from naïve T cells and is important for the induction of RORγ and IL-17 [96]. In summary, rituximab affects Th17 cells locally and systemically, although the mechanism of this effect and its contribution to amelioration of disease remains to be established.

As T cell activation and crosstalk between B cells and CD4+ T cells are important features in pSS pathogenesis, T-cell co-stimulation appears to be a suitable target for treatment. Abatacept, which limits CD28-mediated co-stimulation and thereby activation of T cells, was able to reduce systemic disease activity (ESSDAI scores) in pSS patients in a small open-label study [97]. Although the fraction of circulating Tfh cells was significantly reduced by treatment, circulating Th17 cells (CD4+CD45RA-FoxP3-CXCR5-CXCR3-CCR4+CCR6+) were not affected. Also serum levels of IL-17 did not change at a group level, but two patients with the highest baseline levels did show a reduction in serum IL-17 [8]. Apparently, the clinical efficacy of abatacept is not the result of a significant effect on the Th17/IL-17 axis. It is not known yet whether IL-17-producing cells in glandular tissue of pSS patients are affected by abatacept treatment.

So far IL-17-targeting therapies have not been tested in pSS patients. A case report from a patient with psoriasis and pSS treated with ustekinumab, a monoclonal antibody directed against the p40 protein subunit shared by IL-12 and IL-23, showed beneficial effects not only on cutaneous disease, but also on joint involvement [98]. Effects on other pSS-related symptoms, such as dryness, were not reported. A placebo-controlled trial with the IL-6R antagonist tocilizumab is currently ongoing in pSS (NCT01782235). As naïve CD4+ T cells express IL-6R and IL-6 signaling is important for Th17 differentiation, an effect of tocilizumab on Th17 cells is expected and these results may provide more insight into the contributions of Th17 cells to disease activity.

CONCLUSION

Both human and mouse studies clearly indicate that Th17 cells/IL-17 producing T cells are involved in local inflammation in pSS and SS-like disease. Their contribution to systemic disease is more enigmatic. Th17 cells are elevated in the periphery of a subgroup of pSS patients and higher systemic Th17 cell activity (serum IL-17 level, CD161+RORγ+CD4+ cell frequency) correlates with increased autoantibody titers. However, it remains to be

established if Th17 cells contribute directly to pathogenesis of human pSS. A pathogenic role for Th17 cells is more evident in mouse models of SS, where Th17 cells appear to play a key role in development of the autoimmune phenotype. Pathogenicity of Th17 cells in pSS is possibly linked to plasticity of this cell subset. In particular plasticity towards Th17.1 cells, co-expressing IL-17 and IFN-γ (and CCR6 and CXCR3) may support chronic inflammation and B cell activation in pSS patients (Figure 1&2). Furthermore, mouse models indicate that the major contribution of Th17 cells to disease pathology may be temporal, early and locally in affected tissues. Future studies are needed to clarify Th17 cell phenotypes in glandular infiltrates and to address their contribution to disease onset and progression.

REFERENCES

1 Brito-Zerón P, Baldini C, Bootsma H, *et al.* Sjögren syndrome. *Nat Rev Dis Prim* 2016;**2**:16047.

2 Lessard CJ, Li H, Adrianto I, *et al.* Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjogren's syndrome. *Nat Genet* 2013;**45**:1284–92.

3 Voulgarelis M, Tzioufas AG. Pathogenetic mechanisms in the initiation and perpetuation of Sjogren's syndrome. *Nat Rev* 2010;**6**:529–37.

4 Risselada AP, Looije MF, Kruize AA, *et al.* The role of ectopic germinal centers in the immunopathology of primary Sjogren's syndrome: a systematic review. *Semin Arthritis Rheum* 2013;**42**:368–76.

5 Bombardieri M, Lewis M, Pitzalis C. Ectopic lymphoid neogenesis in rheumatic autoimmune diseases. *Nat Rev Rheumatol* 2017;**13**:141–54.

6 Kroese FG, Abdulahad WH, Haacke E, *et al.* B-cell hyperactivity in primary Sjogren's syndrome. *Expert Rev Clin Immunol* 2014;**10**:483–99.

7 Corneth OBJ, Verstappen GMP, Paulissen SMJ, *et al.* Enhanced Bruton's tyrosine kinase activity in peripheral blood B lymphocytes of autoimmune disease patients. *Arthritis Rheumatol* 2017;**69**:1313-24.

