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BCR and co-receptor crosstalk facilitate the positive selection of self-reactive transitional B cells

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Abstract

The establishment of a diverse B cell repertoire requires fine-tuning of antigen receptor selection during development in order to permit sufficient diversity while reducing the potential for autoimmunity. In this review, we highlight recent studies demonstrating the central role of the B cell antigen receptor (BCR), in coordination with other key pro-survival signals mediated by CD40, BAFF-R, TACI and/or TLRs, in regulating both negative and positive selection of autoreactive B cells. In particular, we show how altered antigen or co-stimulatory signaling can facilitate positive selection of transitional B cells with self-reactive BCRs, ultimately leading to their entry into the mature, naïve B cell compartment. We propose a model wherein altered receptor signals (due to inherited genetic changes) leads: first, to enhanced positive selection of autoreactive cells into the naïve B cell repertoire; subsequently, to an increased probability of pathogenic germinal center responses in individuals with a broad range of autoimmune disorders.

Introduction

Protective immunity against a broad range of pathogens requires the ongoing generation of lymphocytes with diverse antigen receptor specificities. This requirement is achieved via random assortment of germline encoded V(D)J genes in developing lymphocytes. An inherent tradeoff within this process, however, is creation of autoreactive receptors with the potential to elicit an autoimmune response. For B cells, autoreactive specificities are removed or segregated from the developing repertoire at discrete stages in the bone marrow and periphery. Immature B cells with newly rearranged B cell receptors (BCRs) are first tested for reactivity towards self-antigens in the bone marrow. Depending on the strength of BCR signaling and the physical form of the antigen, reactive cells are deleted [1], subjected to receptor editing [2] or rendered functionally anergic [3]. Importantly, despite these processes, up to 40% of transitional B cells (alternatively referred to as recent bone marrow emigrants, or transitional type 1 [T1] or type 2 [T2] cells) continue to exhibit some level of self-reactivity. Splenic transitional B cells are subjected to additional selective processes that

further cull self-reactivity before their maturation and entry into the mature, naïve follicular (FM) or marginal zone (MZ) peripheral B cell compartments. Paradoxically, BCR signals also appear to be necessary for the positive selection of splenic transitional B cells. Importantly, a growing body of recent work indicates that BCR signals synergize with B cell intrinsic, non-antigenic signals, including biochemical events triggered by receptors binding to BAFF, CD40L, and Toll-like receptor (TLR) ligands, and that this interplay is crucial in determining the selection program induced in a given B cell. A better understanding for how the interplay of these BCR and non-BCR signals ultimately shapes the repertoire of the mature, naïve compartment is crucial for a complete understanding of both autoimmune susceptibility, as well as early pathogen-triggered antibody responses to acute infection.

BCR as a master regulator of positive selection

In both central and peripheral B cell development, maintaining an ‘intermediate’ level of BCR signaling is necessary for cell survival and maturation. After emigrating from the bone marrow, tonic BCR signaling is required for the persistence of both transitional and mature B cells via provision of canonical NF κ B-dependent and/or PI3K-dependent pro-survival signals [4–6]. Correspondingly, impaired BCR signaling, such as reduced Btk activity in *xid* mice, results in reduced B cell maturation [7]. Too strong of a BCR signal is equally detrimental, however, as revealed by transitional cell sensitivity to BCR-induced apoptosis [8] as well as recent work in leukemia lines showing that inducing proximal hyperactive pre-BCR signaling components promotes cell death pathways reminiscent of self-reactive BCRs [9]. These combined findings suggest that fluctuations in signaling thresholds directly impacts B cell survival.

Emerging data also imply that this maturation process occurs in conjunction with the tuning of the developing BCR repertoire via direct antigen-engagement. Specifically, recent work [10] revealed that the vast majority of mature, naïve B cells exhibit evidence of direct BCR engagement — presumably via self-antigens and/or possibly, endogenous flora. Using Nur77-GFP reporter mice, this group showed that BCR-ligand engagement first occurs at the T2 B cell stage, implying that entry into the naïve compartment is refined via antigen stimulation [10].

In addition to providing survival signals, BCR specificity also shapes the developing repertoire. VH gene usage is clearly skewed during development, indicating that specific VH families are subjected to preferential positive and negative selection [11]. Consistent with this concept of positive selection, our group previously identified an actively cycling subpopulation within the T2 B cell compartment present in the setting of normal B cell homeostasis [12]. This population is hypothesized to represent transitional B cells that may be positively selected into the mature compartment via clonal expansion upon antigen engagement. Overall, these combined studies indicate that the BCR signaling program both defines and refines the mature, naïve compartment by regulating B cell survival and tolerance processes.

