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Antigen and cytokine receptor signals guide the development of the naïve mature B cell repertoire

Sarah L. Rowland, Kathryn Tuttle, Raul M. Torres, and Roberta Pelanda

Integrated Department of Immunology, University of Colorado School of Medicine, National Jewish Health, Denver, CO, USA

Roberta Pelanda: pelandar@njhealth.org

Abstract

Immature B cells are generated daily in the bone marrow tissue. More than half of the newly generated immature B cells are autoreactive and bind a self-antigen, while the others are nonautoreactive. A selection process has evolved on the one hand to thwart development of autoreactive immature B cells and, on the other hand, to promote further differentiation of nonautoreactive immature B cells into transitional and mature B cells. These negative and positive selection events are carefully regulated by signals that emanate from the antigen receptor, whether antigen-mediated or tonic, and are influenced by signals that are generated by receptors that bind cytokines, chemokines, and other factors produced in the bone marrow tissue. These signals, therefore, are the predominant driving forces for the generation of a B cell population that is capable of protecting the body from infections while maintaining self-tolerance. Here, we review recent findings from our group and others that describe how tonic antigen receptor signaling and bone marrow cytokines regulate the selection of immature B cells.

Keywords

B cell development; B cell selection; B cell tolerance; Bone marrow; BCR signaling; Cytokine

Introduction

B cell generation in the bone marrow of adult mammals occurs through a tightly controlled developmental process. Productive rearrangement of immunoglobulin heavy (IgH)- and light (IgL)-chain gene segments in B lymphocyte precursor cells results in the generation and expression on the cell surface of a mature B cell antigen receptor (BCR). Whereas the pairing of Ig H and L chains determines the antigenic specificity of the newly formed BCR, their association with Ig- α (CD79a) and Ig- β (CD79b) allows the transduction of a signal inside the cell that promotes cell reactivity. Developing B cells first express a BCR on the cell surface in the form of IgM and as such are classified as immature B cells, or fraction E according to the Hardy's nomenclature [1, 2]. It is at the immature B cell stage that the BCR is tested for the first time for reactivity against autoantigens. It is estimated that more than 50 % of all newly generated immature bone marrow B cells in both mice and in humans express a BCR that is specific for an autoantigen [3, 4], and it is important that the development of these cells be constrained to diminish the autoimmune potential of the immune system. Autoreactive immature B cells are eliminated from the naïve repertoire through the process of tolerance, while those expressing a nonautoreactive BCR exit the

bone marrow via the blood and continue their maturation in peripheral tissues to join the naïve mature B cell compartment.

Self-reactive B cells are regulated at several checkpoints throughout their development, and studies have shown that at least four mechanisms function to mediate tolerance to autoantigens in immature B cells: receptor editing, deletion, anergy, and ignorance [5–9]. In contrast, immature B cells that display nonautoreactive BCRs continue to differentiate and progressively acquire expression of surface markers typical of more mature B cells, such as IgD, CD21, and CD23, before and after they travel to the spleen ([10–13] and Fig. 1). The surface expression of a mature and signaling competent BCR is absolutely required for these differentiation events, since genetically altered pre-B cells unable to express mature BCRs and immature B cells expressing a signaling-impaired BCR do not differentiate or leave the bone marrow [14–17]. In addition, deletion of the BCR on immature B cells blocks their further maturation and promotes back-differentiation to earlier developmental stages [18–20]. Furthermore, targeting the Ig- α /Ig- β heterodimer to the cell membrane promotes B cell development in the absence of Ig H and L chains [21]. Overall, these findings suggest that cell surface-assembled nonengaged BCRs transduce signals that promote differentiation of immature B cells into transitional and mature B cells. This antigen-independent BCR signal has been referred to as a tonic or basal signal [22]. In immature B cells, antigen-mediated and antigen-independent BCR signals function to regulate a B cell selection process that mediates the generation of the naïve B cell repertoire. These signals are, therefore, of great importance for the generation of B cell populations that are capable of protecting the body from infections while maintaining self-tolerance.

