PI3K IN LYMPHOCYTE DEVELOPMENT, DIFFERENTIATION AND ACTIVATION

Klaus Okkenhaug and Bart Vanhaesebroeck‡*

Phosphoinositide 3-kinases (PI3Ks) regulate numerous biological processes, including cell growth, differentiation, survival, proliferation, migration and metabolism. In the immune system, impaired PI3K signalling leads to immunodeficiency, whereas unrestrained PI3K signalling contributes to autoimmunity and leukaemia. New insights into the role of PI3Ks in lymphocyte biology have been derived from gene-targeting studies, which have identified the PI3K subunits that are involved in B-cell and T-cell signalling. In particular, the catalytic subunit p110δ seems to be adapted to transmit antigen-receptor signalling in B and T cells. Additional recent work has provided new insights into the molecular interactions that lead to PI3K activation and the signalling pathways that are regulated by PI3K.

PHOSPHOINOSITIDE 3-KINASES (PI3Ks). A family of enzymes that phosphorylate the D3 position of phosphoinositides. The class IA PI3Ks, which are the focus of this review, phosphorylate phosphatidylinositol-(4,5)-bisphosphate to produce phosphatidylinositol-(3,4,5)-trisphosphate.

**Molecular Immunology Programme, The Babraham Institute, Cambridge CB2 4AT, UK. ‡Cell Signalling Group, Ludwig Institute for Cancer Research, 91 Riding House Street, London W1W 7BS, UK*
e-mails: klaus.okkenhaug@bbsrc.ac.uk; bartvanh@ludwig.ucl.ac.uk
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The PHOSPHOINOSITIDE 3-KINASES (PI3Ks) are a family of enzymes that regulate diverse biological functions in every cell type by generating lipid second messengers (for a general review of the PI3K family of enzymes, see REF. 1). On the basis of structural similarities, the PI3K family can be subdivided into three classes — class I, class II and class III (REF. 2). The class IA PI3Ks are involved in signalling by antigen and co-stimulatory receptors, and they are the focus of this review.

Class I PI3Ks

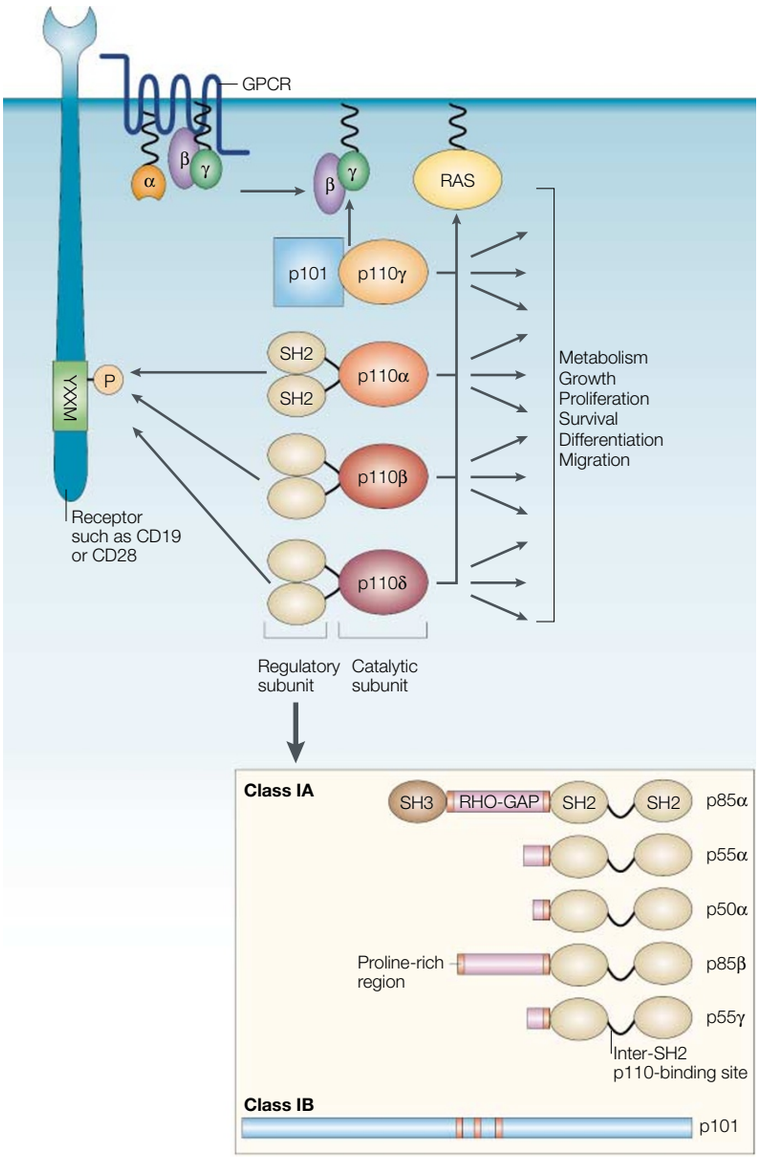
The class I PI3Ks are subdivided into two groups — the class IA and class IB PI3Ks. The class IA PI3Ks are activated by tyrosine-kinase-associated receptors, including antigen, co-stimulatory and cytokine receptors. Class IA PI3Ks are heterodimeric enzymes consisting of a regulatory subunit (p85α, p85β or p55γ) and a catalytic subunit (p110α, p110β or p110δ). Each of the catalytic subunits can associate with all of the regulatory subunits (BOX 1). The class IB PI3K, PI3Kγ, is activated by G-protein-coupled receptors (GPCRs) — a large family of receptor proteins that includes the chemokine receptors. There is only one catalytic subunit and one regulatory subunit for class IB, which are known as p110γ and p101, respectively. The expression of the catalytic p110δ and p110γ subunits is mainly restricted to leukocytes, whereas p110α and p110β are expressed by all cell types.

The function of class I PI3Ks is to convert PHOSPHATIDYLINOSITOL-(4,5)-BISPHOSPHATE ($PtdInsP_2$) to phosphatidylinositol-(3,4,5)-trisphosphate ($PtdInsP_3$) at the inner leaflet of the plasma membrane. $PtdInsP_3$ acts as a binding site for numerous intracellular enzymes that contain PLECKSTRIN-HOMOLOGY DOMAINS (PH domains) with selectivity for this lipid. Perhaps the most important of these is the serine/threonine kinase AKT/PKB, which has an important role in cell proliferation, growth, survival and metabolism in many cell types, and the role of which is conserved through evolution[3,4]. In B and T cells, the PH-domain-containing tyrosine kinases of the TEC family — Bruton agammaglobulinaemia tyrosine kinase (BTK) in B cells and interleukin-2 (IL-2)-inducible T-cell kinase (ITK) in T cells — are also important mediators of PI3K signalling[5].

Class II and class III PI3Ks

Little is known about the role of class II and class III PI3Ks in lymphocytes. Class II PI3Ks can use both PtdIns and PtdIns(4)P as substrates *in vitro*, but their preferred substrate *in vivo* is not known[1]. The class III

Box 1 | **Class I phosphoinositide 3-kinases**



Class IA phosphoinositide 3-kinases (PI3Ks) are heterodimeric enzymes consisting of a regulatory and a catalytic subunit. Three genes encode five regulatory subunits — p85α, p55α, p50α, p85β and p55γ. p55α and p50α are derived from alternatively spliced messenger RNAs from the same gene that encodes p85α. There are also three genes encoding the class IA catalytic subunits p110α, p110β and p110δ. Potential for redundancy exists among the class IA PI3Ks, because each of the catalytic p110 subunits can bind each of the p85, p55 and p50 regulatory subunits, and the different heterodimers seem to be recruited to the same receptors. Class IA PI3Ks are regulated by tyrosine kinases that phosphorylate membrane proteins in Tyr-Xaa-Xaa-Met (YXXM) motifs. In B cells, Tyr-Xaa-Xaa-Met motifs are found in the cytoplasmic domains of CD19 and B-cell PI3K adaptor protein (BCAP). In T cells, Tyr-Xaa-Xaa-Met motifs are found in the cytoplasmic domains of CD28 and T-cell-receptor-interacting molecule (TRIM). Adaptor proteins, such as members of the IRS (insulin-receptor substrate) and GAB (GRB2-associated binding protein) families, can also contain Tyr-Xaa-Xaa-Met motifs. pTyr-Xaa-Xaa-Met (where pTyr = phosphotyrosine) sequences provide the docking sites for the SRC-homology 2 (SH2) domains of the regulatory subunit of PI3K, which brings the p110 catalytic subunit to the membrane, where it catalyses the conversion of phosphatidylinositol-(4,5)-bisphosphate ($PtdInsP_2$) to phosphatidylinositol-(3,4,5)-trisphosphate ($PtdInsP_3$). p85α and p85β also contain proline-rich regions and SH3 domains that can facilitate additional protein–protein interactions, and a RHO-GAP domain that might interact with small GTPases and harbour GTPase-activating protein (GAP) activity. p85 has been reported to bind CDC42 and RAC[137,138], but this apparently did not correlate with GAP activity for these small GTPases. A direct interaction between p110 and activated RAS might also contribute to PI3K activation[139]. The class IB PI3K catalytic subunit p110γ is similar in structure to the class IA catalytic subunits, but it associates with a p101 regulatory subunit and not with p85. The p101 regulatory subunit facilitates the interaction between p110γ and the βγ subunits of the heterotrimeric G proteins that are activated by G-protein-coupled receptors (GPCRs)[140].

