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The long and the short of it: insights into the cellular source of autoantibodies as revealed by B cell depletion therapy

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Abstract

High titers of pathogenic autoantibodies are a hallmark of many autoimmune diseases. However, much remains unknown about the self-reactive plasma cells that are key mediators of disease. We propose a model in which the varying efficacy of precursor B cell depletion for the treatment of humoral autoimmunity can be explained by differences in the relative contributions of pathogenic antibodies by short-lived vs. long-lived plasma cells. Beyond therapeutic considerations, this model suggests that we can infer the cellular source of disease-associated autoantibodies by the durability of serum titers following B cell depletion. Data from clinical trials and animal models across different autoimmune diseases may provide useful insights into the lifespan, lifestyle and fate of autoreactive plasma cells.

Introduction

In multiple autoimmune disorders, high-titer autoantibodies can predict disease onset, serve as biomarkers for diagnosis, or directly promote disease pathogenesis [1,2]. Despite these important roles for autoantibodies in human disease, the biology of plasma cells that are the source of serum immunoglobulin remains poorly understood. During an adaptive immune response to foreign antigen, specific antibodies are produced by both short- and long-lived plasma cell subpopulations [3]. Multiple lines of evidence suggest that similar activation pathways underlie autoimmune pathogenesis. However, since autoreactive plasma cells are rare cells residing in inaccessible locations within the bone marrow, secondary lymphoid organs and inflamed tissues, direct study of plasma cell biology in human autoimmunity is technically challenging.

Over the past two decades, a number of B cell depleting therapies have been trialed in human autoimmunity. The most well-studied agent, rituximab (Rituxan), is a humanized

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monoclonal antibody binding CD20, a B cell surface marker first expressed at the late pre-B cell stage of bone marrow development, maintained throughout peripheral B cell maturation, and downregulated during differentiation into antibody-secreting cells (ASC). Since CD20 expression is lost during plasma cell maturation, treatment with rituximab or related B cell depletion therapies is not predicted to directly target mature plasma cells [3,4]. Rather, these therapies likely impact circulating autoantibody titers by either eliminating autoreactive B cells that are the precursors of pathogenic plasma cells and/or by directly targeting recently generated plasmablasts which can retain low-level CD20 expression [5–7]. Based on these observations, we propose a model in which the impact of B cell ablation on autoantibody titers can be used to infer the characteristics of self-reactive plasma cells in individual diseases. Importantly, therapeutic benefits in B cell depletion frequently precede reductions in autoantibody titers, suggesting that loss of B cell antigen presentation and/or cytokine production contributes to clinical efficacy [2]. However, rather than an exhaustive review of clinical trials of B cell depletion in autoimmunity, in the current manuscript we will focus specifically on the impact of B cell targeting on serum autoantibody titers. As models of distinct mechanisms in autoimmunity, we will highlight data from clinical trials in pemphigus vulgaris, Sjögren's syndrome and systemic lupus erythematosus (SLE); three diseases that we believe exemplify the differential contributions of short- and long-lived plasma cells in autoimmune pathogenesis.

Overlapping contributions of short- and long-lived plasma cells to humoral immunity

During a humoral immune response, antigen-specific B cells differentiate into memory B cells and antibody-producing plasma cells. Memory B cells are antigen-experienced B cells that remain quiescent for prolonged periods before rapid secondary response to antigen rechallenge. In contrast, plasma cells are effector B cells which serve as the source for both protective and pathogenic antibodies. Functionally, plasma cells can be divided into two subsets based on survival kinetics and location: a short-lived population thought to be generated predominantly via extrafollicular B cell activation and to reside in the splenic red pulp or lymph node medullary cords; and long-lived plasma cells (LLPC) that are primarily germinal center (GC)-derived and traffic to bone marrow survival niches [3]. Although considered separately here, short- and long-lived plasma cells are generated concurrently during a T-dependent immune response. After initial antigen challenge, rapid extrafollicular plasma cell responses are followed by the generation of GC-derived, affinity-matured LLPCs, thereby providing overlapping humoral protection from infectious challenge (Figure 1).

