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EXPERT
REVIEWS

B-cell hyperactivity in primary Sjögren's syndrome

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Primary Sjögren's syndrome (pSS) is characterized by mononuclear inflammatory infiltrates and IgG plasma cells in salivary and lacrimal glands which lead to irreversible destruction of the glandular tissue and is accompanied by sensation of dryness of mouth and eyes. B cells play a central role in the immunopathogenesis and exhibit signs of hyperactivity. Hyperactivity of B cells is the consequence of the coordinated and integrated action of stimulation of the B-cell receptor, CD40 and toll-like receptors in the presence of appropriate cytokines. As discussed, overexpression of type I IFN and BAFF on one hand and IL-6 and IL-21 on the other hand are critically involved in the enhanced plasma cell formation in pSS patients. Hyperactivity of B cells results in secretion of autoantibodies and production of various cytokines. These insights in the role of B cells in the pathogenetic process of pSS offer ample targets for successful therapeutical intervention in pSS.

KEYWORDS: autoantibodies • autoimmunity • B cells • chemokines • cytokines • primary Sjögren's syndrome
• pathogenesis • salivary gland

Primary Sjögren's syndrome (pSS) is a chronic systemic inflammatory autoimmune disease that primarily affects the exocrine glands, particularly the salivary and lacrimal glands [1]. The most prominent histopathological finding in salivary gland tissue is the presence of focal mononuclear infiltrates of T and B cells and other cells, including plasma cells, macrophages, myeloid and plasmacytoid dendritic cells (PDCs) and follicular dendritic cells. These infiltrates develop progressively in association with the striated ducts within glandular tissue. The result is impaired function of the glands and ultimately irreversible destruction of glandular tissue. A disturbed function of the salivary and lacrimal glands leads to the characteristic sicca symptoms of pSS: sensation of dryness of mouth (xerostomia) and eyes (keratoconjunctivitis sicca). In a large number (40–50%) of patients, systemic manifestations are observed; almost every organ system (lungs, heart and vessels, kidneys, skin, etc.) can be involved.

Hormonal, genetic and environmental factors are all involved in the initiation of pSS [2]. pSS affects about 10-times more women than men. In most pSS patients, type I interferon (IFN) and type I IFN-induced genes and proteins are overexpressed, resulting in the so-called type I IFN signature of pSS [3–5], and suggest involvement of viruses in its pathogenesis. Genome-

wide association studies show that several genes involved in type I IFN pathway signaling (*IRF5*, *STAT4*, *IL12A*) are risk factors for development of pSS [6]. In fact, the *IRF5* locus, encoding interferon regulatory factor 5, a transcription factor that mediate type I IFN responses, is the locus most strongly associated with pSS, outside the HLA region. A variety of different viruses, for example, EBV, Coxsackie virus and cytomegalovirus, are thought to play a role in onset or triggering of pSS [7]. Especially reactivation of latent EBV in genetically and hormonally susceptible individuals could play a role in the initiation and perpetuation of the chronic inflammatory autoimmune response in the glands. Inoue *et al.* [8] postulated that binding of the exogenous ligand dioxin to the aryl hydrocarbon receptor causes lytic reactivation of EBV in B cells and salivary gland epithelial cells, resulting in immune responses in the salivary glands and possibly pSS. Available evidence indicates that the epithelium plays a dual role in the pathogenesis of pSS. On one hand, epithelial cells are a source of SSA/Ro and SSB/La autoantigens and target of the autoimmune process [9]. On the other hand, these cells serve important immunological functions, such as production and secretion of cytokines and chemokines and MHC class II-mediated presentation of antigen [10].

Table 1. Indicators of B-cell hyperactivity in primary Sjögren's syndrome patients.

Source	Observation	Ref.
Salivary glands	Predominance of B cells with progression of disease	[15]
	Presence of germinal center-like structures	[26,30,31,42,42,44,48–50]
	High frequency of IgG plasma cells	[17]
	High incidence of non-Hodgkin lymphoma	[111]
	Autoreactive B cells and plasma cells	[17,42,59–61]
	Elevated levels of B cell-associated cytokines (e.g., IL-6, IL-21, BAFF, APRIL) and chemokines (e.g., CXCL12, CXCL13)	[17,26,29–32,41,43,71–74,94,95,104]
	Presence of clonal B cell populations and plasma cells	[54,57,109,110]
Saliva, tears, serum	Presence of anti-SSA/SSB autoantibodies	[1,163,164]
	Elevated levels of B cell-associated cytokines and chemokines	[20,21,24,43,65,67–70,94,102,103,105]
Serum	Hypergammaglobulinemia	[1]
	Elevated levels of free light chains and β 2-microglobulin	[165]
	Elevated levels of soluble CD27	[119]
Peripheral blood B cells	Reduced number and frequency of CD27 ⁺ memory B cells	[46,47,115–118]
	Upregulation of CD38 on naïve B cells	[115,116,119,122,123]
Intrinsic B cell abnormalities	Polymorphisms <i>TNIP1</i> and B lymphoid tyrosine kinase	[6,128–130]
	Increased expression of CD19	[123]
	Increased expression of CD72	[132]

APRIL: A proliferation-inducing ligand; BAFF: B cell-activating factor.

The immunopathogenesis of pSS is very complex and is associated with the innate immune system and both arms of the adaptive immune system (for review see [11]). Pronounced B-cell hyperactivity appears to be a hallmark of the disease process. In TABLE 1, the signs of B-cell hyperactivity in pSS patients are summarized. This hyperactivity is reflected at diagnosis of pSS by one or more of following serological manifestations: high serum IgG levels, presence of cryoglobulins, elevated levels of free light chains, β 2-microglobulin and presence of autoantibodies such as rheumatoid factor and autoantibodies to the autoantigens SSA/Ro and SSB/La [1,11]. As discussed in this review, the inflamed glandular tissue of pSS patients is prone to humoral immune responses and generation of plasma cells. Furthermore, patients with pSS show a disturbed composition of B cell subsets in peripheral blood; there is an increased clonal expansion in the exocrine glands and pSS patients display a significantly increased risk for the development of non-Hodgkin lymphoma. The role for B cells is further reflected by the positive effects of B cell-depleting therapy with anti-CD20 monoclonal antibodies (rituximab) on disease activity as measured by the recently introduced disease activity indices European League Against Rheumatism's Sjögren's Syndrome Disease Activity Index (ESSDAI) and Patient-Reported Index (ESSPRI) [12]. Despite the acknowledged role of B cells in pSS, the reason for the hyperactivity of the B cells and their exact contribution to the pathogenetic process is still partly

understood. The coordinated and integrated stimulation of B-cell receptor (BCR), CD40 and Toll-like receptors (TLRs) together with different cytokines are all involved in this process [13,14]. Here the evidence is reviewed illustrating that in pSS, B cells are in an hyperactive state resulting in autoantibody formation and secretion of cytokines.

