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BAFF and the plasticity of peripheral B cell tolerance

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Abstract

BAFF and its receptors play a crucial role in peripheral B cell selection and survival, by dictating the set point for mature primary B cell numbers and adjusting thresholds for specificity-based selection during transitional differentiation. The notion that selective stringency can be varied on the basis of homeostatic demands reveals a previously unappreciated degree of plasticity in B cell tolerance, and suggests a paradigm that unites peripheral negative and positive selection with the maintenance of mature B cell numbers. Moreover, it implies a developmentally regulated coupling of BCR and BAFF receptors at the transitional stages and beyond.

Introduction

Within the confines of clonal selection, acquired tolerance is accommodated by purging autoreactive specificities at key checkpoints during lymphocyte differentiation. Assessments of B cell dynamics are consistent with this idea, inasmuch as only ~5% of newly formed B cells survive to enter mature primary pools. Although BCR signal strength was long assumed the sole factor determining cell death versus survival at these checkpoints; recent evidence reveals a more complex mechanism that integrates tolerogenic elimination, positive selection, and the maintenance of mature B cell numbers. This unifying conceptual advance derives from observations showing that in the penultimate stages of B cell maturation, BCR signaling mediates not only tolerogenic elimination but also survival on the basis of relative fitness to compete for BAFF (BLyS). Herein, we summarize seminal observations leading to these ideas, emphasizing recent literature linking BAFF with B cell selection and homeostasis.

B cells undergo both negative and positive selection as they mature in the periphery

Several observations provided impetus for revisiting the accepted notions of B cell tolerance mechanisms. Key among these was the discovery of a peripheral developmental intermediate, coupled with evidence that BCR-mediated negative and positive selection occur at this stage.

The peripheral differentiative intermediate for primary B cells, now termed the transitional (TR) B cell, was originally characterized by high levels of Heat Stable Antigen (CD24) and has subsequently been refined with additional markers. Several findings emerged from these studies [1,2]. First, these cells resembled neonatal B cells in terms of responses to BCR cross-linking and other signaling characteristics. Second, they displayed rapid turnover, indicating that residency in the TR compartment is short—on the order of days. Last, comparisons of TR

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versus mature B cell production rates indicated that two thirds of TR cells formed die before maturation, suggesting this stage may undergo negative or positive selection.

Historically, deletional versus anergic B cell tolerance mechanisms were considered disparate, yielding rapid cell death in the bone marrow versus a refractory but persistent clone in the periphery. These notions, originally proposed by Nossal and Pike [3,4], were apparently supported by *in vivo* studies in the HEL/anti-HEL transgenic model, where high avidity BCR interactions led to the elimination of HEL-reactive cells in the bone marrow, while low avidity interactions indeed yielded self-reactive cells in the periphery [5]. However, following description of the TR compartment, Fulcher and Basten demonstrated that these ‘anergic’ B cells achieved entrance to the TR pools, but nonetheless died rapidly before entering mature pools [6]. This relationship holds in humans, as demonstrated by studies showing the loss of autoreactive and polyreactive clonotypes at the TR stages [7•], and apparent failure of this checkpoint in SLE patients [8].

Concomitant with these emerging notions of peripheral maturation and tolerance, mounting evidence indicated a positive role for BCR signaling in the development and maintenance of peripheral B cells. The notion that BCR signals are required for peripheral B cell differentiation or survival was first suggested by the studies of Gu *et al.* [9], which revealed clear differences in V_H distribution among immature versus mature bone marrow B cells. Because these findings preceded definition of TR pools, the developmental stage where such bias was imposed remained unclear. Subsequent studies, using analogous assessments of light chain V gene usage, showed that this bias was introduced between the TR and mature peripheral stages [10]. Together, these findings implied that BCR signal strength could specify any of three alternative fates at the TR stage: differentiative failure, continued survival or tolerogenic elimination. This tripartite distribution of BCR-mediated effects was directly demonstrated by Wang and Clarke, using a transgenic BCR model coupled with differing doses of anti-clonotypic antibody [11]. Finally, subthreshold BCR signals are not only essential for continued differentiation at the TR stage, but are also required for survival in the mature peripheral pools. This was definitively shown by studies where induced ablation of either the BCR itself or BCR signaling complex molecules led to the rapid death of most mature B cells [12,13].

