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New insights into B cell biology in SLE and Sjogren's Syndrome

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Abstract

Purpose of review—Our understanding of the physiological and pathogenic functions of B cells in systemic lupus erythematosus (SLE) and Sjogren's syndrome (pSS) continues to expand. In this review, we discuss novel insights published in the last 18 months into the roles of B cells in systemic autoimmunity.

Recent findings—Data has continued to expand the diverse mechanisms by which innate immune signals including toll like receptors (TLR) may regulate the B cell compartment. Localized B cells and long-lived plasma cells have been identified as playing an important role in target tissue including the development of ectopic lymphoid structures in kidney and salivary gland. In addition to pathogenic roles for B cells, there is mounting evidence for regulatory B cell subsets that play a protective role and new insights into the signals that regulate their development.

Summary—The past few years have provided insights into the multiple paths by which innate immune signals can lead to B cell activation in SLE and pSS and the increasingly diverse ways in which B cells contribute to disease expression. Further understanding the imbalance between protective and pathogenic functions for B cells in disease including in understudied target tissue should yield new treatment approaches.

Keywords

B cells; autoimmunity; systemic lupus; Sjogren's Syndrome

Introduction

Systemic lupus erythematosus (SLE) and Primary Sjogren's Syndrome (pSS) are very distinct diseases clinically but share many critical common dysregulated immune pathways including the long known key role of B cells as well as Type I interferon activation and ectopic lymphoneogenesis. Both diseases also have significant unmet needs with SLE still associated with a three- to five-fold increased mortality compared to the general population and pSS lacking disease modifying therapeutic interventions. The need for more effective therapies with less toxic side effects has propelled interest in targeted biologic therapies

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based on an expanding understanding about disease pathogenesis in both diseases. In this review, we will highlight new insights into B cell roles in disease pathogenesis with a focus on novel interactions between the adaptive and innate immune systems and how this has revealed new treatment targets.

Target tissue effects

B cells can provide a critical link between the development of tertiary lymphoid tissue within both the lupus kidney and pSS salivary glands (ectopic lymphoneogenesis) and the propagation of the autoimmune process. This contention has been supported historically by the finding of aggregates of B cells and in some instances germinal center (GC) like structures within target tissue. The precise requirements for the generation of these lymphoid structures, their frequency, and role in the pathogenesis of disease have remained unclear. Clark and colleagues have demonstrated well-circumscribed T:B cell aggregates or GCs containing follicular dendritic cells within lupus nephritis biopsies associated with intrarenal B cell clonal expansion and ongoing somatic hypermutation [1]. Similar structures have been identified within the salivary glands of pSS patients. These results implicate ectopic lymphoneogenesis in the pathogenesis of lupus tubulointerstitial inflammation [1] and possibly antigen-driven B cell selection in pSS salivary gland. In a recent ambitious follow-up study by Clark, novel cell distance mapping approaches were used to identify T follicular helper (TFH) cells in cognate interactions with *in situ* B cells in the lupus kidney, suggesting that this key cell population is instrumental in propagating humoral immunity in target tissue [2].

A key question is what factors may drive the accumulation of TFH, B cells, and ectopic lymphoid structures in target tissue. Studies in rheumatoid arthritis have demonstrated abundant synovial expression of CXCL13 (a major B cell attractant), as well as CCL19 (a T cell attractant), and BAFF (B cell activation factor). CXCL13 can be produced by synoviocytes and follicular dendritic cells in the RA synovium and was also found to be secreted by RA synovial T cells, indicating a direct role for both the inflamed synovial stroma and synovial T cells in the recruitment and organization of B cells [3]. Interestingly, it has recently been shown by Wang et al. [4] that there is an increase in renal expression of B cell survival factors (BAFF, a proliferation-inducing ligand, and IL-6) and B cell and PC attracting chemokines (CXCL13 and CXCL12) coincident with enrichment of autoreactive dsDNA Ab-secreting cells in the kidney of lupus-prone mice. Notably, prolonged B cell depletion altered this B cell survival niche by affecting renal macrophage expression of key survival factors [4]. B cell borne lymphotoxin (LT) α can also have a profound effect on secondary and tertiary lymphoid architecture. Thus, B cell depletion alters lymphoid architecture by eliminating LT α bearing and TNF secreting B cells [5], resulting in a prolonged delay in memory B cell reconstitution [6]. B cells are involved in the formation and/or maintenance of ELS as these tissues do not efficiently develop in B cell deficient mice [7]. Interestingly, studies have revealed the presence of cytokine-producing B cells (IFN γ , IL-6, TNF α , LT α) directly within the ectopic lymphoid tissues [7]. However, the impact of B cell depletion on ELS requires further study.

