Molecular control of PtdIns(3,4,5)P3 signaling in neutrophils

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Abstract

Neutrophils play critical roles in innate immunity and host defense. However, excessive neutrophil accumulation or hyper-responsiveness of neutrophils can be detrimental to the host system. Thus, the response of neutrophils to inflammatory stimuli needs to be tightly controlled. Many cellular processes in neutrophils are mediated by localized formation of an inositol phospholipid, phosphatidylinositol (3,4,5)-trisphosphate (PtdIns(3,4,5)P3), at the plasma membrane. The PtdIns(3,4,5)P3 signaling pathway is negatively regulated by lipid phosphatases and inositol phosphates, which consequently play a critical role in controlling neutrophil function and would be expected to act as ideal therapeutic targets for enhancing or suppressing innate immune responses. Here, we comprehensively review current understanding about the action of lipid phosphatases and inositol phosphates in the control of neutrophil function in infection and inflammation.

See the Glossary for abbreviations used in this article.

Introduction

Neutrophils migrate from the blood to infected tissues in response to inflammatory stimuli, where they protect their host by engulfing, killing, and digesting invading bacterial and fungal pathogens [1–4]. Neutrophils sense invading pathogens in the extracellular environment via several classes of cell surface receptor: G-protein-coupled receptors (GPCRs) to sense chemoattractants, Fc or complement receptors for phagocytosis, adhesion molecules (such as selectins and integrins), cytokine receptors, and innate immune receptors (such as the Toll-like receptor; TLR). When these receptors are bound by their respective ligands, they activate diverse intracellular signal transduction networks that lead to chemotactic migration,

phagocytosis, degranulation, production of reactive oxygen species (ROS), and release of neutrophil extracellular traps [1,5].

Many cellular processes in neutrophils are mediated by localized formation of an inositol phospholipid, phosphatidylinositol (3,4,5)-trisphosphate (PtdIns(3,4,5)P3), at the plasma membrane (Fig 1A). PtdIns(3,4,5)P3 is formed by the phosphoinositide 3-kinase (PI3K) family of enzymes, which transfer the terminal phosphate of adenosine triphosphate (ATP) to PtdIns(4,5)P2 at the D3 position of the inositol ring. PI3Ks are divided class I, II, and III enzymes, but only class I PI3Ks phosphorylate PtdIns(4,5)P2 to form PtdIns(3,4,5)P3 [6,7] (Fig 1B). PtdIns(3,4,5)P3 exerts its function by mediating translocation of various proteins via pleckstrin homology (PH) domains, which is a divergent protein module of approximately 120 amino acids found in many proteins involved in signal transduction [8] and that frequently mediates protein–protein or protein–phosphoinositide interactions. A subset of PH domains, including those in Btk, PKB/Akt, PLC-γ, Gab1, PDK1, Grp1, ARAP3, and multiple Rho guanine nucleotide exchange factors (GEFs), drive membrane translocation of their host proteins via specific, high-affinity recognition of PtdIns(3,4,5)P3 [6,9–11]. This membrane translocation is crucial for PtdIns(3,4,5)P3-mediated cellular processes such as cell survival, proliferation, growth, differentiation, polarization, chemotaxis, cytoskeletal rearrangement, and membrane trafficking [12–15] (Fig 1C).

Negative regulation of PtdIns(3,4,5)P3 signaling in neutrophils

Although neutrophil activation is essential for pathogen killing and clearance, excessive neutrophil accumulation or hyper-responsiveness of neutrophils can be detrimental to the host; the response of neutrophils to inflammatory stimuli therefore needs to be tightly controlled. Over 50 chemokines have been identified in humans, and many of them are constitutively present in blood or tissues. However, neutrophils will only migrate, generate superoxide, or degranulate in response to a strong and stable stimulus, which suggests that inhibitory factors must be present to suppress the positive signals elicited by a weak or unstable chemoattractant signals [16,17]. These intracellular inhibitors establish a threshold for neutrophil responses, and as a result, neutrophils only respond when they receive stimulation that overcomes the negative inhibitory effect. Here, we comprehensively

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Glossary

5ptase IV	phosphoinositide-specific inositol polyphosphate 5-phosphatase IV
COPD	chronic obstructive pulmonary disease
CSE	cigarette smoke extract
G-CSF	granulocyte colony-stimulating factor
GEF	guanine nucleotide exchange factors
GPCR	G-protein coupled receptor
InsP3K	inositol trisphosphate kinase
InsP6K	inositol hexakisphosphate kinase
InsP7	diphosphoinositol pentakisphosphate
IPMK	inositol phosphate multi-kinase
PH-domains	pleckstrin homolog domain
PI3K	phosphatidylinositol 3′-kinases
PLC	phosphatidylinositol-specific phospholipase C
PtdIns(3,4,5)P3	phosphatidylinositol 3, 4, 5 trisphosphate
PtdIns(4,5)P2	phosphatidylinositol 4,5-bisphosphate
PTEN	phosphatase and tensin homologue deleted on chromosome ten
Rock	RhoA-associated kinase
ROS	reactive oxygen species
SHIP1	SH2-containing inositol-5′-phosphatase-1
TLR	Toll-like receptor

review current understanding about the action of lipid phosphatases and inositol phosphates in the control of neutrophil function in infection and inflammation. The role of PtdIns(3,4,5)P3 in infection and inflammation, including in neutrophil biology, has been thoroughly reviewed elsewhere [18–26].

Regulation of PtdIns(3,4,5)P3 signaling by lipid phosphatases

PtdIns(3,4,5)P3 signaling is activated in neutrophils by extracellular ligands such as chemokines or growth factors, while PtdIns(3,4,5)P3 synthesis by PI3K is balanced by the action of lipid phosphatases. The tumor suppressor protein phosphatase and tensin homolog (PTEN), a phosphatidylinositol 3′-phosphatase, converts PtdIns(3,4,5)P3 to PtdIns(4,5)P2, antagonizing the effect of PI3K and inhibiting PtdIns(3,4,5)P3 signaling [27,28]. Similarly, SHIP [27,29] and 5ptase IV [30,31] convert PtdIns(3,4,5)P3 to PtdIns(3,4)P2, thereby also exerting an inhibitory effect (Fig 1C).

PTEN

PTEN is a mammalian phosphatidylinositol 3′-phosphatase known to modulate a range of cellular functions in various cell types,

including neutrophils. PtdIns(3,4,5)P3 accumulates at the leading edge of chemotaxing cells [32–36]. Disruption of PTEN in the amoeba *Dictyostelium discoideum*, a chemotaxis model, results in enhanced and prolonged PtdIns(3,4,5)P3 production, actin polymerization, persistent multiple (or broad) pseudopodia, and impaired directional migration in response to a chemoattractant gradient [33,37]. PTEN's role in hematopoietic cells has been well investigated. Li *et al* [34] demonstrated that PTEN's intracellular localization and activity can be regulated by chemoattractants via the Rho family of GTPases. In their experiments, PTEN and RhoA co-localized at the back of stimulated neutrophils, and active RhoA increased PTEN phosphatase activity via its downstream effector, Rock, which directly phosphorylated PTEN with Cdc42 acting as an enhancer. PTEN phosphorylation is required for its stability and activity [34,38]. Papakonstanti *et al* [39] proposed a pathway in which RhoA is activated via p110δ (see Fig 1B). Inhibition of RhoA resulted in misorientation of PTEN toward the front of the cell where Cdc42 localizes, and knockdown of PTEN in Jurkat T cells impaired chemotaxis in transwell migration assays. It appears that the RhoA activity at the back of polarizing cells is also regulated by PDZrhoGEF, which contains a Galpha12/13 coupled GRS domain and can be directly activated by G12/13. PDZrhoGEF is localized at the back of chemotaxing cells. Cells depleted of this protein exhibit multiple fronts and long tails [40,41]. It is noteworthy that the COOH terminus of PTEN contains a typical PSD-95/Dlg/ZO-1(PDZ) binding motif. Indeed, PTEN has been reported to associate with several PDZ domain-containing proteins [42–47]. It is intriguing to speculate whether PTEN-PDZ domain interaction may play a role in regulating the biological function of PTEN in neutrophil chemotaxis.

Together, the published results allow us to propose a mechanism for PTEN's function in neutrophils: Localization of PTEN at the uropod locally inhibits PtdIns(3,4,5)P3 production at the back of the cell, thereby localizing PtdIns(3,4,5)P3 production and actin polymerization to the cell's leading edge and mediating directional sensing. Disruption of PTEN would therefore be expected to lead to uncontrolled propagation of PtdIns(3,4,5)P3 at the leading edge and consequent formation of multiple pseudopodia, frequent directional changes, and loss of directionality [48]. To investigate the exact role of PTEN in neutrophils, we generated a myeloid-specific PTEN knockout mouse. Stimulation of PTEN$^{-/-}$ mouse neutrophils with chemoattractant resulted in increased PtdIns(3,4,5)P3 synthesis, more exaggerated and sustained F-actin polymerization, elevated sensitivity to chemoattractant, and augmented ROS production [49]. Disruption of PTEN also led to enhanced phagocytosis, consistent with the essential role of phospholipids in phagocytosis [50–57]. Single-cell chemotaxis assays showed that PTEN$^{-/-}$ neutrophils had

Figure 1. Inositol phosphates and phosphoinositides.

(A) Inositol phosphates contain only inositol and phosphate and are therefore water soluble and cytoplasmic. Ins(1,4,5)P3 can be converted to Ins(1,3,4,5)P4 by InsP3K family members [143–145]. InsP7 is a higher inositol phosphate containing energetic pyrophosphate bonds [128,139] and is formed by InsP6K family members [130,136,177]. Phosphoinositides also contain inositol and phosphates, but in addition they contain two hydrophobic fatty acids and are therefore not water soluble and are mainly localized to membrane structures. (B) PI3K isoforms. Class I PI3Ks are heterodimeric proteins with a catalytic subunit and a regulatory subunit. Class IA PI3Ks consist of a catalytic subunit, p110 (α, β, or δ), and an adaptor subunit, p85 (α or β), and they are regulated by receptor tyrosine kinases. The only class IB member is p110γ, which is associated with p101 and p84/p87^{PIKAP} regulatory subunits and is regulated by GPCRs such as chemokine receptors, C5a, and LTB4 [6,9]. (C) PtdIns(3,4,5)P3 signaling pathway. PtdIns(3,4,5)P3 exerts its function by mediating protein translocation via binding to their pleckstrin homolog (PH) domains. PH domain is a divergent protein module of approximately 120 amino acids found in many proteins involved in signal transduction [8,140,178–181]. There are more than 200 PH domains identified in the human genome. PH domains often mediate protein–protein or protein–phosphoinositide interactions. A subset of PH domains, including those in Btk, protein kinase B (PKB)/Akt, PLC-γ, Gab1, PDK1, and Grp1, drive membrane translocation of their host proteins through specific, high-affinity recognition of PtdIns(3,4,5)P3. Only the PH domain-containing protein molecules on the plasma membrane can be activated, which in turn elicit various signal transduction pathways mediating neutrophil functions.
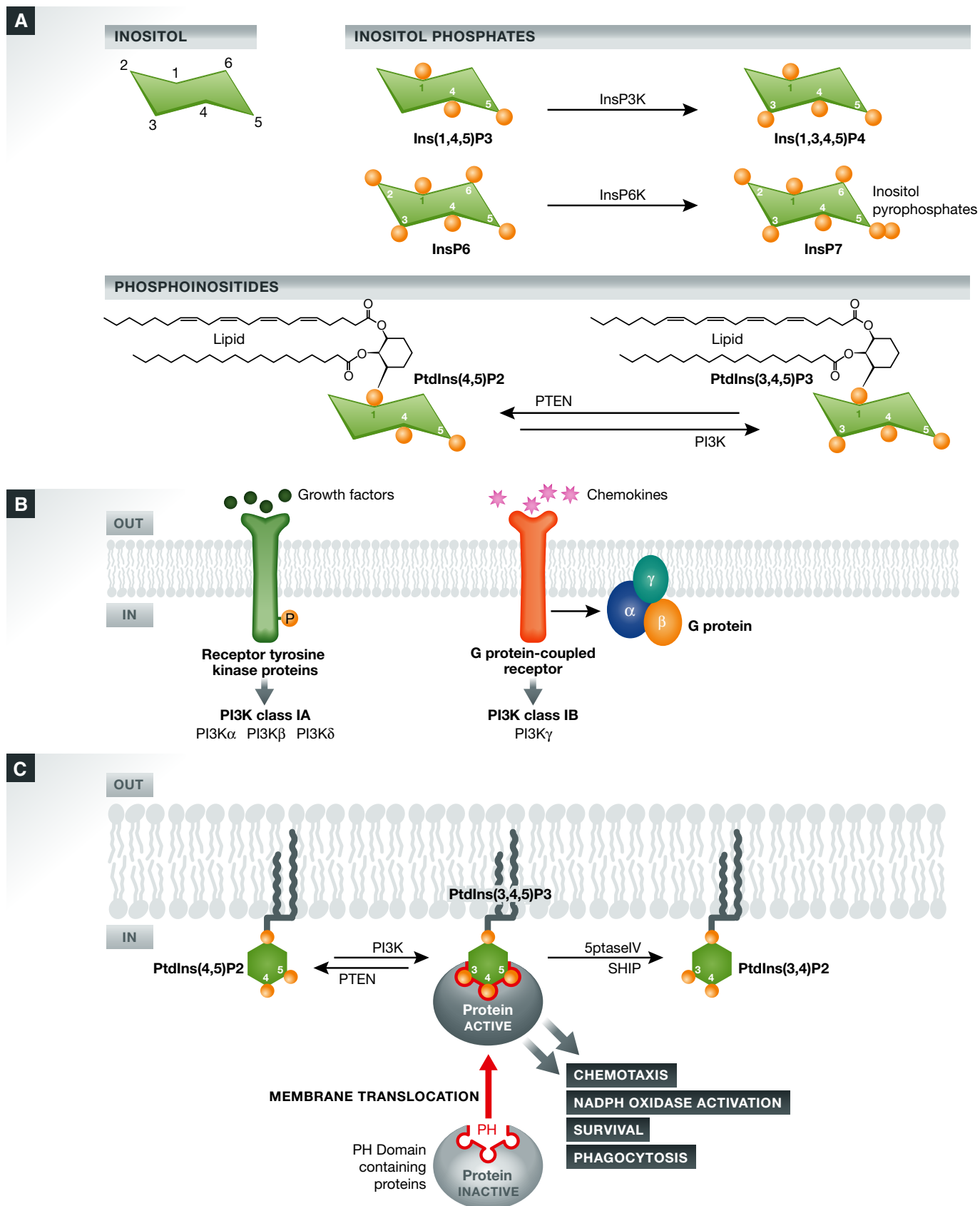
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Figure 1.