Survival and differentiation of B cells is also dependent on cytokines, chemokines, lipids, and other factors that are produced by non-B cells in the bone marrow microenvironment [23–29]. Upon binding their respective receptors on B cells, these factors promote signals that might also influence B cell selection and, therefore, the formation of the mature B cell repertoire. Thus, proper selection of immature B cells requires a microenvironment that provides factors critical for this process. Of note, the factors that act at the immature stage of B cell development to maintain survival and differentiation of immature B cells while they undergo negative and positive selection have not yet been established. The absence of cytokine involvement would be a rather unique case in lymphocyte biology. In this review, we summarize recent findings from our group and others that indicate how tonic BCR and cytokine receptor signaling operate in immature B cells to regulate their negative and positive selection.

BCR signaling in the selection of immature B cells

Tonic BCR signaling has been initially identified and characterized in mature B cells. A number of reports indicate that BCR expression in naïve mature B cells promotes cell survival and that the signaling capacity of the BCR, and not only its expression on the cell surface, is required for peripheral B cell maintenance [19–21, 30]. More recent studies have determined that tonic BCR signaling mediates this survival function through the activity of the PI3K-Akt pathway [31]. Experimental data support the idea that tonic BCR signaling occurs in the absence of antigen binding in early B cell development [21], but that the survival of mature B cells mediated by tonic signals may be dependent on low level of antigen stimulation [32]. These data, thus, suggest that tonic BCR signaling is qualitatively different in immature and mature B cells.

Interruption of tonic BCR signaling in immature B cells causes a developmental regression characterized by the re-expression of genes associated with pro-B and pre-B cell stages of development and, most importantly, the reactivation of V(D)J recombination [18, 33, 34].

Tonic BCR signals are, therefore, involved in mediating Ig allelic exclusion and the generation of B cells expressing a unique Ig H and L chain pair. Studies that further explored the molecular mechanisms of this process found that the activities of PI3K and NF- κ B p50 are required for the cessation of Rag expression and of V(D)J recombination in immature B cells [33, 35].

The function and nature of tonic BCR signaling in the positive selection of immature B cells was initially characterized only in the context of allelic exclusion. However, positive selection of immature B cells implies their further differentiation into transitional B cells with the expression of IgD, CD21, CD23, and other mature B cell markers, and the release of immature and transitional B cells from the bone marrow into the circulation where these cells reach the spleen and undergo final differentiation into mature B cells. To determine the role of tonic BCR signaling in the differentiation of immature B cells into transitional and mature B cells and to resolve the molecular intermediaries of the tonic BCR-signaling pathway in these biological processes, we chose to utilize mice with hypomorphic expression of the BCR [36, 37]. These mice express a wild-type form of the BCR, but only approximately 15 % of its normal level owing to a reduced expression of Ig- α . In initial studies, we have established that optimal tonic BCR signaling requires wild-type levels of surface BCR expression, as indicated by the fact that immature B cells expressing suboptimal BCR levels (BCR-low B cells) have impaired differentiation [37]. Further analyses pointed to the fact that immature B cells must express a very defined high level of surface IgM prior to expressing IgD and other differentiation markers of transitional and mature B cells (unpublished observations), and that immature B cells accumulate this high level of IgM over time [38]. To investigate the molecular components of tonic BCR signaling, we have analyzed differences in the activity of signaling molecules in BCR-normal and BCR-low immature B cells bearing defined Ig H and L chain pairs (3-83H; 3-83 κ and B1-8H;3-83 κ). In addition, we have searched for active genes that, once introduced into BCR-low immature B cells, could complement the tonic BCR-signaling defects of these cells by promoting their differentiation.