While BCR signals serve as the dominant pathway by which both survival and antigen specificity of the naïve repertoire are shaped, access to limited survival and activation signals work in conjunction with the BCR to provide additional fine-tuning mechanisms by

which transitional B cells are able to compete and persist within the naïve compartment [13,14]. Survival signals mediated by CD40, BAFF-R, TACI and TLR receptors can each partially compensate for, and, importantly, may also amplify BCR signal strength to help shape the naïve repertoire.

Crosstalk between the BCR and BAFF-R pathways (Figure 1a)

The importance of cytokine BAFF in B cell survival and homeostasis has been well studied [15] and systemically produced BAFF, generated by fibroblastic reticular cells [16], appears to be particularly important for this process. Although BAFF can bind to three alternative receptors — BAFF-R, TACI, and BCMA — BAFF-R appears to play the dominant role in peripheral B cell maturation. Transitional B cells predominantly express BAFF-R and mice deficient in either BAFF or BAFF-R exhibit an identical developmental block at the T2 stage. By contrast, in TACI or BCMA-deficient animals, B cells are able to develop into the mature compartment [17].

Despite these clear developmental phenotypes, the precise mechanism(s) by which BAFF-R mediates survival of transitional cells remains incompletely defined due to its complex interplay with the BCR, and the dichotomous nature of the BCR itself to promote both negative and positive selection signals. On one hand, evidence supports a model in which BAFF-R engagement can help rescue transitional cells receiving an ‘ineffective’ BCR signal in response to chronic self-antigen encounter. This is achieved through potent activation of the alternative NF κ B pathway, leading to the upregulation of anti-apoptotic members of the Bcl-2 protein family, post-transcriptional modification of the pro-apoptotic member, Bim, and via additional signaling events [15,18,19]. On the other hand, recent studies reveal a novel intersection of BAFF-R with ‘tonic’ BCR signals [20^{**},21]. Schweighoffer *et al.* show that BAFF stimulation promotes the rapid phosphorylation of the proximal BCR signaling components Ig α and Syk. When Syk is inducibly deleted, B cells exhibit impaired survival and fail to respond to BAFF despite the presence of intact alternative NF κ B signals. Survival in this setting is partially restored with ectopic activation of the Erk or PI3 K pathways, implying that BAFF-R signaling likely co-facilitates key BCR survival signals. In addition, BCR signaling has been reported to enhance both BAFF-R transcript and alternative NF- κ B p100 substrate levels [5], which likely modulates NF κ B network states [22] and suggests an additional level of interdependence between these two pathways in promoting survival. Similarly, the BCR co-receptor CD19 has also been recently implicated in regulating BAFF-R levels [17]. Given the emerging evidence that BAFF-R synergizes and intersects with the BCR at multiple points, future studies exploring this crosstalk (particularly under conditions of BCR antigen-dependent versus tonic signaling) should improve our understanding of transitional B cell peripheral selection.

Excess BAFF in autoimmunity (Figure 1b)

Serum, as well as localized, BAFF levels are elevated in many subjects suffering from autoimmune disorders [23]. Transgenic mice overexpressing BAFF (BAFF-Tg) provide useful models to study its impact on disease progression. BAFF-Tg mice display B cell hyperplasia and develop lupus-like disease. Excess BAFF rescues lowaffinity self-reactive transitional cells that are normally outcompeted for entry into B cell follicles (presumably

through BAFF-R survival signaling), allowing self-reactive B cells to mature in ‘forbidden’ splenic zones [24,25]. This relaxed selection model, in concert with the capacity of BAFF-R to synergize with antigen-dependent activation of self-reactive mature, naïve B cells, has been proposed to account for loss of B cell tolerance in BAFF-Tg models [15]. In contrast to this idea, new work suggests that signals mediated through TACI are crucial for generation of autoantibodies and immune complex renal disease in BAFF-Tg mice [26**]. Unlike BAFF-R, TACI predominantly activates the classical NF κ B pathway, leading to transcriptional events that more closely resemble BCR-induced or TLR-induced activation and proliferation [15]. Interestingly, TACI signaling utilizes the adapter protein, MyD88, also essential for TLR signaling, and autoantibody production in BAFF-Tg mice is abrogated by MyD88 deletion. At present, it remains unclear which specific B cell subset(s) is preferentially targeted by TACI activation, although recent work has suggested a specific role for B1b cells in these events [17].

