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Space, Selection, and Surveillance: Setting Boundaries with BlyS

Juli P. Miller, Jason E. Stadanlick, and Michael P. Cancro

The BlyS family of ligands and receptors governs B cell homeostasis by controlling survival, differentiation, and lifespan. This family consists of multiple receptors and ligands, allowing independent regulation of different B cell subsets by varying the combination and levels of receptors expressed. Multiple downstream signaling pathways are implicated in these activities, reflecting this receptor complexity as well as cross-talk with other B cell signaling systems. BlyS levels are associated with multiple forms of humoral autoimmunity and can modulate tolerogenic environments thus balances peripheral selection against cell elimination at the transitional checkpoint. BlyS responsiveness thus varies with different cell numbers, providing an elastic system that varies selective stringency based on homeostatic demands. The Journal of Immunology, 2006, 176: 6405–6410.

Over the last several years, the BlyS family of TNFRs and ligands has emerged as a key player in B cell biology, fostering broad investigation as well as numerous reviews (1–4). In general, this family of molecules controls the magnitude of B cell compartments by modulating survival and differentiation, integrating these homeostatic functions with specificity-based selection.

BlyS was identified through database searches for novel TNF family ligands and, because it was initially reported by multiple groups, is referenced by several names: BlyS (5), B cell-activating factor (BAFF) (6), TALL-1 (7), and THANK (8). Two receptors for BlyS were rapidly identified, transmembrane activator and calcium-modulator and cyclophilin ligand-interactor (TACI), and B cell maturation Ag (BCMA) (9–11). In addition to their interactions with BlyS, these two receptors also bind a proliferation-inducing ligand (APRIL), another TNF family member (9, 12) (Fig. 1). Because neither TACI nor BCMA knockouts showed remarkable phenotypes, an additional BlyS-specific receptor was postulated and subsequently discovered (13, 14). Again, because of its description by several laboratories, this receptor appears in the literature as BlyS receptor 3 (BR3) (13) and BAFF receptor (BAFFr) (14, 15).

Under physiologic conditions, these ligands and receptors form trimeric complexes, similar to other TNF family members. BR3 interacts only with BlyS and contains an extracellular 26-aa sequence critical for downstream signaling (16). While APRIL does not bind BR3, its affinities for TACI and BCMA are ~100-fold greater than those of BlyS (17–19), suggesting APRIL may be the primary ligand for these receptors in vivo. X-ray crystallographic and biochemical studies of BlyS family receptors indicate several differences in the sequences and numbers of cysteine-rich domains of the extracellular regions, as well as differences within the binding sites, that likely account for differences in the strength and nature of ligand interactions (16, 18, 20, 21).

The soluble BlyS trimmer is thought to be the sole biologically active form, although active membrane-bound species remain possibilities. Splice variants have been described for both BlyS and APRIL (22–25) and include -BAFF, which can antagonize BlyS activity (23, 25), as well as TWE-PRIL (24), whose in vivo function remains unclear. BlyS production has been directly demonstrated in a variety of cell types, including neutrophils (26) dendritic cells (27), synovial fibroblasts (28), astrocytes (29), nurse-like cells (28), and T cells (30), and its release is fostered by some inflammatory cytokines (26, 31, 32). In addition, radioresistant sources of BlyS have been inferred (33), which is consistent with the observation that BlyS messenger RNA is present in a wide variety of tissues (34). Thus, multiple tissue sources likely contribute to systemic BlyS levels. Less is known about in vivo sources for APRIL, although it is also produced by dendritic cells, and is found at higher concentrations in certain inflamed sites (35). In addition to their interactions with one another, both APRIL and TACI interact with cell surface sulfated proteoglycans, although the significance of these interactions is not yet clear (36, 37).

