Role of inositol phospholipid signaling in natural killer cell biology

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Natural killer (NK) cells are important for host defense against malignancy and infection. At a cellular level NK cells are activated when signals from activating receptors exceed signaling from inhibitory receptors. At a molecular level NK cells undergo an education process to both prevent autoimmunity and acquire lytic capacity. Mouse models have shown important roles for inositol phospholipid signaling in lymphocytes. NK cells from mice with deletion in different members of the inositol phospholipid signaling pathway exhibit defects in development, NK cell repertoire expression and effector function. Here we review the current state of knowledge concerning the function of inositol phospholipid signaling components in NK cell biology.

Keywords: inositol phospholipid, PIP5K, SHIP, PTEN, PI3K, IFNγ, natural killer cells, INPP4

Unlike T and B lymphocytes, natural killer (NK) cells do not rearrange antigen receptor genes in order to detect their cellular targets (Lanier, 1998). Rather, NK cells utilize an array of activating and inhibitory receptors with the latter largely detecting major histocompatibility complex (MHC) class I ligands, or in the case of 2B4, the signaling lymphocyte activation molecule (SLAM) family ligand CD48. Both activating and inhibitory NK receptors are stochastically expressed with frequencies in the NK compartment determined by their relative promoter strength, and in some cases, survival differences among NK subsets determined by the presence or absence of ligands their receptor array can detect and their relative affinity for that ligand (Manlay et al., 1999; Loeslin-Kroopf and Held, 2000; Wang et al., 2002; Fortenbery et al., 2010). Inhibitory receptors allow for the NK cell to recognize and ignore “healthy-self” cells while activating receptors enable the NK cell to recognize and lyse foreign or “damaged-self” cells or antibody bound cells. In some instances the NK cell may also produce inflammatory cytokines such as interferon (IFN) in response to target cell engagement (Vivier et al., 2011). Individual NK cells in the compartment can express different combinations of activating and inhibitory receptors, but also different levels of certain receptors (Bryceson et al., 2011). The final balance of activating and inhibitory receptors, and the presence or absence of ligands, determines a threshold for activation of an individual NK cell (Lanier, 1998; Bryceson et al., 2011; Vivier et al., 2011). In extreme cases the NK cell may even be energized by unopposed activating signals (Baulier and Vance, 2006). This repertoire diversity in the NK cell compartment of an individual allows for a response to a diverse range of stimuli including an early response to virus-infected cells (Brandstätter and Yang, 2011) and surveillance for residual tumor cells (Vivier et al., 2008).

Phosphatidylinositol (PI) is a membrane lipid found in all cell types that can be phosphorylated to form phosphatidylinositol 3-monophosphate (PI(3)P), PI(4)P or PI(5)P. Each of these PIP species can be further phosphorylated by phosphatidylinositol 3-kinase (PI3K), PI4K, or PI5K to form PI(3,4)P2, PI(3,4,5)P3, or PI(4,5)P2. PI(3,4)P2, PI(3,4,5)P3, and PI(4,5)P2 allow for recruitment to the plasma membrane of pleckstrin homology (PH) domain-containing proteins (several other domains are also able to recruit proteins to these lipids as well and will be discussed below) as shown in Figure 1 and Table 1. PI(4,5)P2 is also important in NK cell signaling by acting as the substrate for phospholipase C (PLC), which hydrolyzes PI(4,5)P2 into diacylglycerol (DAG), to activate PKC and inositol 1,4,5-trisphosphate ([I(1,4,5)P3] which triggers release of intracellular Ca2+ stores. PIP2 and PIP3 can be modified by various phosphatases including inositol polyphosphate 4-phosphatase (INPP4) and SH2 domain-containing inositol 5-phosphatase (SHIP) or modified by phosphatase and tensin homologue deleted on chromosome 10 (PTEN) to create PI(3)P, PI(3,4)P2, or PI(4,5)P2, respectively. These activities can attenuate signaling pathways or, in the case of the SHIP product PI(3,4)P2, activate them by enabling recruitment of proteins with various PH domain-containing proteins to sites of signaling at the plasma membrane (Kerr, 2011). Here we will discuss the role of the above IP modifying enzymes in the context of NK cell biology.