Notably, the paradigm that long-term maintenance of protective antibody titers depends on a small population of LLPCs is a relatively new one. Until twenty years ago, the humoral component of immune memory was believed to be supplied by terminally differentiated plasma cells that had not been observed to survive longer than a week. Since the half-life of an antibody in serum is ~28 days, long-term antibody titers were attributed to the continual homeostatic and/or antigen-driven differentiation of memory B cells into short-lived plasma cells (SLPC). However, in 1998, *Slifka et al.* demonstrated long-term maintenance of virus-

specific antibodies despite memory B cell depletion, establishing the existence of LLPCs maintained without replenishment from the memory B cell pool [8]. A recent study from the same group reported durable anti-tetanus titers in rhesus macaques despite aggressive B cell depletion, with a subset of anti-tetanus plasma cells exhibiting incorporation of BrdU administered at the time of immunization a decade prior [9]. In humans, phenotypic characterization of bone-marrow antibody-secreting cells (ASCs) indicated that durable anti-vaccine titers are produced by analogous plasma cells with a CD19⁻CD38⁺CD138⁺ surface phenotype [4]. Consistent with these data, protective antibody titers were maintained for >6 months in patients undergoing anti-CD19 chimeric antigen receptor (CAR) T cell therapy for B cell malignancy, despite the complete and persistent ablation of CD19⁺ B cells induced by this therapy [10]. Thus, human and animal data confirm that plasma cells can be maintained for decades without ongoing cellular division, with the theoretical lifespan of LLPCs exceeding the life of the host.

Distinct contributions of short- and long-lived plasma cells to humoral autoimmunity

While parallel short- and long-lived antibody responses evolved to resist infection, similar processes theoretically underlie humoral autoimmunity. However, whereas the timing of antigen exposure can be identified after infection or vaccination, humoral autoimmunity occurs stochastically and antigen exposure could be considered continuous. Thus, serum autoantibodies might theoretically be maintained by pathogenic LLPCs, or by the continuous extrafollicular production of autoreactive SLPCs in a persistent inflammatory milieu. This distinction is of clinical importance, since SLPCs may be sensitive to anti-proliferative immunosuppression or depletion of B cell precursors, whereas LLPCs largely resist such treatment [5,11,12]. However, uncovering the relative contribution of each plasma cell population in specific autoimmune diseases is challenging. Secondary lymphoid organs and bone marrow niches, where SLPC and LLPCs are believed to reside, require invasive procedures to access, and plasma cell function is difficult to model *ex vivo* [3,13]. Moreover, the autoimmune state is associated with greater total numbers of plasma cells, made possible by expansion of plasma cell niches in the spleen and at peripheral sites of inflammation [11]. These observations align with a model in which LLPC populations are regulated chiefly by available space in a niche [14,15], but suggest that only studying a particular compartment may miss important PC populations in autoimmunity. Ultimately, the best definition of a long-lived plasma cell may be a functional one: the presence of stable antibody titers for many months after complete depletion of B cell precursors (Figure 2).

Pemphigus: a model for SLPC-driven disease

Pemphigus vulgaris, and the related disorder pemphigus foliaceus, are chronic blistering diseases of the skin and mucous membranes driven by IgG4 autoantibodies targeting epidermal desmoglein proteins [16]. Notably, anti-desmoglein antibodies are both necessary and sufficient for disease pathogenesis, as evidenced by high sensitivity and specificity of autoantibody titers in pemphigus diagnosis [17], and the development of neonatal pemphigus following placental transfer of maternal anti-desmoglein antibodies [18]. Interestingly, animal studies have demonstrated that adoptive transfer of anti-desmoglein

Fab fragments alone can drive blister formation, indicating that disease results from structural disruption of epidermal integrity without the need for Fc-dependent immune activation [19]. These insights drove initial attempts in the use of rituximab as salvage therapy in patients with corticosteroid-refractory pemphigus. In early trials in subjects with severe, treatment-resistant disease, clinical remissions occurred in the majority of patients within three months [20,21]. Subsequently, a larger prospective open label trial demonstrated that rituximab plus short-course prednisone resulted in ~90% complete remission, which correlate with the rapid reduction in anti-desmoglein antibodies [22]. In addition to improving the management of this life-threatening disease, these trials also provide insight into the pathogenesis of pemphigus. Since CD20 expression is lost during plasma cell differentiation, pathogenic anti-desmoglein antibodies are likely derived from continuously generated SLPCs [23]. Consequently, rituximab-mediated depletion of autoreactive CD20⁺ B cell precursors and/or the direct targeting of pathogenic CD20^{low} plasmablasts is sufficient for treatment of pemphigus.

