Role of class IA phosphoinositide 3-kinase in B lymphocyte development and functions

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Abstract
P13K (phosphoinositide 3-kinase) family members control a variety of cellular responses, such as cell growth, survival, cytoskeletal remodelling and the trafficking of intracellular organelles, in many cell types, including lymphocytes. It has been difficult to evaluate the roles of distinct P13Ks in immune responses, because specific inhibitors for each P13K are lacking and most stimuli activate multiple P13Ks. The development of gene-targeted mice has now allowed the elucidation of roles played in vivo by P13K species in the immune system. Studies on mice deficient in catalytic as well as regulatory subunits of class IA P13Ks have shown the importance of this class of P13K in B lineage cells. Here I discuss the role of class IA P13Ks in B lymphocyte development and B cell antigen receptor-mediated signal transduction.

Introduction
P13K (phosphoinositide 3-kinase) is a key enzyme producing phospholipid second messengers, and plays an important role in various signal transduction pathways [1–4]. The P13K family is divided into four groups (IA, IB, II and III) according to structural characteristics and substrate specificity. Of these, class IA heterodimeric P13Ks consist of a catalytic subunit (p110α, p110β, p110γ) and a regulatory subunit (p85α, p56κ, p50α, p85β, p55γ). The regulatory subunits are encoded by at least three genes: the p85α, p56κ and p50α proteins are derived from a single gene by alternative splicing mechanisms, while distinct genes encode p85β and p55γ. Among distinct regulatory subunits, p85α is the most abundantly expressed subunit in most cell types. p110γ, also known as class IB P13K, is structurally similar to p110α, p110β and p110γ, but is associated with a distinct class of regulatory subunit, p101. p110γ is unique in that it is activated via G-protein-coupled receptors [1–4]. Class IA and IB enzymes are involved in receptor-mediated signalling by phosphorylating phosphatidylinositol, PtdIns4P and PtdIns(4,5)P2 to generate PtdIns3P, PtdIns(3,4)P2 and PtdIns(3,4,5)P3 respectively. It should be noted that PtdIns(3,4,5)P3 is generated by this class of enzymes, but not by other class of enzymes in vitro.

All p85 subunits have two SH2 (Src homology 2) domains and a so-called inter-SH2 domain with a coiled-coil structure between the dual SH2 domains. The inter-SH2 domain interacts constitutively with the N-terminal domain of the p110 subunit to maintain the stability of p110 in the cell [5]. The dual SH2 domains are critical for recruiting p110 to tyrosine-phosphorylated proteins at the plasma membrane. The interaction of SH2 domains with phosphotyrosine residues of various adaptor proteins, such as receptor-type tyrosine kinases and insulin receptor substrate family members, releases the p110 kinase activity that is normally blocked by complex formation with p85s [5]. In B lymphocytes, binding of p85 to the cytoplasmic tail of CD19 upon tyrosine phosphorylation of CD19 is believed to be an important step in the activation of class IA P13K [6].

The metabolism of PtdIns species is also controlled by other kinases and phosphatases [1–4]. Among these, two PI phosphatases, namely Pten (phosphatase and tensin homologue deleted on chromosome 10) and SHIP (SH2 domain-containing 5 inositol phosphatase), are important in regulating the amounts of PtdIns(3,4,5)P3 in immune cells [1–4]. Pten catalyses the opposite reaction to P13Ks, while SHIP removes the 5-phosphate from the inositol ring of PtdIns(3,4,5)P3 to generate PtdIns(3,4)P2. A deficiency of Pten or SHIP leads to hyperactivation of P13K-mediated signalling pathways in many cell types and often results in cellular transformation [7–10].

