Modification of Ligand-independent B Cell Receptor Tonic Signals Activates Receptor Editing in Immature B Lymphocytes*

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Received for publication, October 31, 2003, and in revised form, November 19, 2003
Published, JBC Papers in Press, December 10, 2003, DOI 10.1074/jbc.M311970200

Maturation of B lymphocytes strictly depends on the signaling competence of the B cell antigen receptor (BCR). Autoreactive receptors undergo negative selection and can be replaced by receptor editing. In addition, the process of maturation of non-self B cells and migration to the spleen, referred to as positive selection, is limited by the signaling competence of the BCR. Using 3-83Tg mice deficient of CD19 we have shown that signaling incompetence not only blocks positive selection but also activates receptor editing. Here we study the role of ligand-independent BCR tonic tyrosine phosphorylation signals in activation of receptor editing. We find that editing, immature 3-83Tg B cells deficient of CD19 have elevated BCR tonic signals and that lowering these tonic signals effectively suppresses receptor editing. Furthermore, we show that elevation of BCR tonic signals in non-editing, immature 3-83Tg B cells stimulates significant receptor editing. We also show that positive selection and developmental progression from the bone marrow to the spleen are limited to cells capable of establishing appropriate tonic signals, as in contrast to immature cells, splenic 3-83Tg B cells deficient of CD19 have BCR tonic signals similar to those of the control 3-83Tg cells. This developmental progression is accompanied by activation of molecules signaling for growth and survival. Hence, we suggest that ligand-independent BCR tonic signals are required for promoting positive selection and suppressing the receptor-editing mechanism in immature B cells.

Formation and expression of the antigen receptor are hallmarks of T and B lymphocyte development. During early stages of development the expressed receptor signals for both positive and negative selection, whereas in mature cells it signals for activation (1–3). Continuous receptor expression and signaling are also critical for cell survival, as ablation of the antigen receptor results in death of the lymphocytes, which occurs more rapidly in B cells (4, 5). Hence, life and death of lymphocytes depend not only on the specificity of the antigen receptor but also on its signaling capacity.

The B cell antigen receptor (BCR) specificity is important for negative selection, aiming to extinguish self-reactivity (1, 3, 6). The encountering of self-antigen in the bone marrow (BM) stimulates secondary immunoglobulin gene rearrangements to express a new receptor, a mechanism called receptor editing (7, 8). This salvage mechanism allows extended survival and multiple V(D)J recombination attempts for self-reactive B cells and was found to contribute in generating the B cell repertoire (9, 10).

In contrast to negative selection, it is unclear what signals for developmental progression and maturation of non-self B cells, a process also referred to as positive selection (2, 3, 11, 12). Several studies have utilized signaling mutated mice to show that expression of a non-self receptor is not sufficient for B cell maturation. In these mice, B cell development and/or maturation is severely impaired, suggesting that appropriate receptor-signaling capacity is required to promote B cell maturation (13–17). This process has also been shown to be ligand-independent (18). More recently, the signaling-inhibitory function of several BCR intermediary molecules has been identified in activated and in resting B cells (19, 20). These studies have supported the general thought that positive selection signals in B lymphocytes are derived from autonomous “tonic” tyrosine phosphorylation generated by the antigen receptor and regulate developmental progression and maturation from the BM to the spleen (3, 12, 18).

Co-receptors in B lymphocytes function to set BCR-signaling threshold. Thus, while CD19 elevates BCR-signaling threshold, CD22 lowers it (21). Several studies in gene-targeted mice have shown that these molecules are important in determining signaling threshold for negative selection as well as functional responsiveness to antigenic stimulation (for review, see Ref. 22). While most of the published data has probed the role of these co-receptors only upon BCR ligation, little is known about their function in regulating the ligand-independent BCR tonic signals in unstimulated cells. In an earlier study we showed that immature 3-83Tg CD19−/− B cells fail positive selection and undergo intensive receptor editing in an attempt to express a new receptor (23). These studies raised the hypothesis that CD19 expression is important to set BCR tonic signaling for positive selection and provided a possible link between failure of positive selection and activation of receptor editing in immature B cells. The possibility that receptor editing is stimulated by BCR signals that are too high or too low has been suggested (24), but no directly supporting data is available. Using our experimental model of immunoglobulin-transgenic B cells (3-83Tg) that are expressing or deficient of CD19, we directly assess the role of ligand-independent BCR tonic signals in.

