Phosphoinositide Lipid Phosphatases: Natural Regulators of Phosphoinositide 3-Kinase Signaling in T Lymphocytes

Published, JBC Papers in Press, December 10, 2007, DOI 10.1074/jbc.R700044200
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The phosphoinositide 3-kinase signaling pathway has been implicated in a range of T lymphocyte cellular functions, particularly growth, proliferation, cytokine secretion, and survival. Dysregulation of phosphoinositide 3-kinase-dependent signaling and function in leukocytes, including B and T lymphocytes, has been implicated in many inflammatory and autoimmune diseases. As befits a pivotal signaling cascade, several mechanisms exist to ensure that the pathway is tightly regulated. This minireview focuses on two lipid phosphatases, viz. the 3′-phosphatase PTEN (phosphate and tensin homolog deleted on chromosome 10) and SHIP (Src homology 2 domain-containing inositol-5-phosphatase). We discuss their role in regulating T lymphocyte signaling as well their potential as future therapeutic targets.

The PI3K3 pathway plays a central role in regulating many biological processes, primarily via the generation of the potent downstream PI3K effectors including PDK-1 (3′-phosphoinositide-dependent kinase-1), which phosphorylates and activates the AGC protein kinases, including protein kinase B/Akt. Other kinases such as Tec family kinases can also interact directly with PtdIns(3,4,5)P3, as can GTPase-activating proteins and guanine nucleotide exchange factors as well as scaffolding proteins that nucleate the assembly of key signaling complexes (1, 2).

PI3K and T Lymphocytes

T cells express all three class 1A PI3K isoforms (p110α, p110β, and p110δ), which are regulated by protein-tyrosine kinase-coupled receptors, as well as class 1B p110γ, which is activated by G protein-coupled receptors (GPCRs). The T cell antigen receptor (TCR), CD28 family co-stimulatory receptors, and cytokine receptors activate class 1A isoforms (3). Chimeras (by virtue of interacting with GPCRs), activate mainly class 1B PI3K (4). Use of pharmacological tools in leukemic human T cell lines first indicated a possible role for PI3K in T cell activation (5). Mice with a knock-in point mutation of p110δ that abolishes kinase activity exhibit selective impairments in TCR signaling and reduced proliferation in vitro (6) and impaired function of CD4+CD25+Foxp3+ TReg cells (7). Interestingly, mice lacking both p110γ and p110δ show much more profound defects in thymocyte development and survival compared with mice lacking individual isoforms, indicating that these isoforms serve partially redundant functions in thymocytes (8, 9). Pathological consequences of combined p110γδ deficiency includes T cell lymphopenia, which leads to multiple-organ inflammation (10). Surprisingly, mice with T cell-specific loss of class 1A PI3K exhibit largely normal thymocyte development, and peripheral T cell numbers and subsets are unimpaired in young animals (11, 12). In vitro proliferation of T cells from these mice in response to TCR ligation is abrogated, and there is complete loss of PI3K signaling by the TCR and CD28. Although loss of class 1A function in T cells may not lead to significant T cell deficiency, it is worth noting that mice in which T cells are deficient in class 1 PI3Ks develop an autoimmune syndrome that may be related to the impaired TReg Cell function reported in mice expressing a kinase-dead form of p110δ (11, 12).

The studies in gene-targeted mice underline the importance of the PI3K-dependent signaling pathway in T lymphocyte function, and it is therefore important that this pathway is tightly controlled. An important level of regulation is provided by at least two lipid phosphatases, viz. the ubiquitously expressed 3′-phosphatase PTEN and the 5′-phosphatase SHIP, which convert this lipid to PtdIns(4,5)P2 and PtdIns(3,4)P2, respectively. The crucial role of these lipid phosphatases is underlined by the fact that PTEN and SHIP are frequently lost in many leukemias and immortalized leukemic cell lines (13).

PTEN, a Sentinel Phosphatase That Regulates T Lymphocyte Signaling

PTEN is a well-characterized tumor suppressor and is frequently inactivated by mutation, gene deletion, or epigenetic silencing (13). As such, it is widely viewed as the sentinel phosphatase responsible for regulating levels of PtdIns(3,4,5)P3 (Fig. 1). A germ line PTEN knock-out is embryonic-lethal (14), but tissue-specific and heterozygous knock-outs have helped elucidate the function of PTEN as a negative regulator in the immune system. For example, PTEN heterozygous mice develop an autoimmune lymphoproliferation by 9 months of age (15), and T cell-specific PTEN knock-out mice exhibit splenomegaly and an enlargement of the thymus (16). T cell-specific loss of PTEN...
leads to hyperproliferation, auto-reactive T cells, resistance to apoptosis, increased PI3K-dependent signaling events such as phosphorylation of protein kinase B/Akt, and increased secretion of cytokines (16). This is consistent with reports that PTEN also has a role in Th2-driven inflammation (17, 18).

