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# When B cells break bad: development of pathogenic B cells in Sjögren's syndrome

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## ABSTRACT

Primary Sjögren's syndrome (pSS) is often considered a B cell-mediated disease, yet the precise role of B cells in the pathogenesis is not fully understood. This is exemplified by the failure of multiple clinical trials directed at B cell depletion or inhibition. To date, most prognostic markers for severe disease outcomes are autoantibodies, but the underlying mechanisms by which B cells drive diverse disease presentations in pSS likely extend beyond autoantibody production. Here we outline an expanded role of B cells in disease pathogenesis drawing on examples from animal models of SS, and from other autoimmune diseases that share similar clinical or immunological abnormalities. We focus on recent findings from the detailed analysis of pathogenic B cells in patients with pSS to propose strategies for patient stratification to improve clinical trial outcomes. We conclude that an integrated cellular, molecular and genetic analysis of patients with pSS will reveal the underlying pathogenic mechanisms and guide precision medicine.

## Introduction

Primary Sjögren's syndrome (pSS) is a chronic inflammatory autoimmune disease predominantly affecting the salivary and lachrymal glands. The presence of autoantibodies and hypergammaglobulinaemia have led to pSS been considered a B cell-mediated disease. However, the majority of clinical trials testing B cell targeted therapies in pSS, have failed to meet primary endpoints for clinical improvement, indicating the precise role of B cells in disease pathogenesis is not yet completely understood. Compounding clinical trial failure is the heterogeneity of clinical phenotype in patients with pSS, mani-

festing as three major stages. Stage 1 is characterised by autoimmune exocrinopathy with dry eyes and mouth, fatigue, arthralgia, and the presence of serum autoantibodies in >80% of patients, particularly IgG targeting SSA/Ro and SSB/La ribonucleoproteins and IgM rheumatoid factors (1). These autoantibodies are present a median of more than four years before Stage 1 clinical signs (2) (Fig. 1). Disease management of Stage 1 is mainly composed of symptomatic treatment (e.g. artificial tears and saliva, NSAIDs) as disease-modifying treatment options are lacking. Approximately 30–40% of patients progress to stage 2, which is defined by increased disease severity with at least one additional systemic autoimmune manifestation, which may involve the skin, lungs, muscle, kidney, heart, gastrointestinal, central and peripheral nervous systems, and haemopoietic system (3). Systemic autoimmune complications are mediated by immune complex deposition (e.g. cryoglobulinaemic-associated vasculitis and glomerulonephritis) and/or inflammatory cell infiltration of organs and tissues (e.g. interstitial nephritis, constrictive bronchiolitis, interstitial lung disease). Histological examination of affected organs, including the salivary glands, reveal that B cells are a key constituent of the cellular infiltrate (Fig. 2). However, the significance of infiltrating B cells at sites of organ damage in pSS is poorly understood, and there is ongoing debate around whether these hyperactivated B cells are direct initiators of disease pathogenesis or bystanders responding to an inflammatory environment. The latter is, however, unlikely, since the third stage of pSS is progressive development of monoclonal B cell lymphoproliferative disease, most commonly salivary gland mucosa-associated lymphoid tissue (MALT)

lymphomas. B cell malignancies arise in approximately 5% of patients with pSS (4) representing a 20-fold increased risk of lymphoma compared to the general population (3) and the highest risk among individuals affected by all autoimmune diseases (4). There is an increase in non-Hodgkin lymphoma-specific mortality in pSS (standardised mortality ratio 3.25) (5). Rheumatoid factor autoantibodies are closely associated with progression to stage 3 with cryoprecipitable rheumatoid factors being an independent predictor of lymphoma in pSS (6). Moreover, the majority of salivary gland MALT lymphomas express surface IgM comprising stereotypic immunoglobulin rearrangements with rheumatoid factor autoantibody specificity (7). Hence, autoreactive B cells not only contribute to disease progression via production of autoantibodies, but may also become pathogenic through uncontrolled clonal expansion. Multiple B cell intrinsic and extrinsic factors can contribute to this uncontrolled B cell expansion, including polymorphic loci in genes that affect B cell biology (8). In addition to autoantibody production and lymphomagenesis, B cells can contribute to pathogenesis by antigen presentation and pro-inflammatory cytokine production (Fig. 1).

Given the multi-faceted role of B cells in pSS it is likely that therapies directly targeting B cells at various developmental and functional stages and by various pathways, will be required for effective treatment. The aim of this review is to examine the development and function of pathogenic B cells in pSS by evaluating animal and clinical models of pSS and other autoimmune diseases with a focus on recent analysis of pathogenic B cells from patients with pSS. We review potential factors that may contribute to poor clinical trial outcomes and provide a pathway towards precision medicine with a patient vignette and model for patient stratification.

