Summary. Recent studies suggest that developmental check-points in B-lymphopoiesis are set in order to test the B cell receptor signaling competence. In these checkpoints ligand-independent and ligand-dependent receptor signals confer B-lymphopoiesis with positive and negative selection events. As a consequence, B-lymphocytes are forced to make crucial fate decisions to determine developmental progression, survival or apoptosis. In here we review recent progress in unraveling molecular and cellular mechanisms for the role of B cell receptor signaling competence in determination of the B cell fate.

Key words: B cell development, Immunoglobulin gene rearrangement, Antigen receptor signaling, Positive selection, Negative selection, Receptor editing

Introduction

B lymphopoiesis is a process initiating in the bone marrow (BM) and continuing in the periphery. Throughout lifespan there are very small changes in the number of peripheral B cells (Freitas et al., 1986). This homeostasis in the B cell lineage is strictly controlled by processes of proliferation differentiation and apoptosis. Studies have shown that mice produce about 2x10^7 immature B cells per day. Most of these cells die shortly after migration from the bone marrow, and only small number of cells (3%) are selected into the long-lived pool (Osmond, 1991). Since the fate of B lymphocytes primarily depends on appropriate expression and function of the B cell antigen receptor (BCR), it is generally thought that most of B cell death results from receptor incompetence. Early stages of B cell development are guided by immunoglobulin (Ig) gene rearrangements where B cells attempt to assemble a functional receptor. These receptors are then tested for autoreactivity, a process that limits further developmental progression. In peripheral B cells, a continuous flow of tonic receptor signals is essential for long term survival (Lam et al., 1997). Hence, life and death decisions in B lymphopoiesis are determined based on the BCR signaling competence, which is tested throughout multiple positive and negative selection check-points. In here we will review the role of BCR signaling in determining the B cell fate.

The B cell receptor signaling pathway

Assembly and expression of pre-BCR and BCR

The BCR complex is a multiprotein structure composed of immunoglobulin heavy (IgH) and light (IgL) chains that are noncovalently associated with two signal transducing elements Igα (CD79a) and Igβ (CD79b) (Reth et al., 2000; Gauld et al., 2002). B lymphocytes assemble the antigen receptor during development through a highly ordered and well-controlled process that is conducted by the V(D)J recombinase genes (Bassing et al., 2002). Commitment to the B lineage depends on expression of transcriptional factors such as Pax5, E2A and early B cell factor (EBF) (Rolink et al., 2001). Pax-5 is a unique transcription factor that appears to be essential for maintaining B lineage commitment, as in the absence of Pax-5 B cell development is aborted, and, instead giving rise to myeloid and T cell lineage cells (Rolink et al., 2001). The V(D)J recombination initiates at the heavy chain locus in proB cells with DH to JH joining, followed by VH to DHJH recombination. A productive VDJ encodes mH chain that pairs with the surrogate light chain (SLC) components, VpreB and λ5, and is expressed as pre-BCR, a developmental stage defined as large preB (reviewed in: Meffre et al., 2000; Rolink et al., 2001). Signals generated by the pre-BCR are required to suppress V(D)J recombination and to establish allelic exclusion at the HC locus (Constantinescu and Schlissel, 1997; Melchers et al., 1999). The pre-BCR signaling also promotes positive selection and proliferative

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expansion that is IL-7-dependent (Melchers et al., 1999; Rolink et al., 2001). Failure to express the pre-BCR or the SLC components abrogate allelic exclusion and allows ongoing V(D)J recombination (Rajewsky, 1996; Meffre et al., 2000; Rolink et al., 2001). Also, lack or insufficient pre-BCR signaling imposes developmental arrest in several signaling mutated mice (reviewed in: Meffre et al., 2000; Gauld et al., 2002; Niirro and Clark, 2002). Hence, expression and signaling of the pre-BCR confer an important check-point in B lymphopoiesis. After several divisions, large preB cells exit cell cycle and become small preB cells. V(D)J recombinase genes are upregulated and rearrange light chain genes, first at the kappa locus and later at the lambda locus (Rajewsky, 1996; Constantinescu and Schlissel, 1997; Melchers et al., 1999). Productive LC rearrangement leads to BCR assembly and SLC is replaced with the conventional κ or λ, and the cells become immature B cells that first express surface IgM (Fig. 1).

