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BRIEF REVIEWS

# B Cell Positive Selection: Road Map to the Primary Repertoire?

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***Accumulating evidence indicates that positive selection events mediate differentiation, lineage commitment, and longevity of B lymphocytes. The BCR plays a central role, dictating the likelihood that newly formed cells will complete maturation, as well as whether cells persist within mature pools. Competition among B cells for limited, life span-promoting resources, which include self-ligands, lineage-specific cytokines, and innate receptor ligands, underlie these selective processes. Together, these observations suggest that positive selection is a critical feature in the establishment and maintenance of all lymphocyte pools, prompting re-evaluation of the underlying biological rationale for this process. The Journal of Immunology,* 2004, 173: 15–19.**

A central principle of the clonal selection paradigm is that differential cell fate decisions are based on receptor specificity. Although initially advanced to account for the precision of lymphocyte activation and tolerance, the role of receptor-based selection has expanded to encompass primary lymphocytes as well. In these instances, admittance to preimmune repertoires involves the preferential survival of cells whose receptors meet minimum signaling requirements, rather than just avoidance of tolerogenic elimination. The notion that preimmune repertoires are shaped by intrinsic cognitive events first emerged in the network hypothesis (1, 2) and was later expanded to suggest that self-structures provide prospective clues about likely targets for pathogen entry (3–6). The contemporary view of positive selection was keenly influenced by the appreciation of MHC restriction (7), which fostered the idea that T cell repertoires undergo skewing toward haplotype-appropriate self-reactivity to insure efficacy. Because BCR interactions with ligands are not similarly constrained, this perspective implicitly argued against equivalent, self-mediated positive selection among B cells. Nonetheless, findings over the last decade show that repertoire shifts consistent with ligand-mediated selection typify B cell maturation, and that continued subthreshold BCR signaling is required for primary B cell survival. The reprise of positive selection as a feature common to both T and B cell differentiation, combined with recently revealed relationships between positive selection and peripheral survival, prompts reassessment of the fundamental objectives served by such processes.

### *Both negative and positive selection occur among transitional and mature B cells*

After lineage commitment and differentiation through pro- and pre-B cell stages in the bone marrow, acquisition of surface IgM yields immature B cells. These complete maturation as they enter and populate the periphery, navigating several transitional intermediates before joining the mature, preimmune B cell pool (8–12). Although the pre-BCR can engender some skewing of *V* gene usage (13), specificity-mediated selection commences during the immature and transitional stages, inasmuch as these are where a complete Ag receptor is first expressed. Furthermore, although alternative or branched routes may exist (14, 15), and strategies used to subdivide transitional stages vary (11, 12), this is likely the major route of maturation for primary B cells. Evidence for stringent selection at this juncture was first suggested by analyses of production and turnover rates in bone marrow vs peripheral populations (9, 10, 16–19). These revealed that the adult mouse bone marrow produces ~15 million immature B cells per day, but only ~10% exit the marrow, and fewer than half of these new émigrés survive to join the mature peripheral B cell pool (9, 10). These substantial losses, together with the broadly differing turnover rates between transitional and mature B cells (9), indicate that rigorous selective mechanisms operate across this developmental window.

The susceptibility of immature B cells to BCR-induced unresponsiveness in vitro (20–22), as well as in vivo evidence from transgenic models (23, 24), have established negative selection as a key feature of B cell differentiation. This elimination of autoreactive clonotypes (25) may be mediated either through death (20, 22–24), anergy (26–30), or receptor editing (31–35).

Although negative selection accounts for some of the losses observed during these late maturation stages, mounting evidence indicates that BCR signaling also plays a key positive role in both transitional differentiation and mature B cell life span. For example, comparisons of $V_H$ or $V_L$ gene usage within the immature vs mature bone marrow compartments (36), newborn vs adult B cells (37), and transitional vs mature peripheral compartments (38), have all revealed repertoire shifts consistent

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with the notion that BCR specificity mediates recruitment into the primary B cell pools. Furthermore, a crucial role for the BCR in maintaining follicular B cells is evidenced by the rapid diminution of follicular populations following conditional BCR ablation (39).