8 Verstappen GM, Meiners PM, Corneth OBJ, *et al.* Abatacept attenuates T follicular helper-cell-dependent B-cell hyperactivity in primary Sjögren's syndrome. *Arthritis Rheumatol* 2017;**69**:1850-61.

9 Nguyen CQ, Hu MH, Li Y, *et al.* Salivary gland tissue expression of interleukin-23 and interleukin-17 in Sjögren's syndrome: findings in humans and mice. *Arthritis Rheum* 2008;**58**:734–43.

10 Sakai A., Sugawara Y, Kuroishi T, *et al.* Identification of IL-18 and Th17 cells in salivary glands of patients with Sjogren's syndrome, and amplification of IL-17-mediated secretion of inflammatory cytokines from salivary gland cells by IL-18. *J Immunol (Baltimore, Md 1950)* 2008;**181**:2898–906.

11 Katsifis GE, Rekka S, Moutsopoulos NM, *et al.* Systemic and Local Interleukin-17 and Linked Cytokines Associated with Sjögren's Syndrome Immunopathogenesis. *Am J Pathol* 2009;**175**:1167–77.

12 Hirota K, Duarte JH, Veldhoen M, *et al.* Fate mapping of IL-17-producing T cells in inflammatory responses. *Nat Immunol* 2011;**12**:255–63.

13 Ciccia F, Guggino G, Rizzo A, *et al.* Rituximab modulates IL-17 expression in the salivary glands of patients with primary Sjögren's syndrome. *Rheumatology (Oxford)* 2014;**53**:1313–20.

14 Liu R, Gao C, Chen H, *et al.* Analysis of Th17-associated cytokines and clinical correlations in patients with dry eye disease. *PLoS One* 2017;**12**:e0173301.

15 Ciccia F, Guggino G, Rizzo A, *et al.* Potential involvement of IL-22 and IL-22-producing cells in the inflamed salivary glands of patients with Sjogren's syndrome. *Ann Rheum Dis* 2012;**71**:295–301.

16 Blokland SLM, Hillen MR, Kruize AA, *et al.* Elevated CCL25 and CCR9-Expressing T Helper Cells in Salivary Glands of Primary Sjögren's Syndrome Patients: Potential New Axis in Lymphoid Neogenesis. *Arthritis Rheumatol* 2017;**69**:2038-51.

17 Verstappen GM, Kroese FGM, Meiners PM, *et al.* B cell depletion therapy normalizes circulating follicular TH cells in primary Sjögren syndrome. *J Rheumatol* 2017;**44**:49-58.

18 Bikker A, Moret FM, Kruize AA, *et al.* IL-7 drives Th1 and Th17 cytokine production in patients with primary SS despite an increase in CD4 T cells lacking the IL-7Ralpha. *Rheumatology (Oxford)* 2012;**51**:996–1005.

19 Kwok SK, Cho ML, Her YM, *et al.* TLR2 ligation induces the production of IL-23/IL-17 via IL-6, STAT3 and NF-kB pathway in patients with primary Sjogren's syndrome. *Arthritis Res Ther* 2012;**14**:R64.

20 Pollard RP, Abdulahad WH, Bootsma H, *et al.* Predominantly proinflammatory cytokines decrease after B cell depletion therapy in patients with primary Sjogren's syndrome. *Ann Rheum Dis* 2013;**72**:2048–50.

21 Reksten TR, Jonsson M V, Szyszko EA, *et al.* Cytokine and autoantibody profiling related to histopathological features in primary Sjogren's syndrome. *Rheumatology (Oxford)* 2009;**48**:1102–6.

22 Alunno A, Bistoni O, Bartoloni E, *et al.* IL-17-producing CD4-CD8- T cells are expanded in the peripheral blood, infiltrate salivary glands and are resistant to corticosteroids in patients with primary Sjogren's syndrome. *Ann Rheum Dis* 2013;**72**:286–92.

23 Fei Y, Zhang W, Lin D, *et al.* Clinical parameter and Th17 related to lymphocytes infiltrating degree of labial salivary gland in primary Sjogren's syndrome. *Clin Rheumatol* 2014;**33**:523-9.

24 Voigt A, Esfandiary L, Wanchoo A, *et al.* Sexual dimorphic function of IL-17 in salivary gland dysfunction of the C57BL/6.NOD-Aec1Aec2 model of Sjögren's syndrome. *Sci Rep* 2016;**6**:38717.