Pre-BCR and BCR signaling

Both preBCR and BCR form a complex with the Igα and Igβ proteins, linking the extracellular Ig with the intracellular signal transduction pathways. Several protein tyrosine kinases are activated rapidly following engagement of the BCR/preBCR complexes, including members of the Src family (Lyn and Blk), the Syk/ZAP70 family (Syk), and the Tec family Bruton's tyrosine kinase (Btk) (Hsueh and Scheuermann, 2000). The nature of the preBCR signaling is unclear. Several studies suggest that these signals are ligand-independent and are generated from expression and aggregation of the preBCR (Shaffer and Schlissel, 1997; Ohnishi and Melchers, 2003). Other studies, however, have indicated a stromal cell surface ligand, perhaps galectin-1, might interact with the preBCR to trigger its activity (Bradl and Jack, 2001). It is clear that deposition of preBCR on the surface initiates signals that lead to down-regulation of SLC expression and developmental progression (Melchers et al., 1999; Rolink et al., 2001). This down-regulation depends on expression of the adapter protein BLNK as revealed in BLNK deficient model (Flemming et al., 2003), and is mediated via an ERK/MAP kinase-dependent pathway (Fleming and Paige, 2001). Proliferative expansion of preB cells is also abolished in mice deficient of µH transmembrane exon (Kitamura et al., 1991), λ5 (Kitamura et al., 1992) or double-deficient in VpreB1 and VpreB2 (Mundt et al., 2001), and in Ig-αΔC-Ig-βΔC mice (Reichlin et al., 2001). Recently, it has been demonstrated that this signal transduction is also mediated by the non-Ig portion of λ5 and the seven arginine residues in it (Ohnishi and Melchers, 2003). These studies implicate the mandatory requirement of preBCR expression and signaling for this developmental progression.

Immature and mature B cells generate signals through the BCR upon binding of antigen to membrane-Ig, which induces receptor aggregation and phosphorylation of the immunoreceptor tyrosine-based activation motifs (ITAMs) of Igα and Igβ. Phosphorylation of these residues by the SRC-family protein tyrosine kinases (PTK) Lyn, Fyn and Blk results in the recruitment of Syk kinase. The recruitment of Syk to Igα/β facilitates its phosphorylation and activation as well as activation of the TEC-family PTK Btk, leading to activation of downstream signaling cascades (Kurosaki, 1999; Gauld et al., 2002; Niirro and Clark, 2002). Phosphatidylinositol 3-kinase (PI3K) and phospholipase C-γ2 (PLCγ2) are important downstream

Fig. 1. B lineage development from the bone marrow to the periphery. Developmental stages of B lymphopoiesis are illustrated. Cell surface expression of the preBCR (Igμ, λ5 and VpreB) or BCR (Igμ and κ or λ) is shown.
effector molecules of BCR signaling and several adaptor molecules, such as BLANK (Fu et al., 1998), Grb2 (Yankee et al., 2003) and BAM32 (Han et al., 2003), connect the kinases with the effectors. The PI3K pathway is important for survival and is required for activation of downstream kinases such as AKT. Activation of PLCγ2 leads to the release of intracellular Ca^{2+} activation of protein kinase C (PKC), both of which are required for activation of mitogen activated protein kinases (MAPKs) and the RAS-RAF-ERK pathway. PLCγ2 is also required for activation of the NF-κB pathway in BCR-induced B cells (reviewed in: Kurosaki, 1999; Niirro and Clark, 2002). In contrast to these activating intermediates, several inhibitory signaling molecules, such as SH2 domain-containing phosphatidylinositol 5-phosphatases (SHIP) and the RasGAP-binding protein p62<sub>dok</sub> (Dok), have been shown to balance the BCR signaling (reviewed in: Brauweiler et al., 2000).

