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Review

# BLyS receptor signatures resolve homeostatically independent compartments among naïve and antigen-experienced B cells

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**Abstract**

The BLyS family of receptors includes two cytokines, BLyS and APRIL; and three receptors, BR3, BCMA and TACI. Together, these regulate the size and composition of peripheral B cell pools. The multiplicity of ligand–receptor sets, in conjunction with differential receptor expression, alternative binding partners and disparate downstream signaling characteristics, affords the potential to establish independently regulated homeostatic niches among primary and antigen-experienced B cell subsets. Thus, BLyS signaling via BR3 is the dominant homeostatic regulator of primary B cell pools, whereas APRIL interactions with BCMA likely govern memory B cell populations. Short-lived antibody forming cell populations and their proliferating progenitors express a TACI-predominant signature. Further, within each niche, relative fitness to compete for available cytokine is determined by exogenous inputs via adaptive and innate receptor systems, affording intramural hierarchies that determine clonotype composition.


*Keywords:* BLyS; APRIL; B lymphocyte; Homeostasis

## 1. Introduction

Members of the tumor necrosis factor (TNF) superfamily play diverse roles in regulating the activities of both resting and activated lymphocytes [1]. This family includes two closely related cytokines, B lymphocyte stimulator (BLyS) and a proliferation-inducing ligand (APRIL), both of which are now recognized as central players in B cell development and homeostasis. Through differential interactions with several receptors, these two ligands profoundly influence multiple aspects of B cell biology. Their activities include mediating the selection, differentiation and homeostasis of primary B cells; influencing the differentiation of activated B cells; and controlling the generation and longevity of memory B cells. These broad and largely B lineage-specific activities, coupled with clear relevance to both autoimmunity and neoplasia, have focused intense scrutiny on BLyS, APRIL and their corresponding receptors. This concerted activity has already yielded considerable insight into fundamental aspects of B cell biology and has revealed several promising therapeutic targets, prompting extensive review and commentary [2–28].

Currently, the most extensively studied biological activities of BLyS family members are those associated with developing and primary B cells. This focus reflects the striking phenotypic impact that knockouts and transgenics for certain BLyS family members have on primary B cell pools, as well as the presumed relevance of these activities to tolerance and autoimmune disease. In contrast, the nature and mechanisms through which BLyS family members influence antigen-experienced B cell populations remain less extensively explored. Nonetheless, several mechanistic features drawn from studies to date are likely common to all of these interactions. Foremost, the notion of interclonal competition underpins our perception of how this family controls B cell survival and selection. This idea holds that pool sizes can be controlled by limiting the amount or availability of cytokine, such that when cytokine consumption equals availability, steady state pool size is achieved. Further, such competition implies that populations occupying independent homeostatic niches can coexist in the same physical space, so long as each population relies upon and competes for a different cytokine (e.g., APRIL versus BLyS). A second important feature of current understanding is that a B cell's ability to capture these signals is coupled to other cell-intrinsic signaling systems, including innate and adaptive immune receptors. Accordingly, signals via these exogenous sensing systems in aggregate determine a cell's relative fitness compared to others competing for occupation of the same niche. Finally, differen-

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tial receptor binding activities, in combination with changing expression levels influenced by differentiation or cross-talk with other surface receptors, specifies the cytokine-delineated niche within which each B cell competes.

While these general features of BLyS family activities have been revealed through studies of newly formed and primary B cells, only now are analogous properties being defined within activated and antigen-experienced B cell populations. Herein, we first overview the BLyS family members and key aspects of their actions on primary B cell populations. This is followed by a discussion of how activation-driven shifts in BLyS receptor expression are signature events that establish separate, intramurally competing cohorts during the initiation, progress and resolution of immune responses.

## 2. BLyS family of receptors and cytokines

Because of simultaneous initial reports, BLyS is also known as BAFF, TALL-1, zTNF4 and THANK [29–32]. Likewise, APRIL has several aliases: TRDL-1, TALL-2 and TNFSF13A [31,33]. Similar to other TNF members, BLyS and APRIL are type II transmembrane proteins that are proteolytically cleaved to generate active soluble forms. In fact, APRIL appears to be available only in soluble form because cleavage occurs in the Golgi apparatus [34], although alternative splice forms with different properties are now being described (this volume [35]). BLyS also has at least two splice isoforms, one of which can remain membrane bound and appears to inhibit the activity of soluble BLyS [36,37]. Although homotrimers are thought to be the predominant active forms, BLyS and APRIL can also form biologically active heterotrimers both in vitro and in vivo; however, a differential function for such composites remains to be identified [38].

BLyS is able to bind three receptors: transmembrane activator and cyclophilin ligand interactor (TACI), B cell maturation antigen (BCMA) and BAFF receptor 3 (BR3) [39–42]. Two of these receptors, TACI and BCMA, can also bind APRIL [40,43]. These receptors are all type III transmembrane proteins possessing extracellular cysteine-rich domains (CRDs) that mediate ligand binding. While TACI possesses two CRDs, BCMA and BR3 have only a single or a partial CRD, respectively [44,45]. This variation, along with key charge differences in binding site residues, allows widely differing affinities for the respective ligands. Thus, BR3 interacts solely and strongly with BLyS [46], as evidenced by affinity measurements as well as extensive biological findings. BCMA, on the other hand, has up to 1000 times greater affinity for APRIL than for BLyS, making APRIL the more physiologically relevant ligand for this receptor [47,48]. Between these two extremes, TACI interacts appreciably with both cytokines, albeit with a somewhat higher apparent affinity for APRIL [40,49]. Finally, sulfated proteoglycans have been shown to bind APRIL, and while some forms of signaling occur through this interaction, the physiological role of this relationship remains to be clarified [50]. These binding characteristics, coupled with the diverse receptor expression profiles in various B cell subsets, provide a mechanism for establishing non-overlapping niches of independent homeostatic control via BLyS–BR3 versus APRIL–BCMA interactions.

