Review

The Pre-B Cell Receptor and Its Function during B Cell Development

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The process of B cell development in the bone marrow occurs by the stepwise rearrangements of the V, D, and J segments of the Ig H and L chain gene loci. During early B cell genesis, productive IgH chain gene rearrangement leads to assembly of the pre-B cell receptor (pre-BCR), which acts as an important checkpoint at the pro-B/preB transitional stage. The pre-BCR, transiently expressed by developing precursor B cells, comprises the Ig μH chain, surrogate light (SL) chains VpreB and λ5, as well as the signal-transducing heterodimer Igα/Igβ. Signaling through the pre-BCR regulates allelic exclusion at the Ig H locus, stimulates cell proliferation, and induces differentiation to small post-mitotic pre-B cells that further undergo the rearrangement of the IgL chain genes. Recent advances in elucidating the key roles of pre-BCR in B cell development have provided a better understanding of normal B lymphopoiesis and its dysregulated state leading to B cell neoplasia. *Cellular & Molecular Immunology*. 2004;1(2):89-94.

Key Words: B cell development, pre-B cell receptor, surrogate light chains, B cell receptor

Introduction

During postnatal life, mammalian B lymphopoiesis takes place in the bone marrow (BM), where B cell development occurs in a stepwise manner, starting from multipotential progenitors that then give rise to B lineage-committed precursors. Over the past decade, a large body of work has been devoted to characterizing B cell differentiation stages by changes in the expression of phenotypic markers as well as by the status of Ig gene rearrangements (1-3). During B cell ontogeny, progenitor B cells (pro-B) undergo IgH chain gene rearrangement and differentiate into large cycling precursor B cells (pre-B) that synthesize cytoplasmic μH chains and transiently express the pre-B cell receptor (pre-BCR) on their surface membrane. After undergoing three to five rounds of cell division, large pre-B cells differentiate into small non-dividing pre-B cells. Following the rearrangement of IgL chain genes in small pre-B cells, immature B lymphocytes are formed upon the cell surface expression of the IgM molecule as the B cell receptor (BCR) (2, 4-6). After extensive selection against immature B cells with autoreactivity, newly-generated functional B cells then leave the BM and migrate *via* the blood stream to the spleen, where they finally differentiate into mature B lymphocytes. The developmental process of B lineage cells in BM is closely influenced by interaction with their microenvironment, including stromal cells and cytokines such as IL-7 (7). Gene-targeting studies performed on numerous mouse models have demonstrated that developing B cells undergo positive and negative selections at critical checkpoints involving signaling through the pre-BCR and BCR. Extensive studies on the quality control and homeostatic regulation of B lymphopoiesis have provided much insight into the complex process of shaping the functional B cell repertoire (8, 9). Recently, a series of reviews have been published to address how the BCR signaling affects the fate of the immature and mature B cells (9-11). Here we focus on reviewing recent advances in understanding pre-BCR expression and its critical roles in B cell development at the pro-B/pre-B transitional stage.

IgH chain gene rearrangement and the formation of pre-BCR

Substantial progress has been made in recent years towards elucidating the molecular mechanisms by which the primary antibody repertoire is generated and shaped. During B cell development in mice and humans, the Ig genes encoding antibody variable regions are assembled in a stepwise fashion from gene segments termed V, D, and J for the IgH locus and V and J for the IgL locus through a site-specific recombination process regulated by the recombination-activating genes RAG-1 and RAG-2 (12, 13). In pro-B cells, D_H to J_H rearrangements are initiated first, followed by V_H to D_HJ_H-rearrangements at the IgH chain locus. It is now clear that almost all pro-B cells have D_H to J_H rearranged at two alleles, suggesting that both alleles are accessible for V_H to D_HJ_H-rearrangements (14).

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Abbreviations: BM, bone marrow; pre-BCR, pre-B cell receptor; BCR, B cell receptor; pro-B, progenitor B; pre-B, precursor B; SL, surrogate light; EBF, early B cell factor.

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Ig gene rearrangement is an error-prone process that occurs randomly in and out of frame. When the $V_HD_HJ_H$-rearrangements are successful, a μH chain, the product of a rearranged H chain locus, is synthesized and binds to the Hsp70 chaperone called Bip retention protein in the endoplasmic reticulum. The nascent μH chain is further tested for its fitness in association with a surrogate light (SL) chain that is composed of VpreB and λ5 polypeptides (15). Only those μH chains that can pair with an SL chain are selected and then associated with the Igα/Igβ heterodimers, which allows a pre-BCR to be deposited on the surface membrane of large pre-B cells. Shortly after pre-BCRs have been deposited on the cell surface, the recombination machinery and SL synthesis are turned off. It is assumed that the successful generation of a pre-BCR causes the termination of further rearrangements at the other D_HJ_H- rearranged allele.