Syk is an important B-cell signaling molecule as disruption of Syk prevents most downstream BCR signaling (Takata et al., 1994). In Syk-deficient mice most B-lineage cells in the bone marrow are arrested at the pro-B cell stage (Gaud et al., 2002). Lyn-/ mice display normal bone marrow B cell development but B cell maturation beyond the T1 stage is impaired (Meade et al., 2002). Lyn-deficient B cells that do progress to the mature B cell stage exhibit increased autoimmunity (Gaud et al., 2002; Niirro and Clark, 2002) and reduced tyrosine phosphorylation upon BCR ligation (Kurosaki, 1999). Lack of Btk results in significantly reduced activation of PLCγ2 in DT40 B cells and reduced production of inositol trisphosphate (IP3) (Takata and Kurosaki, 1996; Kurosaki, 1999), and impaired Ca^{2+} response in human B cells (Fluckiger et al., 1998). Btk deficiency results in X-linked agammaglobulinemia (XLA) in humans and a related deficiency in mice, xid, characterized with very few mature peripheral B cells, and severe hypogammaglobulinemia of all immunoglobulin isotypes (Kurosaki, 1999; Gauld et al., 2002). SHIP-deficiency results in impaired BM development but increased numbers of mature B cells in the spleen and increased autoantibody production (Kurosaki, 1999; Brauweiler et al., 2000). B cells deficient of SHIP are hypersensitive to BCR-mediated Ca^{2+} (Liu et al., 1998).

In addition to the BCR, B cells express co-receptors that function to set BCR-signaling threshold. CD19 is a B cell specific transmembrane glycoprotein that is expressed from the pro-B cell and elevates BCR-signaling threshold. CD19 interacts with effector molecules downstream of BCR signaling, such as PI3-K and with the adapter proteins Vav, Cbl, and Shc (reviewed in: Fujimoto et al., 2000). Expression of CD19 is required for maximal BCR-mediated PI3K activation and Ca^{2+} mobilization (Buhl et al., 1997). These observations suggest that CD19 is a central regulatory component upon which multiple signaling pathways converge. CD22 is a tansmembrane glycoprotein that is expressed on the surface of mature B cells. CD22-deficient B cells have heightened calcium fluxes, and cell proliferation is obtained at lower ligand concentrations. Mice deficient of CD22 have an augmented immune response and produce increased titers of autoantibodies (O'Keefe et al., 1996). The CD22 molecule has immunoreceptor tyrosine-based inhibitory motifs (ITIMs) that recruit SH2-domain-containing protein tyrosine phosphatase 1 (SHP1) to suppress BCR signaling (Fujimoto et al., 2000; Niirro and Clark, 2002). In the CD22-CD19 interplay, it is suggested that CD19 activates the CD22/SHP1 inhibitory pathway that then acts primarily on CD19 (Fujimoto et al., 1999). CD45 is a transmembrane protein tyrosine phosphatase (PTPase) that positively regulates BCR signaling by association with the Src kinases and dephosphorylation of the inhibitory phosphate on lyn (Law et al., 1996). Lack of CD45 results in poor Ca^{2+} response and decreased proliferation in response to BCR stimuli (Kurosaki, 1999). The FcγRIIB is an inhibitory receptor for the Fc of IgG and contains one ITIM in its cytoplasmic domain (Muta et al., 1994). When FcγRIIB is co-ligated with BCR, SHIP is recruited, leading to the abrogation of BCR signaling by the hydrolysis of phosphatidylinositol-3,4,5-trisphosphate (PtdInsP3) (Brauweiler et al., 2000). Disrupting the balance between the co-receptors leads to diverse abnormalities in B cell development, selection and function as revealed in normal as well as in transgenic (Tg) mouse models (reviewed in: Healy and Goodnow, 1998; Kurosaki, 1999).

The role of ligand-independent signals in positive selection

Ligand-independent signals are vital for B cell selection and survival throughout B-lymphopoiesis. The nature of these signals is not completely understood. It is thought that proper assembly and expression of an oligomeric BCR generates some continuous basal phosphorylation that is required for positive selection and survival (Meffe et al., 2000; Reth et al., 2000; Fuentes-Panana et al., 2004). This basal-tone or steady-state level of signaling is the result of a dynamic equilibrium between positive and negative signaling regulatory molecules and is often referred as "tonic" signals (Fujimoto et al., 2000; Roose et al., 2003).