PI3K, Vps34 (PIK3C3), preferentially phosphorylates PtdIns to yield PtdIns(3)P, which recruits a distinct group of effector proteins with so-called FYVE or Phox (PX) domains[1]. Class II and class III PI3Ks have been shown to regulate various aspects of vesicle trafficking[6], and as such, there is an obvious potential for their involvement in activities such as antigen processing and cytotoxic responses that involve the directed subcellular transport of intracellular vesicles and their cargo.

Dissection of PI3K function

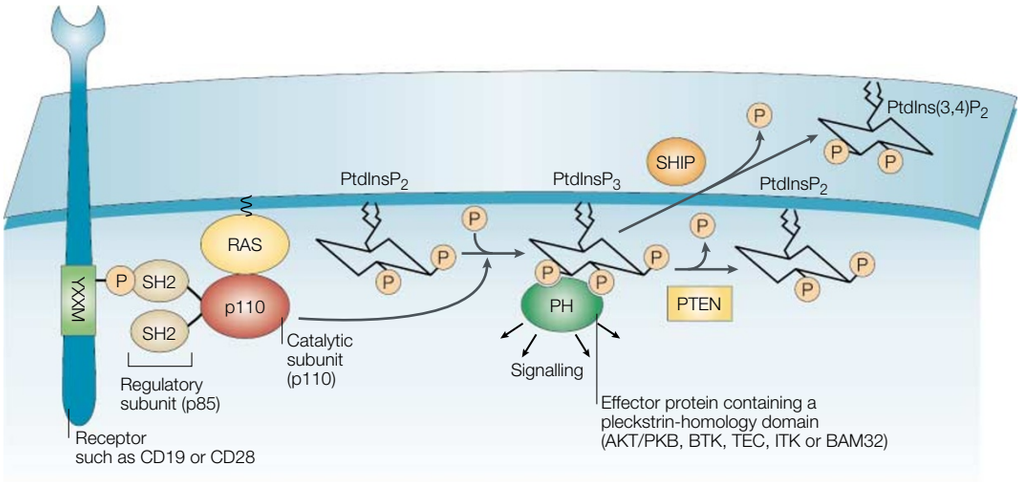
Studies of PI3K signalling in B and T cells using immortalized cell lines have often yielded conflicting results, particularly with respect to T-cell activation and co-stimulation[7]. It is, therefore, essential to study the function of this signalling system in an intact organism, and recent progress using this approach is the focus of this review.

Wortmannin and the structurally unrelated inhibitor LY294002 have high selectivity for PI3Ks[8,9]. The first *in vivo* evidence for the role of PI3Ks in the immune system came from studies in which rats were exposed to wortmannin. These studies indicated that wortmannin is a potent immune suppressor, but that it is also highly toxic[10,11]. Neither wortmannin nor LY294002 discriminate between the different isoforms of PI3K, and as PI3Ks are crucial for all organ systems, other approaches were required to investigate the PI3K subunits that regulate immune function *in vivo*.

PHOSPHATIDYLINOSITOL-(4,5)-BISPHOSPHATE ($PtdInsP_2$). Note that phosphatidylinositol-(3,4)-bisphosphate (resulting from the hydrolysis of $PtdInsP_3$ by SHIP) is sometimes also referred to as $PtdInsP_2$, but this can lead to confusion and should be avoided.

Box 2 | **Genetic dissection of PI3K signalling**

Several genetic approaches have been taken to investigate the functional role of phosphoinositide 3-kinase (PI3K) signalling during B- and T-cell development, maturation and function. These can be divided conceptually into the following categories on the basis of the aspect of PI3K signalling that they affect (molecules to which this strategy has been applied are given in parentheses): mutations in upstream regulators of PI3K (CD19, CD28 and B-cell PI3K adaptor protein, BCAP), PI3K regulatory subunits (p85α), PI3K catalytic subunits (p110α, p110β, p110δ and p110γ), PI3K effectors (AKT/PKB, BTK and ITK) and phosphatidylinositol-(3,4,5)-trisphosphate ($PtdInsP_3$) phosphatases (SH2-domain-containing inositol polyphosphate 5′ phosphatase, SHIP, and phosphatase and tensin homologue, PTEN). There are pros and cons to each of these strategies. Mutating upstream regulators of PI3K, such as CD19 and CD28, is a good way to investigate the contribution of PI3K to the biological function of a receptor. However, these experiments can be difficult to interpret, as the receptors might have alternative means to couple to PI3K. Targeting the regulatory and catalytic subunits of PI3K is perhaps the most direct approach, but it is complicated by the number of different isoforms, the complex regulation of p110 by p85 and the early embryonic lethality that results from mutations of p110α and p110β. Because PI3Ks have pleiotropic roles in development, tissue-specific mutations might be required. In the absence of p85, p110 might also still be activated by RAS. Targeting downstream effectors of PI3K reveals important information regarding specific aspects of PI3K signalling only. Eliminating the $PtdInsP_3$ phosphatases SHIP and PTEN indicates what happens if PI3K signalling is allowed to proceed without restraint, but this might not always correspond to how PI3K signals under normal circumstances.



The analysis of transgenic and gene-targeted mice that have modifications that interfere with or enhance PI3K activation has shed considerable new light on the role of PI3Ks in the development, differentiation and activation of B and T cells, which is the focus of this review. The different approaches that have been taken are outlined in BOX 2. To investigate the role of class I PI3Ks in B and T cells directly, the various PI3K subunits have been targeted by homologous recombination by several groups. Because of the potential for redundancy between the different subunits and because of the complexities involved in the regulation of p110 subunits by p85 subunits, it is worth detailing the different gene-targeting strategies before describing the resulting B- and T-cell phenotypes.

Dissecting PI3K function by gene targeting. The p85α locus (*Pik3r1*) encodes at least three regulatory PI3K subunits (p85α, p55α and p50α) by alternative splicing of a common messenger-RNA precursor. The *Pik3r1* gene was targeted by homologous recombination to either eliminate all three isoforms[12] or eliminate only the long form (p85α) and still allow the expression of p55α and p50α[13]. Not surprisingly, the phenotype of the p85α, p55α and p50α triple-knockout mice is generally more severe than that of the p85α single-knockout mice. Importantly, the triple knockouts die shortly after birth and, therefore, B- and T-cell analysis was carried out by RAG COMPLEMENTATION. For simplicity, where the two phenotypes are comparable, we refer to the single- and triple-knockout mice collectively as p85α-deficient mice. The rationale behind targeting the p85α subunit, which is thought to be the most abundant of the class IA regulatory subunits, was to interfere with the recruitment of the p110 catalytic subunit to tyrosine-phosphorylated receptor complexes. However, there was also a marked reduction in the level of expression of each of the class IA catalytic subunits, which is in keeping with an important role for p85 in protecting the p110 subunits from proteolysis[12–16]. Surprisingly though, in the context of insulin signalling, PI3K-dependent responses were found to be enhanced, rather than abrogated, in p85α-deficient mice, for reasons that are not fully clear[16–18]. This was also observed for p85β-deficient mice[19] (the lymphocyte phenotype of which has not been reported yet). Therefore, although there is little doubt that PI3K

PLECKSTRIN-HOMOLOGY DOMAIN
(PH domain). A non-catalytic modular domain present in more than 180 signalling proteins. Some, but far from all, PH domains bind phosphatidylinositols. Of the proteins described in this review, PDK1, AKT/PKB, BTK, ITK, TEC, BAM32 and the TAPPs contain a signature motif that facilitates binding to $PtdInsP_3$ and/or $PtdIns(3,4)P_2$.

RAG COMPLEMENTATION
Gene-targeted embryonic stem (ES) cells are injected into blastocysts from recombination-activating gene (*Rag*)-knockout mice and implanted into a pseudopregnant female. Any B or T cells in the resulting chimaeric mouse are derived from the injected ES cells.

RHO-GAP DOMAIN
A protein domain of ~200 residues that encodes GTPase-activating protein (GAP) activity for RHO-family members. In p85, this domain is also known as a breakpoint cluster region-homology (BH) domain. It is not clear yet if this domain has RHO-GAP activity in p85.

THYMUS-DEPENDENT ANTIGENS
Antigenic stimuli that require the function of thymus-derived lymphocytes to generate a humoral immune response.