Histopathological basis of B-cell hyperactivity & role of chemokines

The hallmark of pSS is the presence of progressively developing focal lymphoid infiltrates around the striated ducts of salivary glands (lymphocytic sialadenitis; FIGURE 1A). Another histopathological feature of pSS is the development of lymphoepithelial lesions in salivary glands, in particular, in parotid glands. These lesions are formed by hyperplasia of the epithelium in association with lymphocytes. The histopathologic features reflect the autoimmune process and manifestations of local B-cell hyperactivity. More than 90% of the infiltrating cells in minor (labial) and major (parotid) salivary gland biopsies are T and B cells, and a minor proportion other mononuclear cells [15,16]. The occurrence of germinal centers (GCs) is a clear sign of local activation of B cells (FIGURE 1B). In addition to focal infiltrates, another characteristic histopathological feature of pSS and witness of aberrant B cell activity is the marked increase in the number of IgG (but not IgA) secreting plasma cells in the exocrine glands (FIGURE 2) [17]. These IgG plasma cells are

predominantly present within the infiltrate, whereas IgA plasma cells dominate in the parenchyma. Chemokines are the driving force for the recruitment of lymphoid cells to sites of inflammation. As such, these molecules underlie the immunopathological process in exocrine glands and contribute to B-cell hyperactivity, characteristic for pSS.

Infiltration of the glands with lymphoid cells: role of inflammatory chemokines

Different levels of organization of the infiltrates suggest to represent successive stages of development of pSS. Initially, at early stages of the disease, the infiltrates are not organized as ectopic lymphoid tissue, and most lymphoid cells are CD4⁺ Th1 cells [18]. These lymphoid cells are likely attracted to glandular tissue by the proinflammatory chemokine CXCL10 that binds to the chemokine receptor CXCR3, which is expressed on Th1 cells. In a systems biology approach, CXCL10 was identified as one of the major overexpressed genes in parotid glands of pSS patients [19]. CXCL10 levels in saliva and tears from pSS patients are increased [20,21] and higher levels of CXCL10 in saliva are clearly associated with higher salivary gland dysfunction [21]. Patients with 'pre-clinical' SS (i.e., patients with autoimmune disease and focal infiltrates in labial glands who do not yet fulfill SS criteria) have the highest CXCL10 levels in saliva, suggesting that CXCL10 is indeed involved in early inflammatory reactions of salivary glands [20].

CXCL10 is produced by a wide variety of cells and is induced after TLR stimulation. CXCL10 is strongly and synergistically upregulated by type I and type II IFN as well as by proinflammatory cytokines [22,23]. TLR ligands may include microbial (viral) antigens and autoantigens. Type I and II IFN, and proinflammatory cytokines (e.g., IL-1, IL-6, TNF- α) are all significantly overexpressed in salivary gland tissue, saliva and tears of pSS patients [24]. An important source for CXCL10 are ductal epithelial cells; ductal cells of labial salivary glands of pSS patients, but not of control patients, express CXCL10 mRNA [23]. These epithelial cells (as well as other cells) also produce type I IFN and proinflammatory cytokines, which enhance CXCL10 production by epithelial cells in an autocrine fashion [10]. The attracted IFN- γ -producing Th1 cells, further stimulate CXCL10 production, creating some sort of loop. CXCR3 is not only expressed on Th1 cells, but also by other cells including activated B cells/memory cells and IFN- γ -induced IgG plasma blasts [25]. Here-with, CXCL10 may significantly contribute to the presence of IgG plasma cells in exocrine glands. CXCR3 expressing plasma cells are present in the blood of pSS patients, and even in higher numbers than in controls [17]. Plasma cells (IgG and IgA) also express CXCR4, the receptor for the chemokine CXCL12. CXCL12 is constitutively expressed by acinar and ductal epithelial cells and is important for the normal localization of IgA plasma cells in salivary glands. In pSS patients, expression levels of CXCL12 by epithelial cells are, however, strongly elevated and this chemokine is also expressed by interstitial mononuclear cells [17,26]. CXCL12 is the most important chemoattractant for PDCs too and CXCL12 synergizes with CXCL10 in recruitment

Figure 1. Periductal infiltrates in parotid salivary gland biopsy of a primary Sjögren's syndrome patient. (A) Focal lymphoid infiltrate (arrows) in a parotid salivary gland biopsy of a patient with primary Sjögren's syndrome (H&E stain, paraffin section). **(B)** GC-like structure within a large focal infiltrate, associated with a striated duct (asterisk) of a patient with primary Sjögren's syndrome. GC: Germinal center.

of these cells [27]. PDCs are the most potent type I IFN secreting cells. High numbers of PDCs are indeed present in labial salivary gland biopsies of pSS patients [28].

Formation of ectopic lymphoid tissue: role of lymphoid chemokines

At later stages of pSS, with prolonged and continued activation, periductal infiltrates become organized to ectopic lymphoid tissue with segregation of T and B cells and presence of high endothelial venules. At this stage of the development of the lesions, the main target organs of pSS, that is, salivary and lacrimal glands, contain all elements necessary to carry out cellular and humoral immune responses, and to propagate the autoimmune process. The relative number of B cells appears to increase with the severity of the lesions (i.e., infiltration grade and focus score); in severe lesions, the majority of lymphoid cells in the foci are B lymphocytes [15]. Furthermore, numbers of IgG plasma cells are higher in labial salivary gland tissue from patients with higher focus scores [17].

Figure 2. Presence of IgA and IgG plasma cells in parotid salivary gland biopsy of a primary Sjögren's syndrome patient. Immunohistochemically stained paraffin section of a parotid gland biopsy of patient with primary Sjögren's syndrome stained for IgG (brown staining) and IgA (red staining). Numerous intensely stained IgG and IgA plasma cells are present.

Formation of ectopic lymphoid tissue requires expression of the homeostatic lymphoid chemokines CXCL12, CXCL13, CCL19 and CCL21 (reviewed by [29]). These chemokines attract naïve and memory B and T cells to the glands, and play an essential role in the organization of normal (secondary) and ectopic (tertiary) lymphoid tissues. CXCL12, CXCL13, CCL19 and CCL21 protein and mRNA are expressed or strongly upregulated in labial salivary gland tissue of pSS patients [17,26,29–32].