Enabling additional permutations of independent but overlapping control, the downstream mediators of each receptor are distinct but interrelated and involve intersecting pathways used by other key B lineage receptors. TACI interacts with TRAFs 2, 5 and 6 and signals through NF-AT and AP-1 [39,40,45,51]; while BCMA associates with TRAFs 1, 2 and 3 and activates Elk-1, JNK and p38 MAP kinase [45,52]. Both of these receptors also induce elements of the classical NF-κB pathway. In contrast, BR3 appears restricted to using TRAF 3 and preferentially activates the alternative NF-κB pathway [53–56]. Thus, in addition to the differential expression of receptors and the varying strength of each ligand–receptor pair, even the same cytokine binding to different receptors will yield alternative outcomes.

These properties offer multiple levels at which BLyS signaling can be controlled and refined. Certainly, receptor expression and ratio affect outcome, as might cross-talk between BLyS receptors themselves or with other cell surface molecules. Levels of the cytokines themselves may allow further manipulation of the system, however both APRIL and BLyS are expressed ubiquitously [43,57–61], making it unlikely that system-wide variations in cytokine levels differentially control local niche selection. Nonetheless, localized concentration differences in specialized anatomic sites may allow focal control of different populations. Inasmuch as both APRIL and BLyS are produced by inflammatory cells, such local gradients are an appealing possibility but are difficult to interrogate. In contrast, the expression of BLyS family receptors is clearly differentially regulated in terms of developmental stages and subsets, as well as following exogenous activation cues, making this a clear route of control.

## 3. BLyS receptor expression in naïve B cells and their progenitors

Current evidence suggests that BLyS family members play little role in early B lineage commitment and differentiation. Thus, B lineage subsets prior to the bone marrow immature stage (Hardy Fr E) show no BLyS binding activity [62], nor do they express detectable levels of any of the three receptors. In contrast, all B lineage subsets subsequent to successful light chain rearrangement and surface Ig expression can bind BLyS and express one or more of the BLyS family receptors.

Within the immature bone marrow pool, minimal but clear BLyS binding is observed within the $CD23^-$ fraction, whereas somewhat higher levels of BLyS binding are seen in the $CD23^+$ portion of this pool [62]. As these newly formed B cells exit the marrow and pass through the transitional (TR) pools in the spleen, BLyS binding capacity intensifies, reflecting increased levels of both BR3 and TACI [62]. Whether this results from the selection of cells with highest levels of receptor expression, or instead represents bona fide maturation-associated increases in receptor expression on a per cell basis, has not been rigorously interrogated. Regardless of the exact mechanism, mature B cells in the follicular (FO) and marginal zone (MZ) compartments display the greatest and most sharply defined BLyS binding capacities, reflecting uniformly high levels of both TACI

and BR3. Among these developing and mature primary B cell subsets, little if any surface BCMA expression is observed, suggesting BCMA is unlikely to be involved in the homeostatic control of these pools. This view is supported by the observation that no defects in the TR, FO or MZ pools are seen in mice lacking BCMA [62,63].

## 4. BLyS–BR3 interactions govern TR success and primary B cell lifespan

Despite clear TACI expression among TR, FO and MZ B cells, these subsets all appear normal or increased in both TACI and APRIL deficient mice [64–66], indicating that APRIL–TACI interactions are not critical to the generation or maintenance of primary B cells. Nevertheless, more subtle developmental roles for this ligand–receptor pair, such as repertoire selection or differentiation rate, remain possible. In contrast, the BLyS–BR3 axis is vital to the development and homeostatic regulation of virtually all naïve B cell subsets. Elimination of BLyS, either through gene deletion or administration of soluble receptor that sequesters ligand, results in marked reductions of both transitional and mature B cell numbers [42,67]. Similar results are seen with the elimination or mutation of BR3 [68,69]. The major mechanistic activity of BLyS–BR3 signaling appears to be survival: BLyS binding BR3 up-regulates pro-survival factors such as Mcl-1, A1 and Bcl-xL [70]. Without these signals, naïve B cells undergo apoptosis at an accelerated rate. Complementary studies demonstrated that augmentation of BLyS levels, from either exogenous supplementation or inclusion of a transgene, increased peripheral B cell numbers [29,71]. These studies established the BLyS–BR3 axis as an essential modulator of the size of naïve B cell pools. Thus, as B cells complete maturation in the periphery, they increase BR3 expression and become reliant on BLyS for survival [62,68,71–74].

Two sets of experiments yielded definitive evidence that interclonal competition for BLyS signaling through BR3 governs primary pool size by controlling TR differentiation and mature B cell lifespan. These involved mixed marrow chimeras and F1 mice between BR3-sufficient and BR3-insufficient donors or parents, respectively [68]; and showed that BR3-insufficient or haplosufficient B cells compete poorly in the presence of wild-type B cells.

This competition for BLyS–BR3 signaling at the TR stages critically impacts tolerogenic selection, and two studies have directly demonstrated that relative access to BLyS mediates the entrance of autoreactive B cells into the mature pools. Autoreactive B cells have a reduced lifespan when in competition with a diverse B cell repertoire [75], and are normally eliminated at either the immature BM or the TR developmental stages. Thus, when self-reactive cells that are usually eliminated at the TR stage were placed in an environment of reduced BLyS availability, they died at a greater rate than cells that were non-responsive to self [76]. Moreover, in an environment with excess BLyS, either through exogenous administration or through a constitutively active BLyS transgene, these cells were able to survive and compete effectively with non-self-reactive cells [76,77]. Thus, as the required minimum level of competitive fitness is lessened by increased BLyS levels, selective stringency at this checkpoint is relaxed and cells normally eliminated are allowed entrance to the mature naïve niche. The implications of a homeostatically adjustable checkpoint for autoimmunity and therapeutics have been discussed elsewhere [78,79]. Within the context of this review, these findings illustrate the three characteristic features of BLyS family activity: differential receptor expression defining a pool's primary regulatory cytokine (in this case BLyS); the establishment of steady state "set points" for pool size based on interclonal competition once consumption limits available cytokine; integration with antigen receptor systems to modulate relative competitive advantage, coloring the stringency of specificity-based selection. These features, well established within naïve B cell populations, appear to be reiterated in antigen-experienced pools—an observation which demands further attention.

