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Markus Werner
Elias Hobeika
Hassan Jumaa

Role of PI3K in the generation and survival of B cells

Authors' addresses
Markus Werner[1,2], *Elias Hobeika*[2,3], *Hassan Jumaa*[1,2,3]
[1]Faculty of Biology, Department of Molecular Immunology, Albert-Ludwigs-Universität Freiburg, Freiburg, Germany.
[2]Max Planck Institute for Immunobiology, Freiburg, Germany.
[3]Centre for Biological Signalling Studies (Bioss), Albert-Ludwigs-Universität Freiburg, Freiburg, Germany.

Correspondence to:
Hassan Jumaa
Max Planck Institute for Immunobiology
Stuebeweg 51
79108 Freiburg, Germany
Tel.: +497615108437
Fax: +497615108423
e-mail: jumaa@immunbio.mpg.de

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Summary: Engagement of the B-cell antigen receptor (BCR) or its precursor, the pre-BCR, induces a cascade of biochemical reactions that regulate the differentiation, selection, survival, and activation of B cells. This cascade is initiated by receptor-associated tyrosine kinases that activate multiple downstream signaling pathways. Since it is required for metabolism, cell growth, development, and survival, the activation of phosphoinositide 3-kinase (PI3K)-dependent pathways represents a crucial event of BCR/pre-BCR signaling. The phosphorylated substrates of the PI3K promote specific recruitment of selected signaling proteins to the plasma membrane, where important signaling complexes are formed to mediate the above-mentioned biological processes. Here, we review the principles of PI3K signaling and highlight the role of an important PI3K-driven module in VDJ recombination of immunoglobulin (Ig) genes during early B-cell development as compared with class switch recombination of Ig genes in mature B cells after activation by specific antigens. Furthermore, we discuss the role of PI3K in the survival of mature B cells, which is strictly dependent on BCR expression and basal BCR signaling.

Keywords: *B cells, PI3K, survival, basal BCR signal*

PI3K signaling in B cells

Phosphoinositide 3-kinases (PI3Ks) are an evolutionarily conserved family of lipid-modifying enzymes, which can be divided in three classes (class I, II, and III) based on their structure, regulation, and substrate specificity (1). Whereas little is known about class II and III, members of class I play important roles in immune cells and are the only enzymes that are able to generate the lipid second messenger phosphatidylinositol-(3,4,5)-trisphosphate ($PtdInsP_3$) by phosphorylating phosphatidylinositol-(4,5)-bisphosphate ($PtdInsP_2$) (1). Class I PI3Ks are heterodimeric enzymes consisting of a catalytic subunit of approximately 110 kDa, which is constitutively associated with a regulatory subunit required for activation and proper function. Depending on the mechanism of activation, class I PI3Ks can be divided in two subsets: class IA and class IB. Class IA PI3Ks are activated by tyrosine kinase-associated receptors such as antigen receptors, whereas class IB PI3Ks are triggered by G protein-coupled receptors (GPCRs) (2). Additionally, class IA PI3Ks form multiple heterodimers

consisting of one of three different catalytic subunits (p110α, p110β, p110δ) and one of five different regulatory subunits (p85α, p85β, p55γ, p55α, and p50α). Members of the class IB PI3K family, however, possess only one catalytic (p110γ) and one regulatory subunit (p101). The importance of p110γ for immune cells is shown by mice deficient for p110γ, which results in impaired T-cell development and function. B-cell development, in contrast, is not affected, suggesting that class IB PI3Ks are not crucial for proper B-cell development (3).

Class IA PI3Ks play important roles downstream of the pre-B-cell antigen receptor (BCR) and the BCR, both of which contain the signaling transduction subunits Ig-α and Ig-β (immunoglobulin-α and -β) (4). The main difference between the BCR and the pre-BCR is the initiation of receptor signaling. Whereas activation of the BCR depends on antigen binding, pre-BCR signaling is thought to be initiated autonomously upon its expression (5) (*Fig.* 1). Pre-BCR/BCR-dependent activation of class IA PI3Ks is initiated by receptor-associated tyrosine kinases of the Src family (Lyn, Fyn, and Blk) (6) and of the Syk family (Syk and Zap-70) (7). The Src-family protein tyrosine kinases Lyn, Fyn, or Blk phosphorylate the tyrosine residues of the immunoreceptor tyrosine-based activation motifs (ITAMs) located within the cytoplasmic domains of the signal transducing subunits of the BCR-associated Ig-α and Ig-β (4). Phosphorylated ITAMs provide binding sites for Src-homology 2 (SH2) domain-containing proteins, of which the tyrosine kinase Syk is key for subsequent B-cell activation. Recruited from the cytosol and activated upon binding to the phosphorylated ITAM (8, 9), Syk activity is induced by autophosphorylation, further enhanced by additional phosphorylation via Src-kinases and amplified through a positive feedback loop by Syk-mediated ITAM phosphorylation of neighboring pre-BCRs or BCRs (7, 10–12). Cooperatively, activated Syk and Src-family protein kinases promote activation of PI3K by phosphorylating specific proteins such as CD19 or BCAP (B-cell PI3K adapter protein) that mediate the recruitment of PI3Ks to the plasma membrane (2). In fact, the transmembrane protein CD19 is phosphorylated at multiple tyrosine residues, thereby providing docking sites for various signaling intermediates. Especially important for PI3K signaling are tandem YXXM motifs in the cytoplasmic region of CD19, which, if phosphorylated, confer binding to the class IA p85α regulatory subunit (13, 14). However, since the phenotype of p85α-deficient mice is much more severe than that of CD19-deficient mice, other signaling components might compensate for the loss of CD19 (15–19). One such candidate is BCAP, an adapter molecule that is highly expressed in B cells and which provides four potential binding sites for p85α (20). Similar to CD19, loss of BCAP alone did not dramatically impair BCR-mediated activation of PI3K. However, the combined loss of BCAP and CD19 almost completely abolished PI3K activity upon stimulation with antibodies specific for Ig-β (21). This observation suggests that both proteins share overlapping functions and are crucial for BCR-mediated activation of PI3K signaling.

Syk-mediated phosphorylation of CD19 and BCAP leads to the recruitment and subsequent activation of class IA PI3K heterodimers (1, 21). Since these heterodimers are formed by association of a regulatory subunit with a catalytic subunit and since every regulatory subunit is able to associate with every catalytic subunit, the PI3K system has the potential to be highly redundant. Studying the distinct roles of selected regulatory or catalytic subunits could be difficult, since the loss of one subunit might be compensated by the remaining subunits. Based on the phenotypes of knockout mice, however, the p85α regulatory and the p110δ catalytic subunit are thought to be the main class IA PI3Ks acting downstream of the pre-BCR or the BCR (22–25). By binding to the phosphotyrosine (pTyr) residues, as provided by CD19 and BCAP, regulatory subunits control the subcellular localization and membrane recruitment of the catalytic subunits. Moreover, other interactions also contribute to membrane recruitment of the class IA PI3Ks. The different p110 catalytic subunits have a similar overall structure, which includes a Ras binding domain. Hence, in addition to binding to p85, the p110 catalytic subunits can associate with Ras-guanosine triphosphate (GTP) to become activated (26). It is thought that each p110 has a distinct binding capacity to Ras or shows a binding preference for specific Ras isoforms (27, 28). The selective activation of distinct p110s by Ras could therefore modify the downstream effects upon stimulation of an antigen receptor and thus lead to different outcomes.

An initial event upon activation of PI3K is the generation of the lipid second messenger $PtdInsP_3$ by PI3K-mediated phosphorylation of $PtdInsP_2$ (2). $PtdInsP_3$ provides binding sites for proteins that contain a pleckstrin homology (PH) domain. Binding of the PH domain to $PtdInsP_3$ is the molecular mechanism that mediates membrane recruitment and subsequent activation of essential signaling proteins. Important signaling proteins that contain a PH domain are the serine/threonine protein kinase B (PKB or AKT), 3-phosphoinositide-dependent protein kinase 1 (PDK1), or the Tec kinase BTK (Bruton's agammoglobulinemia tyrosine kinase). PI3K-dependent translocation of BTK to the plasma membrane has been shown to be essential for BTK function and is thought to link PI3K activation to calcium signaling through BTK-dependent

activation of phospholipase Cγ (PLCγ). In addition, the phenotypes of mice deficient for p85α, p110δ, BTK, and PLCγ are similar, underlining the fact that PI3K is a central part of a BCR-triggered signalosome (29).