small but significant defects in directionality, consistent with the mechanistic hypothesis that PTEN plays a role in mediating polarized PtdIns(3,4,5)P3 production at the leading edge of chemotaxing cells [34]. However, these neutrophils also moved quickly, and, as a result, overall chemotaxis (which is a function of both speed and directionality) was not affected. Similar results have also been observed in PTEN-deficient T cells [58,59]. PTEN's function in directional sensing in *Dictyostelium* and mammalian cells, including neutrophils, is therefore different. Of note, neutrophils isolated from mice carrying a "knock-in" allele of PI3Kγ showed GPCR-uncoupled PtdIns(3,4,5)P3 accumulation and displayed much greater impairment in directional cell migration in response to chemoattractants. Stimulated mutant macrophages did not polarize PtdIns(3,4,5)P3, and chemoattractant-elicited Rac activation was shortened due to enhanced PI3K-dependent activation of RacGAPs [60].

Consistent with the observed increased responsiveness of PTEN$^{-/-}$ neutrophils *in vitro*, recruitment of PTEN$^{-/-}$ neutrophils to inflamed mouse peritoneal cavities was significantly enhanced *in vivo* [49]. Using intravital video microscopy, we also observed enhanced neutrophil emigration from cremasteric muscle venules in PTEN knockout mice. PtdIns(3,4,5)P3 is a major downstream target of integrins and chemokine receptors and has been implicated in multiple leukocyte trafficking steps [20,61–76]. Unexpectedly, leukocyte adhesion was not increased when PtdIns(3,4,5)P3 signaling was enhanced via PTEN depletion, suggesting that PtdIns(3,4,5)P3 signaling is essential for stimuli-elicited cell adhesion but is not a limiting step. Enhanced neutrophil recruitment to the cremaster muscle in response to different stimuli is most likely due to faster neutrophil movement in the vascular bed, across the vascular endothelium, and into the muscle tissue. Neutrophil rolling influx, rolling speed, and the number of firmly adherent neutrophils were not altered in PTEN knockout mice. PTEN therefore acts as a negative regulator of neutrophil trafficking, and enhanced neutrophil recruitment in PTEN knockout mice is mainly caused by augmented transendothelial migration [77].

Recently, Heit *et al* [78] reported that PTEN is required for prioritizing and integrating responses to multiple chemotactic cues in certain situations. Thus, PTEN's exact function in neutrophils might rely on the number and type of chemoattractants, as well as the relative doses or route used to induce neutrophil inflammatory reactions. In the setting of multiple chemoattractants, neutrophils favor end-target chemoattractants such as fMLP and C5a over intermediary endogenous chemoattractants such as IL-8 and LTB4. Heit *et al* proposed a hierarchical model in which neutrophil migration toward end-target chemoattractants is mediated by p38 MAPK, whereas migration toward intermediary chemoattractants is mediated by PtdIns(3,4,5)P3. When faced with competing gradients of end-target and intermediary chemoattractants, PtdIns(3,4,5)P3 signaling was significantly reduced by p38 MAPK [79], a cross talk that might be mediated by PTEN. When neutrophils were exposed to an IL8 gradient, PTEN localized to the uropod of chemotaxing cells, thereby contributing to accumulation of PtdIns(3,4,5)P3 at the leading edge. In the presence of opposing fMLP gradients, PTEN was distributed throughout the entire cell circumference, thus inhibiting all PI3K activity and permitting "preferential" migration toward fMLP via phospholipase A2 and p38. As a consequence, PTEN$^{-/-}$ neutrophils cannot prioritize chemoattractants and are "distracted" by chemokines when moving toward bacterial chemoattractants

[78]. Another recent study showed that the p38 MAPK p38δ and PKD1 regulate PTEN activity in neutrophils, thereby controlling their extravasation and chemotaxis during acute lung inflammation [80].

PTEN is also a key regulator of neutrophil death. Neutrophils normally have a very short life span and readily undergo spontaneous programmed cell death, which plays a crucial role in neutrophil homeostasis and the resolution of inflammation [81]. PtdIns(3,4,5)P3 signaling dramatically decreases during neutrophil death, and deactivation of PtdIns(3,4,5)P3 signaling has been identified as a critical event in neutrophil spontaneous death. PTEN-depleted neutrophils live much longer than wild-type neutrophils [82], and SHIP1-null neutrophils, in which the PtdIns(3,4,5)P3 pathway is upregulated, have an extended life span [83]. Conversely, activation of SHIP1 by NADPH oxidase-stimulated Lyn leads to enhanced neutrophil apoptosis [84]. These results are consistent with reports that neutrophil apoptosis is enhanced in PI3Kγ-deficient mice with reduced Akt activity [85,86], where Akt is a major survival factor downstream of PtdIns(3,4,5)P3 [87]. PtdIns(3,4,5)P3 production in apoptotic neutrophils is maintained by chemokines in an autocrine manner, which activate PI3Kγ via GPCRs. PI3Kγ, but not class IA PI3Ks, is negatively regulated by gradually accumulating ROS in apoptotic neutrophils, which suppress PI3Kγ activity by inhibiting an actin-mediated positive feedback loop [88]. However, a recent study has shown that granulocyte colony-stimulating factor (G-CSF) activation of Akt is insufficient to prolong neutrophil survival, and neutrophils treated with G-CSF undergo apoptosis even in the presence of high levels of activated Akt. Moreover, inhibition of Akt fails to alter neutrophil survival, suggesting that there are other pathways and factors mediating PtdIns(3,4,5)P3-elicited survival signaling [89].

Of note, some of PTEN's functions may not be mediated by degradation of PtdIns(3,4,5)P3. For instance, Anderson *et al* recently reported that superoxide production elicited by serum-opsonized bioparticles is mainly mediated by complement and CD18. This process requires class III PI3K and its product, PtdIns(3)P, and is independent of class I PI3K and its product, PtdIns(3,4,5)P3 [74,90]. Here, PTEN acts as a lipid phosphatase, removing the D3-positioned phosphate from PtdIns(3,4,5)P3, PtdIns(3,4)P2, and PtdIns(3)P. Therefore, its effect on phagocytosis-associated superoxide production is most likely mediated by its lipid phosphatase activity on PtdIns(3)P.

SHIP

SHIP (or SHIP1) is a 145-kD SH2-containing inositol-5-phosphatase that is widely expressed in hematopoietic cells [27,29,91,92]. It was first identified as a tyrosine phosphoprotein associated with Shc in response to numerous cytokines. Hematopoietic cell-specific SHIP1 is responsible for the majority of phosphatidylinositol 5′-phosphatase activity in neutrophils, while the ubiquitously expressed SHIP2 only plays a minor role in hematopoietic cells. SHIP1 blunts PI3K-initiated signaling and is known to negatively regulate various cellular processes, such as phagocytosis, cell migration, degranulation, cell survival, proliferation, differentiation, and sensitivity to chemokines [93–96]. SHIP1-deficient mice exhibit chronic progressive hyperplasia of myeloid cells, perhaps at the expense of B-cell production. SHIP1 knockout myeloid progenitors show enhanced responsiveness to cytokines and are less susceptible to apoptotic

stimuli *in vitro*, and while increased proliferation and decreased apoptosis may contribute to the expanded hematopoietic stem cell (HSC) compartment in SHIP1 knockout mice, these HSCs are also compromised in their ability to home and repopulate [83,92]. SHIP1 expression was initially thought to be restricted to hematopoietic cells, but more recently it has become apparent that most bone marrow (BM) and blood cells express SHIP1, and Hazen *et al* [97] demonstrated SHIP1 expression and functional activity in non-hematopoietic BM cells. Furthermore, SHIP1 deficiency enables long-term reconstitution of the hematopoietic inductive BM micro-environment [98].

Although excessive PtdIns(3,4,5)P3 production in PTEN$^{-/-}$ neutrophils does not cause significant chemotaxis defects, one report suggests that SHIP1 knockout results in chemotaxis defects [99]. Findings from our own lab show that SHIP1 plays a predominant role in cell adhesion, rather than chemoattractant sensing, during cell migration. SHIP1$^{-/-}$ neutrophils were much more adherent than wild-type cells by engaging integrins. In suspension, PtdIns(3,4,5)P3 production in SHIP1$^{-/-}$ neutrophils remained constant when stimulated with chemoattractant, in contrast to adherent SHIP1$^{-/-}$ neutrophils, which showed marked upregulation of PtdIns(3,4,5)P3 when stimulated. As a consequence, actin polymerization was restricted to the leading edge of suspended SHIP1$^{-/-}$ neutrophils, with cell polarity lost upon adhesion [100]. During migration, SHIP1 appears to act as a negative regulator of PtdIns(3,4,5)P3 formation at the cell–substratum interface, preventing the formation of top-down PtdIns(3,4,5)P3 polarity and facilitating normal cell attachment and detachment during chemotaxis (Fig 2A).

In the same study, we also examined neutrophil migration *in vivo*. Surprisingly, despite the chemotaxis defect, recruitment of SHIP1$^{-/-}$ neutrophils to the inflamed peritoneal cavity was significantly enhanced. We hypothesize that neutrophils passing through the endothelium and entering the tissue control their migration using two independent directional cues: a lateral front-to-back polarity mediated by chemotactic signaling and a dorsal–ventral polarity controlled by adhesion-mediated signaling. SHIP1 activity is regulated by adhesion and plays a critical role in balancing these two types of polarity in neutrophils. Loss of SHIP1 leads to upregulation of dorsal–ventral polarity, preventing neutrophils from effective lateral migration on surfaces. However, this dorsal–ventral polarity promotes top-down migration of neutrophils through soft surfaces such as the gaps between endothelial cells [100] (Fig 2B).