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* This work was supported by the Israel Cancer Research Fund-Rosenwasser Trust, the Israel Ministry of Science jointly with the Deutsches Krebsforschungszentrum (Heidelberg, Germany), the Mars Pittsburgh Foundation for Medical Research, and the Hirshenstrauss-Gutman Medical Research Fund. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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‡ The abbreviations used are: BCR, B cell antigen receptor; BM, bone marrow; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; RT, reverse transcription; PI3K, phosphatidylinositol 3-kinase; Erk, extracellular signal-regulated kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; IL, interleukin; SHIP, Src homology 2 domain containing 5-inositol phosphatase.

This paper is available on line at http://www.jbc.org
activating the receptor-editing mechanism. We show here that modification of tonic tyrosine phosphorylation signals is capable of stimulating or suppressing receptor editing in immature B cells and that positive selection and developmental progression from the BM to the spleen are limited to cells capable of establishing appropriate tonic signals. Hence, we propose that appropriate BCR tonic signals promote positive selection and establishment of allelic exclusion and allow cells to migrate from the BM to the spleen. In contrast, cells that fail to establish appropriate tonic signals are arrested in the BM and activate receptor editing.

**EXPERIMENTAL PROCEDURES**

**Experimental Mice**—The mice in all experiments were 3-83Tg B10.12dnSnJ expressing IgMtgD BCR specific to class I major histocompatibility complex antigens K0 and K1 and 3-83Tg B10.12dnSn/J CD19 cells (CD19-deficient mice) were generated at the laboratory of Klaus Rajewsky (25). Mice were housed and bred at the animal facility of the Technion, Faculty of Medicine (Haifa, Israel), and used at 4–10 weeks of age.

**Cell Culture and B Cell Purification**—Immature B cells were generated from IL-7-driven bone marrow cultures as we have described previously (26). Briefly, BM was obtained from 4–10-week-old mice and cultured in the presence of 50–100 units of IL-7 in Iscove's modified Dulbecco's medium with 10% fetal calf serum. In some experiments B220+ splenic B cells were purified from 4–10-week-old mice using MACS magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). In some experiments the P13K inhibitor wortmannin (Sigma) or a tyrosine phosphatase inhibitor, pervanadate, was added to BM cultures. Pervanadate stock solution was composed of 10 mM sodium orthovanadate (Sigma) and 5 μl of 30% hydrogen peroxide, which were buffered with 20% mg/ml catalase (Sigma). Cell viability was determined by the MTT oxidation method as described previously (27). Briefly, 2.5 × 106 cells grown in BM cultures were incubated for 18 h with different concentrations of wortmannin or pervanadate in 96-well plates. Cultures were pulsed with 10 μl of MTT solution (Sigma, 5 mg/ml) for 4 h following addition of 150 μl of isopropanol, 0.04 N HCl. Plates were read in an enzyme-linked immunosorbent assay reader at 570–630 nm.