Sjögren's syndrome: rituximab-resistant autoantibodies implicate LLPCs in disease pathogenesis

Sjögren's syndrome is an autoimmune condition characterized by exocrine dysfunction of the lacrimal and salivary glands [24]. Disease development is associated with the infiltration of activated B and CD4⁺ T cells into inflamed glands. In addition, plasma cells are frequently identified in salivary glands of Sjögren's patients, in keeping with expansion of plasma cell survival niches outside the BM in other inflammatory conditions [25]. Although autoantibodies have not been directly implicated in disease pathogenesis, Sjögren's syndrome is characterized by autoantibodies targeting Ro/SSA and La/SSB autoantigens. For this reason, rituximab has been studied in several clinical trials in Sjögren's syndrome, with limited clinical utility [26–29]. While the impact of rituximab on serum autoantibody titers is an infrequent clinical endpoint in Sjögren's trials, available data indicate that anti-CD20 B cell depletion does not affect anti-SSA and –SSB titers [30–32]. Notably, this failure to reduce anti-SSA titers was observed despite the apparent depletion of CD19⁺ B cells in both peripheral blood and in salivary gland biopsies [32]. Thus, these data support the idea that the majority of SSA-reactive ASCs in Sjögren's syndrome are likely long-lived, capable of surviving for >5 months in the absence of regeneration from CD20⁺ memory B cell precursors.

SLE – paradigm for contribution of both SLPCs and LLPCs to autoantibody repertoire

In contrast to the above data implying that pemphigus vulgaris and Sjögren's syndrome exhibit distinct contributions of short- vs. long-lived plasma cells, respectively, significant complexity underlies the autoantibody repertoire in systemic lupus erythematosus (SLE). Lupus is a chronic and clinically heterogeneous systemic autoimmune disease characterized by the near universal presence of class-switched autoantibodies against a wide-range of antinuclear antigens. While anti-nuclear antibodies (ANA) are likely insufficient for disease development (since ANA development can predate clinical symptoms by years [33]), autoantibodies contribute to several lupus clinical manifestations, including autoimmune hemolytic anemia and immune complex glomerulonephritis [34]. Importantly, animal models and human clinical studies have indicated that both extra-follicular and GC-

dependent B cell activation pathways likely contribute to autoreactive B cell activation in SLE. For example, spontaneous GCs are observed in murine lupus models and in human clinical samples, and ANA from lupus patients can exhibit extensive somatic hypermutation (SHM) implicating a GC origin [35–37]. In contrast, in MRL.Fas^{lpr} lupusprone mice, spontaneous activation of autoreactive B cells occurs via a predominantly extrafollicular pathway, resulting in GC-independent SHM [38]. These animal models correspond with human studies that show an expansion of circulating plasmablasts in SLE [39,40], with a subset of these plasmablasts exhibiting reduced mutation frequency and prominent clonality that can persist for months [41]. Cumulatively, these data implicate both short- and long-lived plasma cells as likely contributors to the autoantibody repertoire in SLE. Consistent with this model, results from clinical trials have indicated that anti-dsDNA titers decrease following B cell depletion, whereas autoantibodies against RNA-associated nuclear antigens remain unchanged, consistent with production by short- and long-lived plasma cells, respectively [42–45]. Thus, although large randomized controlled trials of rituximab in SLE (EXPLORER [45] and LUNAR [46]) failed to meet their clinical endpoints, data from these and other B cell depletion studies highlight the complexity of SLE and have informed our understanding of lupus biology.