Considered together, these findings carried the overarching implication that a developmental switch transpires during the TR stages, whereby BCR signaling no longer only mediates elimination, but is also required for continued survival.

Peripheral B cells compete for survival—An equally significant contribution to evolving thought about selection in the peripheral B cell compartments has been the notion of competitive survival. Most studies demonstrating such competition relied on mixed bone marrow chimeras. The earliest such studies showed that developing B cells from *xid* mice could persist in the periphery but failed to do so when competing with wild-type B lineage progenitors [14]. More recent studies from the Freitas laboratory indicated that intraclonal competition, in terms of BCR specificity, determined relative competitive advantage among peripheral B cells [15,16]. Thus, comparisons of turnover rates in mixtures of BCR Tg and non-Tg cells revealed that a mature B cell’s lifespan is not absolute, but instead depends on the clonotypic identities of co-existing competitors. Cyster *et al.* showed similar specificity-based competition as cells navigate TR stages [17]. In these studies, B cells bearing an autoreactive BCR transgene persisted in the absence of competition, but were excluded from splenic follicles and died rapidly when competing with B cells of normal receptor diversity.

These findings substantially impacted perceptions of how BCR signals mediate peripheral B cell survival in several ways. First, they showed that B cell survival capacity, and hence

lifespan, is neither fixed nor absolute but variable and relative. Second, they implied that the survival-promoting effects of BCR signals are not cell intrinsic, but are instead coupled to the acquisition of limiting, cell-extrinsic resources.

BAFF/BAFFr signaling integrates primary B cell selection and homeostasis—

The keystone for assembling a conceptual paradigm that could accommodate peripheral selection, survival and competition was provided by the discoveries of BAFF and BAFFr. Initial descriptions of BAFF revealed profound positive effects on mature B cells both *in vivo* and *in vitro* [18,19]. The subsequent discovery of BAFFr, a B-lineage restricted receptor whose sole natural ligand is BAFF, showed that BAFF–BAFFr interactions were crucial to maintaining normal peripheral B cell numbers [20,21].

Striking parallels with the emerging concepts of B cell maturation, survival, and competition in the periphery accumulated rapidly. First, in the absence of BAFF or BAFFr, differentiation failed at the early TR stages, suggesting this is the developmental point where reliance on BAFF is imposed [20–22]. Consistent with this, BAFFr expression begins at the TR stages and increases steadily as cells mature and join the follicular or marginal zone pools [23]. Second, BAFF signaling via BAFFr proved the key resource for which mature peripheral B cells compete, since B cells from BAFFr mutants failed to thrive and were diluted rapidly when competing with BAFFr-sufficient B cells [24]. Last, BAFFr signaling competence dictated mature B cell lifespan, since intermediate turnover rates were observed among B cells heterozygous for a non-functional BAFFr mutation [24]. Enhanced survival has proven the major downstream consequence of BAFF–BAFFr signaling, as demonstrated by *in vivo* labeling showing that the lifespan of TR, follicular (FO) and marginal zone (MZ) cells is reduced in BAFFr mutants [22] and is extended with exogenous BAFF administration [23]. These effects involve upregulation of Bcl-2 family members, prevention of PKC- δ nuclear accumulation, and activation of the non-classical Nuclear Factor of kappa B (NF- κ B) pathway [23,25,26•].

