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New roles for the BLyS/BAFF family in antigen-experienced B cell niches

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Abstract

BLyS family members govern selection and survival of cells in the preimmune B cell compartment, and emerging evidence suggests similar roles in antigen-experienced B cell pools. We review the features of this family, with particular emphasis on recent findings of how BLyS influences affinity maturation in germinal centers, which lie at the intersection of the pre-immune and antigen-experienced B cell compartments. We propose a model whereby tolerogenic selection at the transitional stage and affinity maturation in the germinal center employ the same BLyS driven mechanism.

Keywords

BLyS; BAFF; TACI; germinal center

1. Introduction to the BLyS family of cytokines and receptors

B cells are the effectors of humoral immunity. Quiescent, pre-immune B cells are generated throughout life from hematopoietic stem cells and, when activated by antigen exposure, expand and further differentiate into antibody-forming plasma cells (PCs) or memory B cells (Bmem) that mediate long-term immunity. Members of the B Lymphocyte Stimulator (BLyS, a.k.a. B cell activating factor of the TNF family, BAFF) family of ligands and receptors play unique, lineage-specific roles in B cell development, selection, persistence, and function. Much research and speculation to date has focused on how members of this family govern the size and composition of pre-immune B cell pools. However, more recent evidence reveals roles for this molecular family in dictating the differentiation, selection and persistence of activated and antibody secreting effector cells of the B lineage. Accordingly, we herein briefly overview the basic features of BLyS family members and their roles in

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pre-immune B cell selection and homeostasis. We then provide a more forward thinking and detailed consideration of recently appreciated influences on other B lineage subsets, with emphasis on the selective processes acting on germinal center (GC) B cells.

1.1 BLyS family ligands: BLyS (BAFF) and APRIL

The BLyS family is a subset of the tumor necrosis factor (TNF) superfamily, and includes two ligands (reviewed in detail in (1, 2)). A Proliferation Inducing Ligand (APRIL, TNFSF13a) was the first to be described, and is also termed TALL-2 (TNF- and ApoL-related Leukocyte- expressed Ligand 2) or TRDL-1 (TNF-related Death Ligand-1a). Subsequently, several laboratories simultaneously reported B lymphocyte stimulator (BLyS; TNFSF13b), which also appears in the literature as BAFF (B cell Activating Factor of the TNF superfamily), THANK (a TNF Homologue that activates Apoptosis, Nuclear factor-kappaB, and c-Jun NH2-terminal Kinase), TALL-1 (TNF- and ApoL-related Leukocyte-expressed Ligand 1) and zTNF4. While these two ligands share only ~25% identity with the conserved carboxy-terminal regions of other TNF family members, they share 33% amino acid and 48% DNA homology with each other. Moreover, amino acid sequence homologies for each cytokine between mammals ranges from 80%-97%.

Both APRIL and BLyS are type II transmembrane proteins that are cleaved into soluble forms by protein convertases ((3, 4); reviewed in (2)). Their active forms are composed of homotrimers and, while heterotrimers have been demonstrated, the biological relevance of such hybrid molecules is not yet understood. Nevertheless, heterotrimers are active *in vitro* and are elevated in the serum of some autoimmune patients (for example, (5)). BLyS can also assemble into 60mers, which exhibit distinct binding and signaling characteristics (6). Finally, membrane-bound forms of BLyS have been observed which might reflect incomplete cleavage of the membrane form or the expression of an alternative splice form known as delta BAFF, which lacks the stalk region and consequently cannot be cleaved (7). Unlike BLyS, APRIL is cleaved in the Golgi prior to secretion, precluding expression as a membrane-bound form (4). Nonetheless, it can bind heparan sulfated proteoglycans (HSPG) via its amino terminus, allowing oligomerization and presentation on cell surfaces (8).

