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Phosphoinositide 3-Kinases in Immunity: Lessons from Knockout Mice[1]

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Phosphoinositide 3-kinases (PI3Ks) constitute a family of evolutionarily conserved lipid kinases that phosphorylate the D3 position of the inositol ring of phosphoinositides and produce PI(3)P, PI(3,4)P_2, and PI(3,4,5)P_3. Intense *in vitro* research over the last decade has unequivocally demonstrated that PI3Ks, in particular those belonging to class I, regulate a vast array of fundamental cellular responses. Given the pleiotropic roles of PI3Ks and the lipid product PI(3,4,5)P_3 in plethora of cellular responses, it is pertinent to explore the significance of PI3K signaling *in vivo*. In the past two or three years, the components of this signaling pathway have been genetically manipulated in mouse. This review briefly summarizes the immunological significance of PI3K signaling as revealed by the study of gene-targeted "knockout" mice.

Key words: immune system, knockout mouse, lipid signaling, phosphoinositide 3-kinase, phosphoinositide phosphatases.

PI3Ks and downstream signaling

Phosphoinositide 3-kinases. Since the discovery of a PI3K activity in 1988 (*1*), eight PI3K catalytic subunits have been identified in mammals (*2, 3*). They are divided into three groups on the basis of the phosphoinositides that they preferentially utilize as substrates. All the class I PI3Ks phosphorylate PI, PI(4)P, and PI(4,5)P_2 *in vitro*, but PI(4,5)P_2 is the predominant substrate in cells and the major product of the kinases is PI(3,4,5)P_3. Class II PI3Ks are large proteins, whose catalytic domains are about 50% similar to those of class I PI3Ks. Class II PI3Ks phosphorylate PI and PI(4)P *in vitro*. Which lipids these kinases produce in cells and how their activities are regulated remain to be clarified. Class III PI3Ks can use only PI as a substrate to produce PI(3)P. Disruption of the yeast Class III PI3K Vps34 leads to severe defects in vacuolar protein sorting. PI(3)P is readily detected in resting cells and its amount remains unchanged after receptor stimulation, suggesting that class III PI3Ks function as "housekeeping" proteins that are required for membrane trafficking processes.

Accumulation of PI(3,4,5)P_3 occurs through stimulation of transmembrane receptors, which either possess intrinsic tyrosine kinase activity, are ultimately coupled to tyrosine kinases, or are coupled to heterotrimeric GTP-binding proteins (G-proteins) (Fig. 1). Much attention has been paid to class I PI3Ks since they were found to be the isoforms that are activated upon receptor stimulation. Class I PI3Ks comprise a catalytic subunit with a molecular mass of approximately 110 kDa (p110) and an associated regulatory subunit.

To date, four catalytic isoforms (p110α, β, γ, δ) have been identified in mammal, which are encoded by four separate genes. All these catalytic subunits share the homologous regions including the catalytic domain, PIK domain, C2 domain and Ras-binding domain (*2–4*). Class I PI3Ks are further subdivided into two groups on the basis of the structural features of their adaptor subunit. The Class IA catalytic subunits, p110α, p110β, and p110δ, contain an N-terminal region that constitutively associates with a p85 regulatory molecule. Three genes encoding the regulatory subunit, p85α, p85β, and p55γ, have been identified, and seven adaptor proteins are generated by alternative splic-

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Abbreviations: PI, phosphatidylinositol, PI3K, phosphoinositide 3-kinase, PTEN, phosphatase and tensin homologue, SH2, *src* homology 2, SHIP, SH2-domain containing inositol polyphosphate 5-phosphatase, PKB, protein kinase B, FAK, focal adhesion kinase, TCR, T cell receptor, BCR, B cell receptor, MAPK, mitogen-activated protein kinase, ERK, extracellular signal-regulated kinase; S6K, S6 kinase, fMLP, *N*-formyl-Met-Leu-Phe, DP, CD4 CD8 double positive, IL, interleukin; Tg, transgenic, ES cell, embryonic stem cell, PH, pleckstrin-homology; PDK1, phosphoinositide-dependent kinase 1, Ig, immunoglobulin; GPCR, G-protein–coupled receptor, TKLR, tyrosine kinase–linked receptor, BMMC, bone marrow-derived mast cell; LCMV, lymphocytic choriomeningitis virus, SDF-1, stromal cell–derived factor–1, SCF, stem cell factor; GM-CSF, granulocyte/macrophage colony–stimulating factor; G-CSF, granulocyte colony–stimulating factor; LPS, lipopolysaccharide, PIPase, phosphoinositide phosphatase, GRP1, general receptor for phosphoinositides-1, FKHR, forkhead family transcription factor; GSK, glycogen synthase kinase; PLC, phospholipase C.