Although SHIP1 is thought to be enzymatically active in the cytosol, its activity is ultimately determined by its membrane localization [101]. Recruitment of SHIP1 to the plasma membrane is regulated by its association with adapter proteins (e.g. Shc, Grb2, Dok3), scaffolding proteins (e.g. Gab1/2), and by direct association with tyrosine-phosphorylated receptors via its SH2 domain. These interactions require tyrosine phosphorylation of SHIP1 at the NPXY motif [102–105]. We have observed that cell adhesion, but not chemoattractant stimulation, leads to tyrosine phosphorylation of SHIP1. In addition, SHIP1 can interact with FAK and Lyn upon cell adhesion and β_3 integrin in both suspension and upon cell adhesion. This indicates that adhesion results in the recruitment of SHIP1 to the membrane where it can act on the PtdIns(3,4,5)P3 produced during cell adhesion [100]. It has previously been shown that Lyn, a Src family tyrosine kinase, regulates SHIP1 phosphorylation in integrin $\alpha_{IIb}\beta_3$-mediated adhesion and signaling in platelets [106].

Unlike PTEN-deficient neutrophils, in which ROS production is elevated, loss of SHIP1 leads to reduced ROS production in neutrophils in suspension upon stimulation with chemoattractant. In neutrophils, ROS are mainly produced by the phagocyte NADPH oxidase (also known as the NOX2 complex) [107–111]. During cell activation, the cytosolic components of the enzyme, namely p47phox, p67phox, Rac2, and p40phox, are recruited to the membrane to form a complex with its membrane components, p22phox and gp91 complex. Assembly of the NADPH oxidase complex catalyzes the conversion of molecular oxygen to superoxide, which is known to facilitate the destruction of invading pathogens [81,88,112–114]. Although PtdIns(3,4,5)P3 and PI3Ks have been identified as key regulators of NADPH oxidase activation, recruitment of cytosolic p47phox and p40phox to the NADPH oxidase complex requires the presence of the class III PI3K product, PtdIns(3)P, and the product of SHIP1, PtdIns(3,4)P2 [73,74,87,115–123]. Reduced ROS production in SHIP1$^{-/-}$ neutrophils is primarily due to decreased PtdIns(3,4)P2 levels, which correlates with reduced recruitment of p40phox and p47phox to the NADPH oxidase complex [100]. However, when SHIP1$^{-/-}$ neutrophils are primed and permitted to adhere to fibronectin, they produce very high levels of ROS. This is most likely due to the involvement of SHIP1$^{-/-}$ in integrin-mediated PtdIns(3,4,5)P3 signaling, with increased PtdIns(3,4,5)P3 signaling overriding the effect of decreased PtdIns(3,4)P2 levels [100].

The PtdIns(3,4,5)P3 signaling pathway can be negatively regulated by lipid phosphatases SHIP1 and PTEN in neutrophils. The activity and subcellular localization of these lipid phosphatases are tightly controlled. How temporal and spatial regulation contributes to various neutrophil functions remains ill-defined and needs to be further investigated.

Regulation of PtdIns(3,4,5)P3 signaling by inositol phosphates

PtdIns(3,4,5)P3 signaling was previously thought to be solely dependent on the concentration of PtdIns(3,4,5)P3 in the cell membrane [6]. We discovered that two inositol phosphates, InsP7 and Ins(1,3,4,5)P4, compete with PtdIns(3,4,5)P3 for PH domain binding and suppress PH domain translocation, providing a novel mode of regulation for PtdIns(3,4,5)P3 signaling in neutrophils [124,125] (Fig 3).

InsP7

InsP7 has been implicated in a variety of cellular functions such as vesicular trafficking, apoptosis, endocytosis, DNA repair/recombination, and maintenance of telomere length [126]. InsP7 arises from pyrophosphorylation of InsP6, the most abundant inositol phosphate in mammalian cells [127]. The enzymes that catalyze the synthesis of InsP7 comprise a family of InsP6 kinases (InsP6K) including InsP6K1, InsP6K2, and InsP6K3 [128,129] (Fig 1). InsP6K1 and InsP6K2 are highly expressed in neutrophils, while InsP6K3 is essentially undetectable. In neutrophils, InsP6K1 and InsP6K2 isoforms have non-redundant roles. InsP6K1 seems to be equally distributed in the nucleus and cytoplasm, while, in contrast, InsP6K2 appears to be almost exclusively nuclear in localization [130]. In a recent study, we reported that InsP6K1 could regulate
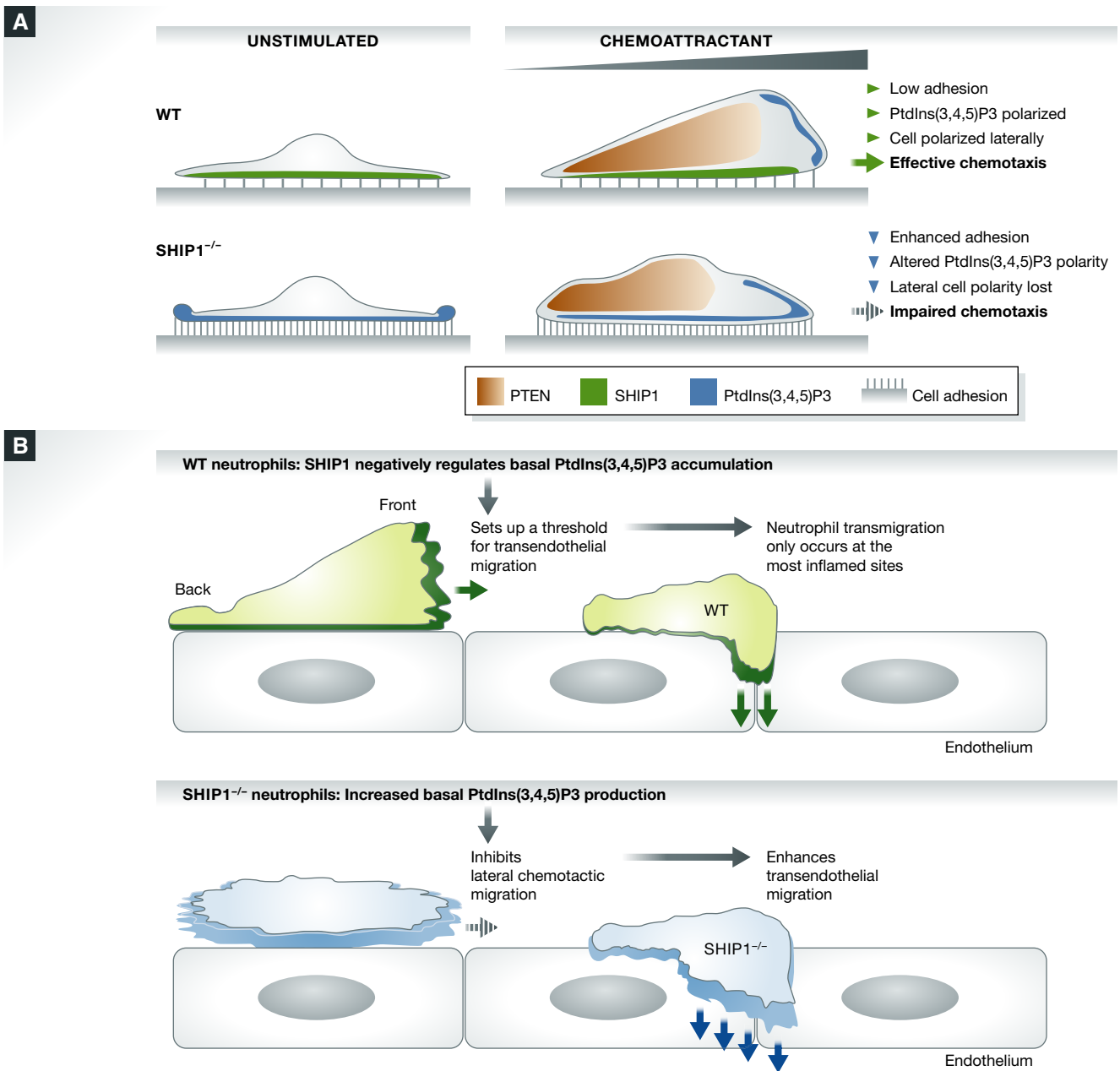
Figure 2. The role of SHIP1 and PTEN in neutrophil chemotactic migration.

(A) SHIP1 and PTEN act via different receptor-regulated processes to control spatial accumulation of PtdIns(3,4,5)P3 and establish a proper anterior–posterior PtdIns(3,4,5)P3 compass. SHIP1 acts as a negative regulator of integrin-mediated cell adhesion in neutrophils. In wild-type neutrophils, integrin-mediated cell adhesion results in PtdIns(3,4,5)P3 production at the sites of cell adhesion. Concurrently, SHIP1 at the cell–substratum interface is engaged, phosphorylated, and activated. This activity is crucial for dephosphorylating the PtdIns(3,4,5)P3 formed during cell adhesion. With the combined actions of both SHIP1 and PTEN, PtdIns(3,4,5)P3 polarity is maintained at the leading edge, neutrophils polarize, and there is effective cell migration. PTEN is localized to the rear of a migrating cell to facilitate the accumulation of PtdIns(3,4,5)P3 at the anterior end, and SHIP1 is active at the cell–substratum interface to abolish the PtdIns(3,4,5)P3 gradient being formed by integrin activation. With loss of SHIP1, adhesion-mediated PtdIns(3,4,5)P3 formation is uncontrolled, resulting in the formation of "top-down" PtdIns(3,4,5)P3 polarity. Increased PtdIns(3,4,5)P3 levels enhance cell adhesion. This leads to activation of various PtdIns(3,4,5)P3 effector proteins and consequently results in impaired chemotaxis. (B) SHIP1 plays a critical role in neutrophil transendothelial migration by balancing the lateral and dorsal–ventral polarity of transmigrating neutrophils.

PtdIns(3,4,5)P3 signaling in neutrophils [131]; disruption of InsP6K1 enhanced PtdIns(3,4,5)P3 signaling, and, as a consequence, these neutrophils exhibited elevated phagocytic and bactericidal capabilities and amplified NADPH oxidase-mediated superoxide production. These findings established a novel role for InsP7 in the regulation of cellular signal transduction pathways in neutrophils and provided a novel mechanism for modulating PtdIns(3,4,5)P3 signaling in mammalian cells [131].
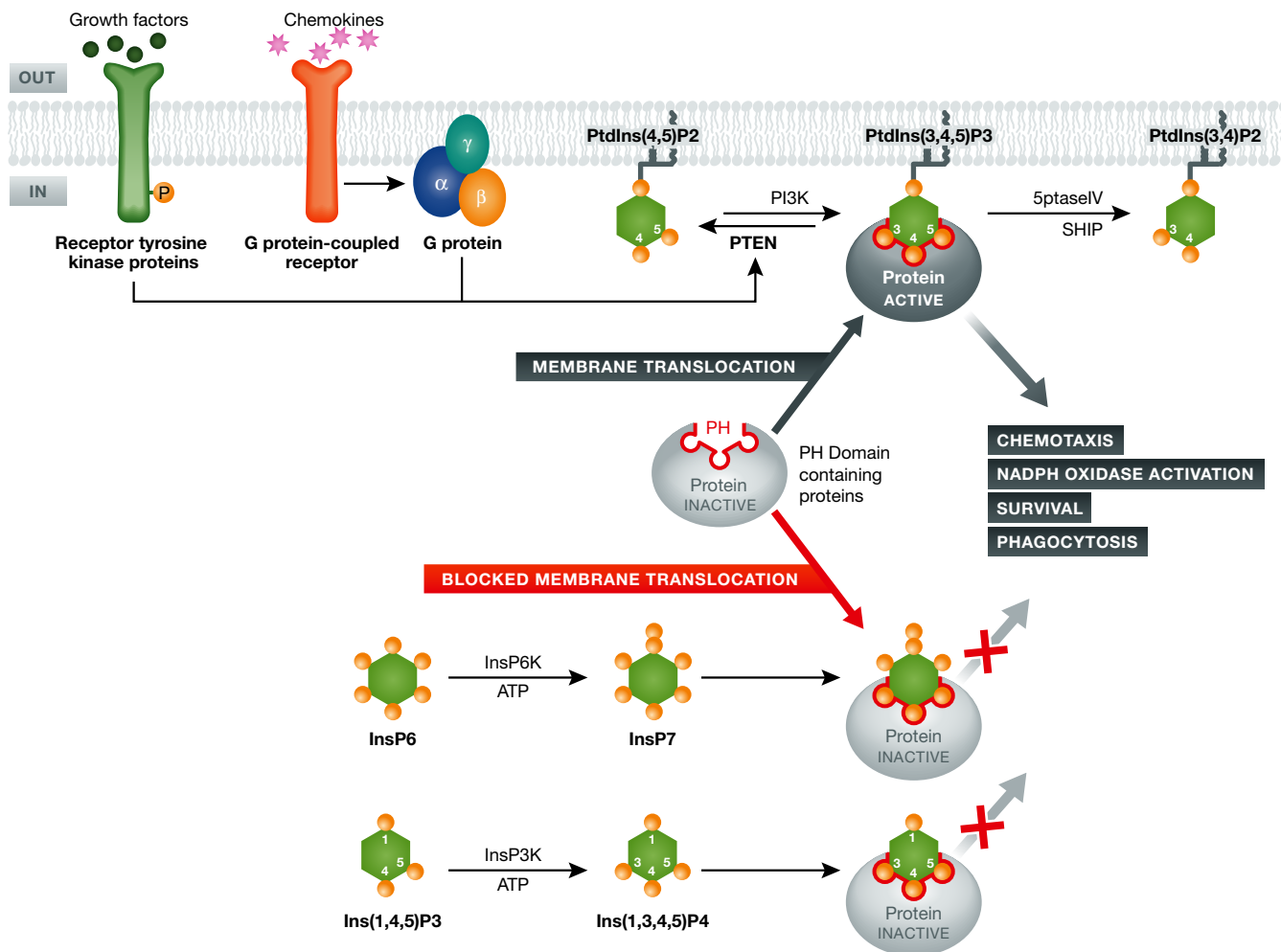
Figure 3. Regulation of PtdIns(3,4,5)P3 signaling by inositol phosphates in neutrophils.
InsP7 or Ins(1,3,4,5)P4 can compete with PtdIns(3,4,5)P3 for PH domain binding and thus prevent their recruitment onto the plasma membrane and consequently inhibit their activation.