THYMUS-INDEPENDENT ANTIGENS
Antigenic stimuli that promote humoral immune responses in the absence of thymus-derived lymphocytes.

activation is affected in one way or another in p85-deficient mice, it is not always clear how. To add an additional element of complexity, some studies have also indicated that p85 might be a signalling protein in its own right, independent of p110, by interacting with small GTPases such as RAC (through its RHO-GAP DOMAIN; also known as a B-cell receptor (BCR)-homology, BH, domain)[20,21], and so it remains possible that p85-deficient cells have phenotypes that are not entirely a consequence of modulated p110 lipid-kinase signalling.

It was, therefore, of interest to investigate the immune system in mice lacking the catalytic PI3K subunits. Bi *et al.*[22,23] have generated p110α- and p110β-deficient mice, both of which die as embryos, after embryonic day (E)9.5 or shortly after implantation, respectively. So, determining a role for p110α and p110β in B and T cells awaits analysis of RAG-complementation studies or tissue-specific knockouts. In contrast to p110α and p110β, p110δ is expressed mainly by leukocytes, and p110δ-deficient mice survive without any gross abnormalities[24–26]. Clayton *et al.*[25] and Jou *et al.*[26] eliminated the expression of p110δ, whereas we[24] introduced a mutation in the p110δ locus that allows the continued expression of a catalytically inactive subunit, $p110\delta^{D910A}$. Retaining the expression of a catalytically inactive form of p110δ might reduce compensation by p110α and p110β, if each of these catalytic subunits, in association with p85, competes for limited access to tyrosine-phosphorylated complexes or RAS. Assuming redundant function and equal expression of the different catalytic subunits, eliminating p110δ would be predicted to eliminate one third of the total tyrosine-kinase-associated PI3K activity in B and T cells, as p110δ is only one of three class IA catalytic subunits that are expressed by lymphocytes. As will be discussed later, p110δ seems to carry out more than this share of PI3K signalling in B and T cells, particularly downstream of antigen receptors.

Mice deficient for p110γ have been reported by three groups[27–29]. The lack of potential redundancy for the catalytic p110γ subunit and the regulatory p101 subunit makes the interpretation of these experiments more straightforward than those involving class IA PI3Ks. These studies have indicated an important role for p110γ in regulating the chemotactic responses of macrophages and neutrophils[27–29]. Important roles for p110γ in mast cells[30], platelets[31] and cardiac myocytes[32] have also been described. Sasaki *et al.*[29] also showed that T-cell development and function were impaired, whereas B cells were unaffected, in p110γ-deficient mice. The role of p110γ in T-cell signalling has recently been reviewed elsewhere[33,34], and is not covered further in this review.

Numerous roles for PI3K in B cells

PI3Ks in B-cell development. B-cell development occurs through defined stages, resulting in at least three distinct lineages of mature B cells (FIG. 1). p85α-deficient mice have a partial block at the pro-B-cell stage and have a reduced number of B cells in the spleen[12,13]. In addition, p85α-deficient mice lack $CD5^+$ B1 cells in the peritoneum (50% reduction in p85α single-knockout mice, and a complete absence in p85α, p55α and p50α triple-knockout mice)[12,13]. p110δ-mutant mice have a similar phenotype, although variations occur between the mouse lines that have been generated[24–26,34]. For example, Okkenhaug *et al.*[24] and Jou *et al.*[26] found a block in B-cell differentiation at the bone-marrow stage, as well as reduced B-cell numbers in the spleens of adult mice. By contrast, Clayton *et al.*[25] did not find any defects in B-cell development in the bone marrow. They observed that 21-day-old mice had a reduced number of B cells in the spleen, but in adult mice, the number of B cells was normal. The reason for these discrepancies is not clear, but they could be due to differences in the targeting strategies used, as well as in genetic background and animal-housing conditions, for example. However, all three groups found that p110δ is required for the development of marginal-zone (MZ) B cells and peritoneal B1 cells. The lack of MZ and B1 cells was of particular interest, as this phenotype is also observed in *Cd19*$^{-/-}$ mice[35,36] (FIG. 1). CD19 is one of the main regulators of PI3K activity in B cells[37] (FIG. 2), and mice expressing a tyrosine-to-phenylalanine mutant of CD19 that cannot bind PI3K also lack B1 and MZ B cells[38]. These results indicate that a PI3K-transmitted signal from CD19 drives the differentiation of B1 and MZ B cells. Alternatively, MZ B cells might develop independently of CD19 and PI3K, but then die rapidly if CD19 and PI3K are lacking and hence escape detection[39]. Although CD19 can act downstream of the complement receptor CD21, this is unlikely to promote the development of MZ B cells as *Cd21*$^{-/-}$ mice have an increased number of MZ B cells[40]. So, it is possible that CD19 interacts with an as-yet-unidentified ligand to promote MZ B-cell development. The importance of CD19 for B1-cell and MZ B-cell development might be related to the ability of CD19 to lower the threshold for activation through the BCR by a factor of as great as 100 (REF. 41), which might somehow favour B1-cell and MZ B-cell development. CD19 is not absolutely required for PI3K activation by the BCR[42,43], but it seems to be required for sustained PI3K activation after BCR stimulation[42]. In the context of insulin signalling, Tengholm *et al.*[44] have recently shown that transient versus sustained PI3K signalling can lead to qualitatively distinct responses by the cell. One possibility, therefore, is that sustained PI3K signalling is required for the development of MZ B cells, and that this depends on both CD19 and p110δ. This conclusion is supported by the observation that phosphatase and tensin homologue (PTEN) deficiency can rescue the development of MZ B cells and B1 cells in *Cd19*$^{-/-}$ mice[45]. PTEN is a lipid phosphatase that removes the D3 phosphate from $PtdInsP_3$, which means that its absence leads to the accumulation of $PtdInsP_3$ and hence sustained PI3K signalling (BOX 2).

Consistent with defects in B-cell activation and development, p85α-deficient and p110δ-deficient mice have reduced antibody concentrations in serum. The p85α single-knockout mice were shown to raise a normal immune response against THYMUS-DEPENDENT ANTIGENS (TD antigens), but failed to respond to THYMUS-INDEPENDENT TYPE-2 ANTIGEN (TI-2 antigens)[13]. By contrast, the p110δ-mutant mice had impaired responses to both TD and

TI-2 antigens[24–26]. However, immune responses have not been reported for the p85α, p55α and p50α triple-knockout mice, which, given their generally more severe phenotype than p85α single-knockout mice, might have attenuated TD and TI responses also. Again, it is of interest to consider the link between CD19 and PI3K in B cells in the context of B-cell responsiveness. TD humoral immune responses are deficient in *Cd19*$^{-/-}$ mice[35]. Moreover, as for MZ B-cell development, the capacity of CD19 to promote TD immune responses is linked to its capacity to recruit and activate PI3K[38].

PI3Ks in B-cell signalling. In B cells, PI3K is activated within seconds of antigen-receptor triggering[46,47]. The tyrosine kinase SYK becomes activated and phosphorylates the co-receptors CD19 and B-cell PI3K adaptor (BCAP), which provide binding sites for PI3Ks[37,48] (FIG. 2). In *Cd19*$^{-/-}$ mouse B cells, IgM-specific antibody-stimulated AKT/PKB phosphorylation is reduced, indicating that CD19 has an important, but not indispensable, role in PI3K activation[42,43]. However, PI3K signalling seems to be important for the function of CD19, as the expression of a mutant form of CD19 that can no longer bind PI3K, expressed on the *Cd19*$^{-/-}$ background, fails to rescue the CD19-deficient phenotype[38]. The role of BCAP in PI3K activation is less clear. Although the production of $PtdIns P_3$ and AKT/PKB phosphorylation are impaired in *Bcap*$^{-/-}$ chicken DT40 B cells[48,49], IgM-specific antibody-stimulated AKT/PKB phosphorylation is unaffected in *Bcap*$^{-/-}$ mouse B cells[50]. One possibility is that BCAP is only required for PI3K signalling in particular B-cell subsets. It is also possible that additional isoforms of BCAP are expressed by mouse B cells[50]. More recently, the guanine nucleotide-exchange factor (GEF) VAV3 has been proposed to contribute to PI3K activation in B cells by a mechanism that might involve