Early biological findings foreshadowed an essential role for BlyS family members in B cell homeostasis and selection. Exogenous BlyS administration rapidly but reversibly doubled peripheral B cell numbers (5), and conversely, soluble BlyS receptor treatment profoundly diminished most peripheral B cell subsets (38). Links with B cell tolerance were quickly forged as
BLyS transgenics developed a broad spectrum of autoantibodies (10), and clinical studies correlated elevated BLyS levels with systemic lupus erythematosus (39–41), rheumatoid arthritis (39, 42), and Sjögren’s syndrome (43, 44). Finally, driven by the obvious implications for cell growth and viability, additional investigations established associations with B cell neoplasms (45). These profound B lineage-specific actions and clear relationships to autoimmunity and cancer have spurred investigation of the BLyS family’s biology and have focused attention on its members as potential therapeutic targets (46–55). Such varied biological activities also suggest that a key feature of the BLyS family is its inclusion of multiple receptors and ligands. This affords the potential for diverse actions through a range of receptor expression patterns in various differentiation contexts. Mechanistic understanding thus requires an appreciation of BLyS receptor expression patterns in different B cell subsets, the developmental cues that determine these patterns, and the downstream mediators of BLyS signaling in each differentiation context.

**BLyS-BR3 interactions govern transitional (TR) differentiation and primary B cell lifespan**

During B cell differentiation, the capacity to bind BLyS appears concomitant with BCR expression. Thus, among emergent bone marrow (BM) B cells (56), only those in the immature subset (Hardy Fr. E) exhibit appreciable BLyS binding capacity, with higher binding activity among the CD23 + cells in this fraction (57). BLyS binding capacity increases through the TR stages (58–60), reflecting increasing expression of both BR3 and TACI, and is highest among cells in the follicular (FO) and marginal zone subsets (57).

Increasing BR3 levels during late maturation match functional studies because BLyS-BR3 interactions dominate at these stages. BR3 is the product of *bcmd* (13, 61), whose mutated form in the A/WySnJ strain yields a paucity of peripheral B cells (62, 63). Studies with this strain (57, 64, 65), as well as experiments using knockout mice or in vivo soluble receptor blockade with TACI-Ig (15, 38), have shown that BLyS-BR3 interactions determine the likelihood that newly emerging B cells will complete TR differentiation, as well as the lifespan of mature FO and marginal zone B cells. For example, in both the A/WySnJ and BAFF knockout mice, most TR cells fail to complete differentiation, and the few FO cells formed have a short lifespan (14, 62, 65). Furthermore, studies with BR3 haploinsufficient mice and mixed marrow chimeras have shown that FO B cells compete for BLyS and that the levels of functional BR3 dictate competitive advantage (65). Coupled with studies showing that the TR differentiation and FO B cell lifespan can be rescued by enforced Bcl-xL expression in BR3 mutants (66), these findings suggest that BLyS-BR3 interactions mainly promote viability. Nonetheless, BLyS can also foster up-regulation of CD21 and CD23 at these stages, suggesting direct influences on differentiation (67, 68). The accumulation of phenotypically immature cells in mice expressing antiapoptotic transgenes in the context of BR3 mutations supports this notion (69). How the induction and ongoing expression of BR3 and TACI are controlled, as well as determinations of the relative roles played by TACI vs BR3 in emerging and primary pools, remain outstanding questions of fundamental importance.

**BLyS family members govern activities of Ag-experienced B cells**

Ag-experienced B cells comprise several homeostatically independent niches, as evidenced by the waxing and waning of activated primary B cell clones and the slower turnover of memory pools. The levels and combinations of BLyS family receptors change among activated B cells and their descendants, suggesting that alternative ligand receptor combinations contribute to the maintenance of these pools.

In vitro studies demonstrate that BLyS promotes the survival of activated B cells, and BCR stimulation or CD40 ligation enhances this effect (70–72). Soluble TACI-Fc administration, following immunization, severely curtails both T cell-dependent and -independent responses (11, 73) and, conversely, exogenous BLyS administration enhances both (71). Furthermore, BR3-deficient and A/WySnJ mice form only rudimentary germinal centers (GCs) and produce abnormally low levels of serum IgG (74–76). The role(s) of BLyS family members among GC B cell subsets remains unclear. For example, while ectopic Bcl-2 expression increased B cell numbers in BR3-deficient mice, GC formation and secondary IgG responses were incompletely rescued (69, 77), suggesting BLyS may act as more than just a survival factor in this setting.