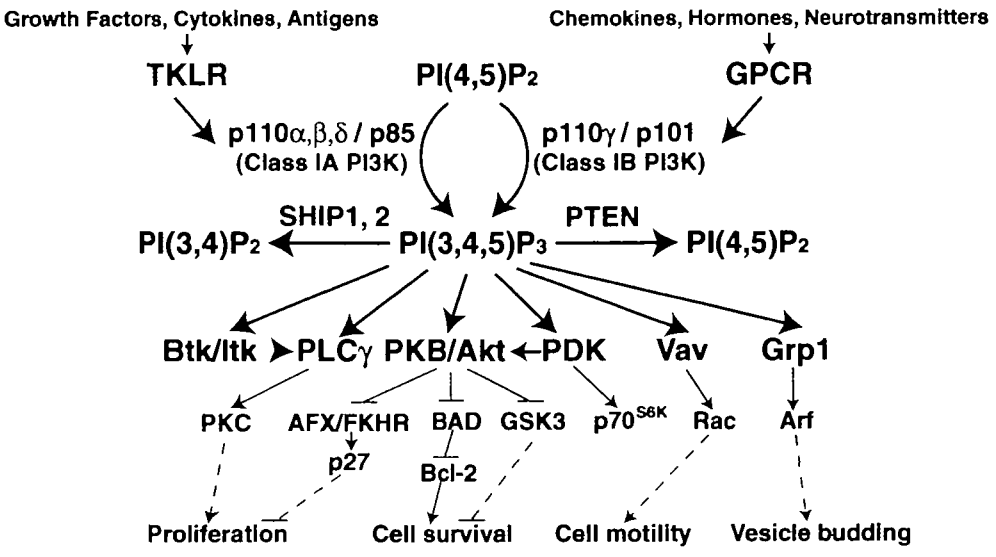

Fig. 1 **Generation of PI-(3,4,5)P_3 and signaling through its targets.** Extracellular factors bind to different types of receptors on the plasma membrane Class IA PI3Ks are indirectly activated by tyrosine kinase receptors and cytosolic tyrosine kinases through binding of the p85 regulatory subunit to tyrosine-phosphorylated proteins. Class IB PI3K is activated by $G_{\beta\gamma}$ subunits liberated from activated GPCRs. The major product, PI(3,4,5)P_3, activates downstream targets such as protein kinases and GDP/GTP *exchanging factors of small* GTPases Some targets are activated by both PI(3,4,5)P_3 and PI(3,4)P_2. PI(3,4,5)P_3 can be dephosphorylated by SHIPs and PTEN, and therefore PI(3,4,5)P_3-dependent signaling pathways are down-regulated Phenotypes of mice with the molecules shown in green letters deleted are discussed in this review.

ing. The SH2 domains of the regulatory subunits bind selectively to phosphotyrosyl residues within the YXXM motif of proteins, which results in increase in the catalytic activity of class IA PI3Ks. Furthermore, the interaction causes the translocation of cytosolic PI3Ks to the source of their phospholipid substrate PI(4,5)P_2 and thus accumulation of intracellular PI(3,4,5)P_3. In addition to the activation by phosphotyrosyl protein/SH2 domain interactions, class IA PI3Ks can be regulated by Ras.

In contrast to class IA, class IB PI3K acts downstream of G-protein–coupled receptors (GPCRs). The sole catalytic subunit of class IB PI3K, p110γ, diverges from those of class IA PI3Ks at its N terminus and cannot associate with p85 family proteins but can, instead, interact with a p101 adaptor molecule (*5, 6*). No functional homology to known proteins has been found in p101 so far and its role remains controversial in terms of p110γ activation. At least *in vitro*, the βγ subunits of G-protein can directly activate p110γ.