BLyS and APRIL are produced by cells of non-hematopoietic as well as hematopoietic origin (reviewed in (9, 10)). Radioresistant stromal cells maintain systemic BLyS levels, with apparently minimal contribution from cells of hematopoietic origin (11). Similar assessments have not yet been made for APRIL. Tumor cell lines derived from non-hematopoietic tissues as well as astrocytes are enriched for APRIL production (12, 13), but the extent of the contribution of these sources to overall APRIL production has not yet been determined. Among hematopoietic cells, myeloid cells/cell lines such as monocytes, eosinophils, osteoclasts, and neutrophils produce both cytokines, albeit with a generally greater propensity to produce APRIL than BLyS (10). Macrophages and dendritic cells express membrane-bound BLyS, and expression levels can be augmented or depressed by cytokines such as IFNγ or IL-4, respectively (3, 14). Further, compared to macrophages and B-1 B cells, resting splenic B-2 cells in mice express neither BLyS nor APRIL message (15). However, TLR agonists or surrogate BCR cross linking *in vitro* may induce transcripts for both cytokines (15, 16). Similarly, quiescent T cells express no BLyS or APRIL,

although expression can be induced by TCR-driven activation in some circumstances (4, 15, 17). Curiously, in autoimmune-prone mice, depletion of CD4 T cells significantly reduces circulating levels of BLyS. Whether this is due to the absence of T cell-derived cytokines (such as IFNγ) that augment BLyS secretion by myeloid cells, or to a significant contribution of BLyS from excessive activated CD4 T cells themselves, is not yet known. Among activated CD4 T effectors, antigen-specific follicular helper T cells (T_{FH}) are enriched for BLyS mRNA expression and express BLyS protein in the germinal center (18), as discussed further in section 3 below.

1.2. BLyS family Receptors: BR3/BAFFR, TACI, and BCMA

BLyS and APRIL can interact with three receptors, BR3 (BLyS Receptor 3, also termed BAFFR), TACI (Transmembrane Activator and Calcium modulator and cyclophilin ligand Interactor) or BCMA (B Cell Maturation Antigen). These interactions are extensively reviewed elsewhere, for both mice and humans ((1, 2, 10, 19), and briefly addressed here. These receptors possess characteristic canonical cysteine rich domains (CRDs) that are comprised of 6 cysteine residues, and transduce TNF Receptor Associated Factor (TRAF)-mediated signals. However, unlike other TNF receptors that express 3-6 CRDs, BR3 has only a partial CRD, BCMA has one CRD, and TACI has two. These atypical structures confer exquisite specificity for BLyS and APRIL, but not for other TNF ligands (20, 21). As noted above, APRIL can passively bind to proteoglycans, though whether such interaction induces downstream signaling events is not yet known (8, 22). Finally, TWE-PRIL, a fusion protein between the intracellular, transmembrane and stalk region of TWEAK (TNF Weak inducer of apoptosis) coupled to the extracellular receptor-binding part of APRIL, recognizes BCMA and TACI (19).

BLyS binds with much higher affinity to BR3 than to BCMA, whereas APRIL has a greater affinity for BCMA and little or no binding capacity for BR3 (23). Moreover, BLyS has a higher affinity for TACI compared to BCMA, and the converse is true for APRIL (21). Nonetheless, BLyS binds to BR3- or TACI-transfected cells with similar strength, and TACI has ~ 25 fold higher affinity for BLyS than for APRIL (24, 25). Therefore, it is conceivable that under steady state conditions, BLyS is largely bound to TACI. Indeed, reagents that detect pre-bound BLyS on circulating? B cells in mice have revealed that TACI is the key receptor involved in binding of BLyS to mature naïve B cells (18). Consistent with the inefficient binding of BLyS to TACI-deficient B cells, elevated levels of circulating BLyS are observed in TACI knockouts (data not shown). Additionally, BLyS 60mers bind to TACI with much higher affinity than BLyS trimers, and thus are readily detectable in the circulation of TACI-deficient mice (26).