These findings fundamentally altered notions of peripheral B cell survival mechanisms because they revealed a second, BCR independent, signaling requirement. Perhaps more importantly, they carried a profound implication for BCR-mediated selection in the periphery: If the likelihood of successful TR differentiation can be modified by BAFF signaling, then the threshold for negative selection at this stage must be variable. This implied plasticity in the stringency of TR selection was directly interrogated in two elegant studies using the HEL transgenic model. The results clearly showed that increased BAFF levels rescued the maturation of autoreactive cells normally lost at the TR checkpoint [27,28••]. Mirroring previous results, the likelihood of such rescue depended on the competing cells, such that when mixed with enough competitors of normal BCR diversity, autoreactive clonotypes failed to persist regardless of excess BAFF [28••]. Importantly, high avidity interactions that yielded deletion at bone marrow immature stage were not influenced by BAFF overexpression, again pinpointing the TR stage as the point where BCR-mediated selection and BAFFr signaling become integrated.

Molecular mechanisms of BAFFr and BCR signal and integration—Overall, these observations suggest a model in which the set point for B cell numbers is reached when free BAFF becomes limiting, and the BCR signal strength suitable for successful TR differentiation varies on the basis of available BAFF [29]. Accordingly, knowledge of the molecular mechanisms integrating BCR and BAFFr signals is fundamental to understanding peripheral B cell tolerance. Clearly, BAFF thwarts death mechanisms driven by strong BCR signals. For example, BCR-driven Bim dephosphorylation and consequent caspase-mediated cell death [30] is blocked by BAFF. While these findings disclose distal events through which BAFF opposes BCR-driven death at the TR stages, the reason why BCR signals are also necessary

for BAFF-mediated survival remains unclear. Several recent studies suggest that cross-talk between members of the NF- κ B family may provide this link. Indeed, disruption of either the classical or non-classical NF- κ B pathways blocks peripheral B cell development [31,32]. Because the BCR primarily if not exclusively, utilizes the classical NF- κ B pathway while BAFFr uses the non-classical NF- κ B pathway, the activation of parallel or interacting downstream systems may be required to yield survival. Recent studies by Sasaki *et al.* demonstrated that constitutive classical NF- κ B pathway activation allowed B cell development in the absence of BAFFr [33•], but BAFF treatment still led to increased survival over that provided by forced classical NF- κ B activation alone, indicating a non-redundant function for BAFFr. A separate study by Enzler *et al.* showed that all major components of the non-classical NF- κ B pathway are crucial for BAFF-mediated survival [26•]. Further, while knockout mice for the components of each pathway differ slightly, all are essentially similar to BAFF^{-/-} or BAFFr^{-/-} mice [20,34–36]. Thus, it seems likely that the BCR and BAFFr are coupled through these two signaling systems, either via simultaneous activation targeting separate promoters of survival, or through direct cross-talk. Regardless of exact mechanism, determining the details of this relationship should eventually afford the opportunity to deliberately manipulate selection and survival within peripheral B cell subsets.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
 - of outstanding interest
1. Allman DM, Ferguson SE, Cancro MP. Peripheral B cell maturation. I Immature peripheral B cells in adults are heat-stable antigenhi and exhibit unique signaling characteristics. *J Immunol* 1992;149:2533–2540. [PubMed: 1383316]
 2. Allman DM, Ferguson SE, Lentz VM, Cancro MP. Peripheral B cell maturation. II Heat-stable antigen (hi) splenic B cells are an immature developmental intermediate in the production of long-lived marrow-derived B cells. *J Immunol* 1993;151:4431–4444. [PubMed: 8409411]
 3. Nossal GJ, Pike BL. Clonal anergy: persistence in tolerant mice of antigen-binding B lymphocytes incapable of responding to antigen or mitogen. *Proc Natl Acad Sci U S A* 1980;77:1602–1606. [PubMed: 6966401]
 4. Nossal GJ, Pike BL. Evidence for the clonal abortion theory of B-lymphocyte tolerance. *J Exp Med* 1975;141:904–917. [PubMed: 47889]
 5. Hartley SB, Crosbie J, Brink R, Kantor AB, Basten A, Goodnow CC. Elimination from peripheral lymphoid tissues of self-reactive B lymphocytes recognizing membrane-bound antigens. *Nature* 1991;353:765–769. [PubMed: 1944535]
 6. Fulcher DA, Basten A. Reduced life span of anergic self-reactive B cells in a double-transgenic model. *J Exp Med* 1994;179:125–134. [PubMed: 8270860]
 - 7••. Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Science. Predominant autoantibody production by early human B cell precursors 2003;301:1374–1377.
 8. Yurasov S, Wardemann H, Hammersen J, Tsuiji M, Meffre E, Pascual V, Nussenzweig MC. Defective B cell tolerance checkpoints in systemic lupus erythematosus. *J Exp Med* 2005;201:703–711. [PubMed: 15738055]