The signaling components I<sub>gk</sub> and I<sub>gβ</sub> are already expressed on the earliest stages, called pro-B, and activation of these molecules is sufficient to drive developmental progression to the small preB stage (Nagata et al., 1997). V(D)J recombination initiates in proB cells by ligation of D<sub>β</sub> and J<sub>β</sub> elements. The DHJH in reading frame 2 (RF2) encodes a truncated form of mIg called D<sub>μ</sub>, which associates with I<sub>gk</sub>/I<sub>gβ</sub> and with the SL chain proteins. Developmental progression of B cells expressing D<sub>μ</sub> is aborted at the proB stage, a phenomenon referred as RF2 counterselection (Gu et al., 1991; Loffert et al., 1996). These cells are either deleted or undergo secondary DH-JH recombination to replace
the truncated receptor (Reth et al., 1986). Studies have shown that Dµ signaling through the Igα/Igß is required for the RF2 counterselection (Gong et al., 1996) (Fig. 2).

The product of a successful VDJ recombination is tested at the large preB stage for its fitness to associate with the SLC components and to form preBCR (Melchers et al., 2000). Testing pre-BCR assembly, expression, and signaling is an important check-point in B cell development as signals from the pre-BCR trigger B cell differentiation, proliferative expansion and heavy chain allelic exclusion. A large body of evidence suggests that these signals are ligand-independent and continuously produced as a consequence of membrane deposition of the preBCR (reviewed in: Meffre et al., 2000; Melchers et al., 2000; Seagal and Melamed, 2003). The preBCR signals are mediated by ITAMs in the cytoplasmic tail of Igα and Igß, as absence of the ITAMs block B cell development at the proB stage (Papavasiliou et al., 1995; Kraus et al., 2001). In addition, lack of the nonredundant signaling intermediaries Syk, BLNK or PI3K impairs proB cell development and the RF2 counterselection (Meffre et al., 2000). Failure to express the preBCR or insufficient preBCR signaling blocks B cell development at the proB stage and abrogate allelic exclusion (reviewed in: Meffre et al., 2000; Melchers et al., 2000; Rolink et al., 2001; Seagal and Melamed, 2003). It has been demonstrated that these cells undergo secondary heavy chain recombination, mainly through VH gene replacement, as reported in several gene targeted mouse models (reviewed in: Nemazee, 2000; Edry and Melamed, 2004) (Fig. 2).