Various proteins containing PH (pleckstrin homology) domains, such as Akt (also known as protein kinase B), PDK1 (phosphoinositide-dependent kinase 1) and Tec family kinases, are thought to function downstream of P13K, because of the ability of their PH domains to bind PtdIns(3,4)P2 or PtdIns(3,4,5)P3, products of P13K [11–13]. For example, Akt and PDK1 are recruited to the plasma membrane via binding to PtdIns(3,4)P2 and PtdIns(3,4,5)P3, respectively, and PDK1 phosphorylates and activates Akt. Although pharmacological inhibitors such as wortmannin and Ly294002 have allowed the examination of the role of P13Ks in various cellular responses in vitro [14], it has been difficult to study the

Key words: Bcl-xL, Bruton’s tyrosine kinase (Btk), nuclear factor-κB, PI3K, PDK1, phosphoinositide-dependent kinase 1, PH, pleckstrin homology, PtdIns, phosphoinositide 3-kinase; Btk, Bruton’s tyrosine kinase; B, B cell antigen receptor; Btk, Bruton’s tyrosine kinase; C, germinal centre; LAB, linker for activation of B cells; LC, marginal zone; NF-κB, nuclear factor-κB; PDK1, phosphoinositide-dependent kinase 1; PH, pleckstrin homology; PtdIns, phosphoinositide 3-kinase; SH2 domain-containing 5 inositol phosphatase, TCR, T cell receptor.

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role of each PI3K in vivo because these reagents inhibit all PI3Ks.

The recent development of genetic approaches with gene-manipulated mouse systems has been a breakthrough in elucidating the roles of individual PI3K members in vivo. Various knockout mice lacking class IA or class IB PI3Ks have been established to date. Of these, p110α and p110β knockout mice die in utero between embryonic days 9.5 and 10.5 [15,16]. The importance of p110α and p110β in cell growth and survival is evident, since it is difficult to even obtain embryonic fibroblast lines from these knockout mice. Cell type-specific gene disruption using cre-loxP system seems to be required for the studies of the in vivo roles of p110α and p110β. In contrast, p110γ and p110δ knockout mice grow normally [17–23]. Two types of p110δ mutant mice have been generated, namely p110δ null mice (p110δ−/− mice) and p110δD910A mice, in which endogenous p110δ is replaced by a functionally inactive mutant of p110δ with a point mutation of Asp at position 910 to Ala. p85α-deficient mice expressing p55α and p50α (p85α−/− mice) are viable, but mice lacking p85α, p55α, and p50α (p85α−p55α−p50α−/− mice) die soon after birth [24–27], indicating that p55α and p50α indeed play a role in vivo. p85β knockout mice are viable and seem to have no apparent defects in the immune system [28]. Knockout mice for SHIP and Pten have also been established. SHIP knockout mice are born but have a shorter life span compared with wild-type mice [9,10]. In contrast, Pten-deficient mice are embryonic lethal, and Pten−/− mice exhibit an increased tumour incidence, indicating that the balance between PI3K and Pten is indeed critical in the regulation of cell growth in vivo [7,8].

Class IA PI3Ks and B lymphocyte development

Studies using the above knockout mice have revealed the importance of class IA PI3Ks in B cell development and functions. B cell development is impaired at an early pre-B stage in p85α−/−, p85α−p55α−p50α−/−, p110α−/− and p110αD910A mice [21–25]. More notably, development of peritoneal CD5+ B cells is severely blocked. BCR (B cell antigen receptor)-, lipopolysaccharide- or CD40-mediated proliferation is also impaired in the absence of these molecules. Reconstitution of Rag-2−/− embryos with p85α−p55α−p50α−/− embryonic stem cells results in B cell phenotypes identical to those of p85α−/− mice, indicating that the defects are intrinsic to B lineage cells [25]. In p110δ−/− as well as p110αD910A mice, generation of MZ (marginal zone) B cells and GC (germinal centre) formation are blocked and in vivo antibody production in response to both T-dependent and -independent antigen is severely impaired [18–20]. On the other hand, p85α−/− as well as p85α−p55α−p50α−/− B cells have defects only in the T-independent antibody response [24,25]. p110δ is the major catalytic subunit of class IA PI3K in B cells, and may utilize regulatory subunits other than p85α in p85α−/− and p85α−p55α−p50α−/− mice. Alternatively, the lack of antibody production may be due to an impairment of T cell function in p110δ mutant mice that is not observed in p85α−/− or p85α−p55α−p50α−/− mice.