PIP5K
Phosphatidylinositol 4-phosphate can be phosphorylated by type 1 phosphatidylinositol phosphate kinases (PIPK1s) to form PI(4,5)P2. Three isoforms of phosphatidylinositol 4-phosphate

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FIGURE 1: Inositol phospholipid family members allow for recruitment of proteins with indicated binding domains. Modification of the inositol phospholipids by the indicated enzymes allows for either the attenuation or promotion of signaling pathways that determine NK cell development, function, survival, and/or trafficking in the host. PIP5K, PLC, phospholipase C, DAG, diacylglycerol, PIP5K, phosphatidylinositol 4-phosphate 5-kinase; PLC, phospholipase C; PTEN, phosphatase and tensin homologue deleted on chromosome 10; SHIP, inositol polyphosphate 4-kinase; PH, pleckstrin homology; ENTH, epsin N-terminal homology; ANTH, AP180 N-terminal homology.

Table 1: PI modifying enzymes (PIP5K, PI3K, PTEN, SHIP1, and INPP4) are either recruited to or activated following signaling through the indicated NK cell receptor.

| Receptors | PIP5K | PI3K | PTEN | SHIP1 | INPP4 |
|-----------|-------|------|------|-------|------|
| CD16      | Ly49A | ?    | Ly49A | ?     |       |
| LFA-1     | Ly49C1|      | Ly49C1|       |       |
| DNAM-1    | KIR2DL1| CDS2|      |       |       |
| NKp46     | KIR2DL3| 2B4 |      |       |       |
| 2B4       | KIR2DL1| FcγRIIB|     |       |       |
| NK02D     | KIR2DL2| NK02D| NK1.1|       |       |
| NK1.1     | NKp46 | Ly49D|       |       |       |

Other important interactions: Talin, Arf6, Downstream pathway/critical effector: PLCγ3, Akt, MAP/Erk, WASp, Munc13-4, JNK1/2 (p110δ−/−), Downstream gene targets: Cytolytic competency, Immune synapse formation, Cytokine production (Vs44KNT), NK development and NKRR formation (Vs14KNT), Serial killing, Cytokine production (Vs14KNT), Cytolytic competency.

Following activation, the enzyme allows for activation of downstream signaling pathways through the production of inositol phospholipid species (as indicated in Figure 1) and leading to specific effector functions as indicated. Receptors listed in orange indicate that a physical interaction between the receptor and given enzyme has not yet been established but that the enzyme is required for proper downstream signaling.
5-kinase have been described (PIP5Kα, PIP5Kβ, PIP5Kγ, Ishi-hara et al., 1996; Liojens and Anderson, 1996; Tobias et al., 1998) with PIP5Kα and PIP5Kγ playing important, non-redundant roles in cell signaling through the production of PI(4,5)P2 in NK cells (Miccucci et al., 2008). PI(4,5)P2 is a major phosphoinositide at the plasma membrane (McLaughlin et al., 2002). It is believed that there are different pools of PI(4,5)P2 in cells, inside and outside of lipid rafts, that control different signaling pathways by allowing for localized changes in PI(4,5)P2 concentration (Pike and Casey, 1996; Hinchliffe et al., 1998; Martin, 2001; McLaughlin and Murray, 2005; Golobieva et al., 2008; Johnson et al., 2008).

Following NK cell activation, ADP-ribosylation factor 6 (Arf6) and talin recruit PIP5Kα and PIP5Kγ to the immunological synapse, respectively (Di Paolo et al., 2002; Galandrini et al., 2008). Expression of both isoforms is required for cytolytic activity of the NK cell (Badour et al., 2003; Mace et al., 2010). This is an important trait of NK cells as they are able to kill multiple target cells (Bhat and Watzl, 2007). PIP5Kα may play a role in serial killing as it is required for the regulation of the soluble N-ethylmaleimide-sensitive factor activating protein receptor (SNARE) protein Munc13-4 that mediates lytic granule recycling. Hence, PIP5Kα+/− NK cells are unable to serially lyse target cells as efficiently as wild type NK cells (Capuano et al., 2012).