In various mouse models of inflammation and autoimmunity, Th17 cells and Th17 cell-derived cytokines, such as IL-17 and IL-22, contribute to the formation of ectopic lymphoid tissue in non-lymphoid organs [33,34]. IL-6 plays an important role in the generation of Th17 cells [35]. IL-6 is also overexpressed in salivary gland tissue of pSS patients (see below). The role of Th17 cells in initiating ectopic lymphoid tissue in humans is still unclear [36]. IL-17 appears to promote formation of ectopic lymphoid tissue by stimulating the production of CXCL13 and CCL19 by stromal cells [33]. At an early phase of the disease, CXCR3-expressing Th17 cells may migrate to the salivary glands under guidance of CXCL10 [37]. In line with a possible notion for Th17 cells in formation of ectopic lymphoid tissue, appreciable numbers of Th17 cells are present in (labial) salivary gland lesions of pSS patients and IL-17 mRNA and protein expression is elevated and progressively increased with higher biopsy focus scores of the glands [38–40]. Also another cytokine produced by Th17 cells, IL-22 (protein and mRNA) is overexpressed in the inflamed salivary gland biopsies of pSS patients [40].

CXCL13 is important for formation and maintenance of the ectopic lymphoid tissue and homing of (naïve) B cells to these sites. CXCL13 binds to its receptor, CXCR5, on the surface of B cells [29]. Interestingly, haplotypes of the gene encoding for CXCR5 are associated with pSS [6]. In salivary glands of pSS

patients, CXCL13 is expressed within focal lymphoid infiltrates, in endothelial cells and in epithelial cells of ducts and acini, whereas CXCL13 is absent in salivary glands of control individuals [26,30,31,41,42]. Higher levels of CXCL13 are found in serum and saliva of pSS patients, compared with controls [43]. Furthermore, pSS patients with xerostomia express higher levels of CXCL13 in whole saliva than patients without xerostomia [43]. The expression levels of CXCL13 within the infiltrate correlate well with the level of lymphoid organization of the infiltrates in labial glands in pSS patients, strongly arguing that CXCL13 is involved in organization of the ectopic lymphoid tissue [30]. Kramer *et al.* [43] observed that blocking CXCL13 in mice with SS-like disease, resulted in reduced inflammation of their salivary glands.

The CXCL13-mediated migration of B cells, in particular memory B cells, is synergistically enhanced by B cell activating factor (BAFF) of the TNF family [44]. Indeed, some studies reported an accumulation of memory (CD27⁺) B cells in minor salivary glands of pSS patients [45–47]. As will be discussed in the section about BAFF, BAFF levels are elevated in salivary gland tissue and blood from pSS patients, and play an important role in the pathogenesis of pSS. The high levels of CXCL13 and BAFF in salivary glands of pSS patients may contribute to preferential homing and sequestration of these CD27⁺ memory B cells in these glands with progression of the disease.

Formation of GCs within the ectopic lymphoid tissue

In approximately 25–30% of the pSS patients, structures resembling GCs of secondary lymphoid organs are found within the (organized) ectopic lymphoid tissue of minor (labial) and major (parotid) salivary glands (FIGURE 1B) [26,30,31,42,44,48–50]. GCs arise after T-cell-dependent antigenic stimulation and presence of these structures obviously reflect local activation of B cells. In salivary glands of pSS patients, GC-like structures are more likely to occur with increased focal infiltration and are associated with more severe disease [48]. Furthermore, their presence is a predictor for malignant lymphoma development [51]. Formation of GCs depends on the chemokines CXCL12 and CXCL13 [52]. As mentioned in the previous paragraph, CXCL12 and CXCL13 are also abundantly present in pSS salivary gland tissues and may contribute to the establishment of GC-like structures in these patients [17,26,31,41,42]. The B cells in these GC-like structures are proliferating and associated with follicular dendritic cell networks and express features of 'regular' GC B cells of secondary lymphoid tissues, including expression of activation-induced deaminase, an enzyme essential for class switching and somatic hypermutation [31,42,53]. Here-with, high-affinity memory B cells can be generated at these sites. B cells in these ectopic GCs undergo an antigen-driven expansion, with somatic hypermutation of immunoglobulin variable region genes followed by selection [54]. Sequence analysis of immunoglobulin variable region genes from pSS patients indicates that the selection process that takes place in these ectopic GCs, might be disordered compared with normal GCs in secondary lymphoid tissues [45,55,56] and signs for classical

antigen-driven selection are lacking [57]. These dysregulated GCs may fail to delete somatically mutated B cells that acquired autoreactivity, which could contribute to systemic humoral autoimmune disease [58]. The microenvironment of the exocrine glands of pSS patients is thus prone to promote formation of ectopic lymphoid tissue with GCs and generation of high-affinity (autoreactive) memory B cells.

Cytokine involvement in B-cell homeostasis, activation, differentiation & longevity of plasma cells

The local microenvironment of inflamed glandular tissue is well equipped to promote B-cell survival, activation and plasma cell formation. In fact, presence of IgG plasma cells in salivary gland tissue is a major histopathologic finding of pSS (FIGURE 2). Activation of B cells and generation of isotype switched cells require stimulation of the BCR and CD40L-binding, in the presence of appropriate cytokines. B cell intrinsic TLR engagement promotes activation of B cells [13]. The salivary glands are considered to be the major site of autoantibody production. Plasma cells within salivary gland tissue also comprise plasma cells producing anti-SSA/Ro or anti-SSB/La autoantibodies, highly characteristic for pSS, as detected by immunohistology [17,42,59–61]. Levels of autoantibody-producing cells correlate well with levels of autoantibodies in serum [42]. (Autoreactive) plasma cells might be derived from memory cells generated in secondary lymphoid tissues, which are recruited from peripheral blood [61,62]. In addition, they may differentiate from memory cells generated in the aforementioned ectopic GCs within the exocrine glands. The relative contribution from both sources to memory cell formation and subsequent differentiation toward plasma cells is still unknown.

Role of the IFN/BAFF/APRIL axis in activation and survival of B cells & plasma cells

Important cytokines involved in B cell survival and activation of B cells are BAFF and a proliferation-inducing ligand (APRIL), both belonging to the TNF-ligand family (reviewed by [63]). BAFF and APRIL are produced by a variety of cells, mainly innate immune cells, such as monocytes, macrophages, dendritic cells and neutrophils. The expression of BAFF and APRIL can be increased in presence of type I IFN, type II IFN (IFN- γ) and other cytokines and TLR ligands. BAFF and APRIL are very complex cytokines, which appear in multiple forms and variants with different effects, which makes interpretation of the observations difficult [64]. Patients with pSS have elevated levels of both BAFF and APRIL in serum and saliva compared with healthy controls [65–70].