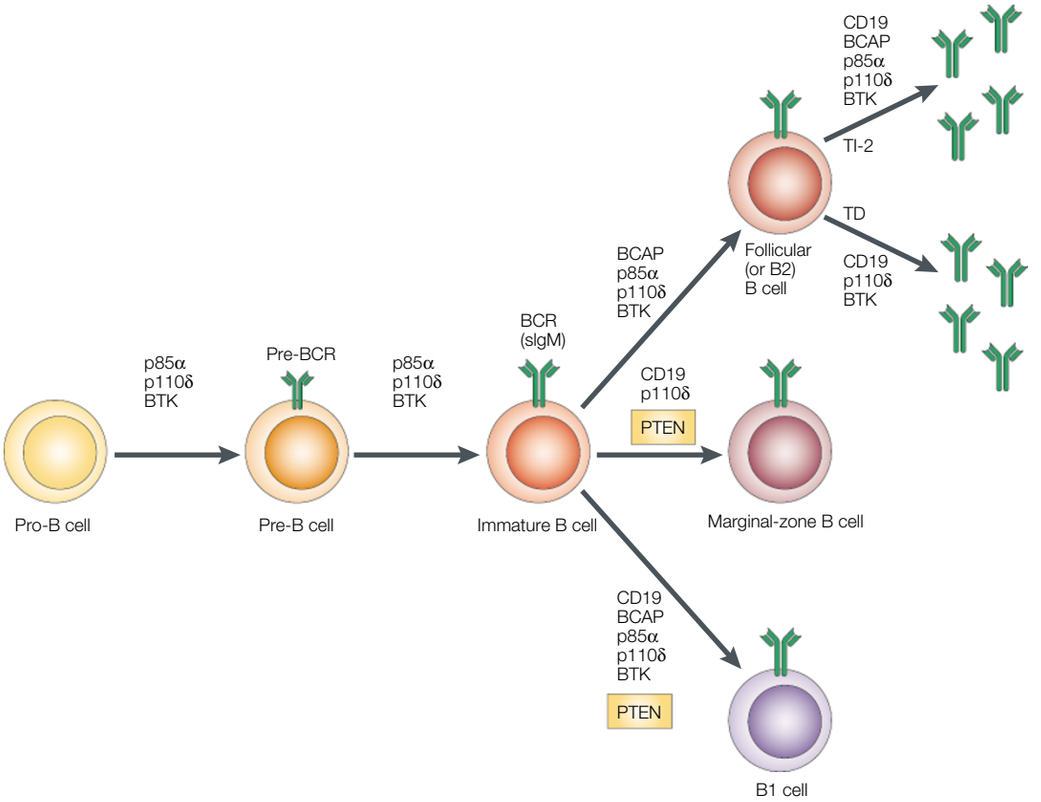


Figure 1 | **Genetic dissection of PI3K signalling in B-cell development.** B-cell development occurs through several discrete stages, at many anatomical locations (reviewed in REF. 141). In adult mice, B-cell development starts in the bone marrow, where recombination-activating gene (Rag)-mediated rearrangement of the μ locus results in the expression of the μ heavy chain, which associates with the surrogate light chain (SLC) and with Igα and Igβ (which transmit signals inside the cell) to form the pre-B-cell receptor (pre-BCR). Signalling through the pre-BCR promotes a proliferative burst, followed by progression stage to the pre-B-cell stage, where Rag-mediated recombination yields the κ and λ light chains. This transition is partially dependent on p85α and p110δ, as well as the downstream effectors BTK (Bruton agammaglobulinaemia tyrosine kinase) and TEC. The rearranged λ or κ light chain replaces the SLC to form the BCR. The BCR signals further development of the immature B cells, which migrate to the spleen (and possibly other locations). There are at least three distinguishable subsets of mature B cells: conventional follicular B2 cells, marginal-zone (MZ) B cells and B1 cells. MZ B-cell and B1-cell development depend on CD19 and p110δ. Whether these subsets follow a common progressive developmental pathway or parallel developmental pathways is not entirely clear, but the strength and quality of BCR signals are thought to influence the lineage decision. After exposure to antigen, B cells undergo terminal differentiation to antibody-secreting plasma cells. PI3K signalling seems to be involved at each of these stages, and is antagonized by phosphatase and tensin homologue (PTEN; shown in a yellow box), as described in the text. BCAP, B-cell PI3K adaptor protein; PI3K, phosphoinositide 3-kinase; TD, thymus dependent; TI-2, thymus independent type 2.

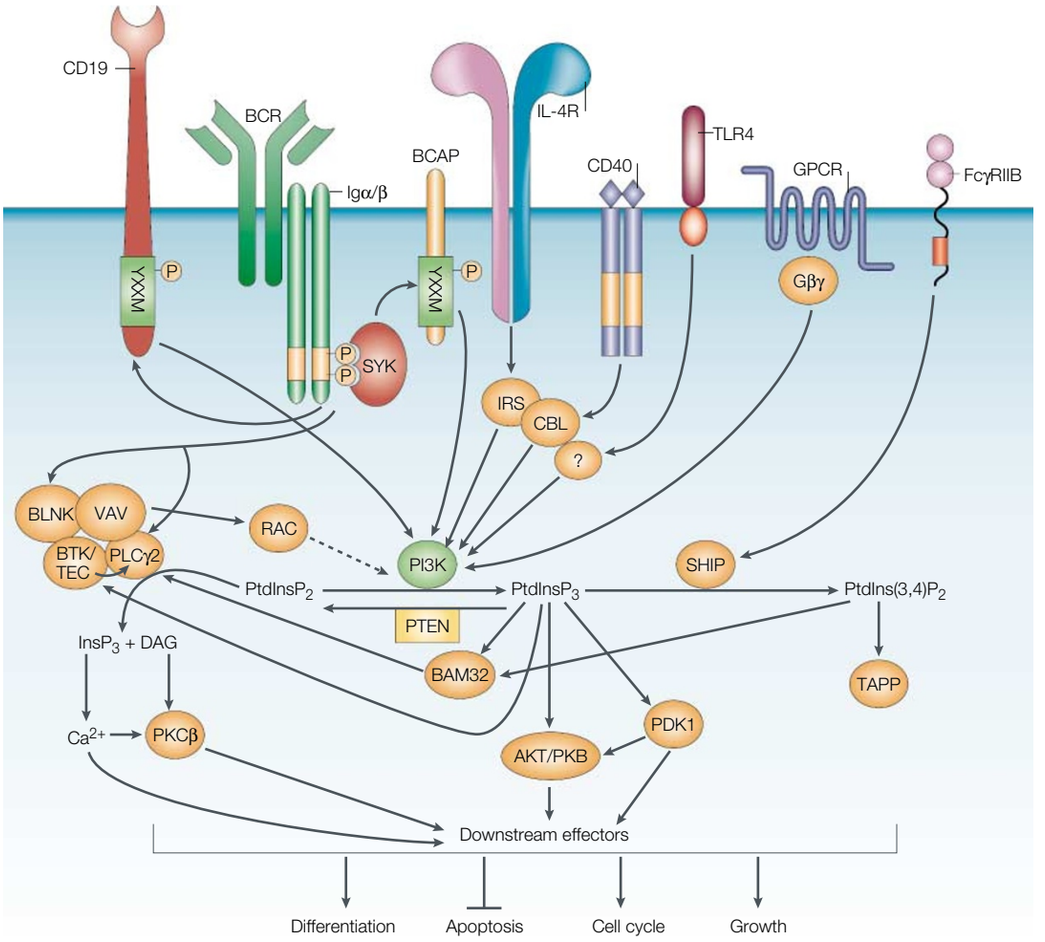


Figure 2 | **PI3K activation and signalling in B cells.** The molecular details of the recruitment and activation of phosphoinositide 3-kinases (PI3Ks) by the B-cell receptor (BCR) is not completely understood, but is at least in part regulated by BCR- and SYK-dependent phosphorylation of CD19 and B-cell PI3K adaptor protein (BCAP), which contain Tyr-Xaa-Xaa-Met (YXXM) sequences[37,48]. More recently, VAV has been proposed to contribute to the activation of PI3K, possibly as a consequence of the capacity of VAV to activate RAC[52]. The interleukin-4 receptor (IL-4R) recruits and activates PI3K through insulin-receptor substrate (IRS) proteins, which have Tyr-Xaa-Xaa-Met motifs[142]. Lipopolysaccharide (LPS)-stimulated activation of PI3K might involve Toll-like receptor 4 (TLR4) and RAC, although the precise mechanism through which LPS stimulation leads to PI3K activation remains to be determined[143,144]. CD40-mediated regulation of PI3K seems to be mediated by CBL[145,146]. B-cell linker (BLNK) is an adaptor protein that becomes tyrosine phosphorylated by SYK and binds to the SRC-homology 2 (SH2) domains of phospholipase Cγ (PLCγ) and BTK, and hence coordinates the activation of these enzymes[147]. BTK contributes to BCR-stimulated calcium signalling by phosphorylating and activating PLCγ. More recently, the adaptor protein BAM32 (B-cell adaptor molecule of 32 kDa) has also been shown to be involved in PI3K-dependent calcium regulation[62,63]. The inhibitory receptor FcγRIIB binds to SH2-domain-containing inositol polyphosphate 5′ phosphatase (SHIP) and stimulates the hydrolysis of phosphatidylinositol-(3,4,5)-trisphosphate ($PtdInsP_3$) to yield $PtdIns(3,4)P_2$. TAPP1 (tandem PH-domain-containing protein 1) and TAPP2 bind preferentially to $PtdIns(3,4)P_2$, but their downstream targets remain undefined[77]. AKT/PKB regulates numerous signalling pathways that promote cell survival, cell-cycle progression and growth[3]. BTK, Bruton agammaglobulinaemia tyrosine kinase; DAG, diacylglycerol; GPCR, G-protein-coupled receptor; PDK1, phosphatidylinositol-dependent kinase 1; PKCβ, protein kinase Cβ; PTEN, phosphatase and tensin homologue.

the activation of RAC, which then binds to p85α through its RHO-GAP domain. This places VAV upstream of PI3K signalling, rather than downstream as had previously been suggested[51,52].