Because tonic BCR signaling has been shown to mediate cell survival in mature B cells, it was possible that the impaired differentiation of BCR-low immature B cells was caused by increased cell death. However, we found that further differentiation of BCR-low immature B cells into transitional and mature B cells was not significantly rescued by constitutive expression of Bcl-2 either in vitro or in vivo despite significantly enhanced cell survival [37]. These results indicate that in immature B cells, tonic BCR signaling activates a differentiation signaling pathway in addition to that promoting cell survival. The specific signaling components of these two pathways and how they differ from those activated downstream of antigen-engaged BCRs to promote tolerance are still unclear. The studies where specific signaling molecules have been evaluated in B cell development and B cell tolerance suggest that tonic and antigen-mediated signals use many of the same signaling intermediaries, but that some intermediates have a predominant role in one or the other process [7, 39–41]. Indeed, whether tonic and antigen-mediated BCR signals differ only quantitatively, or also qualitatively, is a matter of debate. However, the fact that co-expression of nonautoreactive and autoreactive BCRs and, therefore, the simultaneous induction of tonic and antigen-mediated BCR signaling can mediate the differentiation of immature B cells while promoting their tolerance [42–46] suggests that these two signals may activate qualitatively different pathways from their onset, potentially by means of differential receptor aggregation [47, 48].

The extracellular signal-regulated kinases Erk1/2 are classical mitogen-activated protein (MAP) kinases that are activated downstream of antigen receptors, as well as growth factor, cytokine, and G-protein-coupled receptors. The pathway leading to Erk phosphorylation and

activation is initiated with the recruitment to the cell membrane and activation of small GTP-binding proteins of the Ras family. The Ras family consists of 3 genes that encode the ubiquitously expressed proteins H-Ras, N-Ras, and K-Ras. The exchange of GDP for GTP results in the activation of membrane-localized Ras proteins [49], and single amino acid mutations in Ras that interfere with the GTPase activity lead to constitutively active forms with oncogenic potential [50, 51]. Active GTP-bound Ras proteins promote the activation of the Raf kinases, which in turn phosphorylate the MAP kinases MEK1/2, which then phosphorylate and activate Erk1/2 [52]. The Erk pathway has been shown to operate in both B and T cells [39, 53] mediating the development of pre-B cells from pro-B cells [54, 55] and the positive selection of thymocytes [56–60], respectively. In addition, reduced Erk phosphorylation has been previously correlated with developmental arrest and increased receptor editing in CD19-deficient immature B cells [61]. These findings suggested that the Erk pathway might be also activated downstream of tonic BCR signaling to regulate positive selection of immature B cells.

Using flow cytometric and western blot analyses, we found that untreated immature and mature B cells display very low basal levels of phosphorylated Erk1/2 (pErk) that are near the limit of the detection of the assays (unpublished observations). The Erk phosphorylation extent in naïve B cells can be enhanced by treating the cells with compounds such as sodium pervanadate that, by inhibiting tyrosine phosphatases, increase the detectable pool of phosphorylated proteins [62]. Using this method, we detected low pErk levels in immature and transitional B cells, and higher levels in mature B cells [37]. In addition, we found that immature and transitional B cell populations are largely heterogeneous with respect to pErk levels, while all mature B cells have consistently high levels of pErk [37]. Importantly, heterogeneous pErk levels were also found in monoclonal immature B cell populations where all B cells express the same BCR [37], suggesting that the differential Erk activity we observed was related to surface BCR levels and degree of B cell differentiation rather than BCR specificity. In accordance, pErk levels are reduced in BCR-low B cells relative to normal B cells [37]. In addition, we found that the few BCR-low mature B cells that differentiate *in vivo* display levels of pErk comparable to those of BCR-normal mature B cells [37]. These findings suggest that BCR expression and tonic BCR signaling mediate a low degree of Erk activation that is proportional to their levels, and that a threshold level of pErk must be reached in immature B cells to induce their further differentiation. In support of these hypotheses, we have determined that normal immature B cells manifest reduced *in vitro* differentiation into transitional B cells when either MEK or Erk are pharmacologically inhibited with U0126 and FR180204, respectively ([37], and unpublished observations). Conversely, expression of a constitutively active form of N-Ras (N-RasD12) restores the differentiation of BCR-low immature B cells into transitional and mature B cells both *in vitro* and *in vivo*, and through a process dependent upon the activity of MEK [37]. Constitutively active forms of Ras are known oncogenes that prolong the survival of transformed cells [51]. However, we found that normal differentiation of BCR-low immature B cells could only be restored by expression of a constitutively active form of Ras, and not through overexpression of Bcl-2, indicating that the ability of Ras to promote immature B cell development is distinct from its well-known pro-survival function. Dominant negative forms of Ras and MEK1 were previously shown to hinder the developmental progression of pro-B into pre-B cells [54], and a similar defect was also observed in Erk1/2 double-deficient mice [55]. Conversely, the expression of a constitutively active H-Ras form was shown to promote the differentiation of Rag1-deficient pro-B cells despite the absence of Ig expression [63]. These data suggest that pre-BCR signals are transmitted via the Ras-Erk pathway to induce the development of pre-B cells from pro-B cells. As the pre-BCR is also thought to transmit tonic signals [47], these data lend support to our conclusions that the Ras-Raf-MEK-Erk-signaling cascade also