Immunohistological staining and transcript analysis show that in labial salivary gland tissues of pSS patients, BAFF is mainly produced by infiltrating T and B cells, and by ductal epithelial cells [71,72]. Both type I IFN, virus and (viral) TLR ligands can stimulate the production of BAFF in cultured salivary glands epithelial cells, suggesting that viral infection could be responsible for the increase in BAFF production by ductal epithelial cells in pSS patients [73,74]. As such, virus may

contribute directly to the increased BAFF levels seen in pSS patients, and indirectly, via type I IFN.

Also peripheral blood mononuclear cells (monocytes and T cells) appear to be involved in the abnormal BAFF production in pSS patients [75–77]. Blood monocytes of pSS patients showed an abnormally increased production of BAFF after stimulation with type I IFN (IFN- α), compared with peripheral blood mononucleated cells from control individuals [76], emphasizing the role of type I IFN in BAFF production in pSS patients. Indeed, serum of pSS patients with a type I IFN signature induced BAFF-mRNA expression in a monocytoïd cell line [75]. Yoshimoto *et al.* revealed that circulating monocytes from pSS patients produced higher amounts of BAFF than normal monocytes, even in the absence of stimulation with type II IFN (IFN- γ) [77]. Monocytes of pSS patients also produce higher baseline levels of IL-6, which is strongly and abnormally upregulated by IFN- γ . Interestingly, this increase in IL-6 production is partly, in an autocrine fashion, dependent on BAFF produced by the monocytes. Explanation for the dysregulated IL-6 production in pSS monocytes were overexpression of BAFF receptor (BAFF-R) on these cells and overexpression of transcription factors regulating IL-6 production.

In pSS patients, levels of serum (soluble) APRIL and BAFF correlate well with disease parameters, including amount of infiltrates (focus scores) in minor salivary glands, serum IgG levels and autoantibody titers (anti-SSA, anti-SSB, rheumatoid factor) [67,69,70]. These findings strongly argue that APRIL and BAFF are involved in B-cell hyperactivity and B cell autoimmunity. BAFF, but not APRIL, binds to BAFF-R, widely expressed by human peripheral B cells (except for bone marrow plasma cells) [78]. In synergy with tonic triggering of the BCR, this leads to increased expression of anti-apoptotic signals, through activation of the alternative NF- κ B pathway [63]. Herewith, signaling of BAFF-R positively regulates B cell homeostasis. A role for BAFF in B cell homeostasis is further reflected by the finding that B cell depletion therapy with rituximab of pSS patients results in a rise in serum BAFF levels, which decreases again when B cells start to reappear [68]. By contrast, serum APRIL levels are not affected by B cell depletion therapy.

Studies in BAFF-transgenic mice demonstrated that excessive BAFF production results in systemic lupus erythematosus (SLE)-like autoimmunity, caused by a less stringent selection of immature/transitional B cells and subsequent survival of low/intermediate affinity self-reactive B cells [63,79]. Similarly, Groom *et al.* showed that overexpression of BAFF in transgenic mice, as they age, develop pSS-like pathology, manifested by periductal lymphoid infiltrates, destruction of the glandular tissue and reduced saliva production [65]. Elevated BAFF levels could therefore contribute to failure of B cell tolerance in pSS patients, but available evidence is currently lacking.

BAFF and APRIL also can activate B cells by means of interaction with the receptor TACI (a transmembrane activator and calcium-modulating and cyclophilin ligand interacting protein) [63]. This receptor binds BAFF and APRIL, and is predominantly expressed on human CD27⁺ memory B cells,

activated B cells and plasma cells [78]. Ligand binding to TLR9 (recognizing bacterial unmethylated CpG motifs) significantly induces TACI expression on naïve human B cells [78,80]. Engagement of TACI appears to be important for both T-cell-dependent and T-cell-independent class switching, B cell proliferation and plasma cell differentiation, in synergy with MyD88-mediated TLR stimulation [81,82].

BAFF and APRIL can also bind to B cell maturation antigen, a receptor which is expressed by human plasma cells and tonsillar memory/GC B cells [78]. In particular, APRIL appears to be involved in survival of plasma cells [83]. Together, BAFF and APRIL are important co-factors for survival and activation of B cells and longevity of plasma cells and excessive levels of these cytokines, as seen in pSS patients may contribute to autoreactivity. Type I IFN plays a significant role in production of these cytokines, herewith type I IFN contributes indirectly via the IFN/BAFF/APRIL axis to B-cell hyperactivity. In addition, however, type I IFN may also directly enhance BCR-dependent B-cell responses [84].

Role of cytokines in plasma cell differentiation: the IL-6/IL-21 axis

Various cytokines are involved in plasma cell differentiation from antigen-activated B cells including IL-2, IL-6, IL-10, IL-21 [85] and CXCL10 [86]. These cytokines are all overexpressed in pSS patients who may reflect the B-cell hyperactivity. Of these cytokines, IL-21 has been recognized as the most potent cytokine involved in formation of human B cells to plasma cells [87–89]. Besides the many other effects on innate and adaptive immunity, IL-21 induces T-cell (CD40L)-dependent proliferation and differentiation of naïve and memory human B cells toward plasma cells. The synergistic effects of IL-21 and CD40L promote in a STAT3-dependent fashion the expression of Blimp-1 by B cells, which is essential for development of plasma cells [90]. Interestingly, BAFF can replace CD40L for the differentiation of human marginal zone-like B cells, but not naïve or memory B cells [91], bringing the IFN/BAFF axis and the IL-6/IL-21 axis together. Marginal zone-like B cells are indeed present in (minor) salivary glands of pSS patients [50,92,93].

Patients with pSS have higher serum IL-21 levels compared with healthy controls and rheumatoid arthritis (RA) patients [94]. IL-21 levels in pSS patients correlate with serum IgG1 levels and anti-SSA antibody titers. Both IL-21 and IL-21 receptors are expressed in labial salivary glands of pSS patients, but not in salivary glands from healthy controls. Also mRNA levels for IL-21 are higher in labial salivary glands of pSS patients [95].