The serine/threonine kinase AKT is a main mediator of PI3K signaling. By regulating the expression of multiple downstream targets that are involved in the regulation of the cell cycle, the induction of apoptosis and the recombination of Ig genes (discussed below), activation of AKT promotes proliferation, survival and differentiation in B cells (30, 31). Full activation of AKT requires phosphorylation at threonine 308, by PDK1 (32–34), and at serine 473, which might be accomplished by the rapamycin-insensitive mTOR complex 2 (mTORC2, consisting of the kinase mTOR, Rictor, Sin1, and mLST8 complex) (35, 36). In mammalian cells, three isoforms of AKT (AKT1–AKT3) encoded by three different genes, have been described. The three isoforms share more than 80% amino acid sequence identity, and all AKT isoforms share identical or at least overlapping substrate specificity (2, 37), thereby increasing the redundancy of the PI3K-AKT signaling system.

Role of AKT in survival and proliferation

AKT promotes cell survival and proliferation by regulating the activity as well as the expression of proteins involved in these processes. For instance, proapoptotic proteins, such as the Bcl-2 homology domain 3 (BH3)-only proteins, bind directly to and inactivate members of the prosurvival Bcl2-family. AKT-mediated phosphorylation of the BH3-only protein BAD on serine 136 provides a docking site for the adapter 14-3-3, which induces the release of BAD from its target proteins and finally leads to its cytosolic sequestration (38–40). Furthermore, AKT promotes survival of cells by regulating the basal expression of BH3-only proteins through controlling particular transcription factors (41). The forkhead box class O (FOXO) family represents important transcription factors that are regulated by AKT. The activity of the FOXO family members FOXO1, FOXO3a, FOXO4, and FOXO6, all of which are homologs of the *Caenorhabditis elegans* transcription factor DAF-16 (42, 43), is tightly regulated on the post-translational level by modifications including phosphorylation (44, 45). FOXO1, FOXO3a, and FOXO4 are the

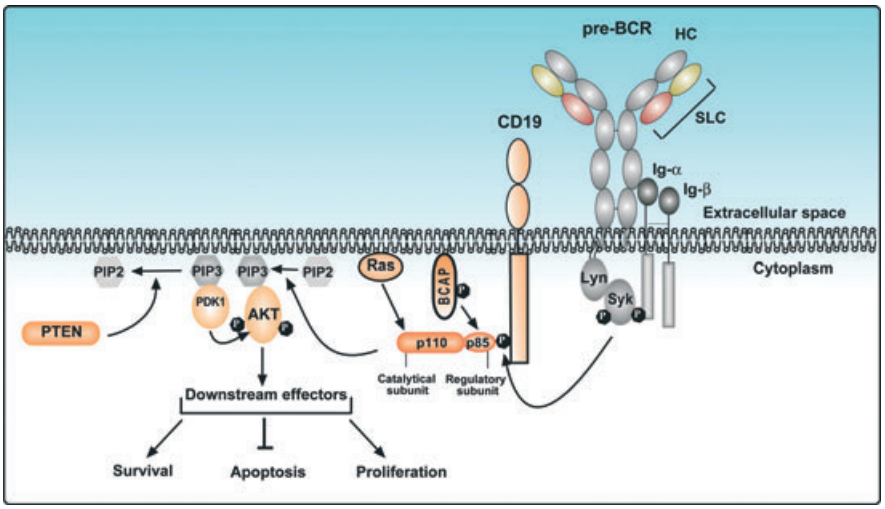

***Fig. 1.* Autonomous pre-BCR signaling activates the PI3K signaling pathway.** The pre-B-cell antigen receptor (pre-BCR) complex consists of two μ heavy chains (HC) that are each associated with a surrogate light chain (SLC). Autonomous signals derived from the pre-BCR are transmitted by the signal transducing elements immunoglobulin-α and immunoglobulin-β (Ig-α and Ig-β) and lead to the activation of Src-kinases (Lyn, Blk, Fyn). These phosphorylate immunoreceptor-tyrosine activation motifs (ITAMs), provided by Ig-α and Ig-β, and thereby recruit and activate the spleen tyrosine kinase (Syk). Activated Syk phosphorylates motifs (YXXM) present in the cytoplasmic tail of the transmembrane protein CD19 and in B-cell adapter of PI3K (BCAP). This leads to recruitment and subsequent activation of class IA phosphoinositide 3-kinase (PI3K) heterodimers, consisting of a regulatory subunit, that is constitutively associated with a p110 catalytic subunit. Alternatively, independent of the regulatory subunit, the several p110s are rendered active upon binding to Ras-GTP. Activated PI3K phosphorylates phosphatidylinositol-(4,5)-bisphosphate (PtdInsP2 or PIP2), to generate the lipid second messenger phosphatidylinositol-(3,4,5)-bisphosphate (PtdInsP3 or PIP3). PtdInsP3 (PIP3) serves as binding sites for proteins that contain a pleckstrin homology (PH) domain such as protein kinase B (PKB or AKT) as well as 3-phosphoinositide-dependent protein kinase 1 (PDK1). Upon PDK1-mediated phosphorylation, by regulating several downstream effectors, AKT-signaling promotes proliferation and survival of pre-B cells. By inactivating FOXO transcription factors (not shown), AKT prevents apoptosis. PI3K signaling is controlled by PTEN that dephosphorylates PtdInsP3 (PIP3) back to PtdInsP2 (PIP2), thereby terminating PI3K signaling.

three isoforms that are expressed during B-cell development. All of them contain three conserved serine/threonine residues that can be phosphorylated by activated AKT. Phosphorylation induces 14-3-3-mediated relocalization of FOXOs from the nucleus to the cytoplasm, where FOXO proteins are subjected to proteasomal degradation (46–48). Thus, activated AKT blocks FOXO-mediated transcription of target genes that have been shown to promote cell cycle arrest and apoptosis (48). Among the numerous targets of FOXO transcription factors that promote apoptosis is the proapoptotic protein BIM, which is responsible for the initiation of apoptosis upon cytokine withdrawal in hematopoietic cells (49). In addition, FOXO transcription factors induce the expression of Fas ligand (FasL), thereby triggering cell death of Fas receptor-expressing cells (50). Since active FOXO transcription factors promote cell death, inactivation of FOXO transcription factors, as observed in many leukemic cells, is considered to be an important event in the pathology of cancer. In line with this, enhanced PI3K-AKT signaling as well as induced deletion of FOXO1, FOXO3a, and FOXO4 in mice result in the development of thymic lymphomas and haemangiomas (26, 52).

Besides the role of AKT in supporting cell survival, activation of AKT also promotes proliferation. For cell cycle progression, cells have to activate specific G1 cyclins and cyclin-dependent kinases (CDKs) that are necessary to initiate cell cycle entry, and downregulate cell cycle inhibitors such as $p27^{Kip1}$. By inactivating glycogen synthase kinase-3β (GSK3β), a negative regulator of c-Myc and cyclin D, PI3K-AKT signaling supports stabilization of these proteins and consequently cell cycle entry (53). Furthermore, AKT signaling negatively regulates the cell cycle inhibitor $p27^{Kip1}$ on the transcriptional as well as on the post-translational level (51, 54).