Structurally resembling the BCR, a pre-BCR comprises IgH and SL chains associated with the Igα/Igβ signaling heterodimer (Figure 1A). In contrast to a conventional IgL chain in BCR, the SL chain is a heterodimer composed of two invariant polypeptides: an Ig V-like sequence called VpreB and an Ig C-like sequence called λ5 (6, 16). The μH chains are covalently attached to two SL chains *via* a disulfide bond between the constant domain of each μH chain and the Ig-like domain of each λ5. In mice, there are two *VpreB* genes named *VpreB1* and *VpreB2* with high structural homology, whereas human has only one *VpreB* gene (17). The VpreB is tightly associated with λ5 through non-covalent interaction. The Igα/Igβ heterodimer responsible for signal transduction is the final component for assembling a functional pre-BCR, which allows transit of the newly assembled pre-BCR through the Golgi apparatus en route to the surface of the pre-B cell. Recently-generated monoclonal antibodies that are capable of distinguishing SL chains in free form from those associated with μH chains have been used for studying the structural components of the pre-BCR and for elucidating the potential functions of SL in signal transduction (18, 19). The pre-BCR complex is expressed only transiently during B cell development, presumably because the expression of λ5 is down-regulated as soon as the pre-BCR complex begins to be assembled (17). Moreover, the level of pre-BCR expression is extremely low on developing B cells, which has hindered a systematic characterization of their expression pattern in the BM. Available data suggest that approximately 5% of murine and 18% of human B cells in the BM express a pre-BCR on their surface (20). Consistent with these findings, we have found that cytoplasmic μ^+ pre-B cells with surface SL expression comprise only 0.9±0.2% of total nucleated cells in mouse BM (Figure 1B). In general, a similar pattern of SL expression has been observed in large cycling μH chain-expressing pre-B cells from both mouse and human BM samples. However, certain discrepancies in SL expression at various differentiation stages exist between mouse and human B cell populations. Studies by Cherayil and Pillai (21) demonstrated SL expression on immature B cells in mouse BM by immunofluorescent microscopy. Similarly, Guelpa-Fonlupt et al. (22) found SL chains expressed by both pre-B cells and $sIgM^+$ immature B cells from human BM. In mouse some pro-B cells express SL chains associated with Igα/Igβ heterodimer to form a surface receptor called the pro-B cell receptor, although it is currently unclear whether this receptor has a signal function in pro-B cell differentiation. In contrast, VpreB and λ5 proteins expressed in human pro-B cells immediately undergo intracellular degradation. Using an enhanced immunofluorescence assay, Wang et al. (23) have not found any pro-B cells bearing SL chain components in humans. Some human B cells coexpress surrogate and conventional L chains ($V\text{-}preB^+L^+$) and display evidence of receptor editing in their unusual H and L chain antibody repertoire. It has been reported that 68% of the antibodies expressed by $V\text{-}preB^+L^+$ B cells are autoreactive, including anti-nuclear antibodies (24). These data indicate that $V\text{-}preB^+L^+$ B cells are a unique subset of normal circulating human B cells that escape central tolerance mechanisms and express self-reactive antibodies. Thus, while SL chains are not exclusively expressed in pre-B cells, they seem predominantly characteristic of this stage. Different patterns of SL chain expression have been observed between normal

