Review

Integrating B Cell Homeostasis and Selection with BLyS

SUSAN HARLESS SMITH and MICHAEL P. CANCRO*

Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA 19104, USA

Abstract. The mechanisms that maintain a pool of B cells that is adequately diverse yet devoid of pathogenic autoreactivity remain poorly understood. B cells complete maturation after migrating to the periphery, where they transit several intermediate developmental stages prior to recruitment into the long-lived primary pool. Since B lineage commitment is not coupled to peripheral B cell numbers and most mature peripheral B cells are quiescent, the sizes of mature peripheral compartments are primarily determined by the proportion of immature B cells that survive transit through later developmental stages, coupled with the longevity of mature B cells themselves. Compelling evidence indicates that the B cell antigen receptor (BcR) plays an essential role in all of these processes, but further findings indicate a similar role for the recently described tumor necrosis factor family member B lymphocyte stimulator (BLyS). Signaling through the BLyS receptor, Bcmd/BR3, controls B cell numbers in two ways: by varying the proportion of cells that complete transitional B cell development, and by serving as the primary determinant of mature B cell longevity. The striking congruence of BcR- and BLyS-mediated effects on B cell selection and survival suggests these pathways may be related. The recent discovery that BcR signaling is selectively coupled to Bcmd/BR3 expression links BcR- and BLyS-mediated activities in transitional and mature B cells, suggesting specificity-based selection and survival may be mechanistically similar processes.

Key words: B lymphocytes; BLyS; homeostasis; development.

Peripheral B Cells Are Maintained through an Interplay of Homeostasis and Selection

The adaptive immune system relies on a primary repertoire of clonally distributed specificities that is sustained at steady state. Given the relatively constant rate of lymphocyte neogenesis, stringent homeostatic processes are clearly implied in the control of peripheral lymphocyte numbers. Further, since antigen receptor diversity is generated through random gene rearrangement and modification processes, mechanisms that selectively control clones bearing potentially autoreactive specificities are likely operative, especially at differentiative stages immediately following antigen receptor expression. Finally, a growing body of evidence suggests that recruitment of newly formed cells into the mature peripheral pool, as well as their subsequent survival, involves active, specificity-dependent, positive selection. Simultaneous regulation of both the magnitude and clonotypic composition of the peripheral B cell pool suggests these two processes might be coupled, but mechanisms capable of integrating homeostasis and specificity-based selection have remained elusive.

The discovery of a novel tumor necrosis factor (TNF) family member, B lymphocyte stimulator
(BLyS), has afforded significant advances in our understanding of B lymphocyte homeostasis. BLyS acts almost exclusively on B cells by virtue of the largely B lineage-restricted expression of BLyS receptors. An essential role for BLyS in peripheral B cell homeostasis and selection is evidenced by the marked increases in steady-state B cell numbers and immunoglobulin levels after BLyS treatment, as well as the severe B cell deficiency seen in either the absence of endogenous BLyS protein or in BLyS receptor mutants. BLyS has also been implicated in a variety of human immune disorders and animal models of autoimmunity, suggesting it plays a key role in specificity-based selection as well. Accordingly, the last several years have witnessed intense investigation of BLyS and its receptors, already yielding numerous reviews and commentaries. Herein, we focus primarily on the role played by BLyS in controlling the lifespan of transitional and mature peripheral B cells, followed by a discussion of mechanisms whereby B cell antigen receptor (BcR) signaling can regulate the relative ability to capture BLyS.

Peripheral B Cell Maturation Relies upon Specificity-Based Selection

B cells are generated throughout life from pluripotent stem cells in fetal liver, neonatal spleen, or adult marrow. After differentiation through pro- and pre-B cell stages, the acquisition of surface IgM yields an immature B cell, which exits the marrow and undergoes final maturation in the periphery. This immature peripheral compartment has been termed the “transitional” stage, and has been further divided into three subsets termed T1, T2, and T3 according to the differential surface expression of CD23 and sIgM. These differentiative stages, as well as their associated surface phenotypes and kinetic properties, are summarized in Fig. 1.

Cytofluorimetric studies have confirmed early observations that newly formed B cells display rapid turnover rates, while mature cells turn over more slowly. Moreover, labeling studies have revealed profound disparities in the production rates of the transitional versus mature B cell populations (Fig. 1). Indeed, the rate at which new cells enter the mature pool accounts for less than 5% of immature cells generated in the bone marrow, suggesting considerable cell death occurs during transitional differentiation.