Consistent with its regulatory role in mediating chemoattractant-elicited signals, InsP7 levels are tightly regulated in neutrophils. Unstimulated neutrophils contain a substantial amount of InsP7, with levels decreasingly markedly and rapidly upon stimulation with chemoattractants [131]. Cigarette smoke extract (CSE) and nicotine also reduce InsP7 levels in aging neutrophils, which subsequently leads to suppression of Akt deactivation and delayed neutrophil death. Delayed neutrophil death contributes to the pathogenesis of CS-induced COPD. Disruption of InsP6K1 consistently augments CS-induced neutrophil accumulation and lung damage [132]. Recently, Chakraborty et al [133] showed that InsP7 also negatively regulates PtdIns(3,4,5)P3/Akt signaling in glucose homeostasis and protein translation. InsP7 inhibits Akt by acting at the PH domain of Akt to prevent its plasma membrane translocation, phosphorylation, and activation by PDK1 [133]. Intriguingly, in contrast to our observation that chemoattractant inhibits InsP7 formation, Chakraborty et al showed that growth factors stimulate InsP7 production. Therefore, the inhibition of Akt signaling by InsP7 may be a general signal transduction phenomenon. However, its regulatory mechanism and resulting physiologic consequences might be cell or system specific.

The mechanisms by which InsP7 production is suppressed in chemoattractant- and CSE-stimulated neutrophils are still largely unknown and need to be investigated further. However, these mechanisms are likely to involve activation of inositol pyrophosphate phosphatase and/or deactivation of InsP6 kinase.

Although InsP6K1 disruption augments PtdIns(3,4,5)P3 signaling, it fails to augment cell adhesion, sensitivity, or migration speed in neutrophils [131]. These results are somewhat different to the migratory phenotypes observed in PTEN-null neutrophils [49]. Although overall chemotactic migration is relatively normal, PTEN disruption in neutrophils results in mildly impaired directionality, enhanced sensitivity to chemoattractant stimulation, and slightly increased migration speed [49]. These distinct effects are likely to occur as a result of different temporal and spatial regulation of PTEN and InsP6K1 in neutrophils. PTEN activity is increased, and its subcellular localization is altered, after chemoattractant stimulation [34]. In contrast, InsP6K1 levels are high in unstimulated neutrophils but are significantly reduced after chemoattractant stimulation. The mechanisms by which PTEN and InsP6K1 regulate PtdIns(3,4,5)P3 signaling are also different; PTEN regulates PtdIns

(3,4,5)P3 levels and controls neutrophil function via a number of downstream pathways, while InsP6K1 deletion is not associated with changes in PtdIns(3,4,5)P3 or its downstream signaling cascades, but instead is limited to its action on Akt. In addition, PTEN appears to act as a housekeeping gene, while InsP6K1 exhibits tissue-specific expression, and there is functional redundancy with InsP6K2 and/or InsP6K3 in some cell types [130,134–136]. These results highlight the complexity of the PtdIns(3,4,5)P3 signaling network and suggest that different temporal and spatial regulation of this signaling molecule can lead to distinct cellular effects.
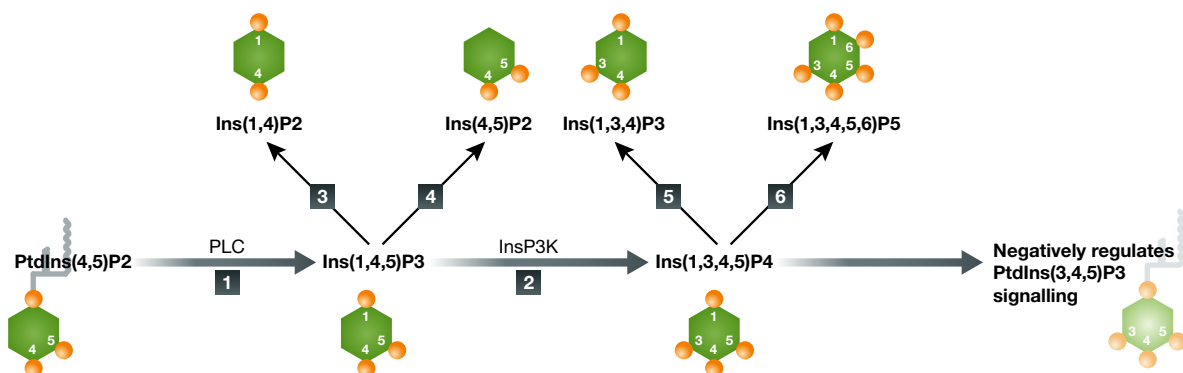
Ins(1,3,4,5)P4

Ins(1,3,4,5)P4 is the predominant InsP4 isoform in neutrophils [137,138], intracellular levels of which are regulated by inositol phosphate kinases and phosphatases [128,139] (Fig 4). The major metabolic pathway generating Ins(1,3,4,5)P4 is via Ins(1,4,5)P3, with Ins(1,4,5)P3 formed from the hydrolysis of PtdIns(4,5)P2 by PLC [116,140–142]. Ins(1,3,4,5)P4 is eventually formed by InsP3Ks, which belong to a highly conserved family of inositol phosphate kinases [128,136,139]. Members of this family include InsP6Ks, InsP3Ks, and IPMK [143–145]. There are three InsP3K isoforms in mammalian cells, designated A, B, and C [143–145]. The gene encoding InsP3KA is exclusively expressed in specific subpopulations of neurons in the central nervous system and in the testis [143], while isoforms B and C are ubiquitously expressed [143–145].

The physiologic functions of InsP3K in hematopoietic cells were recently studied using InsP3K knockout mice [146,147]. The InsP3KB isoform appeared to contribute the majority of InsP3K activity in T and B cells [148–151]. Disruption of InsP3KB led to a dramatic decrease in cellular Ins(1,3,4,5)P4 levels, impaired T-cell development, and defective thymocyte selection. Huang *et al* demonstrated that Ins(1,3,4,5)P4 can bind to the PH domain of the Tec family tyrosine kinase Itk, which plays an important role in TCR signaling. Interestingly, this binding altered the conformation of the Itk PH domain and subsequently promoted, rather than suppressed, PH domain binding to PtdIns(3,4,5)P3 in T cells [152]. Disruption of InsP3KB induced B-cell death and impaired B-cell development [153,154]. Miller *et al* [153] reported that Ins(1,3,4,5)P4 acts by modulating store-operated calcium channels, with elevated calcium

influx detected in InsP3KB null B cells. InsP3K has been reported to be a potential modulator of calcium mobilization, since it can decrease the level of Ins(1,4,5)P3, which mediates calcium release from internal stores by converting it to Ins(1,3,4,5)P4. Surprisingly, no substantial defects in Ins(1,4,5)P3 quantity or calcium mobilization were detected in InsP3KB-null T cells [148,149].

InsP3KB is also the major InsP3K isoform in neutrophils. Disruption of the InsP3KB gene leads to alteration of myelopoiesis, hyperactivation of neutrophils, and dysregulation of innate immunity [125,155,156]. Ins(1,3,4,5)P4 depletion in InsP3KB-deficient neutrophils enhances membrane translocation of the PtdIns(3,4,5)P3-specific PH domain, thus augmenting PtdIns(3,4,5)P3-mediated downstream signaling. This leads to enhanced sensitivity to chemoattractants, elevated superoxide production, and enhanced neutrophil recruitment to inflamed peritoneal cavities in mice [125]. Although loss of InsP3KB leads to elevated neutrophil recruitment and function, bacterial killing was actually reduced *in vivo*. This was mainly due to B- and T-cell defects, which led to significantly reduced levels of immunoglobulin and impaired opsonization and phagocytosis [125]. Disruption of InsP3KB does not affect overall calcium signaling in the presence of extracellular calcium in neutrophils [125]. However, a more detailed investigation revealed significantly decreased calcium release from intracellular stores and enhanced calcium influx through store-operated calcium channels in InsP3KB-null neutrophils stimulated with chemokines. Reduced calcium release from intracellular stores appears to be a result of calcium depletion from the store (Y. Jia and H.R. Luo, unpublished data).

In neutrophils, chemoattractant stimulation triggers a potent and rapid elevation in Ins(1,3,4,5)P4, indicating that Ins(1,3,4,5)P4 levels are regulated by receptor occupancy. InsP3K is not constitutively active in neutrophils, and its activity in unstimulated neutrophils is relatively low. Activity is, however, dramatically increased by chemoattractant stimulation. The low basal InsP3K activity might be necessary for chemoattractant-stimulated neutrophils to generate or maintain an initially high concentration of Ins(1,4,5)P3, which is essential for downstream calcium signaling. Alternatively, the regulation of InsP3K by chemoattractants may simply be another mechanism to finely tune intracellular Ins(1,3,4,5)P4 concentrations.

Figure 4. The metabolic pathways controlling intracellular Ins(1,3,4,5)P4 levels.
1. Phospholipase C (PLC). 2. Ins(1,4,5)P3 kinase (InsP3K). 3. Ins(1,4,5)P3 5-phosphatase. 4. Ins(1,4,5)P3 1-phosphatase. 5. Inositol polyphosphate 5-phosphatase. 6. Ins(1,3,4,5)P4 6-kinase. All these enzymes are ubiquitously expressed.

The molecular mechanism underlying the chemoattractant-induced enhancement of InsP3K activity is still largely unknown. Recent studies have shown that all three InsP3K isoforms contain an F-actin binding domain, and InsP3KB is highly localized to the leading edge of polarized neutrophils [144,157–159]. Whether the actin–InsP3KB interaction plays any role in modulating InsP3KB activity needs to be determined. In addition, since all three isoforms contain a calmodulin binding motif, the InsP3KB activity in neutrophils may also be regulated by calcium [125].