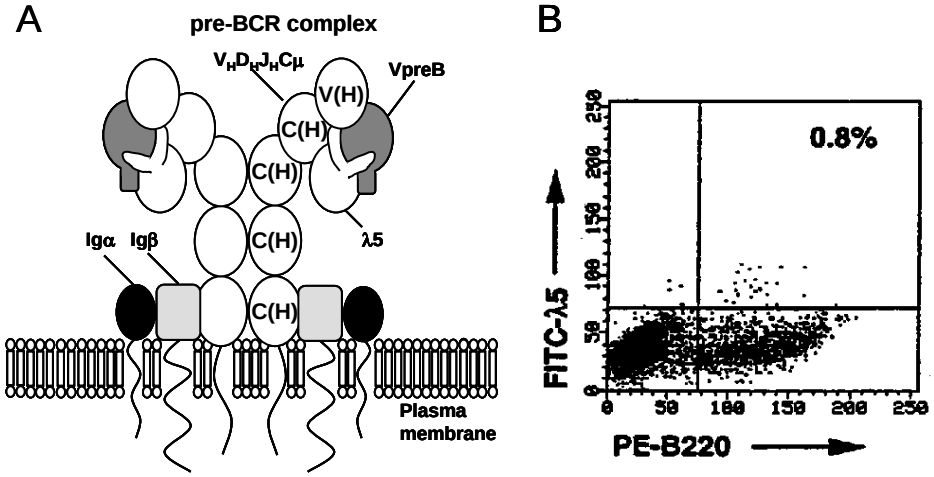

Figure 1. (A). Scheme of the pre-BCR complex, composed of Ig μH chain, SL chains VpreB and λ5 and the signal-transducing heterodimer Igα/Igβ. (B). Flow cytometric analysis of λ5 expression on $B220^+$ B cells from freshly prepared normal C3H/HeJ mouse BM. The incidence of $B220^+$ $\lambda5^+$ B cells is indicated in the representative profile of five separate experiments (Lu L and Osmond DG, unpublished).

primary and transformed cells at various differentiation stages as well as between different species. All Abelson leukemia virus transformed mouse cell lines at various development stages express SL chains (25). Moreover, the pre-BCR expressed in these cell lines can transduce signals when triggered with anti-μ antibodies. These pre-BCR-expressing cell lines have provided useful tools for delineating the potential functions of the pre-BCR in B cell development.

Pre-BCR signaling and its regulation

Pre-BCR signaling is necessary for pre-B cell differentiation, but the nature of pre-BCR signaling has long been an enigma in B cell biology. Whether pre-BCR surface expression is sufficient to initiate a constitutive signal or if pre-BCR signaling is triggered by receptor-ligand interaction has been under intensive investigation. Several observations have pointed to the existence of a ligand from the BM microenvironment that binds to either the μH chain or the SL components of the pre-BCR. Consistent with early findings that soluble pre-BCR binds to mouse ST2 stromal cells, Gauthier et al. (26) have recently identified a stromal cell-expressed ligand, Galectin-1, which can specifically bind to recombinant SL and the pre-BCR. This interaction mediates synapse formation between pre-B cells and stromal cells and initiates intracellular tyrosine kinase activity. Accumulating data also suggest that the pre-BCR signals in a ligand-independent fashion. It has been observed that proliferation can occur from a single pre-B cell suspended in liquid culture without any external ligands for the pre-BCR (27). Moreover, monoclonal antibodies specific for the pre-BCR components, i.e. VpreB, λ5 or μH chains, do not perturb early B cell development when administered *in vivo* whereas antibodies against μH chains can significantly affect B cell development at the immature B cell stage. A new study by Ohnishi and Melchers (28) has demonstrated that retroviral transduced expression of various λ5 mutants lacking the N terminus in λ5 deficient but μH chain expressing pre-B cell lines resulted in increased display of the pre-BCR, delayed pre-BCR internalization, and reduced amount of phosphotyrosinated proteins. Thus, these results suggest that appropriate pre-BCR signaling might depend upon the unique non-Ig-like region of λ5. Since these cells are clonally expanded in cultures lacking stromal cells and any exogenous cytokines, Ohnishi and Melchers propose that either pre-B cells express a ligand that specifically interacts with and cross-links the non-Ig portion of SL or that SL chains can directly interact with each other to result in aggregation and signaling of the pre-BCR. Despite the compelling evidence presented by Melchers and his colleagues (28), the important question remains whether the signaling *via* the pre-BCR in virally transformed pre-B cell lines reflects signal transduction in normal developing B cells. Hence, studies on primary pre-B cells from SL chain gene-targeted mutant mice will be necessary to corroborate these findings.