participates downstream of tonic BCR signaling to regulate the differentiation of immature B cells into transitional and mature B cells.

The heterogeneous levels of pErk observed in immature and transitional B cell populations, in contrast to the homogeneous higher level found in mature B cells [64], suggest the existence of a selection checkpoint based on a threshold of Erk phosphorylation. Wild-type immature and transitional B cells display a range of surface IgM levels, from low to progressively high, and the pErk levels appear to correlate with those of surface IgM in these cells. These observations suggest that a critical level of IgM must be expressed to achieve the basal threshold of pErk that induces cell differentiation (Fig. 2). Thus, Erk may function to transform an analog signal into a digital output. This pattern of Erk activation is reminiscent of that observed in *Xenopus* during oocyte maturation in response to progesterone [53, 65], and it has also been suggested to occur in thymocyte positive selection and T cell activation [66, 67].

Further studies are needed to determine the pathway by which tonic BCR signaling regulates phosphorylation of Erk1/2 in immature B cells. The fact that pErk levels in BCR-low immature B cells are reduced relative to those in BCR-normal cells [37] suggests that BCR-low developing B cells have reduced expression and/or activity of kinases upstream of Erk. Because a constitutively active form of Ras restores the differentiation of BCR-low immature B cells, the signaling pathway between Ras and Erk is likely functional in these cells. Thus, the defect of BCR-low B cells probably originates from the defective activation of signaling molecules upstream of Ras or of Ras itself. Determining the level of expression and activity of the Ras forms and of the signaling pathway elements upstream of Ras and downstream of Erk in BCR-low and BCR-normal immature B cells will be instrumental for mapping the tonic BCR-signaling pathway that leads to the differentiation of immature B cells into transitional and mature B cells.

While our data support the idea that the Ras-Erk-signaling pathway is basally activated by tonic BCR signals, we cannot exclude an alternative model in which this pathway is activated downstream of other surface proteins that are expressed and/or function following tonic BCR signaling. CD19 [61] and BAFFR [68] are potential candidates in this context, although data from our laboratory discount a primary role for BAFFR (see below), and that of CD19 seems unlikely because BCR-low B cells express normal levels of this molecule (unpublished observations). Ras is also known to activate PI3K [69], and PI3K has been shown to be activated downstream of tonic BCR signaling to mediate survival of mature B cells and allelic exclusion in immature B cells [31, 33]. Thus, it remains possible that Ras mediates the differentiation of immature B cells into transitional and mature B cells by activating the PI3K pathway as well, a possibility that remains to be explored. Because the PI3K pathway regulates cell survival via Akt, it will be important to test a role of PI3K in differentiation independent of its effects on cell survival.

The selection events that occur at the immature B cell stage represent an important checkpoint for the elimination of autoreactive cells and the differentiation of B cells capable of reacting with foreign antigens. Our findings support a model in which sufficient BCR levels must be reached and maintained to achieve tonic BCR signals that activate Erk, in a Ras-dependent manner, to a level that specifically promotes positive selection of nonautoreactive immature B cells and, therefore, their differentiation into transitional and mature B cells (Fig. 2). Dysregulation of tonic BCR signals through altered activation of the Ras-Erk signaling cascade could, therefore, have a profound impact on the peripheral B cell repertoire. Regarding this, it is of interest that B cells of the lupus prone strain B6.Sle1 display superphysiological levels of pErk in the absence of stimulation [70], and that the *Sle1* locus has been suggested to increase selection of immature B cells into transitional B

cells [71]. In immature B cells that react with low avidity self-antigens, both antigen-dependent and independent signals are simultaneously transduced. It will be important to determine, therefore, whether Erk activation plays a role in the differentiation and bone marrow egress of anergic B cells, and whether enforced activation of Ras can induce the positive selection of autoreactive immature B cells despite the presence of antigen-mediated BCR signals.