In addition to tempering the magnitude of humoral responses, BLyS and APRIL are both implicated in isotype switching (27, 78–82). BLyS receptor-deficient mice exhibit varying degrees of impaired isotype switching, with defects in the APRIL/TACI axis impacting T cell-independent responses most severely (83, 84). Whether BLyS and APRIL simply increase the probability of isotype switching by prolonging daughter cell survival within activated clones, or instead directly regulate class switch recombination per se, remains an open question.

Although BR3 expression dominates among primary and recently activated cells, TACI and BCMA subsequently emerge as the predominant species, implying a shift from BLyS to APRIL dependency (85). Analyses of BCMA-deficient mice are in accord with this view: although BCMA is dispensable for primary B cells (86), BCMA−/− mice lack long-lived BM plasma cells thought to be critical for humoral memory (87). Thus, shifts in predominance between BR3, TACI, or BCMA expression likely represent key milestones in humoral immune responses, affording independent homeostatic regulation of Ag-experienced subsets.

**BLyS-mediated effects involve multiple, overlapping pathways**

How BLyS and APRIL influence B cell physiology poses a complex problem because primary B cells and cell lines express multiple BLyS family receptors that use pathways common to other fundamental B cell signaling systems. Thus, it is not surprising that several signaling cascades, as well as multiple downstream...
mediators of survival, death, and cell cycle, have been implicated as consequences of BlyS- and APRIL-driven signals.

The most receptor-proximal players are likely TNFR-associated factors (TRAFs) (reviewed in Ref. 88). Studies using both cell lines and primary cells show that TRAF-1, -2, and -3 can associate with BCMA, whereas TRAF-2, -5, and -6 associate with TACI (89). BR3 appears to interact solely with TRAF-3 (90), and this interaction is conformationally unique compared with other known TNFR/TRAF3 interactions, suggesting specialized signaling properties (90). Despite this knowledge, the specifics of TRAF/BlyS receptor interactions and their consequences remain puzzling. For example, while the A/WySnJ mutation yielding B cell deficiency encodes a BR3 molecule lacking the TRAF3 binding sequence (13, 66, 91), B lineage-specific TRAF-3−/− mice have enlarged peripheral B cell compartments (G. Bishop, unpublished observation).

Downstream mediators of BlyS and APRIL clearly include both the classical (NF-κB1) and nonclassical (NF-κB2) NF-κB pathways (9, 16, 27, 71, 92). It is tempting to speculate that the biological impact of the various BlyS receptors may roughly conform to the notion that classical NF-κB signaling chiefly mediates inflammatory responses and innate immunity, whereas the nonclassical pathway chiefly governs responses to TNF family cytokines and facilitates adaptive immunity (93). In accord with this, BlyS binding to BR3 robustly activates the nonclassical NF-κB pathway, although rapid and transient classical pathway activity has been observed in vitro (92, 94–96). In contrast, in vitro studies indicate that TACI signaling favors the classical pathway (Ref. 97 and our own unpublished observations). Knockouts of NF-κB pathway components and isolated BlyS receptor systems, as well as examinations of cross-talk between BlyS receptors and other exogenous stimuli, should yield a more comprehensive picture of these relationships.

Although a precise description of BlyS-derived intracellular signals is pending, their ultimate result is antiapoptotic. In this regard, BlyS modulates several Bcl-2 family members, including Bcl-xL, Mcl-1, A-1, Bcl-2, and Bim, via survival-promoting kinase systems such as Pim 1/2 or Erk (34, 57, 70, 71). BlyS signaling also reduces proapoptotic protein kinase Cθ nuclear accumulation, and this survival is dependent on c-Myb (98, 99). Furthermore, BlyS-mediated survival is enhanced by BCR stimulation and CD40 ligation (70–72, 100), directly suggesting cross-talk among the involved signaling cascades. Finally, BlyS regulates the G1 cell cycle checkpoint protein p27kip (101), suggesting that BlyS lowers the threshold for cell cycle entry, thus releasing naive cells from homeostatic restraints.