Challenges with inferring the contributions of short vs. long-lived PCs in autoimmunity

An important caveat to our proposed model is that our ability to infer the plasma cell subset producing specific autoantibodies requires complete ablation of peripheral B cell precursors following B cell targeting. In human clinical trials, rituximab results in the rapid depletion of circulating CD20⁺ B cells that is maintained for >3 months in the majority of subjects. However, resident B cells in the tissues and secondary lymphoid organs are less efficiently cleared, as evidenced by the long-term persistence of splenic vaccinia virus-specific memory B cells and robust amnestic tetanus toxoid immunization responses following rituximab treatment [47–49]. Independent of autoimmunity, inflammatory conditions are also associated with a shortened therapeutic half-life and reduced efficacy of CD20-depletion in mouse models [50]. Consistent with these data, presumably-autoreactive B cells can persist in synovial membranes in rheumatoid arthritis and the salivary glands in Sjögren's syndrome following rituximab treatment [51,52].

Among the humoral autoimmune diseases, lupus B cells are particularly resistant to therapeutic depletion. For example, the Shlomchik group reported that splenic B cells resist anti-CD20 ablation in independent models of murine lupus, with longer treatment required for equivalent B cell depletion as control mice [53]. In addition, distinct B cell subsets within the spleen and lymph nodes exhibited variable sensitivity to anti-CD20 antibodies. In murine models, peritoneal B1 B cells, GC B cells and to a lesser degree marginal zone B cells resist B cell ablation [53,56]; findings of particular relevance to autoimmunity since these B cell subpopulations have been identified as potential sources of pathogenic ASCs [57–59]. Moreover, SLE is characterized by increased serum titers of the B cell survival cytokine B-cell-activating factor of the tumor-necrosis-factor family (BAFF), which promotes resistance to B cell depletion [60,61]. A recent study reported that increased serum

BAFF following B cell depletion also extended the survival of splenic plasma cells in both wild-type and lupus prone mice; findings which correlated with plasma cell maturation and the adoption of a “long-lived” gene expression profile [62].

Together, these data may explain the incomplete clinical efficacy of B cell depletion in autoimmunity, and suggest caution in attributing a cellular source to distinct autoantibody specificities. Although anti-desmoglein autoantibodies in pemphigus are almost certainly derived from SLPCs, it is currently not clear whether apparently “rituximab resistant” autoantibody specificities in SLE are derived from true LLPCs. Given incomplete B cell ablation, pathogenic SLPCs might continue to be generated from residual memory B cells. Alternatively, increased serum BAFF levels following peripheral B cell depletion may result in the maturation and/or long-term survival of self-reactive plasma cells otherwise destined to be short-lived (**Figure 3**). In this context, recent results from a clinical trial of combined rituximab and BAFF inhibition (belimumab, Benlysta) for severe lupus nephritis are particularly interesting [63]. In this study, anti-dsDNA and RNA-associated autoantibody titers were both reduced by ~50%, whereas titers of LLPC-derived vaccine antibodies remained unchanged. While this trial included relatively few patients, these data suggest that SLPCs and LLPCs contribute to both DNA- and RNA-reactive autoantibodies in SLE, with increased serum BAFF contributing to the differential maintenance of autoreactive plasma cells or memory B cell precursors [64]. Results from a large ongoing randomized trial in lupus nephritis comparing rituximab and cyclophosphamide with and without belimumab (CALIBRATE; NCT02260934) should be particularly informative in this regard.

Finally, the careful study of clinical data from anti-CD19 CAR T cell trials is likely to yield additional insights into autoimmune plasma cell biology. The surface marker CD19, targeted by recently approved CAR T cell therapies for B cell malignancy, is also expressed by nonmalignant B cell subsets in a manner largely overlapping with rituximab’s cognate antigen, CD20 [65]. Evidence of striking anti-tumor efficacy in subjects where rituximab had previously failed to control disease suggests that anti-CD19 CAR T cells are likely more potent and/or penetrant in the depletion of endogenous B cells. Consistent with this model, patients achieving long-term remission following anti-CD19 CAR T cell transfer exhibit profound B cell aplasia that can persist for years after a single treatment [10]. Thus, antibody titers maintained long-term under these conditions may reasonably be ascribed to LLPC, while declining antibody specificities likely derive from SLPC or from CD19⁺ ASC populations that have been observed in human vaccination studies [66]. One caveat is that current CAR T cell protocols require lymphodepleting conditioning, with treatment provided as salvage therapy following multiple rounds of cytotoxic chemotherapy and/or rituximab treatment. Additionally, the malignancy itself may perturb the dynamics of hematopoiesis, B cell development, and the bone marrow microenvironment. Nevertheless, the small amount of existing data on antibody titers in antiCD19 CAR T cell-treated patients suggests that LLPC are strikingly resilient. In patients with profound B cell aplasia extending >180 days, both vaccine-induced anti-body titers to measles and HSV1/2 titers arising from pre-treatment natural infection have been found to be maintained with remarkable stability [10].