Cellular targets. The recent identification of molecular targets for lipid products of PI3Ks has elucidated the mechanisms by which PI3K activation leads to a number of cellular responses. Of particular relevance to PI3K signaling is the pleckstrin-homology (PH) domain, a protein module of around 120 amino acids found in about 200 intracellular proteins (*7, 8*). Each PH domain binds with different affinity to various phosphoinositides, and a subset of PH domains has been shown to recognize the lipid products of PI3Ks. For example, PI(3,4)P_2 and PI(3,4,5)P_3 interact with the PH domain of the serine/threonine kinase Akt/PKB. High affinity binding of these lipids to Akt/PKB leads to the recruitment of the kinase to the plasma membrane, where it undergoes a conformational change and is phosphorylated by PDK1, becoming fully activated (*9*). In turn, Akt/PKB phosphorylates a wide variety of proteins that have been implicated in suppression of apoptosis, glucose metabolism, protein synthesis and promotion of cell growth (*10, 11*). Other molecules that bind to and are possibly regulated by the lipid products of PI3Ks include guanine nucleotide exchanging factors for small GTPases, PKCs and Tec-family tyrosine kinases (Fig. 1). Therefore, the extreme diversities of receptors that are coupled to PI3K activation and the proteins that bind to PI(3,4,5)P_3 provide a rationale for how PI3Ks regulate so many diverse physiological functions.

PI(3,4,5)P_3 phosphatases. Rapid and efficient attenuation of PI3K-controlled signaling pathways is crucial to preventing side-effects caused by hyperactivation of the pathways Dephosphorylation of the lipid products of PI3Ks by phosphoinositide phosphatases (PIPase) is the most obvious candidate for such negative regulation. Two major routes for degradation of PI(3,4,5)P_3 have been demonstrated: one is mediated by PTEN, which hydrolyzes the 3′ phosphate to produce PI(4,5)P_2, and the other involves its conversion to PI(3,4)P_2 by SHIPs.

PTEN is deleted or mutated in a variety of sporadic tumors, and germline transmission of mutations in *PTEN* gene are also observed in Cowden disease and Bannayan-Zonana syndrome, are the rare autosomal dominant syndrome with multiple hamartomas of the skin, intestine, breast, and thyroid, and high risk of malignant tumors such as breast and thyroid cancers (*12*). PTEN contains the invariant signature motif Cys(X)$_5$Arg. This motif was initially described as the consensus of the protein tyrosine phosphatase family, but after the discovery of PTEN as a PIPase, several proteins with this motif such as myotubularin, Sac1, inositol polyphosphate 4-phosphatases, and PTEN2 have been identified as PIPases (*13*). Although only PTEN hydrolyzes PI(3,4,5)P_3, these phosphatases potentially act as negative regulators of PI3K signaling, as they all utilize 3-phosphorylated inositol lipids as substrates.

SHIP1 is a hematopoietic-specific PIPase (*14, 15*). It has been shown to be tyrosine-phosphorylated in response to multiple cytokines and to B cell receptor engagement. Recently identified SHIP2 is widely expressed and involved in growth factor and insulin signaling (*16*). SHIPs may operate as a "seesaw," that is, they shunt signaling away from PI(3,4,5)P_3-dependent effectors toward targets that are exclusively driven by PI(3,4)P_2.

Immunity in mice with deleted PI3Ks

B cell function and development. Mice lacking p85α (*p85α*$^{-/-}$) (*17, 18*) or p85α together with its spliced variants p55α and p50α (*p85-p55-p50α*$^{-/-}$) (*19, 20*) have been reported. Both strains express p85β and p55γ proteins, and p55α and p50α are still expressed in the former. Whereas *p85α*$^{-/-}$ mice are viable, *p85-p55-p50α*$^{-/-}$ mice die from liver degeneration within days after birth *p85-p55-p50α*$^{-/-}$ mice in a more outbred background survive longer, suggesting that genetic factors influence the life-span in the absence of all p85α isoforms.

