A Crucial Role for the p110δ Subunit of Phosphatidylinositol 3-Kinase in B Cell Development and Activation

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Abstract
Mice lacking the p110δ catalytic subunit of phosphatidylinositol 3-kinase have reduced numbers of B1 and marginal zone B cells, reduced levels of serum immunoglobulins, respond poorly to immunization with type II thymus-independent antigen, and are defective in their primary and secondary responses to thymus-dependent antigen. p110δ−/− B cells proliferate poorly in response to B cell receptor (BCR) or CD40 signals in vitro, fail to activate protein kinase B, and are prone to apoptosis. p110δ function is required for BCR-mediated calcium flux, activation of phospholipaseCγ2, and Bruton’s tyrosine kinase. Thus, p110δ plays a critical role in B cell homeostasis and function.

Key words: Akt • Btk • calcium • gene targeting • p110δ

Introduction
B lymphocyte development, selection, and activation are critically dependent on signal transduction events mediated by the B cell antigen receptor (BCR)* (1). The BCR is tightly associated with nonpolymorphic subunits, CD79a and b, which contain immunoreceptor tyrosine–based activation motifs. Immunoreceptor tyrosine–based activation motifs serve to recruit tyrosine kinases of the Src and Syk families that initiate the signal transduction cascade by phosphorylation of multiple substrate proteins (2).

A number of studies have provided evidence for a role for phosphatidylinositol 3-kinase (PI3-K) and the second messenger phosphatidylinositol 3,4,5-trisphosphate (PIP-3) in BCR signal transduction. PI3-K is activated downstream of the BCR for antigen (3, 4) and the CD19 coreceptor (5, 6). The class IA PI3-K’s consist of three catalytic subunits p110α, β, and δ that are encoded by distinct genes and interact with a family of adaptor proteins that regulate location and enzyme activity (7). Mice deficient in the p85α or p85/55/50/δ adaptor proteins (8, 9) of PI3-K display an immune defect similar to xid mice in which the Pleckstrin homology domain of Bruton’s tyrosine kinase (Btk) can no longer bind PI3-K (10). Btk, a member of the TEC family of tyrosine kinases, is thought to be a major effector of PI3-K in B cells. Btk acts, in part, to regulate calcium flux through the phosphorylation and activation of phospholipaseCγ2 (PLCγ2; reference 11). The regulation of Btk membrane association and activation by phoshoisotides plays an important role in regulating B cell responses (12).

B cells from mice deficient in the breakdown of PIP-3, such as those lacking the SH2 domain-containing inositol polypophosphate 5′-phosphatase 1, display accelerated development and are hyperresponsive to BCR stimulation (13–15). Furthermore, mice deficient in the phosphatase and tensin...
homolog gene that encodes a PIP-3 3' phosphatase develop B cell hyperplasia, lymphoma, and hypergammaglobulinemia (16).

Each of the three class IA catalytic subunits of PI3-K are expressed in B cells, however their relative roles in B cell development and function are unknown. Mutant mice lacking p110α (17) and p110β (18) have previously been reported to have lethal phenotypes that precluded analysis of immune cell development and function. p110δ is the most recently identified PI3-K catalytic subunit with expression reported to be highest in hematopoietic cells (19, 20). We have used gene-targeting to generate mice that lack p110δ function. Our analysis of B cell development in these mice revealed an essential role for p110δ in the development of B1 and marginal zone (MZ) B cells. Furthermore, B cell responses to thymus-dependent and -independent antigens required p110δ function. Analysis of BCR signal transduction revealed an important role for p110δ in the regulation of proliferation and calcium flux. These defects can be attributed to a failure to activate protein kinase B (PKB) and Btk.

Materials and Methods

Generation of p110δ Knockout Mice. The structure of murine pik3δd genomic clones isolated from a 129/5v genomic library has been described previously (21). The targeting vector consists of LoxP flanked neomycin and hygromycin-resistance cassettes cloned 7.5 kb apart into the EcoRV and XhoI sites respectively of the pik3δd genomic clone. This strategy was adopted in an attempt to generate a conditional allele of pik3δd. The targeting vector was transfected into PC3 mouse embryonic stem cells (22) and analyzed for the targeting event using Southern blotting of KpnI-digested DNA with probe A (a 700-bp EcoRI fragment). Correctly targeted clones were injected into blastocysts to produce chimaeric mice. The resulting chimaeras, which express the Cre enzyme in the male germline (22), were bred to obtain lines of mice harboring a pik3δd gene which had undergone Cre-mediated recombination and thus deleted exons 1–9 encoding the first gene-targeting to generate mice that lack p110δ/H9254 was from Santa Cruz Biotechnology, Inc., anti-Bcl-XL was from BD Transduction Laboratories, anti-p85α was from Upstate Biotechnology, anti-PLCγ2 PY 759 antibody will be described previously (unpublished data).