Augmented and sustained PI3K-AKT signaling in developing B cells would therefore promote uncontrolled cellular expansion and malignant transformation, whereas reduced or deficient activation of PI3K could lead to lymphopenia and immunodeficiency. Based on this, a tight regulation of PI3K-AKT activity is mandatory. Important negative regulators of PI3K signaling are PTEN (phosphatase and tensin homolog deleted on chromosome 10) that removes the 3′-phosphate of $PtdInsP_3$, thereby generating $PtdIns(4,5)P_2$ and the phosphatase SHIP (SH2 domain-containing inositol 5′-phosphatase) that removes the 5′-phosphate to generate $PtdIns(3,4)P_2$ (2, 55). Also, AKT is subjected to negative regulation. A phosphatase that counteracts AKT activity is PHLPP (PH domain leucine-rich

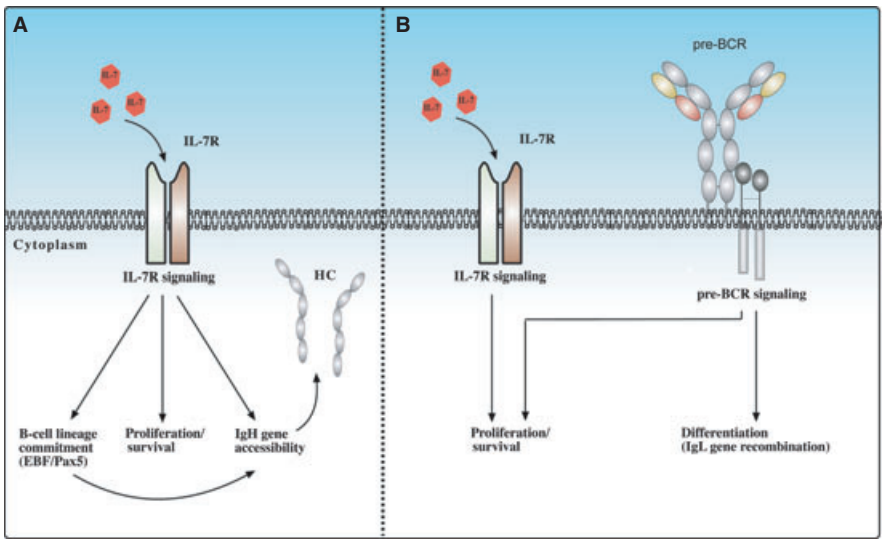

***Fig. 2.* The IL-7R and the pre-BCR.** (A) The interleukin-7 receptor (IL-7R) complex comprises the IL-7Rα chain associated with the common cytokine γ chain (γc). Upon binding of IL-7, the two IL-7R chains heterodimerize and signaling is initiated. An important function of IL-7R signaling is the promotion of survival and proliferation of developing B cells. A further role of IL-7R signaling is commitment to the B-cell lineage through induction of a B-cell-specific transcriptional network that includes the essential factors early B-cell factor (EBF) and Pax5. As IL-7R derived signals regulate the accessibility of immunoglobulin heavy chain (IgH) genes, the IL-7R promotes generation and expression of a μ heavy chain (HC), leading to assembly of the pre-B cell antigen receptor (pre-BCR), the second important receptor for early B-cell development. (B) Autonomous pre-BCR signaling leads to upregulation of IL-7R expression and thus to a synergistic effect of both receptors on survival and proliferation of pre-B cells. However, pre-BCR-driven signals also cause the downregulation of IL-7R expression, thereby presumably decreasing the proliferative capacity but promoting differentiation of pre-B cells.

repeat protein phosphatase), which dephosphorylates AKT at serine 473, providing thereby a further mechanism for PI3K-AKT signal termination (56).

The adapter protein SLP-65 (SH2-domain-containing leukocyte protein of 65kDa) (also known as BLNK or BASH) was recently identified as another important regulator of PI3K signaling in pre-B cells. Activation of an inducible form of SLP-65 results in markedly lowered levels of phosphorylated AKT (57), suggesting that the pre-BCR-induced Syk-dependent activation of the adapter protein SLP-65 inhibits PI3K signaling. The precise mechanism, as well as the components that accomplish this SLP-65-dependent negative regulation of PI3K signaling, are currently not known. However, this observation raises the question as to how the pre-BCR steers the balance between the activation of the PI3K-proliferation/survival system and the SLP-65-dependent differentiation pathways.

Coreceptors in proliferation and survival of early B cells

The survival, proliferation, and differentiation during early B-cell development are critically dependent on interleukin-7 (IL-7), its receptor (IL-7R), and the pre-BCR (58, 59) (*Fig.* 2). Numerous experiments indicate that the signaling pathways downstream of these receptors share intense crosstalk and, together, provide a synergistic system that is crucial for the

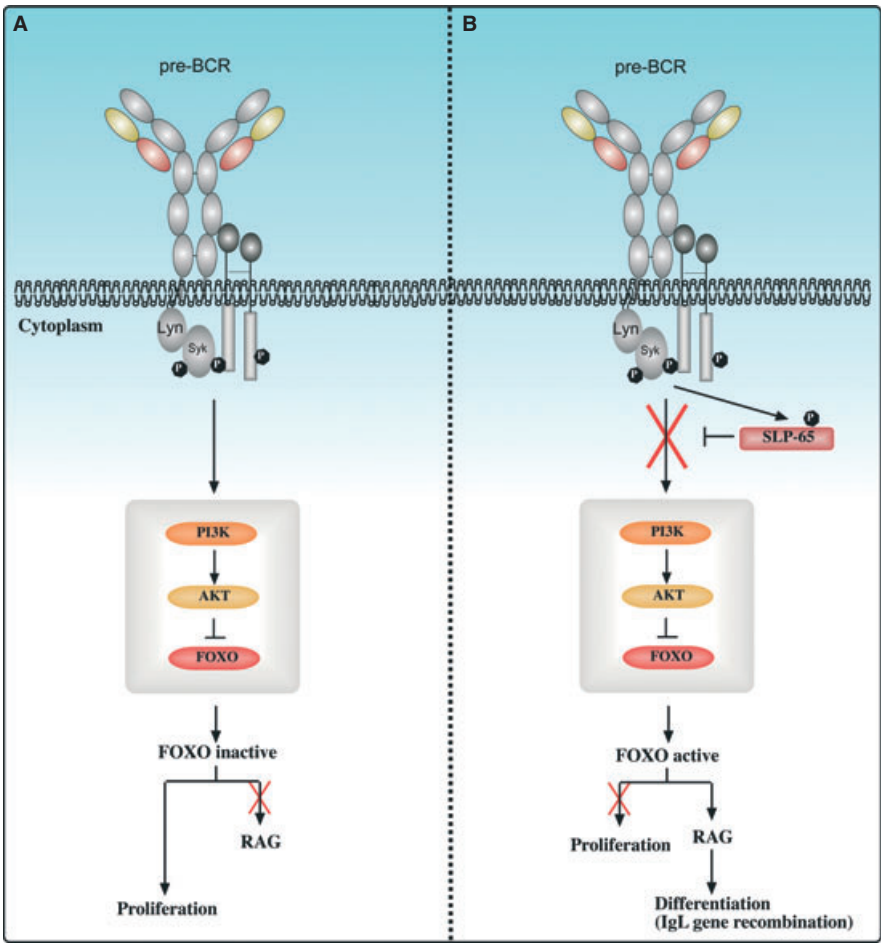

***Fig.* 3. PI3K regulates IgL gene recombination.** (A) Expression of a signaling-competent pre-B-cell antigen receptor (pre-BCR) on the cell surface induces the phosphoinositide 3-kinase (PI3K) pathway through autonomous signaling that is mediated by Syk (spleen tyrosine kinase). PI3K activates protein kinase B (PKB or AKT) that phosphorylates forkhead box O (FOXO) transcription factors, thus leading to their proteasomal degradation. Under these conditions, the recombination-activating gene (RAG) proteins are transcribed at very low levels, and therefore, recombination processes at the immunoglobulin light chain (IgL) genes are suppressed. Due to the inactivation of FOXOs, pre-BCR expressing cells are primed for proliferation. (B) However, pre-BCR-mediated activation of SH2-domain-containing leukocyte protein of 65kDa (SLP-65) inhibits PI3K activity. FOXO transcription factors are therefore released from AKT-mediated negative regulation and able to induce the expression of their target genes. Active FOXOs promote exit of the cell cycle and induce expression of the RAG proteins. As a consequence of enhanced RAG expression, recombination of the IgL genes is initiated.