Several parallels exist between positive- and negative-selection processes. First, cells within the immature marrow and transitional peripheral subsets are the targets of these events (28, 29, 40–42). Second, whether and when selection transpires varies according to several concurrent parameters. Although BCR signal strength seems to be the principal determinant (20, 43–45), the milieu of competing clonotypes also plays a strong role in dictating the outcome of BCR ligation events (42, 46). For example, autoreactive cells that evade elimination until transitional stages when developing in an oligoclonal repertoire, die at earlier differentiation stages when competing with a cohort of normal diversity. Thus, ultimate selective fate decisions—either positive or negative—are based on the composite influence of selecting ligands, BCR signal strength, clonotypic composition of the developing cohort, and cellular microenvironment.

### *BCR specificity governs relative fitness to compete for viability-promoting resources*

Results indicating that the propensity for maturation and survival depend on BCR signaling first came from experiments in which mixed-marrow chimeras were constructed from normal and *xid* donors. In these studies, *xid*-derived peripheral B cells could persist only in the absence of normal (Btk-sufficient) progenitors (47). Moreover, several studies indicate that BCR specificity dictates relative competitive advantage among extant clonotypes. Thus, in mixed-marrow chimeras that used different combinations of Ig transgenic progenitors, a given clonotype's ability to mature and persist varied, depending on the clonotypic makeup of the competing cohort (48–51). Together, these findings indicate that limited, survival-enabling resources govern both recruitment into, as well as persistence within, mature lymphocyte pools (49, 51, 52), and that adequate BCR signaling determines the ability to capture these resources. These considerations indicate that factors influencing steady-state surface BCR levels, such as intrinsic turnover rates and efficiency of H-L chain pairing, might influence clonal fitness.

Recent studies indicate that it may be possible to mimic these signals, and that final B cell maturation can be accomplished without BCR ligation (53), suggesting that signal strength is the fundamental determining factor, rather than specificity per se (54). Nonetheless, acknowledgment of a BCR signaling requisite for maturation and survival implies that beyond a minimal threshold, gradations of clonal fitness will be tied to the aggregate signal afforded by the milieu of selecting ligands. This will ultimately be dictated by the $V_LV_H$ pair, such that within a repertoire of significant diversity, a given clone's ability to marshal viability-promoting resources will be tightly coupled with specificity.

Parabiosis experiments have extended these concepts to include the notion that limited viability-promoting resources actually calibrate steady-state B cell numbers by controlling life span. In these experiments, normal mice were conjoined with one or more B cell-deficient partners. The results showed that a single marrow equivalent can establish and maintain multiple peripheral compartments at normal steady-state levels (55, 56), despite a relatively constant marrow production rate.

The limiting resources governing B cell maturation and survival remain unclear. In models positing selection on self-ligands, the availability of appropriate self-epitopes may serve as the limiting factor, with associated BCR signaling providing direct survival signals. This possibility has been suggested and modeled (49, 51, 57), and is supported by the observation that most competition appears to be intra- rather than interclonal.

Alternatively, lineage-specific cytokines may also serve as limiting determinants of B cell positive selection and survival. The recently described TNF family member B lymphocyte stimulator (BLyS)[2] is an attractive candidate for such activity (58–62). Excess BLyS increases the proportion of transitional cells completing differentiation, as well as the longevity of mature follicular B cells (58, 62), whereas reduced BLyS signaling has opposite effects (63–65). Moreover, elevated BLyS levels are associated with humoral autoimmunity (66, 67), and ectopic BLyS expression can alter the stringency of negative selection in late transitional B cells (68, 69), indicating a critical role for BLyS in selection during transitional differentiation.

Finally, BCR interactions might indirectly promote survival by enhancing the capture of other survival or activation factors. For example, BCRs that bind scavenger receptor activators such as phospholipids derived from apoptotic cells, LPS, or CpG, could selectively focus these onto B cell surfaces. Recent reports that IgG-specific B cells can be activated through a TLR9-mediated mechanism by binding chromatin-specific IgG Abs may reflect such a mechanism (70, 71). Furthermore, possibly in combination with innate receptors, BCRs specific for self- or bacteria-associated molecules may select canonical marginal zone (MZ) B cell clones over others for survival (72–74). Analogous to enabling the capture of innate receptor ligands, the BCR may enhance the ability to secure viability-promoting cytokines. Recent findings indicate that BCR signaling can selectively up-regulate BLyS receptors (75, 76), perhaps affording a selective advantage to clones with higher levels of basal BCR signaling and hence BLyS receptor expression.