IL-21 is produced by CD4⁺ T cells; CD4⁺ Tfh cells are the main producers of this cytokine (reviewed by [96]). In infiltrates of salivary gland tissue of pSS patients, IL-21-positive cells express CXCR5 (receptor for CXCL13), but not CD20, indicating that the cells that produce IL-21 are indeed Tfh cells. Tfh cells play a pivotal role in humoral immunity. Long-lived T-cell-dependent humoral immunity depends on the action of this small subpopulation of T cells, by providing help to

differentiation of antigen-specific B cells to memory and plasma cells (reviewed by [96]). Abnormal activation of Tfh cells is involved in autoimmunity, including pSS. pSS patients with extraglandular manifestations had higher proportions of Tfh cells in peripheral blood than pSS patients without extraglandular manifestations and healthy controls [97]. Furthermore, the proportions of Tfh cells were higher in patients with anti-SSA or anti-SSB antibodies and in patients with elevated levels of serum IL-21, and correlated with focus scores in labial gland biopsies, indicating that Tfh cells are involved in pSS.

Tfh cells differentiate from Th0 cells under the influence of IL-21 together with IL-6, and other cytokines (e.g., IL-27) (reviewed by [98]). IL-6 participates not only in differentiation of human Tfh cells, but importantly also triggers the production of IL-21 by human CD4⁺ T cells as demonstrated in *in vitro* cultures by Diehl *et al.* [99]. These authors conclude that the IL-6-induced antibody production by B cells relies on their capacity to induce IL-21 by CD4⁺ T cells. In line with this notion, treatment of RA patients with an IL-6R blocking agent (tocilizumab) causes a selective decrease of IL-21 and IgG4 autoantibodies [100].

IL-6 is an important proinflammatory cytokine, which can be produced after TLR activation [101]. Many cell types can produce this cytokine, but the primary sources of IL-6 are monocytes/macrophages at sites of inflammation. In patients with pSS, IL-6 is overexpressed, promoting the IL-21-mediated plasma cell differentiation. Elevated levels of IL-6 in pSS patients are observed in serum, saliva, tears and salivary glands [17,102–105]. The number of IL-6-producing cells in labial salivary glands is generally higher in pSS patients with a higher focus scores [17]. As mentioned in the previous paragraph, peripheral blood monocytes from pSS patients exhibit aberrant production of IL-6, even without further stimulation [77]. Although IL-21 is clearly derived from CD4⁺ T cells, the source for IL-6 might be activated B cells [96]. IL-6-producing B cells have been observed in labial salivary glands of pSS patients [106]. Furthermore, the elevated serum levels of IL-6 drop after B cell depletion therapy of pSS patients with rituximab, but not after placebo treatment [103]. These observations suggest that in pSS patients B cells are, indeed, an important source for IL-6.

The inflamed salivary gland environment of pSS patients not only supports formation of memory B cells and plasma cells, but also greatly facilitates long-term survival of plasma cells, including autoreactive (anti-SSA/SSB) plasma cells [17,42,59–61]. These long-lived plasma cells drive the lasting autoinflammatory response [107]. Longevity of plasma cells in salivary glands of pSS patients is clearly illustrated by the observation that even up to 1 year after B cell depletion with rituximab, persisting clonally related immunoglobulin sequences can be observed in these glands [57]. Several cytokines and cell surface molecules on the plasma cells are involved in their long-term survival. Among the cytokines, APRIL, IL-6 and CXCL12 appear all to be critically involved in this long-term survival [83,107,108]. Evidently, CXCL12 is not only important for localization of

CXCR4-expressing plasma cells, but also for survival. Of these plasma cells. In bone marrow, CXCL12 is secreted by stromal cells and APRIL and IL-6 by other cells including eosinophils and megakaryocytes, creating so-called survival niches [83,107,108]. Szyszko *et al.* [17] observed that also in labial salivary glands of pSS patients, plasma cells are located in the vicinity of CXCL12 and IL-6-producing cells (epithelial cells, mononuclear cells), likely representing analogs of survival niches in the bone marrow. The plasma cells located here are non-proliferating and express Bcl-2, and thus probably represent long-lived plasma cells. Whether these plasma cells were formed within the salivary glands itself and/or were derived from plasmablasts/plasma cells generated outside the glands remains to be seen.

B-cell hyperactivity: clonal expansions in salivary gland tissue

Immunoglobulin heavy chain variable region gene DNA and mRNA sequence analysis reveals the presence of polyclonal expansions of B cells and plasma cells within the salivary glands of pSS patients [54,57,109,110]. Clonal expansions of B cells and plasma cells are increased in the salivary glands of pSS patients compared with those observed in control salivary glands and are composed of IgA and/or IgG expressing cells [57]. Almost all obtained IgG and IgA sequences are somatically hypermutated, suggesting a post-GC origin of the cells. The occurrence of these clonally related cells as well as the intraclonal switching implies local activation and proliferation of B cells within the glandular tissue. Neoplastic transformations of clonally expanded cells may ultimately lead to the development of lymphoma in 5–10% of the pSS patients [111]. Most of these lymphomas are of the mucosa-associated lymphoid tissue-type, which are believed to be derived from marginal zone-like B cells [112]. Such B cells appear to be accumulated in salivary glands of pSS patients [50,92,93]. The observed expression of the sphingosine 1-phosphate receptor 1 on these cells may contribute to this accumulation [50].

Currently, it is unclear what the selective forces are that drive particular B cells in pSS patients to clonal proliferation. Stimulation of the BCR by (auto-)antigen, in conjunction with CD40 and TLR engagement in the presence of cytokines, all contribute to proliferation and clonal expansions of B cells. Immunoglobulins produced by lymphoma cells from pSS patients frequently exhibit rheumatoid factor reactivity [111], suggesting a role for autoantigen in clonal expansions. Alternate binding to the BCR, for example, by superantigens [113] or lectins to carbohydrate moieties of immunoglobulins [114] could also be involved in selective expansion of B cells.

Disturbed B cell subsets in peripheral blood of pSS patients exhibit signs for B-cell hyperactivity

B cell homeostasis is markedly disturbed in patients with pSS; the composition of B cell subsets in the peripheral blood is altered compared with healthy controls and patients with other systemic autoimmune disease. The most pronounced disturbance

is that in pSS the proportion and absolute numbers of CD27⁺ memory B cells in peripheral blood is significantly reduced, compared with healthy controls and patients with SLE and RA [46,47,115–118]. Both IgM⁺IgD⁺CD27⁺ B cells (natural effector cells) and IgM⁺IgD⁺CD27⁺ B cells (class switched memory cells) are affected (FIGURE 3). The reason for the diminished number and frequency of CD27⁺ memory cells remains unclear. Hansen *et al.* observed significantly increased numbers of CD27⁺ expressing CD19⁺ B cells in inflamed tissues and therefore speculated that increased recruitment and retention of memory cells could well explain the lower numbers of memory cells in blood [45–47]. Overexpression of CXCL12 and CXCL13 in inflamed tissues may contribute to this recruitment and the accumulation of CXCR4⁺ and CXCR5⁺ expressing CD27⁺ B cells in salivary glands.