Targeting PtdIns(3,4,5)P3 signal pathway in infectious and inflammatory diseases

Excessive neutrophil accumulation or hyper-responsiveness of neutrophils can be detrimental to the host system, and the response of neutrophils to inflammatory stimuli needs to be tightly controlled. Neutrophils contribute to the pathogenesis of a number of autoimmune diseases such as rheumatoid arthritis, Crohn's disease, and systemic lupus erythematosus (SLE). Since receptor-mediated signal transduction in neutrophils relies heavily on PI3K isoforms, small molecule inhibitors to class I PI3Ks should counteract the undesirable pro-inflammatory effects of neutrophils in inflammatory conditions. Over the last few years, a number of isoform-specific PI3K inhibitors have been developed, and some of them are already being tested in clinical trials for various inflammatory diseases [68,75,160–167]. Since SHIP1 inhibition leads to massive myeloid infiltration of the lungs and progressive inflammation, pharmacological activation of SHIP1 has emerged as a therapeutic strategy for inflammatory pulmonary diseases. A small molecule SHIP1 activator AQX-1125, which binds to the C2 domain of SHIP1 to increase its catalytic activity, is currently in Phase II clinical trials. Since SHIP1 expression is restricted to hematopoietic cells, targeting SHIP1 is expected to limit unwanted off-target side effects. AQX-1125 suppresses Akt phosphorylation, reduces cytokine production, and inhibits neutrophil activation and chemotaxis [168,169].

There are also cases in which enhanced neutrophil recruitment and function would be expected to be clinically beneficial. For example, neutropenia and related infections are the most important dose-limiting toxicities of cytotoxic anti-cancer chemo- and radiotherapies, which impact on both the quality and quantity of life. One obvious strategy for treating neutropenia-related infections is to administer broad-spectrum antibiotics to neutropenic patients. However, not all patients respond to antibiotic treatments, and this method carries the risk of inducing antibiotic resistance. An alternative approach is G-CSF (filgrastim) treatment [170], which is now used clinically to restore neutrophil counts in neutropenia-related pneumonia patients via stimulation of the BM to produce more neutrophils. However, this therapy does not always work if the BM has not recovered from chemotherapy, and treatment is associated with side effects such as bone pain, headache, fatigue, and nausea. Long-term use of G-CSF might also increase the risk of leukemia [170–173]. Therefore, directly elevating innate immunity by promoting neutrophil recruitment and function is a reasonable alternative strategy under these circumstances. We have recently shown that neutrophil function can be enhanced in neutropenic conditions by activating intracellular PtdIns(3,4,5)P3 signaling [174]; significantly, more neutrophils were recruited to inflamed lungs during neutropenia-associated pneumonia in myeloid-specific PTEN knock-out mice. Using an adoptive transfer technique, we demonstrated that enhanced neutrophil recruitment was caused directly by PTEN depletion in neutrophils. In addition, disruption of PTEN increased recruitment of macrophages and elevated levels of pro-inflammatory cytokines/chemokines in the inflamed lungs, which might also have contributed to enhanced neutrophil recruitment. Finally, we provided direct evidence that the enhanced neutrophil function caused by elevated PtdIns(3,4,5)P3 signaling can alleviate pneumonia-associated lung damage and decrease pneumonia-elicited mortality. Furthermore, we showed that activation of PtdIns(3,4,5)P3 signaling by PTEN disruption or using the SF1670 PTEN inhibitor increased the efficacy of granulocyte transfusion in neutropenia-related pneumonia [175]. It is noteworthy that although we consistently observed enhanced neutrophil migration in PTEN-deficient mice suffering from *E. coli* pneumonia, Schabbauer *et al* recently reported that the absence of myeloid cell-associated PTEN dampens pulmonary inflammation, reduces neutrophil influx, and augments the phagocytic properties of macrophages, which ultimately resulted in decreased tissue injury and improved survival during murine pneumococcal pneumonia. PTEN may differentially regulate the attraction of neutrophils depending on the inducing agent [176].

Elevating PtdIns(3,4,5)P3 can augment neutrophil recruitment and function in host defense responses. However, hyperactivation of neutrophils can lead to unwanted tissue damage and inflammation. In addition, some activators of PtdIns(3,4,5)P3 signaling may not be appropriate therapeutic targets for clinical intervention due to their carcinogenetic or other harmful effects. This balance between benefit and harm remains a challenge in targeting PtdIns(3,4,5)P3 signaling in inflammatory conditions. The PtdIns(3,4,5)P3 signaling pathway is negatively regulated by lipid phosphatases and inositol phosphates, which consequently play a critical role in controlling neutrophil function and thus would be expected to act as ideal therapeutic targets for enhancing or suppressing innate immune responses.

Sidebar A: In need of answers

(i) How are the activity and subcellular localization of PTEN and SHIP1 regulated in neutrophils? How do temporal and spatial regulation contribute to various neutrophil functions?

(ii) What molecular mechanism underpins chemoattractant-induced enhancement of InsP3KB activity in neutrophils? Do actin–InsP3KB interactions play any role in modulating InsP3KB activity?

(iii) The mechanisms by which InsP7 production is suppressed in chemoattractant- and CSE-stimulated neutrophils.

(iv) When targeting PtdIns(3,4,5)P3 signaling in the clinical setting, how can a balance between the beneficial and detrimental effects be achieved?

References

1. Nauseef WM, Borregaard N (2014) Neutrophils at work. *Nat Immunol* 15: 602–611

2. Nathan C (2006) Neutrophils and immunity: challenges and opportuni-ties. *Nat Rev Immunol* 6: 173–182

3. Phillipson M, Kubes P (2011) The neutrophil in vascular inflammation. *Nat Med* 17: 1381–1390

4. Kolaczkowska E, Kubes P (2013) Neutrophil recruitment and function in health and inflammation. *Nat Rev Immunol* 13: 159–175

5. Dale DC, Boxer L, Liles WC (2008) The phagocytes: neutrophils and monocytes. *Blood* 112: 935–945

6. Cantley LC (2002) The phosphoinositide 3-kinase pathway. *Science* 296: 1655–1657

7. Vanhaesebroeck B, Stephens L, Hawkins P (2012) PI3K signalling: the path to discovery and understanding. *Nat Rev Mol Cell Biol* 13: 195–203

8. Lemmon MA (2003) Phosphoinositide recognition domains. *Traffic* 4: 201–213

9. Cantrell DA (2001) Phosphoinositide 3-kinase signalling pathways. *J Cell Sci* 114: 1439–1445

10. Krugmann S, Anderson KE, Ridley SH, Risso N, McGregor A, Coadwell J, Davidson K, Eguinoa A, Ellson CD, Lipp P *et al* (2002) Identification of ARAP3, a novel PI3K effector regulating both Arf and Rho GTPases, by selective capture on phosphoinositide affinity matrices. *Mol Cell* 9: 95–108

11. Enomoto A, Murakami H, Asai N, Morone N, Watanabe T, Kawai K, Murakumo Y, Usukura J, Kaibuchi K, Takahashi M (2005) Akt/PKB regu-lates actin organization and cell motility via Girdin/APE. *Dev Cell* 9: 389–402

12. Rickert P, Weiner OD, Wang F, Bourne HR, Servant G (2000) Leukocytes navigate by compass: roles of PI3Kgamma and its lipid products. *Trends Cell Biol* 10: 466–473

13. Hemmings BA (1997) PtdIns(3,4,5)P3 gets its message across. *Science* 277: 534

14. Corvera S, Czech MP (1998) Direct targets of phosphoinositide 3-kinase products in membrane traffic and signal transduction. *Trends Cell Biol* 8: 442–446

15. Downward J (1998) Mechanisms and consequences of activation of protein kinase B/Akt. *Curr Opin Cell Biol* 10: 262–267

16. Foxman EF, Campbell JJ, Butcher EC (1997) Multistep navigation and the combinatorial control of leukocyte chemotaxis. *J Cell Biol* 139: 1349–1360

17. Zigmond SH (1989) Chemotactic response of neutrophils. *Am J Respir Cell Mol Biol* 1: 451–453

18. Sapey E, Greenwood H, Walton G, Mann E, Love A, Aaronson N, Insall RH, Stockley RA, Lord JM (2014) Phosphoinositide 3-kinase inhibition restores neutrophil accuracy in the elderly: toward targeted treatments for immunosenescence. *Blood* 123: 239–248

19. Ferguson GJ, Milne L, Kulkarni S, Sasaki T, Walker S, Andrews S, Crabbe T, Finan P, Jones G, Jackson S *et al* (2007) PI(3)Kgamma has an impor-tant context-dependent role in neutrophil chemokinesis. *Nat Cell Biol* 9: 86–91

20. Heit B, Liu L, Colarusso P, Puri KD, Kubes P (2008) PI3K accelerates, but is not required for, neutrophil chemotaxis to fMLP. *J Cell Sci* 121: 205–214

21. Andrews S, Stephens LR, Hawkins PT (2007) PI3K class IB pathway in neutrophils. *Sci STKE* 2007: cm3

22. Hawkins PT, Stephens LR, Suire S, Wilson M (2010) PI3K signaling in neutrophils. *Curr Top Microbiol Immunol* 346: 183–202

23. Hannigan MO, Huang CK, Wu DQ (2004) Roles of PI3K in neutrophil function. *Curr Top Microbiol Immunol* 282: 165–175

24. Cadwallader KA, Condliffe AM, McGregor A, Walker TR, White JF, Stephens LR, Chilvers ER (2002) Regulation of phosphatidylinositol

3-kinase activity and phosphatidylinositol 3,4,5-trisphosphate accumu-lation by neutrophil priming agents. *J Immunol* 169: 3336–3344

25. Condliffe AM, Hawkins PT, Stephens LR, Haslett C, Chilvers ER (1998) Priming of human neutrophil superoxide generation by tumour necrosis factor-alpha is signalled by enhanced phosphatidylinositol 3,4,5-trisphosphate but not inositol 1,4,5-trisphosphate accumulation. *FEBS Lett* 439: 147–151

26. Germena G, Hirsch E (2013) PI3Ks and small GTPases in neutrophil migration: two sides of the same coin. *Mol Immunol* 55: 83–86

27. Di Cristofano A, Pandolfi PP (2000) The multiple roles of PTEN in tumor suppression. *Cell* 100: 387–390

28. Maehama T, Dixon JE (1999) PTEN: a tumour suppressor that functions as a phospholipid phosphatase. *Trends Cell Biol* 9: 125–128

29. Rauh MJ, Kalesnikoff J, Hughes M, Sly L, Lam V, Krystal G (2003) Role of Src homology 2-containing-inositol 5′-phosphatase (SHIP) in mast cells and macrophages. *Biochem Soc Trans* 31: 286–291

30. Kisseleva MV, Cao L, Majerus PW (2002) Phosphoinositide-specific inositol polyphosphate 5-phosphatase IV inhibits Akt/protein kinase B phosphory-lation and leads to apoptotic cell death. *J Biol Chem* 277: 6266–6272

31. Astle MV, Horan KA, Ooms LM, Mitchell CA (2007) The inositol poly-phosphate 5-phosphatases: traffic controllers, waistline watchers and tumour suppressors? *Biochem Soc Symp* 74: 161–181

32. Iijima M, Devreotes P (2002) Tumor suppressor PTEN mediates sensing of chemoattractant gradients. *Cell* 109: 599–610

33. Funamoto S, Meili R, Lee S, Parry L, Firtel RA (2002) Spatial and tempo-ral regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. *Cell* 109: 611–623

34. Li Z, Dong X, Wang Z, Liu W, Deng N, Ding Y, Tang L, Hla T, Zeng R, Li L *et al* (2005) Regulation of PTEN by Rho small GTPases. *Nat Cell Biol* 7: 399–404

35. Wang F, Herzmark P, Weiner OD, Srinivasan S, Servant G, Bourne HR (2002) Lipid products of PI(3)Ks maintain persistent cell polarity and directed motility in neutrophils. *Nat Cell Biol* 4: 513–518

36. Suire S, Condliffe AM, Ferguson GJ, Ellson CD, Guillou H, Davidson K, Welch H, Coadwell J, Turner M, Chilvers ER *et al* (2006) Gbetagammas and the Ras binding domain of p110gamma are both important regu-lators of PI(3)Kgamma signalling in neutrophils. *Nat Cell Biol* 8: 1303–1309

37. Iijima M, Huang YE, Devreotes P (2002) Temporal and spatial regula-tion of chemotaxis. *Dev Cell* 3: 469–478

38. Vemula S, Shi J, Hanneman P, Wei L, Kapur R (2010) ROCK1 functions as a suppressor of inflammatory cell migration by regulating PTEN phosphorylation and stability. *Blood* 115: 1785–1796

39. Papakonstanti EA, Ridley AJ, Vanhaesebroeck B (2007) The p110delta isoform of PI 3-kinase negatively controls RhoA and PTEN. *EMBO J* 26: 3050–3061

40. Wong K, Van Keymeulen A, Bourne HR (2007) PDZRhoGEF and myosin II localize RhoA activity to the back of polarizing neutrophil-like cells. *J Cell Biol* 179: 1141–1148