### **B cell mediated pathology in pSS**

#### *Direct contributions of B cells to pathogenesis*

##### • *Autoantibodies*

Serum autoantibodies provide substantive evidence for autoimmune-mediated

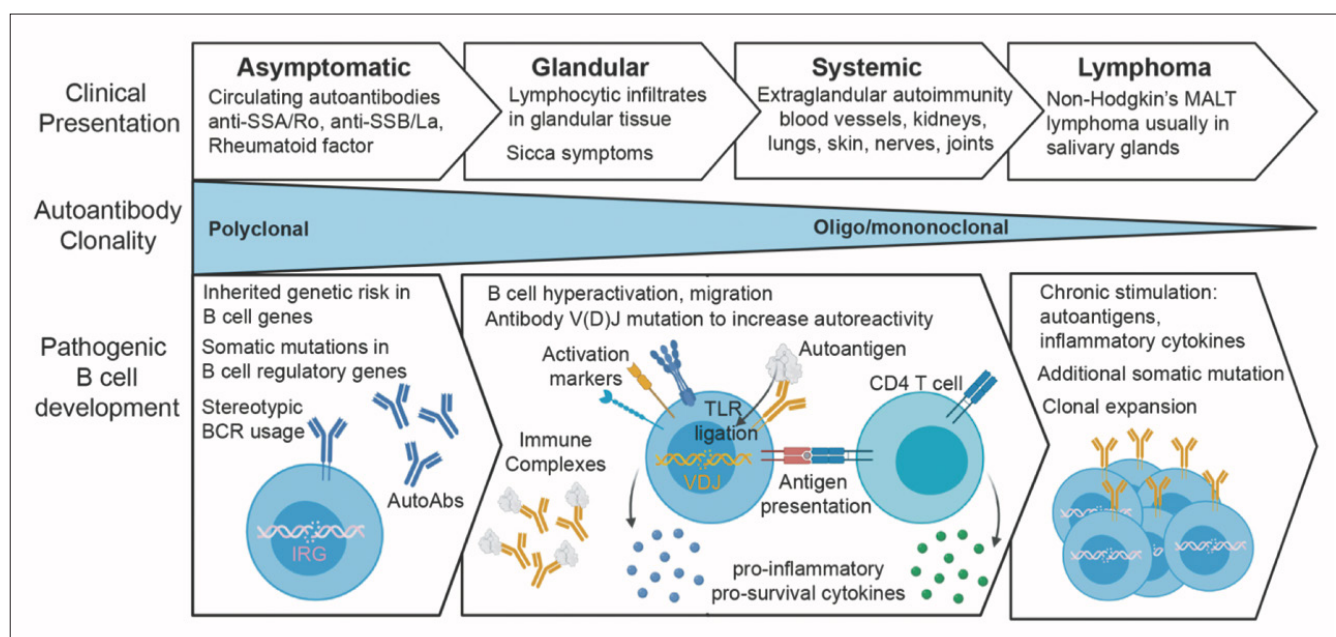
exocrine gland pathology in pSS. Anti-52kD SSA/Ro (Ro52) and anti-60kD SSA/Ro (Ro60) autoantibodies are most common in pSS and hence one of the key objective criteria for diagnosis (9). Other common autoantibodies in pSS include anti-SSB/La and rheumatoid factor. While anti-Ro/La and rheumatoid factor autoantibodies occur in a majority of patients, they are not exclusive to pSS and can occur in individuals with other autoimmune diseases. Likewise, many autoantibodies associated with other systemic autoimmune diseases are also found in pSS, such as anti-centromere, anti-cyclic citrullinated peptides, anti-mitochondrial and anti-DNA antibodies (10). In addition, a range of autoantibodies not routinely tested in diagnostic laboratories have been detected in patients with pSS, including anti-M3 muscarinic acetylcholine receptors (M3R), anti-alpha-fodrin, anti-salivary gland protein 1, anti-carbonic anhydrase 6 and anti-parotid secretory protein antibodies. The incidence and clinical significance of these minor autoantibodies has been previously reviewed (10).

The presence and increased titres of anti-Ro, anti-La and rheumatoid factor serum autoantibodies are correlated with more severe disease in patients with pSS (11). However, the occurrence of these same autoantibodies in asymptomatic individuals years prior to developing clinical signs of disease, poses questions about their pathogenicity (2, 12). Anti-Ro52 and anti-Ro60 autoantibodies are almost universally implicated in neonatal lupus, a passively acquired autoimmune syndrome manifesting as a transient rash, cytopenia, mild hepatic derangement, or congenital heart block (13); and are also strongly associated with subacute cutaneous lupus erythematosus in adults with pSS, systemic lupus erythematosus (SLE), or isolated cutaneous lupus erythematosus (14). Only 2% of anti-Ro positive mothers give birth to a child with neonatal lupus, however the risk in later pregnancies following an affected neonate rises to 17% (15), suggesting that specific attributes define pathogenic autoantibodies, such as epitope specificity, and/or other factors

such as foetal microenvironment and genetics may contribute to autoantibody pathogenicity (16-20). Interestingly, neonatal lupus overwhelmingly occurs in offspring of asymptomatic women, 30% of whom later develop pSS (21), possibly related to the increased prevalence of pSS in relation to SLE (both diseases exhibiting anti-Ro antibodies), the frequently delayed and older age at diagnosis of pSS, and disease-specific differences between these antibodies (22-24) – an important area for future research.

Rheumatoid factor autoantibodies targeting IgG Fc present a similar scenario: seemingly benign autoantibodies are detected in a majority of patients, but are directly linked to the development of severe autoimmune pathology caused by type II mixed cryoglobulinaemia, in only a few. Type II mixed cryoglobulinaemia most often presents as glomerulonephritis and cutaneous vasculitis, caused by deposition of immune complexes composed of IgM rheumatoid factor and polyclonal IgG precipitating at low ambient temperatures in small to medium sized blood vessels. The pathogenicity of rheumatoid factors in complex with IgG is well-established and was first demonstrated in passive transfer to rodents, to cause cutaneous vasculitis where lower temperature is a co-factor or glomerulonephritis when undefined co-factors trigger precipitation in the mesangium (25-28).