## 5. BLyS receptor expression changes among antigen-experienced B cells

While the necessity for BLyS–BR3 signaling among naïve B cells is well-documented, the exact roles played by BLyS family members in activated cells continue to unfold. B lymphocytes express a variety of surface receptors that govern the likelihood of activation and induction of primary humoral responses. Ligation of the prototypical B cell surface protein, the B cell receptor (BCR), results in widely divergent outcomes based on the avidity and extent of BCR–ligand interaction per se, as well as the availability and timing of additional signals, including cognate T cell help, innate immune receptor ligation and the engagement of other cell-intrinsic regulatory or costimulatory systems.

T-dependent (TD) responses generally involve FO B cells and arise following BCR ligation and concomitant CD40–CD154 interaction [80]. These responses are characterized by the emergence of several functionally and phenotypically distinct B cell pools and structures [81]: relatively short-lived primary antibody forming cells (AFCs); germinal centers (GCs), where extensive isotype switching and affinity maturation occur; and long-lived memory B cells. In contrast T-independent (TI) responses do not involve cognate T help and fall into two groups: TI-1 responses that are induced by polyclonal activators such as Toll-like receptor (TLR) ligands; and TI-2 reactions that involve extensive BCR cross-linking, usually by large polysaccharide molecules [82–84]. TI responses typically involve marginal zone or B1 B cells and result in rapid AFC formation with limited isotype switching. Further, TI responses lack robust GCs, and exhibit neither affinity maturation nor the establishment of effective memory.

Based on these general features, it is clear that both TD and TI responses involve the formation of B cell populations whose kinetics, selective criteria and lifespans differ strikingly from those of primary B cells. This necessarily implies that each of these newly created populations have, through ongoing antigen-driven differentiation, entered niches that are under independent homeostatic control and prompts the question of what mechanisms underlie transit to these independent niches,

as well as what role BLyS family members play in these processes.

## 6. Distinct BLyS receptor signatures are displayed by GC and memory subsets generated during TD responses

TD immunization elicits both rapid plasma cell differentiation and antibody secretion, as well as the initiation of germinal centers within the splenic B cell follicles [85–90]. During the GC reaction, these rapidly dividing B cells undergo somatic hypermutation, generating novel BCR specificities. Those B cells expressing BCRs with a high affinity for antigen relative to the other cells in that GC are selectively preserved, while cells with mutations that either lower relative antigen affinity or yield self-reactivity perish [91–96], indicating competition within the circumscribed enviornment of each GC. While the molecular basis for targeted, high-frequency point mutation events is now being elucidated [97–99], the mechanisms underlying subsequent affinity-dependent competitive survival remain unclear.

Compelling evidence supports the notion that BLyS and its receptors play a role in these phenomena. Studies in the BR3 mutant A/WySnJ mouse revealed normal primary IgM responses for both TI and TD antigens, but poor secondary humoral responses and low serum antigen-specific IgG levels [100]. Moreover, poorly evolved, rudimentary GCs formed in these mice following immunization [74,101]. These data are consistent with other reports indicating compromised GC formation in BLyS knockout mice, and when BLyS signaling is impeded or neutralized [101,102]. A role for BLyS in the appropriate evolution of primary humoral responses also comes from findings that suggest both BLyS and APRIL influence isotype switching, either directly via mediators of switch recombination or indirectly by extending cell survival within expanding primary B cell clones [103,104]. We have recently examined BLyS receptor expression patterns on B cells following in vitro costimulation and on ex vivo GC B cells. Our results are in accordance with a role for BLyS–BR3 interactions in GC formation and evolution, since BR3 is maintained at levels similar to or higher than on resting FO B cells (Fig. 1), whereas TACI is down regulated and BCMA remains essentially unexpressed.

Based on these observations, as well as the parallels between selective processes operative during TR and GC differentiation, it is tempting to speculate that the GC comprises a second venue for BLyS–BR3 mediated competitive survival; where the ability to compete for limited BLyS is based on optimal BCR ligation. While attractive, this model nonetheless raises several conundrums. First, inasmuch as the competition fostering affinity maturation occurs within, rather than between, GCs [105–107], it requires a local mechanism for limiting or supplying BLyS. The GC may afford tethering, sequestration and presentation of BLyS by follicular dendritic cells, or these cells might themselves be rich sources of the cytokine. Second, while BCR stimulation can directly modulate BR3 levels [72], it seems unlikely that this mechanism mediates selection in GCs, since we have failed to observe GC cells expressing low levels of BR3 (J.E. Crowley, unpublished results). While this could reflect the rapid removal of apoptotic cells, the critical determinants may instead involve cross-talk between BR3-driven intracellular signals and those of the BCR and other exogenous receptors. For instance, potent negative regulators of BCR signaling, such as FcγRIIB coligation [108,109], likely play a role in the negative selection within a GC and thus may intersect BR3-driven survival pathways either directly or indirectly. Examination of BLyS production, utilization and downstream signaling systems among GC B cells should provide insight into these and other possibilities.