IgM-specific antibody-stimulated AKT/PKB phosphorylation was nearly abolished in p85α- and p110δ-deficient B cells, which indicates that these are the main class IA regulatory and catalytic isoforms acting downstream of the BCR[24,25,53]. Indeed, using a new approach[54] to measure the production of $PtdInsP_3$ in primary, unlabelled cells, Clayton *et al.*[25] documented complete loss of $PtdInsP_3$ production in p110δ-deficient B cells in response to IgM-specific antibody-mediated stimulation. Furthermore, antibody-stimulated calcium flux was attenuated in p110δ-deficient B cells[24–26]. Antibody-stimulated proliferation was also markedly attenuated in p85α- and p110δ-deficient mice. B-cell proliferation stimulated by IL-4, lipopolysaccharide or a CD40-specific antibody was reduced, but not as markedly as that stimulated by an IgM-specific antibody[12,13,24–26].

It is of interest to note, however, that whereas p85α-deficient B cells proliferate normally in response to co-stimulation with IL-4 and a CD40-specific antibody[12], p110δ-deficient B cells do not[24,25]. This raises the possibility that IL-4 and CD40 together can engage p110δ independently of p85α, perhaps by engaging p85β-associated p110δ.

PI3K-mediated regulation of BTK

The TEC-family kinases are thought to link PI3K activation to calcium signalling. BTK, a TEC-family kinase expressed by B cells, has a PH domain that binds $PtdInsP_3$ with high affinity. A mutation in the BTK PH domain that disrupts binding of $PtdInsP_3$ is the cause of the mouse Xid phenotype, which is as severe as the *Btk*$^{-/-}$ phenotype and is similar to the p85α- and p110δ-deficient phenotypes, indicating that PI3K-dependent regulation of BTK translocation to the plasma membrane is essential for BTK function[55,56]. The phosphorylation of phospholipase Cγ (PLCγ) by BTK is thought to be required to yield the threshold levels of $InsP_3$ that allow calcium flux from extracellular stores into the cytosol, which is required for a sustained calcium response[57]. After recruitment of BTK to the cell membrane mediated by the association of the PH domain of BTK with $PtdInsP_3$, tyrosines 551 and 223 (Tyr551 and Tyr223) of BTK are thought to be phosphorylated sequentially by LYN and by autophosphorylation, respectively[58,59]. Indeed, Clayton *et al.*[25] reported attenuated phosphorylation of Tyr551 and Tyr223 of BTK in IgM-specific antibody-stimulated p110δ-deficient B cells using site-specific phosphopeptide-specific antibodies, confirming a role for PI3K in regulating the activation of BTK. However, Jou *et al.*[26] found no effect of p110δ deficiency on BTK phosphorylation using the general phosphotyrosine-specific antibody 4G10. Similarly, BTK phosphorylation as detected using 4G10 was normal in p85α-deficient B cells, as well as in wild-type B cells inhibited by wortmannin or LY294002 (REF. 53). Moreover, the kinase activity of BTK seemed to be unaffected by PI3K inhibition[53], and PLCγ2 phosphorylation was unaffected in p110δ-deficient cells[25,26]. These results might be reconciled, at least in part, by the observation that the phosphorylation of Tyr551 of BTK is partially inhibited by pre-treatment of the cells with wortmannin, whereas phosphorylation detected using 4G10 is not inhibited, which indicates that sites other than Y233 and Y551 might be dominantly recognized by 4G10 (REF. 60). Nevertheless, the phenotype of BTK and p85α double-deficient mice was more severe than that of either knockout alone[53], and BTK is required for the transcription of a small subset of genes that are not regulated by PI3K[61]. Therefore, although there is overwhelming evidence of a role for PI3K in the regulation of BTK, PI3K-independent functions of BTK need to be considered also.

In contrast to p110δ-deficient mice, *Btk*$^{-/-}$ mice still develop MZ B cells, and their TD immune responses are less affected than in p110δ-deficient mice[36]. It is, therefore, pertinent to consider the role of additional PI3K effectors in BCR signalling. B-cell adaptor molecule of 32 kDa (BAM32) is an adaptor protein with a high-affinity $PtdInsP_3$-binding PH domain that seems to have a role in BCR-dependent calcium flux, although the mechanism by which this occurs is unknown[62,63]. A role for other PI3K effectors, including AKT/PKB, in B-cell signalling is also anticipated[64], but has yet to be shown *in vivo*. VAV might be regulated, in part, by PI3K activation, as $PtdInsP_3$ can regulate VAV GEF activity *in vitro*[51,65]. Moreover, BCR-stimulated calcium flux depends on VAV, which indicates that VAV could provide a link between PI3K and PLCγ activation. However, in contrast to the PH domains of BTK, AKT/PKB and BAM32, the VAV PH domain does not contain crucial residues required to bind $PtdInsP_3$ with sufficient affinity to lead to the membrane recruitment of VAV[66,67]. In this context, it is of interest to note that T-cell lymphoma invasion and metastasis 1 (TIAM1), a VAV-related GEF, has been proposed to be regulated by PtdIns(3)P, rather than by $PtdInsP_3$ (REF. 68). The precise role of the VAV PH domain, however, remains unknown[65]. For an excellent review on PH domains, see REF. 69.

Co-clustering of the BCR and FcγRIIB by antigen–antibody complexes inhibits B-cell activation. This inhibition is mediated by FcγRIIB, which uses the lipid phosphatase SH2-DOMAIN-CONTAINING INOSITOL POLYPHOSPHATE D5 PHOSPHATASE (SHIP) to convert $PtdInsP_3$ to $PtdIns(3,4)P_2$, thereby modulating PI3K signalling. Co-ligation of FcγRIIB inhibits both AKT/PKB[70,71] and BTK[72,73] phosphorylation, which indicates that both of these kinases require $PtdInsP_3$ for activation *in vivo*. In *Ship*$^{-/-}$ mice, the lack of inhibition of B-cell signalling contributes to the development of autoimmune disease (note that abnormal activation of myeloid cells also contributes to autoimmune disease in this model)[74–76]. The amplitude of the sustained phase of IgM-specific antibody-stimulated calcium flux in B cells from these mice is markedly increased, and this cannot be inhibited by FcγRIIB signalling[73,74]. It is worth pointing out, however, that although the conversion of $PtdInsP_3$ to $PtdIns(3,4)P_2$ can terminate BTK- and AKT/PKB-dependent signalling, the PI3K signal might then be 'taken over' by the recently discovered tandem PH-domain-containing protein 1 (TAPP1) and TAPP2 adaptor proteins, which have binding specificity for $PtdIns(3,4)P_2$ (REFS 77,78). Whether these proteins propagate negative signalling pathways, or have other functions, remains to be determined.

Numerous roles for PI3Ks in T cells

T-cell development takes place in the thymus, where thymocyte precursors are selected by self-peptides presented by MHC molecules. $CD4^+$ and $CD8^+$ T cells that avoid being eliminated by reacting too strongly with self-peptides migrate to the lymph nodes and the spleen, where peptides are presented by dendritic cells (DCs). Recognition of self-peptides in the spleen and lymph nodes is thought to keep the T cells in an alert state through sub-optimal T-cell receptor (TCR) signalling[79]. After presentation of a foreign peptide antigen by an activated DC, T cells start to secrete IL-2, undergo rapid proliferation and differentiate. T cells exposed to

SH2-DOMAIN-CONTAINING INOSITOL POLYPHOSPHATE D5 PHOSPHATASE (SHIP). A lipid phosphatase that removes the D5 phosphate from $PtdInsP_3$ to yield $PtdIns(3,4)P_2$.

IL-12 tend to differentiate to interferon-γ-secreting T helper 1 (T_H1) cells, whereas T cells exposed to IL-4 tend to differentiate to T_H2 cells, which can secrete IL-4, IL-5 and, in some cases, IL-10 (REF. 80). Subsequent to the proliferative burst, most of the T cells die by apoptosis, but some survive as long-lived memory T cells[81]. CD28 has a crucial role during T-cell activation, by associating closely with the TCR at the interface between the T cell and the antigen-presenting cell (APC)[82]. APCs express B7 molecules (including CD80 and CD86), which are ligands for CD28, and the capacity of an APC to activate a T cell depends, to a large extent, on its expression of B7 (or related co-stimulatory) ligands[83].