Cytokine receptors in the selection of immature B cells

At present, it is unclear which cytokines and growth factors operate on immature B cells to regulate their survival and selection. Yet, it appears that immature B cells spend up to 2–3 days at this stage of differentiation [72–74] and may require specific signals from the bone marrow environment to fulfill their fate. Autoreactive immature B cells may undergo several attempts of receptor editing before expressing an innocuous BCR [75], while nonautoreactive immature B cells must accumulate enough surface IgM to receive proper tonic BCR signaling and undergo further differentiation [37, 38]. Whether these two events take place in the same or different environmental niches and if they are under the influence of cytokines and other factors are questions just starting to be addressed.

Interleukin-7 (IL-7) is produced in the bone marrow by stromal cells [27, 76, 77] and promotes the proliferation and survival of pro-B and pre-B cells and the differentiation of pro-B cells into pre-B cells [29]. It is known that wild-type immature B cells express IL-7R, but the level of expression and the signaling capacity of this cytokine receptor are reportedly lower than those of pre-B cells ([29, 78] and ImmGen website). How IL-7R expression and function relate to BCR specificity and BCR level is currently unclear. Autoreactive immature B cells retain similarities to pre-B cells, as seen by ongoing V(D)J recombination and marker profile [73, 79–81]. In accord, using the 3-83Ig mouse model, we found that RNA levels of the *il7r* gene are 1.5- to 2-fold higher in autoreactive than nonautoreactive immature B cells and higher expression is also present in BCR-low immature B cells (Fig. 3a). This suggests that IL-7 might play a role in extending the survival of autoreactive immature B cells. In fact, sorted autoreactive 3-83Ig⁺ immature B cells tend to survive better than nonautoreactive immature B cells in the presence of IL-7 in vitro (Fig. 3b). Potentially, therefore, IL-7 might sustain the survival of autoreactive immature B cells while they undergo receptor editing, and also that of nonautoreactive immature B cells while they accumulate surface BCR (Fig. 2). This cytokine, however, does not promote or halt further differentiation of IgM⁺ immature B cells into transitional and mature B cells [38, 64, 82]. Thus, other factors must exist in the bone marrow to promote further differentiation of nonautoreactive immature B cells into transitional B cells in synergy with tonic BCR signaling.

The interaction of the cytokine BAFF with the BAFF receptor, BAFFR, has been well characterized as an essential survival signal for peripheral B cells [83–91]. In addition, some evidences suggest that BAFF also functions at earlier B cell stages, as shown by the fact that BAFFR is already expressed on immature B cells [87, 88, 90–92]. We have extended those findings to show that BAFFR expression on immature B cells is positively regulated by tonic BCR signaling and correlates with BCR levels [64]. Indeed, we found that BAFFR levels on nonautoreactive immature B cells are significantly higher than those on BCR-low cells. Moreover, BAFFR levels can be increased on BCR-low cells by the expression of the constitutively active N-RasD12 [64], which mimics tonic BCR signals [37]. Autoreactive immature B cells, which internalize their BCR following antigen engagement and, therefore, have little surface BCR and tonic BCR signaling like BCR-low cells, express low to no BAFFR [64]. Accordingly, another group has shown that BAFFR expression inversely correlates with Rag2 expression and receptor editing in wild-type immature B cells [93].

Differential expression of BAFFR by nonautoreactive and autoreactive immature B cells suggests that these cells may display different sensitivity to BAFF. Indeed, sorted nonautoreactive 3-83Ig⁺immature B cells tend to survive better than autoreactive immature B cells in the presence of BAFF in vitro (Fig. 3b). Overall, these findings suggest that BAFF and BAFFR signaling play a role in the positive selection of nonautoreactive immature B cells into the peripheral mature B cell compartment (Fig. 2).