Crosstalk between the BCR and CD40 pathways (Figure 2a)

Emerging data suggest that CD40 signaling, similar to BAFF-R, may also modulate transitional B cell selection in concert with the BCR program. CD40 engagement promotes both the alternative and classical NF κ B pro-survival pathways [27]. In addition to the well-described upregulation of CD40L following T cell activation, CD40L is also constitutively expressed by naïve CD4 T cells [28]. A range of *in vitro* observations have long implicated CD40 signals in modulating transitional B cell survival including, for example, data demonstrating CD40-dependent rescue of BCR-induced apoptosis in transitional cells [8]. Additionally, CD40 exhibits a compensatory role in the absence of BCR signals, as revealed by the severely reduced transitional B cell compartment size in Btk and CD40 doubly deficient mice [7]. Notably, although CD40-deficient mice have normal transitional B cell numbers, several lines of evidence suggest a more nuanced role for CD40 in altering transitional cell selection [13,29]. Consistent with this hypothesis, using a range of approaches (including mixed chimera models, single cell BCR cloning and high-throughput sequencing) Schwartz *et al.* recently showed that CD40 facilitates B cell positive selection and a broader antigen specificity profile within the mature B compartment, particularly within the MZ B cell repertoire [30*].

Excess CD40 signals in autoimmunity (Figure 2b)

An autoimmune risk variant in the phosphatase, PTPN22, can be used as a model for understanding how dual enhanced BCR and CD40 signaling may shape transitional B cell function and selection. Subjects with the PTPN22-R620W risk variant exhibit altered B cell selection and increased B cell CD40 expression and activation [31]. In addition, human subjects with the risk allele and the knock-in murine model share many features, including an increase in the size of the transitional B cell compartment and reduced sensitivity of transitional B cells to apoptosis [31,32]. One key unresolved issue is whether the variant promotes a BCR signal that is hyporesponsive (supported by human data) or hyper-responsive (supported by murine data). However, as described in a recent review, preliminary evidence suggests that chronic antigen stimulation may be responsible for the unexpected, blunted antigen receptor signals observed in humans [33]. At any rate, these combined data support an emerging model in which enhanced BCR signals function, in part,

to facilitate CD40 signaling, culminating in events that may promote increased survival of transitional cells. Future mechanistic studies that directly assess the intersecting roles for the CD40 and BCR pathways are likely to provide additional insights into peripheral B cell selection.

Crosstalk between the BCR and TLR pathways (Figure 3a)

A cardinal feature of the autoimmune disorder, systemic lupus erythematosus (SLE), is generation of autoantibodies reactive towards a relatively restricted spectrum of self-antigens, including nucleic acids (dsDNA, RNA) and proteins co-associated with other apoptotic cell materials. Crosstalk between the BCR and TLR signaling pathways play a crucial role in orchestrating loss of peripheral B cell tolerance to such antigens [34,35]. BCR engagement with self-antigens facilitates their trafficking to TLR-resident endosomal compartments, leading to engagement of specific TLRs by associated ligands and synergistic B cell activation [36,37].

Conversely, new work strongly suggests that coordinate BCR and TLR signals also play a crucial role in tolerance to nuclear self-antigens. Recent studies clearly demonstrate a role for DNA-specific TLRs (specifically, TLR9) in preventing spontaneous activation of peripheral auto-reactive B cells [38]. Likewise, humans deficient in either *IRAK-4* or *MYD88* exhibit increased autoreactivity within the naïve B cell compartment, implying that innate signaling pathways are necessary for the elimination of autoreactive BCRs during B cell development [39,40]. Finally, genetic studies using an engineered CD45 lupus risk allele in B6 versus BALB/c mice demonstrate additional complexity with respect to TLR9 signal strength in B cell tolerance. Strikingly, strain-specific variance in TLR9 signal strength can serve to *either* promote central tolerance via negative selection *or*, alternatively, facilitate loss of peripheral B cell tolerance (presumably via enhanced positive selection) [41*]. Importantly, the latter outcome is consistent with the emerging idea that dual BCR/TLR signals both promotes positive selection of self-reactive transitional B cells and concurrently facilitates loss of B cell tolerance via triggering of spontaneous and self-reactive germinal center responses (as discussed below).

Excess TLR signals in autoimmunity (Figure 3b)

Dissecting the role of dual TLR and BCR signals in regulating positive selection of the naïve B cell compartment is best understood in the setting of the primary immunodeficiency, Wiskott–Aldrich syndrome (WAS), whereby complete or partial loss-of-function mutations in the WAS protein (WASp) affects actin polymerization and cytoskeleton organization. WAS subjects exhibit a high prevalence of spontaneous systemic autoimmunity, and consistent with this human data, B cell-intrinsic WASp deficiency leads to spontaneous autoimmune GC responses and production of a broad range of class-switched autoantibodies in mice [42,43]. Notably, WASp-deficient B cells exhibit a modest enhancement in both BCR and TLR signaling, and these changes in signaling strength appear to be sufficient to mediate this autoimmune cascade even in the autoimmune-resistant B6 mouse strain.

