Members of the B-lymphocyte stimulator (BLyS) family play key roles in B-lymphocyte survival, selection, and differentiation. Recent US FDA approval of the neutralizing anti-BLyS antibody, belimumab, for the treatment of systemic lupus erythematosus brings members of this cytokine family further into the spotlight as targets for treating autoimmune disease. Here, we briefly review the current understanding of BLyS family roles from extensive research in mice, and note similarities and differences with humans. We summarize the significant insight into human B-cell biology that has been derived from clinical trials. Finally, we discuss how future clinical trials might be designed to afford a deeper understanding of processes governed by the BLyS family, in order to better predict clinical success with BLyS family-targeted therapies.

Keywords: autoimmune disease • B-cell activating factor • B-cell subsets and selection • belimumab • BLyS • systemic lupus erythematosus

As therapeutics targeting B lymphocytes enjoy growing success, the varied roles of these cells in the immune system are increasingly appreciated. In addition to producing antibodies, B cells function as potent antigen-presenting or cytokine-producing cells, and have regulatory roles in both normal immune responses and autoimmunity. Accordingly, understanding the mechanisms that govern selection, survival, and differentiation in pre-immune and antigen-experienced B-cell pools is critical to identifying therapeutic targets that will yield increased specificity and efficacy.

The B-cell antigen receptor (BCR) and its associated signaling systems have long been recognized as drivers of B-cell selection and survival, both during initial development and following antigen encounter. Indeed, until recently, the BCR was largely viewed as the sole mediator of these processes. However, the last decade has witnessed emergence of a second molecular family – whose prototypical member is B-lymphocyte stimulator (BLyS) – as an essential and complementary player. This subset of the tumor necrosis factor/receptor superfamily consists of two ligands and three receptors \[1,2\] (Figure 1). The ligands are BLyS, also known as B-cell activating factor (BAFF) \[3–5\]; and a proliferation-inducing ligand (APRIL) \[6\]. Despite their monikers, neither ligand causes primary B cells to divide. Instead, through interaction with their receptors, BLyS and APRIL regulate B-cell survival and differentiation. The receptors in this family are BLyS receptor 3 (BR3; also termed BAFF-R) \[7–9\], transmembrane activator and CAML interactor (TACI) \[10,11\], and B-cell maturation antigen (BCMA) \[12\]. BLyS can bind to all three receptors, whereas APRIL binds TACI and BCMA, but not BR3. Although the full array of functions performed by BLyS family members is not yet elucidated, it is clear that they play critical roles in the homeostasis and selection of pre-immune B-cell pools. Moreover, accumulating evidence indicates similarly essential roles in the selection, differentiation, and lifespan of antigen-experienced B-lineage subsets.
Targeting the BlyS family in autoimmunity

Since BlyS is implicated in the pathology of some autoimmune diseases, several BlyS-targeted therapies are in current clinical trials [13]. Belimumab, a neutralizing anti-BlyS monoclonal antibody, yielded positive results in Phase III clinical trials for systemic lupus erythematosus (SLE), and was recently approved by the US FDA for therapeutic use in this disease [301]. Further insight into processes governed by the BlyS family, derived both from clinical trial results and animal research, should continue to prove fruitful for identifying therapeutic targets and the patients most likely to benefit from a particular therapy.

Many currently held concepts in B-cell biology derive from extensive studies with inbred mice. Nonetheless, thanks in part to observations made in clinical trials involving B-cell- ablative therapies, it is increasingly clear that many of the processes governing B-cell selection and homeostasis are similar – albeit not completely congruent – between mice and humans. In particular, emerging evidence suggests that BlyS family members are important factors in the development of therapies for autoimmunity, and may have potential in other arenas where manipulating B-cell function may be beneficial. Accordingly, in this review we first summarize development and selection of primary (naive) and antigen-experienced B cells, with a comparison of subsets and processes between mice and humans. We focus on the roles played by BlyS family members in these processes in health and autoimmunity, suggesting that BlyS and other members of this TNF family are promising targets in the treatment of autoimmune disease. We close with a look ahead at designing future studies to afford a deeper insight into processes governed by the BlyS family, with an eye towards refining therapeutic targets and further individualizing treatment options for autoimmunity.

Primary B-cell subsets and selection in mice & humans

■ Generation of naive mature B-cell subsets

B lymphocytes arise from hematopoietic stem cells in the bone marrow (BM). Commitment to the B-cell lineage is characterized by initiation of gene rearrangements at the immunoglobulin heavy-chain locus and expression of the B cell-specific transcription factor Pax5 [14]. After expression of a pre-BCR, consisting of the successfully rearranged heavy chain gene product, surrogate light-chain and signaling components, pre-B cells undergo a proliferative burst, followed by light chain gene rearrangement. This culminates in the assembly of a complete BCR, whose cell surface expression identifies the immature B cell. These processes are reviewed in detail elsewhere [15–17].