25 Wanchoo A, Voigt A, Sukumaran S, *et al.* Single-cell analysis reveals sexually dimorphic repertoires of Interferon-γ and IL-17A producing T cells in salivary glands of Sjögren's syndrome mice. *Sci Rep* 2017;**7**:12512.

26 Lin X, Rui K, Deng J, *et al.* Th17 cells play a critical role in the development of experimental Sjögren's syndrome. *Ann Rheum Dis* 2015;**74**:1302–10.

27 Iizuka M, Tsuboi H, Matsuo N, *et al.* A crucial role of RORγt in the development of spontaneous Sialadenitis-like Sjögren's syndrome. *J Immunol* 2015;**194**:56–67.

28 Lee B, Carcamo WC, Chiorini JA, *et al.* Gene therapy using IL-27 ameliorates Sjögren's syndrome-like autoimmune exocrinopathy. *Arthritis Res Ther* 2012;**14**:R172.

29 Contreras Ruiz L, Mir FA, Turpie B, *et al.* Thrombospondin-derived peptide attenuates Sjögren's syndrome-associated ocular surface inflammation in mice. *Clin Exp Immunol* 2017;**188**:86–95.

30 Coursey TG, Bian F, Zaheer M, *et al.* Age-related spontaneous lacrimal keratoconjunctivitis is accompanied by dysfunctional T regulatory cells. *Mucosal Immunol* 2017;**10**:743–56.

31 Iizuka M, Wakamatsu E, Tsuboi H, *et al.* Pathogenic role of immune response to M3 muscarinic acetylcholine receptor in Sjögren's syndrome-like sialoadenitis. *J Autoimmun* 2010;**35**:383–9.

32 Tahara M, Tsuboi H, Segawa S, *et al.* RORγt antagonist suppresses M3 muscarinic acetylcholine receptor-induced Sjögren's syndrome-like sialadenitis. *Clin Exp Immunol* 2017;**187**:213–24.

33 Nguyen CQ, Yin H, Lee BH, *et al.* Pathogenic effect of interleukin-17A in induction of Sjögren's syndrome-like disease using adenovirus-mediated gene transfer. *Arthritis Res Ther* 2010;**12**:R220.

34 Lee JS, Tato CM, Joyce-Shaikh B, *et al.* Interleukin-23-Independent IL-17 Production Regulates Intestinal Epithelial Permeability. *Immunity* 2015;**43**:727–38.

35 Dudakov JA, Hanash AM, van den Brink MRM. Interleukin-22: Immunobiology and Pathology. *Annu Rev Immunol* 2015;**33**:747–85.

36 Abusleme L, Moutsopoulos N. IL-17: overview and role in oral immunity and microbiome. *Oral Dis* 2017;**23**:854-65.

37 Vakrakou AG, Polyzos A, Kapsogeorgou EK, *et al.* Impaired anti-inflammatory activity of PPARγ in the salivary epithelia of Sjögren's syndrome patients imposed by intrinsic NF-κB activation. *J Autoimmun* 2018;**86**:62-74.

38 Iwakura Y, Ishigame H, Saijo S, *et al.* Functional Specialization of Interleukin-17 Family Members. *Immunity* 2011;**34**:149–62.

39 van Hamburg JP, Asmawidjaja PS, Davelaar N, *et al.* Th17 cells, but not Th1 cells, from patients with early rheumatoid arthritis are potent inducers of matrix metalloproteinases and proinflammatory cytokines upon synovial fibroblast interaction, including autocrine interleukin-17A production. *Arthritis Rheum* 2011;**63**:73–83.

40 Pérez P, Kwon Y-J, Alliende C, *et al.* Increased acinar damage of salivary glands of patients with Sjögren's syndrome is paralleled by simultaneous imbalance of matrix metalloproteinase 3/ tissue inhibitor of metalloproteinases 1 and matrix metalloproteinase 9/tissue inhibitor of metalloproteinases 1 ratios. *Arthritis Rheum* 2005;**52**:2751–60.

41 Durbin K, Casola SS, Rajewsky K, *et al.* Pulmonary Neutrophilia Changes in the Airway and Drives Derived IL-17 Mediates Epithelial – T Cell T Cell–Derived IL-17 Mediates Epithelial Changes in the Airway and Drives Pulmonary Neutrophilia. *J Immunol* 2017;**8**:3100–11.