A productive light chain recombination and synthesis promotes developing B cells into the immature stage. The light chain protein replaces the SLC components and, if pairs with the heavy chain, is deposited on the cell membrane as a complete BCR. Failure to pair results in developmental block as demonstrated in 20% of preB cells expressing light chain proteins in the cytoplasm but not on the surface (Rolink et al., 2001). Direct biochemical evidence for ligand-independent signaling through the BCR comes from the studies with the protein tyrosine phosphatase inhibitor pervanadate, which stabilizes signals that are either weak, transient, or both on the ITAMs of Igα and Igß, retaining the evidence that such signals have been generated (Wienands et al., 1996). The requirement of these signals to the process of positive selection and developmental progression is supported by demonstrating that B cell maturation is severely impaired in many BCR-signaling mutated mice such as Btk, SLP-65, Lyn, Vav and in mice lacking functional Igß cytoplasmic tail (reviewed in: Gauld et al., 2002; Niiro and Clark, 2002; Fuentes-Panana et al., 2004). Also, B cell maturation is accompanied with stage-dependent differences in BCR signaling molecules and in the BCR ability to enter into lipid rafts (Sproul et al., 2000; Benschop et al., 2001). Collectively, the obtained results suggested that ligand-independent signaling through the BCR is required for positive selection and developmental progression, although a direct evidence for this was still missing. Recently, Monroe and
colleagues designed a chimeric protein consisting of a fusion of the cytoplasmic domains of Igα and Igβ that is targeted to the intracellular face of the plasma membrane, which is called ‘MAHB’. MAHB does not contain any extracellular domains and signals transduced by this protein are therefore not resulting from interaction with any extracellular ligand. Retroviral administration of MAHB into precursor B cells derived from Igμ or Rag deficient mice, where B cell development is aborted at the proB stage, allowed developmental progression of preB, immature, and mature peripheral B cells. This directly indicates that surface expression of the signaling domains of Igα and Igβ and generation of basal signaling is sufficient to promote positive selection and developmental progression (Bannish et al., 2001; Fuentes-Panana et al., 2004). Lately, we and others have shown that in B and T lymphocytes, tonic activity of signaling pathways independent of receptor ligation determines the physiologic gene expression programs and the induction of RAG genes. In both systems these ligand-independent signals are mediated by Erk kinase (Roose et al., 2003; Keren et al., 2004). To study the fate of B cells that fail to fulfill appropriate basal signaling we used Ig-Tg mice (3-83) deficient of CD19. The CD19 is an important positive regulator of BCR signaling and lack of CD19 results in receptor signaling incompetence (Buhl et al., 2000). We found that immature 3-83Tg CD19-/- B cells fail positive selection and undergo developmental arrest. These cells upregulate recombinase genes and undergo receptor editing, as demonstrated in vivo and in vitro (Shivtiel et al., 2002). Similar results were also reported for 3-83Tg mice deficient of Lyn (Meade et al., 2002). By modification of the basal signal strength we have recently shown that receptor editing is directly stimulated by inappropriate tonic signaling in immature B cells (Keren et al., 2004). Thus, failure of positive selection activates secondary light chain gene recombination in order to edit and express a new receptor that is signaling competent (Fig. 2).

Signaling through the BCR is important also for differentiation of B1 and B2 cells. Earlier studies suggested that BCR specificity and self reactivity determine B1 and B2 cell differentiation (Berland and Wortis, 2002). However, recent studies have shown that BCR signal strength, rather than BCR specificity, regulates B1 cell development. Disruption of CD19, Btk, PKCβ, Vav or PLCγ results in decreased BCR signaling and decreased B1 cell compartment. In contrast, disruption of CD22 or SHP-1 increases BCR signaling and results in increased B1 compartment (reviewed in: Berland and Wortis, 2002). In mature B cells, BCR expression is mandatory for survival. Loss of BCR expression by conditional IgM ablation results in rapid cell death (Lam et al., 1997). This indicates that already in the absence of antigen, the BCR continuously transmits survival signals (Fig. 2).

The role of ligand-dependent signals in negative selection

With the expression of IgM, immature B cells become susceptible to tolerogenic processes upon binding of self antigen. Negative selection of autoreactive cells prevents maturation of B cells with an autoimmune potential. Autoreactivity is extinguished by clonal selection imposed by apoptosis of cells binding self-tissue or by functional inactivation (anergy). It was later demonstrated that anergic B cells are short lived (Fulcher and Basten, 1994). Receptor selection is an alternative tolerogenic mechanism that spares the life of the cell and allows multiple Ig gene rearrangements to replace an autoreactive receptor with a new, non-autoreactive one, a mechanism referred as receptor editing. Thus, B cell negative selection is mainly imposed by receptor editing, apoptosis and anergy. Studies with normal mice treated with anti-IgM antibodies or with Ig-Tg mice confirmed the contribution of all three mechanisms in B cell tolerance (Lawton et al., 1972; Nemazee and Burki, 1989; Hartley et al., 1991; Gay et al., 1993; Tiegs et al., 1993).