**Immunoprecipitation and Western Blot Analysis**—Analysis of tyrosine phosphorylation in B cells was performed as described previously (28). Briefly, B cells from BM cultures or purified from spleen were lysed in 1% Nonidet P-40 lysis buffer (150 mM NaCl, 50 mM Tris (pH 7.5), 50 mM NaF, 2 mM sodium orthovanadate, 20 mM sodium pyrophosphate (pH 7.5), 1 mM aprotinin, 1 mM antitrypsin, 1 mM leupeptin, and 2 mM phenylmethylsulfonyl fluoride). Supernatants were used for immunoprecipitation or used as total cell lysate for phosphorylation analysis. Protein lysates were separated on SDS-PAGE and transferred to polyvinylidene fluoride membrane (Millipore, Bedford, MA). In some experiments, cells grown in culture were stimulated with anti-BCR antibody (F(ab)2 rabbit anti-mouse IgG (heavy and light), Zymed Laboratories, San Francisco, CA) for 3 min before lysis. For immunoprecipitation of Igα (CD79a) and Igκ we used polyclonal rabbit antisera, generously provided by Prof. John C. Cambier (National Jewish Medical and Research Center, Denver, CO). Blots were probed with antiphosphotyrosine (4G10), followed by protein-specific antibodies. Tyrosine phosphorylation of Erk1/2 and Lyn and was performed using rabbit antiphospho-Erk1/2 and rabbit antiphospho-Lyn (Cell Signaling Technology, Beverly, MA), followed by rabbit anti-Erk1/2 (Cell Signaling) and rabbit anti-Lyn (kindly provided by J. C. Cambier). Visualization of bound antibodies was performed by enhanced chemiluminescence reagent. To determine levels of tyrosine phosphorylation of specific signaling intermediaries, densitometry analysis was performed using the UVidoc gel documentation system and UVidoc analysis software (UVitec, Cambridge, UK), and the ratio between the phosphorylated and the corresponding total band was calculated.

**Flow Cytometry and Sorting**—Antibodies used for cell staining were as follows: anti-mouse α-fluorescein isothiocyanate; anti-mouse IgM-phycocerythrin (PE); anti-B220-PE, RA3–6B2 (CALTAG, Burlingame, CA); anti-IgD–biotin, AMS 9.1; and anti-3-83 idiotypic 54.1-biotin. Biotinylated antibodies were visualized with streptavidin SPRD (Southern Biotechnology Associates, Birmingham, AL). In some experiments cells were sorted based on their surface markers and collected for further analysis. Cell sorting and data collection were performed using FACScalibur™ and CELLquest™ software (BD Biosciences, Immunocytometry Systems, Mountain View, CA).

**RESULTS**

**Ongoing Receptor Editing in 3-83Tg CD19–/– BM Cultures**—We have shown previously that immature 3-83Tg CD19–/– B cells undergo receptor editing in BM in vivo and in BM cultures in vitro (23). To determine the extent of receptor editing in 3-83Tg CD19–/– cells developing in vitro, V(D)J recombination markers (RAG-2 and a light chain expression) were measured at various times throughout the culture period. In 3-83Tg and 3-83Tg CD19–/– BM cultures immature B cells grow equally well as reflected by absolute numbers (23) and their relative proportion reaching 90–95% purity after 5 days (% B220+ cells, Fig. 1A, top). However, in contrast to the 3-83Tg cultures, receptor editing is activated in 3-83Tg CD19–/– cultures as revealed by the accumulation of cells expressing endogenous λ light chain (Fig. 1A, middle) and RAG-2 expression (Fig. 1A, bottom). Despite the increasing B cell numbers, levels of RAG-2 expression did not significantly change during the 3-83Tg CD19–/– culture period, suggesting that ongoing V(D)J recombination is a general characteristic in this cell population. To support this, RAG-2 was determined in sorted 3-83Tg CD19–/– B cell populations differing by BCR level of expression. Sorting gates were set as we have described previously (9). As shown in Fig. 1B, RAG-2 is significantly expressed in all sorted IgMhi/IgD–, IgMhi/IgD–, IgMhi/IgD–, and IgMhi/IgD– B cell populations, but levels of expression dropped with developmental progression as determined by BCR expression. The detection of RAG-2 in all sorted cell populations may suggest that developmental progression in vitro is not sufficient for termination of V(D)J recombination in 3-83Tg CD19–/– BM cultures.