It is known that tissue specific expression of LT α in the kidney and pancreas causes inflammation and formation of ELS in the mouse with promotion of antigen-specific responses [8]. In combination with LT β , LT α enhances ectopic lymphoid neogenesis. Thus, evidence in patients with pSS and animal models have demonstrated that ectopic lymphoneogenesis is dependent on the ectopic expression of LT and lymphoid chemokines CXCL13, CCL19, CCL21 and CXCL12. Infiltrating immune cells as well as resident epithelial and stromal cells can produce these factors during chronic inflammation in pSS [9]. In pSS animal models spontaneous development of splenic GCs precedes the lymphocytic infiltration of the salivary gland suggesting that tertiary lymphoid structures may arise from trafficking of activated T and B cells to sites of inflammation [10]. Interestingly, blockade of CXCL13 was sufficient to disrupt follicular architecture of the spleen but did not impact tertiary lymphoid structures in the salivary gland [11]. A recent study also implicated EBV dysregulation in the formation of ectopic lymphoid structures in pSS with evidence of EBV infection in B cells and plasma cells in the salivary gland of pSS patients with ectopic structures. Importantly, perifollicular plasma cells displaying Ro52 reactivity were frequently infected by EBV, and transplantation of pSS salivary gland tissue to SCID mice supported the production of Ro antibodies and anti-EBV antibodies [12].

Recent data has also elucidated unique cellular initiators of lymphoneogenesis possibly also in ectopic locations, including hematopoietic derived lymphoid tissue inducer cells (LTi cells), IL17 secreting CD4 T cells, and T follicular helper cells [13]. More is known about the role of LTi cells in secondary lymphoid organogenesis. Thus, the accumulation and survival of LTi cells is coordinated by the expression of CXCL13, RANKL, and IL7. There is a critical interplay between LTi cells and mesenchymal derived lymphoid organizer stromal cells, the latter producing IL7 and RANKL and inducing the expression of LT α 1 β 2 on LTi cells, in turn engaging the LT β receptor on lymphoid tissue organizer cells. LT β receptor signaling on lymphoid tissue organizer cells, leading to the production of critical chemokines such as CCL19, CCL21, and CXCL12 [14], plays a key role in lymph node development. Stromal tissue cells may acquire lymphoid tissue organizer cell-like properties within the RA synovium [15]. However, the relevance of these findings to ectopic lymphoid structures in SLE and pSS remains to be defined. It is even unclear whether the development of tertiary lymphoid structures is dependent on LTi cells. Thus, an interesting recent paper demonstrated that robust tertiary lymphoid tissue development occurs in the lung and during colitis in ROR γ t-deficient mice, which lack both LTi and Th17 cells, and was dependent on LT β expressed by B cells [16]. These findings raise the intriguing possibility that B cells may assume an LTi function.

The B cell- innate immune system interface

Data has continued to expand the mechanisms by which innate immune signals may regulate the B cell compartment [17]. A recent elegant study described a population of neutrophils normally colonizing the human splenic MZ that promote B cell activation and differentiation through the production of BAFF, APRIL, and IL21, termed 'B helper neutrophils' [18]. It was proposed that this unique neutrophil subset may be important for rapid humoral immune responses to blood-borne microorganisms. Whether the function of this unique neutrophil population is dysregulated in autoimmune disease remains to be defined. Intriguingly,

splenic neutrophil location was perturbed in a single lupus spleen examined with infiltration into the GC, suggesting that B helper neutrophils may provide aberrant B cell help in SLE. The Cerutti group has also recently demonstrated that ROR γ t + innate lymphoid cells (ILC) (LTi cells are part of this expanding cell family) can enhance antibody production by MZ B cells in the human spleen [19]. Interestingly, ILC were able to stimulate circulating neutrophils to acquire a B helper phenotype and induced neutrophil extracellular trap (NET) formation [19]. The investigation of ILCs in autoimmunity including SLE and pSS is an important area for future study.