In mice, immature B cells exit the BM and continue to mature, passing through the ‘transitional’ developmental stages before entry into one of the primary, pre-immune B-cell subsets [18,19]. Transitional B cells recirculate in the blood but not the lymphatics, and can be further subdivided into several categories, termed T1, T2 and T3 [20]. The T1 subset consists of the earliest BM émigrés, and these cells are phenotypically identical to immature BM B cells [20,21]. Their successors, the T2 subset, gain CD23 expression, and are believed to be the common precursor of both follicular (FO) and marginal zone (MZ) B cells [22]. A third transitional subset, T3, has been distinguished in both mice and humans [20,23,24]. While most T3 cells are probably derived directly from the T2 pool, empirical data in mice and mathematical models suggest that the T3 transitional pool may be a ‘death niche’ for any B cells undergoing anergic elimination [25–27]. The human T3 stage appears to be a developmental intermediate in mature naive B-cell maturation, and it also may include some anergic cells [23].
B-cell development in humans and mice share many features: as in mice, human B lymphocytes arise from hematopoietic stem cells in the BM and fetal liver, where precursors undergo immunoglobulin gene rearrangement, and immature BM B cells express a complete BCR [28–30]. Studies where peripheral B-cell reconstitution has been followed after B-cell depletion or hematopoietic stem cell transplant indicate that human B cells develop across a continuum from the first cells to exit the BM (early transitional/T1), through stages characterized by changing surface phenotype, leading to mature B-cell subsets [23,24,31–33]. Most observations of B cells in humans are made with peripheral blood, and are thus confined to circulating subsets. Moreover, the variety of phenotypic subclassing approaches used by different groups makes direct comparisons between different human studies, as well as between human and mouse, challenging. In Figure 1, surface phenotype similarities and differences are shown, highlighting some of the ambiguities in identifying equivalent subsets between mice and humans based on phenotype alone. For example, in mice, transitional B cells are best identified by expression of CD93 (clone AA4.1) [34], whereas anti-CD93 reagents do not stain human B cells. On the other hand, both murine and human transitional B cells express CD24 (heat-stable antigen), the marker with which they were originally defined [35,36]. The evaluation of additional markers on human subsets, as well as the increasing use of cell sorting and functional and gene expression assays, should help to further distinguish human B cell subsets [37].

Primary B-cell homeostasis: throughput, survival & selection
B-lymphocyte homeostasis involves maintaining an array of clonal specificities sufficiently diverse to enable protective immunity, yet devoid of pathogenic autoreactivity. Research in mice indicates that newly formed B cells encounter two major checkpoints, where potentially autoreactive clones are purged, before joining the FO or MZ pre-immune pools. The first is at the immature BM stage, where avid BCR ligation yields receptor editing or death [38,39], resulting in a 90% loss of immature B cells [35]. This negative selection process is B cell intrinsic, and cannot be tempered by exogenous survival factors such as BlyS. The transitional stages are the second checkpoint, where additional selection based on BCR specificity occurs. Under normal circumstances, selection at this checkpoint yields additional losses, such that only approximately one-third of transitional B cells survive to maturity [35]. However, in contrast to immature BM selection, the extent of selective losses at this stage may be tempered by BlyS [40] (Figure 2). Murine B cells acquire the BR3 and TACI receptors as they pass through transitional stages and join the FO or MZ pre-immune pools [41]. Through their integration with BCR signaling systems, BlyS signals via BR3 govern continued survival at the transitional and mature B-cell stages [9,42,43]. Thus, tonic signaling levels dictated by BCR specificity determine a particular cell’s relative ‘fitness’ to process BlyS signals for survival, thereby regulating the throughput of transitional cells and the overall size of the FO and MZ pools [44]. Since BlyS can modulate the stringency of transitional selection, increases in available BlyS allow a higher proportion of transitional cells to mature, thereby increasing the representation of potentially autoreactive clones in mature pools [45–47]. Indeed, exogenous administration or overexpression of BlyS in mice not only leads to increased primary B-cell numbers, but also to autoimmune manifestations [46,48].