These striking losses of B lymphocytes between their formation in the bone marrow and entry into the mature peripheral B cell pool, together with the widely differing turnover rates between transitional and mature peripheral B cells, suggests that both selective and homeostatic mechanisms active during transitional differentiation influence the composition and size of the B cell pool. Deletion of self-reactive B cells likely contributes to attrition at the marrow-periphery interface, and a variety of in vitro and transgenic models have provided compelling evidence consistent with specificity-based deletion. Nonetheless, such mechanisms fail to account for all developmental losses, especially given recent insights regarding alternative fates for potentially autoreactive clones. Instead, a portion of these losses probably reflects the absence of a viability-promoting event, as evidenced by disparate clonotype distributions between immature and mature B cells, skewed population dynamics in Ig-transgenic mixed-marrow chimeras, and blocked B cell differentiation in some gene knockout mice.

Consistent with the possibility that signals through the BcR underlie the recruitment and survival of mature splenic B cells, the repertoire of Ig V-gene usage among mature B cells appears skewed, presumably towards genes that encode for immunoglobulin specificities best fit to transmit a minimum basal signal to afford final maturation. For example, while V_{H} genes...
among immature bone marrow B cells were distributed stochastically across the J558 family, recirculating mature B cells preferentially utilize two related J558 family genes. Similarly, Levine et al. showed a truncation among light chain variable regions in transitional versus mature peripheral B cells. These results suggest that BcR specificity plays a central role in determining which developing B cell clones survive to maturity.

Mature B Cell Lifespan, the Primary Determinant of Peripheral B Cell Numbers, Is Also Influenced by Specificity-Based Selection

The rate of pro-B cell genesis is not coupled to peripheral B cell numbers, speaking against feedback regulation of B lineage commitment in the bone marrow as a homeostatic control mechanism. Further, most mature peripheral B cells are quiescent. Thus, the steady-state size of the mature peripheral compartment is primarily determined by the proportion of immature B cells surviving transit through later developmental stages, coupled with the longevity of mature B cells themselves. Accordingly, the lifespan of mature peripheral B cells is largely responsible for the population size at steady-state, given the apparent “inefficiency” with which newly formed cells enter the peripheral pool. Primary B cells display a relatively long average lifespan of 80–120 days, but the factors controlling mature lifespan have remained poorly understood.

Whether continued specificity-based selection among mature B cells is integrated with the factors controlling mature B cell homeostasis has remained obscure. Nonetheless, the BcR clearly plays a major role in maintaining the viability of mature B cells. For example, conditional ablation of BcR expression subsequent to the establishment of steady-state B cell numbers results in the death of most peripheral B cells. The exact mechanism through which BcR signaling influences B cell longevity remains to be resolved, but it appears that, analogous to its role in transitional B cell selection, basal or “tonic” signaling via the BcR remains necessary to afford continued mature B cell survival. Moreover, BcR specificity plays a role in determining the relative fitness of mature peripheral B cells to survive. This has been best demonstrated through experiments employing mixed-marrow chimeras from the Freitas laboratory. An important conceptual conclusion derived from these studies is that mature B cells are competing for lifespan-promoting factor(s) and that their relative competitive advantage is dictated by their BcR specificity.

The BLyS/BLyS Receptor Subfamily

BLyS, its receptors, and its related cytokine APRIL, are recent additions to the TNF family. BLyS appears in the literature under multiple names: BAFF, TALL-1, THANK, zTNF4 and TNFSF13B. Herein we will apply the terms BLyS and APRIL for the ligands; and B cell maturation antigen (BCMA), transmembrane activator and calcium modulating cyclophilin ligand interactor (TACI), and Bcmd/BR3 for the three receptors, although Bcmd/BR3 is also referred to as BAFF-R.

APRIL is a 250-amino-acid TNF family member whose exact function remains somewhat controversial. Nonetheless, soluble APRIL enhances B cell proliferation in vitro, and in vivo administration of APRIL increases the representation of B cells in secondary lymphoid organs. APRIL has also been implicated in isotype switching and may act as a costimulatory or survival factor for some T lymphocytes. However, a dominant role for APRIL in B cell development and homeostasis seems unlikely, since endogenous APRIL fails to rescue B cell defects in either BLyS-deficient mice or Bcmd/BR3-deficient mice.

BLyS is a 285 amino-acid transmembrane protein that was simultaneously reported by several groups, primarily generated by furin cleavage and appears to be induced by bacterial lipopolysaccharide and some inflammatory cytokines. Compelling evidence indicates that BLyS plays a dominant, lineage-specific role in the survival of B lymphocytes.