Evidence for an expanded role of B cells in pSS pathogenesis beyond production of autoantibodies comes from clinical trials with the chimeric anti-CD20 monoclonal antibody rituximab. Rituximab results in almost complete depletion of CD20-expressing B cells (including naïve and memory B cell compartments) and short-lived plasmablasts in both the periphery and salivary glands, reduced numbers of T follicular helper cells and has a variable effect on long-lived plasma cells, which typically have low expression of CD20 (29). Clinical efficacy of open-label rituximab measured by decreased ESSDAI score and increased salivary flow was achieved in the presence of sustained anti-Ro/La autoantibody titres (30), suggesting an expanded role for B cells



**Fig. 1.** Proposed development and function of pathogenic B cells during Sjögren's syndrome disease progression.

Multiple germline and somatic variants in immune regulatory genes (IRG) enable B cells bearing stereotypic, autoreactive B cell receptors to evade tolerance pathways, survive, proliferate and differentiate into autoantibody secreting cells. Autoreactive B cell hyperactivation, antibody variable region (VDJ) mutation and autoantibody secretion can occur via T cell independent or dependent pathways. For T cell-independent activation, self-antigens associated with nucleic acids (e.g. Ro ribonucleoproteins) provide a dual signal, stimulating the B cell receptor (BCR) and ligating endosomal Toll-like receptors (TLR). For T cell-dependent activation, autoreactive B cells present autoantigen via MHC Class II to CD4<sup>+</sup> helper T cells resulting in differentiation into T effector populations including T follicular helper cells that promote antibody somatic hypermutations to enhance autoantibody affinity. Secreted autoantibodies form pathogenic immune complexes with autoantigens that stimulate additional inflammatory signalling including TLR activation and Type I interferon. Chronic self-antigen stimulation, inflammatory microenvironment and accumulating somatic mutations provide the necessary conditions for uncontrolled proliferation of autoreactive B cells leading to clonal expansion and lymphoma.

in disease pathogenesis. Inconsistencies in clinical efficacy for rituximab reported in different trials (31-33) supports a hypothesis where B cells at different developmental stages, in different microenvironments, contribute to the broad clinical phenotypes observed across patients with pSS and unpredictable responses to treatment. Hence, understanding how B cells contribute to disease pathogenesis and specifically how and where pathogenic B cells develop and differentiate is imperative to successful B cell targeting.

#### • Antigen presentation

A beneficial clinical response to rituximab has also been reported in patients with autoimmune diseases where the pathogenesis was originally thought to be T-cell mediated or not caused by autoantibodies: multiple sclerosis (34) and type I diabetes (35). Findings from both the non-obese diabetic (NOD) mouse model of diabetes and experimental autoimmune encephalitis (EAE) model of multiple sclerosis demonstrated that

B cells, but not secreted autoantibody, are essential for disease development (36, 37). In these models, B cells initiate disease via MHC class II restricted antigen presentation, as deficiency in class II molecules on B cells, but not other antigen presenting cells, prevented CD4<sup>+</sup> T cell activation and diabetes in NOD mice (38). Similarly, selective deletion of MHC Class II on B cells rendered mice resistant to EAE due to a B cell dependent block in T<sub>H</sub>1 and T<sub>H</sub>17 development (37). More recent experiments evaluating human B cells *ex vivo* obtained from patients with multiple sclerosis support these findings by demonstrating that antigen experienced memory B cells directly stimulate proliferation and activation of CD4<sup>+</sup> T cells (39). Likewise, transglutaminase 2 specific B cells from patients with coeliac disease showed a dominant antibody response to an N-terminal epitope that coincided with antigen presentation to CD4<sup>+</sup> T cells and disease onset (40). The main genetic risk associations for pSS are in genes assigned to antigen

presentation pathways, particularly the human leukocyte antigen-DR and -DQ isotypes that are positively associated with disease pathogenesis and control the diversification of the Ro/La autoantibody response (41). It would be interesting to determine whether the approximate 20% of patients with pSS that are "serologically negative" (e.g. lacking anti-Ro/La autoantibodies) and diagnosed by lymphocytic infiltrates in salivary gland biopsies show a clinical response to B cell targeted therapies. Often this sub-group of patients are excluded from clinical trials targeting B cells as their disease pathogenesis is considered to be B cell-independent or do not constitute a large enough sample size for correlations to be made. However, it is tempting to speculate that B cells infiltrating the salivary glands mediate pathogenesis by presenting autoantigens to T cells and promoting inflammation in a similar manner to pathogenic B cells in multiple sclerosis and type I diabetes. Further research, in animal models of pSS and clinical



disease is needed to determine whether autoreactive B cells function as antigen presenting cells to activate autoreactive T cells in pSS.

#### • Cytokine production

A third potential mechanism of B cell pathogenicity in pSS is via endogenous secretion of pro-inflammatory cytokines. In particular, Toll-like receptor (TLR)-activated B cells express a variety of pro-inflammatory (*e.g.* IL-6, TNF- $\alpha$ ) and anti-inflammatory (*e.g.* IL-10) cytokines (42). A potential role of B cells as a major source of pro-inflammatory cytokines is reinforced by the observed decline in serum IL-6 levels following B cell depletion therapy with rituximab in pSS patients (43). Moreover, in the EAE mouse model of multiple sclerosis, a B cell-specific IL-6 deficiency reduced disease severity and rituximab treatment ameliorated disease only in mice with IL-6-sufficient B cells at baseline (44). While IL-6-producing B cells may be actively involved, numbers and/or suppressive function of IL-10-producing (regulatory) B cells are often decreased in autoimmune conditions (45). However, current evidence indicates no functional impairment of IL-10-producing B cells in pSS (46). In addition to IL-10, B cells can exert regulatory function via production of IL-35 (IL-12p35/Ebi3) (47). Interestingly, serum levels of IL-35 were decreased in pSS patients and IL-12p35 and Ebi3 mRNA transcripts in blood were detected only in B cells, suggesting that B cells in pSS produce lower amounts of IL-35 (48). Whether this can be directly translated to B cells in the target organs of pSS remains to be investigated, but it is plausible that a disturbed balance between pro- and anti-inflammatory B cell-derived cytokines contributes to the ongoing inflammation in these organs.