PI3Ks in T-cell development and differentiation. T-cell development in the thymus does not seem to be affected in p85α-deficient, p110δ-deficient or $p110\delta^{D910A/D910A}$ mice, although positive and negative selection were not specifically examined[24,26,84]. When compared with T cells from wild-type littermates, peripheral $p110\delta^{D910A/D910A}$ T cells express lower levels of CD44 and higher levels of CD62L, which is consistent with a more naive phenotype[24] (K.O., unpublished observations). Moreover, $p110\delta^{D910A/D910A}$ mice develop a mild form of inflammatory bowel disease, characterized by infiltration of leukocytes in segments of the large intestine. As the intestinal flora, from the point of view of the immune system, is foreign, mechanisms need to be in place to suppress unnecessary inflammation of the intestine. This is mediated, in part, by regulatory T cells that secrete IL-10 or transforming growth factor-β (TGF-β), which suppress immune responses against the harmless intestinal flora[85]. In $p110\delta^{D910A/D910A}$ mice, it is possible that IL-10- or TGF-β-secreting regulatory T cells do not become activated, thereby allowing infiltration of the large intestine by leukocytes to occur unchecked. In terms of T-cell differentiation, it is also of interest to note that two PI3K effector proteins, AKT/PKB and ITK, have been proposed to regulate differentiation along the T_H1- and T_H2-cell lineages, respectively[86–88]. Together, these results indicate possible roles for PI3K in the differentiation of naive T cells to effector, regulatory and memory T cells.

PI3Ks in T-cell signalling. PI3K activation occurs within seconds of T-cell activation[89,90], even preceding calcium flux[91]. One study, in which the $PtdInsP_3$ reporter construct green fluorescent protein (GFP)–AKT/PKB-PH was expressed in the T cells of TCR-transgenic mice[90], yielded some surprising results: after encounter of a T cell with an APC, PI3K remained active for nine hours or more[90]. Moreover, using the PI3K inhibitor LY294002, it was shown that PI3K activation during the first nine hours of T-cell stimulation is essential for T-cell proliferation, after which time PI3K is no longer strictly required. As might be predicted, the accumulation of $PtdInsP_3$ was localized to the contact area between the T cell and APC[90,91]. An unanticipated result was that $PtdInsP_3$ also accumulated at the rear of the T cell, away from the APC[90]. The mechanism or significance of this posterior accumulation of $PtdInsP_3$ is not clear. However, these studies are an important technical breakthrough, as PI3K activity has previously been difficult to measure using limited numbers of primary T cells, especially as activated by APCs.

There are several possible mechanisms for the activation of PI3K in T cells[92]. TCR-interacting molecule (TRIM) is a transmembrane protein that has a short extracellular domain that is unlikely to bind any ligand, but that has a cytoplasmic domain containing Tyr-Xaa-Xaa-Met motifs that might be phosphorylated after TCR stimulation[93] (FIG. 3). The transmembrane adaptor linker for activation of T cells (LAT) might also couple to PI3K, although probably indirectly as it does not contain a Tyr-Xaa-Xaa-Met motif[94]. Analogous to the situation in B cells, VAV1 has recently been proposed to regulate PI3K activation in thymocytes, placing VAV upstream, rather than downstream, of PI3K signalling[95]. The co-stimulatory receptors CD28 and inducible T-cell co-stimulator (ICOS) also contain a Tyr-Xaa-Xaa-Met motif that can function as a docking site for the SRC-HOMOLOGY 2 DOMAINS (SH2 domains) of p85 and can also contribute to PI3K activation[83].

p85α-deficient T cells develop normally, and show no defects in proliferation in response to stimulation with a CD3-specific antibody[12]. CD3-specific antibody-stimulated proliferation was nevertheless inhibited by LY294002, which indicates that PI3Ks have a role in TCR-dependent proliferation[12]. In fact, no evidence was presented to indicate that TCR-coupled PI3K activation was impaired in p85α-deficient T cells; therefore, a compensatory role of p85β, for example, could not be excluded. In $p110\delta^{D910A/D910A}$ T cells, AKT/PKB phosphorylation was nearly abrogated in response to TCR stimulation[24]. CD3-specific antibody-stimulated proliferation of purified $p110\delta^{D910A/D910A}$ $CD4^+$ T cells was reduced by about 50% (REF. 24). Curiously, $p110\delta^{D910A/D910A}$ T cells stimulated with CD3-specific and CD28-specific antibodies showed normal, or even enhanced, proliferation and secreted normal levels of IL-2. Therefore, these results supported previous studies indicating an important role for PI3K in TCR signalling independently of CD28 (REFS 96,97). Jou *et al.*[26] observed a less marked reduction in proliferation in response to stimulation through CD3 in p110δ-deficient T cells compared with $p110\delta^{D910A/D910A}$ T cells. This observation might reflect a greater capacity for p110α and p110β to compensate in the absence of p110δ expression compared with when the 'kinase-dead' p110δ is expressed. However, differences in the experimental protocols could also be of importance. Jou *et al.* used cultures of unfractionated spleen cells in their assay, in which co-stimulation is provided by B7 ligands expressed by B cells, macrophages and DCs[26]. Under these conditions, $p110\delta^{D910A/D910A}$ T cells are also only mildly affected (K.O., unpublished observations), which is consistent with a non-essential role for p110δ in CD3-dependent proliferation in the presence of co-stimulation.

Similar to BTK in B cells, the related tyrosine kinase ITK connects TCR signalling to calcium flux in T cells[98]. Interestingly, $Itk^{-/-}$ mice are refractory to stimulation by CD3-specific antibody, but hyperproliferate in response

SRC-HOMOLOGY 2 DOMAIN (SH2 domain). A non-catalytic modular protein domain that binds to phosphotyrosines in specific sequence motifs.

to co-stimulation through CD28 (REF. 99), which is consistent with results obtained for p110δ-deficient mice[24]. T cells also express a second TEC-family kinase, RLK (resting lymphocyte kinase; also known as TXK), which lacks a PH domain and is uncoupled from regulation by PI3K[100]. The capacity of RLK to be activated independently of PI3K could be one reason that T-cell proliferation in response to antigen-receptor stimulation is less affected in p85α- and p110δ-mutant mice than the proliferation of B cells[101].

Shi *et al.*[102] showed that IL-2 production by T cells is sensitive to wortmannin in response to antigen stimulation by APCs, regardless of whether co-stimulatory signals through CD28 are provided, but is independent of PI3K when induced by antibodies specific for CD3. They proposed that PI3K is required to form APC–T-cell conjugates. More recent evidence, however, indicates that the formation of such complexes does not depend on PI3K[90]. Nevertheless, TCR-transgenic $p110\delta^{D910A/D910A}$ T cells stimulated with a peptide ligand