survival and the clonal expansion of developing B cells (59, 60). Available data suggest that IL-7R signaling, in addition to promoting survival and differentiation of early B-cell progenitors, is necessary for the commitment of these cells to the B-cell lineage by initiating the expression of a B-cell-specific transcriptional network that includes essential factors such as EBF (early B-cell factor) and PAX5 (64). The expression of the IL-7R complex is induced at the common lymphoid progenitor stage (CLP) and sustained until the pre-B-cell stage. Accordingly, mice that are deficient for elements of the IL-7 signaling pathway show a blocked B2 B-cell development at the transition from the CLP stage to the pro-B cell stage (62, 63). Generation of B1 and marginal zone (MZ) B cells, however, is not affected in mice deficient for IL-7 (64). In humans, notably, B-cell development of all subsets is independent of IL-7, as genetic deficiencies of IL-7R signaling do not result in B-cell deficiency or lymphopenia (65).

The IL-7R complex comprises the IL-7Rα-chain (IL-7Rα), also known as CD127, and the common cytokine-receptor γ

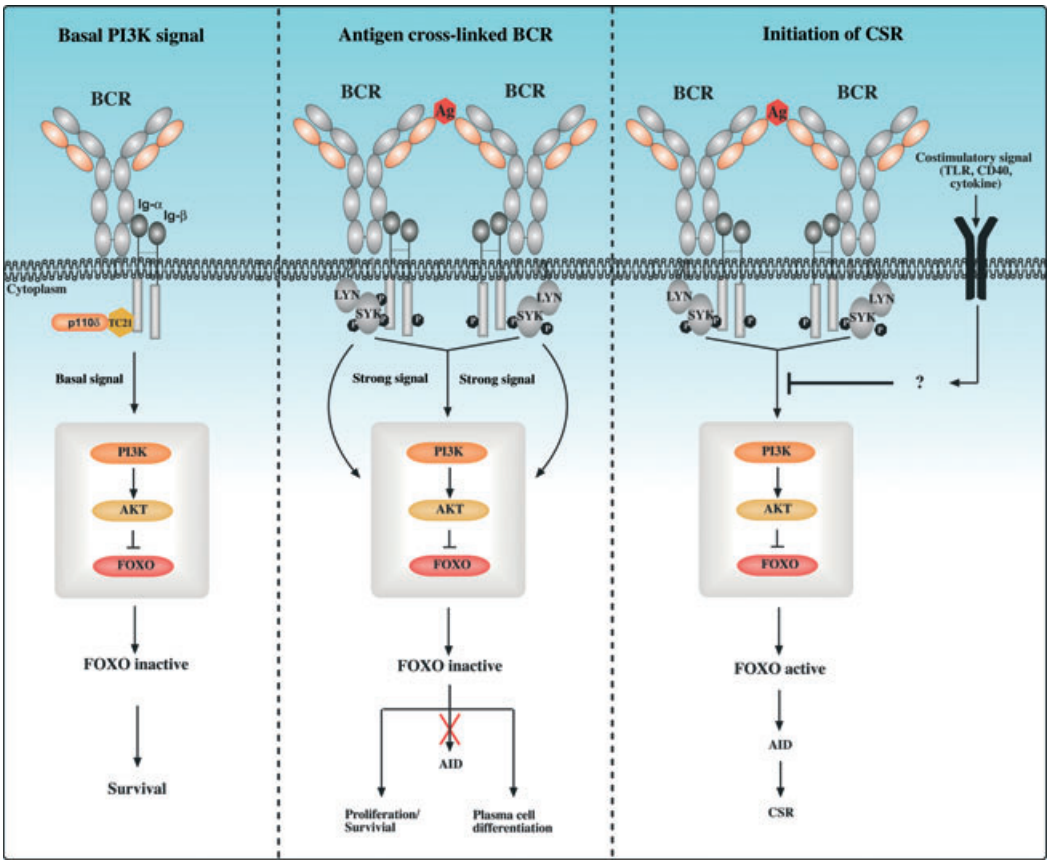

***Fig. 4.* Mature B cells and PI3K.** (A) The B-cell antigen receptor (BCR) induces a basal phosphoinositide 3-kinase (PI3K) signal. Establishment of this basal signal seems to not depend on activation of Src-kinases (Lyn, Blk, Fyn) or kinases of the Syk family (Syk and Zap-70). It has been proposed that the small Ras-GTPase TC21 (or rRas-2) binds simultaneously and preferentially to non-phosphorylated immunoreceptor-tyrosine activation motifs (ITAMs) and to the PI3K p110δ subunit. The interaction between the non-phosphorylated ITAM, TC21, and p110δ mediates the recruitment of PI3K to the plasma membrane, its activation, and the initiation of PI3K signaling. Subsequent phosphorylation and activation of protein kinase B (PKB or AKT) leads to the degradation of FOXOs and thereby to survival of mature B cells. (B) Antigen-mediated cross-linking of the BCR leads to the activation of Src-kinases and the Syk kinase, which phosphorylate CD19 and B-cell adapter of PI3K (BCAP), thereby initiating PI3K signaling (not shown). PI3K in turn activates AKT that phosphorylates forkhead box O (FOXO) transcription factors, thus leading to their proteasomal degradation. Due to the inactivation of FOXOs, expression of activation-induced cytidine deaminase (AID) is low and class switch recombination (CSR) is inhibited. Notably, the PI3K signaling strength downstream of a cross-linked BCR is substantially higher than the one of resting BCRs (basal signal). Therefore, due to the high PI3K activity, antigen-stimulated naive B cells are primed for proliferation. (C) Since in the presence of high PI3K activity, FOXOs are not active and AID expression is not sufficient to induce CSR (see B), it appears likely that for the initiation of CSR the activity of PI3K has to decrease. As the PI3K-AKT-FOXO signaling module downstream of the BCR is strikingly similar to the one downstream of the pre-BCR, it is possible that a mechanism, similar to the SH2-domain-containing leukocyte protein of 65kDa (SLP-65)-mediated drop of PI3K activity in pre-B cells, exists in mature B cells. To prime antigen-stimulated naive B cells to initiate CSR, costimulatory signals provided by T-helper cells are required. It is therefore conceivable that signals driven by these costimulatory receptors [CD40, Toll-like receptor (TLR), cytokine receptors] lead to the activation of a signaling pathway that counteracts PI3K activity, thereby rendering FOXOs active. This would promote the expression of AID and the initiation of CSR.

chain (γ_c), also known as CD132, that is also shared by the receptors for the cytokines IL-2, IL-4, IL-9, IL-15, and IL-21(59). Binding of IL-7 to the IL-7R promotes heterodimerization of these two receptor chains and leads to the activation of the receptor-associated Janus kinases JAK1 (IL-7Rα) and JAK3 (γ_c) (66). Activated JAKs phosphorylate specific tyrosine residues present on the IL-7R chains, thereby providing binding sites for STATs (signal transducers and activators of transcription) that are recruited via their SH2-domain (66). Subsequently, JAK-mediated phosphorylation of STATs, specifically the two STAT5 isoforms STAT5a and STAT5b, enables them to dimerize and to translocate into the nucleus. They act as transcriptional modulators of genes that are involved in the regulation of survival and proliferation, such as the anti-apoptotic protein Bcl-XL, as well as the cell cycle regulators c-Myc and D-type cyclins (67–69). Thus, IL-7R signaling activates a similar set of pro-survival and proliferation-inducing elements as PI3K. Moreover, expression of a constitutively active form of STAT5 promotes PI3K-dependent activation of AKT, thereby establishing an additional synergistic crosstalk between pre-BCR and IL-7R signaling (70).