#### Factors contributing to B cell hyperactivity

##### • Genetic risk factors contributing to enhanced B cell activation

In addition to HLA type II polymorphisms, multiple risk loci for pSS have been established in genes involved in B cell response, including *BLK*, *IRF5*,

*TNIP1*, *STAT4* and *CXCR5*, albeit with relatively low odds ratios (49) as well as *BAFF* (with an odds ratio of 2.6) (50). A germline variant of *TNFAIP3* (A20), a negative regulator of NF- $\kappa$ B signalling, was specifically linked to pSS-associated lymphoma (6) and more recently, to pSS risk in general (51). An additional contribution of genetics to disease initiation in pSS may be X chromosome dosage, since X chromosome aneuploidies are in excess among patients with pSS (52, 53). Candidate genes that mediate the X dosage effect are *TLR7* and *CXorf21*, an IFN-inducible gene of which expression correlates with *TLR7* (54). These genes participate in innate immune responses and have the ability to escape X-inactivation (55, 56). Biallelism of these (and other) genes may contribute to the strong female bias seen in euploid patients with pSS and SLE. Epistatic genetic interactions have also been defined as risk factors for pSS, such as those between HLA and the RCA alpha block (Regulators of Complement Activation), a region important for the non-inflammatory clearance of immune complexes (57). Together, the combination of multiple known and as yet undiscovered genetic polymorphisms can predispose the B cells to hyper-responsiveness.

##### • T-cell independent B cell activation via IFN and BAFF

TLR signalling is central to T-cell independent B cell responses and can be greatly enhanced by type I interferon (IFN) (58, 59). TLRs are not only capable of pathogen recognition, but may also recognise self-antigens, in particular nuclear antigens released from apoptotic cells. Dual engagement of the B cell receptor (BCR) and TLR signalling pathway by nuclear antigens is thought to play a central role in breaking tolerance and autoimmunity (59). For example, pSS-associated autoantigens Ro52, Ro60, and La form complexes with RNA and as such may directly engage specific TLRs (TLR3, 7, 8) expressed by B cells (60, 61). The importance of TLR signalling is further supported by the finding that in lupus-prone mice both formation of anti-nuclear antibodies and tissue infil-

tration of T cells depend on intact TLR signalling in B cells (62). Of particular interest to pSS, type I IFN specifically enhances TLR7-mediated activation of naïve B cells (63). In line with this finding, pre-incubation of naïve B cells from healthy donors with IFN- $\alpha$  could recapitulate the observed hypersensitivity of naïve B cells from pSS patients to TLR7, but also TLR9 agonists (64). In addition to TLR upregulation, increased expression of IFN-stimulated genes (ISGs) has been observed in blood B cells and in salivary gland tissue of a majority of patients with pSS and correlates with anti-Ro/La expression. Chronic inflammation due to TLR and type I IFN signalling may be caused or exacerbated by genetic polymorphisms in *IRF5*, a transcription factor that induces pro-inflammatory cytokines including IFN- $\alpha$  (65). Patients carrying the *IRF5* risk variants had increased expression of *IRF5* mRNA and type I IFN inducible genes (66). Furthermore, presence of a 'type I IFN signature' in peripheral blood is strongly associated with increased B cell activity (*e.g.* higher levels of autoantibodies); reviewed by (67). Interestingly, a recent study showed that *EPSTII1*, an ISG involved in NF- $\kappa$ B pathway activation, is elevated in B cells from pSS patients (68). Thus, type I IFN broadly enhances innate signalling pathways and thereby contributes to continued B cell activation.

In addition to its direct effects on B cells, type I IFN can also indirectly influence B cells via induction of B cell activating factor (BAFF) secretion by various cell types. BAFF promotes survival of B cells at immature and mature stages of B cell development (69, 70). BAFF transgenic mice develop autoimmunity with pathologic features of both SLE and pSS (71, 72). BAFF is produced by cultured salivary gland epithelial cells (SGEC) upon stimulation with IFN and SGECs are probably a major local source of BAFF *in vivo* (73). In the salivary glands, BAFF can enhance the survival of memory B cells and plasmablasts. The involvement of BAFF in the pathogenesis of pSS in humans is illustrated by elevated systemic BAFF levels and *BAFF* gene

expression in the inflamed salivary gland tissue (72, 74, 75). Interestingly, anti-BAFF treatment with belimumab resulted in a larger decrease in serum immunoglobulin levels in pSS patients with a type I IFN 'high' versus 'low' signature (76).

In addition to sustaining B cell activation, high levels of BAFF in peripheral blood of patients with pSS may also contribute to breaches of tolerance at early stages of B cell development. This is exemplified in BAFF transgenic mice, which appear to have less stringent selection of newly emigrant/transitional B cells and subsequent survival of low affinity self-reactive B cells (72). Also, in patients with pSS, central and peripheral tolerance checkpoints seem impaired, since both newly emigrant/transitional B cells and naïve B cells harbor an increased proportion of polyreactive cells (77). Notably, patients with pSS have an expanded population of CD21<sup>-low</sup> B cells that are enriched for autoreactive cells. These cells respond to activation via TLRs, but not via their BCR, and can undergo clonal expansion (78, 79). In addition to BAFF, increased type-I IFN expression and the concomitant increase in ISG expression can also contribute to breaks in tolerance checkpoints. In patients with SLE, transitional B cells from untreated patients with or without flares show overexpression of ISGs and the highest IFN score was seen in early transitional cells (80). In line with this finding, resting naïve B cells from SLE patients showed epigenetic modifications in IFN-stimulated genes, indicating early-stage priming and perturbed B cell development in the bone marrow (81). Although not yet proven, similar perturbations in transitional and naïve B cells are probably involved in pSS. Interestingly, resting naïve B cells from patients with pSS further show elevated Bruton's tyrosine kinase (BTK) and CD86 expression, inclining a lower activation threshold (82). Together, (poly)genetic changes in B cells, chronic type I IFN activation and subsequent secretion of BAFF by innate immune cells and epithelial cells in pSS can promote B cell activation and survival in a T cell-independent manner.