The lower levels of CD27⁺ memory cells could also be attributed to overactive differentiation to plasma cells. This is illustrated by elevated levels of soluble CD27 (sCD27) in serum of patients with pSS and patients with other systemic autoimmune disease [119]. Serum sCD27 levels correlate with concentrations of serum IgG (but not serum IgM or IgA). This sCD27 might be derived from (memory) B cells that is released from the cell surface by metalloproteinases or by differential splicing of the mRNA [120,121]. Thus, higher sCD27 levels may reflect increased IgG plasma cell differentiation, resulting in higher IgG levels. Activation toward plasma cells may occur in the inflamed glandular tissues upon recruitment from blood [61,62,116]. Aqrabi *et al.* found only low numbers of CD27⁺CD20⁺ memory B cells in salivary glands and elevated levels of CD27⁺⁺ expressing plasma blasts and plasma cells [62]. Furthermore, the SSA-specific cells that could be detected in salivary gland tissue appear to represent differentiating plasma cells [61]. As discussed above, the microenvironment of inflamed salivary glands express high levels of cytokines that promote plasma differentiation.

In addition to reduced numbers of CD27⁺ memory B cells in peripheral blood of pSS patients, remaining B cells in blood express significantly elevated surface levels of CD38 (FIGURE 3) [115,116,119,122,123]. In healthy individuals, resting mature naïve B cells express only very low levels of CD38 and expression of CD38 is one of the early markers of B cell activation. The high levels of CD38 on peripheral blood B cells may obviously reflect the hyperactivation state of (naïve) B cells in pSS patients. IgD/CD38 staining allows identification of various B cell subsets and was initially designed to classify B cell subsets in secondary lymphoid organs, such as tonsils. Due to absence of GC B cells in peripheral blood, interpretation of IgD/CD38-based classification of B cell subsets should be done with care. Nevertheless, staining for IgD/CD38 not only reveals significantly elevated levels of CD38 on naïve B cells (CD27⁺IgM⁺IgD⁺ B cells; FIGURE 3), but with the concomitant low numbers of memory B cells in blood, this results in a unique IgD/CD38 profile of peripheral blood B cells in pSS patients. A ratio of IgD⁺/CD38^{+/++} naïve B cells to IgD⁺/CD38^{-/+} memory B cells above 5 is considered to be diagnostic for pSS [122,124]. Interestingly, this abnormal B cell profile correlates with serum levels of Fms-like tyrosine kinase 3 ligand (Flt-3L), a cytokine implicated in B cell survival and

Figure 3. Reduced frequency of CD27⁺ memory B cells and elevated expression of CD38 on B cells in primary Sjögren's syndrome patients. Peripheral blood samples from pSS patients and HC were stained for IgM, IgD, CD19, CD27 and CD38. Flow cytometric CD27/IgD expression profiles of CD19⁺ B cells show a significantly reduced frequency of both CD27⁺ IgD⁺ and CD27⁺ IgD⁻ and B cells in pSS patients, compared with HCs (upper panels). Numbers indicate the proportion of cells displaying a particular phenotype among CD19⁺ B cells. The other panels show the CD38 expression levels of various B cell subsets, which are clearly increased: CD27⁺ IgM⁺ IgD⁺ B cells (effector memory cells), CD27⁺ IgM⁻ IgD⁻ B cells (isotype switched memory cells) and CD27⁻ IgM⁺ IgD⁺ B cells (mostly naïve B cells). HC: Healthy controls; pSS: Primary Sjögren's syndrome.

proliferation [125]. Serum Flt-3L levels are increased in pSS patients compared with healthy controls and levels are higher in pSS patients with a history of lymphoma compared with those without lymphoma [125,126]. In peripheral blood, expression of the receptor for this cytokine (Flt-3) is largely restricted to a fraction of IgD⁺/CD38^{+/++} naïve B cells and B cells from pSS patients appear to express higher levels of Flt-3 relative to healthy controls [125]. These data may point to a role for Flt-3L in abnormalities in B cell homeostasis and progression to lymphoma, as seen in pSS patients.

Another sign of disturbed homeostasis in pSS is that among the CD27⁻ B cells the proportion and absolute numbers of CD21^{-low} B cells are increased in pSS patients, compared with healthy controls [118]. This B cell subset is also expanded in patients with RA and SLE. B cells that belong to this subset express mostly mutated IgM antibodies, exhibiting signs for antigen selection, and many of these autoantibodies are autoreactive. These B cells could not be activated by BCR ligation, but remained responsive to TLR stimulation. They were therefore considered to be anergic B cells, that are not eliminated and which remain in peripheral blood. Such anergic B cells may contribute to autoimmunity after stimulation by (endogenous) TLR ligands.

Intrinsic B cell factors involved in B-cell hyperactivity

In addition to exogenous triggers, also B cell intrinsic (genetic) factors could play a role in hyperactivity of B cells in pSS pathogenesis. In particular, dysregulation of BCR signaling events could attribute to pSS or severity of pSS. After binding of antigen to BCR, the proximal signaling machinery starts, involving kinases such as the Src-family protein tyrosine kinase Lyn, spleen tyrosine kinase and, via the BCR co-receptor CD19, also activation of phosphatidylinositol-3 kinase [14]. This initial activation is followed by activation of more downstream kinases B lymphoid tyrosine kinase and Bruton's tyrosine kinase (Btk). Hypersignaling of BCR may result in systemic autoimmune disease, as experimentally shown in mice with transgenic overexpression of Btk [127]. These mice exhibit spontaneous development of GCs, increased plasma cell numbers, formation of autoantibodies and infiltration of various organs, including salivary glands, with lymphoid cells. Btk also activates the transcription factor NF- κ B that regulates genes involved in proliferation and survival of B cells. Polymorphisms in *TNIP1* (ABIN1), a repressor of NF- κ B, are associated with autoantibody (anti-SSA and/or anti-SSB) positive pSS patients [128]. Also polymorphisms of the gene encoding for B lymphoid tyrosine kinase, another downstream kinase involved in BCR signaling, is associated with pSS, as shown by genome-wide association studies [6,129,130].