Significant toxicity and expense are likely to prohibit the near-term application of CAR T cell therapy in autoimmunity. However, since patients with humoral autoimmune diseases,

including SLE, rheumatoid arthritis and Sjögren's syndrome, are at increased risk of developing malignancy, in particular B cell-lineage lymphomas [67–70], anti-CD19 CAR T cells will inevitably be offered to subjects with pre-existing autoantibodies. Thus, the measurement of autoantibody titers following CAR T cell treatment has the potential to greatly expand our understanding of plasma cell biology, to add insights into the long-term effects of B cell aplasia, and to stimulate new approaches to therapy for patients with autoimmunity.

Concluding remarks

Careful analysis of both animal models and human clinical trials of B cell depletion has resulted in many insights into the biology of autoreactive plasma cells. However, important questions remain regarding the cellular identity, plasticity and durability of autoantibody-secreting cells. First, what are the mechanisms that drive the formation of short- vs. long-lived plasma cells in autoimmunity? What disease-specific B cell activation pathways or tolerance mechanisms prevent the establishment of pathogenic LLPCs in pemphigus, but not in Sjögren's syndrome or SLE? What role do BAFF-mediated survival signals play in the long-term persistence of autoantibody titers in systemic autoimmunity? Finally, would a more robust depletion of tissue-resident B cells, for example using anti-CD19 CAR T cells or bi-specific antibodies [65,71], result in greater therapeutic efficacy in humoral autoimmunity?

Ultimately, while targeting B cell precursors has given immunologists important information about the cellular sources of circulating autoantibodies, direct plasma cell depletion may be required for effective treatment of antibody-mediated disease. Many of the features that enable LLPC to provide stable, life-long protective antibody titers also render plasma cells resistant to therapeutic ablation. However, recent advances in the treatment for multiple myeloma, a plasma cell malignancy, hold the promise of more effective tools in the near future [72,73]. As agents for the selective depletion of malignant plasma cells improve in potency and safety, their use will likely extend to autoimmunity, providing new options for treatment and advancing our understanding of disease mechanisms [74,75].

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Highlights:

- Pathogenic autoantibodies can be derived from short- or long-lived plasma cells
- Therapeutic B cell depletion is a common treatment approach in humoral autoimmunity
- B cell ablation strategies target surface markers absent on mature plasma cells
- Distinct autoimmune diseases exhibit variable clinical responses to B cell depletion
- Autoantibody titers after B cell depletion correspond with plasma cell lifespan