41. Xu J, Wang F, Van Keymeulen A, Herzmark P, Straight A, Kelly K, Takuwa Y, Sugimoto N, Mitchison T, Bourne HR (2003) Divergent signals and cytoskeletal assemblies regulate self-organizing polarity in neutrophils. *Cell* 114: 201–214

42. Takahashi Y, Morales FC, Kreimann EL, Georgescu MM (2006) PTEN tumor suppressor associates with NHERF proteins to attenuate PDGF receptor signaling. *EMBO J* 25: 910–920

43. Wu Y, Dowbenko D, Spencer S, Laura R, Lee J, Gu Q, Lasky LA (2000) Interaction of the tumor suppressor PTEN/MMAC with a PDZ domain

of MAGI3, a novel membrane-associated guanylate kinase. *J Biol Chem* 275: 21477–21485

44. Wu X, Hepner K, Castelino-Prabhu S, Do D, Kaye MB, Yuan XJ, Wood J, Ross C, Sawyers CL, Whang YE (2000) Evidence for regulation of the PTEN tumor suppressor by a membrane-localized multi-PDZ domain containing scaffold protein MAGI-2. *Proc Natl Acad Sci USA* 97: 4233–4238

45. Adey NB, Huang L, Ormonde PA, Baumgard ML, Pero R, Byreddy DV, Tavtigian SV, Bartel PL (2000) Threonine phosphorylation of the MMAC1/PTEN PDZ binding domain both inhibits and stimulates PDZ binding. *Cancer Res* 60: 35–37

46. Valiente M, Andres-Pons A, Gomar B, Torres J, Gil A, Tapparel C, Antonarakis SE, Pulido R (2005) Binding of PTEN to specific PDZ domains contributes to PTEN protein stability and phosphorylation by microtubule-associated serine/threonine kinases. *J Biol Chem* 280: 28936–28943

47. Wu H, Feng W, Chen J, Chan LN, Huang S, Zhang M (2007) PDZ domains of Par-3 as potential phosphoinositide signaling integrators. *Mol Cell* 28: 886–898

48. Billadeau DD (2008) PTEN gives neutrophils direction. *Nat Immunol* 9: 716–718

49. Subramanian KK, Jia Y, Zhu D, Simms BT, Jo H, Hattori H, You J, Mizgerd JP, Luo HR (2007) Tumor suppressor PTEN is a physiologic suppressor of chemoattractant-mediated neutrophil functions. *Blood* 109: 4028–4037

50. Aderem A, Underhill DM (1999) Mechanisms of phagocytosis in macrophages. *Annu Rev Immunol* 17: 593–623

51. Yeung T, Ozdamar B, Paroutis P, Grinstein S (2006) Lipid metabolism and dynamics during phagocytosis. *Curr Opin Cell Biol* 18: 429–437

52. Vieira OV, Botelho RJ, Rameh L, Brachmann SM, Matsuo T, Davidson HW, Schreiber A, Backer JM, Cantley LC, Grinstein S (2001) Distinct roles of class I and class III phosphatidylinositol 3-kinases in phagosome formation and maturation. *J Cell Biol* 155: 19–25

53. Cox D, Berg JS, Cammer M, Chinegwundoh JO, Dale BM, Cheney RE, Greenberg S (2002) Myosin X is a downstream effector of PI(3)K during phagocytosis. *Nat Cell Biol* 4: 469–477

54. Ai J, Maturu A, Johnson W, Wang Y, Marsh CB, Tridandapani S (2006) The inositol phosphatase SHIP-2 down-regulates FcgammaR-mediated phagocytosis in murine macrophages independently of SHIP-1. *Blood*, 107: 813–820

55. Horan KA, Watanabe K, Kong AM, Bailey CG, Rasko JE, Sasaki T, Mitchell CA (2007) Regulation of FcgammaR-stimulated phagocytosis by the 72-kDa inositol polyphosphate 5-phosphatase: SHIP1, but not the 72-kDa 5-phosphatase, regulates complement receptor 3 mediated phagocytosis by differential recruitment of these 5-phosphatases to the phagocytic cup. *Blood* 110: 4480–4491

56. Botelho RJ, Teruel M, Dierckman R, Anderson R, Wells A, York JD, Meyer T, Grinstein S (2000) Localized biphasic changes in phosphatidylinositol-4,5-bisphosphate at sites of phagocytosis. *J Cell Biol* 151: 1353–1368

57. Fairn GD, Ogata K, Botelho RJ, Stahl PD, Anderson RA, De Camilli P, Meyer T, Wodak S, Grinstein S (2009) An electrostatic switch displaces phosphatidylinositol phosphate kinases from the membrane during phagocytosis. *J Cell Biol* 187: 701–714

58. Gao P, Wange RL, Zhang N, Oppenheim JJ, Howard OM (2005) Negative regulation of CXCR4-mediated chemotaxis by the lipid phosphatase activity of tumor suppressor PTEN. *Blood* 106: 2619–2626

59. Lacalle RA, Gomez-Mouton C, Barber DF, Jimenez-Baranda S, Mira E, Martinez AC, Carrera AC, Manes S (2004) PTEN regulates motility but not directionality during leukocyte chemotaxis. *J Cell Sci* 117: 6207–6215

60. Costa C, Barberis L, Ambrogio C, Manazza AD, Patrucco E, Azzolino O, Neilsen PO, Ciraolo E, Altruda F, Prestwich GD *et al* (2007) Negative feedback regulation of Rac in leukocytes from mice expressing a constitutively active phosphatidylinositol 3-kinase gamma. *Proc Natl Acad Sci USA* 104: 14354–14359

61. Totani L, Piccoli A, Manarini S, Federico L, Pecce R, Martelli N, Cerletti C, Piccardoni P, Lowell CA, Smyth SS *et al* (2006) Src-family kinases mediate an outside-in signal necessary for beta2 integrins to achieve full activation and sustain firm adhesion of polymorphonuclear leucocytes tethered on E-selectin. *Biochem J* 396: 89–98

62. Mocsai A, Abram CL, Jakus Z, Hu Y, Lanier LL, Lowell CA (2006) Integrin signaling in neutrophils and macrophages uses adaptors containing immunoreceptor tyrosine-based activation motifs. *Nat Immunol* 7: 1326–1333

63. Lowell CA, Fumagalli L, Berton G (1996) Deficiency of Src family kinases p59/61hck and p58c-fgr results in defective adhesion-dependent neutrophil functions. *J Cell Biol* 133: 895–910

64. Mocsai A, Ligeti E, Lowell CA, Berton G (1999) Adhesion-dependent degranulation of neutrophils requires the Src family kinases Fgr and Hck. *J Immunol* 162: 1120–1126

65. Carbo C, Duerschmied D, Goerge T, Hattori H, Sakai J, Cifuni SM, White GC, 2nd, Chrzanowska-Wodnicka M, Luo HR, Wagner DD (2010) Integrin-independent role of CalDAG-GEFI in neutrophil chemotaxis. *J Leukoc Biol* 88: 313–319

66. Dash-Koney M, Deevi RK, McFarlane C, Dib K (2011) Exchange protein directly activated by cAMP 1 (Epac1) is expressed in human neutrophils and mediates cAMP-dependent activation of the monomeric GTPase Rap1. *J Leukoc Biol* 90: 741–749

67. Lefort CT, Rossaint J, Moser M, Petrich BG, Zarbock A, Monkley SJ, Critchley DR, Ginsberg MH, Fassler R, Ley K (2012) Distinct roles for talin-1 and kindlin-3 in LFA-1 extension and affinity regulation. *Blood* 119: 4275–4282

68. Camps M, Rückle T, Ji H, Ardissone V, Rintelen F, Shaw J, Ferrandi C, Chabert C, Gillieron C, Françon B *et al* (2005) Blockade of PI3Kgamma suppresses joint inflammation and damage in mouse models of rheumatoid arthritis. *Nat Med* 11: 936–943

69. Smith DF, Deem TL, Bruce AC, Reutershan J, Wu D, Ley K (2006) Leukocyte phosphoinositide-3 kinase {gamma} is required for chemokine-induced, sustained adhesion under flow *in vivo*. *J Leukoc Biol* 80: 1491–1499

70. Liu L, Puri KD, Penninger JM, Kubes P (2007) Leukocyte PI3Kgamma and PI3Kdelta have temporally distinct roles for leukocyte recruitment *in vivo*. *Blood* 110: 1191–1198

71. Puri KD, Doggett TA, Douangpanya J, Hou Y, Tino WT, Wilson T, Graf T, Clayton E, Turner M, Hayflick JS *et al* (2004) Mechanisms and implications of phosphoinositide 3-kinase delta in promoting neutrophil trafficking into inflamed tissue. *Blood* 103: 3448–3456

72. Puri KD, Doggett TA, Huang CY, Douangpanya J, Hayflick JS, Turner M, Penninger J, Diacovo TG (2005) The role of endothelial PI3Kgamma activity in neutrophil trafficking. *Blood* 106: 150–157

73. Sasaki T, Irie-Sasaki J, Jones RG, Oliveira-dos-Santos AJ, Stanford WL, Bolon B, Wakeham A, Itie A, Bouchard D, Kozieradzki I *et al* (2000) Function of PI3Kgamma in thymocyte development, T cell activation, and neutrophil migration. *Science* 287: 1040–1046

74. Hirsch E, Katanaev VL, Garlanda C, Azzolino O, Pirola L, Silengo L, Sozzani S, Mantovani A, Altruda F, Wymann MP (2000) Central role for

G protein-coupled phosphoinositide 3-kinase gamma in inflammation. *Science* 287: 1049–1053

75. Barber DF, Bartolomé A, Hernandez C, Flores JM, Redondo C, Fernandez-Arias C, Camps M, Rückle T, Schwarz MK, Rodríguez S *et al* (2005) PI3Kgamma inhibition blocks glomerulonephritis and extends lifespan in a mouse model of systemic lupus. *Nat Med* 11: 933–935

76. Sadhu C, Masinovsky B, Dick K, Sowell CG, Staunton DE (2003) Essential role of phosphoinositide 3-kinase delta in neutrophil directional movement. *J Immunol* 170: 2647–2654

77. Sarraj B, Massberg S, Li Y, Kasorn A, Subramanian K, Loison F, Silberstein LE, von Andrian U, Luo HR (2009) Myeloid-specific deletion of tumor suppressor PTEN augments neutrophil transendothelial migration during inflammation. *J Immunol* 182: 7190–7200

78. Heit B, Robbins SM, Downey CM, Guan Z, Colarusso P, Miller BJ, Jirik FR, Kubes P (2008b) PTEN functions to "prioritize" chemotactic cues and prevent "distraction" in migrating neutrophils. *Nat Immunol* 9: 743–752

79. Heit B, Tavener S, Raharjo E, Kubes P (2002) An intracellular signaling hierarchy determines direction of migration in opposing chemotactic gradients. *J Cell Biol* 159: 91–102

80. Ittner A, Block H, Reichel CA, Varjosalo M, Gehart H, Sumara G, Gstaiger M, Krombach F, Zarbock A, Ricci R (2012) Regulation of PTEN activity by p38delta-PKD1 signaling in neutrophils confers inflammatory responses in the lung. *J Exp Med* 209: 2229–2246

81. Luo HR, Loison F (2008) Constitutive neutrophil apoptosis: mechanisms and regulation. *Am J Hematol* 83: 288–295

82. Zhu D, Hattori H, Jo H, Jia Y, Subramanian KK, Loison F, You J, Le Y, Honczarenko M, Silberstein L *et al* (2006) Deactivation of phosphatidyl-inositol 3,4,5-trisphosphate/Akt signaling mediates neutrophil spontaneous death. *Proc Natl Acad Sci USA* 103: 14836–14841

83. Liu Q, Sasaki T, Kozieradzki I, Wakeham A, Itie A, Dumont DJ, Penninger JM (1999) SHIP is a negative regulator of growth factor receptor-mediated PKB/Akt activation and myeloid cell survival. *Genes Dev* 13: 786–791

84. Gardai S, Whitlock BB, Helgason C, Ambruso D, Fadok V, Bratton D, Henson PM (2002) Activation of SHIP by NADPH oxidase-stimulated Lyn leads to enhanced apoptosis in neutrophils. *J Biol Chem* 277: 5236–5246