1.3. BLyS family members govern B lineage homeostasis and selection

Homeostasis in the various functional B cell compartments is achieved by regulating generation rates, selection thresholds, and cellular lifespan. Steadily accumulating evidence over the last decade has revealed that BLyS family members play critical and non-redundant roles in all of these processes. A key feature of the BLyS family is that it includes multiple receptors and ligands with different binding preferences. We and others have posited that differential expression of the three BLyS family receptors affords coexisting, yet distinct and

independently regulated, homeostatic niches for mature naïve, antibody-secreting, and memory B cell subsets (27). Consistent with this idea, the various pre-immune and antigen-experienced B cell subsets express different combinations of BLyS receptors at varying levels (reviewed in (28, 29)), and display differential reliance on the two cytokines (summarized in Table 1). The following sections expand on this theme, and review evidence supporting this idea for resting, activated, and antibody secreting B cell populations. Moreover, we propose a model based on recent findings that suggests transitional (TR) and germinal center (GC) B cell selection proceed via the same, BLyS-mediated, mechanism.

2. The BLyS-BR3 axis governs pre-immune B-2 cell selection and homeostasis

B cells are broadly divided broadly into two categories, B-2 and B-1 B cells. Cells of the B-2 lineage are continuously replenished from bone marrow (BM) precursors, exhibit extensive B cell antigen receptor (BCR) diversity, and comprise the bulk of recirculating B cells in the blood and associated secondary lymphoid organs. Following lineage specification from lymphoid progenitors, developing B-2 cells undergo RAG-mediated rearrangement of their immunoglobulin heavy and light chain genes during the pro- and pre-B cell stages, respectively, culminating in the expression of a functional B cell antigen receptor (BCR). The immature B-2 cells thus formed undergo negative and positive selection based on BCR specificity, and then exit the BM as transitional (TR) B cells. After further BCR-mediated selection, cells that successfully complete TR differentiation enter the mature, quiescent B-2 pools as either follicular (FO) or marginal zone (MZ) B cells. Negative selection during immature and TR differentiation purge many autoreactive BCR specificities, eliminating ~95% of all incipient B-2 cells. In contrast to these B-2 characteristics, cells of the B-1 lineage are derived largely from progenitors in the fetal liver and neonatal spleen, display a restricted BCR repertoire, and are generally confined to particular anatomic locales, including the peritoneal cavity and some mucosal sites. Emerging evidence that BLyS family members are involved in B-1 lineage homeostasis has recently been reviewed elsewhere (30), so we focus herein on roles for the BLyS family in the B-2 lineage.

2.1 BLyS receptor expression commences at the TR developmental stages

Within the B2 lineage, neither pro- nor pre-B cells express BLyS family receptors. The CD23+ subset of immature BM B cells express BR3 and TACI transcripts, but show minimal surface protein. As immature B cells emigrate from the BM and continue maturation in the periphery as TR B cells, surface expression of both BR3 and TACI increases (28). Early TR B cells (T1 subset) express surface BR3 but little TACI, while later TR subsets (T2, T3) maintain surface BR3 and up regulate TACI message and surface protein (29, 31, 32). These TR B cells in turn give rise to mature naïve B cells of the FO or MZ subsets, which express the highest levels of surface BR3 and TACI (33). Thus, as B cells progress through peripheral maturation stages, both BR3 and TACI are up regulated on the cell surface, and are stably maintained on mature quiescent B cells. BCMA transcript and surface protein are low to undetectable on all pre-immune human and murine B cell subsets (32, 34).

2.2 BLyS signaling through BR3 governs transitional differentiation and mature B cell survival

BLyS signaling via BR3 is indispensable for successful TR differentiation and for mature pre-immune B cell survival. Genetic deficiency in either BLyS or BR3 stalls B cell development at the late TR stages (reviewed in (1, 35)), and yields profound reductions in FO and MZ B cells. Similar effects are observed after treatment with BLyS neutralizing antibody or soluble decoy receptors (20, 21, 36, 37). Conversely, administration of exogenous BLyS or over-expression of BLyS in transgenic mice significantly increases FO and MZ B cell numbers (38, 39). In contrast, TACI or BCMA knockouts, as well as APRIL-deficient mice, have no defects in pre-immune B-2 B cell maturation (40-42).