To investigate the role of BAFFR signals in immature B cell development, we studied the in vitro differentiation of nonautoreactive 3-83Ig⁺immature B cells in the presence or absence of BAFF. We determined that BAFF enhances, but is not required for the differentiation of 3-83Ig⁺ immature B cells into CD21⁺CD23⁻ transitional T1 B cells [64]. Moreover, the differentiation of transitional T1 B cells is primarily due to the pro-survival activity of BAFF [94], as evidenced by the fact that this population is partly generated in the absence of BAFF when cell survival is increased through transgene-mediated expression of Bcl-2 [64]. A more specific developmental role of BAFF was detected in the differentiation of T1 into T2 transitional B cells. Specifically, we found that BAFF induces the differentiation of CD23⁻ T1 transitional B cells into CD23⁺ T2 transitional B cells in vitro [64], and in accordance with similar findings in wild-type B cells [95]. In addition, we found that the capacity of BAFF to mediate the development of CD23⁺ transitional B cells is independent of that promoting cell survival, as it could not be complemented by superphysiological expression of Bcl-2 [64]. Thus, these data indicate that BAFF must possess a pro-maturation function in addition to the well-known function of promoting cell survival.

While in vitro experiments are important for dissecting mechanisms and specific functions, they are also contrived and potentially miss additional effects provided by the more complex lymphoid tissue environment present in vivo. Thus, we also performed in vivo studies to investigate the role of BAFFR in the differentiation of immature B cells with a wild-type Ig repertoire. Previous studies have shown a mild reduction in the number of transitional T1 B cells in the spleens of BAFF- and BAFFR-deficient mice [87, 88, 91], and a reduced export of bone marrow B cells into the spleen of BAFFR-mutant mice [83]. These data suggested a role for BAFF in the differentiation of transitional B cells in vivo. We investigated further this possibility using a mixed bone marrow chimera approach to increase the sensitivity of the assay. Extending earlier findings and our in vitro data, we found that both the T1 and T2 transitional wild-type B cells in the spleen are dependent on BAFF as they are significantly reduced in numbers in the absence of BAFFR. In contrast, only the CD23⁺, but not the CD23⁻, transitional B cell population in the bone marrow is affected by the absence of BAFFR [64]. Our data, thus, support a role for BAFF in the differentiation of immature into transitional B cells both in vitro and in vivo and suggest that a cytokine different from BAFF, or a different BAFF receptor, may also operate in the bone marrow environment to assist this process. A proliferation-inducing ligand (APRIL), hemokinin-1 (HK-1), macrophage migration inhibitory factor (MIF), and thymic stromal lymphopoietin (TSLP) are cytokines expressed in the bone marrow tissue and shown to operate at various B cell developmental stages [26, 96–99]. We found that none of these factors, however, aided the differentiation of immature B cells into transitional B cells in vitro in the absence of BAFF [64] and, thus, are not likely the factors that promote the differentiation of CD23⁻ transitional B cells in the bone marrow of BAFFR-deficient mice.

An important question is whether BAFFR signaling could drive the differentiation of immature B cells in the absence of proper tonic BCR signaling. If so, autoreactive immature B cells could be positively selected into the circulating mature B cell population by a malfunctioning BAFFR-signaling pathway and/or superphysiological BAFF levels. However, we found that increasing BAFFR levels is not sufficient to restore the differentiation of either BCR-low or autoreactive immature B cells [64]. These data indicate

that the Ras-Erk cascade is not activated downstream of the BAFFR-signaling pathway. Instead, expression of BAFFR is an event promoted by BCR expression and N-Ras signaling [64]. Our data, therefore, indicate that tonic BCR signaling is the primary driving force in the differentiation of immature B cells into transitional and mature B cells, and that BAFFR signaling requires tonic BCR signaling to reinforce B cell differentiation.