Immunofluorescence Staining of Tissue Sections. Spleens were harvested and immediately frozen by dipping in liquid nitrogen. Spleens were mounted in OCT and 8-μm sections were cut, air-dried, and stored at –20°C until use. Sections were fixed in ice-cold acetone for 15 min, air-dried briefly, rehydrated in PBS and then blocked with 5% normal rat serum for 15 min. Sections were stained with FITC-conjugated MOMA-1 antibody (rat IgG2a; Serotec), and biotinylated anti-IgM (clone R6–60.2, rat IgG2a; BD BioSciences), or control FITC-conjugated rat IgG2a (BD BioSciences) for 1 h at room temperature. Sections were washed in PBS and stained with a 1:200 dilution of streptavidin–TRITC (Jackson ImmunoResearch Laboratories) for 1 h. Sections were washed and mounted in Aqua PolyMount (Polysciences, Inc.) and viewed with an Olympus BX–40 epifluorescence microscope using appropriate filters. Images were digitally captured using a high-resolution CCD camera (F–View) using analySIS® software (SIS, GmhB) and processed using Adobe Photoshop® v.7.0.

Antibody Responses. Staining of cells with fluorescent antibodies was performed as described previously (24). All antibodies were purchased from BD Pharmingen except anti-iGm–Cy5 and anti-IgD–PE (Jackson ImmunoResearch Laboratories). Serum IgG in naive mice were determined by ELISA using antibodies purchased from BD Pharmingen. For immune responses, 8-wk-old mice were injected intraperitoneally with either 5 μg DNP-Ficoll in a solution of PBS or 50 μg DNP conjugated to KLH in a solution of PBS. Serum antibody levels were determined by ELISA as described previously (24). The relative units of DNP-specific antibodies are shown as optical density values. A dilution series of the serum samples was measured and for each isotype a single dilution factor which fell in the linear part of the curve is represented for all time points. Serum dilution factors were as follows: thymus-independent responses 1:800 for IgM and 1:800 for IgG3. For thymus-dependent responses, the dilutions were 1:800 for IgM, IgG3/H9251, and IgG3/H9252; 1:1,600 for IgG1, and 1:200 for IgG2a.

B Cell Proliferation and Apoptosis Assays. Purified B cells were cultured for 72 h at an initial concentration of 10^6 cells per milliliter with the indicated doses of polyclonal anti-IgM or monoclonal anti-IgM (clone B7.6), monoclonal anti-CD40 (clone 3/23) and recombinant murine IL-4 in RPMI 1640 supplemented with 10% FCS, 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 5 × 10^{-5} M 2-mercaptoethanol. Proliferation was measured by incorporation of [3]thymidine following a 16-h pulse. For analysis of apoptosis, B cells were cultured in the above media but without antibodies or IL-4 and apoptosis determined by flow cytometry of permeabilized cells stained with propidium iodide as described previously (26).

Calcium Flux Analysis. Purified splenic B cells were loaded for 30 min at R.T in the dark with 3 μM Fluo-4 a.m. (Molecular Probes) at a density of 6 × 10^6 cells per milliliter in 0.5% BSA/PBS. The cells were washed in indicator free medium and then resuspended at 3 × 10^6 cells per milliliter in 0.5% BSA/PBS containing 1 mM CaCl_2. After a further incubation of 30 min to low complete deesterification of intracellular Fluo-4 a.m. ester, the variations in absorbance were measured using a Perkin-Elmer LS55 Luminescence Spectrometer. [Ca^{2+}] was calculated as described previously (27).
Results