p85-p55-p50α$^{-/-}$ ES cells were injected into *rag2*$^{-/-}$ blastocysts to explore the function of p85α gene products in lymphocytes (*19*). As rag2-deficient mice lack mature B and T cells, any lymphocytes in the chimeras must be deficient in all p85 gene products. Such chimeras and *p85α*$^{-/-}$ mice both exhibit impaired B cell development at the pro-B cell stage, have reduced numbers of peripheral mature B cells and peritoneal CD5$^+$ B cells, and decreased serum immunoglobulin The few B cells that do develop have diminished proliferative responses to anti-IgM, LPS, and CD40 The phenotype in B cells resembles defects observed in *xid* mice and knockout mice lacking Bruton's tyrosine kinase (Btk). It has been shown that the PH domain of Btk binds to PI(3,4,5)P_3 and is responsible for recruitment of this kinase to the plasma membrane. The mutation of Btk in *xid* (R28C) is in the PH domain, resulting in defective binding of its mutated PH domain to PI(3,4,5)P_3 (*21*).

Involvement of PI3Ks and PI(3,4,5)P_3 in B cell function is further underscored by the phenotype in *ship1*$^{-/-}$*rag1*$^{-/-}$ chimeric mice (*22*). Immune complexes consisting of antigen and IgG antibodies are potent inhibitors of humoral immune responses. One of the receptors for IgG, FcγRIIB, delivers the signal to SHIP1 through its immunoreceptor tyrosine–based inhibitory motif (ITAM), which becomes tyrosine-phosphorylated in response to BCR plus FcγRIIB colligation (*23*). *ship1*$^{-/-}$*rag1*$^{-/-}$ chimeric mice have increased basal serum Igs, and *ship1*$^{-/-}$ B cells exhibit increased proliferation, prolonged Ca^{2+} influx and MAPK activation upon BCR-FcγRIIB colligation These phenotypes would be attributed to elevated PI(3,4,5)P_3 level in the absence of SHIP1 (*24*). SHIP1 does not just function as a negative regulator of PI3K signaling because it can also modulate the balance between PI(3,4,5)P_3-dependent and PI(3,4)P_2-dependent signaling pathways. In this respect, PI(3,4)P_2 may also be important for B cell development, as is PI(3,4,5)P_3, because *shup1*$^{-/-}$ mice have decreased percentage of B220$^+$ cells in the bone marrow and sIgD$^+$sIgM$^+$ mature B cells in spleen, and pre-B colony forming cell numbers are reduced (*22*).

T cell function and development. Previous studies have shown that PI3Ks play a role in T cell responses. For instance, proliferation of primary T cells is blocked by PI3K inhibitors (*19*). The PI3K isoforms biochmically linked to T cell activation are the class IA PI3Ks. The costimulatory receptor CD28 possesses a cytoplasmic YXXM motif and has been recognized as the major activator of class IA PI3Ks in T cells. However, no apparent defects in T cell activation or development are observed either in *p85α*$^{-/-}$ or *p85-p55-p50α*$^{-/-}$ knockout mice. Likewise, Okkenhaug *et al.* reported that CD28 promotes T cell activation, proliferation and cytokine production independently of its association with p85, based on studies using Tg mice expressing a CD28 mutant that fails to bind to class IA PI3Ks in CD28-deficient background (*25*). Hence, in T cells, class IA catalytic subunits may become activated independently of the regulatory subunits, *e.g.*, in a Ras-dependent manner. Alternatively, other PI3K(s) may play a pivotal role in T cell activation

Three groups, including ours, reported a gene knockout for the p110γ catalytic subunit of class IB PI3K in mice (*26–28*). p110γ-deficient mice are viable and exhibit a decreased number of splenic CD4$^+$ T cells. Proliferation of *p110γ*$^{-/-}$ T cells is reduced in response to anti-CD3ε antibody, and cytokine production is also diminished. Moreover, *p110γ*$^{-/-}$ mice exhibit defects in T cell function following LCMV challenge and hapten immunization, indicating that p110γ is required to generate effective, CD8$^+$ T cell–dependent antiviral responses and functional T-helper cell–dependent responses to hapten antigens *in vivo* (*26*). In contrast to mutation of *p85α*, which leads to developmental and functional defects in B cells, but not T cells, deletion of *p110γ* has no effect on B lymphocytes, except that production of antibodies containing lambda light chains in response to T cell-independent antigens is altered (*27*). The decreased CD4/CD8 ratio in p110γ-deficient spleen and impaired T cell proliferation is reminiscent of the phenotype of mice lacking Itk, a Tec family kinase with a PH domain (*29, 30*). While another Tec family molecule, Rlk, that lacks a PH domain is also expressed in T cells, Rlk-deficient mice do not show similar T cell phenotypes to those observed in *p110γ*$^{-/-}$ and *Itk*$^{-/-}$ mice (*30*).