Less is known about mechanisms of primary B-cell homeostasis in humans. However, many observations suggest similarity with these processes in mice. For example, BR3/BAFF-R is expressed on human transitional and mature B-cell subsets [23,49–51], and naive human B cells have BlyS bound to their surface [52]. Also as in mice [53,54], human B cells acquire responsiveness to BCR stimulation and increasing sensitivity to BlyS as they mature through transitional stages [23,24]. A homozygous BR3 deletion in humans blocks B-cell development at the transitional stage, and severely reduces numbers of all mature B cells [55], as occurs in knockout or mutant mice lacking either BR3 or BlyS [7,8,56]. Moreover, treatment of either mice or humans with anti-BlyS reduces transitional and mature B cells [57,58]. In addition, there is evidence that immature and transitional B-cell selection in humans is based on BCR specificity, and that either or both of these checkpoints are defective in humoral autoimmune diseases [59–63]. Taken together, these observations indicate that human primary B cells require BlyS/BR3 to survive, and that signals from both BR3 and the BCR influence human B-cell selection, as is the case in mice. Further clarification of which human subsets undergo selection, as well as the underlying molecular mechanisms, will prove important in predicting the success of therapeutic approaches that are centered on B cells. Box 1 summarizes these and other questions regarding the generation and selection of pre-immune human B cells.

An additional B-lineage population, the B1-subset, is phenotypically and functionally distinct from the conventional ‘B2’ subsets discussed so far. B1 cells are generated in the fetal liver and are primarily sustained by self-renewal in the periphery, although some BM production has been reported in a mouse adoptive transfer system [64–67]. In mice, B1 cells preferentially localize to the peritoneal cavity [65,68]. B1 cells secrete so-called
natural antibodies: IgM and IgA isotypes that are present before intentional challenge, recognize pathogen-associated antigens and some self-antigens such as cellular debris, and activate the complement system upon infection [69–71]. A B1-like (CD5+) subset may be expanded in some patients with rheumatoid arthritis (RA) and Sjögren’s syndrome (e.g., [72,73]). Human B1 cells have recently been characterized in umbilical cord blood and adult peripheral blood; they spontaneously secrete IgM \textit{ex vivo} and harbor mostly unmutated immunoglobulin gene rearrangements as do those in mice; and most, but not all, express CD5 [71]. The specific characterization of the human B1-cell phenotype as distinct from CD5+ and memory subsets represents a significant advance [71], paving the way for more detailed studies of B1 roles in human health and disease. Interestingly, anti-BLyS treatment in mice ablates splenic B1 cells but spares those in the peritoneal cavity, and has no effect on serum natural antibody titers [57]. It will thus be of particular interest to see whether similar differential effects are observed in humans.

**Generation of antigen-experienced B-cell subsets in mice & humans**

- **Humoral immune responses**

Antigen-mediated BCR ligation initiates the intracellular and intercellular processes that culminate in a humoral immune response. Two general categories of
antigens elicit responses with distinct characteristics. T-dependent (TD) antigens consist of proteins that can be internalized, processed, and presented as peptides in the context of MHC class II, engendering cognate T–B interactions. By contrast, T-independent (TI) antigens are often polysaccharides that display densely repeating epitopes or contain moieties capable of stimulating innate sensors such as Toll-like receptors.

In both TD and TI responses in mice, antigen-specific plasma cells are generated in extrafollicular regions within a few days of challenge [74,75]. Since they persist for only 1–2 weeks, these cells are termed short-lived plasma cells (SLPCs) or early antibody-forming cells [74,76]. These cells secrete the predominantly unswitched (IgM), low-affinity antibody characteristic of early primary TD responses and in TI responses [77]. Whereas most TI responses culminate with the decay of the extrafollicular SLPC response, TD responses concomitantly generate transient structures in secondary lymphoid tissue (spleen, tonsil and lymph nodes) known as germinal centers (GCs). GCs form in lymphoid follicles, at the interface between B-cell and T-cell zones. Within a GC, responding B cells undergo successive rounds of somatic hypermutation of immunoglobulin genes and selection for increased antigen affinity (affinity maturation reviewed in [74,77–80]). Antibody isotype switching also occurs in GC evolution, and experimental evidence in mice bears this out. Although GCs form in the absence of B-cell subsets and LLPCs will continue to be aided by the use of multiple phenotyping markers for flow cytometry, gene expression arrays and proteomic approaches. This information will be invaluable for tracking these subsets in healthy and autoimmune individuals, and for the identification of subset-specific or disease-specific targets for treating autoimmune disease.

- **Emerging roles for the BlyS family in activated & antigen-experienced B-cell subsets**

The selection and homeostatic regulation of antigen-experienced B-cell subsets is poorly understood. Current conjecture is that long-lived pools, such as the memory and plasma cell subsets, must be free from competition with the much larger primary pool, and therefore are under independent homeostatic regulation [88,89]. There is increasing evidence that BlyS family members play important and nuanced roles in the generation, selection, and maintenance of antigen-experienced B-cell pools.