Initially, decreased levels of PIP5Kα or PIP5Kγ have no impact on IFNγ production and do not alter PTK signaling in NK cells as measured by Akt activation and Vav-1 phosphorylation (Miccucci et al., 2008). Thus, PIP5Ks are required for sustained cytolytic competence, but are dispensable for cytokine production by NK cells.

Having different pools of PI(4,5)P2 in the membrane may allow diverse cell functions to be compartmentalized via production of key second messengers [e.g., DAG, IP3 and PI(3,4,5)P3] by diverse cell functions to be compartmentalized via production of key second messengers [e.g., DAG, IP3 and PI(3,4,5)P3] by selective recruitment of different signaling proteins with PI(4,5)P2 binding domains and the regulation of ion channels preferentially localized to these compartments (Smeyers and Shapiro, 2007). There are several domains that enable PI(4,5)P2 binding by a protein: PH, epsin N-terminal homology (ENTH), AP180 N-terminal homology (ANTH), FERM, and C2 domains (Ferguson et al., 1995; Hamada et al., 2000; Ford et al., 2001). PH domains enable signaling proteins to selectively bind different PI(4,5)P2 species while ENTH and ANTH domain-containing proteins have a higher affinity for PI(4,5)P2 than for other inositol phospholipids (Ford et al., 2001; Itoh et al., 2001). Wiskott–Aldrich syndrome protein (WASP), the clathrin adaptor AP1, the actin nucleating protein Arp2/3, and talin make use of ENTH and ANTH domains for recruitment to the plasma membrane. As NK cells are required to reorganize actin to form an immunological synapse before lysing a target cell (Rak et al., 2011). There is recent evidence that clathrin and AP1 aid in this process in T cells (Alvarer Arias et al., 2010; Calabia-Linares et al., 2011). After NK cell activation, increased PI(4,5)P2 levels recruit WASp to the membrane which in turn activates the actin nucleating protein complex Arp2/3 allowing for actin rearrangement and formation of the NK immunological synapse (Badour et al., 2003; Mace et al., 2010). Thus, PI(4,5)P2 also plays a critical role in actin reorganization and creation of the immune synapse.

**PI3K**

There are three different classes of PI3K enzymes; class I enzymes exist as a heterodimer between a catalytic subunit and a regulatory subunit. Class I PI3K enzymes are p110α (PI3KCα), p110β (PI3KCβ), and p110δ (PI3KCδ), which can pair with one of five regulatory subunits p55γ, p50γ, p50α (alternatively spliced from PI3KRIIγ), p85α (PI3KIIα), and p85γ (PI3Kγ). There is one PI3K class Ib enzyme: p105γ (PI3KCγ) which heterodimerizes with either p101 (PI3KRIIβ) or p87 (PI3KRIIα). There are three class II enzymes (PI3K-C2α, PI3K-C2β, and PI3K-C2γ) that have a poorly defined role in cell signaling and do not heterodimerize with a regulatory subunit. One PI3K class III enzyme (VPS4) has recently been identified which heterodimerizes with its regulatory subunit VPS15 to catalyze the formation of PI(3)P from PI. Class I PI3K enzymes (in leukocytes primarily p110γ and p110β) are the main enzymes responsible for the phosphorylation of the D-3 position of PI(4,5)P2 to create PI(3)P. PI3K expression in NK cells is required for lymphocyte function- associated antigen-1 (LFA-1) adherence to intercellular adhesion molecule-1 (ICAM)-expressing cells and thus, important for formation of the NK immune synapse (Barber and Long, 2003) and for facilitating signaling through various NK activating receptors (Barber et al., 2004). In addition, the 2B4 and killer cell immunoglobulin-like receptors (KIR; that sense self-ligands CD48 and MHC class I, respectively) can also recruit PI3K (Marti et al., 1998; Aukaty and Jan, 2002; Eisemann et al., 2003), and this may enable these receptors to have self-licensing roles (Fortenbery et al., 2010).