It has also been described recently that neutrophils can be primed by interferon and autoantibodies to release NETs as a source of immunogenic DNA, histones, and neutrophil proteins contributing to pDC activation [20,21]. The development of autoantibodies to both self-DNA and antimicrobial peptides in NETs suggests that NETs could also serve as autoantigens to trigger B cell activation. NETs clearly can trigger pDCs to produce large quantities of IFN α , a key cytokine acting at the center of an amplification loop in SLE, indirectly contributing to B cell abnormalities and promoting the differentiation of activated B cells into plasmablasts. Notably, IFN can act in conjunction with toll-like receptor (TLR) stimulation to trigger B cell expansion [22] and a lowered activation threshold for autoreactive B cells [23]. In turn, B cells can produce anti-DNA and anti-RNA autoantibodies further promoting NETosis. A novel role for IFN α activation has also been reported in the bone marrow of SLE patients by decreasing B cell lymphopoiesis, contributing to B cell lymphopenia, and theoretically decreasing the stringency of B cell negative selection [24].

It is notable that the induction of NETosis and activation of pDCs via NETs is dependent on Fc receptors and TLR signaling delivered in part via RNA- or DNA-containing autoantigen/ autoantibody immune complexes. This adds to the accumulating evidence that B cell derived autoantibodies can play an active role in propagating the autoimmune process in SLE, through TLR dependent immune cell activation [25]. In addition to the well-described activation of pDCs by costimulation of TLRs and FcRs via immune complex binding, relevant for both SLE and pSS, B cell responses can also be modified by TLR signaling. The concept is that RNA and DNA-associated autoantigens are bound by the BCR and transported to a TLR-compartment where TLR detection of DNA or RNA provides a second signal promoting B cell activation. Intriguingly, a recent paper demonstrated the surprising finding in UNC-93B deficient lupus-prone mice that the development of non-nuclear autoantibody specificities, including anti-red blood cell and in other contexts anti-MPO, was dependent on TLR signaling in B cells [26]. One possible explanation for the dependence of non-nuclear antibodies on nucleic acid-TLR signaling is that they act as auto-antigens only when associated with nucleic acids, for example on apoptotic cells or neutrophil extracellular traps.

Recent literature has also uncovered new findings about the impact of both TLR7 and TLR9 on autoantibody formation and disease progression in murine models of SLE. The contribution of TLR7 to SLE has been more straightforward, correlating positively with autoimmunity as exemplified by the TLR7 duplication Yaa lupus susceptibility locus in mice and the abrogation of disease in TLR7 deficient mice. On the other hand, there has

been conflicting evidence regarding whether the role of TLR9 is protective or deleterious in SLE. Although TLR9 is required for development of anti-dsDNA and anti-chromatin consistent with the importance of BCR-delivered TLR stimulation, TLR9 deficiency surprisingly enhances disease in multiple murine lupus models [27]. This paradox was partially resolved by recent literature demonstrating that TLR9 promotes tolerance by restricting survival of anergic anti-DNA B cells but is also essential for their activation and differentiation to antibody secreting cell in extra-follicular reactions [28]. The differential outcome of TLR9 signaling remains unexplained but could relate to B cell developmental state, location, and/or other extrinsic signals. An unsuspected tolerogenic role for TLR signaling was also supported by recent work demonstrating defective central and peripheral B cell tolerance checkpoints in patients deficient for interleukin-1 receptor-associated kinase 4 (IRAK-4), myeloid differentiation factor 88 (MyD88), and UNC-93B, key for signaling via multiple TLRs [29]. In mice, the exacerbation of clinical disease and production of anti-RNA antibodies in the absence of TLR9 was dependent on Type I IFN signaling [30] and was B cell intrinsic [31,32]. The enhanced production of anti-RNA antibodies is consistent with other data that TLR9 deficiency up-regulates TLR7 in B cells [33]. Adding to the complexity, TLR8 was also reported to negatively regulate murine SLE possibly by restraining TLR7 responses [34].