BLyS also regulates an elastic threshold for BCR-mediated selection during TR development. B cells with relatively high BCR signal strengths are normally eliminated through negative selection at this TR checkpoint. However, elevated BLyS levels relax selective stringency, broadening the range of acceptable BCR signal strength and allowing more TR cells to survive, effectively increasing the production rate of mature FO and MZ B cells ((43); reviewed in (44, 45)). Likewise, reduced BLyS levels increase the stringency of TR selection, allowing fewer cells through than normal. Thus, BLyS levels dictate homeostatic “space” for pre-immune B-2 cells by governing both the proportion and quality of TR B cells that complete differentiation, as well as the lifespan of mature B cells themselves, thereby controlling the overall size and composition of FO and MZ B cell populations.

These observations also suggest that BCR and BR3 signals are integrated and non-redundant, since the absence of either signaling arm severely curtails peripheral B cell maturation and maintenance (reviewed in (46, 47)). The molecular mechanisms of interplay between BCR and BR3 signaling pathways appear complex (32, 48, 49), but likely involve cross-talk between downstream signaling and transcriptional regulatory systems including non-classical NF-κB, PI3-Kinase, and Syk (32, 49-52). More recent work indicates that the BCR/Igα function as adapter proteins in the BR3 signaling pathway, instead of delivering an independent survival signal (48). Regardless of the exact molecular mechanisms involved, the ability of BLyS to serve as a limited, and therefore competitive, resource in specificity-driven selection of the developing pre-immune B2 repertoire raises the question of whether it plays a similar role in other competitive selection events of this lineage, such as affinity maturation during the GC reaction (see below).

3. BLyS family members play multiple roles among antigen-experienced B cells

Upon antigen challenge and BCR cross-linking, mature pre-immune B cells may differentiate directly into plasma cells. Alternatively, they may become germinal center (GC) B cells that will alter their BCRs through somatic hypermutation (SHM) and undergo further specificity-based selection, subsequently giving rise to either Bmem or long-lived plasma cells (LLPC) (reviewed in (53, 54)). The propensity for adopting these alternate fates depends on the so-called second signals received following BCR ligation, that direct the

ensuing expansion and differentiation. If the second signals do not include cognate CD4 T cell help (T cell independent or TI responses), then the predominant fate is direct differentiation to relatively short-lived plasma cells (SLPCs). In contrast, activated B cells that receive cognate CD4 T cell help (T cell dependent or TD responses) are directed to adopt GC B cell characteristics, undergo affinity maturation, and subsequently give rise to long lived subsets including LLPCs and Bmem cells.

Marked alterations in BLyS receptor expression occur after all types of antigen encounter and activation, but the exact expression patterns varying, depending on the fate adopted (summarized in Table 1). In the following sections, we first consider the changes observed in BLyS family receptor expression and ligand dependence as cells enter antibody secreting PC pools, then focus on the recently revealed role for BLyS family members in affinity maturation during the GC reaction.

3.1 TACI and BCMA may distinguish alternative routes to antibody-secreting effectors

Accumulating evidence indicates that local sources of APRIL are key in the establishment and maintenance of antibody-secreting B cells. Accordingly, these interactions appear to be mediated primarily through the ARIL-binding receptor partners, TACI, BCMA, and HSPGs. However, the distribution of these receptors differs among PC subsets and, while not absolute, these may reflect distinct receptor expression patterns among SLPC versus LLPC.

In general, TI antigens circumvent the need for cognate T help either by engaging innate receptors such as Toll-like receptors (TLRs) or through extensive BCR ligation. Such antigens trigger B cell proliferation and accumulation at extrafollicular sites within 4-5 days (55), and largely result in SLPCs that persist for days to weeks (56). TLR stimulation up regulates TACI on both FO and MZ B cells, and the SLPC generated during TI responses up-regulate TACI (16). Further, both the overall TI responses as well as class switching are blunted in TACI-deficient mice (41, 57, 58). Similarly, APRIL-deficient mice are also impaired in their ability to induce TI responses and in class switching to IgA (42, 56). Moreover, HSPG assist APRIL binding to TACI, thereby promoting IgA production (59). Extrafollicular PCs reside next to macrophages that are rich sources of APRIL (60). Together, these observations indicate an important role for APRIL interactions with TACI, and possible tripartite interactions between APRIL, TACI and HSPGs, in establishing or maintaining cells in the SLPC niche.