The PTEN- and SHIP-null Jurkat leukemic T cell line has been used extensively to assess the ability of these lipid phosphatases to functionally oppose PI3K in TCR and CD28 signaling (4). In keeping with its proposed role as a sentinel phosphatase, restoration of PTEN expression in Jurkat cells is unable to oppose PI3K activity during acute TCR stimulation, but is important for maintaining low basal levels of PtdIns(3,4,5)P$_3$ before and after TCR signaling (19, 20). This would allow cells to discriminate between transient and persistent TCR stimulation and the presence or absence of co-stimulating signals, thus setting the threshold for cellular activation via the TCR. Accordingly, in PTEN-null T cells, TCR stimulation alone (in the absence of co-stimulation) is sufficient to induce hyperactivation of the PI3K pathway and enhanced IL-2 production. Thus, PTEN imposes a requirement for CD28 co-stimulation (19, 20), consistent with defects in central and peripheral tolerance observed in T cell-specific PTEN-null mice (16). PTEN can be down-regulated after T cell activation, and this TCR priming facilitates IL-2-induced proliferation of CD4$^+$CD25$^+$ T$_{Reg}$ cells. Hence, T$_{Reg}$ cells deficient in PTEN can be expanded in response to IL-2 alone without the need to stimulate the TCR and without loss of suppressor function (21).

A Fleet of Multitasking SHIP Molecules

SHIP-1 is a 145-kDa protein largely confined to hematopoietic cells, whereas the 150-kDa SHIP-2 is more widely expressed. SHIP-1 (henceforth referred to as SHIP) is a well characterized inhibitory molecule that is recruited by engagement of the inhibitory Fc$\gamma$ type IIB receptor in B cells and mast cells or by engagement of Fce type I or Fc$\varepsilon$ type III, cytokine, and growth factor receptors in myeloid cells (22). Once recruited to the plasma membrane by signaling complexes, its enzymatic activity depletes PtdIns(3,4,5)P$_3$ and prevents membrane localization of some PH domain-containing effectors, ultimately leading to impaired PI3K-dependent signaling events (23).

In addition to a core catalytic domain that is responsible for the hydrolysis of the 5'-phosphate on PtdIns(3,4,5)P$_3$, SHIP contains multiple structural domains that facilitate protein-protein interactions (Fig. 2). Hence, these structural domains are able to support the relocalization of SHIP from the cytosol to the plasma membrane, where its catalytic activity regulates PtdIns(3,4,5)P$_3$ accumulation (22, 24). In addition, these structural features and binding motifs allow SHIP to serve a scaffolding role for the recruitment of other proteins to the plasma membrane independent of catalytic function.

Multiple forms of SHIP have been reported with molecular masses of 145, 135, 130, 125 and 110 kDa (23, 25) that may arise from alternative mRNA splicing, protein degradation, or post-translational modification such as phosphorylation. As a consequence, the different forms of SHIP exhibit different protein binding profiles defined by the absence or presence of distinct binding motifs (Fig. 1). For example, s-SHIP (expression of which is restricted to murine embryonic stem cells) and its human homolog, SIP-110, are truncated at the N terminus and lack the SH2 domain, which is restricted to murine embryonic stem cells (26, 27).

Is SHIP a Terminator or a Gatekeeper?

Although many PH domains (e.g. those within Grp-1) are recruited exclusively to PtdIns(3,4,5)P$_3$, some such as DAPP1 (dual adaptor of phosphotyrosine and 3-phosphoinositides 1) can interact with both PtdIns(3,4,5)P$_3$ and PtdIns(3,4)P$_2$. In addition, TAPP (tandem PH domain-containing protein) contains PH domains that can exhibit selectivity toward PtdIns(3,4)P$_2$ (28). The ability of PH domain-containing pro-
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**Role of PTEN and SHIP in T Lymphocyte Migration**

The ability of naive and activated T cells to migrate toward chemoattractants is a key feature of basic immunosurveillance and the adaptive immune response. Several lines of evidence support an evolutionarily conserved role for PI3K in cell motility and directional migration toward chemoattractants. Chemokine interaction with GPCRs on T lymphocytes has been shown to depend predominantly on G<sub>1</sub> proteins, and these receptors are predominantly coupled to the βγ-dependent p110γ isoform (38). PI3K is activated by most chemokine receptors expressed on T cells, yet paradoxically, it is now clear that activation of PI3K by chemokines can be a dispensable signal for directional migration of T cells (3, 39).