Intriguingly, TLR7 and TLR9 also appear to have opposing roles in the formation of spontaneous germinal centers, with B cell intrinsic TLR7 expression essential for the development of GCs and breaking of GC-tolerance checkpoints and TLR9 providing a negative regulatory function [35]. The mechanisms driving these differences in response are not clear. TLR7 overexpression in B cells was also recently shown to promote the proliferation and plasma cell differentiation of transitional type 1 B cells [36], highlighting the multiple stages of B cell development that may be affected by TLR signaling.

Contribution of dysregulated B cells to disease expression via cytokines

Another mechanism by which B cells can contribute to SLE and pSS is via the production of cytokines. Similar to T cells, cytokine-producing human B cells can be polarized, and actually induce Th1 and Th2 cells (termed Be1 and Be2 cell respectively) [37]. CD27⁺ Be1 cells may accumulate in the salivary glands in pSS [38]. A recent elegant paper in a different autoimmune disease, experimental autoimmune encephalomyelitis, demonstrated that B cell production of IL6 drives Th17 responses, and moreover B cell depletion alleviates disease through ablation of IL6 producing B cells [39]. As detailed earlier cytokine-producing B cells can also regulate lymphoid tissue formation and maintenance, including the formation and/or maintenance of tertiary lymphoid tissues [7]. On the other hand, growing evidence is accumulating for regulatory B cells capable of preventing or suppressing autoimmunity in different mouse models [40]. This protective role can be mediated by inducing T cell anergy during antigen presentation or inducing Treg expansion or activity, [40]. B cells may also directly suppress Th1 and Th17-mediated diseases [41]. These activities are mediated, at least in part, by the production of IL10 or TGF β and may control a variety of autoimmune diseases including: inflammatory arthritis, inflammatory bowel disease, autoimmune diabetes, SLE, and pSS [42].

Recent data has further elucidated the nature of both murine and human Bregs. Mouse Breg activity has been variously assigned to cells with a transitional (in particular, T2-Marginal Zone Precursors – T2/MZP), Marginal Zone (MZ) or B1/transitional/MZ intermediate phenotype and lupus resistance has been associated with expansion of MZ cells [42,43]. Our observation that expansion of transitional B cells correlates with long-term remission in SLE patients treated with B cell depletion therapy is consistent with a regulatory nature [44]. Two recent papers have further characterized the human counterpart of the regulatory B cell. Thus, Iwata et al. identified a small fraction of CD24^{hi}CD27⁺ B cells in human peripheral blood that express IL10 and regulate monocyte production of TNF α [45]. In contrast, Mauri and colleagues identified a CD19⁺CD24^{hi}CD38^{hi}CD1d⁺ B cell, likely a transitional subset, with high propensity to produce IL10 and suppress T cell cytokine production. Notably, the suppressive function of this cell subset appeared to be defective in SLE, but not in pSS [46].

Additional data has further defined the factors that control the development and function of B regulatory cells. For example, Rosser et al. have found that gut microbiota promote the differentiation of Bregs in the spleen and mesenteric lymph nodes in an IL6 and IL1 dependent fashion [47]. This was important in the regulation of inflammatory arthritis in a mouse model, but the relevance for SLE and pSS remains unclear. Another paper demonstrated that Tim-1, a type I transmembrane glycoprotein important in apoptotic cell clearance, is critical for IL10 production by regulatory B cells. Thus, Tim-1 mutant mice particularly on an autoimmune prone background developed accelerated systemic autoimmunity with accumulation of double-negative T cells and autoantibodies to a number of lupus-associated autoantigens [48]. The authors suggest that one possible mechanism by which Tim-1 maintains Breg IL10 production may be through the interaction of Tim-1 with apoptotic cells. Of note, apoptotic cells have been shown to promote Bregs, which inhibited collagen induced arthritis in an IL10dependent manner. A more recent extension of this study found that IL10 production by Bregs in response to apoptotic cells was dependent on recognition of DNA-containing complexes via TLR9, and CD27⁺ human B cells also responded to DNA-bearing apoptotic cells by secreting IL10 [49]. This seems to challenge the existing paradigm that TLR9 ligation must generate an inflammatory signal. Bregs may also mediate their effects by mechanisms other than cytokine production. For example, Khan et al. recently identified a PD-L1 (the ligand for an inhibitory programmed death receptor) expressing B cell within the mouse MZ and human naïve compartment that suppressed TFH but was not IL10 mediated [50].