While the impact of synergistic BCR and TLR signals have been examined in detail within the GC, the effects of increased signaling in these pathways on the positive selection of

naïve B cells is unclear. In this context, preliminary studies have revealed that while central tolerance mechanisms remain intact, robust proliferation of the T2 B cells is observed [44^{**}]. Interestingly, in both murine and human models of WASp-deficiency, we observed preferential selection for autoreactive-associated VH families (VH10, VH4-34 in murine and human, respectively) at the late transitional to mature, naïve B cell transition. Notably, VH10 and VH4-34 family antibodies have previously been shown to exhibit increased binding affinity to self-antigens that serve as TLR ligands, including dsDNA [45^{*},46], apoptotic debris [47,48], and carbohydrates [49]. Consistent with these predicted specificities, enhanced transitional B cell cycling and altered repertoire selection were antigen-specific and MyD88-dependent-supporting a model in which heightened BCR and TLR signals function coordinately to promote the positive selection of self-reactive specificities into the naïve repertoire.

Surprisingly, VH4-34 specific BCRs represent 5–10% of B cells within the naïve repertoire in healthy controls [50], although cells utilizing this VH family are normally negatively selected during GC responses and contribute minimally to isotype switched antibody responses. This observation suggests that natural IgM derived from VH4-34 naïve B cells may serve a beneficial role to the host in the context of clearance of apoptotic debris [48]. However, in autoimmune prone settings, including both SLE and WAS, VH4-34 B cells are enriched within the GC and memory B cell compartment, as well as in a recently described activated naïve B cell compartment [44^{**},51^{**}]. Correspondingly, these subjects frequently exhibit high serum titers of VH4-34 specific IgG, demonstrating that alterations within the naïve compartment may further exacerbate GC positive selection of self-reactive specificities, leading to severe and pathogenic functional consequences.

Conclusions

We have summarized how the BCR signal pathway modulates negative and positive selection mechanisms in transitional cells through its integration with additional peripheral factors (Figures 1a, 2a and 3a), as well as evaluated the role that altered signal pathways play in contributing to the loss of B cell tolerance in various autoimmune diseases (Figures 1b, 2b and 3b). Although Figures 1–3 provide a simplified overview of the crosstalk between dual BCR and individual peripheral factor signaling pathways, continued work exploring shared signaling regulators will help further define the nuances of B cell tolerance mechanisms [52]. At any rate, we hypothesize that reduced or enhanced signaling variants that impact the sensitivity of BCR, CD40, BAFF-R, TACI or TLR signals may adjust tolerance thresholds by relaxing negative selection and/or by promoting positive selection and preferential expansion of cells with self-reactive specificities. Importantly, recent data provides direct evidence that altering tolerance thresholds in transitional cells is able to skew the mature, naïve repertoire towards autoreactivity [30^{*},31,44^{**}], thereby increasing the risk of generating a pathogenic or self-reactive germinal center response. A more complete understanding of how these key signals integrate and modify the positive selection process is likely to provide valuable insights into a broad range of autoimmune disorders, and may also lead to development of novel therapeutic interventions for autoimmune disorders associated with common signaling variants.

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Figure 1.

BCR, BAFFR and TACI crosstalk. **(a)** Under normal conditions, BAFFR synergizes with the BCR signal to promote pro-survival and metabolic signals via the alternative NFκB pathway and by facilitating BCR-mediated activation of Syk in transitional cells. **(b)** In an autoimmune setting of excess serum BAFF, increased levels of BAFF 60-mers are present. These multimers specifically bind to TACI, driving aberrant activation of the classical NFκB pathway and, possibly, directly impact transitional B cell activation.

Figure 2.

BCR and CD40 crosstalk. **(a)** Under normal conditions, CD40 synergizes with BCR signals to promote the pro-survival program in transitional cells via both the classical and alternative NFκB pathways. **(b)** In carriers of an autoimmune risk variant in PTPN22, enhanced CD40 signaling likely combines with enhanced BCR signals to promote increased survival of transitional cells with specific self-reactive properties.

Figure 3.

BCR and TLR crosstalk. **(a)** Under normal conditions, antigen-bound BCRs trigger BCR signaling and co-coordinately deliver self-ligands to a subset of TLRs located within endosomes, allowing TLR signals to promote either apoptosis or survival of transitional cells, depending on the context. **(b)** In subjects with the autoimmune disorder, WAS, enhanced TLR and BCR signaling drives preferential selection and aberrant cycling of autoreactive transitional B cells with a distinct set of antigen specificities into the naïve mature B cell compartment.