The marked similarities between primary B-cell maturation and GC B-cell differentiation, in terms of selection processes based on BCR specificity, suggest that the BlyS/Br3 axis may also play a key role in GC evolution, and experimental evidence in mice bears this out. Although GCs form in the absence of BlyS/BR3 signaling, they do not evolve correctly, as they are shorter-lived and generate reduced titers of class switched, high-affinity antibodies [90–92]; recall (secondary) responses are also impaired [93,94]. The BlyS/BR3 axis thus plays a role in normal evolution of the murine GC, and there is evidence that it does so in humans as well [95,96].

The general nature of humoral immune responses is similar in mice and humans; for example, vaccines or pathogens that are expected to elicit a TD response can confer protection for years or decades [83–85]. Memory responses in both mice and humans are heterogeneous, as several subsets of B cells can respond to a single antigen or class of antigens, memory cells can express either unswitched (IgM) or class switched antigen receptors, and variable region genes may be unmutated or highly mutated [37,75,86]. There are at least five memory B-cell subsets in mice [87], and at least seven in humans [87]; examples for each species are shown in Figure 1. Definitive characterization of memory B-cell subsets and LLPCs will continue to be aided by the use of multiple phenotyping markers for flow cytometry, gene expression arrays and proteomic approaches. This information will be invaluable for tracking these subsets in healthy and autoimmune individuals, and for the identification of subset-specific or disease-specific targets for treating autoimmune disease.

**Box 1. Generation and selection of human pre-immune B cells.**

- What are the major B-cell lineages (B1, B2, Breg and others)?
- How do B cells progress from one phenotype to the next?
- How does B-cell subset phenotype correlate to function – for example, immunoglobulin repertoire with antigen affinity?
- Are B cells of a given phenotype in the blood functionally the same as cells of that phenotype in other tissues/anatomic compartments?
- How do B-cell subset representation, phenotype, and function differ in autoimmune versus healthy people, and can these characteristics serve as biomarkers to identify disease subtypes or predict therapeutic success?
Human plasmablast or plasma cell subsets from tonsil and spleen express either TACI or BCMA [51,95]. All of these receptor expression patterns are generally congruent with those observed in mice [94,96–98]. Further, the use of different blocking reagents indicates that BlyS/BR3 signaling may be more important in the generation of human plasma cell precursors, whereas signaling via TACI or BCMA is more important for plasma cell generation [95], which is again generally consistent with findings in mice [94].

The BCMA knockout mouse shows that BCMA plays a key role in long-lived plasma cell survival [94]. Moreover, simultaneous neutralization of both BlyS and APRIL results in a significant reduction in LLPC [99], and it appears that either cytokine alone is sufficient for LLPC maintenance [57,99]. Treatment of human SLE and RA patients with atacicept, a therapeutic agent that neutralizes both BlyS and APRIL, leads to significant reductions in total serum immunoglobulin and autoantibody levels, indicating that plasma cells are likely affected [100–103]. Further, there is evidence that APRIL/TACI and BlyS/TACI interactions mediate isotype switching in both mice and humans [104–107], and signaling through TACI and CD40 promotes plasmablast differentiation in vitro [97]. BlyS and APRIL can also induce CD40-independent class switch and differentiation to antibody-secreting cells (ASCs) [108]. APRIL is implicated in plasma cell homing to or retention in BM [109]. Taken together, these results suggest that both BlyS and APRIL operate during plasma cell generation and maintenance, most likely at different stages during and after the GC reaction as well as in TI responses.

TACI is associated with isotype switching in humans as well as mice, since missense mutations in TACI are found in – and apparently contribute to – common variable immunodeficiency (CVID) and possibly IgA deficiency [105,110]. BCMA and BR3, but not TACI, were observed on human plasmablasts in one study [111], and as noted above, TACI and BCMA expression increase as plasma cells differentiate in vitro [95]. BlyS (along with inflammatory cytokines) can drive human memory cells to differentiate into antibody-secreting plasmablasts [52,111–113] and induce BCMA expression [111], suggesting an important function for BlyS in recall responses and for BCMA in plasma cell differentiation or maintenance.

In mice, neither BlyS nor APRIL is required for memory B-cell maintenance [99]. Consistent with this, memory B cells are relatively resistant to BlyS neutralization, though switched (IgG) cells are less dependent on BlyS than unswitched (IgM+) cells [57]. In vitro work with CD27+ (memory) human B cells suggests that BlyS plays a critical role during the differentiation of memory cells in secondary responses: whereas BlyS attenuates ASC formation under TI-like stimulation, it enhances ASC formation with TD-like stimulation [52]. Human memory B cells have been reported to express all three BlyS receptors, though the receptor signature varies with subset, and may depend upon tissue location [51,95,114]. Activated human memory B cells lose BR3 expression but upregulate BCMA, and this ‘inverse’ expression pattern appears to be a consequence of differentiation into ASCs [114].