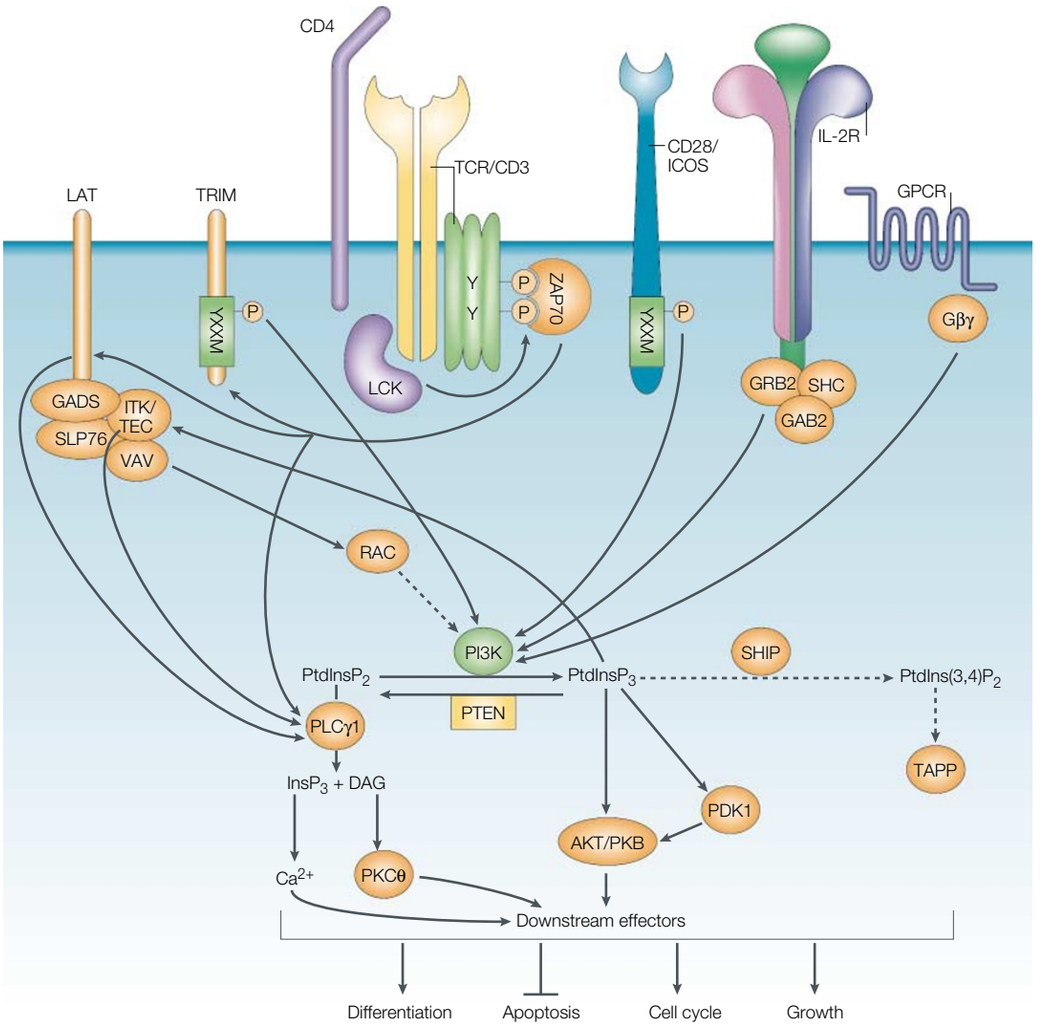


Figure 3 | **PI3K activation and signalling in T cells.** As for the B-cell receptor, signalling through the T-cell receptor (TCR) is known to involve phosphoinositide 3-kinase (PI3K), but the molecular mechanism by which the TCR is coupled to PI3K remains unclear. The co-stimulatory receptors CD28 and inducible T-cell co-stimulator (ICOS) contain consensus PI3K-binding motifs (YXXM), and might either contribute to or complement TCR-dependent PI3K signalling. The transmembrane adaptor protein TCR-interacting molecule (TRIM) also contains Tyr-Xaa-Xaa-Met motifs that might facilitate the coupling of the TCR to PI3K signalling. In addition, VAV might regulate PI3K signalling upstream of RAC[95,148]. Phospholipase Cγ (PLCγ) is regulated by a pathway involving LAT–GADS–SLP76 and VAV. PI3K, through the membrane recruitment of interleukin-2-inducible T-cell kinase (ITK), contributes to calcium signalling downstream of the TCR[98]. In contrast to B cells, it is at present speculative whether inhibitory receptors signal through SH2-domain-containing inositol polyphosphate D5 phosphatase (SHIP) in T cells[131,132]. AKT/PKB connects PI3K to signalling pathways that promote cytokine transcription, survival, cell-cycle entry and growth[3,149,150]. DAG, diacylglycerol; GAB2, GRB2-associated binding protein 2; GPCR, G-protein-coupled receptor; ITK, interleukin-2-inducible T-cell kinase; LAT, linker for activation of T cells; PDK1, phosphatidylinositol-dependent kinase 1; PKC, protein kinase C; PtdIns, phosphatidylinositol; PTEN, phosphatase and tensin homologue; SLP76, SH2-domain-containing leukocyte protein of 76 kDa; TAPP, tandem PH-domain-containing protein; ZAP70; ζ-chain-associated protein kinase 70 kDa.

presented by wild-type B cells acting as APCs had markedly reduced proliferative responses and a reduced capacity to produce IL-2, even though under these conditions, co-stimulation would be provided by the APCs[24]. Together, these results show that T cells probably depend on p110δ to amplify signals stimulated by peptide antigen, but that the strong stimulus provided by CD3-specific antibody in conjunction with co-stimulation through CD28 can bypass this requirement.

One mechanism whereby signals through the TCR are thought to be amplified involves the accumulation of lipid rafts at the T-cell–APC interface[103]. Lipid rafts are sphingolipid- and cholesterol-rich microdomains in the plasma membrane. Lipid rafts are enriched in SRC-family kinases and small GTPases, and therefore, their accumulation at the T-cell–APC interface might provide a high local concentration of crucial signalling molecules that lead to either amplified or sustained signalling[104]. The accumulation of lipid rafts can also be induced by CD3-specific and CD28-specific antibodies bound to cell-sized polystyrene beads, and can be visualized using fluorescently labelled cholera toxin protein, which binds to the GM1-glycosphingolipid that is found in rafts[103]. Interestingly, $p110\delta^{D910A/D910A}$ T cells had a reduced capacity to form such lipid rafts in response to stimulation with CD3-specific and CD28-specific antibodies[24]. Although this defect did not affect the capacity of the T cells to proliferate or produce IL-2 in response to antibody stimulation (which involves high-affinity receptor interactions), low-affinity interactions formed between the TCR and peptide–MHC on the APC might be crucially dependent on raft recruitment for signalling to take place or to be sustained. Consistent with this notion, p110δ-deficient T cells respond poorly to peptide antigen presented by APCs[24] or to stimulation with phytohaemagglutinin, which is a relatively weak TCR agonist for mouse T cells[26].

PI3K and CD28 co-stimulation

CD28 provides an essential co-stimulatory signal during T-cell activation, which augments the production of IL-2, increases T-cell proliferation and prevents the induction of anergy and cell death. Much attention has been devoted to the potential of CD28 to stimulate PI3K in T cells. The observations that CD28 can activate PI3K independently of the TCR[89] and that CD28 contains a Tyr-Xaa-Xaa-Met motif in its cytoplasmic domain that can bind the SH2 domains of p85 led several groups to consider whether co-stimulation through CD28 involves PI3K activation[105]. Initial experiments, using various T-cell lines transfected with mutant forms of CD28, yielded conflicting results with respect to the role of PI3K downstream of CD28 (REF. 105). Some of these studies are difficult to interpret because transformed T-cell lines were used, many of which have been recognized since to be transformed, in part, because they no longer express the $PtdInsP_3$ phosphatase PTEN[7]. Without PTEN, cells accumulate high levels of $PtdInsP_3$, even in the absence of stimulation, and as such, PI3K signalling is constitutively switched on. Also, in contrast to primary T cells, transformed T-cell lines proliferate independently of signals from the TCR and CD28. In addition, transformed T-cell lines cannot be used to investigate the role of CD28 in the prevention of anergy.

More recently, transgenic approaches have been used to investigate the role of PI3K signalling downstream of CD28 in primary T cells. Replacing the tyrosine of the Tyr-Xaa-Xaa-Met motif with phenylalanine abrogates PI3K binding to CD28 (REF. 106). Transgenic mice were generated expressing such mutant CD28(Tyr170Phe) on the *Cd28*$^{-/-}$ background[107,108]. Alternatively, retroviral transduction was used to express CD28(Tyr170Phe) proteins in *Cd28*$^{-/-}$ primary T cells *in vitro*[109]. These experiments showed that CD28 can co-stimulate T-cell proliferation and IL-2 production independently of its association with PI3K[107–109], although one group argued that proliferation and IL-2 production were delayed in the mutant mice[108]. However, the capacity of CD28 to provide co-stimulation *in vivo* was not affected, as determined by the ability of CD28(Tyr170Phe)-expressing T cells to provide help for B cells during an antiviral immune response and to prevent the induction of anergy[107]. In contrast to IL-2 production, the capacity of CD28 to promote survival through the expression of $BCL\text{-}X_L$ was abrogated in CD28(Tyr170Phe)-expressing T cells[107,109], which might have led to the failure of CD28(Tyr170Phe)-expressing T cells to survive after engraftment into a non-irradiated host[108]. These results are in line with the important role of AKT/PKB in promoting the expression of $BCL\text{-}X_L$ downstream of PI3K activation in T cells[110]. Although the capacity of CD28 to upregulate $BCL\text{-}X_L$ expression helps to promote T-cell survival[107,108,111,112], CD28 clearly engages additional signals to promote T-cell proliferation and to prevent the induction of anergy.

Overexpression of an activated form of AKT/PKB in *Cd28*$^{-/-}$ $CD4^+$ primary T cells recovered their capacity to secrete IL-2 in response to peptide antigen[86]. This study seems to be in conflict with the observation that CD28(Tyr170Phe)-expressing $CD4^+$ T cells, which cannot stimulate AKT/PKB phosphorylation[24], produce normal levels of IL-2 in response to stimulation with peptide antigen[109]. However, although the capacity of CD28 to recruit PI3K might be dispensable for the regulation of IL-2 production, it is still possible that CD28 could influence the capacity of the TCR to couple to PI3K and AKT/PKB activation independently of the Tyr170 site. In the cytoplasmic domain of CD28, there are two proline-rich regions distal to the Tyr-Xaa-Xaa-Met PI3K-binding site that can bind proteins with SRC-HOMOLOGY 3 DOMAINS (SH3 domains)[113–116] (FIG. 4). The most carboxy-terminal of these is essential for the capacity of CD28 to co-stimulate proliferation of and IL-2 production by primary $CD4^+$ T cells[109]. Although it has been proposed that this proline-rich region facilitates the recruitment of p85 through its SH3 domain[117], this is inconsistent with the complete loss of CD28-associated PI3K activity in CD28(Tyr170Phe) mutants[106,118–120], with the failure of the Tyr170Phe mutant to activate AKT/PKB[107] and with the lack of interaction between a recombinant p85 SH3 domain and the CD28 protein *in vitro*[114]. However, the CD28 carboxy-terminal

SRC-HOMOLOGY 3 DOMAIN (SH3 domain). A non-catalytic modular protein domain that binds to proline-rich sequences.