Through regulation of the recombination processes at the IgH gene locus, IL-7R signaling is mandatory to induce the generation and expression of a pre-BCR. This is demonstrated by mice with impaired IL-7R signaling that show a developmental arrest at the pro-B-cell stage with impaired V to DJ recombination at the IgH gene locus (59). As these cells are not able to generate a μ heavy chain (HC), they cannot express a pre-BCR and consequently fail to establish the synergistic pre-BCR/IL-7R pro-survival system. Successful generation of a productive heavy chain transcript, however, enables late pro-B cells to express a pre-BCR and to transit to the large pre-B cell stage. The pre-BCR complex consists of two μHCs, which are both associated with the surrogate light chain (SLC) components λ5 and VpreB, and the signal transducing elements Ig-α and Ig-β (71, 72). Expression of a functional pre-BCR marks an important checkpoint in B-cell development, as only B cells that express a pre-BCR are positively selected and able to mature further. Autonomous signals derived from the pre-BCR have been shown to induce the PI3K-AKT signaling axis (57) as well as the activation of Erk1 and Erk2 (73) both of which are positively linked to the survival and proliferation of developing B cells. Furthermore, pre-BCR-derived autonomous signals lead to upregulation of IL-7R expression and thus to higher responsiveness of pre-B cells to IL-7. This supports the survival as well as the selective expansion of pre-BCR-expressing cells (60, 74).

However, as shown by using mice that are transgenic for the SLC, prolonged pre-BCR expression beyond the pre-B-cell stage does not lead to an increased pre-B-cell proliferation (80). Instead, B-cell development in such mice is blocked at the immature B-cell stage, suggesting that the pre-BCR-induced proliferation is restricted to large pre-B cells. Possibly, only large pre-B cells express sufficient IL-7R and are thus responsive to IL-7, whereas later stages of B-cell development have reduced IL-7R surface expression and consequently a decreased proliferative capacity. In fact, the pre-BCR-induced activation of SLP-65 has been shown to result in downregulation of IL-7R surface expression (74–76). This might limit the expansion of pre-B cells but support the initiation of pre-BCR-mediated differentiation processes. The importance of SLP-65-mediated IL-7R-downregulation in pre-B cells is demonstrated by several observations. Unrestrained IL-7R signaling triggers proliferation of pro- and pre-B cells in the bone marrow as well as the migration of these populations to secondary lymphoid organs such as the spleen, lymph nodes, and the blood (59). Mice with enhanced IL-7R signaling are prone to develop pro- and pre-B-cell tumors, and deregulated activation of STAT5 contributes to the development of leukemia and solid tumors (77, 78). The role of SLP-65 as a tumor suppressor is further supported by the finding that activated SLP-65 is able to bind to JAK3, thereby suppressing the JAK3-mediated activation of STAT5 (79). In line with this, the JAK3/STAT5 signaling axis is constitutively active in pre-B leukemia cells derived from SLP-65-deficient mice (79).

Role of PI3K in early B cells

Autonomous pre-BCR-induced signaling activates PI3K and is important for survival and cell cycle progression of pre-BCR-expressing cells. It is therefore conceivable that diminished or missing pre-BCR-mediated PI3K activation leads to developmental defects during early stages of B-cell development and to a reduced amount of mature B cells due to the impaired survival and proliferation of B-cell progenitors in the bone marrow.

As mentioned above, activation of PI3K in pre-B cells is dependent on Src- and Syk-kinases that phosphorylate the adapter proteins BCAP and CD19. Considering this, it is not surprising that mice lacking either components of the pre-BCR, Syk, CD19, BCAP, or distinct PI3K subunits demonstrate strikingly similar phenotypes (discussed below). The importance of pre-BCR expression for the induction of clonal proliferation and survival is clearly shown when particular components of the pre-BCR complex are missing. For instance, developing B cells that are not able to express a func-

tional μHC are blocked at the pro-B-cell stage, and a two fold enrichment of pro-B cells in the bone marrow is observed (81). Mice lacking one of the signaling components Ig-α or Ig-β exhibit a complete block at the pro-B-cell stage, due to the inability to express a signaling-competent pre-BCR complex (82, 83). Mice deficient for the SLC, either λ5 or both VpreB proteins (VpreB1 and VpreB2) show a partial block at the pro-B-cell stage, resulting in a marked decrease of mature B cells in the periphery (84, 85). The incomplete block in SLC-deficient mice indicates that pre-B cells are partially able to overcome the absence of a normal pre-BCR and the accompanied absence of survival and proliferation signals. Expression of a BCR with a conventional IgL chain (86) and the expression of receptors that contain the μHC but not the SLC or conventional LC on the surface (87–89) were shown to overcome the absence of a pre-BCR, possibly through providing sufficient PI3K activation that mediates the survival and further development of these cells.

B cells that are deficient for Syk are not able to promote pre-BCR-mediated activation of PI3K and therefore show an almost complete developmental block at the pre-B-cell stage (90, 91, E.H., unpublished data). This finding suggests that activation of Syk is the first important event in pre-BCR-mediated signaling. Absence of the transmembrane protein CD19, which has been shown to enhance signaling through the pre-BCR (92), also results in reduced pre-B-cell numbers (93). The levels of phosphorylated AKT upon stimulation of a BCR are reduced in $CD19^{-/-}$ cells, and the fact that expression of a CD19 mutant that cannot bind PI3K is not able to restore the $CD19^{-/-}$ phenotype suggests, that CD19 plays an important role in bridging pre-BCR-mediated activation of Syk to the activation of PI3K (13, 19, 94). A similar phenotype has been reported for BCAP-deficient mice (95). Whereas the loss of BCAP or CD19 alone has shown to result in partial defects due to compensation by the other protein, combined loss of both results in a severely impaired AKT activation upon BCR stimulation (21). The combined deficiency of BCAP and CD19 leads to an accumulation of large pre-B cells, whereas the numbers of small pre-B cells are reduced (21). It is not clear whether the accumulation of large pre-B cells is due to IL-7R-mediated signals and whether IL-7R expression is increased in the BCAP/CD19 double deficient pre-B cells. Alternatively, pre-BCR signals that promote activation of PI3K are required for differentiation of large pre-B cells towards small pre-B cells, and in the absence of both BCAP and CD19, these PI3K-dependent differentiation signals are interrupted.

Activation of PI3K requires its recruitment to pTyrs provided by CD19 and BCAP. This membrane localization is mediated by the SH2-domain containing class IA PI3K regulatory subunits that are constitutively associated with a catalytic subunit. Therefore, B cells deficient for the regulatory subunit p85α show strongly impaired PI3K activation and exhibit a partial block at the pro-B-cell stage, accompanied by markedly reduced numbers of mature B cells in the spleen (18). The role of the distinct catalytic subunits for B-cell development is rather difficult to study. The catalytic subunits p110α and p110β are ubiquitously expressed, and mice deficient for these two subunits die during embryonic development (96, 97). Therefore, to study the contribution of these subunits in lymphocyte development, lineage-specific deletions, which have not been reported, are required. In contrast to p110α and p110β, expression of the catalytic p110δ subunit is mainly restricted to leukocytes, and the development of mice lacking p110δ is not severely impaired. Different strategies were used to generate p110δ-deficient mice (22–24). Whereas two groups eliminated the expression of p110δ (23, 24), one group introduced a mutation in the catalytic domain, thereby allowing the expression of a catalytically inactive p110δ subunit (22). In light of the great redundancy of the PI3K system, the advantage of the latter strategy might be the reduction of compensatory effects by the remaining p110 subunits, with respect to p85-binding and the competition for Ras.