9. Gu H, Tarlinton D, Muller W, Rajewsky K, Forster I. Most peripheral B cells in mice are ligand selected. *J Exp Med* 1991;173:1357–1371. [PubMed: 1903427]
10. Levine MH, Haberman AM, Sant'Angelo DB, Hannum LG, Cancro MP, Janeway CA Jr, Shlomchik MJ. A B-cell receptor-specific selection step governs immature to mature B cell differentiation. *Proc Natl Acad Sci U S A* 2000;97:2743–2748. [PubMed: 10688906]
11. Wang H, Clarke SH. Evidence for a ligand-mediated positive selection signal in differentiation to a mature B cell. *J Immunol* 2003;171:6381–6388. [PubMed: 14662836]
12. Lam KP, Kuhn R, Rajewsky K. In vivo ablation of surface immunoglobulin on mature B cells by inducible gene targeting results in rapid cell death. *Cell* 1997;90:1073–1083. [PubMed: 9323135]
13. Kraus M, Alimzhanov MB, Rajewsky N, Rajewsky K. Survival of resting mature B lymphocytes depends on BCR signaling via the Igalpha/beta heterodimer. *Cell* 2004;117:787–800. [PubMed: 15186779]
14. Sprent J, Bruce J. Physiology of B cells in mice with X-linked immunodeficiency (xid). III Disappearance of xid B cells in double bone marrow chimeras. *J Exp Med* 1984;160:711–723. [PubMed: 6381637]
15. Freitas AA, Rosado MM, Viale AC, Grandien A. The role of cellular competition in B cell survival and selection of B cell repertoires. *Eur J Immunol* 1995;25:1729–1738. [PubMed: 7615000]
16. McLean AR, Rosado MM, Agenes F, Vasconcellos R, Freitas AA. Resource competition as a mechanism for B cell homeostasis. *Proc Natl Acad Sci U S A* 1997;94:5792–5797. [PubMed: 9159153]
17. Cyster JG, Hartley SB, Goodnow CC. Competition for follicular niches excludes self-reactive cells from the recirculating B-cell repertoire. *Nature* 1994;371:389–395. [PubMed: 7522305]
18. Moore PA, Belvedere O, Orr A, Pieri K, LaFleur DW, Feng P, Soppet D, Charters M, Gentz R, Parmelee D, et al. BLYS: member of the tumor necrosis factor family and B lymphocyte stimulator. *Science* 1999;285:260–263. [PubMed: 10398604]
19. Schneider P, MacKay F, Steiner V, Hofmann K, Bodmer JL, Holler N, Ambrose C, Lawton P, Bixler S, Acha-Orbea H, et al. BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. *J Exp Med* 1999;189:1747–1756. [PubMed: 10359578]
20. Schiemann B, Gommerman JL, Vora K, Cachero TG, Shulga-Morskaya S, Dobles M, Frew E, Scott ML. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. *Science* 2001;293:2111–2114. [PubMed: 11509691]
21. Yan M, Brady JR, Chan B, Lee WP, Hsu B, Harless S, Cancro M, Grewal IS, Dixit VM. Identification of a novel receptor for B lymphocyte stimulator that is mutated in a mouse strain with severe B cell deficiency. *Curr Biol* 2001;11:1547–1552. [PubMed: 11591325]
22. Lentz VM, Cancro MP, Nashold FE, Hayes CE. Bcmd governs recruitment of new B cells into the stable peripheral B cell pool in the A/WySnJ mouse. *J Immunol* 1996;157:598–606. [PubMed: 8752907]
23. 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;168:5993–5996. [PubMed: 12055205]
24. Harless SM, Lentz VM, Sah AP, Hsu BL, Clise-Dwyer K, Hilbert DM, Hayes CE, Cancro MP. Competition for BLYS-mediated signaling through Bcmd/BR3 regulates peripheral B lymphocyte numbers. *Curr Biol* 2001;11:1986–1989. [PubMed: 11747827]
25. Mecklenbrauker I, Kalled SL, Leitges M, Mackay F, Tarakhovsky A. Regulation of B-cell survival by BAFF-dependent PKCdelta-mediated nuclear signalling. *Nature* 2004;431:456–461. [PubMed: 15361883]
26. Enzler T, Bonizzi G, Silverman GJ, Otero DC, Widhopf GF, Anzelon-Mills A, Rickert RC, Karin M. Alternative and classical NF-kappa B signaling retain autoreactive B cells in the splenic marginal zone and result in lupus-like disease. *Immunity* 2006;25:403–415. [PubMed: 16973390] These authors used a powerful series of knockout and mutant mice to demonstrate the crucial importance of non-classical NF-kB components in BAFFr function.
27. Lesley R, Xu Y, Kalled SL, Hess DM, Schwab SR, Shu HB, Cyster JG. Reduced competitiveness of autoantigen-engaged B cells due to increased dependence on BAFF. *Immunity* 2004;20:441–453. [PubMed: 15084273]