Impaired BCR Stimulated PIP-3 Production in the Absence of p110δ. To address the function of p110δ in B cells, we used gene targeting to produce p110δ null mice (Fig. 1, A and B). Thymocytes and B cells from these mice lacked p110δ protein (Fig. 1 C and unpublished data). B cells from control and mutant mice expressed similar levels of p110α and p110β catalytic subunits, but showed a small reduction in the levels of the p85α and p55/50α adaptor subunits (Fig. 1 D). To determine the impact of this mutation on BCR-induced PIP-3 production we employed a novel assay that permits determination of PIP-3 levels in primary cells, without the need for biosynthetic labeling. We observed that BCR-stimulated B cells from p110δ-deficient mice produced little PIP-3 (Fig. 1, E and F). In control B cells, PIP-3 levels peaked after 1 min of BCR stimulation and returned to baseline by 10 min, in mutant mice no increases in PIP-3 levels were observed within 10 min of stimulation (Fig. 1 F). These data indicate p110δ was responsible for most of the BCR-induced PIP-3 production.

B1 and MZ B Cells Require p110δ. Flow cytometric analysis of the bone marrow of p110δ−/− mice did not reveal any major blocks in B cell development (Table I). However, we observed a marked reduction in the B1 subset resident in the peritoneal cavity (Fig. 2 A and Table I). Furthermore, CD21hi CD23lo MZ B cells of the spleen were also significantly reduced in number (Fig. 2 B and Table I). This conclusion was further supported by noting the absence of the splenic B220+ CD1hi population in the mutants (unpublished data). Examination of frozen splenic tissue sections stained with anti-IgM and anti–MOMA-1 (which stains MZ metallophilic-macrophages), revealed that in the p110δ mutant there were very few B cells beyond the marginal sinus (delineated by MOMA-1 staining), supporting the conclusion that the MZ B cell population was severely reduced. The number of conventional B2 B cells was significantly reduced in young p110δ−/− mice (Table I). By contrast, older p110δ−/− mice had only marginally reduced numbers of B2 cells (Table I). Subdivision

Figure 1. p110δ mutant B cells fail to produce PIP-3. (A) Gene targeting strategy. (B) Southern blot analysis of tail DNA from wild-type (+/+), heterozygous (+/−), and homozygous (−/−) mutant mice using probe A. (C) Western blot analysis of wild-type, heterozygous, and homozygous mutant thymocytes using antibody against p110δ. The blot was reprobed with anti–Vav-1 to demonstrate equal protein loading. (D) Western blot analysis of wild-type and homozygous mutant B-lymphocytes for levels of p110α, p110β, the p85/55/50α subunits of PI3-K, and PLCγ2. (E) BCR-stimulated PIP-3 production in wild-type and mutant B cells. Wild-type is represented by black bars, mutant by white bars. (F) Time course of PIP-3 production in wild-type and mutant B cells stimulated with 10 µg/ml anti-IgM F(ab)2. In E and F, error bars represent the variance of triplicate determinations.
Immunized P110° mice had near normal numbers of IgM+ IgDlo B cells, which are mature recirculating follicular (RF) B cells (Fig. 2 B and Table I). The level of surface IgM staining on follicular B cells was not different between control and mutants mice (Fig. 2 B). Thymocyte development, and the number of CD4+, CD8+ T cells, and Mac1+ macrophages in the spleens of P110°/− mice were not different from littermate controls (Table I and unpublished data).

Significantly reduced when compared with that of control independent type II antigen, DNP-Ficoll. The hapten-specific IgG2α, and IgG3 were significantly reduced, whereas of splenic B cells using IgM and IgD staining revealed that number of CD4+ T cells, and Mac1+ macrophages in the spleens of P110°/− mice were not different from littermate controls (Table I and unpublished data).