The PI3K signaling cascade has emerged as an essential intra- cellular signaling pathway in NK cell biology. The spleen tyrosine kinase (Syk) is able to activate the PI3K→ Rac1→ PAK1→ MEK→ ERK signaling pathway leading to NK cell degranulation (Iang et al., 2000, 2002, 2003). We believe that PI3K might also promote Bruton’s tyrosine kinase (Btk) activation in NK cells given that increased PI(3,4,5)P3 levels in other hematopoietic cell types lead to Btk activation (Kawakami et al., 2000; Saito et al., 2001) and that Btk has recently been shown to be required for proper NK cell activation (Bao et al., 2012). Interestingly, Btk has been shown to regulate PIP5Ks (and thus PI(4,5)P2 production) in B cells (Saito et al., 2003). Thus, the interaction between Btk and the inositol phospholipid signaling pathway in NK cells merits further investigation.
Deletion of specific P13K subunits has allowed for determining their individual contributions to inositol phospholipid signaling in NK cells. Kim et al. (2008) found that PIK3RT1 (p110δ) NK cells (NK cells lacking p85α, p55α, and p50α) have a severely disrupted NK cell compartment. They showed that PIK3RT1−/− NK cells are decreased in number in the bone marrow and liver but not the spleen. Moreover, NK cells that were present were cytologically incompetent against both "missing-self" and NKGD2 (an activating receptor expressed by both human and mouse NK cells) ligand-expressing target cells and had a skewed Lyn receptor repertoire compared to wild type (WT) NK cells. This cytolytic defect could be due to improper formation of the NK immune synapse. Activation of NK cells via NKGD2 requires interaction between the Rho guanine triphosphatase Cdc42 (Carlín et al., 2011), the adaptor protein CskL (Segovis et al., 2009) and DAP10 (Wu et al., 1999; Bailleul et al., 2003; Upshaw et al., 2006) with p58α required for proper formation of the immunological synapse. Thus, multiple inositol phospholipid signaling events are required for proper microtubule and actin cytoskeleton rearrangement.

The PI3K class Iα and class Iβ subunits p110α and p110β seem to have non-redundant roles in NK cell signaling. In vitro cytolytic assays indicate that either p110α (Kim et al., 2007; Saudemont et al., 2007; Tassi et al., 2007) or p110γ (Kim et al., 2007; Tassi et al., 2007) enzymes are dispensable for target cell lysis. However, two papers have shown that there is decreased ability for NK cell rection of tumor cell in vivo, at least in the case of p110δ deficiency (Saudemont et al., 2007; Gao et al., 2008). Further investigations are required to understand why p110δ is required for in vivo target cell lysis but not for NK cytolytic activity in vitro. Data regarding the requirement of p110δ and p110γ for cytokine production are more contradictory. Two studies found that p110δ is required for cytokine production (Kim et al., 2007; Gao et al., 2008) and one went on to show that p110γ is dispensable (Kim et al., 2007) for the production of cytokines including IFNγ, tumor necrosis factor (TNFα), and granulocyte-macrophage colony-stimulating factor (GM-CSF). However, two different studies have shown that p110γ is in fact required for NK cell cytokine production (Tassi et al., 2007; Orr et al., 2009). At least part of the discrepancy may be due to the use of different mouse genetic backgrounds. Kim et al. (2007) made use of B10D2 mice (H-2b) background whereas most of the other mutants were on a C57BL/6 genetic background. Further, the mice used by Tassi et al. (2007) were incompletely backcrossed to the C57BL/6 background such that only ~80% of all alleles were C57BL/6 homogenous, with other alleles remained from the original 129Sv background. This is potentially problematic as 129Sv mice have hypersensitive NK cells and thus these 129Sv allelic loci may act as genetic modifiers of PI3K mutations (Belanger et al., 2008). For a more detailed and nuanced discussion of the differences observed in the different PI3K mutant studies please see Kerr and Cohucci (2011). Interestingly, p110ε, a PI3K isozyme found in many cell types but not in leukocytes, is required for the upregulation of the NKGD2 ligand RAE-1 following murine cytomegalovirus (MCMV) infection (Tokiyma et al., 2011). Thus, PI3K may regulate NK cell behavior not only in a cell intrinsic manner, but also via regulation of activating ligands expressed by target cells.