PTEN knockout mice have provided further insight into the importance of PI3K signaling in T cell function and homeostasis (*31–34*). *Pten*$^{+/-}$ mice, especially females, begin to show polyclonal lymphoid hyperplasia at 28 weeks, which progresses to T cell lymphoma in some cases, and half of these mice die within a year of birth. We have recently generated a T cell–specific deletion of the *Pten* gene (*Pten*$^{flox/-}$) using a Cre-LoxP system (*35*). As expected, *Pten*$^{flox/-}$ mice develop tumors much earlier and with higher incidence than *Pten*$^{+/-}$ mice, all the mutant mice die of malignant T cell lymphoma within 17 weeks. Most of T cell tumors in *Pten*$^{flox/-}$ mice are classified as CD4$^+$ T cell lymphomas, and CD8$^+$ lymphomas have not been observed. In some tumors, monoclonal T cell expansion was also detected. T cells are hyperproliferative in *Pten*$^{flox/-}$ mice even before they develop lymphomas. In addition, activated *Pten*$^{flox/-}$ T cells produce higher levels of both Th1 and Th2 cytokines. Enhanced level of serum IgG and increased B cell number observed in *Pten*$^{flox/-}$ mice can be attributed to the increase in IL-4 and IL-10.

Besides lymphoma development, both *Pten*$^{+/-}$ and *Pten*$^{flox/-}$ mice develop spontaneous autoimmunity characterized by auto-antibody production, infiltration of activated lymphocytes into some of the organs, and parenchymal damage. It is well established that defects in thymic and/or peripheral tolerance leads to uncontrolled T cell responses directed against self-rather than environmental antigens. Indeed, *Pten*$^{flox/-}$ mice exhibit defects in both central and peripheral tolerance, as shown in HY-TCR transgenic mice and superantigen-induced deletion assays. Moreover, loss of PTEN confers protection against apoptosis in thymocytes and peripheral T cells *in vitro*. It is also reported that *Pten*$^{+/-}$ T cells are resistant to Fas apoptotic stimulation, and that susceptibility is restored by inhibit-

ing PI3Ks with wortmannin (*36*). It is worth noting that a $p65^{PI3K}$ Tg mice that express a constitutively active truncated form of p85α in T cells exhibit phenotypes very similar to those of *Pten*$^{flox/-}$ mice (*37*). Lymphoproliferation and autoimmune disease characterized by an increased number of T cells, particularly $CD4^+$ cells, are observed in $p65^{PI3K}$ Tg mice. Furthermore, $p65^{PI3K}$ Tg mice are predisposed to T cell lymphoma, that is, the Tg mice develop tumors in a *p53*$^{-/-}$ background. Therefore, the abnormalities mentioned above are most likely due to increased intracellular PI-(3,4,5)P_3 level rather than defective dephosphorylation of the previously reported protein substrates of PTEN such as FAK or Shc.

Thymocytes. One of the gross phenotypic characteristics of *p110γ*$^{-/-}$ mice is that their thymus is reduced by half in size (*26*). Absence of p110γ results in a reduced capacity of double-positive (DP; $CD4^+CD8^+$) cells to survive after anti-CD3 antibody administration *in vivo*. Meanwhile, adenosine is an extracellular factor that plays a pivotal role in thymi, and its receptor expressed on thymocytes is a GPCR (*38*). Human patients with mutations in adenosine deaminase and mice lacking the enzyme exhibit severe combined immunodeficiency and a defect in thymus development (*39*). *p110γ*$^{-/-}$ thymocytes exhibit enhanced apoptosis when stimulated with anti-CD3ε plus adenosine analogues. Thus, p110γ may have a role in the maintenance of homeostasis of the thymus by regulating TCR- and GPCR-induced cell death.