**Table I. Lymphocyte Populations in p110°/− Mice**

| Tissue/cell type | Control       | p110°/−       |
|------------------|---------------|---------------|
| Bone Marrowa     |               |               |
| Fraction A–C     | 8.6 x 10^6 (2.7) | 7.5 x 10^6 (1.5) |
| Fraction D       | 4.1 x 10^6 (1.3) | 3.1 x 10^6 (0.8) |
| Fraction E       | 9.6 x 10^6 (4.0) | 7.2 x 10^6 (1.4) |
| Fraction F       | 6.6 x 10^6 (2.4) | 9.6 x 10^6 (4.2) |
| Spleen (8–10 wk old) |               |               |
| Large Mac1+      | 1.5 x 10^7 (0.2) | 1.2 x 10^7 (0.3) |
| CD4+             | 2.5 x 10^6 (0.5) | 2.5 x 10^6 (0.9) |
| CD8+             | 1.3 x 10^7 (0.3) | 1.1 x 10^7 (0.4) |
| CD21hi CD23lo    | 3.7 x 10^6 (0.8) | 0.6 x 10^6 (0.05)b |
| B220+            | 4.4 x 10^6 (1.3) | 3.1 x 10^6 (0.7) |
| IgMhi IgDlo      | 3.6 x 10^6 (1.1) | 2.6 x 10^6 (0.8) |
| IgMlo IgDhi      | 1.1 x 10^7 (0.3) | 1.1 x 10^7 (0.2) |
| IgMlo IgDlo      | 2.6 x 10^7 (0.8) | 1.6 x 10^7 (0.4) |
| Spleen (21-d old) |               |               |
| IgMhi IgDlo      | 2.7 x 10^6 (0.8) | 10^6 (0.1)c |
| IgMhi IgDhi      | 9 x 10^6 (1.4) | 2.2 x 10^6 (0.4)c |
| IgMlo IgDhi      | 3.2 x 10^6 (0.2) | 0.8 x 10^6 (0.2)c |
| Peritoneum       |               |               |
| B1 total         | 10^6 (0.2) | 0.08 x 10^6 (0.02)c |
| B1a              | 2.4 x 10^6 (0.2) | 0.1 x 10^6 (0.02)c |
| B1b              | 1.9 x 10^5 (0.3) | 0.07 x 10^5 (0.01)c |
| B2               | 5.7 x 10^5 (2.9) | 4.9 x 10^5 (3.6) |

Values are given as mean with SD in parenthesis. n = 9 for determination of B cell numbers in the spleen of adult mice; n = 4 for all other determinations, except the mutant peritoneum where n = 3.

aFractions assessed using the criteria of Hardy. Significant differences are highlighted in bold, significance assessed by Student’s t test.

bP < 0.02.

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ylation of IκBα that was blocked by the PI3-K inhibitor wortmannin (Fig. 4 F). B cells from p110δ-deficient mice did not show detectable phosphorylation of IκBα after BCR stimulation (Fig. 4 F). Bcl-xL levels in freshly isolated B cells from mutant mice were lower than control cells (Fig. 4 G). Furthermore, when cultured for 24 h in the presence of antibodies to the BCR, Bcl-xL levels increased in control but not in p110δ-deficient B cells (Fig. 4 G).

*p110δ is Required for Normal Function of Btk and PLCγ2.* The mobilization of intracellular calcium (Ca^{2+}) after the addition of antibodies against IgM was reduced in p110δ−/− B cells at all doses of agonist tested (Fig. 5 A). This was evident in both the initial peak response and the sustained response. To further evaluate the level at which calcium mobilization was impaired we measured BCR-induced production of inositol 3,4,5 trisphosphate (IP3), the second messenger that triggers calcium release from intracellular stores. BCR stimulation of IP3 production was reduced in p110δ−/− B cells (Fig. 5 B). This observation could reflect defective activation of PLCγ2, as this lipase requires phosphorylation by Btk, a tyrosine kinase whose activity is dependent on PIP-3. Therefore, we measured the phosphorylation and activation of Btk using phosphorylation-site specific mAbs. In control mice Btk was phosphorylated on tyrosine 551 in response to BCR cross-linking (Fig. 5 C). This reflects phosphorylation in trans by Src and Syk family kinases (31–33). By contrast, phosphorylation of tyrosine 551 was not detected in p110δ-deficient B cells. Tyrosine 223 is a major autophosphorylation site of Btk, its phosphorylation can thus be used as an indicator of whether Btk has become activated. B cells from p110δ−/− mice showed no detectable phosphorylation of Btk at position 223. These results suggest that Btk activation requires the function of p110δ. Within PLCγ2 tyrosines 753 and 759 have been identified as important Btk substrates (34, 35). To establish whether PLCγ2 was phosphorylated by Btk on tyrosine 759 we employed a phosphospecific mAb. BCR stimulation of control B cells induced phosphorylation of PLCγ2 on tyrosine 759, however there was little phosphorylation of this residue in stimulated B cells from p110δ-deficient mice.