Phosphoinositide 3-kinase is also required for NK cell chemotaxis to various chemokines. These include lymphotactin, CC-chemokine ligand (CCL)2, CCL5, IFN-inducible protein-10 (CXCL10) and stromal-derived factor-1 alpha (SDF-1α; Al-Awukat et al., 1999). When the function of individual PI3K isoforms in NK chemotactic behavior was examined it was found that both p110α and p110γ are required for chemotaxis to CXCL12 and CCL3 both in vitro and in vivo. However, only p110β was found to be required for chemotaxis to CXCL10 and the G-protein-coupled receptor (GPCR) sphingosine 1-phosphate receptor 5 (SIP5), a receptor known to influence NK cell tissue distribution. Additionally, p110β was found to be sufficient to mediate NK cell extravasation to tumors and steady state NK cell distribution to the spleen, lymph nodes, and liver (Saudemont et al., 2009).

PTEN
Phosphatase and tension homologue deleted on chromosome 10 (PTEN) is one of the most commonly mutated genes in human cancers and is the underlying genetic etiology of Cowden syndrome, a disease characterized by the development of multiple hamartomas (Lynch et al., 1997). PTEN reverses the PI3K reaction by hydrolyzing PI(3,4,5)P3 to PI(4,5)P2. It is unclear if this reaction contributes meaningfully to the PI(4,5)P2 pool or if the importance of PTEN rests solely on antagonizing PI(3,4,5)P3 production. PTEN has not been extensively investigated in the context of NK cells. One study found that PTEN-deficient Vα14iNKT cells, a subpopulation of NKT cells accounting for about half of NKT cells, are not able to produce IFNγ efficiently as WT Vα14iNKT cells. Moreover, compared to mice without deletion of PTEN these mice were unable to mount an effective response to melanoma (Kishimoto et al., 2007). Recent preliminary work from the Caligiuri lab has indicated that PTEN may decrease NK cell activation through the attenuation of the Akt and ERK1/2 signaling pathways through decreased availability of PI(3,4,5)P3. NK-92 cells transduced with a lentivirus expressing PTEN have decreased cytotoxicity against target cells and primary NK cells over-expressing PTEN exhibit decreased CD107a surface expression upon stimulation (Biercheck et al., 2012). The data from the Caligiuri lab indicate that PTEN may play a conventional role in most NK cells by limiting Akt activation; however, perhaps at least in the Vα14iNKT subpopulation of NK cells PTEN plays a role in NK cell activation through the creation of PI(4,5)P2 pools. Thus, PTEN appears to have an important role in both NK types and thus should be investigated more thoroughly in the context of NK cell biology, perhaps through the creation of mice with NK-specific deletion of PTEN.

SHIP1
There are two paradigms of SHIP. SHIP1 (Dammen et al., 1996; Kavanagh et al., 1996; Kerr et al., 1996; Lioubin et al., 1996; Ono et al., 1996) which is expressed in hematopoietic cells, pluripotent stem cells (Yu et al., 2011) and osteoblast lineage cells (Hazen et al., 2009), and SHIP2 (Pesesse et al., 1997) which is expressed in a wide array of cell types and tissues. SHIP1 contains an
N-terminal SH2 domain which allows it to bind to phosphory- 
tine motifs, a inositol-5-phosphatase enzymatic domain allowing 
for removal of the 5-phosphate from PI(3,4,5)P3 and PI(3,4)P2 to 
produce PI(3,4)P2 and IL-12, respectively, and two C- 
terminal NPXY motifs which, when tyrosine phosphorylated, 
allow for ITAM domain binding. SHIP1 also contains a C2 domain 
that binds its product PI(4,5)P2 triggering an allosteric change 
that can enhance SHIP1 enzyme activity (Ong et al., 2007), as 
well as a PH-like domain that recognizes its substrate PI(3,4,5)P3 
(Ming-kam et al., 2012). The conversion of PI(3,4,5)P3 to 
PI(3,4)P2, allows for the attenuation of signaling pathways where 
PH domain-containing PI3K effectors exhibit selective recruit- 
ment to PI(3,4,5)P3 while also enabling the activation of other 
PI3K effectors whose PH domains allow recruitment to PI(3,4)P2 
(Kerr, 2011).