Regardless of the mechanisms through which they are initially selected, the ultimate products of the GC reaction – long-lived memory B cells – appear to have shifted markedly from the BLyS–BR3 dominated primary pool to a BCMA-dependent niche. The first evidence of this came from BCMA knockout mice that, while displaying no abnormalities in primary B cell numbers or immune responses, lacked long-lived bone marrow plasma cells [110]. In accord with these findings, antigen-specific memory B cells that emerge from the GC reaction during an ongoing TD response have clearly down-regulated BR3 and TACI, and up-regulated BCMA (Fig. 1). Accordingly, it is highly likely that long-lived memory B cells are freed from competition with primary pools since BCMA, while able to interact with BLyS, likely uses APRIL as its primary ligand. The effects of eliminating either APRIL or BLyS on pre-existing humoral memory pools will be required to definitively interrogate this possibility. Should this prove the case, however, the potential to selectively eliminate primary, but not memory B cell compartments or vice versa might afford attractive therapeutic strategies in a variety of clinical scenarios.

## 7. Short-lived AFCs and their immediate progenitors in both TD and TI responses display predominant TACI expression

In contrast to the kinetics of cells destined for memory compartments via GC mediated selection and differentiation, AFCs are formed early in TD responses and wane rapidly. Interestingly, these cells express a unique BLyS receptor signature that is opposite that seen in GC cells: TACI is markedly up-regulated whereas BR3 is down-regulated (Fig. 1). Interestingly, rapid expansion and differentiation into AFCs are hallmarks of TI responses, and a similar BLyS receptor signature is observed among these cells as well. Indeed, accumulating evidence links TACI with antibody formation and TI responses. TACI knockouts fail to produce normal IgG3 and IgA levels when stimulated with the TI-2 antigen NP-Ficoll [41,65], although their responses to both TI-1 and TD antigens appear normal. Moreover, APRIL knockout mice have reduced IgA switching [104] and, conversely, APRIL transgenics show enhanced TI-2 responses [111]. Since TACI is the only APRIL-binding BLyS family receptor expressed on primary B cells, these findings strongly suggest a role for TACI–APRIL interactions during TI activation. Consistent with this notion, humans with TACI mutations that preclude APRIL binding fail to generate antibodies to pneumococcal vaccine—a classic TI-2 polysaccharide antigen [112,113]. The role of TACI in TI-1 responses is less clear. For example, while responses to TNP–LPS are normal in TACI defi-

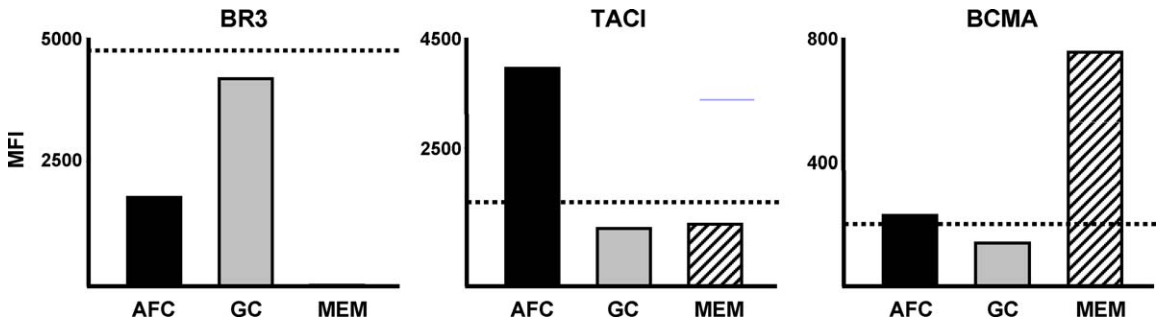


Fig. 1. BLyS receptor expression on antigen-responding B cell populations. Mouse splenocytes were analyzed by flow cytometry following NP-CGG immunization and the following NP-binding B cell populations were discerned: short-lived antibody forming cells (AFCs) (day 7 post-immunization), germinal center B cells (GCs) (day 7 post-immunization) or memory cells (MEM) (day 14 post-immunization) (based on the staining scheme of Driver et al. [81]). Mean fluorescence intensity (MFI) was calculated for surface expression of each BLyS receptor as the difference over isotype control staining for each cell type. Dotted line represents the MFI of each BLyS receptor on naïve follicular B cells.

cient mice [64,65], we have found that TACI is preferentially up-regulated following TLR-9 stimulation (L. Treml, unpublished results).

While the exact role of TACI in B cell biology remains somewhat enigmatic, the conspicuous association of a TACI-predominant signature with rapidly expanding, short-lived populations suggests a role in controlling the extent and quality of AFC differentiation and/or expansion. In this regard, Huang et al. have shown that BLyS stimulation allows splenic B cells arrested at the G0/G1 checkpoint to enter G1, although concomitant BCR signaling was required for progression to S phase [114]. This cell cycle effect appears to function independently from the survival effects of BLyS, as the increase in cyclin D2 and phosphorylated Rb occurred even in cells transgenic for the survival factor, Bcl-2. These studies demonstrate that BLyS receptors are involved in cell cycle control; however, because resting B lymphocytes express both TACI and BR3 [69], determining the exact BLyS receptors and B cell populations involved remain important questions.

## 8. Summary and perspective

Further analyses of the BLyS family should yield an understanding of the molecular mechanisms that afford independent homeostatic control of B naïve and antigen-experienced pools. These insights should in turn yield the ability to precisely manipulate or intervene in these homeostatic processes, suggesting novel diagnostic, prognostic and therapeutic opportunities in immune deficiency, autoimmunity, neoplasia and vaccine development.