Clues about the roles of BlyS receptors will be provided through further analyses in the more heterogeneous genetic context seen in humans. For example, both of the siblings carrying the homozygous BR3 deletion noted above had reduced serum IgM and IgG and an impaired TI response to pneumococcal polysaccharides, yet only one sibling developed recurrent infections [55]. TACI is highly polymorphic in humans [115–118], and genetic variations – including those that result in TACI deficiency – are associated primarily with impaired immunoglobulin production and CVID [105,117,119,120], consistent with the observed role for murine TACI in class switching. In addition to TACI and BAFF-R, several other genes may be mutated in some CVID patients [121–123], indicating multiple contributing factors to CVID.

Box 2 summarizes some questions about the generation of antigen-experienced B-cell subsets in humans.

- **Biological sources of BlyS**
  Numerous cell types express BlyS and/or APRIL, including myeloid cells, activated B- and T-cells, osteoclasts, placental mesenchymal cells, and airway and intestinal epithelial cells [124–129]. A potential difference between mice and humans in the cellular sources of APRIL – macrophages in mice, neutrophils in humans – is emerging [130]. In mice, a radiation-resistant stromal cell population is the source of the BlyS required for B-cell homeostasis; BM-derived cellular sources are sufficient to support antibody responses, but not normal B-cell numbers [133]. Regulation of BlyS and APRIL production at either the cellular or organismal level is not yet well understood. One conjecture is that systemic BlyS is generated through constitutive expression by a persevering cell type, such as a radiation-resistant pool [134], whereas ‘point’ sources such as myeloid cells are a localized source of BlyS or APRIL required for ongoing immune responses [88]. This would afford relatively constant steady-state BlyS levels tied to organism volume that provide homeostatic control of quiescent primary pools, while allowing local fluctuations to support transient expansion and selection of activated B cells.
Therapeutic Perspective

Therapeutic Perspective

Therapeutic Perspective

Therapeutic Perspective

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Therapeutic Perspective

BlyS family receptors & responses among non-B cells

BR3 and TACI are expressed on subsets of resting and activated T cells and dendritic cells, and BlyS modulates T-cell function (reviewed in [132]). Notably, BlyS or APRIL can cause T cells to produce cytokines and thereby contribute to autoimmunity in mouse models [133,134]. In humans, BlyS and APRIL can be produced in organs or sites associated with the pathology of autoimmune disease: for example, by B cells, T cells, and macrophages in salivary glands (Sjogren’s); by glomeruli or mesangial cells in kidney (lupus nephritis); and in inflamed joints (inflammatory arthritis) [135–138]. Production of BlyS and APRIL by dendritic cells induces processes such as class switching or plasma cell differentiation, and may thus link or integrate innate and adaptive immune responses [108,139–141]. Therefore, some of the benefits engendered by the therapeutic targeting of BlyS may turn out to be rooted in effects on non-B cells.

B cells & BlyS family members in autoimmune disease

B cells and autoantibodies are clearly involved in the immunopathology of autoimmunity. Autoantibodies may arise in the setting of dysregulated primary B-cell maturation and selection; from dysregulated selection in GCs; and the antibodies themselves may contribute to tissue inflammation and organ malfunction [61,62,142–147]. For example, autoreactive B cells (9G4 Id+) that are normally excluded early in GC reactions successfully progress through them in some individuals with SLE, to ultimately join memory and plasma cell compartments [147]. Interestingly, this phenomenon is not observed in RA [147]. In addition, there are several examples of specific B-cell subsets that are associated with disease activity in SLE, including transitional B-cells, plasmablasts, and specific memory subsets [145,148,149].

B cells also may serve either protective or pathogenic functions mediated via antibody-independent mechanisms such as cytokine secretion [150–152] or T-cell activation [153–155]. Indeed, regulatory B cells (Bregs) that produce IL-10 are associated with immune suppression in infection as well as in autoimmunity [156–159]. In mice, there is evidence that BlyS can promote expansion of regulatory B cells [160] as well as regulatory T cells [161]). Moreover, selective targeting of regulatory B cells within the T2 transitional subset improves symptoms and survival in lupus-prone mice [162]. An IL-10-producing B-cell subset that phenotypically and functionally parallels mouse Bregs was recently characterized in humans [163]. These cells appeared at elevated frequencies in patients with various autoimmune diseases compared with age-matched controls; however, there was no clear correlation with disease status [163].