**Immature 3-83Tg CD19–/– Cells Have Increased Tonic Tyrosine Phosphorylation**—Because 3-83Tg CD19–/– BM cultures lack the 3-83 cognate antigen, we hypothesize that ongoing receptor editing results from generation of inappropriate ligand-independent tonic signals. To test this we measured tyrosine phosphorylation in unstimulated immature 3-83Tg CD19–/– cells grown in BM cultures relative to control 3-83Tg cultures. Initial tyrosine phosphorylation analysis in total cell lysates clearly showed increased tonic phosphorylation in immature 3-83Tg CD19–/– cells (Fig. 2A). Similar results were obtained when analysis was performed on gels loaded with equal amounts of proteins rather then cell equivalent (data not shown). To further assess tyrosine phosphorylation of specific signaling intermediaries that are proximal to the BCR, immunoprecipitation and phosphotyrosine immunoblotting were performed. We found that immature 3-83Tg cells deficient of CD19 have an increased tyrosine phosphorylation of Igα and Lyn (Fig. 2B). Quantitation analysis revealed that tonic Igα phosphorylation is increased by 1.7–2-fold, whereas that of Lyn is increased by 30–50% (Fig. 2C). Finally, to confirm that both cell populations can elevate tyrosine phosphorylation levels in response to BCR ligation, cells were stimulated with anti-BCR antibodies. The results in Fig. 2D show that anti-BCR antibodies stimulated significant tyrosine phosphorylation in 3-83Tg and in 3-83Tg CD19–/– cells as measured in total cell lysates. Hence, we conclude that immature 3-83Tg CD19–/– cells have increased tonic phosphorylation of signaling intermediaries.

**Elevation of Tonic Tyrosine Phosphorylation Stimulates Receptor Editing in 3-83Tg Cells**—To study whether increased
Modified BCR Tonic Signals Activate Receptor Editing

Establishment of Appropriate Tonic Tyrosine Phosphorylation Signals in 3-83Tg CD19−/− Splenic B Cells—Despite the impaired development, a significant population of 3-83Tg CD19−/− B cells was capable of compensating for CD19 deficiency and undergoing positive selection. This suggests that appropriate tonic tyrosine phosphorylation signals are established in these cells to facilitate such developmental progression. To test this, splenic B cells were sorted and analyzed for tonic tyrosine phosphorylation. We found that 3-83Tg CD19−/− splenic B cells effectively lowered tonic signals to levels that are not significantly different relative to the control 3-83Tg splenic B cells, as revealed in both total tyrosine phosphorylation and specific IgM/D expression (Fig. 6, A and B, respectively). Establishment of appropriate tonic signals that promote positive selection should also be revealed in downstream BCR-signaling molecules. As Ras/MEK/Erk is a major pathway linked to the BCR and was shown to signal for growth and survival of B cells (30, 31), we studied Erk phosphorylation in immature and splenic 3-83Tg and 3-83Tg CD19−/− B cells.
Interestingly, our results clearly show that editing 3-83Tg CD19−/− immature B cells have significantly reduced tonic levels of phosphorylated Erk (Fig. 6C, left), but this is in agreement with the fact that efficient Erk phosphorylation depends on CD19 (31, 32). In contrast, tonic Erk phosphorylation in splenic B cells from 3-83Tg CD19−/− mice was indistinguishable from that of the control 3-83Tg splenic B cells (Fig. 6C, right). Thus, splenic B cells from 3-83Tg CD19−/− that effec-

Fig. 2. Immature 3-83Tg CD19−/− B cells have increased tonic tyrosine phosphorylation. Immature 3-83Tg and 3-83Tg CD19−/− B cells were grown in BM cultures and lysed to determine tonic tyrosine phosphorylation. A, immunoblot analysis of tyrosine phosphorylation in total cell lysates (5×10⁶ cell equivalents/lane). The blot shown is a representative of at least five different experiments. B, Ig phosphorylation was determined by immunoprecipitation (IP) (10×10⁶ cells). Membranes were probed with antiphosphotyrosine, stripped, and reprobed with anti-Ig-specific antibody. Lyn phosphorylation was directly determined in total cell lysates using specific antibodies to phosphorylated Lyn and total Lyn as described under "Experimental Procedures." Blots shown are representative of four different experiments. C, relative phosphorylation of Igo and Lyn. Blots shown in B were subjected to densitometry analysis. To determine relative phosphorylation, values obtained for a phosphorylated band were divided by the values corresponding to the total band. Results shown are mean ± S.E. of four experiments. D, immunoblot analysis of tyrosine phosphorylation in total cell lysates (2×10⁶ cell equivalents/lane). Cells were either unstimulated or stimulated with anti-BCR antibody (10 μg/ml F(ab')₂ rabbit anti-mouse IgG heavy and light) for 3 min. The blot shown is a representative of five different experiments.