D'Arbonneau *et al.* [123] observed that expression of the BCR co-receptor molecule CD19 is increased on B cells from pSS patients compared with normal individuals, which might be due to the higher BAFF levels seen in pSS patients. Higher CD19 levels are associated with prolonged translocation of the BCR into lipid rafts upon activation of B cells. This extended

retention in lipid rafts is explained by the notion that the CD19/CD21 co-receptor retains BCR in lipid rafts [131]. Prolongation of BCR in lipid rafts may enhance signaling of BCR and could contribute to hyperactivity of B cells in patients with pSS. Also the B cell-associated molecule CD72 is expressed at higher levels on B cells from patients with pSS, in contrast to B cells obtained from healthy controls, RA patients and SLE patients [132]. When ligated, CD72 is a positive regulator of B cell signaling and herewith upregulation of CD72 may stimulate B cell activity in pSS patients [132,133].

Pathogenetic function of B cells in pSS

The production and secretion of a wide variety of autoantibodies by B cells and plasma cells are presumed to play a main role in the pathogenesis of pSS. These autoantibodies are associated with different clinical manifestations of pSS [134]. Autoantibodies are, however, well present before clinical onset of pSS, they can be detected more than 15 years before symptoms are recognized [135], indicating that merely presence of autoantibodies is not sufficient for clinical disease.

The pathogenic role of autoantibodies in pSS remains still largely obscure. The best known autoantibodies related to pSS are anti-SSA/Ro and anti-SSB/La autoantibodies, directed against ribonucleoproteins. These autoantibodies also are a diagnostic criterion for pSS [136,137]. The glandular epithelium is an important source for these autoantigens (for reviews see [138,139]). SSA/Ro and SSB/La autoantigens are upregulated in salivary gland tissue of pSS patients. Type I IFN (IFN- α) upregulates the SSA/Ro52 autoantigen. Increased apoptosis of glandular ductal and acinar epithelial cells as well as release of exosomes (small membrane vesicles) lead to exposure of these autoantigens to the immune system in an immunogenic fashion. Anti-SSA and anti-SSB autoantibodies may induce apoptosis of epithelial cells by enhancement of transcription levels and activation of caspase-8 [140].

Immune complexes formed by autoantibodies and RNA-containing autoantigens (ribonucleoproteins) can activate PDCs to produce type I IFN after interaction with TLR7 or TLR9 [28,141]. In turn, type I IFN upregulates TLR7 on B cells, which may contribute to the activation of B cells, upon ligand binding [13]. Herewith, these autoantibodies stimulate B cell responses further and create some sort of loop. Thus, although initially type I IFN production might be induced by, for example, viral antigens, autoimmune response might be sustained by autoantigen-containing immune complexes, resulting in some form of vicious circle. Direct cell–cell contact between B cells and PDCs significantly enhance the immune complex-induced IFN- α production by PDCs [142]. This stimulation of the type I IFN response is not the only *direct* role ascribed to B cells in pSS pathogenesis. For example, direct interaction of (tonsillar) B cells with a human salivary gland cell line induces caspase-3-mediated apoptosis of epithelial cells, which was not caused by CD95/Fas-CD95L/FasL-dependent interaction [143]. Herewith, infiltration of glandular tissue with B cells could play a role in the enhanced apoptosis of epithelial cells seen in pSS patients.

Besides their classical role as antibody-producing cells, activated B cells also have the ability to produce and secrete cytokines that are able to modulate immune responses [144–146]. Herewith, B cells also play an antibody-independent role in tolerance and autoimmunity. The signals that are required for human B cells to develop to cytokine-producing cells are not fully understood, but TLR signaling appears to be critically involved [144]. Two subsets of cytokine secreting B cells can be identified, regulatory B cells and effector B cells. Regulatory B cells produce mainly IL-10 and TGF- β and effector B cells produce cytokines such as IL-2, IL-4, IL-6, IL-12, IFN- γ and TNF- α [146]. IL-10-producing regulatory B cells are thought to play important roles in dampening immune responses. In a recent study, Furuzawa-Carballeda *et al.* showed that patients with pSS have an increased frequency of IL-10-producing circulating regulatory B cells, defined as CD19⁺CD38^{hi}CD24^{hi}IL-10⁺ cells, compared with controls [147]. Importantly, the proportion of regulatory B cells was higher in clinically inactive pSS patients, compared with clinically active pSS patients suggesting that these cells may downregulate autoimmune inflammation to induce homeostasis. Whether these regulatory B cells are also functionally normal in pSS remains, however, to be seen. A reduced TLR9-mediated IL-10 production has, for example, been shown in patients with another autoimmune disease, multiple sclerosis [148].

Effector B cells, producing the proinflammatory cytokines IFN- γ or IL-6, have been detected in infiltrates of salivary glands of pSS patients [106]. We have observed that B cell depletion therapy with rituximab affects serum cytokine levels [103] and was associated with a decrease in ESSDAI and ESSPRI [12]. In our study, elevated serum levels of GM-CSF, IL-1Ra, IL-6, IL-10, IFN- α , TNF- α , CCL4 and CXCL9 declined after rituximab treatment of pSS patients, but not after placebo treatment [103]. Some of these cytokines (e.g., GM-CSF, IL-6, IL-10, TNF- α) can be produced by activated B cells and data suggest that this decrease in cytokine levels could, at least in part, be explained by a direct effect of depletion of cytokine secreting B cells. In particular, B cell-derived IL-6 might be responsible for amelioration of disease activity after rituximab treatment, as was shown by Barr *et al.* [149] in a mouse model of experimental autoimmune encephalomyelitis. Potential roles of IL-6 in pSS pathogenesis include the aforementioned generation of Th17 and Tfh cells and the induction of IL-21 secretion, mediating plasma cell differentiation. Recently, a novel role for B cell-derived IL-6 has been described. Direct cell–cell contact between macrophages and B cells results in secretion of CXCL10 by macrophages in response to IL-6 derived from B cells, stimulating directly the differentiation of B cells to (IgG and IgA) plasma cells [86].