Receptor ligation in immature B cells imposes developmental arrest (Hartley et al., 1993; Melamed and Nemazee, 1997). In vivo and in vitro studies have shown that immature B cells encountering self antigen undergo rapid apoptosis (Carsetti et al., 1995; Norvell et al., 1995). Activation of the apoptotic pathway depends on Ca²⁺ mobilization (Aagaard-Tillery and Jelinek, 1995), NFκB and c-Myc (Wu et al., 1996; Sonnenshein, 1997). However, intracellular signaling by antigen receptors are not all or nothing event and are affected by qualitative and quantitative factors, including the antigen valency, BCR specificity and BCR signaling threshold (Healy and Goodnow, 1998). In the hen egg lysozyme (HEL) Tg system, membrane HEL expression results in deletion of anti-HEL B cells (Hartley et al., 1991), whereas soluble HEL (sHEL) expression imposes anergy (Goodnow et al., 1988). As BCR signaling can be modified by absence or overexpression of signaling intermediates or BCR co-receptors, studies were performed to probe for the role of BCR signal quality and quantity in regulating B cell negative selection. It was demonstrated that in the absence of Lyn, SHP-1 or CD22, chronic exposure to sHEL resulted in developmental arrest and apoptosis of HEL-specific B cells rather than anergy in the signaling-sufficient counterparts (Cyster and Goodnow, 1995, 1997; Cornall et al., 1998). In contrast sHEL promotes positive selection of HEL-specific B cells deficient of CD45 (Cyster et al., 1996), and development of autoimmunity when CD19 is over-expressed (Inaoki et al., 1997) (Fig. 3).

Receptor ligation in immature B cells can also stimulate secondary recombination and receptor editing. At the kappa locus, secondary recombination replaces the entire pre-existing VκJκ by recombination of an upstream Vκ to a downstream Jκ (Nemazee, 2000;
Seagal and Melamed, 2003). Such mechanism of secondary recombination is not applicable at the HC locus, as no more D regions are left available after primary VDJ formation (Nemazee, 2000). Instead, receptor editing at the HC locus utilizes cryptic recombination signal sequences, embedded in many VH genes, to allow upstream VH genes to recombine with the existing VDJ and to produce hybrid VH genes (Kleinfield and Weigert, 1989). Receptor editing at the HC locus is thought to occur during preBCR formation and, unlike light chain editing, is not tolerance mediated (Nemazee, 2000). Stimulation of receptor editing was first demonstrated in H+L Ig Tg mouse systems expressing BCR specific to DNA or to MHC (Gay et al., 1993; Tiegs et al., 1993). B cell development in these models was limited to very small number of cells that effectively inactivated the Tg receptor and expressed an endogenous, non-self receptor. Receptor editing was not activated in these mouse models when bred on RAG-deficient background, confirming that alternation of BCR specificity is due to a secondary recombination (Spanopoulou et al., 1994). A broad recognition of receptor editing as a main mechanism in negative selection has been established utilizing different in vitro culture systems (Hertz and Nemazee, 1997; Melamed and Nemazee, 1997). Immature B cells encountering self-antigen undergo developmental arrest, and, as a consequence, the majority of these cells (>60%) are stimulated to undergo light chain receptor editing (Hertz and Nemazee, 1997; Melamed and Nemazee, 1997). A better in vivo estimation for the efficiency of receptor editing emerged from autoantibody targeted mouse models (knock-in), where the transgenic receptor is inserted into its physiological gene context. These mouse models have shown that primary autoreactive-encoding VκJκ is inactivated by RS recombination or replaced by new recombination of an upstream Vκ to downstream Jκ (Chen et al., 1997; Pelanda et al., 1997; Pewzner-Jung et al., 1998). These mice have essentially normal B cell numbers and genetic analysis revealed that 85-98% of the cells have undergone light chain editing (Chen et al., 1997; Pelanda et al., 1997). Thus, receptor editing is very efficient in rescuing autoreactive B cells, although multiple rearrangements are often required until appropriate Vκ editor is selected (Li et al., 2001). High frequency of receptor editing was also demonstrated in normal mouse B cells (Rettet and Nemazee, 1998). Nussenzweig and colleagues have estimated that at least 25% of the antibody molecules are produced by light chain gene replacement, providing receptor editing with a major contribution in generating the antibody repertoire (Casellas et al., 2001). It is not completely understood how receptor editing is regulated in negative selection. Clearly, BCR signaling following interaction with antigen is profoundly different relative to the tonic signals required to promote positive selection, both in level of phosphorylation and pattern of phosphorylated proteins (Keren et al., 2004). Modification of these signals by lack of CD19 or CD45 co receptors or Btk have no effect on receptor editing stimulated by multivalent membrane-bound antigen (Buhl et al., 2000; Dingjan et al., 2001; Shivtiel et al., 2002). It was also efficiently stimulated by very low affinity antigens (Lang et al., 1996). This activation appears to be independent of c-Myc expression, an important oncoprotein that is activated in BCR-induced apoptosis (Leider and Melamed, 2003). Utilizing an in vitro culture system and an Ig-Tg model we found that immature B cells progress from receptor editing competent, apoptosis resistant stage into receptor editing