Three receptors, all of which are preferentially expressed by B cells, have been described for BLyS and/or APRIL: BCMA, TACI, and BLyS-receptor-3, the product of the previously defined bcmd locus (Bcmd/BR3). BLyS interacts with all three receptors, whereas APRIL interacts with TACI and BCMA, but not Bcmd/BR3. The preferential interaction of Bcmd/BR3 with BLyS, coupled with recent studies in Bcmd/BR3 signaling mutants, suggest that it is the primary mediator of BLyS activities in peripheral homeostasis and selection.

BLyS Acts on Transitional and Mature B Lymphocytes

The activities of BLyS and its receptors among transitional and mature B cells are summarized in Fig. 2.
BlyS plays little role in early B cell genesis, since neither BlyS binding nor BlyS receptor message is detectable among marrow B cell progenitors prior to the immature B cell stage (Hardy fraction E); and neither the size nor dynamics of these early differentiative stages are affected in BlyS transgenics, BlyS knockouts, BlyS receptor knockouts, or BlyS receptor mutants.

In contrast, all B cell subsets including and subsequent to the immature marrow stage bind BlyS and respond to BlyS either per se or to manipulations that influence signaling via BlyS receptors. Thus, either ectopic expression or exogenous administration of BlyS quickly yields increases in all except the B1 B cell subset, accompanied by aberrant splenic architecture and elevated serum IgM and IgA. Conversely, animals treated with the soluble BlyS receptor, TACI-Ig, which acts to reduce endogenous BlyS levels, exhibit a rapid diminution of all mature peripheral pools except the B1 subset. Similarly, in BlyS knockout mice, all mature peripheral B cells except for the B1 subsets are vastly reduced. Finally, bcmd mutations also yield extensive reductions in all peripheral B cells except the early T1 and B1 subsets.

In addition to excessive peripheral B cell numbers, BlyS transgenic mice develop humoral autoimmune symptoms by ~8 weeks of age, including serum rheumatoid factors, Ig complex depositions in the kidneys, and the appearance of various autoantibodies. Combined, these observations suggest that BlyS also plays a strong role in specificity-based selective events during B cell differentiation that normally act to avert pathogenic autoreactivity.

BlyS Governs Successful Transitional B Cell Differentiation

The transitional B cell pools (Fig. 1) are the progenitors of mature follicular B cells, and are thought to be the differentiative stage where specificity-based selection is first imposed. Thus, the finding that exogenously administered or ectopically expressed BlyS not only increased B cell numbers but fostered autoantibody formation was an indication that BlyS likely influences transitional B cells. In accord with this early evidence, subsequent findings have firmly established the transitional pools as key targets of BlyS-mediated activity. Several lines of evidence indicate that the receptor primarily responsible for BlyS-mediated effects within transitional cell pools is Bcmd/BR3: First, the bcmd locus, which encodes a mutated form of this receptor, was originally discovered in the A/WySnJ mouse and was so-named (B cell maturation defect) for a phenotype of severely impaired transitional B cell differentiation and shortened mature B cell lifespan. Moreover, neither the TACI nor BCMA knockouts exhibit phenotypes with substantially altered transitional B cell differentiation.

Recent studies have probed the effect of BlyS on transitional populations both in vitro and in vivo. Rolefink et al. have shown that the addition of BlyS to supplemented cultures of transitional B cells afforded extended survival and led to the appearance of phenotypically and functionally mature B cells. These findings led to the suggestion that BlyS provides an inductive signal for maturation. However, in vivo kinetic analyses revealed that transit rates across peripheral developmental subsets were identical in BlyS-treated versus control mice, suggesting that prolonged survival, rather than induction of more rapid differentiation, was responsible for enhanced transitional cell differentiation, presumably by altering the proportion of transitional cells surviving to complete maturation in the periphery.

The expression of BlyS receptors also differs substantially between each of the transitional subsets and mature B cell populations. Cells within the T1 compartment predominantly express BCMA and very low levels of TACI and Bcmd/BR3. In
marked contrast, the T2, T3, and mature follicular populations show the opposite pattern of expression, primarily expressing TACI and Bcmd/BR3 with essentially undetectable levels of BCMA. The basis and significance of this observation remain unclear, although they are consistent with a developmental shift in BlyS responsiveness associated with cells undergoing recruitment into the mature peripheral pool.