**Discussion**

B cells from mice deficient in p110δ produce little PIP-3 after BCR engagement. This finding indicates that p110δ is mainly responsible for the bulk of PIP-3 production downstream of the BCR and that the p110α and β subunits, although expressed at normal levels in p110δ-deficient B cells, cannot compensate for loss of p110δ. This suggestion is further substantiated by the observations that the well characterized PI3-K effectors Btk and PKB are not, or only weakly, activated in response to BCR stimulation in p110δ-deficient B cells. Our results do not exclude roles for...
the p110α and β subunits in BCR signal transduction because weak phosphorylation of PKB could be detected in p110δ−/− B cells after high levels of BCR cross-linking. Furthermore, the PI3-K inhibitor wortmannin was able to mediate additional inhibition of BCR-stimulated calcium flux in p110δ−/− B cells (unpublished data). Our measurements of total cell PIP-3 do not take account of small, highly localized, concentrations of PIP-3 that may be gen-

Figure 3. Immune function in p110δ mutant mice. (A) Serum Ig levels in naive mice were measured by ELISA. (B) DNP-specific Ig of the indicated isotypes were determined by ELISA of “preimmune” collected before immunization, and “immune” sera collected 7 d after immunization with DNP-Ficoll. (C) DNP-specific Ig was measured in preimmune serum, and in serum taken 7 and 21 d after immunization with DNP-KLH. Mice were then reimmunized at 21 d and bled 7 d later to measure the secondary response. In each set of graphs, the relative unit (absorbance) value for individual control animals is represented by a filled circle, individual p110δ-deficient mice are represented by open circles. Bars represent the average and SD for the group. In the TD responses, arrows represent the points at which mice were immunized (days 1 and 21). P values denote the levels of significance between sera of control and p110δ−/− mice as determined by the Student’s t test. a, P < 0.01; b, P < 0.05; c, not significant; d, P < 0.001. (D) Quantitation of germinal center formation in spleens taken 10 d after immunization. The results are expressed as the ratio of PNA+ germinal centers/B220+ follicles. Black bar, wild type; white bar, mutant. Data was compiled from two control mice and four mutant mice. (E) Flow cytometric analysis of lymphocytes from Peyer’s patches. Activated B cells are gated as B220+ GL7+, the numbers refer to the percentage of lymphocytes falling within the indicated gate.
Methodological limitations preclude our measuring the spatio-temporal accumulation of PIP-3 in primary B cells, but such measurements will greatly increase our understanding of PI3-K function in B cell signaling. We have also noted a difference in B cell phenotype between p110α-deficient mice and mice deficient in the p85α/p55/p50α adaptor subunits of PI3-K (8, 9). Unlike p85α and p85/p55/p50α-deficient mice, the spleens of adult p110α-deficient mice contain normal numbers and proportions of IgMlo IgDhi RF B cells. These observations presumably reflect the participation of the p110α or β subunits, coupled via the p85α adaptor, into a pathway that regulates the maturation of RF B cells. Alternatively, there may exist additional functions for the p85α subunit that are independent of the catalytic subunits. The functional interrelationships between the catalytic and regulatory subunits are poorly understood in complex systems, as exemplified by the phenotype of P85α-deficient mast cells. These cells display reduced expression of p110α but normal levels of p110β and δ (37). Intriguingly, c-kit signaling is impaired in P85α-deficient mast cells, whereas signaling through the high affinity IgE-receptor (that is also dependent on PI3-K activation) is normal (37, 38). Taken together with studies using manipulated cell lines (39), these data argue for selective roles for the individual p110 catalytic subunits.

Our results show that p110α is required for the development and/or survival of the B1 and MZ B cell subsets. A number of previous studies have highlighted similarities between...
was then stripped and reprobed with antisera specific to PLC

detectable class switching to IgG1 and severely impaired
and secondary immune response. Although we observed a
tated by B2 cells, was significantly impaired in both the primary