In contrast to the SLPC generated in TI responses, LLPC are mainly products of GC reactions (reviewed in (53)). However, our understanding of events that occur between GC resolution and LLPC establishment is limited. Nevertheless, BCMA clearly mediates survival of LLPC in mouse bone marrow, with both APRIL and BLyS likely required for establishment and maintenance of the LLPC niche (reviewed in (46, 61)). BCMA is the only BLyS family receptor expressed on most murine BM LLPC, though a small subset express TACI (Quinn et al., submitted). HSPG are expressed on plasma cells and indeed may assist APRIL binding to BCMA in the bone marrow, thereby promoting PC retention (8, 22, 62). Moreover, many BM resident and recirculating cell types express HSPG and/or produce APRIL, including osteoclasts, RANKL-stimulated macrophages and eosinophils (Quinn et al., submitted; (63-65). Taken together, these observations suggest that APRIL elaborated

and sequestered by BM stromal cells creates a survival niche for BCMA-expressing BM plasma cells (61, 63, 66).

3.2 Memory B cells are largely BLyS independent

Memory B cells are also thought to arise mainly or solely from GC reactions, and although they do not secrete antibody, are mentioned here because of evidence from mouse models that establishment and maintenance of the memory B cell niche are independent of both BLyS and APRIL (37, 67). For example, in studies where the memory pool was allowed to establish for 6-8 weeks before ablating systemic BLyS, there was no impact on either memory B cell numbers or the ability to respond to secondary challenge. Further subsetting however revealed a small but consistent impact o on non-switched memory, suggesting some Bmem subsets may be BLyS sensitive (37). Whether these distinctions between switched and unswitched Bmem reflects differential BLyS receptor expression on these subsets of cells is not known.

3.3 Systemic and locally produced BLyS play distinct roles in GC maintenance and selection

Germinal centers are transient structures that form at the T-B interface of lymphoid follicles in the spleen and lymph nodes following antigen encounter. Somatic hypermutation in GC B cells results in activated B cell clones with novel BCR specificities that subsequently undergo both positive selection for higher affinity antigen-specific clones, as well as negative selection against incipient autoreactive somatic variants (reviewed in (53, 54)). The mechanisms mediating this specificity-driven selection remain unclear, but likely involve a combination of cellular interactions and competitive processes that, in concert, favor the survival of appropriate clonotypes. Substantial evidence indicates this process relies on interactions with accessory cells such as T follicular helper (T_{FH}) cells (reviewed in (68)) and follicular dendritic cells (FDCs). T_{FH}, a subset of activated helper T cells that are critical for GC responses, up regulate the chemokine receptor CXCR5 and migrate into the light zone of the germinal center (69). The requisite for cognate help in optimal evolution of the GC response as well as for normal GC selection has long been appreciated (68). Follicular dendritic cells (FDCs) display immune complexes on their surface and are thought to be a source of antigen for GCB cells; however, the role of immune complex presentation on FDCs is still controversial, since mice lacking the ability to secrete antibody show minimal impact on affinity maturation (70).

Several lines of evidence suggest roles for BLyS and BR3 in the GC reaction. First, mice that are BLyS-deficient or lack BR3 signaling capacity can form GCs, but are unable to maintain them (71, 72). Further, neutralization of BLyS alone or both BLyS and APRIL impairs GC maturation and function (72, 73). In contrast, APRIL-deficient mice have normal GC responses (42). Although these studies point to a role for BLyS-mediated signals in sustaining GCs, the B lymphopenia accompanying global systemic BLyS or BR3 deficiency thwarts straightforward interpretation. For example, GCs are constantly fed by naïve B cells (74), and antigen-presenting B cells are critical for complete T_{FH} differentiation (75-78). Further, BLyS knockout mice also exhibit impaired FDC network maturation (72). These considerations raise the possibility that BLyS plays only an indirect