#### • *T-cell dependent B cell activation via IL-21*

T-cell dependent activation of B cells also contributes to the development of pathogenic B cells (83). The proportion of activated T follicular helper (Tfh) cells in blood from pSS patients correlates with systemic disease activity and the frequency of Tfh cells in the inflamed salivary glands correlates with lymphocytic focus score and B cell activity parameters (84-86). Furthermore, IL-21 transcript levels are enriched in labial gland biopsies with ectopic lymphoid structures and in parotid MALT lymphoma (87). IL-21 is a potent inducer of IgG and IgA antibody secreting cell differentiation (88). Interestingly, IL-21 is also a critical cytokine for the differentiation of a pathogenic, extrafollicular B cell subset in patients with lupus nephritis. These pathogenic B cells are characterised by a CXCR5<sup>+</sup>CD21<sup>lo</sup>CD11c<sup>hi</sup>T-bet<sup>+</sup> phenotype and their activation and differentiation into antibody secreting cells depends on TLR7 and IL-21 signalling (89, 90). Although not yet formally proven, pathogenic B cells with a similar phenotype are likely involved in the pathogenesis of pSS and these cells will be further discussed below.

#### Examples of pathogenic B cell subsets

##### *Rogue B cells producing pathogenic cryoglobulins*

Recent advances in single cell omics and mass spectrometry technologies have enabled the "rogue" B cells responsible for producing pathogenic cryoprecipitating rheumatoid factors to be identified, isolated and compared with other circulating B cells in patients with pSS complicated by type II cryoglobulinaemia (91). Rogue B cell clones in 4 patients showed immunophenotypes resembling memory B cells and plasmablasts with increased antigen presenting molecule expression (CD86, HLA) compared to polyclonal memory B cell and plasmablast counterparts from the same patient. Consistent with the pathogenic extrafollicular B cell subset in patients with lupus nephritis, CD21 expression was low; however, CD11c expression was variable. Single cell molecular analysis

of clonal rheumatoid factors enabled the construction of evolutionary trees, which revealed that pathogenic cryoglobulins arise from B cells bearing benign soluble rheumatoid factors that accumulate mutations in both lymphocyte regulatory genes and immunoglobulin V(D) J gene segments. Rogue B cells from all 4 patients carried one or multiple somatic mutations in genes found recurrently as drivers of lymphoma including *CARD11*, *TNFAIP3*, *CCND3*, *ID3*, *BTG2* and *KLHL6*. These genes normally function in regulating B cell activation, differentiation, proliferation and antibody V-region mutation. Somatic hypermutation to the antibody itself bestowed rheumatoid factor pathogenicity by increasing the propensity to form insoluble immune complexes with IgG. Further, lymphocyte regulatory gene mutations appeared to precede pathogenic antibody variable (V)-region mutations, which led to the hypothesis that lymphoma driver mutations are required to evade the tolerance checkpoints that normally prevent B cells from secreting a pathogenic autoantibody. Whether this shared pathogenic process with lymphoma is unique to B cells producing cryoprecipitating rheumatoid factors, thereby explaining the increased risk of lymphoma in pSS patients with cryoglobulinaemia, or common for other autoreactive lymphocytes remains to be established and has important implications for treating pSS and other autoimmune diseases by repurposing existing lymphoma therapies.

The accumulation of antibody V-region mutations to produce a pathogenic autoantibody appeared to be a gradual process, taking over 3 years in one patient with progressing disease from stage 1 glandular features to stage 2 type II cryoglobulinaemia. Moreover, this disease process appeared to be stochastic with one patient's cryoprecipitating rheumatoid factor accumulating additional V-region mutations that ultimately restored rheumatoid factor solubility. This gradual and stochastic process of V-region mutation altering autoantibody pathogenicity could explain the presence of autoantibodies in asymptomatic individuals, sporadic onset of symptoms and variability in disease course

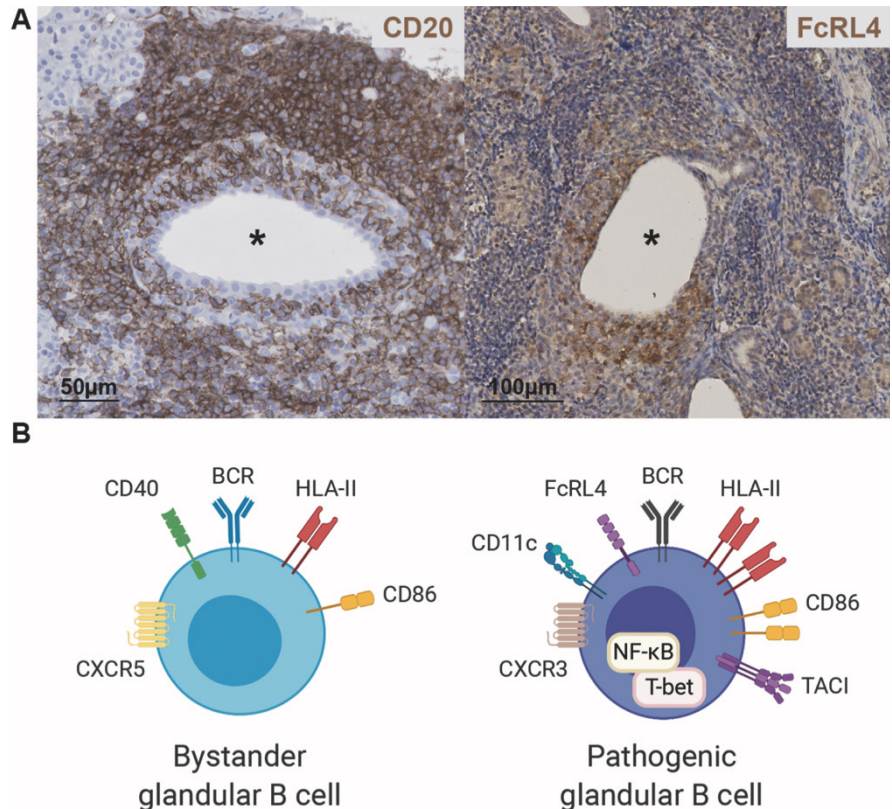
over time. Previous studies that have sequenced and cloned anti-Ro52 and anti-La autoantibodies from patient peripheral blood have also indicated a central role for immunoglobulin V-region mutation in producing or enhancing auto-reactivity (92, 93). What enables the B cells producing anti-Ro/La autoantibodies to bypass tolerance checkpoints and secrete high titre, high affinity autoantibodies remains undefined. It is possible that these B cells may acquire somatic mutations in lymphoma driver genes similar to rheumatoid factor B cells. Alternatively, extrinsic changes in the inflammatory milieu that promote B cell activation and/or change the quantity, quality or specificity of autoantibody, could also contribute to the emergence of pathogenic clones.