85. Yang KY, Arcaroli J, Kupfner J, Pitts TM, Park JS, Strassheim D, Perng RP, Abraham E (2003) Involvement of phosphatidylinositol 3-kinase gamma in neutrophil apoptosis. *Cell Signal* 15: 225–233

86. Webb PR, Wang KQ, Scheel-Toellner D, Pongracz J, Salmon M, Lord JM (2000) Regulation of neutrophil apoptosis: a role for protein kinase C and phosphatidylinositol-3-kinase. *Apoptosis* 5: 451–458

87. Chen J, Tang H, Hay N, Xu J, Ye RD (2010) Akt isoforms differentially regulate neutrophil functions. *Blood* 115: 4237–4246

88. Xu Y, Loison F, Luo HR (2010) Neutrophil spontaneous death is mediated by down-regulation of autocrine signaling through GPCR, PI3Kgamma, ROS, and actin. *Proc Natl Acad Sci USA* 107: 2950–2955

89. Souza LR, Silva E, Calloway E, Cabrera C, McLemore ML (2013) G-CSF activation of AKT is not sufficient to prolong neutrophil survival. *J Leukoc Biol* 93: 883–893

90. Anderson KE, Boyle KB, Davidson K, Chessa TA, Kulkarni S, Jarvis GE, Sindrilaru A, Scharffetter-Kochanek K, Rausch O, Stephens LR *et al* (2008) CD18-dependent activation of the neutrophil NADPH oxidase during phagocytosis of *Escherichia coli* or *Staphylococcus aureus* is regulated by class III but not class I or II PI3Ks. *Blood*, 112: 5202–5211

91. Damen JE, Liu L, Rosten P, Humphries RK, Jefferson AB, Majerus PW, Krystal G (1996) The 145-kDa protein induced to associate with Shc by multiple cytokines is an inositol tetraphosphate and phosphatidylinositol 3,4,5-triphosphate 5-phosphatase. *Proc Natl Acad Sci USA* 93: 1689–1693

92. Helgason CD, Damen JE, Rosten P, Grewal R, Sorensen P, Chappel SM, Borowski A, Jirik F, Krystal G, Humphries RK (1998) Targeted disruption of SHIP leads to hemopoietic perturbations, lung pathology, and a shortened life span. *Genes Dev* 12: 1610–1620

93. Huber M, Helgason CD, Damen JE, Liu L, Humphries RK, Krystal G (1998) The src homology 2-containing inositol phosphatase (SHIP) is the gatekeeper of mast cell degranulation. *Proc Natl Acad Sci USA* 95: 11330–11335

94. Sly LM, Ho V, Antignano F, Ruschmann J, Hamilton M, Lam V, Rauh MJ, Krystal G (2007) The role of SHIP in macrophages. *Front Biosci* 12: 2836–2848

95. Leung WH, Tarasenko T, Bolland S (2009) Differential roles for the inositol phosphatase SHIP in the regulation of macrophages and lymphocytes. *Immunol Res* 43: 243–251

96. Parry RV, Harris SJ, Ward SG (2010) Fine tuning T lymphocytes: a role for the lipid phosphatase SHIP-1. *Biochim Biophys Acta* 1804: 592–597

97. Hazen AL, Smith MJ, Desponts C, Winter O, Moser K, Kerr WG (2009) SHIP is required for a functional hematopoietic stem cell niche. *Blood* 113: 2924–2933

98. Liang OD, Lu J, Nombela-Arrieta C, Zhong J, Zhao L, Pivarnik G, Mondal S, Chai L, Silberstein LE, Luo HR (2013) Deficiency of lipid phosphatase SHIP enables long-term reconstitution of hematopoietic inductive bone marrow microenvironment. *Dev Cell* 25: 333–349

99. Nishio M, Watanabe K, Sasaki J, Taya C, Takasuga S, Iizuka R, Balla T, Yamazaki M, Watanabe H, Itoh R *et al* (2007) Control of cell polarity and motility by the PtdIns(3,4,5)P3 phosphatase SHIP1. *Nat Cell Biol* 9: 36–44

100. Mondal S, Subramanian KK, Sakai J, Bajrami B, Luo HR (2012) Phospho-inositide lipid phosphatase SHIP1 and PTEN coordinate to regulate cell migration and adhesion. *Mol Biol Cell* 23: 1219–1230

101. Phee H, Jacob A, Coggeshall KM (2000) Enzymatic activity of the Src homology 2 domain-containing inositol phosphatase is regulated by a plasma membrane location. *J Biol Chem* 275: 19090–19097

102. Tu Z, Ninos JM, Ma Z, Wang JW, Lemos MP, Desponts C, Ghansah T, Howson JM, Kerr WG (2001) Embryonic and hematopoietic stem cells express a novel SH2-containing inositol 5′-phosphatase isoform that partners with the Grb2 adapter protein. *Blood* 98: 2028–2038

103. Zhang S, Mantel C, Broxmeyer HE (1999) Flt3 signaling involves tyrosyl-phosphorylation of SHP-2 and SHIP and their association with Grb2 and Shc in Baf3/Flt3 cells. *J Leukoc Biol* 65: 372–380

104. Lamkin TD, Walk SF, Liu L, Damen JE, Krystal G, Ravichandran KS (1997) Shc interaction with Src homology 2 domain containing inositol phosphatase (SHIP) *in vivo* requires the Shc-phosphotyrosine binding domain and two specific phosphotyrosines on SHIP. *J Biol Chem* 272: 10396–10401

105. Lemay S, Davidson D, Latour S, Veillette A (2000) Dok-3, a novel adapter molecule involved in the negative regulation of immuno-receptor signaling. *Mol Cell Biol* 20: 2743–2754

106. Maxwell MJ, Yuan Y, Anderson KE, Hibbs ML, Salem HH, Jackson SP (2004) SHIP1 and Lyn kinase negatively regulate integrin alpha IIb beta 3 signaling in platelets. *J Biol Chem* 279: 32196–32204

107. Henderson LM, Chappel JB (1996) NADPH oxidase of neutrophils. *Biochim Biophys Acta* 1273: 87–107

108. Heyworth PG, Cross AR, Curnutte JT (2003) Chronic granulomatous disease. *Curr Opin Immunol* 15: 578–584

109. Babior BM, Lambeth JD, Nauseef W (2002) The neutrophil NADPH oxidase. *Arch Biochem Biophys* 397: 342–344

110. Segal BH, Leto TL, Gallin JI, Malech HL, Holland SM (2000) Genetic, biochemical, and clinical features of chronic granulomatous disease. *Medicine* 79: 170–200

111. Dinauer MC (2005) Chronic granulomatous disease and other disorders of phagocyte function. *Hematology Am Soc Hematol Educ Program*, 2005: 89–95

112. Subramanian KK, Luo HR (2009) Non-classical roles of NADPH-oxidase dependent reactive oxygen species in phagocytes. In *Granulocytes: Classification, toxic materials produced and pathology*, Hagg R, Kohlund S (eds), pp. 137–148. Hauppauge NY: Nova Science Publishers, Inc

113. Dahlgren C, Karlsson A (1999) Respiratory burst in human neutrophils. *J Immunol Methods* 232: 3–14

114. Hattori H, Subramanian KK, Sakai J, Jia Y, Li Y, Porter TF, Loison F, Sarraj B, Kasorn A, Jo H *et al* (2010) Small-molecule screen identifies reactive oxygen species as key regulators of neutrophil chemotaxis. *Proc Natl Acad Sci USA* 107: 3546–3551

115. Roberts AW, Kim C, Zhen L, Lowe JB, Kapur R, Petryniak B, Spaetti A, Pollock JD, Borneo JB, Bradford GB *et al* (1999) Deficiency of the hematopoietic cell-specific Rho family GTPase Rac2 is characterized by abnormalities in neutrophil function and host defense. *Immunity* 10: 183–196

116. Li Z, Jiang H, Xie W, Zhang Z, Smrcka AV, Wu D (2000) Roles of PLC-beta2 and -beta3 and PI3Kgamma in chemoattractant-mediated signal transduction. *Science* 287: 1046–1049

117. Ding J, Vlahos CJ, Liu R, Brown RF, Badwey JA (1995) Antagonists of phosphatidylinositol 3-kinase block activation of several novel protein kinases in neutrophils. *J Biol Chem* 270: 11684–11691

118. Vlahos CJ, Matter WF, Brown RF, Traynor-Kaplan AE, Heyworth PG, Prossnitz ER, Ye RD, Marder P, Schelm JA, Rothfuss KJ *et al* (1995) Investigation of neutrophil signal transduction using a specific inhibitor of phosphatidylinosit
    ol 3-kinase. *J Immunol* 154: 2413–2422

119. Sue AQAK, Fialkow L, Vlahos CJ, Schelm JA, Grinstein S, Butler J, Downey GP (1997) Inhibition of neutrophil oxidative burst and granule secretion by wortmannin: potential role of MAP kinase and renaturable kinases. *J Cell Physiol* 172: 94–108

120. Condliffe AM, Davidson K, Anderson KE, Ellson CD, Crabbe T, Okkenhaug K, Vanhaesebroeck B, Turner M, Webb L, Wymann MP *et al* (2005) Sequential activation of class IB and class IA PI3K is important for the primed respiratory burst of human but not murine neutrophils. *Blood* 106: 1432–1440

121. DeLeo FR, Renee J, McCormick S, Nakamura M, Apicella M, Weiss JP, Nauseef WM (1998) Neutrophils exposed to bacterial lipopolysaccharide upregulate NADPH oxidase assembly. *J Clin Invest* 101: 455–463

122. Welch HC, Condliffe AM, Milne LJ, Ferguson GJ, Hill K, Webb LM, Okkenhaug K, Coadwell WJ, Andrews SR, Thelen M *et al* (2005) P-Rex1 regulates neutrophil function. *Curr Biol* 15: 1867–1873

123. Lemmon MA (2008) Membrane recognition by phospholipid-binding domains. *Nat Rev Mol Cell Biol* 9: 99–111

124. Luo HR, Huang YE, Chen JC, Saiardi A, Iijima M, Ye K, Huang Y, Nagata E, Devreotes P, Snyder SH (2003) Inositol pyrophosphates mediate chemotaxis in Dictyostelium via pleckstrin homology domain-PtdIns (3,4,5)P3 interactions. *Cell* 114: 559–572

125. Jia Y, Subramanian KK, Erneux C, Pouillon V, Hattori H, Jo H, You J, Zhu D, Schurmans S, Luo HR (2007) Inositol 1,3,4,5-tetrakisphosphate

126. Saiardi A (2012) Cell signalling by inositol pyrophosphates. *Subcell Biochem* 59: 413–443

127. Shears SB, Weaver JD, Wang H (2013) Structural insight into inositol pyrophosphate turnover. *Adv Biol Regul* 53: 19–27

128. Shears SB (2004) How versatile are inositol phosphate kinases? *Biochem J* 377: 265–280

129. Burton A, Hu X, Saiardi A (2009) Are inositol pyrophosphates signalling molecules? *J Cell Physiol* 220: 8–15

130. Saiardi A, Nagata E, Luo HR, Snowman AM, Snyder SH (2001) Identification and characterization of a novel inositol hexakisphosphate kinase. *J Biol Chem* 276: 39179–39185

131. Prasad A, Jia Y, Chakraborty A, Li Y, Jain SK, Zhong J, Roy SG, Loison F, Mondal S, Sakai J *et al* (2011) Inositol hexakisphosphate kinase 1 regulates neutrophil function in innate immunity by inhibiting phosphatidylinositol-(3,4,5)-trisphosphate signaling. *Nat Immunol* 12: 752–760

132. Xu Y, Li H, Bajrami B, Kwak H, Cao S, Liu P, Zhou J, Zhou Y, Zhu H, Ye K *et al* (2013) Cigarette smoke (CS) and nicotine delay neutrophil spontaneous death via suppressing production of diphosphoinositol pentakisphosphate. *Proc Natl Acad Sci USA* 110: 7726–7731

133. Chakraborty A, Koldobskiy MA, Bello NT, Maxwell M, Potter JJ, Juluri KR, Maag D, Kim S, Huang AS, Dailey MJ *et al* (2010) Inositol pyrophosphates inhibit Akt signaling, thereby regulating insulin sensitivity and weight gain. *Cell* 143: 897–910