BlyS family members are dysregulated in autoimmune disease, and therefore have been targeted in the development of novel therapeutics. Elevated serum BlyS and/or APRIL is observed in a number of autoimmune diseases including SLE, RA, Sjogren’s syndrome, idio-pathic thrombocytopenic purpura, and bullous pemphigoid; in many studies, this characteristic correlates with disease activity [164–168]. Moreover, BlyS is associated with the “rescue” of autoreactive B cells in mouse models, and with elevated autoantibodies in human disease [143,169–174]. BlyS receptor expression also may be altered. For example, BCMA expression appears to be increased on autoantibody-secreting cells in SLE patients, though B-cell subsets were not rigorously distinguished in this study [175]. Another study found similar BR3 expression levels on B cells from peripheral blood, spleen, and tonsils of SLE patients and healthy controls, but consistent occupancy of this receptor in SLE [54]. In another report, BR3 expression on naive and memory B cells from peripheral blood is reduced in Sjogren’s and SLE patients, and this correlates with disease activity [176]. A final important note is that in RA patients who had undergone B cell depletion therapy, BR3 expression was significantly reduced on both naive and memory B cells at relapse, independent of serum BlyS or B-cell levels [177]. Going forward, it will be important to characterize BlyS receptor expression patterns on both naive and antigen-experienced B-cell subsets, and understand how these are altered in various autoimmune diseases, in order to consider the development of additional targeted therapeutics.

Box 2. Antigen-experienced and antibody-producing human B-cell subsets.

Which B-cell subsets are best able to respond to which classes of pathogens or other immunologic stimuli?

What are the optimal stimuli to promote long-lived protective B-cell immunity without inducing autoreactivity?

What is the role of each B-lymphocyte stimulator receptor/ligand interaction in the generation or maintenance of memory B cells and plasma cells? What is the B-lymphocyte stimulator receptor signature of these subsets?

What is the functional significance of the various memory B-cell subsets?

How is memory B–cell subset representation or function altered in autoimmune disease?

Which memory B-cell subset(s) can be targeted safely (without excessive compromise of protective immunity) in therapies for autoimmunity?
Insights from clinical trials
Understanding developmental relationships between B-cell subsets, how selection is governed and modulated, and how antigen-experienced B cells are generated and maintained, have important implications for interventions targeting B cells or BlyS family members. This importance is highlighted by the experience with rituximab, where extensive studies have revealed valuable insights into human B-cell biology. Rituximab is an antibody (anti-CD20) that directly kills B cells including immature, naive and memory B cells, but not plasma cells that do not express CD20 [178–180]. Indeed, although rituximab treatment results in nearly complete depletion of peripheral B cells, total serum immunoglobulin and pathogen-specific antibody as well as autoantibody titers show more modest or variable reductions [178,181–183]. Effects on antibody levels depend, in part, upon the length of depletion, turnover of antibody-producing B-cell subsets, and baseline autoantibody profile [184,185]. IgM antibodies produced by short-lived plasmablasts are reduced earlier than IgG antibodies; similarly, with limited periods of rituximab treatment, primary IgM antibody responses are significantly more reduced than memory B-cell responses [186]. In addition, rituximab is primarily effective in seropositive (anticitrullinated peptide antibody and rheumatoid factor) RA, indicating that certain patients may stand to benefit from certain therapies [187,188]. Very limited data raise the intriguing possibility that B-cell depletion with rituximab or other agents may be followed by recovery of immunological tolerance [189]. Although successfully tested and widely used to treat RA, multiple sclerosis, seropositive (anti-neutrophil cytoplasmic antibody-positive) vasculitis, and type 1 diabetes [178,186,188,190,191], two rituximab Phase II/III clinical trials for SLE failed to meet endpoints [181,185].

When considered in the context of mouse studies, where anti-CD20 treatment is effective in ameliorating symptoms and extending lifespan [182,192], the failure to meet endpoints in the rituximab trials for human SLE suggests possibilities for future clinical study design or prediction of therapeutic success. Genetic predisposition and/or defects acquired during disease progression may determine the degree of B-cell sensitivity to depletion – as autoimmune-prone strains are refractory to anti-CD20 treatment when compared with wild-type strains [182], and microenvironmental factors such as BlyS signals clearly play a role [193]. Another observation is that anti-CD20 treatment may be more effective when given prophylactically, or during disease progression, rather than after development into a chronic condition [192]. Finally, depletion of the mature B-cell pool with rituximab may lead to a period of time when BlyS levels are high, so that the threshold for transitional selection is altered, allowing cells that would normally be selected against to proceed into mature pools [89].