Recent evidence suggests that early B cell development is critically regulated by a group of transcriptional factors, including EBF (early B cell factor) and Pax-5 that activate genes required for B cell differentiation (29). EBF-deficient progenitor B cells do not express VpreB and λ5. Functional EBF-binding sites have also been identified in the promoters for Pax-5, VpreB and λ5 (30). In agreement with these findings, *in vitro* studies have shown that ectopic expression of EBF in cell lines can activate transcription of the endogenous λ5 gene. In Pax-5-deficent mice, B cell development is arrested at the early pro-B cell stage due to severely impaired V_H to D_HJ_H-rearrangements at the IgH locus (31). Expression of functionally rearranged Igμ or chimeric Igμ/Igβ transgenes in Pax-$5^{-/-}$ mice fails to advance B cell development to the pre-B cell stage. Although pre-BCR expression is detected on Igμ$^+$ Pax-$5^{-/-}$ pro-B cells, there is no signaling response. Collectively, these data suggest that Pax-5 controls B cell genesis at an early developmental stage that is unresponsive to pre-BCR signaling. Recently, BLNK, a signaling adaptor protein, has been found to be a target gene of Pax-5 that induces intracellular calcium flux in response to pre-BCR signaling. Studies by Schebesta et al. (32) have demonstrated that expression of BLNK in Igμ$^+$ Pax-$5^{-/-}$ pro-B cells can restore pre-BCR signaling and induce cell proliferation. The signals generated from the pre-BCR originate within a juxta-membrane signaling complex around the Igα/Igβ heterodimer. These proteins possess several immuno-receptor tyrosine-based activation motifs (ITAM) that recruit and activate the protein tyrosine kinase Syk. Activated Syk leads to protein kinase C activation and calcium mobilization. Additionally, signaling through the pre-BCR regulates the activation of NF-κB, a critical transcriptional factor involved in enhancing cell survival and suppressing apoptosis (33). Recent knock-out mouse studies have demonstrated the essential role of the Src-family tyrosine kinases Blk, Fyn and Lyn in pre-BCR- mediated NF-κB activation signaling during B cell development (34, 35). It has also been recognized that cooperation between IL-7 and the pre-BCR plays a critical role in B cell selection (36), and there is new evidence that pre-BCR signaling mediates a selective proliferative response of pre-B cells to IL-7 *via* an ERK/MAP kinase-dependent pathway(37). Hence, these recent findings provide a better understanding of the roles and mechanisms of pre-BCR signaling in regulating B cell development.

Roles of pre-BCR in B cell development

Studies on cell population dynamics by Osmond and his colleagues (38) provided the first *in vivo* evidence of substantial cell loss among precursor B cells in normal mouse BM. Subsequently, we have demonstrated that B cell apoptosis occurs most markedly at two developmental stages associated with IgH chain gene rearrangements and BCR expression (39). Our data from apoptotic kinetics studies have suggested that as many as 75% of large pre-B cells in BM are deleted by apoptosis during development, which is in good accordance with the findings that only 20% of the $V_HD_HJ_H$-rearrangements in precursor B cells are productive (13, 14). It is now well recognized that the pre-BCR expression on precursor B cells serves as a critical checkpoint during early B cell development (40).

Recent studies on gene-modified mice have shown that surface deposition of the pre-BCR initiates signaling that can induce proliferation of pre-B cells and down-regulate the SL chain expression. In μMT mice, the μH chain transmembrane gene segment is deleted by gene-targeting. In these animals, B cells can no longer express the membrane bound form of μH chain, causing a complete block in B cell development at the pro-B/pre-B transitional stage in BM (41). As a result of abolished proliferative expansion of pre-B cells, the population size of pre-B cells is severely diminished and no immature or mature B lymphocytes are found in blood, spleen or lymph nodes of these mutant mice. Findings of normal B cell development and maturation in mice with targeted deletion of the μH chain constant region have reinforced the conclusion that it is the membrane-bound deposition of a μH chain that is critical for pre-B cell differentiation and proliferation (42). Mice deficient in the SL component λ5 also show impaired B cell development at the pro-B/pre-B transitional stage (43). Pre-B cells from $\lambda5^{-/-}$ mice do not enter the proliferative phase of expansion. However, λ5 deficiency does not cause a complete block in B cell development as low numbers of pre-B cells and immature B lymphocytes are detected in BM. A similar phenotype has been observed in mice lacking the VpreB1 gene or VpreB1/VpreB2 genes (44). By using a transgenic mouse model in which the expression of an Ig μH chain gene is solely driven by a tetracycline-controlled transactivator, Hess et al. (45) have found that the *de novo* synthesis of μH chain in transgenic pro-B cells not only induces differentiation but also proliferation of the pro-B cells. This elegant study also provides direct evidence that proliferation of pre-B cells induced by *de novo* expressed μH chain depends on the presence of the SL chain paired with the μH chain to form a pre-BCR. These data demonstrate a crucial function of the pre-BCR in inducing the proliferative expansion of pre-B cells, following the production of a functional μH chain that pairs with an SL chain. A recent study by Su et al. (46) has shown that certain pre-B cells from BLNK/$\lambda5^{-/-}$ mouse BM express a novel receptor complex that contains a μH chain but lacks any SL chain. Anti-μ treatment of these pre-B cells induces tyrosine phosphorylation of substrate proteins and a strong calcium release. Furthermore, expression of this novel pre-BCR is associated with a high differentiation rate toward κLC-positive cells. Given that B cell development is only partially blocked and allelic exclusion is unaffected in SL-deficient mice, these findings suggest that the SL-lacking-pre-BCR might share important functions with or be able to replace the conventional pre-BCR in regulating B cell development. It is of interest to note that prematurely expressed Igκ-transgenes can rescue B cell development in λ5-deficient mice. It is thought that the transgenes substitute for the SL-chain receptor in inducing proliferative expansion of pre-B cells that produce functional μH chains (47, 48). Although the underlying signaling mechanism remains to be determined, these findings suggest the importance of surface-expressed intact pre-BCR in driving proliferative expansion of pre-B cells and their progression to later stages of development.