Fig. 3. Elevation of tonic signals activates receptor editing in 3-83Tg immature B cells. Immature 3-83Tg cells grown in BM cultures for 5 days were cultured for 18 h in the presence of the indicated concentrations of pervanadate. A, immunoblot analysis of tyrosine phosphorylation in total cell lysates from cells treated with pervanadate (5×10⁶ equivalent cells/lane). Lysates run on the right lane are from the same cells treated with anti-IgM (5 μg/ml) for 10 min. The blot shown is a representative of three different experiments. B, survival rate of pervanadate-treated cells as determined by the MTT assay in three experiments. C and D, relative Rag-2 expression in 3-83Tg immature B cells treated with 20 μM pervanadate for 18 h. A representative blot is shown in C, and summarized semiquantitative results from three different experiments are presented as mean ± S.E. in D.

Fig. 4. Lowering of tonic signals suppresses receptor editing in 3-83Tg CD19−/− immature B cells. Immature 3-83Tg CD19−/− cells were cultured for 18 h in the presence of different concentrations of wortmannin. A, immunoblot analysis of tyrosine phosphorylation in total cell lysates from cells treated with 10 μM wortmannin (5×10⁶ equivalent cells/lane). The blot shown is representative of three experiments. B, survival rate of wortmannin-treated cells as determined by the MTT assay in three experiments. C and D, relative Rag-2 expression in 3-83Tg CD19−/− immature B cells treated with 10 μM wortmannin for 18 h. A representative blot is shown in C, and summarized semiquantitative results from three different experiments are presented as mean ± S.E. in D.
tively compensated for the lack of CD19 by establishing appropriate tonic tyrosine phosphorylation for positive selection could also activate Erk to signal for growth and survival in the periphery. These experiments lead us to conclude that positive selection and developmental progression from the BM to the spleen are limited to cells capable of fulfilling appropriate tonic signaling requirements. This, however, may still not be sufficient for extended survival in the periphery.

**DISCUSSION**

Autonomous, or tonic, tyrosine phosphorylation signals that are generated upon expression of BCR are critical for B cell development, selection, and survival (3, 12, 18). These signals are ligand-independent and therefore may have profound differences relative to ligand-dependent signaling cascade in B cells, which is well described in the literature. The nature and function of these tonic signals are poorly described. In the present study we show that establishment of appropriate tonic signals is important in promoting B cell development as tonic signals that are too high activate receptor editing in immature B cells and block the process of positive selection and developmental progression from the BM to the spleen.

Receptor editing is a salvage mechanism allowing B cells to escape deletion by rearranging and expressing a new receptor (7, 8). Although this mechanism has so far been applied to negative selection and central tolerance, the data presented here implicate receptor editing with immature B cells that fail to establish appropriate BCR tonic signals. Several studies have shown that signaling-incompetent BCR fails to promote B cell development and maturation in vivo (13–17). This failure has been attributed to a process of positive selection (2, 3, 11, 12), which is thought to be regulated by ligand-independent BCR tonic signals (11, 18, 33, 34). Thus, signaling-incompetent BCR fails to promote positive selection, and such B cells undergo developmental arrest. In mature B cells failure to generate these signals as a result of BCR ablation results in rapid death (5). However, unlike mature B cells, developmentally arrested immature B cells are competent to undergo receptor editing (35, 36). A recent study by our laboratory has shown that 3-83Tg immature B cells expressing signaling-incompetent BCR, deficient of CD19, fail positive selection and undergo developmental arrest. These cells undergo intensive receptor editing both in vivo and in vitro (23), thereby suggesting that in positive selection, receptor editing allows secondary recombination to express a new receptor that can compensate for the insufficient positive selection signal. Thus, receptor editing in developing B cells is a major mechanism in both negative and positive selection.