As noted above, baseline BlyS levels appear to be elevated in many autoimmune patients. Following rituximab treatment for several autoimmune diseases (SLE, RA and Sjogren’s syndrome), serum BlyS levels increase two-to-threefold, and then gradually return to pretreatment levels [194–197]. This pattern would be expected if mature naive B cells, the major ‘consumers’ of BlyS, are initially ablated, and there is no immediate negative regulation of BlyS production in the body [194,197]. However, such a temporary increase in BlyS raises several possible concerns. One is the potential for increased transitional cell throughput and/or relaxed selection against autoreactive transitional B cells [47,194,199], as seen in BlyS transgenic mice [47]. This could occur in humans as a result of altered transitional subset representation following rituximab treatment, particularly in comparison to baseline levels [23,198]; reduced BR3 expression on both naive and memory B cells has been observed at relapse [177]; or other effects on B-cell subsets or BlyS receptors that are associated with selection checkpoints. Improved survival of self-reactive plasmablasts or plasma cells is another potential concern: enhanced survival of plasmablasts is seen in vitro when human memory cells are treated with BlyS [111], and BlyS upregulates expression of an antiapoptotic gene in murine LLPC [94]. In addition, there are associations between elevated BlyS and lymphomas (e.g., [199]). On the other hand, elevated BlyS levels may either directly or indirectly favor Breg and Treg generation [160,161], potentially compensating for deficiencies in such subsets that might accompany SLE [158]. Therefore, it is of critical importance to understand the roles played by B cells and BlyS in different autoimmune diseases, as well as how B cell-mediated processes are dysregulated in different patients.

Additional insight has been derived from belimumab clinical trials. Belimumab is an antibody that binds to and neutralizes soluble BlyS [200]. Two Phase III clinical trials of belimumab for SLE recently met primary endpoints [13,201]. Detailed descriptions such as patient stratification and dosage comparisons are provided elsewhere [13,181,201]. Belimumab treatment results in significant reductions in naive, transitional, activated, plasmacytoid B cells, and some memory B-cell subsets [201,202]. However, serum immunoglobulin and anti-dsDNA antibody titers show only modest decreases, indicating that plasma cells and some memory subsets are spared [112,181,201,202]. These results are consistent with observations in anti-BlyS-treated mice [57]. Since seropositive (anti-nuclear antibody-positive) but not seronegative patients showed a significant clinical response in later trials [202,203], there may be different subgroups
of patients who stand to benefit from therapy [13,203]. Taken together, results to date suggest that despite clinical improvements, BlyS neutralization does not fully correct defects in selection, generation, or maintenance of many antigen-experienced B-cell subsets [201].

Three additional BlyS-targeted therapies are currently in trials for SLE [13,200]: A-623 and LY2127399, both of which target BlyS; and atacicept, which neutralizes both BlyS and APRIL. Experience with atacicept has also been informative, particularly with regard to human plasma cells. Atacicept is a TACI-Ig fusion protein that binds to and neutralizes both BlyS and APRIL [13,200]. As summarized above, studies in mice indicate that LLPCs probably require both BlyS and APRIL at certain stages of generation or for maintenance [94,99]. Indeed, atacicept treatment leads to rapid and significant reductions in serum Ig in healthy individuals and in serum Ig and autoantibody levels in individuals with SLE, RA and multiple sclerosis, indicating that plasma cells are affected by this therapy [100–103,144]. Although atacicept clearly has potential for treating diseases involving pathogenic autoantibodies, it may also affect production of protective antibodies [204]. Indeed, one trial of atacicept in SLE was terminated early due to an increased risk of severe infections (ClinicalTrials.gov identifier NCT00573157). Trials of atacicept in multiple sclerosis have been suspended due to an increase in inflammatory disease activity [144].

In summary, targeting BlyS or BlyS family members (as with belimumab or atacicept) may provide a more nuanced, immunomodulatory approach to treatment, as opposed to more broadly directed B-cell depletion [195,204,205]. Combining BlyS inhibition with B-cell depletion may promote synergistic therapeutic effects, particularly if BlyS levels are elevated. In addition, concerns about the increase in BlyS following rituximab raise the possibility that complementary belimumab treatment may benefit patients treated with rituximab, as suggested previously [195]. Of course, prolonged B-cell depletion or combination therapies raise the concern of immunodeficiency – for example, depletion of protective plasma cells or antibodies. Clearly, some therapies are more effective in treating certain autoimmune disorders compared with others (e.g., rituximab), and more effective for certain patients compared with others. Clinical trials and additional research in both mice and humans will help in the identification of the most effective BlyS family targets for treating various autoimmune diseases, and greatly enhance the ability to identify those patients most likely to benefit from a particular therapy.

Box 3 summarizes these questions and other areas of active investigation in human B-cell/BlyS family biology.