Phenotypes of mice lacking class IA PI3K are similar to those originally observed in Xid mice, which are deficient in Btk (Bruton’s tyrosine kinase) function [29–31]. Xid-like deficiency has been observed in many knockout mice, such as those lacking BLNK (B cell linker protein; also known as SLP65) [32], protein kinase Cβ [33], phospholipase C-γ2 [34], Vav-1/2 [35] and CD19 [36,37] in addition to class IA PI3Ks [21–25], suggesting that these molecules function in the same signalling pathway. However, the functional relationship between these molecules in BCR signalling is not fully understood (see below). As expected from its enzymic activity, SHIP deficiency results in B cell hyperactivation. An inhibitory Fcy receptor, FcγRIIB, recruits SHIP when antigen–antibody complex triggers both BCR and FcγRIIB, and down-regulates the PI3K pathway in B cells [38,39].

Although Pten deficiency results in embryonic lethality [7,8], lymphocyte-specific disruption of Pten has been performed [40–42]. B cell-specific deletion of Pten leads to the preferential generation of MZ B and CD5+ B cells [40,41]. Pten-deficient B cells are hyperproliferative in response to various stimuli, and exhibit a lower activation threshold through the BCR [40,41]. Interestingly, inactivation of Pten rescued GC formation, MZ B cells and CD5+ B cells in CD19−/− mice, demonstrating that recruitment and activation of PI3K are the major role of CD19 [40]. This is consistent with the observation using CD19−/− mice expressing a mutant CD19 as a transgene that tyrosine residues for p85α binding are critical for GC formation and generation of MZ B cells [6]. Intriguingly, the lack of Pten leads to impaired GC formation, as observed in mice deficient for class IA PI3K. Pten-deficient mice also show defects in immunoglobulin class switch associated with decreased induction of activation-induced cytidine deaminase, which is critical for class switch recombination [41]. Since SHIP-deficient mice show intact immunoglobulin class switch [10], regulation of GC formation and class switch recombination may be independent of PtdIns(3,4,5)P3.

Only subtle phenotypic changes have been observed in the T cells of class IA PI3K knockout mice. T cell development is affected only slightly, if at all, in class IA PI3K mutant mice [21–25]. The proliferative response to TCR (T cell receptor) stimulation alone or in combination with CD28 stimulation is also unaffected in both p85α−/− and p110α−/− T cells in vitro [21–25]. In contrast, stimulation with cognate antigen using DO11.10 TCR transgenic mice on the p110α mutant background results in a poor proliferative response, suggesting that this class IA PI3K may determine the TCR signalling threshold [21]. In fact, T cell-specific deletion of the Pten gene results in impaired self-tolerance and autoimmunity [42]. In addition, forced expression of a truncated form of p85α named p65(P13K) that can constitutively activate p110s enhances thymocyte differentiation and increases the CD4/CD8 ratio [43]. These results indicate that PtdIns species also contribute to T lymphocyte development and functions (see [4]).
Figure 1 | Functional relationship between PI3K and Btk in BCR-mediated signal transduction

Btk has several distinct structural domains, including PH, TH (Tec homology), SH3, SH2 and a kinase domain. Btk seems to have two conformations, namely 'closed (inactive)' and 'open (active)'. Once Btk is recruited to the plasma membrane, conformational change and tyrosine phosphorylation within the kinase domain by Lyn or Syk activates Btk's kinase activity.
Class IA PI3Ks and BCR signal transduction

Cross-linking of the surface BCR evokes sequential activation of a variety of protein and lipid kinases, including Src family kinases (Lyn, Fyn, Blk), Syk, Btk, Akt and PI3K [44–49]. Although activation of PI3K is observed upon BCR stimulation, signalling events upstream and downstream of PI3K are not necessarily well characterized. In B cells, Lyn, c-Cbl, CD19 and BCAP (B-cell adaptor for phosphoinositide 3-kinase) bind the p85α subunit of PI3K, suggesting that these molecules are upstream activators of PI3K [44–49].