It is intriguing to compare the thymi of p110γ-deficient mice with those of mice deficient in PTEN or Akt1 (PKBα), or Akt1 Tg mice. PTEN-deficient thymocytes are resistant to apoptosis triggered by adenosine analogues, γ irradiation and UV irradiation. Accordingly, there is a dramatic increase in the DP compartment in PTEN-deficient thymus (*35*). Chen *et al.* reported a significant spontaneous apoptosis in thymi of *akt1*$^{-/-}$ mice and that thymocytes derived from the mutant animals are more susceptible to a variety of apoptotic stimuli (*40*). Conversely, DP thymocytes expressing a constitutively active form of Akt1 display a survival advantage (*41*). Hence it is likely that p110γ and Akt1 operate in concert to oppose apoptosis in the thymus and that PTEN antagonizes the anti-apoptotic pathway.

Besides the compromised negative selection mentioned above, lineage commitment of $CD8^+$ cells is also impaired in HY-TCR Tg *Pten*$^{flox/-}$ mice, suggesting that DP precursors may preferentially develop into $CD4^+$ cells. Consequently, the $CD4^+$ subset is increased as a result of T cell–specific deletion of *Pten* (*35*). This increase is reminiscent of $p65^{PI3K}$ Tg mice, and in sharp contrast with Itk deficiency or p110γ deficiency in mice (*26, 29, 30*). This comparison implies that these molecules act in a common pathway regulating CD4/CD8 differentiation. A plausible candidate that is activated downstream of PI3Ks-PI(3,4,5)P_3-Itk and counteracted by PTEN is ERK. Development of $CD4^+$ cells is increased when ERK activity is increased, and that of $CD8^+$ cells is increased when ERK is decreased (*42*). Since ERK activation is upregulated in *Pten*$^{flox/-}$ T cells (*35*), it is possible that activated ERK signaling biases T cell differentiation toward $CD4^+$ and away from $CD8^+$.

Mast cells, granulocytes, and macrophages. SHIP1 has been postulated to negatively regulate cytokine signaling in myeloid cells (*14*). SHIP1-deficient mice exhibit splenomegary, lymphoadenopathy, and myeloid infiltration of vital organs (*24, 43*). These symptoms are probably a consequence of hyperresponsiveness to stimulation by various cytokines, including GM-CSF, IL-3, M-CSF, and SCF, that regulate myeloid cell proliferation and survival. It is worthy of note that the lung is a major site of GM-CSF production (*44*), and the most likely cause of premature death of *ship1*$^{-/-}$ mice is dysfunction of the lung with myeloid cell infiltration.

In SHIP1-deficient bone marrow-derived mast cells (BMMCs), intracellular levels of PI(3,4,5)P_3 and Akt activity are significantly upregulated when cells are stimulated with IL-3, SCF, or IgE (*24, 45*). This suggests that PI-(3,4,5)P_3 has a more predominant role than PI(3,4)P_2 in terms of activation of Akt in the cells. Prolonged activation of Akt would account for decreased sensitivity of *ship1*$^{-/-}$ mast cells to multiple death stimuli (*24*). It has been shown that inhibitors of PI3Ks abrogate both histamine release and calcium entry in mast cells. *ship1*$^{-/-}$, but not the wild-type BMMCs undergo degranulation in response to SCF (*46*). In addition, IgE-FcεR complex can induce degranulation without being ligated by its antigen. The enhanced degranulation correlates with higher and more sustained intracellular calcium concentration. As neither PLCγ2 phosphorylation, IP_3 levels nor intracellular calcium release is altered in ship1-deficient cells (*46*), it appears that PI(3,4,5)P_3 acts on machinery downstream of calcium release regulating entry of extracellular calcium. Taken together, these results indicate that SHIP1 regulates thresholds for induction of cell death and histamine release in mast cells.

The function of p110γ in neutrophils, macrophages and mast cells has been described (*26–28*). Biochemically, p110γ deficiency leads to a complete blockade of PI(3,4,5)P_3 production and PKB/Akt activation in response to the GPCR agonists including C5a, fMLP, and IL-8 In contrast, loss of p110γ does not impair the signaling pathways following stimulation of receptors for GM-CSF, IgG, IL-3, or SCF, which is linked to tyrosine kinases or is itself a tyrosine kinase.