Looking ahead for BlyS-targeted therapies

We should extend studies in mice because, as argued herein, many discoveries made with mouse models are relevant to human health and disease. Not only the broad features, but some emerging details of BlyS family members’ roles in B-cell biology are sufficiently similar in mice and humans, that studies in mice will continue to serve as a passport to the complex and often empirically less tractable human sphere. In addition, clinical-trial design can be modified to afford a better understanding as to why BlyS family-targeted therapies work or fail, and to allow more accurate prediction of clinical success. For example, researchers could:

- Determine a broader immune phenotype of patients at baseline. This could include a B-cell subset profile with standardized phenotypes [37] and wider use of multidimensional immunophenotyping data analysis approaches such as cytometric fingerprinting [206], to include BlyS receptor expression, for improved

<table>
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<tr>
<th>Box 3. Targeting B cells and BlyS family members in autoimmune disease.</th>
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<tr>
<td>Do individual patients with autoimmunity harbor different alterations in their antigen-experienced B-cell subsets, pathogenic or protective pools, or tolerance checkpoints? Can these alterations be used to predict responsiveness to particular forms of B-cell targeted therapy?</td>
</tr>
<tr>
<td>How are B-lymphocyte stimulator (BlyS) and a proliferation-inducing ligand levels regulated locally and systemically in health and in autoimmune disease? Do particular BlyS or B-cell targeted therapies affect the regulation of BlyS expression?</td>
</tr>
<tr>
<td>What are normal BlyS receptor expression patterns on naive and antigen-experienced B cells? How are they altered in autoimmunity, and how might this be exploited in the development of novel BlyS-family-targeted therapies?</td>
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<tr>
<td>Do particular B-cell targeted therapies result in immune stimulation, increased antibody production, altered frequencies of class switching or somatic mutation, immune complex disease/serum sickness, or cytokine storm?</td>
</tr>
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<td>What are the effects of prolonged B-cell depletion on mature B-cell subsets and function? Does prolonged B-cell depletion effectively re-set the antibody repertoire, or do autoantibody-producing clones persist?</td>
</tr>
<tr>
<td>Which, if any, manifestations of BlyS activity in non-B-cell compartments contributes to the etiology or pathology of autoimmune disease?</td>
</tr>
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Our understanding of the development, selection, and maintenance of naive and antigen-experienced B-cell subsets has expanded substantially in the last decade. B-lymphocyte stimulator (BLyS) family members play key roles throughout these processes in both mice and humans. BLyS family members are useful targets for treating autoimmune disease, as they afford a means of modulating specific B-cell subsets and adjusting the stringency of peripheral tolerance mechanisms. Clinical trials provide significant opportunities to obtain a deeper insight into human B-cell biology. Future research and clinical trials with B cell and BLyS family-targeted therapies should allow refinement of treatment options designed to understand B-cell maturation, selection, or function in normal individuals; dysfunction of these processes in disease is useful information, but we also need to know how they work ‘normally’.

- Carry through with broader immune phenotype characterization during and after B-cell- or BLyS-targeted therapies. Since autoimmune diseases are often chronic, long-term treatment may be essential, and long-term follow-up of effects on B-cell subsets and serological responses are likely to be critical to determine therapeutic benefit [141,204]. For example, it would be informative to know if long-term therapy with belimumab can ‘reset’ selection checkpoints, or if it will erode specific memory or plasma cell subsets;

- Attempt to correlate clinical outcome measures with B-cell-centric outcome measures. Include studies designed to understand B-cell maturation, selection, and function in normal individuals; dysfunction of these processes in disease is useful information, but we also need to know how they work ‘normally’;

- Derive some of the above information from existing clinical trial data sets or existing samples. Access to raw data would permit rigorous statistical analyses and correlations between, for example, serum BLyS level and autoantibody titer [208].

**Future perspective**

In the short term, we will be able to target B-cell subsets with increasing specificity, for longer periods of time, and analyze results at the B-cell level with ever-increasing parameters and therefore discrimination. In the long term, we will be able to predict which patients will most likely benefit from which therapies.

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**Executive summary**

- Our understanding of the development, selection, and maintenance of naive and antigen-experienced B-cell subsets has expanded substantially in the last decade. B-lymphocyte stimulator (BLyS) family members play key roles throughout these processes in both mice and humans.
- BLyS family members are useful targets for treating autoimmune disease, as they afford a means of modulating specific B-cell subsets and adjusting the stringency of peripheral tolerance mechanisms.
- Clinical trials provide significant opportunities to obtain a deeper insight into human B-cell biology.
- Future research and clinical trials with B cell and BLyS family-targeted therapies should allow refinement of treatment options for diseases with clinical manifestations as varied as those associated with systemic lupus erythematosus, rheumatoid arthritis, Sjogren’s syndrome and others.

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Targeting the BLyS family in autoimmunity

Therapeutic Perspective


Therapeutic Perspective

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**Therapeutic Perspective**

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**FDA news release. FDA approves Benlysta to treat lupus**

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