Btk, a Tec family kinase, is activated by tyrosine phosphorylation and plays a critical role in BCR signalling [29–31]. Btk−/− mice as well as mice with the Xid mutation [a natural mutation in the PH domain of Btk whereby an arginine residue critical for binding to PtdIns(3,4,5)P3 is replaced by cysteine] show deficiencies in the development and activation of B cells. In humans, deficiency of Btk leads to Bruton’s type agammaglobulinaemia [30,31]. Stimulation of B cells. In humans, deficiency of Btk leads to X-linked Bruton’s type agammaglobulinaemia [30,31]. Stimulation-dependent membrane localization of a chimaeric protein comprising the Btk PH domain and green fluorescent protein in transient transfection systems has been demonstrated, and such membrane recruitment is blocked by a PI3K inhibitor, wortmannin [50,51]. Overexpression of the p110α PI3K catalytic subunit in a B cell line results in Btk tyrosine phosphorylation [52]. It has been proposed from these observations that PI3K is responsible for the activation of Btk by bringing Btk to the plasma membrane through interactions between the PH domain of Btk and PtdIns(3,4,5)P3 [11–13], leading to tyrosine phosphorylation of Btk by other protein tyrosine kinases such as Lyn or Syk (Figure 1A). It was thus not surprising that mice deficient in class IA PI3K show a phenotype similar to that of Btk−/− or Xid mice.

Using p85α−/− and Btk−/− mice, as well as wortmannin- or Ly294002-treated B cells, the roles of PI3K and Btk in the BCR-mediated cell signal transduction pathway were extensively investigated [53]. According to the widely accepted model as mentioned above (Figure 1A), it was expected that the activation of Btk would be abrogated in p85α−/− and wortmannin- or Ly294002-treated B cells. Surprisingly, however, both BCR-induced activation of Btk and recruitment of Btk to the plasma membrane were unaffected by a lack of PI3K or by PI3K inhibitors [53]. On the other hand, BCR-induced activation of Akt was normal in Btk−/− B cells, but severely impaired in p85α−/− B cells. Furthermore, p85α−/−Btk−/− double deficient mice showed more severe phenotypes than either single deficient mouse [53]. This biochemical and genetic evidence shows that PI3K and Btk function independently in BCR signal transduction pathways.

The above observations also suggest the presence of an alternative mechanism for Btk recruitment to the plasma membrane. In fact, recent studies have raised the possibility that Btk is recruited to the plasma membrane through a mechanism independent of PtdIns(3,4,5)P3 generation. An adaptor protein, BLNK, is phosphorylated by Syk and provides Btk with docking sites to bring them into close proximity [31,49]. Syk then activates Btk. At the same time, BLNK is recruited to the plasma membrane via distinct adaptor proteins, which leads to the recruitment of Btk to the plasma membrane (Figure 1B). Candidates for such adaptor proteins include a subunit of the BCR complex, Igα [54,55], and a recently identified adaptor molecule, LAB (linker for activation of B cells) [56].

Among downstream events, activation of NF-κB (nuclear factor-κB) and induction of Bcl-xL and cyclin D2 were impaired in both p85α−/− and Btk−/− single deficient B cells [53,57–59]. Forced expression of Bcl-xL restored development and the proliferative responses of B cells in p85α−/− mice [53]. These results indicate that class IA PI3K and Btk have distinct roles in BCR signal transduction (Figure 1C).

Concluding remarks

The phenotypes of p85α−/−, p85α−p55α−p50α−/−, p110δ−/− and p110δ+p90α−/− and p110δ+p90α mice are generally similar, but there are some differences. The lack of p85α results in augmented expression of p55α and p50α in some cell types of p85α−/− mice [24,26]. In this regard, the p110δ+p90α mouse represents a better model to examine the role of p85α/p110δ among class IA PI3Ks in the immune system without affecting the expression levels of other family members. Since p85 is associated with p110α, p110δ and p110β, the lack of p85 probably affects the functions of p110α and p110β as well. In fact, p85α−/−, p85α−p55α−p50α−/− and p85β−/− mice show increased glucose metabolism, resulting in hypoglycaemia [26–28].

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Furthermore, p85α−/− mice show particular phenotypes in immune cells other than lymphocytes, as p85α−/− mice are impaired in the development of gastrointestinal mast cells and the Th2 response upon nematode infection [60]. Signals downstream of c-kit are paralysed in the absence of p85α. In addition, interleukin-12 production is enhanced in p85α−/− dendritic cells as well as in wormmann-treated wild-type dendritic cells, indicating the presence of a negative feedback regulation of interleukin-12 expression by PI3K [61,62]. There is, however, little information on whether p110δ is involved in these mechanisms. In order to examine whether the effect of deficiency is due to the impairment of p85/p110δ enzyme, the recently developed p110δ-specific inhibitor, IC87114 [63], will be a valuable tool in future studies.

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