Concluding remarks

As we discussed above, positive selection of immature B cells into the peripheral mature B cell pool is primarily regulated by tonic BCR signaling and secondarily by BAFFR signaling. These signals promote the differentiation of immature B cells into transitional and mature B cells, and they are later essential to the survival of mature B cells. Without tonic BCR signaling, or even with reduced levels, immature B cells are not incorporated into the peripheral B cell pool. An intriguing issue to solve will be to determine whether negative selection of autoreactive immature B cells occurs because of antigen-mediated BCR signaling or rather due to the absence of appropriate tonic BCR signaling. Moreover, it will be important to investigate whether mice that spontaneously develop autoimmunity (such as those of the MRL/*lpr* and B6.Sle123 strains) display an altered threshold for the positive selection of immature B cells that may contribute to the development of a larger autoimmune B cell repertoire. As several groups have reported, B cell selection is influenced by environmental cues in the form of cytokines, chemokines, and other factors. How the bone marrow environment influences the central selection of immature B cells is just starting to be appreciated. Future challenges will be to increase our knowledge of the bone marrow microenvironment and to establish whether specific and distinct niches exist for the processes of negative and positive selection of immature B cells, the environmental factors that affect these processes and whether and how these factors are compartmentalized. Furthermore, it appears that in developing B cells (like in developing thymocytes), positive selection is initiated only once pErk has reached a set threshold. A major challenge will be to determine what factors regulate and define this threshold that separates negative and positive selection. Finally, these findings will have to be applied to our understanding of diseases, to determine whether individuals prone to either B cell-mediated autoimmunity or B cell deficiency display abnormal tonic BCR-signaling tuning and/or aberrant bone marrow microenvironments.

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Fig. 1.

Schematic representation of central B cell selection. B cell development in the bone marrow results in the generation of immature B cells each expressing a different antigen receptor. A fraction of immature B cells is autoreactive, binds self-antigens, and undergoes negative selection (tolerance). Immature B cells that do not bind self-antigens (nonautoreactive cells) continue their differentiation into transitional and mature B cells and are positively selected into the circulation and the peripheral lymphoid compartment

Fig. 2.

A model for positive selection of immature B cells. Surface BCR levels vary on immature B cells depending on their developmental progression and avidity for self-antigen. Differentiation of immature B cells (IgM^+) into transitional and mature B cells (IgM^+IgD^+) and entry into the peripheral B cell population depends on BCR expression and tonic BCR signaling. Tonic BCR signaling translates into Erk phosphorylation, and a threshold level of pErk may be necessary to set in motion these biological events. The cytokine IL-7 may be important for maintaining the survival of immature B cells while they attempt to reach the threshold level of BCR expression and tonic BCR signaling required for positive selection. Once the BCR has reached the threshold level for positive selection, BAFFR is also expressed and immature B cells react to available BAFF. BAFFR signaling contributes to the differentiation of immature B cells into transitional and mature B cells

Fig. 3.

Autoreactive and nonautoreactive immature B cells display differential response to IL-7 and BAFF. **a** Relative *il7r* RNA levels in 3-83Ig⁺ autoreactive, nonautoreactive, and BCR-low immature B cells. Bone marrow B220⁺IgD⁻CD23⁻CD43⁻ immature B cells were sorted from nonautoreactive 3-83Igi,H-2^d (white bar), autoreactive 3-83Igi,Rag1^{-/-},H-2^b (black bar), and 3-83Igi-low mice and subjected to whole genome microarray analyses. The bars represent the arithmetic mean and SD of the normalized processed signal for the *il7r* gene from three independent samples and microarrays reporting 3 different probes. The signals detected in autoreactive and low cells were normalized to those of nonautoreactive cells that were set to one. **p* = 0.008 (two-sample randomization paired test). Additional information about this analysis and the mice can be found in [64]. **b** Survival of 3-83Ig⁺ autoreactive and nonautoreactive immature B cells in culture. Bone marrow immature B cells were sorted as B220⁺IgD⁻CD23⁻CD43⁻ from nonautoreactive 3-83Igi,H-2^d mice (white bar) and as B220⁺IgM⁻IgD⁻CD23⁻CD43⁻ from autoreactive 3-83Igi,H-2^b (black bar) mice. Sorted cells were cultured at equal numbers in complete medium with or without IL-7 or BAFF. After 3 days of culture, live and dead cells were counted following trypan blue staining. The bars represent the arithmetic mean and SD of live cell numbers from 3 independent experiments. The difference between autoreactive and nonautoreactive cells was not statistically significant at each condition, but the trend was the same in each of the three experiments. Additional information about the mice can be found in [75]