role in the GC reaction, by maintaining sufficient naive B cell numbers for replenishment, antigen presentation, and organogenesis. Alternatively, BLyS might be directly involved in GC B cell selection, since BCR occupancy – and presumably signal strength - is linked to a survival advantage, analogous to TR selection. However, this raises the conundrum of how cells within the GC could compete for BLyS independently from naïve cells in the physically proximal and much larger FO and MZ pools -- unless the GC provides a unique local microenvironment that enables this.

Recent findings from our laboratory (18) have now separated the roles played by systemic BLyS versus locally produced BLyS in GC maintenance versus selection respectively. These studies *in toto* show that: (1) GC B cells downregulate TACI, resulting in their inability to sequester BLyS; (2) this in turn creates a BLyS-poor microenvironment within GCs; (3) T_{FH} are a major source of BLyS within the GC; and (4) T_{FH}-derived BLyS is required to promote the survival of high-affinity GC B cells. An intriguing aspect of these findings was that GCs are largely devoid of BLyS, despite a lack of physical barriers separating them from neighboring follicles where ample systemic BLyS is available. Moreover, this sequestration of systemic BLyS into the follicles and out of GCs correlated with complete loss of TACI on GC B cells; and TACI down regulation is driven by IL-21 in the context of BCR and CD40 signals (18).

This somewhat surprising set of observations has several implications. First, it indicates that even though naïve B cells rely on BR3 and not TACI for their BLyS-mediated survival signals, the TACI receptor is nonetheless critical for retention of systemic BLyS on FO and MZ B cell surfaces. Second, it suggests TACI down-regulation is a distinguishing feature of GC B cells, which may prove useful in identifying cells that have participated in GC reactions. Third, it implies that within the GC, BLyS availability is highly limited. Finally, since GC B cells maintain surface BR3 (18), this is the sole receptor through which BLyS can act.

Thus, the GC provides a unique microenvironment that is largely separated from any influence of systemic BLyS, and that contains activated B cells with a unique, BR3 only, receptor profile. A key observation suggesting how this might be leveraged to influence GC selection was revealed in histological and gene expression analyses: at the peak of the GC response, T_{FH} cells are a local source of BLyS within the otherwise BLyS-poor GC. Moreover, when T cells are BLyS-deficient but systemic BLyS and B cell numbers are normal, GC size, structure and kinetics, as well as total serum antibody levels, are unimpaired, yet high-affinity antibody production is compromised. Together, these results indicate that while T_{FH}-derived BLyS is dispensable for GC initiation and maintenance, it is critical to the persistence of high-affinity clones and efficient affinity maturation.

4. A unified model for TR and GC B cell selection

Because selection within the GC is based on BCR specificity, it has been called a “second window” of tolerogenic selection (79, 80) and termed “neoteny (81),” thus suggesting similarity to BCR-mediated selection during the TR stages of pre-immune B cell development. A long-standing caveat in this analogy has been the question of why GC B

cells with increased affinity for the immunizing antigen – and therefore highest BCR occupation and signal strength – would undergo positive selection, contrasting TR selection where the strongest BCR signals yield death. This line of reasoning is further complicated by the simultaneous negative selection of GC B cells that have acquired self reactivity, inasmuch as these should also be experiencing high BCR occupation by the self antigen.