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## References

[1] Locksley RM, Killeen N, Lenardo MJ. The TNF and TNF receptor superfamilies: integrating mammalian biology. Cell 2001;104:487–501.
[2] Ware CF. APRIL and BAFF connect autoimmunity and cancer. J Exp Med 2000;192:F35–8.
[3] Ambrose CM. Baff-R. J Biol Regul Homeost Agents 2002;16:211–3.
[4] Do RK, Chen-Kiang S. Mechanism of BLyS action in B cell immunity. Cytokine Growth Factor Rev 2002;13:19–25.
[5] Kalled SL. BAFF: a novel therapeutic target for autoimmunity. Curr Opin Investig Drugs 2002;3:1005–10.
[6] Mackay F, Browning JL. BAFF: a fundamental survival factor for B cells. Nat Rev Immunol 2002;2:465–75.
[7] Nardelli B, Moore PA, Li Y, Hilbert DM. B lymphocyte stimulator (BLyS): a therapeutic trichotomy for the treatment of B lymphocyte diseases. Leuk Lymphoma 2002;43:1367–73.
[8] Stohl W. B lymphocyte stimulator protein levels in systemic lupus erythematosus and other diseases. Curr Rheumatol Rep 2002;4:345–50.
[9] Cancro MP, Smith SH. Peripheral B cell selection and homeostasis. Immunol Res 2003;27:141–8.
[10] Carter RH. A role for BLyS in tissue inflammation? Arthritis Rheum 2003;48:882–5.
[11] Harless Smith S, Cancro MP. BLyS: the pivotal determinant of peripheral B cell selection and lifespan. Curr Pharm Des 2003;9:1833–47.
[12] Harless Smith S, Cancro MP. Integrating B cell homeostasis and selection with BLyS. Arch Immunol Ther Exp (Warsz) 2003;51:209–18.
[13] Mackay F, Ambrose C. The TNF family members BAFF and APRIL: the growing complexity. Cytokine Growth Factor Rev 2003;14:311–24.
[14] Medema JP, Planelles-Carazo L, Hardenberg G, Hahne M. The uncertain glory of APRIL. Cell Death Differ 2003;10:1121–5.
[15] Schneider P, Tschopp J. BAFF and the regulation of B cell survival. Immunol Lett 2003;88:57–62.
[16] Cancro MP. The BLyS family of ligands and receptors: an archetype for niche-specific homeostatic regulation. Immunol Rev 2004;202:237–49.
[17] Cancro MP. Peripheral B-cell maturation: the intersection of selection and homeostasis. Immunol Rev 2004;197:89–101.
[18] Mackay F, Tangye SG. The role of the BAFF/APRIL system in B cell homeostasis and lymphoid cancers. Curr Opin Pharmacol 2004;4:347–54.
[19] Stohl W. Targeting B lymphocyte stimulator in systemic lupus erythematosus and other autoimmune rheumatic disorders. Expert Opin Ther Targets 2004;8:177–89.
[20] Crowley JE, Treml LS, Stadanlick JE, Carpenter E, Cancro MP. Homeostatic niche specification among naive and activated B cells: a growing role for the BLyS family of receptors and ligands. Semin Immunol 2005;17:193–9.
[21] Jelinek DF, Darce JR. Human B lymphocyte malignancies: exploitation of BLyS and APRIL and their receptors. Curr Dir Autoimmun 2005;8:266–88.