Although B-cell development in p110δ-deficient mice is not completely abrogated, B cells that lack a functional p110δ subunit show a partial block at the pro-/pre-B-cell transition, as indicated by a drastic reduction of the pre-B to pro-B cell ratio in mutant mice compared to the corresponding wildtype littermates (1:1 and 4:1, respectively) (22). This finding suggests that p110δ plays an important role for pre-BCR-mediated survival or differentiation of pre-B cells. An interesting approach for future studies would be the generation of conditional knockout mice, allowing the tissue-specific deletion of particular p110 catalytic subunits in mice. Recent data showed that upon stimulation of the BCR, p110δ and p110α but not p110β were selectively recruited to the cytoplasmic tails of CD19 (98). Based on this observation, it would be interesting to investigate whether the combined deletion of p110α and p110δ has a more severe effect on B-cell development and thus exceeds the phenotype observed in p110δ-deficient mice.

The impact of AKT1 and AKT2 on B-cell development has recently been reported (99). Since mice deficient for AKT1/AKT2 die shortly after birth (100), examination of B-cell development was accomplished by injection of fetal liver cells from AKT1/AKT2-deficient mice into irradiated

mice. Regarding early stages of B-cell development, AKT1/AKT2 double deficient B cells seem to accumulate at the pro- and the pre-B-cell stage (three fold and four fold increase, respectively) as well as to a lesser extent at the immature B cell stage (two fold increase). The numbers of mature B cells, however, are strongly reduced (99). The finding that pro- and pre-B as well as immature B cells are present at higher numbers in these mice indicates that AKT1/AKT2-deficient cells in early B-cell development have no general problem in survival. Although the AKT3 isoform is still expressed in these cells and might be sufficient for survival and proliferation, the phenotype of AKT1/AKT2-deficient B cells clearly suggests a role of PI3K-dependent signaling pathways in the activation of pre-B cell differentiation, which would be in agreement with the accumulation of large pre-B cells in CD19/BCAP double deficient mice.

Role of PI3K in regulating VDJ recombination

Large pre-B cells that have undergone the phase of extensive proliferation move to the small pre-B-cell stage and recombine the IgL gene loci to form a BCR (101). Interestingly, both the proliferation as well as the differentiation of pre-B cells are induced by pre-BCR-driven signals. The two seemingly opposite Syk-dependent pathways that are activated downstream of the pre-BCR are the PI3K proliferation/survival pathway and the SLP-65-dependent differentiation pathway. The pre-BCR-induced activation of PI3K-AKT promotes proliferation and counteracts differentiation processes such as immunoglobulin light chain (IgL) recombination, whereas pre-BCR-mediated activation of SLP-65 and the concomitant downmodulation of PI3K signaling has been shown to promote LC gene recombination and thus differentiation of developing B cells (30, 57). In line with these findings, Syk-deficient B cells with non-autoreactive BCRs are severely impaired in triggering PI3K signaling and therefore show evidence for ongoing LC gene recombination and receptor editing at the IgL gene (104). Thus, in the complete absence of Syk-mediated signals, VDJ recombination is constitutively active without additional triggering. Supporting this, Syk/ZAP-70 double deficient pre-B cells show loss of allelic exclusion and uncontrolled VDJ recombination of the μHC locus (105). In SLP-65-deficient mice, in contrast, IgL chain gene recombination is impaired, which leads to compromised receptor editing and to an increased antibody response to immunization with a self-antigen (106). This suggests that SLP-65 is necessary to prevent development of autoreactive B cells by suppressing PI3K-AKT signaling. Most likely, SLP-65 induces a regulated downmodulation of PI3K-AKT, thereby inducing the activity of FOXO proteins that promote secondary LC gene recombination to remove autoreactive BCR specificities. Deletion of the AKT downstream target FOXO1 at the early pro-B-cell stage leads to a complete block in B-cell development and, whereas the levels of D_H to J_H gene recombination are normal, FOXO1-deficient B cells exhibit impaired V_H to DJ_H recombination (107). Since FOXO1 has been shown to induce the expression of the essential recombination-activating genes (RAGs) (102), low RAG expression might explain the impaired V_H to DJ_H recombination observed in FOXO1-deficient B cells. In PI3K signaling-deficient B cells, in contrast, FOXO1 is not subjected to AKT-mediated negative regulation. It would be therefore interesting to investigate whether the activity of FOXO proteins is increased in Syk-deficient cells, thereby leading to high RAG activity. Similarly, RAG activity is expected to be high in AKT1/AKT2-deficient pre-B cells, which might result in increased and continuous LC recombination or loss of allelic exclusion. In line with this idea, immature $CD19^{-/-}$ B cells or p85α-deficient B cells, which are compromised in PI3K signaling, exhibit increased expression of both Rag1 and Rag2 and consequently undergo intensive receptor editing (108, 109).

The described link between the autonomous pre-BCR-initiated PI3K-AKT-FOXO signaling and the regulation of RAG-mediated recombination processes suggests a model how primary and secondary IgL chain gene recombination are regulated by the pre-BCR and the BCR during the course of B-cell development (*Fig.* 3). Upon successful recombination of a μHC, late pro-B cells assemble a pre-BCR complex on the surface and autonomous signals derived from the pre-BCR trigger PI3K, initiated by Syk-mediated phosphorylation of CD19 and BCAP. This leads to the activation of AKT and finally results in the degradation of FOXO transcription factors. In the absence of SLP-65, pre-BCR-expressing cells remain in this proliferative state and are not able to induce IgL gene recombination. Instead, B cells deficient for SLP-65 are prone to develop pre-B-cell tumors, supporting the important role of SLP-65 in preventing malignant transformation and leukemia (110–112). Activation of SLP-65 leads to downmodulation of PI3K signaling and the stabilization of FOXO proteins that induce RAG expression. Hence, SLP-65 is the crucial component for the initiation of IgL chain gene recombination in pre-B cells, as it mediates the switch from proliferation towards the initiation of differentiation. An obvious question in this context is how the pre-BCR ensures several rounds of proliferation before SLP-65 is activated, PI3K-AKT signaling is downmodulated and IgL gene recom-

bination is initiated. The fact that BCAP/CD19-deficient mice show increased large pre-B-cell numbers but reduced small pre-B-cell numbers suggests that PI3K signaling is involved in the initiation of pre-B-cell differentiation. It is conceivable that PI3K transmitted signals influence the activation or expression of signaling components of the SLP-65 dependent pro-differentiation pathway. If so, the PI3K-mediated proliferation signals would precede those inducing differentiation thereby allowing proliferation to take place before the differentiation machinery is activated.

The initial recombination processes of the IgL chain genes can lead to three possible outcomes. First, the recombination processes at the IgL chain genes result in the generation of a LC that is able to associate with a μHC to form a conventional BCR. Expression of such a BCR establishes a basal level of PI3K activity, which leads to the inactivation of FOXOs. This prevents further IgL chain gene recombination and promotes further development. Second, due to a non-productive V to J gene segment recombination, developing B cells may not form a functional BCR. In this case, no basal BCR-mediated activation of PI3K is established, FOXO transcription factors stay active and promote further recombination processes at the IgL gene loci. Third, the inherent variability in the IgL gene recombination process can result in the generation of a BCR with an autoreactive specificity. Self-antigen-mediated cross-linking of such a BCR leads to constitutive receptor-internalization and possibly to the removal of basal BCR signaling (103, 113). The signaling pathways downstream of an autoreactive BCR triggered by self-antigen are presumably similar to those downstream of a pre-BCR (114). Hence, self-antigen-mediated cross-linking of the autoreactive BCR might activate SLP-65, which promotes further secondary recombination of the IgL chain genes to create a BCR with non-autoreactive specificity. Since the activity of FOXO transcription factors is also linked to the induction of apoptotic programs (115), a certain time frame is given in which secondary LC gene recombination and the assembly of a conventional BCR can take place. If cells are not able to change the autoreactive receptor specificity to a non-autoreactive one by secondary recombination of the IgL chain gene, the absence of BCR-mediated basal PI3K activation and therefore the sustained presence of activated FOXO proteins might result in the death of the cell.

Mature B cells and a BCR-derived PI3K-mediated basal signal

The successful recombination of the IgL gene enables developing B cells to express a conventional BCR and to differentiate to the immature stage. Immature B cells exit the bone marrow and migrate to the spleen where they are subjected to additional selection processes at early and late transitional stages before they become mature B cells.