Studies in neutrophil-like cell lines and the amoeba Dictyostelium discoideum have shown that distinct localization of PI3K and PTEN amplifies the distribution of PtdIns(3,4,5)P<sub>3</sub> with respect to the chemoattractant gradient (40). Hence, PI3Ks are recruited to the plasma membrane at the front of cells, resulting as well as excessive myeloid cell activation (34, 35). However, the SHIP<sup>−/−</sup> mice were generated using a strategy that leaves s-SHIP expression intact, and likewise, the ubiquitously expressed SHIP-2 also remains and may compensate for lack of SHIP, contributing to the mild phenotype.

Closer analysis of peripheral T cells from SHIP-null mice reveals that they are constitutively activated and give rise to increased numbers of CD4<sup>+</sup>CD25<sup>+</sup> T<sub>Reg</sub> cells, suggesting a key regulatory role for SHIP in T<sub>Reg</sub> cell development (36). However, this may be a consequence of the inflammatory environment brought about by SHIP-deficient myeloid cells rather than a T cell defect per se. To avoid the pleiotropic effects of the SHIP-null deletion, more refined and T cell lineage-specific deletion strategies have been employed. Interestingly, T cell-specific deletion of SHIP does not alter T cell development, activation state, or number of T<sub>Reg</sub> cells (37). Instead, this approach has uncovered a failure to skew toward Th2 in response to challenge with Schistosoma mansoni. This is most likely a consequence of enhanced sensitivity to cytokines that mediate induction of the Th1-associated transcription factor T-bet. SHIP-null CD8<sup>+</sup> T cells also show more efficient cytotoxic responses consistent with elevated T-bet levels (37). It appears therefore that, in T cells, SHIP negatively regulates cytokine-mediated activation in a way that allows effective Th2 responses and limits T cell cytotoxicity.

**Insights into the Role of SHIP in T Lymphocytes from Gene Targeting Strategies**

Several lines of evidence indicate a prominent regulatory role for SHIP in T cell biology. For example, SHIP is tyrosine-phosphorylated in response to TCR and CD28 ligation (29). Moreover, expression of a constitutively active SHIP mutant in the leukemic T cell line Jurkat regulates constitutive PtdIns(3,4,5)P<sub>3</sub> levels and CD28-activated PI3K effectors (30). In addition, SHIP interacts with Tec kinase and inhibits its function in T cells (31) as well as participate in a negative signaling complex by associating with the adaptor protein LAT (32). SHIP has also been shown to have a protective role in T lymphocytes exposed to oxidative stress (33). Despite this evidence, the impact of SHIP deletion on T cell biology was initially thought to be rather mild, although these mice exhibited defects in B cell development as proteins to discriminate between different 3′-phosphoinositide lipids suggests that SHIP can act as a switch to redirect PI3K-dependent signaling toward another set of distinct effectors that are temporally and functionally separate from PtdIns-(3,4,5)P<sub>3</sub>-dependent events. Hence, although the role of PTEN that are temporally and functionally separate from PtdIns-dependent signaling toward another set of distinct effectors...
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in localized production of PtdIns(3,4,5)P_3. Conversely, PTEN is recruited to the back and sides of cells (41, 42). Similar redistribution of PTEN in mammalian leukocytes remains controversial, and although some evidence exists for accumulation of PtdIns(3,4,5)P_3 at the leading edge of neutrophils (43), it has not so far been demonstrated in T lymphocytes. It is worth noting that neutrophils and T cell lines deficient in PTEN are still able to migrate toward chemoattractants (3, 39). In fact, both PTEN-null neutrophils and T cell lines in which PTEN expression has been suppressed by small interfering RNA exhibit enhanced directional migration (44, 45), whereas reconstitution of PTEN expression in Jurkat cells down-regulates CXCL12-stimulated cell migration (44). Interestingly, recent studies performed in SHIP-null neutrophils revealed that PtdIns(3,4,5)P_3 failed to localize to the front of the cell, suggesting that SHIP governs polarization and the formation of the leading edge. Introduction of constitutively active SHIP into leukemic cell lines normally deficient in SHIP abrogates CXCL12-mediated chemotaxis (46). Together, these observations suggest that both PTEN and SHIP can negatively regulate leukocyte migration, but are dispensable for directional sensing.