[22] Kalled SL. The role of BAFF in immune function and implications for autoimmunity. Immunol Rev 2005;204:43–54.
[23] Mackay F, Sierro F, Grey ST, Gordon TP. The BAFF/APRIL system: an important player in systemic rheumatic diseases. Curr Dir Autoimmun 2005;8:243–65.
[24] Noelle RJ, Erickson LD. Determinations of B cell fate in immunity and autoimmunity. Curr Dir Autoimmun 2005;8:1–24.
[25] Salzer U, Grimbacher B. TACItly changing tunes: farewell to a yin and yang of BAFF receptor and TACI in humoral immunity? New genetic defects in common variable immunodeficiency. Curr Opin Allergy Clin Immunol 2005;5:496–503.
[26] Schneider P. The role of APRIL and BAFF in lymphocyte activation. Curr Opin Immunol 2005;17:282–9.
[27] Stohl W. BlySfulness does not equal blissfulness in systemic lupus erythematosus: a therapeutic role for BLyS antagonists. Curr Dir Autoimmun 2005;8:289–304.
[28] Szodoray P, Jonsson R. The BAFF/APRIL system in systemic autoimmune diseases with a special emphasis on Sjogren's syndrome. Scand J Immunol 2005;62:421–8.
[29] Moore PA, Belvedere O, Orr A, Pieri K, LaFleur DW, Feng P, et al. BLyS: member of the tumor necrosis factor family and B lymphocyte stimulator. Science 1999;285:260–3.
[30] Mukhopadhyay A, Ni J, Zhai Y, Yu GL, Aggarwal BB. Identification and characterization of a novel cytokine, THANK, a TNF homologue that activates apoptosis, nuclear factor-kappaB, and c-Jun NH2-terminal kinase. J Biol Chem 1999;274:15978–81.
[31] Shu HB, Hu WH, Johnson H. TALL-1 is a novel member of the TNF family that is down-regulated by mitogens. J Leukoc Biol 1999;65:680–3.
[32] Schneider P, MacKay F, Steiner V, Hofmann K, Bodmer JL, Holler N, et al. BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. J Exp Med 1999;189:1747–56.
[33] Kelly K, Manos E, Jensen G, Nadauld L, Jones DA. APRIL/TRDL-1, a tumor necrosis factor-like ligand, stimulates cell death. Cancer Res 2000;60:1021–7.
[34] Lopez-Fraga M, Fernandez R, Albar JP, Hahne M. Biologically active APRIL is secreted following intracellular processing in the Golgi apparatus by furin convertase. EMBO Rep 2001;2:945–51.
[35] Bossen C, Ingold K, Tardivel A, Bodmer JL, Gaide O, Hertig S, et al. Interactions of tumor necrosis factor (TNF) and TNF receptor family members in the mouse and human. J Biol Chem 2006;281:13964–71.
[36] Gavin AL, Duong B, Skog P, Ait-Azzouzene D, Greaves DR, Scott ML, et al. deltaBAFF, a splice isoform of BAFF, opposes full-length BAFF activity in vivo in transgenic mouse models. J Immunol 2005;175:319–28.
[37] Gavin AL, Ait-Azzouzene D, Ware CF, Nemazee D. DeltaBAFF, an alternate splice isoform that regulates receptor binding and biopresentation of the B cell survival cytokine, BAFF. J Biol Chem 2003;278:38220–8.
[38] Roschke V, Sosnovtseva S, Ward CD, Hong JS, Smith R, Albert V, et al. BLyS and APRIL form biologically active heterotrimers that are expressed in patients with systemic immune-based rheumatic diseases. J Immunol 2002;169:4314–21.
[39] Yan M, Marsters SA, Grewal IS, Wang H, Ashkenazi A, Dixit VM. Identification of a receptor for BLyS demonstrates a crucial role in humoral immunity. Nat Immunol 2000;1:37–41.
[40] Marsters SA, Yan M, Pitti RM, Haas PE, Dixit VM, Ashkenazi A. Interaction of the TNF homologues BLyS and APRIL with the TNF receptor homologues BCMA and TACI. Curr Biol 2000;10:785–8.
[41] Yan M, Brady JR, Chan B, Lee WP, Hsu B, Harless S, et al. Identification of a novel receptor for B lymphocyte stimulator that is mutated in a mouse strain with severe B cell deficiency. Curr Biol 2001;11:1547–52.
[42] Schiemann B, Gommerman JL, Vora K, Cachero TG, Shulga-Morskaya S, Dobles M, et al. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. Science 2001;293:2111–4.
[43] Hahne M, Kataoka T, Schroter M, Hofmann K, Irmler M, Bodmer JL, et al. APRIL, a new ligand of the tumor necrosis factor family, stimulates tumor cell growth. J Exp Med 1998;188:1185–90.
[44] Hymowitz SG, Patel DR, Wallweber HJ, Runyon S, Yan M, Yin J, et al. Structures of APRIL-receptor complexes: like BCMA. TACI employs only a single cysteine-rich domain for high affinity ligand binding. J Biol Chem 2005;280:7218–27.
[45] von Bulow GU, Bram RJ. NF-AT activation induced by a CAML-interacting member of the tumor necrosis factor receptor superfamily. Science 1997;278:138–41.
[46] Day ES, Cachero TG, Qian F, Sun Y, Wen D, Pelletier M, et al. Selectivity of BAFF/BLyS and APRIL for binding to the TNF family receptors BAFFR/BR3 and BCMA. Biochemistry 2005;44:1919–31.
[47] Patel DR, Wallweber HJ, Yin J, Shriver SK, Marsters SA, Gordon NC, et al. Engineering an APRIL-specific B cell maturation antigen. J Biol Chem 2004;279:16727–35.
[48] Pelletier M, Thompson JS, Qian F, Bixler SA, Gong D, Cachero T, et al. Comparison of soluble decoy IgG fusion proteins of BAFF-R and BCMA as antagonists for BAFF. J Biol Chem 2003;278:33127–33.
[49] Wu Y, Bressette D, Carrell JA, Kaufman T, Feng P, Taylor K, et al. Tumor necrosis factor (TNF) receptor superfamily member TACI is a high affinity receptor for TNF family members APRIL and BLyS. J Biol Chem 2000;275:35478–85.
[50] Ingold K, Zumsteg A, Tardivel A, Huard B, Steiner QG, Cachero TG, et al. Identification of proteoglycans as the APRIL-specific binding partners. J Exp Med 2005;201:1375–83.
[51] Xia XZ, Treanor J, Senaldi G, Khare SD, Boone T, Kelley M, et al. TACI is a TRAF-interacting receptor for TALL-1, a tumor necrosis factor family member involved in B cell regulation. J Exp Med 2000;192:137–43.
[52] Hatzoglou A, Roussel J, Bourgeade MF, Rogier E, Madry C, Inoue J, et al. TNF receptor family member BCMA (B cell maturation) associates with TNF receptor-associated factor (TRAF) 1, TRAF2, and TRAF3 and activates NF-kappa B, elk-1, c-Jun N-terminal kinase, and p38 mitogen-activated protein kinase. J Immunol 2000;165:1322–30.
[53] Claudio E, Brown K, Park S, Wang H, Siebenlist U. BAFF-induced NEMO-independent processing of NF-kappa B2 in maturing B cells. Nat Immunol 2002;3:958–65.
[54] Kayagaki N, Yan M, Seshasayee D, Wang H, Lee W, French DM, et al. BAFF/BLyS receptor 3 binds the B cell survival factor BAFF ligand through a discrete surface loop and promotes processing of NF-kappaB2. Immunity 2002;17:515–24.
[55] Gordon NC, Pan B, Hymowitz SG, Yin J, Kelley RF, Cochran AG, et al. BAFF/BLyS receptor 3 comprises a minimal TNF receptor-like module that encodes a highly focused ligand-binding site. Biochemistry 2003;42:5977–83.
[56] Hatada EN, Do RK, Orlofsky A, Liou HC, Prystowsky M, MacLennan IC, et al. NF-kappa B1 p50 is required for BLyS attenuation of apoptosis but dispensable for processing of NF-kappa B2 p100 to p52 in quiescent mature B cells. J Immunol 2003;171:761–8.
[57] Roth W, Wagenknecht B, Klumpp A, Naumann U, Hahne M, Tschopp J, et al. APRIL, a new member of the tumor necrosis factor family, modulates death ligand-induced apoptosis. Cell Death Differ 2001;8:403–10.
[58] Nardelli B, Belvedere O, Roschke V, Moore PA, Olsen HS, Migone TS, et al. Synthesis and release of B-lymphocyte stimulator from myeloid cells. Blood 2001;97:198–204.
[59] Scapini P, Nardelli B, Nadali G, Calzetti F, Pizzolo G, Montecucco C, et al. G-CSF-stimulated neutrophils are a prominent source of functional BLyS. J Exp Med 2003;197:297–302.
[60] Gorelik L, Gilbride K, Dobles M, Kalled SL, Zandman D, Scott ML. Normal B cell homeostasis requires B cell activation factor production by radiation-resistant cells. J Exp Med 2003;198:937–45.
[61] Craxton A, Magaletti D, Ryan EJ, Clark EA. Macrophage- and dendritic cell-dependent regulation of human B-cell proliferation requires the TNF family ligand BAFF. Blood 2003;101:4464–71.
[62] Hsu BL, Harless SM, Lindsley RC, Hilbert DM, Cancro MP. Cutting edge: BLyS enables survival of transitional and mature B cells through distinct mediators. J Immunol 2002;168:5993–6.
[63] Xu S, Lam KP. B-cell maturation protein, which binds the tumor necrosis factor family members BAFF and APRIL, is dispensable for humoral immune responses. Mol Cell Biol 2001;21:4067–74.