134. Huang CF, Voglmaier SM, Bembenek ME, Saiardi A, Snyder SH (1998) Identification and purification of diphosphoinositol pentakisphosphate kinase, which synthesizes the inositol pyrophosphate bis(diphospho) inositol tetrakisphosphate. *Biochemistry* 37: 14998–15004

135. Saiardi A, Erdjument-Bromage H, Snowman AM, Tempst P, Snyder SH (1999) Synthesis of diphosphoinositol pentakisphosphate by a newly identified family of higher inositol polyphosphate kinases. *Curr Biol* 9: 1323–1326

136. Saiardi A, Caffrey JJ, Snyder SH, Shears SB (2000) The inositol hexakisphosphate kinase family. Catalytic flexibility and function in yeast vacuole biogenesis. *J Biol Chem* 275: 24686–24692

137. Stuart JA, Anderson KL, French PJ, Kirk CJ, Michell RH (1994) The intracellular distribution of inositol polyphosphates in HL60 promyeloid cells. *Biochem J* 303: 517–525

138. Bradford PG, Rubin RP (1986) Quantitative changes in inositol 1,4,5-trisphosphate in chemoattractant-stimulated neutrophils. *J Biol Chem* 261: 15644–15647

139. Irvine RF, Schell MJ (2001) Back in the water: the return of the inositol phosphates. *Nat Rev Mol Cell Biol* 2: 327–338

140. Rhee SG (2001) Regulation of phosphoinositide-specific phospholipase C. *Annu Rev Biochem* 70: 281–312

141. Wu D, Huang CK, Jiang H (2000b) Roles of phospholipid signaling in chemoattractant-induced responses. *J Cell Sci* 113(Pt 17): 2935–2940

142. Cicchetti G, Allen PG, Glogauer M (2002) Chemotactic signaling pathways in neutrophils: from receptor to actin assembly. *Crit Rev Oral Biol Med* 13: 220–228

143. Choi KY, Kim HK, Lee SY, Moon KH, Sim SS, Kim JW, Chung HK, Rhee SG (1990) Molecular cloning and expression of a complementary DNA for inositol 1,4,5-trisphosphate 3-kinase. *Science* 248: 64–66

144. Nalaskowski MM, Bertsch U, Fanick W, Stockebrand MC, Schmale H, Mayr GW (2003) Rat inositol 1,4,5-trisphosphate 3-kinase C is enzymatically specialized for basal cellular inositol trisphosphate

negatively regulates phosphatidylinositol-3,4,5- trisphosphate signaling in neutrophils. *Immunity* 27: 453–467

phosphorylation and shuttles actively between nucleus and cytoplasm. *J Biol Chem* 278: 19765 – 19776

145. Dewaste V, Roymans D, Moreau C, Erneux C (2002) Cloning and expression of a full-length cDNA encoding human inositol 1,4,5-trisphosphate 3-kinase B. *Biochem Biophys Res Commun* 291: 400 – 405

146. Sauer K, Cooke MP (2010) Regulation of immune cell development through soluble inositol-1,3,4,5-tetrakisphosphate. *Nat Rev Immunol* 10: 257 – 271

147. Sauer K, Park E, Siegemund S, French AR, Wahle JA, Sternberg L, Rigaud S, Jonsson AH, Yokoyama WM, Huang YH (2013) Inositol tetrakisphosphate limits NK cell effector functions by controlling PI3K signaling. *Blood* 121: 286 – 297

148. Pouillon V, Hascakova-Bartova R, Pajak B, Adam E, Bex F, Dewaste V, Van Lint C, Leo O, Erneux C, Schurmans S (2003) Inositol 1,3,4,5-tetrakisphosphate is essential for T lymphocyte development. *Nat Immunol* 4: 1136 – 1143

149. Wen BG, Pletcher MT, Warashina M, Choe SH, Ziaee N, Wiltshire T, Sauer K, Cooke MP (2004) Inositol (1,4,5) trisphosphate 3 kinase B controls positive selection of T cells and modulates Erk activity. *Proc Natl Acad Sci USA* 101: 5604 – 5609

150. Marechal Y, Queant S, Polizzi S, Pouillon V, Schurmans S (2011) Inositol 1,4,5-trisphosphate 3-kinase B controls survival and prevents anergy in B cells. *Immunobiology* 216: 103 – 109

151. Pouillon V, Marechal Y, Frippiat C, Erneux C, Schurmans S (2013) Inositol 1,4,5-trisphosphate 3-kinase B (Itpkb) controls survival, proliferation and cytokine production in mouse peripheral T cells. *Adv Biol Regul* 53: 39 – 50

152. Huang YH, Grasis JA, Miller AT, Xu R, Soonthornvacharin S, Andreotti AH, Tsoukas CD, Cooke MP, Sauer K (2007) Positive regulation of Itk PH domain function by soluble IP4. *Science* 316: 886 – 889

153. Miller AT, Sandberg M, Huang YH, Young M, Sutton S, Sauer K, Cooke MP (2007) Production of Ins(1,3,4,5)P4 mediated by the kinase Itpkb inhibits store-operated calcium channels and regulates B cell selection and activation. *Nat Immunol* 8: 514 – 521

154. Marechal Y, Pesesse X, Jia Y, Pouillon V, Pérez-Morga D, Daniel J, Izui S, Cullen PJ, Leo O, Luo HR *et al* (2007) Inositol 1,3,4,5-tetrakisphosphate controls proapoptotic Bim gene expression and survival in B cells. *Proc Natl Acad Sci USA* 104: 13978 – 13983

155. Jia Y, Loison F, Hattori H, Li Y, Erneux C, Park SY, Gao C, Chai L, Silberstein LE, Schurmans S *et al* (2008) Inositol trisphosphate 3-kinase B (InsP3KB) as a physiological modulator of myelopoiesis. *Proc Natl Acad Sci USA* 105: 4739 – 4744

156. Jia Y, Schurmans S, Luo HR (2008) Regulation of innate immunity by inositol 1,3,4,5-tetrakisphosphate. *Cell Cycle* 7: 2803 – 2808

157. Schell MJ, Erneux C, Irvine RF (2001) Inositol 1,4,5-trisphosphate 3-kinase A associates with F-actin and dendritic spines via its N terminus. *J Biol Chem* 276: 37537 – 37546

158. Schell MJ, Irvine RF (2006) Calcium-triggered exit of F-actin and IP(3) 3-kinase A from dendritic spines is rapid and reversible. *Eur J Neurosci* 24: 2491 – 2503

159. Dewaste V, Moreau C, De Smedt F, Bex F, De Smedt H, Wuytack F, Missiaen L, Erneux C (2003) The three isoenzymes of human inositol-1,4,5-trisphosphate 3-kinase show specific intracellular localization but comparable Ca^{2+} responses on transfection in COS-7 cells. *Biochem J* 374: 41 – 49

160. Billottet C, Grandage VL, Gale RE, Quattropani A, Rommel C, Vanhaesebroeck B, Khwaja A (2006) A selective inhibitor of the p110delta isoform of PI 3-kinase inhibits AML cell proliferation and

161. Rommel C, Camps M, Ji H (2007) PI3K delta and PI3K gamma: partners in crime in inflammation in rheumatoid arthritis and beyond? *Nat Rev Immunol* 7: 191 – 201

162. Ito K, Caramori G, Adcock IM (2007) Therapeutic potential of phosphatidylinositol 3-kinase inhibitors in inflammatory respiratory disease. *J Pharmacol Exp Ther* 321: 1 – 8

163. Park SJ, Min KH, Lee YC (2008) Phosphoinositide 3-kinase delta inhibitor as a novel therapeutic agent in asthma. *Respirology* 13: 764 – 771

164. Venable JD, Ameriks MK, Blevitt JM, Thurmond RL, Fung-Leung WP (2010) Phosphoinositide 3-kinase gamma (PI3Kgamma) inhibitors for the treatment of inflammation and autoimmune disease. *Recent Pat Inflamm Allergy Drug Discov* 4: 1 – 15

165. Blajecka K, Borgstrom A, Arcaro A (2011) Phosphatidylinositol 3-kinase isoforms as novel drug targets. *Curr Drug Targets* 12: 1056 – 1081

166. Blunt MD, Ward SG (2012) Targeting PI3K isoforms and SHIP in the immune system: new therapeutics for inflammation and leukemia. *Curr Opin Pharmacol* 12: 444 – 451

167. Foster JG, Blunt MD, Carter E, Ward SG (2012) Inhibition of PI3K signaling spurs new therapeutic opportunities in inflammatory/autoimmune diseases and hematological malignancies. *Pharmacol Rev* 64: 1027 – 1054

168. Stenton GR, Mackenzie LF, Tam P, Cross JL, Harwig C, Raymond J, Toews J, Wu J, Ogden N, MacRury T *et al* (2013) Characterization of AQX-1125, a small-molecule SHIP1 activator: part 1. Effects on inflammatory cell activation and chemotaxis *in vitro* and pharmacokinetic characterization *in vivo*. *Br J Pharmacol* 168: 1506 – 1518

169. Stenton GR, Mackenzie LF, Tam P, Cross JL, Harwig C, Raymond J, Toews J, Chernoff D, MacRury T, Szabo C (2013) Characterization of AQX-1125, a small-molecule SHIP1 activator: part 2. Efficacy studies in allergic and pulmonary inflammation models *in vivo*. *Br J Pharmacol* 168: 1519 – 1529

170. Anderlini P, Przepiorka D, Champlin R, Korbling M (1996) Biologic and clinical effects of granulocyte colony-stimulating factor in normal individuals. *Blood* 88: 2819 – 2825

171. Joos L, Tamm M (2005) Breakdown of pulmonary host defense in the immunocompromised host: cancer chemotherapy. *Proc Am Thorac Soc* 2: 445 – 448

172. Viscoli C, Varnier O, Machetti M (2005) Infections in patients with febrile neutropenia: epidemiology, microbiology, and risk stratification. *Clin Infect Dis* 40(Suppl. 4): S240 – S245

173. Leung AN, Gosselin MV, Napper CH, Braun SG, Hu WW, Wong RM, Gasman J (1999) Pulmonary infections after bone marrow transplantation: clinical and radiographic findings. *Radiology* 210: 699 – 710

174. Li Y, Jia Y, Pichavant M, Loison F, Sarraj B, Kasorn A, You J, Robson BE, Umetsu DT, Mizgerd JP *et al* (2009) Targeted deletion of tumor suppressor PTEN augments neutrophil function and enhances host defense in neutropenia-associated pneumonia. *Blood* 113: 4930 – 4941

175. Li Y, Prasad A, Jia Y, Roy SG, Loison F, Mondal S, Kocjan P, Silberstein LE, Ding S, Luo HR (2011) Pretreatment with phosphatase and tensin homolog deleted on chromosome 10 (PTEN) inhibitor SF1670 augments the efficacy of granulocyte transfusion in a clinically relevant mouse model. *Blood* 117: 6702 – 6713

176. Schabbauer G, Matt U, Günzl P, Warszawska J, Furtner T, Hainzl E, Elbau I, Mesteri I, Doninger B, Binder BR *et al* (2010) Myeloid PTEN promotes inflammation but impairs bactericidal activities during murine pneumococcal pneumonia. *J Immunol* 185: 468 – 476

survival and increases the cytotoxic effects of VP16. *Oncogene* 25: 6648 – 6659

177. Schell MJ, Letcher AJ, Brearley CA, Biber J, Murer H, Irvine RF (1999) PiUS (Pi uptake stimulator) is an inositol hexakisphosphate kinase. *FEBS Lett* 461: 169−172

178. Lemmon MA, Ferguson KM (2000) Signal-dependent membrane targeting by pleckstrin homology (PH) domains. *Biochem J* 350(Pt 1): 1−18

179. Maffucci T, Falasca M (2001) Specificity in pleckstrin homology (PH) domain membrane targeting: a role for a phosphoinositide-protein co-operative mechanism. *FEBS Lett* 506: 173−179

180. Rebecchi MJ, Scarlata S (1998) Pleckstrin homology domains: a common fold with diverse functions. *Annu Rev Biophys Biomol Struct* 27: 503−528

181. Kavran JM, Klein DE, Lee A, Falasca M, Isakoff SJ, Skolnik EY, Lemmon MA (1998) Specificity and promiscuity in phosphoinositide binding by pleckstrin homology domains. *J Biol Chem* 273: 30497−30508