Earlier studies suggest that these ligand-independent signals are necessary to promote developmental progression (18, 34) and may be generated by the constitutive activity of CD45, which is able to activate Src proteins (37), and balanced by positive and negative BCR regulators (18). The absence of CD19 results in elevated tonic tyrosine phosphorylation signals in immature 3-83Tg cells (Fig. 2) and increased tonic PI3K activity in non-transgenic cells (38). In addition immature 3-83Tg CD19^{−/−} B cells fail positive selection and undergo rapid apoptosis in the periphery (23). The possibility that increased tonic signals are a result of elevated levels of BCR is unlikely because unlike in the spleen, immature 3-83Tg and 3-83Tg CD19^{−/−} B cells developing in the BM or in BM cultures have indistinguishable levels of surface BCR (23, 39). We have also considered the possibility that the elevated tonic signals may result from increased responsiveness to IL-7 stimulation because both CD19 receptor signaling and IL-7 receptor signaling have been shown to play an important role in modulating RAG gene expression and repertoire diversification in human B cell development (40). Although we have not specifically monitored phosphorylation of proteins linked to the IL-7 receptor signaling, this possibility is unlikely because elevation in total tyrosine phosphorylation and in Igα phosphorylation was obtained in 3-83Tg CD19^{−/−} cells cultured for 24–48 h in the absence of IL-7 without significant increase in cell death (data not shown). These observations suggest that failure of positive selection and activation of receptor editing in these cells are due to inappropriately increased tonic signals. It is difficult to define what should be the appropriate levels of tonic signals. As 3-83Tg cells have normal development and establish efficient allelic exclusion, we postulated that these cells do produce appropriate levels and therefore can be used as a reference. Indeed, elevation of the tonic signals by treatment with the protein-tyrosine phosphatase inhibitor pervanadate stimulated receptor editing in these cells (Fig. 3). This notion was further confirmed in the reciprocal experiment when lowering the tonic signals in 3-83Tg CD19^{−/−} cells (using the PI3K inhibitor wortmannin) effectively down-regulated receptor editing (Fig. 4). We currently have no direct explanation of how PI3K inhibition suppresses tonic tyrosine phosphorylation. Although the canonical signaling cascade of PI3K in many cells has been characterized, the signaling events upstream and downstream of PI3K in B lymphocytes are not well known (31, 41). Clearly, in B lymphocytes PI3K activation is a central event in BCR signaling and is directly linked to CD19 (28). Evidence from several studies shows that through multiple cross-talk mechanisms the PI3K activation is integrated directly or indirectly, via adaptor proteins, with other BCR-signaling pathways such as Ras-Raf and PLCγ2 (31, 41–43). Hence, it is possible that inhibition of PI3K results in reduced activation of kinases that are linked to these pathways, effectively lowering tonic tyrosine phosphorylation in these cells. This may be further clarified in current experiments using specific protein-tyrosine kinase inhibitors. Similar to the CD19 deficiency, Lyn deficiency alters BCR-signaling threshold and activates receptor editing in 3-83Tg Lyn^{−/−} mice (44). Hence, immature B cells expressing receptors that generate signals that are too high or too low will fail to develop and may activate receptor editing in an attempt to express a new receptor. This, however, is difficult to show in a polyclonal repertoire but becomes apparent in a monoclonal repertoire that is restricted by an immunoglobulin transgene such as 3-83. In addition, binding self-antigen may elevate signals that are too low to promote positive selection, thereby rescuing autoreactive cells and promoting autoimmunity, as has been suggested for CD22- and SHIP-deficient mice (22). In these studies, however, tonic signals have not been measured directly. In contrast, lack of CD45...
has no effect on B cell development and positive selection (45).