*FcRL4-expressing B cells in salivary gland tissue*

Advanced stages of salivary gland disease in pSS are characterised by an increase in infiltrating B cells (Fig. 2A) and differentiated plasma cells (94). A characteristic histopathologic feature of pSS involving B cells is the formation of lympho-epithelial lesions (LELs). LELs consist of proliferative intraepithelial B cells and hyperplastic ductal epithelium, with the influx of intraepithelial B cells a likely first step in LEL formation (95). Importantly, the development of MALT lymphoma ('stage 3' of pSS) is strongly associated with the presence of LELs (96). Below, we discuss the potential pathogenic role of a FcRL4-expressing B cell subset that is associated with LELs and MALT lymphoma in pSS.

• *Pathogenic role of tissue-based FcRL4<sup>+</sup> B cells*

Fc receptor-like (FcRL) 4 is an immunoregulatory receptor expressed by a specific subset of tissue-based memory B cells (97, 98). FcRL4 is a receptor for IgA and recent findings show that it binds specifically to systemic and mucosal IgA, but not secretory IgA (sIgA; IgA attached to a secretory component) (99). Interestingly, expression and ligation of FcRL4 inhibits BCR-mediated and promotes TLR-mediated activation of B cells (100). *In vitro*, B cells up-



**Fig. 2.** B cell involvement and pathogenic B cells in salivary gland tissue. **A:** Immunohistochemical staining for CD20 (left) and FcRL4 (right) on parotid gland tissue sections illustrate the involvement of B cells in tissue pathology (positive staining, brown; x20 actual magnification). CD20<sup>+</sup> B cells are abundantly present in periductal infiltrates and also within lympho-epithelial lesions (asterisk). FcRL4<sup>+</sup> cells are predominantly found within the lesions. **B:** Proposed phenotypical differences between bystander glandular B cells and pathogenic glandular B cells that infiltrate the ductal epithelium.

regulate FcRL4 expression after stimulation with soluble CD40 ligand and/or CpG DNA, indicating that FcRL4 is also a marker of recent activation (101). However, the mechanisms involved in FcRL4<sup>+</sup> B cell formation *in vivo* remain elusive.

FcRL4-expressing B cells were first linked to pSS pathogenesis in the context of MALT lymphomas, where FcRL4 was mainly expressed by tumour cells involved in LELs (102). Haacke *et al.*, showed that in addition to pSS-associated MALT lymphomas, FcRL4 is expressed by intraepithelial B cells and subepithelial B cells in inflamed salivary gland tissue of pSS patients without lymphoma (Fig. 2A) (103). This study also found that FcRL4<sup>+</sup> cells are proliferating within LELs. Further characterisation of FcRL4<sup>+</sup> B cells isolated from parotid gland tissue of patients with pSS showed that these cells are chronically activated,

pro-inflammatory B cells. Glandular FcRL4-expressing B cells contained, for example, higher transcript levels of genes encoding CD11c, T-bet, TACI, IL-6, and NF-κB1/p50, compared with FcRL4-negative counterparts (104). The transcriptional profile of glandular FcRL4<sup>+</sup> B cells shows similarities to the double negative (CD27<sup>-</sup>/IgD<sup>-</sup>) CD11c<sup>hi</sup> memory B cell subset associated with the pathogenesis of lupus nephritis (89) (Fig. 2B). However, in contrast to CD11c<sup>hi</sup> B cells in lupus, glandular FcRL4<sup>+</sup> B cells do not express markers associated with antibody-secreting cells, such as IRF4 or Blimp-1 (103) suggesting that FcRL4<sup>+</sup> B cells contribute to pathogenesis by mechanisms other than antibody secretion. Further, the finding that FcRL4<sup>+</sup> B cells are proliferating within the ducts, together with the observation that clonal expansions are present among intraductal B cells (105), suggests that FcRL4<sup>+</sup> B



**Table I.** Clinical outcomes of randomised controlled trials with B-cell targeted therapies in primary Sjögren's syndrome.

Trial	Drug	Target	n (treatment/ placebo)	Primary outcome	Primary outcome met	Improved secondary outcomes*	Comments
Meijer <i>et al.</i> , 2010 (123)	Rituximab	CD20	20/10	SWSF (mean change from baseline)	Yes	- UWSF - Lissamin green test - VAS dryness - MFI general fatigue - SF-36	Post-hoc analyses showed improvement in ESSDAI score and SG morphology (124, 125)
Devauchelle-Pensec <i>et al.</i> , 2014 (126)	Rituximab	CD20	60/60	VAS scores week 24 (improvement of at least 30 mm in 2 of 4 VASs)	No	- VAS fatigue - VAS dryness	Post-hoc analyses showed improvements in composite endpoint and SG ultrasound score (127, 128)
Bowman <i>et al.</i> , 2017 (129)	Rituximab	CD20	67/66	VAS scores week 48 (30% reduction in either fatigue or oral dryness)	No	- UWSF	Post-hoc analysis showed improvement in SG ultrasound score (130)
Dörner <i>et al.</i> , 2020 (131)	Ianalumab	BAFF-R	190**	ESSDAI week 24 (change from baseline)	Yes	- PhGA	Endpoint was met for 300mg dose only. Second blinded treatment period ongoing

SWSF: stimulated whole salivary flow; UWSF: unstimulated whole salivary flow; VAS: Visual Analogue Scale; MFI: Multidimensional Fatigue Index; SF-36: Short Form (36) Health Survey; ESSDAI: EULAR Sjögren's syndrome disease activity index; SG: salivary gland; PhGA: physician's global assessment.