Investigating the importance of BCR expression for the survival of naive mature B cells *in vivo* has been made possible by the invention of conditional gene targeting using the Cre/loxP system (116). Taking advantage of this technology, BCR components can be inducibly deleted in mature B cells (117–119). In early studies, the role of the BCR in the survival of mature B cells was investigated in transgenic mice in which the μHC can be inducibly ablated. The results showed that mature B cells that have passed through early checkpoints in the bone marrow and were selected into the long-lived peripheral pool still depend on the expression of the BCR for their maintenance. Ablation of the BCR led to deletion of mature B cells within 1–2 weeks, demonstrating that BCR expression is indispensable for the long-time survival of mature B cells. However, it was not clear whether B cells die because they need BCR assembly as such to survive or whether the loss of BCR surface expression leads to cell death because of improper processing of its different components in the endoplasmic reticulum. Alternatively, survival of mature B cells could depend on a low level of steady-state signals triggered by the BCR and propagated downstream of the BCR by the signaling machinery. The answer to these questions came from studies done by the same group, where Ig-α, a signaling subunit of the BCR, was deleted (118). In this work, the effects of BCR ablation by induced Ig-α-inactivation were compared to that of interference with BCR signaling by the inducible deletion of the Ig-α cytoplasmic tail. As in the case of the μHC inactivation, BCR depletion through Ig-α deletion as well as induced deletion of the Ig-α signaling tail also resulted in the loss of the majority of mature B cells. Thus, interference with BCR signaling through deletion of the Ig-α tail leads to a loss of mature B cells, similar to BCR ablation through induced μHC or Ig-α inactivation. These results demonstrated that survival of resting mature B cells depends on BCR signaling through the Ig-α/Ig-β heterodimer. The BCR can activate multiple signaling cascades, however, the nature of the major pathway involved in mature B-cell survival was only recently described (119). Simultaneous depletion of BCR and activation of candidate signaling pathways in mature B cells *in vivo* provided evidence that B cells without BCRs survive and accumulate in the presence of a constitutively active PI3K pathway. This was accomplished by the expression of a constitutively active p110α catalytic subunit or by deletion of PTEN, the negative regulator of PI3K. In

contrast, the establishment of either a constitutive mitogen-activated protein kinase pathway, a constitutive Rac1 pathway, or a constitutive non-canonical NF-κB pathway was not able to rescue B cells depleted of BCRs. Interestingly, deletion of FOXO1 alone also rescued B cells without BCRs, which might fit with the hypothesis presented above that FOXO activation induces death of immature B cells that fail to express a functional BCR. These results suggest that the PI3K-AKT-FOXO axis is crucial in supporting BCR-driven signals that mediate survival of B cells. However, although the activation of PI3K and its downstream effectors is well studied upon stimulation of the BCR, it still remains unclear how this pathway is activated during basal BCR signaling in the absence of antigen binding. One possibility is that during basal BCR signaling, a baseline ITAM phosphorylation, established by the different Src kinases (Lyn, Fyn, Blk) or the Syk kinase family (Syk and ZAP-70), ensures sufficient activation of PI3Ks to promote a constitutive survival signal. Nonetheless, deletion of Lyn as well as the combined deletion of Syk and ZAP-70 does not lead to complete loss of mature B cells, suggesting that in some B cells these kinases are not mandatory to induce basal BCR survival signaling (E.H., unpublished data). However, remaining Syk/ZAP-70 deficient B cells could not be activated by anti-IgM or anti-Ig-β antibodies (E.H., unpublished data). This finding suggests that for the basal BCR-mediated activation of PI3K signaling, alternative pathways exist that recruit the PI3K to the membrane to promote their activation. Recently, Delgado *et al.* (120) showed that the PI3K p110δ subunit interacts with components of the BCR. This BCR-PI3K interaction was lost in B cells derived from the TC21-deficient mouse strain. TC21, known also as rRas-2, is a small Ras GTPase, which was shown to be involved in cellular transformation and tumorigenesis (121). Interestingly, TC21 binds simultaneously and preferentially to non-phosphorylated ITAMs and to the PI3K p110δ subunit (122). This alternative interaction between non-phosphorylated ITAMs, TC21, and p110δ allows the recruitment of PI3K to the plasma membrane, which may render it active, and lead to $PtdInsP_3$ generation (*Fig.* 4A). Subsequently, phosphorylation and activation of AKT leads to the degradation of FOXO and thereby to survival of B cells. It is possible that mature resting B cells may receive basal survival signals by activating the PI3K pathway downstream of the BCR via the unconventional TC21 route. In contrast, deletion of Syk results in a nearly complete block in B-cell development at the pro-B to pre-B-cell stage, while $TC21^{-/-}$ mice show no defect at that stage. This observation suggests that early B-cell development depends merely on the activation of the common PTKs (protein tyrosine kinases) downstream of the pre-BCR rather than on TC21.

Coreceptors have synergistic effects on B-cell survival and PI3K signaling

As discussed above, the survival of B cells is sustained by a constant basal signal derived from the BCR. In addition, survival of B cells is supported by the B-cell activating factor of the TNF family (BAFF) (also called BLyS, TNFSF13b, THANK, zTNF4 or TALL-1), which is produced by stromal cells and various cell types of myeloid origin. The expression of its receptor (BAFFR) (also known as BR3, TNFRSF13c, CD268) is low on newly formed immature B cells but increases continuously during further maturation (123). Genetic ablation of BAFF or mutational inactivation of BAFFR leads to a decrease in the number of mature B cells (124–126). By contrast, transgenic mice that overexpress BAFF have an expansion of mature B cells and elevated levels of serum immunoglobulins, anti-DNA antibodies, and immune complexes in the kidney (127, 128). This indicates that signals generated by BAFF and its receptor are necessary for survival and homeostasis of mature B cells.

Similar to the pre-BCR-mediated upregulation of IL-7R expression in pre-B cells, there is evidence that BCR-driven signals regulate the expression of BAFFR (129). Interestingly, this mechanism is dependent on BCR-mediated PI3K signaling. In fact, inhibition of PI3K with the inhibitor wortmannin or deletion of p110δ abrogates this BCR-mediated increase in BAFFR expression (130). Therefore, it appears likely that the BCR may also control B-cell survival by inducing the expression of the prosurvival BAFFR. Mice that were defective in both BCR and BAFFR signaling revealed that signaling pathways downstream of both receptors interact and contribute to the survival of mature peripheral B cells. These mice showed a complete block at the transitional (T1) stage (131), suggesting that the signaling mechanisms during selection at transitional stages and survival after maturation rely on a cross-talk between BCR and BAFFR signaling pathways (132). The signaling pathways that are initiated upon interaction of BAFF with its receptor are numerous and are mainly involved in the regulation of survival, growth, and proliferation. The NF-κB signal transduction cascade has emerged as a critical player in the cross-talk downstream of BCR and BAFFR signaling (133, 134). NF-κB activation can accordingly be subdivided into two main branches, which have been termed canonical and alternative pathway. Mammalian cells contain five members of the NF-κB/Rel transcription factor family: NF-κB1/p50, NF-κB2/p52, RelA, cRel, and RelB. The p50 and p52 proteins are

produced through partial proteolytic degradation from their inactive precursors p105 and p100, respectively. Stimulation of receptors inducing canonical NF-κB, such as the BCR, results in rapid activation of the IκB kinase (IKK) complex, which consists of the IKKα and IKKβ catalytic subunits and the NEMO (NF-κβ essential modulator)/IKKγ regulatory protein. Induction of this complex leads to phosphorylation and subsequent proteasomal degradation of IκB proteins, liberating the associated NF-κB dimers to activate transcription of target genes. The generation of p52 from p100, also known as p100 processing, is an indicator of activation of the alternative NF-κB pathway, which occurs downstream of the BAFFR (133, 134). It has been shown that binding of BAFF to BAFFR is required and sufficient to sustain the p100 processing needed for B-cell survival (135, 136). It initiates the transcription of anti-apoptotic genes such as members of the Bcl-2 family. Thus, the main role of the alternative NF-κB pathway in mature B cells appears to be the transmission of a survival signal elicited by BAFF, whose limited availability regulates the size of the peripheral B-cell pool. In addition, the nuclear accumulation of pro-apoptotic protein kinase C δ (PKCδ) and the accumulation of the pro-apoptotic molecule BIM are both blocked by BAFF (137, 138).