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Fig. 3. Ligand-dependent signals in tolerance induction and activation of B lymphocytes. IgM expression allows B lymphocytes to interact with antigen. Immature B cells that bind self-antigen undergo unproductive selection mainly by receptor editing, anergy or apoptosis. Transitional B cells in the spleen undergo rapid apoptosis upon interaction with self-antigen. BCR ligation in mature B cells, in the presence of appropriate T cell help, results in activation proliferation and differentiation to antibody-producing plasma cells.
incompetent, apoptosis sensitive stage (Melamed et al., 1998). Since c-Myc responsiveness to BCR ligation increases with developmental progression (Leider and Melamed, 2003), we suggest that B cell development compartmentalizes receptor editing from apoptosis (Melamed et al., 1998). Other studies have utilized the HEL system and suggested that receptor editing in immature B cells can also be stimulated in response to soluble Ag at a later developmental stage (Tze et al., 2003). Also, studies with non-Tg immature B cells suggested that receptor editing stimulation depends on the site of antigen encounter and the presence of Thy1full cells in the BM (Sandel et al., 2001). Using a different anti-DNA targeted mouse model Eilat and colleagues have shown that receptor editing efficiency depends on the number of available Jk on the expressed Vx allele (Yachimovich et al., 2002). Although several studies suggested that secondary rearrangements can be stimulated in mature B cells (referred as receptor revision), several RAG-GFP indicator mice have shown that such activation of receptor editing occur in peripheral B cells with an immature phenotype, and not in mature B cells (reviewed in: Nemazee, 2000; Seagal and Melamed, 2003). Hence, the competence to undergo receptor editing is lost with developmental progression and maturation. In contrast, BCR stimulation in the presence of appropriate T cell help, provided by CD40L, results in cell activation proliferation and differentiation to antibody-producing plasma cells (Fig. 3).

It remains to be elucidated why signals generated by the BCR are tolerogenic in immature B cells and stimulate immune responsiveness in mature B cells. Recent data suggest that both qualitative and quantitative differences in BCR signaling do exist in immature relative to mature B cells. First, B cell maturation is accompanied with significant stage-dependent differences in BCR signaling molecules (Benschop et al., 2001). Second, differential activation of BCR signaling intermediates have been found between mature and immature stimulated B cells (Koncz et al., 2002). Third, in mature B cells, signaling through the B cell antigen receptor(BCR) is initiated from within rafts whereas in immature B cells, the BCR is excluded from rafts (Sproul et al., 2000). Lipid rafts are important in regulating B cell responses as they concentrate the Src family kinase Lyn that plays a key role in the initiation of BCR signaling cascade (Cheng et al., 1999). An important role for CD19/CD21 has been shown for prolong residency and signaling of the BCR from the rafts (Cherukuri et al., 2001). Thus, differential responsiveness of immature and mature B cells to BCR ligation appears to be developmentally regulated from both qualitative and quantitative aspects.

Conclusions

It is now clear that signals generated by the BCR are critical for determination of the B cell fate throughout multiple developmental check-points. Knockout studies have shown that receptor expression with signaling capacity is mandatory for B cell development and survival. Ligand-independent signals that are generated by the preBCR and the BCR are required for positive selection developmental progression and survival of proB, immature and mature B lymphocytes. Ligand-dependent signals set BCR signaling threshold for negative selection. Developmental progression of B lymphocytes that fail to fulfill appropriate receptor signaling requirements is aborted. Such cells undergo receptor editing, a mechanism thought to "repair" receptor genes encoding a defective and/or self-reactive BCR.

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