Genetic studies have revealed that approximately half of the pro-B cells initiate IgH chain gene rearrangements at only one allele (VDJ_H/DJ_H) whereas the other half are VDJ_H/VDJ_H rearranged at both alleles (6, 49). In cells with VDJ_H/DJ_H rearranged, the functional rearranged VDJ_H allele encodes a μH chain to pair with an SL chain as the pre-BCR expressed on the cell surface while the V_H to DJ_H rearrangements at the second allele are inactivated by allelic exclusion. In VDJ_H/VDJ_H rearranged pro-B cells, only one allele can encode a functional μH chain that is capable of pairing with SL chain for the pre-BCR assemblage. In μMT mice, however, the deletion of the transmembrane exon causes completely loss of allelic exclusion, highlighting the critical role of membrane bound μH chain in signaling this mechanism (50). Allelic exclusion is not restored by the secreted form of μH chain, introduced as a transgene in mice. These findings favor the notion that the pre-BCR needs to be expressed on the surface of precursor B cells in order to signal IgH allelic exclusion. Interestingly, the IgH loci are allelically excluded in $VpreB1^{-/-}/VpreB2^{-/-}$ deficient mice (51). Since it has been shown that λ5 can pair with H chain in the presence of VpreB3 and several other proteins in pre-B cell lines, it remains to be examined whether λ5, in the absence of VpreB1 and VpreB2, can form a modified pre-BCR that is able to signal IgH allelic exclusion at the stage of pre-B cells (52). Hence, λ5 and VpreB may play differential roles in signaling allelic exclusion during B cell development.

Recent studies have provided strong evidence for a direct involvement of pre-BCR signaling in the repression of RAG-1, RAG-2 and terminal deoxynucleotidyl transferase gene during the pro-B to pre-B cell transition (32). Comparative analysis of large and small pre-B cells has demonstrated that cessation of pre-BCR expression after SL chain gene silencing is linked to the cell cycle exit of large pre-B cells and to the onset of L chain gene rearrangement driven by upregulated RAG-1 and RAG-2 gene expression in small pre-B cells (23). During B lymphopoiesis the transitional expression of pre-BCR in precursor B cells coincides with a dramatic change in the cells' requirement for growth factors. While pro-B and large pre-B cells require both stem cell factor and IL-7 for their survival and proliferation, the small pre-B cells become unresponsive to these growth factors due to loss of the corresponding cytokine receptors (53). It has been shown that the pre-BCR is involved in the down-regulation of the IL-7 receptor and stem cell factor receptor (c-kit) expression. However, a full understanding of how cytokines such as IL-7, thymic stromal lymphopoietin (TSLP) and HK-1 regulate growth and survival of precursor B cells at the pro-B/pre-B transitional stage is still an important challenge, as these factors play important roles not only in normal B cell development but also in the course of B cell diseases (54, 55).

Although much remains to be studied about the precise mechanism for SL in signaling selection of developing B cells, it is clear that expression of the pre-BCR leads to activation of the adaptor protein BLNK and the cytoplasmic kinase Btk. Several lines of evidence has suggested that dysregulated pre-BCR signaling is involved in the development of B cell malignancy. Recent findings of a high incidence of pre-B cell lymphoma in BLNK deficient mice demonstrate that BLNK acts as a tumor suppressor *in vivo* (56). Moreover, Btk/BLNK double mutant mice have a much higher incidence of pre-B cell

tumor (57). Around 50% of childhood pre-B acute lymphoblastic leukemias show a complete loss or a drastic reduction of BLNK expression (58). Future studies of cooperation between BLNK and Btk will shed new light on the function of pre-BCR signaling. It has become clear that the pre-BCR, acting as a biological sensor that regulates pre-B cell differentiation, is essential for the growth and survival of precursor B cells. Further knowledge of the pre-BCR signaling pathways and effector molecules will provide a fuller understanding of B lymphopoiesis. Such information should be valuable for uncovering novel mechanisms of dysregulated pre-BCR signaling in the development of humoral immunodeficiency as well as neoplasia.

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