We suggest that the previously unappreciated sequestration of systemic BLyS out of GCs, combined with local production by T_{FH}, reconciles these caveats; and we propose a unified model whereby TR and GC selection employ the same mechanism. This model is schematized in Figure 1. At the TR checkpoint (Fig. 1 left panel), selection based on BCR signal strength is modulated by *systemic* BLyS levels (purple trimers), such that cells with sub-threshold BCR signaling are better competitors for BLyS and survive; whereas cells with the highest BCR signal strength are negatively selected (large X) except when BLyS levels are abnormally high. Because systemic BLyS is uniformly available to all emerging TR B cells, an upper BCR signaling threshold for negative selection is thereby evenly applied across the population. A central feature of this model is that within the GC (Fig. 1 right panel), cells with strong BCR signal strength are, in fact, similarly disadvantaged, and must rely on BLyS-mediated rescue to survive. However, because systemic BLyS is meager within the GC and GC B cells are unable to retain surface BLyS, only cells engaged in sustained contact with T_{FH} will acquire this locally produced BLyS. Accordingly, cells that acquire self reactivity will die because they no longer capture the immunizing antigen and are unable to present to and engage in sustained interactions with T_{FH}. In contrast, cells that have mutated to higher affinity for the immunizing antigen will capture and present antigen efficiently, and can thus be rescued by T_{FH}-produced BLyS, despite their high BCR signal strength. This model for GC selection is consistent with a requirement for competitive B cell antigen capture from FDC as a key event in the process, as well as with reports that T_{FH} interactions are crucial for GC positive selection. Furthermore, it predicts that, as with TR selection, failures in BLyS family components – such as BLyS overproduction by T_{FH}, inappropriate BR3 upregulation or TACI down regulation -- could thwart GC selection, resulting in poor protective humoral responses or autoimmunity.

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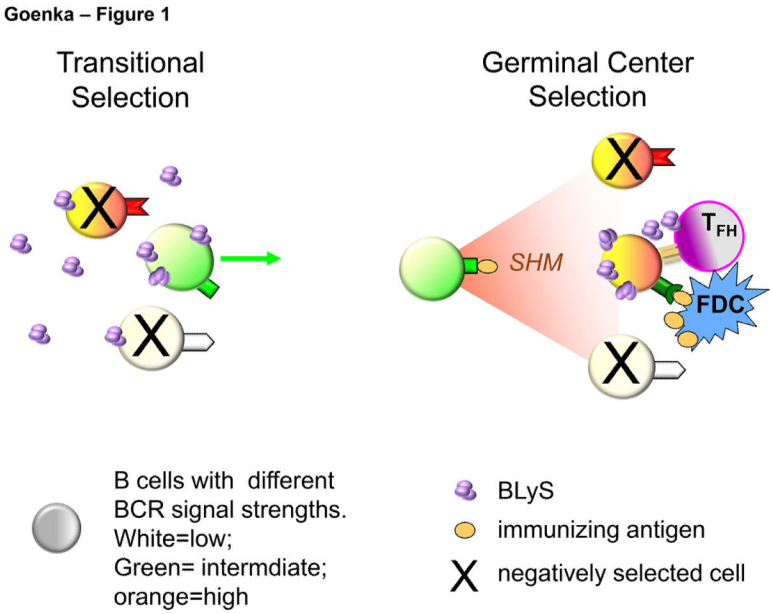

Figure 1.

Systemic BLyS levels afford survival of only B cells with sub-threshold BCR signals at the transitional checkpoint (left), while T_{FH}-produced BLyS selectively spares antigen-presenting GC B cells with very strong BCR signals (right).

Table 1

BLyS family receptor expression patterns and cytokine dependence for developing, pre-immune, and antigen-experienced B cell pools

	Differentiation Stage	Receptor Expression[1]			Cytokine Dependence[2]	
		BR3	TACI	BCMA	BLyS	APRIL
Developing and Pre-immune pools	Bone marrow Pro-B and Pre-B	−	−	−	N	N
	Bone marrow immature	+	±	−	Y (?)	N
	Transitional	+	+	−	Y	N
	Follicular	+	+	−	Y	N
	Marginal zone	+	++	−	Y	N
	Germinal center	+	↓	−	Y	N
Antigen-experienced pools	Short-lived plasma cell	↓	↑	−	N (?)	Y
	Long-lived plasma cell	↓	↓	↑	Y	Y
	Memory B cell	↓	+	↑	N (?)	N (?)

[1] No expression (−), relative expression level (± or +), downregulation (↓), or upregulation (↑) compared to pre-immune pools

[2] Y = yes, N = no evidence/not known