B cell targeted immunotherapies

The data above begins to highlight potential novel therapeutic approaches in SLE and pSS as well as insights into the variable success of B cell depletion therapy. One issue may be that B cells in the target tissue are incompletely depleted, particularly within the local inflammatory microenvironment of ectopic lymphoid structures. In accord with this, studies have shown a clear discrepancy between peripheral blood B cell depletion and depletion in the salivary gland in pSS. One study found clonal expansions of IgA and IgG-expressing cells in pSS parotid tissue and even higher mutational load after rituximab. These results suggest that local somatic hypermutation, isotype switch, and plasma cell differentiation continue to occur in residual tissue cells in ectopic locations after rituximab treatment [51].

More efficacious ways to interrupt ectopic lymphoneogenesis are under investigation, including blockade of LT β receptor signaling with the decoy receptor baminercept. Although baminercept failed to show clinical efficacy in early phase clinical trials in rheumatoid arthritis, its utility in pSS is current under investigation in a trial sponsored by the Autoimmunity Centers of Excellence (National Institute of Allergy and Infectious Diseases). Combination therapies, for example B cell depletion and LT β receptor blockade or inhibition of B cell attracting chemokines (e.g. CXCL13) or B cell survival cytokines (e.g. BAFF or IL6) may also have merit. Given the recently elucidated roles of Th17 and TFH cells in ectopic lymphoid structures, the cytokines IL21 and IL17 are additional attractive targets.

Another issue with B cell depletion is that it may indiscriminately deplete pro-inflammatory B cells and Breg populations. On the other hand, the recently described PD-L1 expressing Bregs appear to be relatively resistant to B cell depletion therapy, possibly due to increased expression of BAFF receptors (BAFF-R, TACI and BCMA) [50]. Understanding the imbalance between opposing B cell functions in disease and how this imbalance may be restored after targeted biologic therapies remains an important area of investigation. Another potential reason for variable efficacy of B cell depletion is the persistence of long-lived CD20 negative plasma cells and pathogenic autoantibodies. This observation has raised interest in the possibility of targeting the plasma cell compartment. Bortezomib is a proteasome inhibitor that effectively depletes even long-lived plasma cells, has efficacy in the treatment of murine lupus [52], and was recently reported to have beneficial effects in a small open label trial in human SLE [53]. Novel immunoproteasome inhibitors with a lower toxicity profile were also recently described to create a powerful synergistic effect in the treatment of murine SLE by targeting plasma cells and IFN α production by pDCs [54].

Conclusions

Given the disappointing clinical trial results with targeted B cell depletion, a better understanding of the multiple pathways leading to autoreactive B cell activation and the diverse roles for B cells in both SLE and pSS is clearly important. New studies defining the functional interactions between B cells and other critical cell populations within target tissue have great potential to yield novel insights into disease pathogenesis and unveil new treatment targets. Combination therapy directed at multiple steps in disease pathogenesis needs to be considered.

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*Of special interest

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**Of outstanding interest

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Key Points

1. Ectopic lymphoid structures (ELS) are collections of B and T cells sometimes in very organized germinal center structures in inflamed tissues that can contribute to disease pathogenesis by generating autoreactive clonal B cell expansion and somatic hypermutation in target tissues such as the lupus kidney and Sjogren's salivary gland.
2. Lupus and Sjogren's RNA and DNA-associated autoantigens can act as TLR7, 8, and 9 ligands being bound by the B cell receptor and transported to a TLR-compartment thus providing a second signal promoting B cell activation.
3. In addition to apoptotic cells, neutrophils in the form of extracellular traps can be a source of immunogenic DNA and histones, providing nucleic acid containing TLR ligand signals to B cells and contributing to B cell dysregulation in systemic autoimmunity by multiple mechanisms: loss of B cell tolerance by lowering autoreactive B cell activation, breaking of germinal center tolerance check-points, and promotion of B cell proliferation and plasma cell differentiation.
4. B cells can contribute to lupus and Sjogren's via autoantibody independent mechanisms such as the production of cytokines, with recent examples provided by CD27+ memory B cells driving Th17 responses, LT α expression driving ELS, and defects in IL10 expressing regulatory B cells.