It will be important to establish the relative roles of these mechanisms. For example, it is unclear whether BCR vs BLyS signals independently regulate selection vs peripheral pool size, or whether, instead, the two are integrated through the coupling of BLyS receptor expression to BCR signaling. Furthermore, determining whether particular selection strategies are characteristic or prevalent within different functional subsets should also yield important insights.

### *Positive selection and recruitment to functional niches are related processes*

The notion that requisite active selection affords access to peripheral subsets is not limited to follicular B cells, inasmuch as triage to the B1 or MZ compartments can also be influenced by BCR specificity (73, 77–85). The origins of these functional subsets remain controversial, revolving around whether they are derived from predetermined precursors early in life, or whether marrow-derived B cells undergo a final lineage commitment step in the periphery. Important determinants of functional lineage fate include where emigrant cells contact ligand, and what functional changes they undergo after such encounters. Until recently, the pathways and sites of splenic entry for newly

[2] Abbreviations used in this paper: BLyS, B lymphocyte stimulator; MZ, marginal zone; FO, follicular.

formed B cells have been poorly understood. Recently, LFA-1/ICAM-1 and $\alpha_4\beta_1$/VCAM-1 interactions were implicated in T and B cell movement from blood to follicular or MZ regions (86). Because MZ B cells express higher levels of these integrins, they may bind more avidly to ICAM-1- and VCAM-1-expressing cells in the MZ, fostering residence (86, 87). Although higher integrin expression may engender MZ retention, prior events may have predestined circulating B cells for the MZ, altering their relative capacity to bind homing ligands on endothelial cells of the marginal sinus or metallophilic macrophages (88). Indeed, MZ B cells and B1 cells express several unique molecules, including the scavenger receptor molecule CD36, and a newly described FcRH receptor with a scavenger domain motif (J. F. Kearney, unpublished data), as well as CD9 (89). These might provide additional signaling to MZ B cells via ligands encountered either en route to the spleen or within the MZ microenvironment per se, initiating preferential up-regulation of chemokine receptors or integrins. Further studies comparing homeostatic bone marrow-spleen migration with BCR-Ag influenced entrance and migration within the splenic environment will be revealing.

*Do self-molecules provide positively selecting epitopes?*

Establishing the predominant selecting ligands for B cell positive selection and homeostasis poses a difficult problem. It is now evident that positively selecting ligands for T lymphocytes are primarily presented self-peptides (90–92), and that these same self-peptide/MHC combinations can promote homeostatic survival or proliferation among at least some mature T cell subsets (93). Whether an active skewing toward self-reactivity occurs among B cells is difficult to address experimentally, because the genetic manipulation of presentation molecules is not an option.

Nonetheless, several studies have shown that self-ligands can mediate selection into the primary repertoire. For example, recent studies in a double-transgenic model indicate that follicular cells displaying low-avidity interactions with self-ligand enjoy the highest likelihood of maturation and survival (57, 94). Similarly, there are several well-documented examples of self-Ags that afford enrichment or survival of B1 and MZ B cell clonal populations. Thus, Thy-1 may act as a selecting ligand for B1 cells (79), and recent studies suggest that early developmental dominance of the T15 clonotype may reflect reactivity to modified phospholipids associated with oxidized self-low-density lipoprotein (95, 96). Although it is not possible to make low-density lipoprotein-free mice, dominance of the T15 clone in Ag-free mice is consistent with a self-mediated selection mechanism. Finally, the observation that natural IgM levels (97) as well as MZ B cell numbers are comparable in Ag-free and conventional mice (98), coupled with the high frequency of autoreactivity among these Abs (99, 100), similarly supports self-mediated positive signaling and activation.

If self-epitopes are the predominant selecting elements for the primary repertoire, the question arises as to how memory B cells are sustained despite the potential loss of sufficient self-cognition during affinity maturation. Recent studies have shown that B cell memory can be maintained by constitutive TLR expression or noncognate T cell help (101), and that long-lived marrow plasma cells express BCMA, a receptor that interacts with APRIL as well as BLyS (102). Thus, Ag-driven differentiation may lead to altered survival requisites, releasing memory cells from enforced competition with primary follicular cells.