Expert commentary

Obviously, in patients with pSS B cells are hyperactive, as serologically manifested by hypergammaglobulinemia, presence of autoantibodies and cryoglobulins, the elevated levels of free light chains and β 2-microglobulin and the frequent occurrence

of lymphoproliferative disease. Both antibody-dependent and antibody-independent mechanisms are likely involved in immunopathogenesis of pSS. B cell activation is the consequence of coordinated activation of BCR in conjunction with co-stimulatory signals delivered by cytokines, CD40L and TLR ligands. As discussed above, essential cytokines for B cell activation and differentiation, viz. the IFN/BAFF axis and the IL-6/IL-21 axis, are overexpressed and overactive in pSS. B cell activation *per se* does not lead to generation or secretion of autoreactive antibodies. Aberrant selection of B cells due to altered BCR signaling appears to play an important role in breaking tolerance and development of systemic autoimmune disease, including pSS [13,14,150–152]. In particular, engagement of TLRs could be involved in this process. Binding of ligands to TLRs, possibly with simultaneous CD40 stimulation, may set another threshold for BCR activation and could lead to interference with negative selection of autoreactive B cells in GCs [14,127]. These TLR ligands may be derived from microorganisms and/or from autologous nucleic acid containing cellular material from dying cells, which can bind to TLR7 and TLR9 expressed by B cells [152]. As mentioned before, TLR engagement is also important for generation of cytokine-producing B cells [144], but the signals that are required for the differentiation of B cells to either regulatory B cells or effector B cells remain unclear. Only limited data are available on the role of TLR activation of B cells in pSS pathogenesis. Zheng *et al.* showed an upregulation of TLR7 and TLR9 mRNA in peripheral blood mononucleated cells of pSS patients [153] and Guerrier *et al.* speculated that TLR9 might be implicated in development of autoreactive marginal zone like B cells in salivary gland tissue of pSS patients [154]. In summary, in conjunction with B cell intrinsic factors, TLR engagement may contribute to aberrant BCR signaling and development of autoimmune disease seen in pSS patients, which is further sustained by the high levels of cytokines involved in B cell activation and differentiation.

Given the role of the humoral immune system in pathogenesis of pSS, B cells and plasma cells are important targets for effective and rationale treatment of pSS. Many biological disease-modifying antirheumatic drugs (DMARDs) have been developed and applied to treat successfully other rheumatic diseases. Some of these biologicals have been conducted now in Phase II and III trials to treat also pSS patients resulting in temporarily relief of clinical symptoms. These trials mainly focused on depletion of B cells by anti-CD20 therapy (rituximab) [155–157] and inhibition of their T-cell-dependent activation by blocking co-stimulation of CD4⁺ Th cells (abatacept) [158,159]. Larger trials with rituximab and abatacept are currently underway.

Five-year view

Understanding of the pathogenetic mechanisms of pSS in general, and the role of B cells and plasma cells, in particular, is rapidly expanding. Proteomic approaches will help to elucidate further the complexity of the pathogenesis of pSS and to

establish known and novel biomarkers for early diagnosis, measurement of disease activity and definition of subgroups of pSS patients who might be susceptible to a particular treatment.

Many biological DMARDs are currently available and in development to target various molecules involved in the cascade of hyperactive B cells and plasma cells including biologicals that can interfere with a large number of relevant cytokines and chemokines. In addition, non-biological drugs that inhibit BCR signaling molecules and cytokine receptors have become available. Because BCR signaling plays such an important role in the autoimmune process, targeting important molecules of this pathway, such as spleen tyrosine kinase and Btk [160], respectively, is presumed to be a promising new approach for treatment of pSS too.

A major potential disadvantage of all these therapies is that not only the harmful autoimmune responses are affected, but also beneficial humoral responses. Although the pathogenic role of autoantibodies remains enigmatic and not all autoantigens have been identified yet, tolerizing specifically autoreactive B cells is an exciting new development. For example, Macauley *et al.* [161] used liposomal nanoparticles displaying antigen and ligands for CD22. Enforced ligation of the BCR with the inhibitory BCR co-receptor CD22 results in apoptosis of exclusively antigen-specific B cells.

Besides understanding the pathogenetic process and the availability of biological and synthetic DMARDs, assessment of disease activity in pSS is an essential step in the development of novel therapeutic approaches [162]. With the development

and validation of the ESSDAI and ESSPRI, important tools have become available for rating the disease activity and patients' complaints in pSS patients. Both indices are complementary and should be used together in addition to objective measurements of dryness and biological markers of disease activity. ESSDAI and ESSPRI were shown to well characterize patients at baseline as well as to show effects of an intervention therapy at various times after treatment [12]. By applying ESSDAI and ESSPRI, it will become easier to compare the results of studies in which the same or different intervention treatments are assessed. This way, it can be decided which therapy is indeed effective for the treatment of pSS and/or in which (sub)group of pSS patients this therapy is worth trying. The increased knowledge on the way to assess patients with pSS, along with the emergence of new targeted therapy, will stimulate the conduction of clinical trials and development of effective treatment options in pSS.

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Key issues

- Periductal lymphoid infiltrates in salivary glands and presence of IgG plasma cells are the major histopathological finding in primary Sjögren's syndrome (pSS). With time, the organization grade of the infiltrates increase, leading to ectopic lymphoid tissue with a dominance of B cells during disease progression and presence of ectopic germinal centers.
- Chemokines are the driving force of the recruitment of lymphocytes. Initially proinflammatory chemokines such as CXCL10 are involved, while at later stages the homeostatic chemokines CXCL12, CXCL13, CCL19 and CCL21 play a critical role in maintaining the inflammatory infiltrate.
- Besides B-cell receptor/CD40/Toll-like receptor engagement, cytokines are critically involved in B-cell activation. Key cytokines involved in B cell activation, proliferation and differentiation are all overexpressed in salivary gland tissue, saliva and serum. Both the type I interferon/B cell-activating factor/a proliferation-inducing ligand axis and the IL-6/IL-21 axis contribute to the hyperactivity of B cells in pSS patients.
- B-cell hyperactivity is further reflected by presence of clonal expansions in salivary gland tissue, which may culminate in neoplastic transformations, leading to non-Hodgkin lymphoma.
- Changes in B cell subset distribution in peripheral blood also reflect increased hyperactivity. Increased expression of CD38 on B cells and reduced numbers and frequencies of CD27⁺ memory B cells may be suggestive of their activated state.
- Aberrant signaling of the B-cell receptor might be involved in breaking tolerance and development of autoimmune disease. Both B cell intrinsic factors and Toll-like receptor engagement may play a role in this process.
- Hyperactivation leads to increased antibody production, resulting in hypergammaglobulinemia, elevated levels of free light chains and β 2-microglobulin and production of autoantibodies directed against SSA/Ro and SSB/La autoantigens and rheumatoid factor. In addition to this classical role of B cells, they are an important source of cytokine production.
- Understanding the role of B cells in pSS pathogenesis, availability of biological disease-modifying antirheumatic drugs to a variety of targets involved in B cell activation and development of disease activity indices European League Against Rheumatism Sjögren's syndrome disease activity index and patient reported index are crucial for the development beneficial therapies for treatment of pSS patients.

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