42 Mitsdoerffer M, Lee Y, Jager A, *et al.* Proinflammatory T helper type 17 cells are effective B-cell helpers. *Proc Natl Acad Sci U S A* 2010;**107**:14292–7.

43 Subbarayal B, Chauhan SK, Di Zazzo A, *et al.* IL-17 Augments B Cell Activation in Ocular Surface Autoimmunity. *J Immunol* 2016;**197**:3464–70.

44 Pfeifle R, Rothe T, Ipseiz N, *et al.* Regulation of autoantibody activity by the IL-23–TH17 axis determines the onset of autoimmune disease. *Nat Immunol* 2016;**18**:104–13.

45 Hsu HC, Yang P, Wang J, *et al.* Interleukin 17-producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice. *Nat Immunol* 2008;**9**:166–75.

46 Rangel-Moreno J, Carragher DM, de la Luz Garcia-Hernandez M, *et al.* The development of inducible bronchus-associated lymphoid tissue depends on IL-17. *Nat Immunol* 2011;**12**:639–46.

47 Peters A, Pitcher LA, Sullivan JM, *et al.* Th17 cells induce ectopic lymphoid follicles in central nervous system tissue inflammation. *Immunity* 2011;**35**:986–96.

48 Barone F, Nayar S, Campos J, *et al.* IL-22 regulates lymphoid chemokine production and assembly of tertiary lymphoid organs. *Proc Natl Acad Sci U S A* 2015;**112**:11024–9.

49 Patel DD, Kuchroo VK. Th17 Cell Pathway in Human Immunity: Lessons from Genetics and Therapeutic Interventions. *Immunity* 2015;**43**:1040–51.

50 Kang EH, Lee YJ, Hyon JY, *et al.* Salivary cytokine profiles in primary Sjögren's syndrome differ from those in non-Sjögren sicca in terms of TNF-α levels and Th-1/Th-2 ratios. *Clin Exp Rheumatol*;**29**:970–6.

51 Noordenbos T, Blijdorp I, Chen S, *et al.* Human mast cells capture, store, and release bioactive, exogenous IL-17A. *J Leukoc Biol* 2016;**100**:453–62.

52 Wolk K, Kunz S, Witte E, *et al.* IL-22 increases the innate immunity of tissues. *Immunity* 2004;**21**:241–54.

53 Ciccia F, Guggino G, Rizzo A, *et al.* Interleukin (IL)-22 receptor 1 is over-expressed in primary Sjogren's syndrome and Sjögren-associated non-Hodgkin lymphomas and is regulated by IL-18. *Clin Exp Immunol* 2015;**181**:219–29.

54 Bettelli E, Carrier Y, Gao W, *et al.* Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006;**441**:235–8.

55 Szyszko E a, Brokstad K a, Oijordsbakken G, *et al.* Salivary glands of primary Sjögren's syndrome patients express factors vital for plasma cell survival. *Arthritis Res Ther* 2011;**13**:R2.

56 Kwok SK, Lee J, Yu D, *et al.* A pathogenetic role for IL-21 in primary Sjogren syndrome. *Nat Rev* 2015;**11**:368–74.

57 Korn T, Bettelli E, Oukka M, *et al.* IL-17 and Th17 Cells. *Annu Rev Immunol* 2009;**27**:485–517.

58 Zohar Y, Wildbaum G, Novak R, *et al.* CXCL11-dependent induction of FOXP3-negative regulatory T cells suppresses autoimmune encephalomyelitis. *J Clin Invest* 2014;**124**:2009–22.

59 Ogawa N, Ping L, Zhenjun L, *et al.* Involvement of the interferon-gamma-induced T cell-attracting chemokines, interferon-gamma-inducible 10-kd protein (CXCL10) and monokine induced by interferon-gamma (CXCL9), in the salivary gland lesions of patients with Sjögren's syndrome. *Arthritis Rheum* 2002;**46**:2730–41.

60 Mavragani CP, Crow MK. Activation of the type I interferon pathway in primary Sjogren's syndrome. *J Autoimmun* 2010;**35**:225–31.

61 Hirota K, Yoshitomi H, Hashimoto M, *et al.* Preferential recruitment of CCR6-expressing Th17 cells to inflamed joints via CCL20 in rheumatoid arthritis and its animal model. *J Exp Med* 2007;**204**:2803–12.

62 Annunziato F, Cosmi L, Santarlasci V, *et al.* Phenotypic and functional features of human Th17 cells. *J Exp Med* 2007;**204**:1849–61.