*What is the goal of continuous self-oriented positive selection?*

Obligate self-mediated positive selection in the maturation, lineage determination, and homeostasis of peripheral B cells, coupled with parallels in T lymphocyte development and homeostasis, compels consideration of the underlying biological rationale. One possibility is that sufficient repertoire diversity is ensured through positive selection and continuous intraclonal competition. The reasoning for this argument is that if intraclonal competition for self-epitopes determines fitness, then overlapping specificities will compete but nonoverlapping specificities will not, thus favoring diversity. Whether diversity per se is the ultimate end point of positive selection might be questioned from the standpoint that the random genetic processes used for receptor generation will likely insure diversity given adequate pool size and turnover rates. Furthermore, mice bearing a single $V_H$ gene were able to generate high-affinity Abs to several T-dependent protein Ags using CDR3 variability and somatic hypermutation (103). Nonetheless, these mice could not respond to conserved carbohydrate Ags, suggesting a dichotomy may exist between evolutionarily conserved $V_H$ genes, such as those characteristic of conserved carbohydrate responses, vs those reliant on somatic diversity-generating mechanisms.

Subthreshold signaling on self-epitopes might minimize the Ag concentrations required for lymphocyte activation. This has been suggested for T cells (104), based on experiments showing that even single MHC-peptide complexes can induce signaling in T cells, presumably through the formation of signaling complexes by TCR interactions with neighboring self-peptide/MHC complexes. This scenario seems less likely for B cells, given relatively high initial BCR affinities found in early primary responses. Furthermore, because B cells bind native pathogen structures rather than processed peptides, multideterminant arrays of B cell epitopes yielding high-avidity interactions are likely the norm.

An alternative rationale for positive selection stems from the notion that randomly generated preimmune repertoires are prone to failure without intrinsic algorithms for anticipating protective utility. This suggests functional subsets will vary in their dependence on self-mediated positive selection, based on whether evolutionary vs somatic assessments of protective efficacy are used. In this model, reminiscent of "layering" suggested by Herzenberg and colleagues (105, 106), B cells expressing receptors that bind unchanging aspects of infectious agents—especially those that afford capture of additional viability-promoting ligands—will be least subject to competitive BCR-mediated selection: Because this repertoire's targets are consistent, it can be retrospectively refined and evolutionarily fixed, obviating any need for further selection based on BCR specificity. Moreover, because protective efficacy of these receptors is assured a priori, a small number of cells can effectively fill this niche. An obvious candidate is the B1 pool, whose BCR repertoire is skewed to conserved germline specificities that capture scavenger receptor ligands, and much of which is established without TdT-mediated junctional additions (107, 108). Furthermore, unlike the follicular (FO) and MZ compartments, B1 cells are independent of BLyS (64). Similarly, B cells whose receptors are not part of an evolutionarily conserved cluster, but that fortuitously capture ligands for alternative life span-promoting circuits, might be shuttled into these compartments as well, perhaps explaining the occasional inclusion of such V regions in the MZ and B1 pools (109).

Outside of evolutionarily tested and fixed specificities, the potential of microbes for rapid genetic variation and consequent immune evasion requires a means to prospectively evaluate probable protective utility. This provides a rationale for positive selection in the follicular pool, whereby continuous competition for subthreshold self-reactivity minimizes potential evasion by rapid microbial evolution. The conceptual underpinnings of this idea are that efficient surveillance should be focused toward structures that can interact with the host (self), and that the aggregate of self is a circumscribed array of ligand-receptor sets, thus providing intrinsic structural mimics of potentially relevant pathogen structures. Accordingly, the more comprehensive and avid the array of subthreshold self-reactivity, the smaller the unaccounted structural space between self and nonself, and the lower the probability of immune evasion. This scenario predicts that with age, the FO repertoire will truncate toward specificities that best fit these criteria, and average FO B cell life span should increase, both of which occur (110, 111). Furthermore, it should be possible to experimentally vary the representation of clonotypes by conditional induction or ablation of selecting self-ligands. Such experimental systems might eventually include assessment of disease susceptibility, if selecting ligands relevant to pathogen tropism can be identified. An attractive consequence of this model is the visualization of positive selection as an ongoing, dynamic repertoire refinement process, whereby clonal survival—regardless of functional maturity—is tied to maximal subthreshold self-reactivity, such that potential protective value is continuously reassessed relative to competitor clones.

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