[64] Yan M, Wang H, Chan B, Roose-Girma M, Erickson S, Baker T, et al. Activation and accumulation of B cells in TACI-deficient mice. Nat Immunol 2001;2:638–43.
[65] von Bulow GU, van Deursen JM, Bram RJ. Regulation of the T-independent humoral response by TACI. Immunity 2001;14:573–82.
[66] Varfolomeev E, Kischkel F, Martin F, Seshasayee D, Wang H, Lawrence D, et al. APRIL-deficient mice have normal immune system development. Mol Cell Biol 2004;24:997–1006.
[67] Gross JA, Dillon SR, Mudri S, Johnston J, Littau A, Roque R, et al. TACI-Ig neutralizes molecules critical for B cell development and autoimmune disease. impaired B cell maturation in mice lacking BLyS. Immunity 2001;15:289–302.
[68] Harless SM, Lentz VM, Sah AP, Hsu BL, Clise-Dwyer K, Hilbert DM, et al. Competition for BLyS-mediated signaling through Bcmd/BR3 regulates peripheral B lymphocyte numbers. Curr Biol 2001;11:1986–9.
[69] Shulga-Morskaya S, Dobles M, Walsh ME, Ng LG, MacKay F, Rao SP, et al. B cell-activating factor belonging to the TNF family acts through separate receptors to support B cell survival and T cell-independent antibody formation. J Immunol 2004;173:2331–41.
[70] Do RK, Hatada E, Lee H, Tourigny MR, Hilbert D, Chen-Kiang S. Attenuation of apoptosis underlies B lymphocyte stimulator enhancement of humoral immune response. J Exp Med 2000;192:953–64.
[71] Khare SD, Sarosi I, Xia XZ, McCabe S, Miner K, Solovyev I, et al. Severe B cell hyperplasia and autoimmune disease in TALL-1 transgenic mice. Proc Natl Acad Sci USA 2000;97:3370–5.
[72] Smith SH, Cancro MP. Cutting edge: B cell receptor signals regulate BLyS receptor levels in mature B cells and their immediate progenitors. J Immunol 2003;170:5820–3.
[73] Gross JA, Johnston J, Mudri S, Enselman R, Dillon SR, Madden K, et al. TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. Nature 2000;404:995–9.
[74] Rahman ZS, Manser T. B cells expressing Bcl-2 and a signaling-impaired BAFF-specific receptor fail to mature and are deficient in the formation of lymphoid follicles and germinal centers. J Immunol 2004;173:6179–88.
[75] Cyster JG, Hartley SB, Goodnow CC. Competition for follicular niches excludes self-reactive cells from the recirculating B-cell repertoire. Nature 1994;371:389–95.
[76] Lesley R, Xu Y, Kalled SL, Hess DM, Schwab SR, Shu HB, et al. Reduced competitiveness of autoantigen-engaged B cells due to increased dependence on BAFF. Immunity 2004;20:441–53.
[77] Thien M, Phan TG, Gardam S, Amesbury M, Basten A, Mackay F, et al. Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches. Immunity 2004;20:785–98.
[78] Miller JP, Stadanlick JE, Cancro MP. Space, selection, and surveillance: setting boundaries with BLyS. J Immunol 2006;176:6405–10.
[79] Cancro MP. Tipping the scales of selection with BAFF. Immunity 2004;20:655–6.
[80] Grewal IS, Flavell RA. CD40 and CD154 in cell-mediated immunity. Annu Rev Immunol 1998;16:111–35.
[81] Driver DJ, McHeyzer-Williams LJ, Cool M, Stetson DB, McHeyzer-Williams MG. Development and maintenance of a B220-memory B cell compartment. J Immunol 2001;167:1393–405.
[82] Mond JJ, Mongini PK, Sieckmann D, Paul WE. Role of T lymphocytes in the response to TNP–AECM–Ficoll. J Immunol 1980;125:1066–70.
[83] Mond JJ, Lees A, Snapper CM. T cell-independent antigens type 2. Annu Rev Immunol 1995;13:655–92.
[84] Jeurissen A, Ceuppens JL, Bossuyt X. T lymphocyte dependence of the antibody response to 'T lymphocyte independent type 2' antigens. Immunology 2004;111:1–7.
[85] Tsiagbe VK, Linton PJ, Thorbecke GJ. The path of memory B-cell development. Immunol Rev 1992;126:113–41.
[86] MacLennan IC, Liu YJ, Johnson GD. Maturation and dispersal of B-cell clones during T cell-dependent antibody responses. Immunol Rev 1992;126:143–61.
[87] Kroese FG, Timens W, Nieuwenhuis P. Germinal center reaction and B lymphocytes: morphology and function. Curr Top Pathol 1990;84(Pt 1):103–48.
[88] Nieuwenhuis P, Opstelten D. Functional anatomy of germinal centers. Am J Anat 1984;170:421–35.
[89] MacLennan IC, Gray D. Antigen-driven selection of virgin and memory B cells. Immunol Rev 1986;91:61–85.