tion. The response to TD antigen, which is principally medi-

in B cells (unpublished data). Between these subsets, which appear to play important roles in
immunity through the production of natural antibodies, and
by being able to rapidly respond to antigenic challenge (40).
Indeed, the ability to mount T cell–independent type II anti-
gen responses has been attributed to MZ B cells (41). Our
finding that the levels of IgM and IgG1 in the serum of naive
mice were significantly reduced and that antibody responses to
DNP-Ficoll were severely impaired is consistent with the
properties of these cells. We found that p110δ was required
for the generation of normal numbers of B2 B cells, particu-
larly in young mice, but not their maturation. Taken together
with the increased tendency of p110δ−/− B cells toward apop-
tosis this observation may reflect increased turnover of B2
cells. Confirmation of this will require further experimenta-
tion. The response to TD antigen, which is principally medi-
ated by B2 cells, was significantly impaired in both the primary
and secondary immune response. Although we observed a
modest primary antigen-specific IgM response, there was no
detectable class switching to IgG1 and severely impaired
switching to the other IgG subtypes. Consistent with this, ger-

tinal center formation was profoundly impaired. The pheno-
type of p110δ−/− mice shows similarities with CD19−/− mice
that also lack B1 and MZ B cells, but have apparently normal
development of B2 cells which display impaired function (42–
44). However, splenic B cells from CD19−/− mice are less ser-
iously impaired in their ability to activate PKB (45, 46) and
Btk (47) than are p110δ−/− B cells. It will be interesting to de-
termine whether CD19 employs p110δ as a signal transducer.
The defective proliferation of p110δ−/− B cells to anti-CD40 is
also a feature shared with CD19−/− B cells (48) and suggests that
the mutant B cells may be unable to respond to T cell help.
CD40 ligation on B cells activates PI3-K (49), and CD40-
mediated B cell proliferation is blocked by PI3K inhibitors or
p85α deficiency (8, 9). Preliminary experiments suggest CD86
upregulation is also defective in p110δ−/− B cells (unpublished
data), therefore cognate T–B interactions required for the
normal humoral response may be defective. It will also be in-
teresting to determine whether the ability of p110δ−/− T cells
to provide help is defective. p110δ was not required for mitoge-
nic responses to all stimuli, as 110δ−/− B cells proliferated nor-
mally in response to LPS which also activates PI3-K (50) and
requires the function of p85α to exert its mitogenic effect (8, 9).
Our results thus suggest that catalytic subunits other than
p110δ may be mediating the mitogenic LPS signal. IL-4–
mediated proliferation is also sensitive to PI3-K inhibitors or
p85α deficiency (8, 9), but preliminary experiments determi-
ning the ability of IL-4 to mediate sur-
vival of cultured B cells has not revealed a difference be-
tween wild-type and p110δ−/− B cells (unpublished data).
PI3-K signaling has been intimately linked with cell survival in a number of systems by virtue of its ability to regulate PKB (28). p110β-deficient B cells were impaired in their ability to survive after in vitro culture and expressed less of the ant apoptotic protein Bcl-xL. After activation through the BCR, p110β-deficient B cells failed to appreciably activate PKB and also displayed defective phosphorylation of IkBα. In addition, the increased expression of Bcl-xL that follows BCR stimulation required the function of p110β. Taken together, these results are consistent with the suggestion that p110β is important for the activation of survival pathways in B cells.

A number of studies have described mice lacking components of the BCR signaling pathway that share B cell developmental phenotypes. These include xid mice as well as mice deficient in Btk, B cell linker protein, PLCγ2, Vav-1/Vav-2, and B cell adaptor for PI3-K (BCAP) (51). The similarities in phenotypes between these mice, taken together with evidence of physical association, has led to the suggestion that these molecules act as a molecular machine or “signalosome” (52, 53). One model for BCR activation of calcium flux places emphasis on Btk-mediated phosphorylation and activation of PLCγ2. Activation of Btk is dependent on PI3-K (12, 54), asPIP-3 regulates Btk function by regulating both the location (55, 56) and the catalytic activity of Btk (57). Our data are consistent with such a model as we found that Btk was not phosphorylated and activated as assessed using phosphospecific antibodies. In addition, we found PLCγ2 phosphorylation and activation was defective in p110β−/− B cells. A recent study also implicated p110β in the regulation of PLCγ2 function in FcεRI-stimulated RBL-2H3 cells by injecting p110β-specific inhibitory antibodies (58). Besides activation of Btk, additional PIP-3–mediated mechanisms may contribute to PLCγ2 activation as PIP-3 has been reported to bind directly to and activate PLCγ2 (58–60). Taken together, our results suggest that p110β is the key PI3-K catalytic subunit required for the function of the signalosome in B cells.

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