\*Improvement over time compared with placebo.

\*\*190 patients allocated 1:1:1 over placebo and three different doses of ianalumab.

cells are continuously activated by the ductal epithelium. Furthermore, expression of TACI provides a local survival mechanism for these cells, since activated salivary gland epithelial cells produce high levels of the TACI ligand BAFF (73). In turn, FcRL4<sup>+</sup> B cells themselves produce cytokines, such as IL-6, that support inflammation of the epithelium. Finally, we speculate that transient expression of AID by chronically activated FcRL4<sup>+</sup> B cells may result in off-target mutations (*i.e.* outside of Ig genes) and incidental acquisition or accumulation of lymphoma-driver mutations observed in cryoglobulin producing rogue B cells (91).

### Avenues for targeting pathogenic B cells

Although two large randomised controlled trials (RCTs) of B cell depletion therapy using rituximab did not meet their primary endpoint in pSS, there are indications that a -yet to be stratified- subgroup of pSS patients benefit from this treatment (33). Excitingly, preliminary results of dual BAFF receptor inhibition and B cell depletion with ianalumab suggest clinical efficacy in pSS patients with moderate-to-high systemic disease activity (106). Observed clinical improvements in

RCTs with B cell targeted therapies are summarised in Table I. Other promising approaches currently under investigation are sequential treatment with belimumab (anti-BAFF) and rituximab to enhance depletion of B cells in affected tissues and inhibition of BTK, which sits at the crossroads of BCR and TLR signalling (107, 108). Of further interest, a *post-hoc* analysis of an RCT with epratuzumab (anti-CD22) in SLE patients with associated SS, suggesting a greater pathogenic role of CD22-expressing B cells in this subgroup (109). Besides the aforementioned B cell targeted approaches, drugs that block costimulatory proteins, such as anti-CD40, and more broadly acting immunosuppressive treatments, such as combined use of hydroxychloroquine and leflunomide, may affect pathogenic B cells in pSS (110-112). Furthermore, therapies that have recently shown clinical efficacy in SLE trials (*i.e.* anti-IL-12/23 p40 and anti-IFN- $\alpha$  receptor antibodies) may also be useful for (partial) inhibition of B cell activation (113, 114). With the availability of multiple promising new therapeutics, the challenge remains to identify responders *a priori* and to achieve this, we may need to rethink patient stratification. For

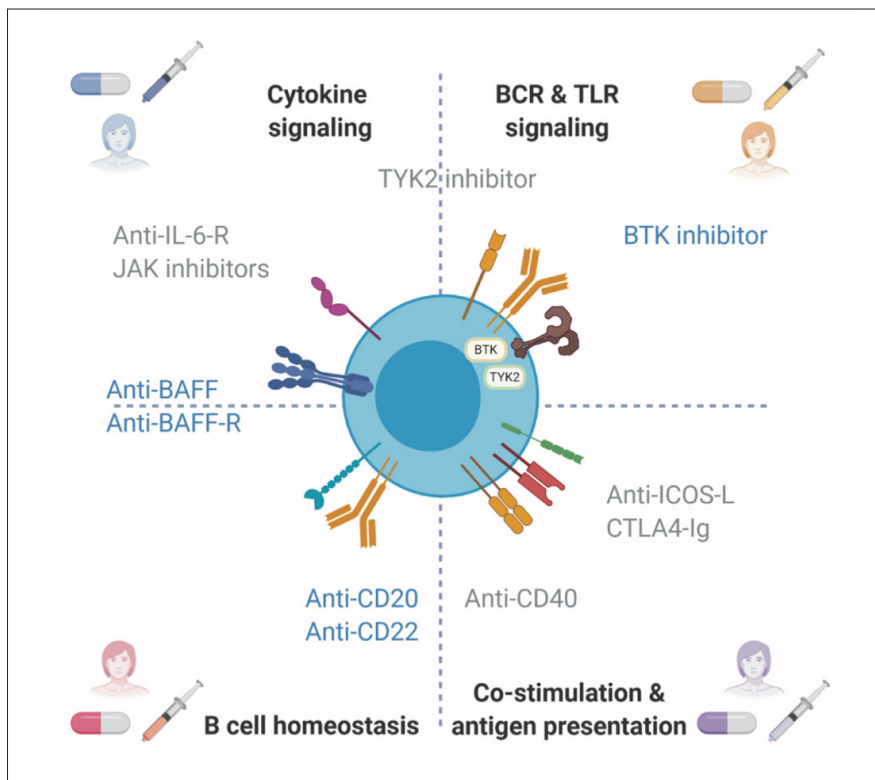
example, an elegant new approach of clinical phenotyping based on patient-reported symptoms was recently proposed by Tarn *et al.* (115). However, given the heterogeneity of pSS, it is likely that patients with similar clinical phenotypes may exhibit a range of underlying pathologies. Therefore, stratification of patient groups should also consider disease endotypes, similar to other chronic diseases. To define a 'B cell dominant' endotype, patient stratification will need to extend from autoantibody positivity alone to standardised assays to measure hyper-responsiveness of B cells, phenotypical and functional markers to define pathogenic B cells, and surrogate biomarkers that reflect pathogenic B cell involvement in affected tissues as well as genomic markers of disease. Since the roles of B cells in disease pathogenesis are diverse, the specific target for treatment may need to be adjusted at the individual patient level (Fig. 3). It is unlikely that different B cell targeting therapies will be equally effective in heterogeneous groups of patients, or that a single, uniform targeted strategy will be effective as illustrated by a recent case series of pSS patients with cryoglobulinaemic vasculitis refractory to rituximab alone, but successfully treated by the com-



bination of rituximab and belimumab (anti-BAFF) (116). Below we provide an additional example that highlights the complexity and promise of molecularly defining a pathogenetic B cell response, to guide precision medicine approaches.