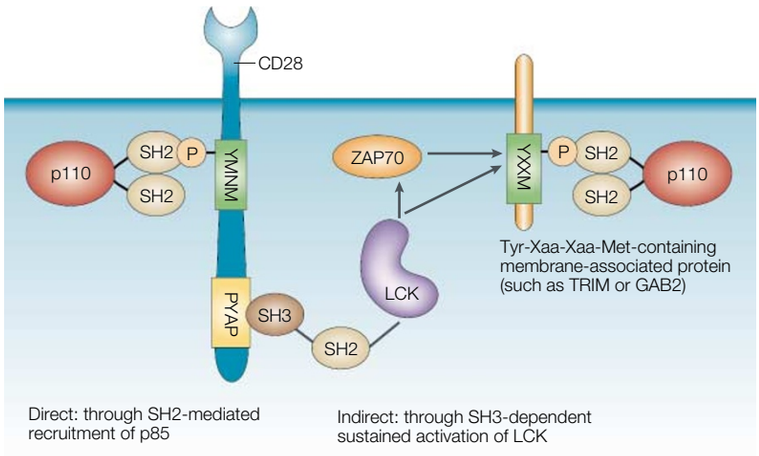


Figure 4 | **Two modes of CD28-dependent activation of PI3K.** In the direct mode, the p85 SRC-homology 2 (SH2) domains bind the phosphorylated Tyr-Met-Asn-Met (YMNM) motif in the cytoplasmic domain of CD28 (REF. 106). This is analogous to the manner in which growth-factor receptors, such as platelet-derived growth factor receptor and c-KIT, recruit and activate class IA phosphoinositide 3-kinases (PI3Ks). The second mode of activation is more speculative at this time, and is based on the observation that the proline-rich region of CD28 (PYAP) is required for CD28 to sustain LCK in its activated state (the initial activation is controlled by the TCR and CD4)[115,121]. The link between LCK and PI3K could involve the phosphorylation of TCR-interacting molecule (TRIM; a transmembrane adaptor protein with Tyr-Xaa-Xaa-Met, YXXM, motifs), linker for activation of T cells (LAT; which lacks Tyr-Xaa-Xaa-Met motifs, but which might couple to PI3K indirectly through GAB) or other linker proteins with Tyr-Xaa-Xaa-Met motifs. LCK could phosphorylate these targets directly or through its activation of ζ-chain-associated protein kinase 70 kDa (ZAP70). Either way, CD28 could contribute to T-cell-receptor-dependent PI3K activation by virtue of its capacity to influence the kinetics of LCK activation.

proline-rich region does seem to be required to prolong the activation of LCK in response to TCR stimulation[121], possibly by binding to the LCK SH3 domain, and hence relieving the inhibitory effect that the LCK SH3 domain imposes on the LCK kinase domain[121,122]. Indeed, this mechanism of activation of tyrosine kinases had previously been shown for CD28-mediated regulation of ITK, which depends on the membrane-proximal proline-rich motif of CD28 and the ITK SH3 domain[116,123]. Sustained activation of LCK and/or ITK could thereby contribute to sustained tyrosine-kinase-dependent activation of PI3K by the TCR, for example by phosphorylating TRIM or another protein containing Tyr-Xaa-Xaa-Met motif(s). Therefore, expressing AKT/PKB in *Cd28*$^{-/-}$ T cells does not necessarily bypass the requirement for CD28 to recruit and activate PI3K directly, but might instead bypass the requirement for CD28 to promote TCR-dependent PI3K activation by maintaining LCK or ITK in an active configuration (FIG. 4). In support of this model, PI3K does seem to have an integral role in TCR signalling[24,96,97]. Moreover, the requirement for co-stimulation through CD28 is circumvented in *motheaten* T cells (which have defects in the gene that encodes SH2-domain-containing protein tyrosine phosphatase 1, SHP1), in which the lack of SHP1-associated tyrosine-phosphatase activity led to sustained tyrosine-kinase activity independent of CD28 (REF. 124).

The relative contributions of the TCR and CD28 to PI3K signalling remain to be determined. In addition, PI3K might have important roles in transmitting signals from other co-stimulatory receptors and cytokine receptors at different stages of the immune response[92]. In particular, PI3Ks are thought to be important for regulation of the cell cycle and cell survival by the IL-2 receptor[92] (FIG. 3), although the specific PI3K subunits that are required for IL-2-receptor signalling *in vivo* remain to be identified. Recently, Frauwirth *et al.*[125] have proposed that PI3K could have an important role downstream of CD28 in regulating glucose metabolism in a manner analogous to how the insulin receptor uses the PI3K signalling pathway. Whether this function is unique to CD28 remains to be determined, but these studies do reveal an often-neglected aspect of T-cell activation in which PI3K might have an important role. There is clearly much yet to be learned about PI3K activation in T cells.

Consequences of unrestrained PI3K signalling

PTEN, a $PtdInsP_3$ phosphatase that removes the phosphate group from the D3 position of the inositol ring, is frequently mutated in tumours[126,127]. *Pten*$^{-/-}$ mice die as embryos, and *Pten*$^{+/-}$ heterozygous mice survive and develop autoimmunity, which is thought to result, in part, from increased resistance to Fas-mediated apoptosis[128]. Cre-mediated deletion of PTEN restricted to T cells results in a fatal lymphoproliferative disease, with 100% mortality by 17 weeks of age[129]. These mice also suffer autoimmunity as a consequence of impaired negative selection in the thymus[129]. Although PTEN was deleted in both $CD4^+$ and $CD8^+$ T cells, it was mainly the $CD4^+$ T cells that underwent uncontrolled proliferation[129]. These studies indicate that constitutive PI3K activation protects T cells from apoptosis, both in the thymus during negative selection and in the periphery, by resulting in their failure to undergo activation-induced cell death. Consistent with these conclusions is the phenotype of mice with T-cell-restricted transgenic expression of a p85α-deletion mutant that lacks negative regulatory sequences[130]. These mice have a milder lymphoproliferative disease that develops with age, and they are predisposed to developing leukaemia. In contrast to the profound effects of PTEN deficiency on T cells, T cells from mice that lack SHIP, another $PtdInsP_3$ phosphatase (but which, in contrast to PTEN, removes the D5-phosphate from the inositol ring), seem to be normal, with the exception of a slight increase in the number of $CD4^+$ T cells[74]. It is nevertheless possible that SHIP could regulate T-cell activation to some extent[131,132].

Concluding remarks

In summary, PI3Ks are important regulators of adaptive immunity. Too little PI3K activity leads to immunodeficiency, whereas too much activity leads to autoimmunity and leukaemia. The molecular basis for the preferential requirement for p110δ in B and T cells is not known. Possible explanations include distinct subcellular localization, different capacities to interact with RAS or other small GTPases, and different kinetics of activation compared with p110α and p110β. The molecular basis for PI3K activation in B and T cells needs to be further elucidated before such questions can be

answered. In addition, tissue-specific knockouts will probably provide information about any roles for p110α and p110β in the regulation of adaptive immunity, as the phenotype of p110δ-deficient mice does not exclude compensatory or complementary functions for these kinases. The precise role of p110γ in B and T cells has also yet to be carefully examined, particularly in the context of chemotactic responses, which involve GPCRs. Finally, virtually nothing is known about the function, if any, of the class II and class III PI3Ks in lymphocyte biology. Considerable progress has been made in understanding the contributions of PI3Ks to lymphocyte biology since their initial description nearly 15 years ago[133]. It is hoped that, in the next 15 years, this knowledge will contribute to the development of treatments for immune-related diseases, including autoimmunity, leukaemia and graft rejection. Indeed, the development of PI3K isoform-selective inhibitors is currently being pursued by several pharmaceutical companies[134]. In this context, it is particularly encouraging to note the recent development of p110δ-specific inhibitors[135,136].

Note added in proof

Since the submission of this manuscript, Suzuki *et al.*[151] have shown enhanced B1 and marginal-zone B-cell production in mice lacking PTEN expression in B cells.

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Online links

DATABASES

The following terms in this article are linked online to:

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
AKT/PKB | B7 | BAM32 | BCAP | BTK | CBL | *Cd19* | CD28 | CD44 | CD62L | GADS | ICOS | IL-2 | IL-4 | IL-10 | IL-12 | ITK | LAT | LYN | p55γ | p85α | p85β | p101 | p110α | p110β | p110γ | p110δ | *Pik3r1* | PLCγ2 | PTEN | RAC | RLK | SHIP | SLP76 | TAPP1 | TAPP2 | TGF-β | TRIM | VAV1 | VAV3 | Vps34

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Bart Vanhaesebroeck's lab homepage:
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