63 Xanthou G, Polihronis M, Tzioufas AG, *et al.* "Lymphoid" chemokine messenger RNA expression by epithelial cells in the chronic inflammatory lesion of the salivary glands of Sjögren's syndrome patients: possible participation in lymphoid structure formation. *Arthritis Rheum* 2001;**44**:408–18.

64 Blokland S, Hillen M, Meller S, *et al.* THU0241 Decreased circulating CXCR3+CCR9+ th cells are associated with elevated levels of their ligands CXCL10 and CCL25 in the salivary gland of patients with SJÖGREN'S syndrome to potentially facilitate concerted migration. *Ann Rheum Dis* 2017;**76**:295–295.

65 Tandon M, Perez P, Burbelo PD, *et al.* Laser microdissection coupled with RNA-seq reveal cell-type and disease-specific markers in the salivary gland of Sjögren's syndrome patients. *Clin Exp Rheumatol* 2017;**35**:777-85.

66 Barone F, Bombardieri M, Rosado MM, *et al.* CXCL13, CCL21, and CXCL12 expression in salivary glands of patients with Sjogren's syndrome and MALT lymphoma: association with reactive and malignant areas of lymphoid organization. *J Immunol* 2008;**180**:5130–40.

67 Krausgruber T, Blazek K, Smallie T, *et al.* IRF5 promotes inflammatory macrophage polarization and TH1-TH17 responses. *Nat Immunol* 2011;**12**:231–8.

68 Chen Y, Chauhan SK, Tan X, *et al.* Interleukin-7 and -15 maintain pathogenic memory Th17 cells in autoimmunity. *J Autoimmun* 2017;**77**:96–103.

69 Bikker A, van Woerkom JM, Kruize AA, *et al.* Increased expression of interleukin-7 in labial salivary glands of patients with primary Sjögren's syndrome correlates with increased inflammation. *Arthritis Rheum* 2010;**62**:969–77.

70 Sisto M, Lorusso L, Lisi S. TLR2 signals via NF-κB to drive IL-15 production in salivary gland epithelial cells derived from patients with primary Sjögren's syndrome. *Clin Exp Med* 2017;**17**:341–50.

71 Cosmi L, De Palma R, Santarlasci V, *et al.* Human interleukin 17–producing cells originate from a CD161 $^{+}$ CD4 $^{+}$ T cell precursor. *J Exp Med* 2008;**205**:1903–16.

72 Zhao L, Nocturne G, Haskett S, *et al.* Clinical relevance of RORγ positive and negative subsets of CD161+CD4+ T cells in primary Sjögren's syndrome. *Rheumatology (Oxford)* 2017;**56**:303–12.

73 Li XXY, Wu ZB, Ding J, *et al.* Role of the frequency of blood CD4(+) CXCR5(+) CCR6(+) T cells in autoimmunity in patients with Sjogren's syndrome. *Biochem Biophys Res Commun* 2012;**422**:238–44.

74 Vogelsang P, Brokstad KA. Abstracts Meeting Abstracts from The 13th International Symposium on Sjögren's Syndrome Meeting abstracts A Tissue-Based Map of the Human. *Scand J Immunol* 2015;**81**:385.

75 Alunno A, Carubbi F, Bartoloni E, *et al.* Unmasking the pathogenic role of IL-17 axis in primary Sjogren's syndrome: A new era for therapeutic targeting? *Autoimmun Rev* 2014;**13**:1167-73.

76 Cha S, Nagashima H, Brown VB, *et al.* Two NOD *Idd* -associated intervals contribute synergistically to the development of autoimmune exocrinopathy (Sjögren's syndrome) on a healthy murine background. *Arthritis Rheum* 2002;**46**:1390–8.

77 You I-C, Bian F, Volpe EA, *et al.* Age-Related Conjunctival Disease in the C57BL/6.NOD-*Aec1Aec2* Mouse Model of Sjögren Syndrome Develops Independent of Lacrimal Dysfunction. *Investig Opthalmology Vis Sci* 2015;**56**:2224.

78 Lin X, Song J -x., Shaw P-C, *et al.* An autoimmunized mouse model recapitulates key features in the pathogenesis of Sjogren's syndrome. *Int Immunol* 2011;**23**:613–24.

79 Cha S, Brayer J, Gao J, *et al.* A dual role for interferon-gamma in the pathogenesis of Sjogren's syndrome-like autoimmune exocrinopathy in the nonobese diabetic mouse. *Scand J Immunol* 2004;**60**:552–65.