Although the role of CD19 in mediating BCR signaling upon receptor ligation has been studied intensively, its contribution in maintaining appropriate tonic signals for selection and survival has not been shown. Clearly, analysis of tonic signaling should be different from analysis of signals generated upon stimulation. For example, in CD19-deficient B cells, tonic activity of PI3K is elevated relative to control cells (38), but upon stimulation, PI3K activity in CD19−/− cells is remarkably reduced (28, 38). Thus, in unstimulated cells appropriate tonic signals should continuously be generated and balanced due to the function of stimulatory and inhibitory signaling intermediaries. It is possible that the increased tonic signaling in immature 3-83Tg CD19−/− B cells is due to inefficient activity of the CD22 inhibitory pathway as optimal CD22 inhibitory function depends on CD19 expression (21). Studies by Tedder and colleagues suggest that the reciprocal function and the appropriate balance of CD19 and CD22 activity are important for B cell signaling (46). The CD22 activity is mediated by the recruitment of activated SHIP (22, 38). The inhibitory function of SHIP has been shown to be mediated by association with Dok (19, 20), and this interaction correlates with increased tyrosine phosphorylation of Dok and inhibition activity (20). In addition, Dok is colocalized to the BCR together with CD19 and CD22 (47). In agreement with this we find reduced levels of activated Dok in immature 3-83Tg CD19−/− B cells (Fig. 5), suggesting that increased tonic signals in these cells result from reduced CD22-mediated inhibitory balancing activity. This hypothesis implies that in resting cells, CD19 functions not only to generate tonic signals but also to be involved in activation of inhibitory molecules that can balance these signals, as has been suggested previously (21).

Despite the impaired development, a significant number of CD19−/− B cells are found in spleens of 3-83Tg and non-transgenic mice (23), suggesting that these cells express a BCR that can signal for positive selection and developmental progression. We find that tonic tyrosine phosphorylation signals in these cells were effectively lowered to levels that are not different relative to control. This supports our previous study showing that elevated BCR level of expression, or expression of an endogenous BCR composed of a light chain, is able to promote positive selection and developmental progression of 3-83Tg CD19−/− B cells in vivo and in vitro (23). Such compensating mechanisms may increase tonic activation of the BCR-signaling inhibitory molecules, such as Dok, that function to lower tonic signals and to promote positive selection of immature 3-83Tg CD19−/− B cells. Establishing appropriate tonic signals and positive selection is also revealed in downstream signaling molecules. Thus, while the editing, immature 3-83Tg CD19−/− B cells have significantly reduced phosphorylated Erk, positively selected splenic B cells from 3-83Tg CD19−/− mice establish normal levels of tonic Erk phosphorylation. The Erk/mitogen-activated protein kinase also signals for growth and survival and is primarily activated through the PLCγ2 and Ras-Raf pathways (30, 31). As efficient Erk activation depends on CD19 (32), it is not surprising to find significantly reduced Erk phosphorylation in immature 3-83Tg CD19−/− B cells (Fig. 6), and these cells undergo rapid apoptosis in adoptive transfer experiments (23). In contrast, splenic 3-83Tg CD19−/− B cells that are able to compensate for CD19 deficiency have normal levels of phosphorylated Erk, suggesting that positive selection of immature B cells is also associated with generation of survival signals. This, however, does not agree with recent data showing that CD19-deficient B cells have inappropriate Akt activation (48), reduced survival in vivo, and a more transitional phenotype (39, 49). Because final stages of B cell maturation take place in the spleen (11), it is possible that BCR tonic signals that promote positive selection and migration from the BM are not sufficient for complete maturation and selection into the long-lived pool. Data showing that survival signals and selection into the follicle and marginal zone are CD19-dependent support this hypothesis (33). Hence, positive selection and migration from the BM to the spleen are regulated by appropriate ligand-independent tonic signals. However, further selection into the long-lived pool may depend on additional cellular functions, such as appropriate responsiveness to chemokines (50), which may require effective activation of the PI3K pathway and Akt phosphorylation (31). This can explain the ability of CD19−/− cells to migrate from the BM and their failure to survive in the periphery. The interrelationship between all these survival pathways and their regulation during positive selection is yet to be determined.

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