**BlyS levels vary TR selection stringency, defining an elastic checkpoint**

Maintaining pool sizes by controlling throughput and longevity, as well as establishing distinct homeostatic niches by varying the spectrum of expressed receptors, present important but relatively straightforward mechanistic puzzles. In contrast, understanding the relationship between these processes and the control of autoreactive B cells—fundamental to deciphering the link between BlyS and autoimmunity—poses a key conceptual challenge. Recent findings not only provide a critical piece of this puzzle but may explain how the disparate triumvirate of immunological pressures—maximizing diversity, minimizing autoreactivity, and guaranteeing enough cells for adequate surveillance—are accommodated. Indeed, these have long posed a conundrum: if entry into primary pools is tied only to maintaining enough cells for effective surveillance, then autoreactive specificities will survive. On the other hand, if surveillance capacity is ignored in favor of tolerogenic elimination, then cell numbers could fall below the minimum for competent surveillance. Recent findings suggest that the acquisition of BlyS responsiveness reconciles these demands by dividing selection into two stages: a first in which decisions are B cell intrinsic, based solely on BCR signal strength, and a second in which the BCR signaling threshold for elimination varies based on current surveillance capacity, with BlyS as the metric for unoccupied space.

Two categories of tolerogenic elimination have long been appreciated (102). The first involves deletion of high-affinity autoreactive B cells at the immature BM stage (103–105). In the second, lower avidity interactions yield B cells (106, 107) that migrate to the periphery but die at TR stages (108, 109). With the exception of receptor editing (110, 111) or gene replacement (112–114) events that change BCR specificity, no means of sparing autoreactive clones at the BM selection step have been revealed. In contrast, TR B cells slated for elimination can be rescued under certain conditions, and importantly, the key determinant of TR cell fate is interclonal competition (115, 116). Two recent reports have now shown that BlyS is the limiting resource for which TR cells compete, dictating the extent to which autoreactive B cell clones survive this space-dependent checkpoint (34, 117). Taken together, these studies showed that while elevated BlyS could not influence BM deletion, it

![FIGURE 2. BlyS responsiveness defines an elastic checkpoint.](http://www.jimmunol.org/)

A. During selection at the marrow-periphery interface, high-affinity autoreactive cells (red) are deleted in the BM immature subset. Following acquisition of BlyS responsiveness, cells enter the TR subsets and undergo further selection. As schematized here by the bracketed alternative scenarios, TR selection is elastic and depends on available BlyS depicted by varying violet intensity. When BlyS is in excess, all clonotypes mature. As free BlyS becomes limiting, low-avidity autoreactive clones (pink) are eliminated and only the most fit (green > yellow) clonotypes survive. B. At normal steady state (I), far more TR cells are formed than can be supported by existing free BlyS, so selective stringency is high. Events that yield increases in available BlyS reduce selective stringency increasing throughput. These include peripheral lymphopenia (II), reduced BM output (III), administration or overproduction of BlyS (IV), or combinations of these factors.
could rescue TR elimination. Furthermore, the likelihood of rescue varied based on avidity for self and the degree of competition from concurrently emerging clonotypes.

These considerations suggest that the TR stage is an elastic checkpoint where thresholds for negative selection are homeostatically adjusted by free BLYS concentration. A consequence of this elasticity is that the number of cells entering such checkpoints must greatly exceed throughput or selection will fail. Thus, events which compromise this rule—such as acute drops in incoming and resident cell numbers or precipitous increases in free BLYS—will stress the system beyond acceptable limits and circumvent selection (Fig. 2). Lymphopenia, reduced marrow output, and elevated BLYS have all been associated with homeostatically stressed elastic checkpoint may prove the common thread. Whether arising through idiopathic mechanisms such as hormonal shifts and inflammatory processes or through therapies targeting mature or emerging B cells, a reexamination of such associations from this perspective warrants consideration.

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Disclosures

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