It is now widely recognized that the plasma membrane is not uniform but divided into signaling sub-compartments. In this context, one recent striking finding is of a dynamic local accumulation of PI(3,4,5)P_3 at the leading edge of migrating neutrophils (*47*) and slime mold (*48*). *p110γ*$^{-/-}$ neutrophils show decreased migration in response to a wide variety of GPCR agonists such as fMLP, C5a, IL-8, and chemokines such as SDF-1 and RANTES *in vitro*. In contrast, SHIP1-deficient cells, in which PI(3,4,5)P_3 is not degraded to PI(3,4)P_2 by this enzyme, migrate to the chemokine SDF-1 better than wild-type cells (*49*). In *p110γ*$^{-/-}$ mice, neutrophils and macrophages are poorly recruited in a septic peritonitis and *Listeria* infection model. In addition, bone marrow neutrophils show severe defects in oxidative burst. As a result, clearance of viable bacteria from the peritoneal cavity is severely impaired in p110γ-deficient mice (*28*). Although granulopoiesis is normal in p110γ-null mice, they show increased numbers of neutrophils, eosinophils and monocytes in the blood (*26, 28*). The increase in circulating granulocytes coupled with the inability of chemotaxis is reminiscent of mice lacking small GTPase Rac2 (*50*). Although the mechanistic basis for p110γ-mediated migration needs to be studied further, p110γ may mediate actin rearrangement at the leading

TABLE I **The phenotypes of PI3K signaling molecule knockout mice.**

Targeted Gene	Locus (Human)	Relevant Phenotype	Ref
p85α/p55α/p50α	5q13	Perinatal lethal (p85α/p55α/p50α KO)	19, 20
p85α		B cell development & activation ↓	17, 19
		Hypoglycemia	18, 20
		Insulin sensitivity ↑	
		Anti-viral responses ↑	52
p85β	19q13.2	Insulin sensitivity ↑	53
p110α	3q26	Embryonic lethality	54
		Defective cell proliferation	
p110β	3q23	Embryonic lethality	55
p110γ	7q22	Chemotaxis ↓	26-28
		T cell development & activation ↓	
		Oxidative burst ↓	
		Thrombopoiesis ↓	58
		Inflammation ↓	60
		Tumorigenesis	61
		Heart function & blood pressure ↑	
		(Crackower, M., unpublished data)	
p110δ	1q36	Viable (Ihle, J., personal communication)	
PTEN	10q23	Embryonic lethality	31, 32
		Autoimmune disease	35, 36
		Tumorigenesis	56, 57, 62
		Defective T cell development ↑	35
		T cell activation ↑	
		Chemokinesis/chemotaxis ↑ (Suzuki, A., unpublished data)	
		Self-renewal/proliferation of neuronal stem cells ↑	63
SHIP1	2q36	Myeloid infiltration into various organs	24, 43
		Myeloid cell proliferation/survival ↑	
		Mast cell degranulation ↑	45, 46
		B cell activation ↑	22
		Chemotaxis ↑	49
SHIP2	11q23	Perinatal lethality	16
		Hypoglycemia	
		Insulin sensitivity ↑	

edge by chemokines *via* small GTPases, since $PI(3,4,5)P_3$ can activate GEFs (*51*), and the actin cytoskelton is dynamically reorganized at the leading edge of migrating cells.

Conclusion

In addition to the immune responses mentioned above, the knockout mice provide information about PI3K signaling in various physiological and pathological processes, such as embryonic development, self-renewal of neuronal stem cells, glucose metabolism, tumorigenesis, and heart size and function (Table I) (*16, 18, 20, 31–33, 52–64*). Although many issues remain to be resolved, drugs that specifically inhibit or stimulate the individual enzymes can be therapeutically useful for diseases, especially immune diseases, and such drugs are now in development

These genetic mutant mice are invaluable tools not only for confirming a proposed function of a particular gene in an *in vivo* setting, but also for uncovering novel functions of a gene that were not anticipated from conventional experiments. This is an area of research that will bear more fruit in years to come

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