**Patient vignette and concluding remarks**

Here, we present a case vignette as an illuminating example of how understanding the role of B cells in disease pathogenesis could drive the application of precision medicine. The case was one of the 4 patients with cryoglobulinaemia found to exhibit lymphoma driver mutations, reported above (91). A 23-year-old woman was taken to the emergency department with quadriplegia, one week following an upper respiratory tract infection. The cause of her paralysis was identified as severe hypokalaemia due to distal renal tubular acidosis. Following review by a renal physician with an interest in pSS, the cause was determined as tubulointerstitial nephritis secondary to stage 2 pSS. In fact, the patient had presented to her primary physician 6 years earlier at the age of 17, in stage 1 pSS with severe fatigue, salivary glandular swelling and sicca symptoms, but was diagnosed only with depression. Her disease progressed over the following decade, manifesting as severe sicca symptoms, inflammatory polyarthritis, ongoing type I distal renal tubular acidosis complicated by nephrocalcinosis and frequent hospital admissions with urosepsis; despite treatments including hydroxychloroquine, corticosteroids, methotrexate and mycophenolate mofetil. Serum parameters revealed high rheumatoid factor, low C3 and C4, hypergammaglobulinaemia, IgA kappa and IgM kappa paraproteins, and type II mixed cryoglobulinaemia. Cyclophosphamide, and plasmapheresis were used sequentially to treat cryoglobulinaemic vasculitis, which manifest with cutaneous and gallbladder vasculitis, and bilateral ulnar nerve palsy. Several courses of rituximab, given as 2 doses of 1 g infusions two weeks apart, led to minimal improvement in clinical symptoms or serum cryoglobulin levels. Sin-



**Fig. 3.** Model for precision medicine with proposed B cell-targeted therapies in Sjögren's syndrome. Four aspects of B cell biology and conforming treatment options are displayed. We hypothesize that each patient has an individual B cell activation signature and that patients may be categorised in different profiles (colours) to guide precision treatment decisions. Therapies that target B cells directly are shown in blue and therapies that target B cells indirectly are shown in grey. More broadly acting, immunomodulatory drugs such as hydroxychloroquine and leflunomide are not displayed, but can also target B cells. Additional therapies that have recently shown clinical efficacy in systemic lupus erythematosus trials (*i.e.* anti-IL-12/23 p40 or anti-IFN- $\alpha$  receptor antibodies) may also be useful for inhibition of B cell activation.

gle cell analysis revealed a plausible explanation for the failure of rituximab in this patient: the majority of her B cells producing the pathogenic cryoglobulin had differentiated into plasmablasts with very low to no surface CD20. These rogue clones represented more than one third of the patient's CD27<sup>+</sup> memory B cell and plasmablast compartment 3 months after treatment with rituximab. The cryoglobulin-producing B cells also carried a *CARD11* mutation at a site frequently mutated in diffuse large B cell lymphoma causing hyperactivation of inflammatory mediator NF- $\kappa$ B and differentiation into plasmablasts (91, 117). While the combined cellular, molecular and genetic analysis of B cells producing pathogenic autoantibody, as outlined here, is important to understand disease pathogenesis, it also provides insight into treatment selection. BTK inhibitors such as ibrutinib are a lym-

phoma treatment that has recently entered clinical trials for pSS. Importantly, lymphomas carrying *CARD11* gain-of-function mutations are resistant to treatment with ibrutinib because the *CARD11* mutation causes a constitutively active *CARD11* downstream of BTK, which activates NF- $\kappa$ B independent of BCR signalling and BTK (118). Therefore, ibrutinib would not perturb the pathogenic cryoglobulin producing B cells in this patient and should be avoided. Along similar lines, comes the great anticipation that detailed analyses of pathogenic B cells will identify novel therapeutic targets and pathways. One of the most differentially expressed genes in this patient's rogue cells compared to her developmentally matched polyclonal B cells was *CD86*, which encodes a surface protein that interacts with CD28 on T cells to stimulate immune responses. Previous studies in mice have shown that overexpression

of CD86 on self-reactive B cells is sufficient to evade a tolerance checkpoint of T cell-mediated apoptosis and instead receive T cell help to drive autoantibody secretion (119). Abatacept, a CTLA4-Ig fusion protein registered for the treatment of rheumatoid arthritis, blocks CD86-CD28 interaction and may provide an avenue for eliminating these rogue B cells. However, as with most other trials of biologic therapies for pSS (120), abatacept failed to meet its primary endpoints in two phase 3 randomised controlled trials (112, 121), possibly due to inadequate stratification of clinical trial subjects with respect to pathologic B cell phenotypes. The challenge moving forward is to develop personalised treatment strategies for patients with pSS via stratification according to the pathogenic mechanisms governing their various clinical manifestations (122). This will require the development of technology to apply a detailed cellular and molecular genomics-based analysis to pathogenic B cells in more patients to link pathophysiological mechanisms of disease with immune function, and realise the potential of precision medicine. The development of new cellular, molecular and genetic biomarkers will better determine the efficacy of treatments for individual subjects in clinical trials and, importantly, will identify those most at risk of developing progressive disease, allow early rationalised interventions, and prevent complications of undertreated disease including progression to stage 3 pSS.

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