[90] Jacob J, Kelsoe G, Rajewsky K, Weiss U. Intraclonal generation of antibody mutants in germinal centres. Nature 1991;354:389–92.
[91] Berek C. The development of B cells and the B-cell repertoire in the microenvironment of the germinal center. Immunol Rev 1992;126:5–19.
[92] Berek C, Berger A, Apel M. Maturation of the immune response in germinal centers. Cell 1991;67:1121–9.
[93] Apel M, Berek C. Molecular analysis of germinal centre B cells. Res Immunol 1991;142:220–2.
[94] Nossal GJ. The molecular and cellular basis of affinity maturation in the antibody response. Cell 1992;68:1–2.
[95] MacLennan IC, Liu YJ, Oldfield S, Zhang J, Lane PJ. The evolution of B-cell clones. Curr Top Microbiol Immunol 1990;159:37–63.
[96] Gray D, Leanderson T. Expansion, selection and maintenance of memory B-cell clones. Curr Top Microbiol Immunol 1990;159:1–17.
[97] Muramatsu M, Kinoshita K, Fagarasan S, Yamada S, Shinkai Y, Honjo T. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. Cell 2000;102:553–63.
[98] Petersen-Mahrt SK, Harris RS, Neuberger MS. AID mutates *E. coli* suggesting a DNA deamination mechanism for antibody diversification. Nature 2002;418:99–103.
[99] Martin A, Bardwell PD, Woo CJ, Fan M, Shulman MJ, Scharff MD. Activation-induced cytidine deaminase turns on somatic hypermutation in hybridomas. Nature 2002;415:802–6.
[100] Miller DJ, Hanson KD, Carman JA, Hayes CE. A single autosomal gene defect severely limits IgG but not IgM responses in B lymphocyte-deficient A/WySnJ mice. Eur J Immunol 1992;22:373–9.
[101] Rahman ZS, Rao SP, Kalled SL, Manser T. Normal induction but attenuated progression of germinal center responses in BAFF and BAFF-R signaling-deficient mice. J Exp Med 2003;198:1157–69.
[102] Vora KA, Wang LC, Rao SP, Liu ZY, Majeau GR, Cutler AH, et al. Cutting edge: germinal centers formed in the absence of B cell-activating factor belonging to the TNF family exhibit impaired maturation and function. J Immunol 2003;171:547–51.
[103] Litinskiy MB, Nardelli B, Hilbert DM, He B, Schaffer A, Casali P, et al. DCs induce CD40-independent immunoglobulin class switching through BLyS and APRIL. Nat Immunol 2002;3:822–9.
[104] Castigli E, Scott S, Dedeoglu F, Bryce P, Jabara H, Bhan AK, et al. Impaired IgA class switching in APRIL-deficient mice. Proc Natl Acad Sci USA 2004;101:3903–8.
[105] Jacob J, Miller C, Kelsoe G. In situ studies of the antigen-driven somatic hypermutation of immunoglobulin genes. Immunol Cell Biol 1992;70(Pt 2):145–52.
[106] Jacob J, Kelsoe G. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. II. A common clonal origin for periarteriolar lymphoid sheath-associated foci and germinal centers. J Exp Med 1992;176:679–87.
[107] Jacob J, Kassir R, Kelsoe G. In situ studies of the primary immune response to (4-hydroxy-3-nitrophenyl)acetyl. I. The architecture and dynamics of responding cell populations. J Exp Med 1991;173:1165–75.
[108] Nimmerjahn F, Ravetch JV. Fcgamma receptors: old friends and new family members. Immunity 2006;24:19–28.
[109] Ravetch JV, Bolland S. IgG Fc receptors. Annu Rev Immunol 2001;19:275–90.
[110] O'Connor BP, Raman VS, Erickson LD, Cook WJ, Weaver LK, Ahonen C, et al. BCMA is essential for the survival of long-lived bone marrow plasma cells. J Exp Med 2004;199:91–8.
[111] Stein JV, Lopez-Fraga M, Elustondo FA, Carvalho-Pinto CE, Rodriguez D, Gomez-Caro R, et al. APRIL modulates B and T cell immunity. J Clin Invest 2002;109:1587–98.
[112] Salzer U, Chapel HM, Webster AD, Pan-Hammarstrom Q, Schmitt-Graeff A, Schlesier M, et al. Mutations in TNFRSF13B encoding TACI are asso-

ciated with common variable immunodeficiency in humans. Nat Genet 2005;37:820–8.

[113] Castigli E, Wilson SA, Garibyan L, Rachid R, Bonilla F, Schneider L, et al. TACI is mutant in common variable immunodeficiency and IgA deficiency. Nat Genet 2005;37:829–34.

[114] Huang X, Di Liberto M, Cunningham AF, Kang L, Cheng S, Ely S, et al. Homeostatic cell-cycle control by BLyS: induction of cell-cycle entry but not G1/S transition in opposition to p18INK4c and p27Kip1. Proc Natl Acad Sci USA 2004;101:17789–94.