- 28••. Thien M, Phan TG, Gardam S, Amesbury M, Basten A, Mackay F, Brink R. Excess BAFF rescues self-reactive B cells from peripheral deletion and allows them to enter forbidden follicular and marginal zone niches. *Immunity* 2004;20:785–798. [PubMed: 15189742] These studies employed transgenic mice and mixed marrow chimeras to examine the fate of autoreactive clones in a BAFF overexpressing environment. The results showed that autoreactive clones can be rescued from death at the transitional stages by excess BAFF, but competitors of normal BCR diversity reduced the likelihood of rescue.
29. Miller JP, Stadanlick JE, Cancro MP. Space, selection, and surveillance: setting boundaries with B₂L₂. *J Immunol* 2006;176:6405–6410. [PubMed: 16709796]
30. Craxton A, Draves KE, Gruppi A, Clark EA. BAFF regulates B cell survival by downregulating the BH3-only family member Bim via the ERK pathway. *J Exp Med* 2005;202:1363–1374. [PubMed: 16301744]
31. 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;3:958–965. [PubMed: 12352969]
32. Pasparakis M, Schmidt-Supprian M, Rajewsky K. IkappaB kinase signaling is essential for maintenance of mature B cells. *J Exp Med* 2002;196:743–752. [PubMed: 12235208]
- 33•. Sasaki Y, Derudder E, Hobeika E, Pelanda R, Reth M, Rajewsky K, Schmidt-Supprian M. Canonical NF-kappaB activity, dispensable for B cell development, replaces BAFF-receptor signals and promotes B cell proliferation upon activation. *Immunity* 2006;24:729–739. [PubMed: 16782029] These authors showed that constitutive classical NF-kB activation could mimic some functions associated with BAFF signaling. In conjunction with the paper by Enzler *et al.*, these findings implied downstream interactions of the classical and non-classical NF-kB pathways.
34. Beinke S, Ley SC. Functions of NF-kappaB1 and NF-kappaB2 in immune cell biology. *Biochem J* 2004;382:393–409. [PubMed: 15214841]
35. Claudio E, Brown K, Siebenlist U. NF-kappaB guides the survival and differentiation of developing lymphocytes. *Cell Death Differ* 2006;13:697–701. [PubMed: 16528380]
36. Sasaki Y, Casola S, Kutok JL, Rajewsky K, Schmidt-Supprian M. TNF family member B cell-activating factor (BAFF) receptor-dependent and -independent roles for BAFF in B cell physiology. *J Immunol* 2004;173:2245–2252. [PubMed: 15294936]