**Competition for BlyS Mediates Longevity of Mature B Cells**

The earliest observation suggesting a role for BlyS in maintaining viability among mature B cells was the finding that treatment of mice with soluble BlyS receptors resulted in the rapid loss of most mature peripheral B cells. The two-week time frame of these reductions was too short to be explained by senescent attrition of mature B cells alone, since the average lifespan of cells in this pool is 80–120 days. Instead, these results were most consistent with the notion that BlyS is requisite for continued mature B cell viability.

Studies in the bcmd mutant mouse strain A/WySnJ confirmed and extended this idea in two ways: First, in vivo BrdU labeling studies demonstrated that mature B cell lifespan was severely compromised in the absence of Bcmd/BR3 signaling. Second, similar kinetic studies in (A/WySnJ × BALB/c)F1 mice showed that B cells heterozygous for the bcmd defect have an intermediate but uniform life span, indicating that follicular B cell viability requires continuous signaling via this pathway. In addition, mature A/WySnJ-derived B cells failed to compete effectively for survival, since they did not maintain their proportional representation upon reconstitution in mixed-marrow chimeras. Together, these findings not only definitively established BlyS signaling via the Bcmd/BR3 pathway as the dominant mediator of quiescent peripheral B cell survival, but also indicated that stochastic competition for available BlyS dictates the lifespan of resting mature B cells, thereby mediating homeostatic control of the peripheral B cell pool.

**BlyS Promotes Viability through the Regulation of Bcl-2 Family Members**

In vitro studies from Chen-Kiang and colleagues suggested that a primary activity of BlyS is the attenuation of apoptosis, since BlyS alone did not engender mitogenesis, yet increased the relative representation of several anti-apoptotic Bcl-2 family members. The idea that BlyS acts primarily to enhance survival is further supported by in vivo kinetic and cell-cycle analyses. These studies showed that BlyS affords increased proportional survival in the late transitional developmental subsets, such that the efficiency of transit into the mature B cell compartment is vastly increased; but does so without inducing division within these normally quiescent pools.

It is well established that Bcl-2 family members influence the survival and lifespan of normal and neoplastic lymphocytes. The expression of these genes is clearly regulated during normal lymphocyte development, and experiments in transgenic mice or targeted deletion mutants have demonstrated that alterations in bcl-2 family gene expression affect B lymphocyte survival and differentiation. The possibility that BlyS might affect the viability and lifespan of B cells through the alteration of Bcl-2 family members was initially proposed by Do et al., who found that in conjunction with CD154 (CD40L), BlyS upregulated anti-apoptotic members of the Bcl-2 family among splenic B cells, apparently through an NFκ-B/relB pathway. Moreover, peripheral blood B cells of BlyS transgenic mice have increased expression of anti-apoptotic bcl-2 family members. Conversely, an increase in pro-apoptotic bcl-2 family members has been reported in BlyS receptor mutants, further suggesting a connection between BlyS-mediated survival and the expression of bcl-2 family genes.

More recently, the influence of BlyS on several bcl-2 family members was assessed among transitional and mature cells following short-term culture with recombinant BlyS. These studies showed that mature B cells strongly upregulated both Bcl-xL and A1 in response to BlyS, whereas the upregulation of these genes in bulk transitional populations was low. Finally, in addition to extending mature B cell lifespan, the forced expression of Bcl-xL in the Bcmd/BR3 mutant A/WySnJ mouse fully rescues transitional cell differentiation.

**BlyS Influences Aspects of Mature B Cell Activation**

A role for BlyS in the ligand-driven activation of mature B cells has been posited since early observations that BlyS, in conjunction with mitogenic stimuli, yields enhanced thymidine incorporation. This led to an
initial interpretation that BLyS serves as a costimulatory factor for B cells, perhaps acting in a fashion similar to CD154 (CD40L), and prompted the suggestion that BLyS might induce proliferation among B lymphocytes, leading to the increases in B cell numbers and Ig levels associated with in vivo BLyS treatment. Again, the Bcmd/BR3 receptor appears to be the dominant receptor for this activity, inasmuch as the Bcmd/BR3 mutant A/WySnJ mice do not exhibit enlarged B cell compartments upon exogenous BLyS administration, and B cell proliferation assays on CD23+ B cells from these mice fail to display increased thymidine uptake when supplemented with recombinant BLyS. Nevertheless, subsequent studies have favored a dominant role for BLyS in mediating survival, rather than any co-mitogenic activity per se, under such stimulatory conditions. Following BcR ligation, there is increased proportional survival within initial daughter cohorts of activated mature B cells, resulting in increased 3H-dR uptake, without altering division number on a per cell basis (Smith et al., unpublished). If a similar activity exists in vivo, it would suggest that BLyS may determine the proliferative burst size reached by responding primary B cell clones. This would likely influence the magnitude of primary humoral responses, as well as the cells available to initiate germinal centers as memory progenitors. The generally elevated IgM levels in BLyS treated mice or in BLyS transgenics, as well as impaired memory responses in Bcmd/BR3 mutants are consistent with these ideas.