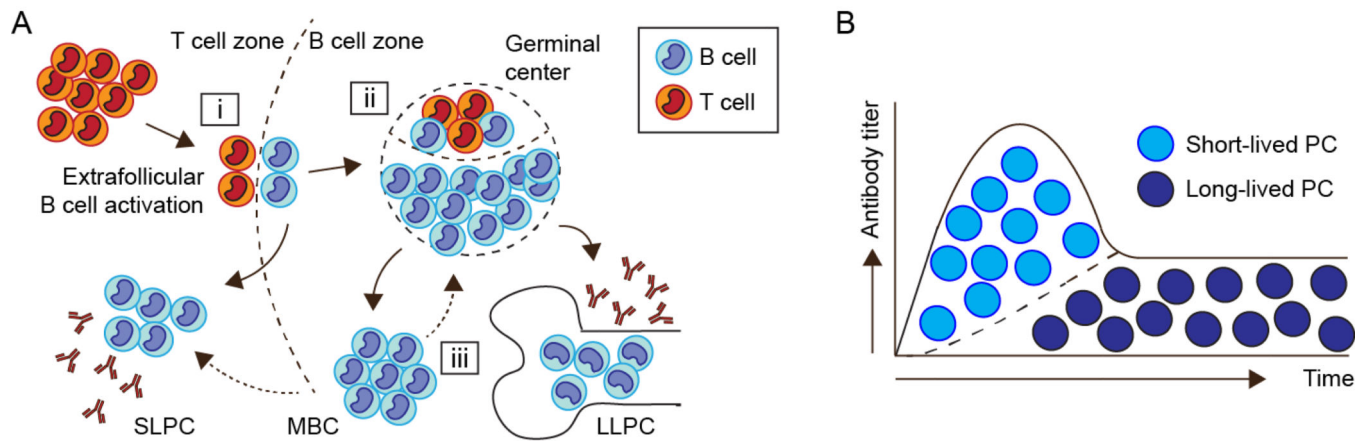


Figure 1. T cell-dependent humoral immune response:

(A) (i) After antigen exposure, antigen-specific B cells and $CD4^+$ T cells migrate to the T cell:B cell border. These initial cognate interactions promote B cell proliferation and facilitate the rapid differentiation of short-lived plasma cells/plasmablasts which are the source for early, low-affinity protective antibody titers. (ii) Subsequently, continued B cell:T cell co-stimulatory and cytokine crosstalk drives T follicular helper (Tfh) cell differentiation and the formation of germinal centers (GC). Within the GC, iterative rounds of B cell somatic hypermutation and affinity maturation ultimately results in the formation of high-affinity memory B cells (MBC) and plasma cells, a subset of which are able to engraft into the long-lived bone marrow compartment. (iii) Of particular relevance to autoimmunity, MBC exhibit lower thresholds for antigen-dependent activation, resulting in either GC re-entry or rapid differentiation into antibody-producing plasma cells. (B) Theoretical model indicating the relative contributions of short- vs. long-lived plasma cells to serum antibody titers over time. The initial increase in antibody titers following T-dependent antigen exposure is believed to be produced predominantly by SLPCs, with LLPC being responsible for the long-term maintenance of protective antibody levels.

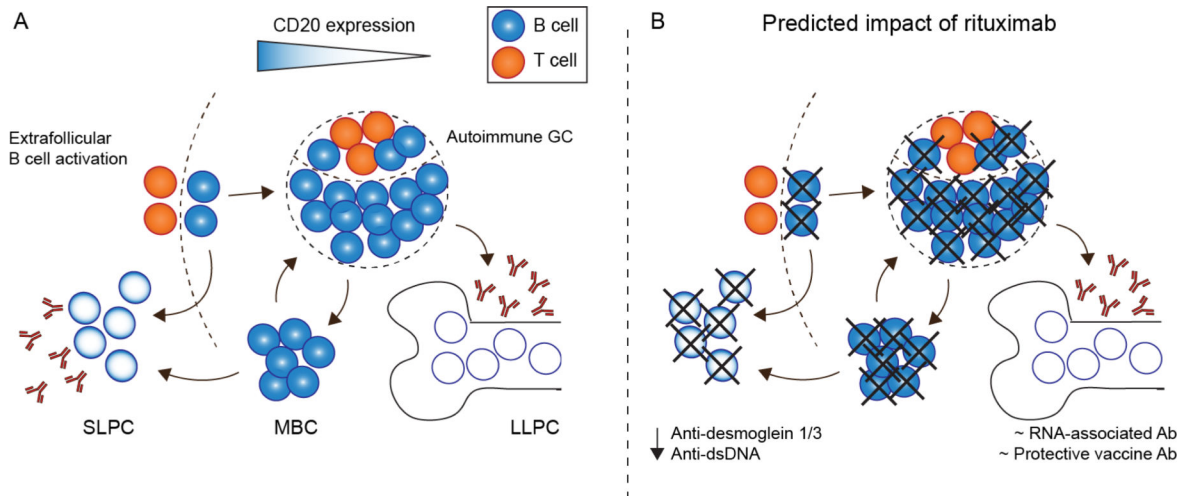


Figure 2. Theoretical impact of anti-CD20 B cell depletion on plasma cell subsets:

(A) During humoral autoimmunity, autoantibodies might be derived from either short- or long-lived plasma cell subsets. In this model, the degree of surface CD20 expression is indicated by a blue gradient, including: CD20⁺ naïve B cells, GC B cells and MBC; CD20^{low/neg} short-lived plasmablasts/plasma cells; and, CD20^{neg} LLPC. (B) Based on surface CD20 expression, rituximab treatment is predicted to spare CD20^{neg} LLPC, but deplete SLPC via either direct CD20 targeting or indirectly via ablation of CD20⁺ B cell precursors. Thus, protective vaccine titers and RNA-associated autoantibodies are preserved following B cell depletion, whereas SLPC-derived anti-desmoglein 1/3 and anti-dsDNA autoantibodies decline.

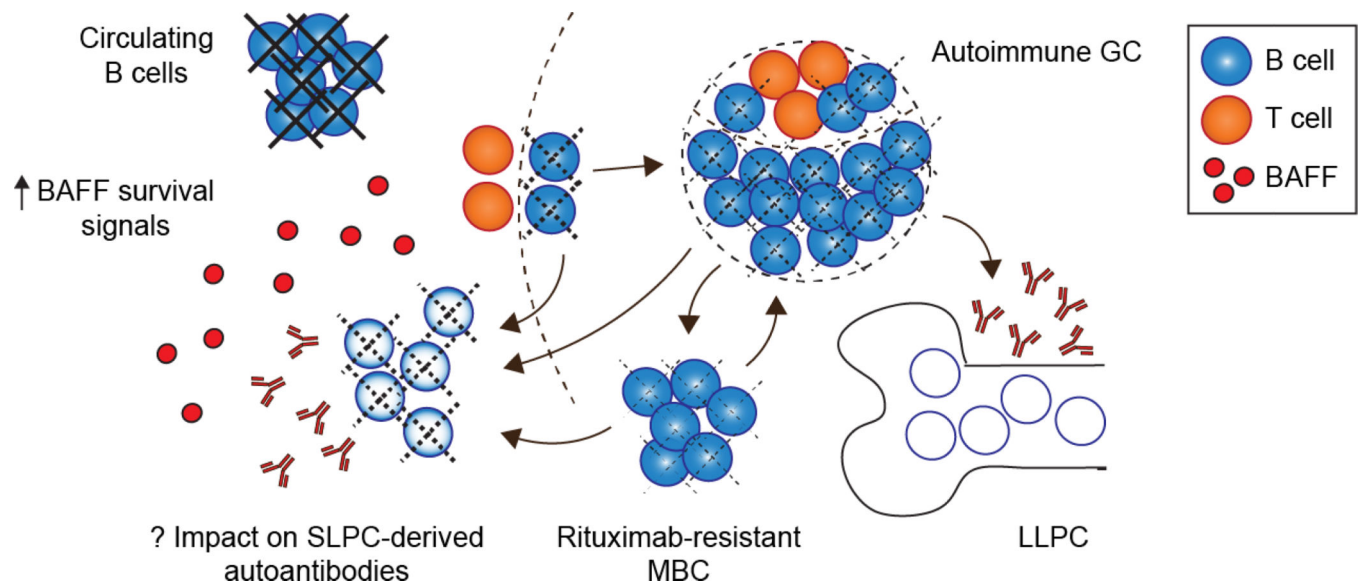


Figure 3. Incomplete anti-CD20 B cell depletion and elevated serum BAFF complicates the interpretation of B cell depletion trials:

Despite near-complete ablation of circulating B cells, autoreactive GC B cells and MBC (as well as MZ and B1 B cells, not shown) are relatively resistant to rituximab-mediated depletion (dashed lines indicate partial ablation). Thus, persistent serum autoantibodies following rituximab treatment might be produced by pathogenic SLPCs continuously generated from MBC, GC and/or other resistant B cell subsets. Moreover, increased serum BAFF levels following B cell depletion may promote the long-term survival of SLPC in autoimmunity.