Evidence for a Role of Other Lipid Phosphatases in T Lymphocyte Activation

Although PTEN and SHIP are the best characterized phosphoinositide lipid phosphatases, there are at least 28 phosphoinositide lipid phosphatases in humans, a number of which show activity toward 3′-phosphoinositide lipids (47, 48). MTMR6, a member of the myotubularin family of 3′-phosphatases with specificity for PtdIns(3)P and PtdIns(3,5)P_2 (Fig. 1), binds the K_cα3.1 calcium-activated potassium channel through coiled-coil domain interaction (49). The K_cα3.1 channel is essential in the maintenance of the membrane potential that drives Ca^{2+} influx during activation of naive and memory CD4^{+} T cells. MTMR6 is postulated to regulate T cell activation by dephosphorylating the PtdIns(3)P, which is required for activation of the K_cα channel. Accordingly, overexpression of MTMR6 inhibits calcium influx in CD4^{+} T cells and reduces their proliferation in response to antigen. These studies suggest that MTMR6 may have a role in maintaining tolerance and preventing autoimmune disease.

Lipid Phosphatases Are Exploited by Pathogens

Given the potential of 3′-phosphoinositide lipid phosphatases to modulate acquired immune responses, it is not surprising that they are targeted by pathogens to evade immune detection and subsequent destruction. For example, cytotoxic distending toxin subunit B (CdtB) is an immunotoxin produced by Actinobacillus actinomycetemcomitans that can hydrolyze PtdIns(3,4,5)P_3 to PtdIns(3,4)P_2 (50). Exposure to CdtB leads to cell cycle arrest and death by apoptosis, consistent with the down-regulation of proliferation observed upon overexpression of SHIP in leukemic cell lines (51). Lymphocytes are considerably more sensitive to CdtB than are other cell types. The lipid phosphatase activity of CdtB activity may therefore result in reduced immune function, facilitating chronic infection with Actinobacillus and other enteropathogens that express Cdt proteins (50).

The measles virus evades destruction by the immune system at least in part by targeting negative regulation of PI3K/Akt signaling. It induces expression of SIP-110, which depletes the cellular PtdIns(3,4,5)P_3 pools, suggesting that the threshold for activation signals for induction of T cell proliferation is raised (52, 53).

Lipid Phosphatases as Targets for Drug Discovery

Because of its widespread and diverse function in the immune system, there is a growing appreciation of the therapeutic potential of inhibitors of the PI3K pathway in inflammatory and autoimmune diseases. This has stimulated intense interest in compounds with suitable pharmacological profiles. These are primarily directed toward PI3K isoforms, with many small-molecule ATP-competitive inhibitors currently in development (54). However, achieving isoform selectivity of PI3K inhibitors has proven to be nontrivial, and current thinking suggests that selective pan-isoform inhibitors may actually prove to be therapeutically useful (54). It is therefore worth highlighting that activation of the lipid phosphatases that regulate cellular levels of PtdIns(3,4,5)P_3 offers the same therapeutic benefits as inhibition of PI3K and that phosphatases may be more tractable targets in the long term.

With its expression largely restricted to hematopoietic cells, drug-mediated activation of SHIP would offer the ability to target hematopoietic and immune disorders in which the PI3K pathway is dysregulated, such as autoimmune syndromes and inflammatory conditions. Conversely, inhibitors of lipid phosphatases might be expected to improve T lymphocyte function either during immunodeficiency syndromes or as part of anti-tumor therapies. Of particular note is the recent identification of pelorol, a meroterpenoid originally isolated from the marine sponge Dactylospongia elegans as an activator of SHIP (24). More potent synthetic analogs of pelorol have been shown to exhibit anti-inflammatory effects in in vitro and in vivo models (24). Moreover, these derivatives appear to act through allosteric activation of SHIP by binding to a putative C2 domain. Conceptually, use of an allosteric regulator reduces the chances of off-target effects compared with compounds that target the conserved ATP-binding domain in kinases (24).

Conclusion

PTEN and SHIP provide tight regulation of the PI3K pathway and are essential not only for normal immune system development and responsiveness but also for prevention of immunopathology. Indeed, unchecked activation of the PI3K pathway in T cells induces lymphoproliferation and systemic autoimmune disease. To date, however, the interest in these lipid phosphatases as pharmacological targets has been limited, perhaps because phosphatases per se have been perceived to be less druggable than the kinome. The recent validation of small-molecule entities that can modulate both PTEN and SHIP demonstrates that modulation of lipid phosphatase function is now becoming a tractable proposition (24, 55). The power of this approach to immunomodulation is demonstrated in nature by the observation that some pathogens utilize phosphatases to effect immune escape. Therefore, we cannot afford to neglect
lipid phosphatases during either the study of biological processes or drug discovery.

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