Other aspects of primary follicular B cell activation may be facilitated or even induced by BLyS signaling as well. In a recent study, Litrnksi et al. demonstrated that dendritic cell derived BLyS or APRIL can facilitate isotype switching in a CD40-independent manner, if supplemented with appropriate interleukins.

**BLyS May Influence the Formation or Maintenance of Memory B Cells**

Although the effects of BLyS on naïve B cell populations are well documented and accepted, whether BLyS or its receptors play a role in the generation, selection, or maintenance of memory B cell populations has not yet been addressed in detail. The nature of the A/WySnJ defect at bcmd has afforded initial interrogation of this possibility, since some mature B cells are indeed formed in these mice, and can thus be stimulated to participate in immune responses. Ongoing studies in this model indicate that although the BLyS receptor mutant strain can initiate similar numbers of germinal centers (GC), the characteristic GC enlargement between days 6 and 12 post immunization fails to occur. These results, coupled with earlier studies that showed a relatively normal primary IgM response but unusually low secondary serum responses and diminished IgG titers, strongly suggest that BLyS signaling via the Bcmd/BR3 receptor is instrumental in establishing or maintaining humoral memory. Aberrant secondary responses in A/WySnJ mice might reflect compromised survival in GC B cells such that fewer cells remain viable to enter the memory B cell pool, and thus memory cell formation is compromised. Alternatively, this could reflect a failure to meet selective criteria, analogous to those required for transitional B cell success. Finally, whether memory B cells require BLyS for continued survival in a fashion similar to the maintenance of the naïve B cell compartment remains unexplored.

**BcR-Mediated Bcmd/BR3 Expression May Integrate Selection and Homeostasis**

It is clear that BLyS plays a pivotal role in the selection, homeostasis, and activation of peripheral B cells. In aggregate, the evidence to date suggests that BLyS controls peripheral B cell numbers both by varying the proportion of cells that successfully complete late transitional B cell development and by serving as the primary determinant of longevity among mature follicular B cells. As indicated above, the BcR also plays a similar crucial role in B cell differentiation and survival: immature B cells are targets of BcR-mediated selection, as evidenced by the significant cell losses and repertoire shifts associated with late B cell maturation; continued BcR expression is requisite for mature B cell survival; and both BcR specificity per se as well as altered BcR signaling can dictate relative survival advantage in the periphery. These conspicuous parallels suggest a potential relationship between BcR signaling and peripheral survival via the BLyS-Bcmd/BR3 pathway, and this possibility has recently been probed. Ongoing studies (Smith and Cancro, in press) have revealed that BcR ligation upregulates expression of the BLyS receptor Bcmd/BR3, but not other known BLyS receptors. Further, this coupling of BcR signaling with Bcmd/BR3 expression is limited to late transitional and mature B cells.

It is yet unclear whether the coupling of BcR signaling with Bcmd/BR3 expression is a maturation-induced developmental switch, or is instead a constitutive association observed only in clonotypes whose BcRs...
yield a minimum requisite level of Bcmd/BR3 expression. Nonetheless, the coupling of BCR signaling with Bcmd/BR3 expression during transitional cell differentiation suggests that specificity-based selection in part results from BCR-driven Bcmd/BR3 expression determining each clone’s relative fitness. This relationship suggests a mechanism, outlined in Fig. 3, whereby the basal BCR signaling requisite among mature B cells reflects a necessary minimum level of Bcmd/BR3 expression and that a further survival advantage is afforded clones whose BCRs yield the highest relative levels of Bcmd/BR3. This model consolidates specificity-based selection in part with continuous, specificity-based interclonal competition in the mature repertoire\(^3, 33\) with continuous, specificity-based transitional selection\(^33, 48\) with continuous, specificity-based interclonal competition in the mature repertoire\(^44, 87\).

Further, it predicts a gradually evolving landscape of specificities; whose intrinsic survival advantage is continuously reevaluated relative to extant competitor clones. Thus, the mature primary repertoire will eventually truncate in terms of fitness, and possibly diversity. Elucidating the details of this integrated regulatory system, the selecting ligands involved, and the consequences of protracted repertoire truncation during an individual’s lifetime will likely yield insights regarding the establishment and maintenance of immunity and tolerance.

References


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