80 Geng J, Yu S, Zhao H, *et al.* The transcriptional coactivator TAZ regulates reciprocal differentiation of TH17 cells and Treg cells. *Nat Immunol* 2017;**18**:800–12.

81 Christodoulou MI, Kapsogeorgou EK, Moutsopoulos NM, *et al.* Foxp3+ T-Regulatory Cells in Sjögren's Syndrome. *Am J Pathol* 2008;**173**:1389–96.

82 Diller ML, Kudchadkar RR, Delman KA, *et al.* Balancing Inflammation: The Link between Th17 and Regulatory T Cells. *Mediators Inflamm* 2016;**2016**:1–8.

83 Ellis JS, Wan X, Braley-Mullen H. Transient depletion of $CD4^+$ $CD25^+$ regulatory T cells results in multiple autoimmune diseases in wild-type and B-cell-deficient NOD mice. *Immunology* 2013;**139**:179–86.

84 Thrombospondin-1 Is a Major Activator of TGF-β1 In Vivo. *Cell* 1998;**93**:1159–70.

85 Turpie B, Yoshimura T, Gulati A, *et al.* Sjögren's syndrome-like ocular surface disease in thrombospondin-1 deficient mice. *Am J Pathol* 2009;**175**:1136–47.

86 Muranski P, Restifo NP. Essentials of Th17 cell commitment and plasticity. *Blood* 2013;**121**:2402–14.

87 Peters A, Lee Y, Kuchroo VK. The many faces of Th17 cells. *Curr Opin Immunol* 2011;**23**:702–6.

88 Ramesh R, Kozhaya L, McKevitt K, *et al.* Pro-inflammatory human Th17 cells selectively express P-glycoprotein and are refractory to glucocorticoids. *J Exp Med* 2014;**211**:89–104.

89 Paroni M, Maltese V, De Simone M, *et al.* Recognition of viral and self-antigens by TH1 and TH1/TH17 central memory cells in patients with multiple sclerosis reveals distinct roles in immune surveillance and relapses. *J Allergy Clin Immunol* 2017;**140**:797-808.

90 Arbelaez CA, Glatigny S, Duhen R, *et al.* IL-7/IL-7 Receptor Signaling Differentially Affects Effector $CD4^+$ T Cell Subsets Involved in Experimental Autoimmune Encephalomyelitis. *J Immunol* 2015;**195**:1974–83.

91 Matsui M, Motomura D, Karasawa H, *et al.* Multiple functional defects in peripheral autonomic organs in mice lacking muscarinic acetylcholine receptor gene for the M3 subtype. *Proc Natl Acad Sci U S A* 2000;**97**:9579–84.

92 Bacman S, Berra A, Sterin-Borda L, *et al.* Muscarinic acetylcholine receptor antibodies as a new marker of dry eye Sjögren syndrome. *Invest Ophthalmol Vis Sci* 2001;**42**:321–7.

93 Moutsopoulos NM, Katsifis GE, Angelov N, *et al.* Lack of efficacy of etanercept in Sjogren syndrome correlates with failed suppression of tumour necrosis factor and systemic immune activation. *Ann Rheum Dis* 2008;**67**:1437–43.

94 Bootsma H, Kroese FGM, Vissink A. Editorial: Rituximab in the Treatment of Sjögren's Syndrome: Is It the Right or Wrong Drug? *Arthritis Rheumatol* 2017;**69**:1346–9.

95 Verstappen GM, van Nimwegen JF, Vissink A, *et al.* The value of rituximab treatment in primary Sjögren's syndrome. *Clin Immunol* 2017;**182**:62-71.

96 Kimura A, Naka T, Kishimoto T. IL-6-dependent and -independent pathways in the development of interleukin 17-producing T helper cells. *Proc Natl Acad Sci* 2007;**104**:12099–104.

97 Meiners PM, Vissink A, Kroese FG, *et al.* Abatacept treatment reduces disease activity in early primary Sjogren's syndrome (open-label proof of concept ASAP study). *Ann Rheum Dis* 2014;**73**:1393-6.

98 Chimenti MS, Talamonti M, Novelli L, *et al.* Long-term ustekinumab therapy of psoriasis in patients with coexisting rheumatoid arthritis and Sjögren syndrome. Report of two cases and review of literature. *J Dermatol Case Rep* 2015;**9**:71–5.