Recent evidence has shown that BAFF enhances the metabolic fitness of B cells and induces cell growth, as B cells cultured *in vitro* in the presence of BAFF showed increased cell size and protein content over time. This BAFF-mediated effect was induced by PI3K signaling, as BAFF treatment of B cells resulted in phosphorylation of AKT as well as the induction of genes involved in the regulation metabolism and cell cycle progression (139, 140). If BAFFR signaling activates PI3K, then the question arises as to why the deletion of the BCR cannot be compensated for by BAFFR-induced activation of PI3K.

One possible explanation for the elimination of B cells upon deletion of BCR expression could be that BAFFR signaling through PI3K is not strong enough to compensate for the loss of BCR-driven basal PI3K activity. Alternatively, BAFFR-mediated PI3K signals could have specific functions different from those induced by the BCR. It was shown that BAFF signaling in B cells leads mainly to better metabolic fitness and that BAFF-mediated phosphorylation of AKT was dependent on PKCβ. In line with this, PKCβ-deficient B cells were only partially responsive to the survival action of BAFF and showed severe defects in cell growth despite BAFF treatment (139, 140). Together, it appears that BAFF-induced AKT activation preferentially regulates the basal metabolic state of B cells. In addition, it is still possible that deletion of the BCR downmodulates the expression of the PI3K machinery and thus leads to decreased downstream signaling by BAFF through AKT. On the other hand, B cells with impaired BAFFR signaling cannot be rescued by BCR-driven signals, even though the PI3K-mediated basal signaling, guaranteed by BCR expression, is still intact in these cells. Indeed, neutralization of BAFF by the treatment of mice with anti-BAFFR showed a decrease in mature B-cell cellularity (123, 141) supporting the importance of BAFFR signaling for the maintenance of mature B cells.

Taken together, mature B-cell survival seems to be regulated by the achievement of a certain activation state induced by a non-redundant combination of basal BCR and BAFF-BAFFR signaling.

PI3K regulates AID-dependent processes in mature B cells

In addition to the important role in regulating the recombination of Ig genes in early stages of B-cell development and ensuring the survival of mature B cells, PI3K signaling is also essential in later stages of B-cell development with respect to class switch recombination (CSR) and somatic hypermutation (SHM). Accordingly, a common aspect downstream of numerous surface receptors, involved in B-cell differentiation and CSR, such as the BCR, Toll-like receptors (TLRs), CD40, and cytokine receptors, is activation of PI3K signaling(142).

CSR and SHM take place in the germinal centers (GCs) and give rise to the production of antibodies with different isotypes as well as to the formation of high affinity BCRs (143). CSR causes the exchange of the Cμ/δ-region with a downstream C region. This results in the generation and secretion of antibodies of different isotypes (IgG, IgA, and IgE), which recognize the same antigen but have distinct effector functions (144, 145). To increase the affinity of a BCR and the secreted antibodies for a certain antigen, B cells utilize SHM. Through SHM, point mutations and to a lesser extent deletions or insertions are introduced into both the V_H and the V_L regions of immunoglobulin genes, thereby potentially increasing the affinity for the respective antigen (146, 147).

Prior to CSR and SHM, naive B cells that encounter antigen are positively selected and undergo a phase of intense proliferation (*Fig. 4B*). Upon this proliferation, antigen-triggered B cells develop either into antibody-secreting cells (ASCs) or enter GCs to undergo CSR and SHM (148).

High PI3K activity, which is necessary to induce the selective expansion of antigen-triggered naive B cells, inhibits CSR but promotes differentiation towards an ASC. Accordingly, mice deficient for PTEN, the negative regulator of PI3K signaling, generate increased numbers of ASCs and show defective CSR, indicated by strongly elevated serum levels of IgM and

markedly reduced levels of IgA and IgGs (149, 150). Inhibition of p110δ in the abovementioned PTEN-deficient B cells, however, was able to restore CSR upon stimulation of these cells (149). These data demonstrate a crucial role for PI3K in the decision either to become an IgM ASC or to undergo CSR. The fine-tuning of these opposing differentiation decisions is a delicate matter, as genetic mutations that lead to increased PI3K activity have been linked to the production of autoantibodies and to the development of autoimmune diseases. For instance, mice overexpressing CD19 produce high amounts of autoantibodies, suggesting that tolerance mechanisms are dependent on a tightly regulated PI3K activity (151, 152).

The B-cell-specific activation-induced cytidine deaminase (AID), which is highly expressed in B cells located within GCs, is indispensable for CSR and SHM (153, 154). Regulation of AID expression and activity occurs at the transcriptional as well as the post-translational level and ensures that action of AID is mainly restricted to GC B cells (155). Recent work identified FOXO1 as a positive regulator of AID gene expression and therefore CSR (107, 149). However, the potency of constitutively active FOXO1 in inducing CSR is strongly enhanced when PI3K activity is concomitantly inhibited. This suggests that in addition to the FOXO1-dependent expression of AID, other signals derived from PI3K influence AID-mediated effects. The potency of ectopically expressed AID to promote CSR is consequently increased upon combination with PI3K inhibitor-treatment, whereas expression of a constitutively active form of AKT weakens it (149). These observations point to a potential mechanism by which PI3K signaling might counteract CSR in a way that activation of PI3K results in AKT-mediated inactivation of FOXOs and impaired CSR and SHM.

This kind of regulation is reminiscent of the PI3K-mediated regulation of RAG expression in pre-B cells (discussed above). Since high pre-BCR-mediated PI3K activity suppresses RAG expression and IgL chain gene recombination in pre-B cells, it appears likely that for the induction of AID expression in antigen-stimulated B cells, PI3K activity must also be downregulated. It is not clear how antigen-stimulated B cells can downmodulate PI3K signaling, thereby stabilizing FOXO activity and promoting AID expression and function. A signaling mechanism similar to the SLP-65-mediated PI3K inhibition in pre-B and immature B cells probably occurs downstream of the BCR upon antigen encounter (*Fig. 4C*). However, since mice deficient for SLP-65 show no general defect in CSR (156), other mechanisms must exist and lead to downregulation of PI3K activity, which is necessary to promote the expression of AID and the initiation of CSR and SHM. Antigen-triggered mature B cells must receive costimulatory signals provided by a T-helper cell to undergo CSR. It is therefore conceivable that signals transmitted via T/B-cell interactions may induce a signaling pathway that downregulates PI3K activity and thereby promotes AID-dependent CSR and SHM.

Concluding remarks

It is remarkable that an evolutionarily conserved signaling pathway, which regulates cell metabolism and survival, plays a central role in highly tissue-specific processes such as VDJ gene recombination and AID-mediated CSR and SHM in B cells. This link might be required to ensure that survival signals are generated only in those cells that express functional receptors, which are able to organize survival-specific signaling pathways or sense environmental signals. The overlapping synergistic effects of the different receptors ensure that efficient activation of survival and proliferation can only be achieved if both receptors function properly. This might be required to avoid uncontrolled survival/proliferation signals by aberrant activation of only one receptor system. In this context, it is noteworthy that the survival of mature B cells is somehow equally dependent on intrinsic basal BCR signals and on environmentally derived BAFFR signals. Other signaling machineries such as cytokine receptors or cell adhesion molecules provide additional modulation to the PI3K-mediated BCR signals, even though the molecular mechanisms are not yet well characterized. Likewise, it is unclear how PI3K-mediated signals activate the differentiation of B cells, while at the same time playing an essential role in cell cycle progression and proliferation. Clearly, a hierarchy and well-timed activation of the different signaling cascades are required.

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