SH2 domain-containing inositol-5-phosphatase 1 has been 
shown to play an important role in NK cell biology in several 
different studies albeit with some discordant findings. SHIP1- 
deficient mice were initially shown to have increased NK cell 
numbers due to increased survival of certain subsets that expressed 
poly-specific Ly49 receptors resulting in a skewed NK receptor 
repertoire and thus an inability to reject an H-2d mismatched 
bone marrow transplant (Wang et al., 2002; Fortenbery et al., 
2010). Subsequently it was shown that SHIP1−/− NK cells are 
hyposesponsive for target cell lysis on an H2b MHC-I background due 
to inappropriate recruitment of SHP-1 to B24 resulting in an 
imbalance of inhibitory signals (Wahle et al., 2006, 2007; Forten- 
bery et al., 2010). Mice that are deficient in both 2B4 and SHIP1 
have restored ability to lyse target cells lending further evidence 
for the inhibitory dominance of 2B4 in SHIP1-deficient mice (Fortenbery et al., 2010). NK cells from SHIP1−/− deficient mice were 
also shown to produce IFNγ inefficiently following stimulation and 
to have inappropriate expression of Ly49B, a poly-specific 
MHC-I receptor (Scarpellino et al., 2007) normally expressed by 
myeloid cells (Fortenbery et al., 2010). Interestingly, unlike mice 
with an H2b MHC-I genetic background, SHIP1−/− mice on an 
H2a homozygous background are able to kill MHC-I-mismatched 
target cells at levels comparable to WT NK cells (Fortenbery et al., 
2010). This was proposed to occur due to increased NK licensing 
due to over-expression of Ly49A that was observed in the H2d 
SHIP1+/− NK cell compartment. These studies led to the ques- 
tion of whether the NK defects in SHIP1−/− mice are NK cell 
intrinsic or due to the inflammatory milieu present in these mice. 
Banch et al. (2012) showed that based upon CD27 and CD11b 
expression NK cells from SHIP1−/− mice are less mature than 
those from wild type mice. Contradictory to previous studies 
(Wang et al., 2002; Trotta et al., 2005), they showed that SHIP1- 
deficient mice have decreased numbers of NK cells and that NK 
cells from these mice do have decreased production of IFNγ when 
co-stimulated with interleukin (IL)-12 and IL-18. In bone mar- 
row chimera experiments Banch et al. (2012) saw no difference 
in IFNγ production when NK activating receptors were cross- 
linked, but saw a difference only when NK cells were co-stimulated 
with IL-12 and IL-18 leading to the conclusion that SHIP1 does 
not play an intrinsic role in NK cell cytokine production. How- 
ever, in a mouse model with NK cell-specific deletion of SHIP1 
we saw a significant impairment in IFNγ production following 
activation receptor cross-linking (Gambleton and Kerr, unpub- 
lished data). In another study, NK cells with over-expression 
of SHIP1 had decreased IFNγ production, and SHIP1-deficient 
mice produced greater IFNγ whenstimulated with IL-12 and 
anti-CD16 antibody, indicating that perhaps SHIP1 plays an 
inhibitory role in the context of IL-12 co-stimulation (Furrath et al., 
2005).

In contrast to mouse NK cells where an absence of SHIP1 leads 
to decreased IFNγ production and cellular cytotoxicity indicat- 
ing SHIP1 plays a role in NK cell activation, in human NK cells 
SHIP1 was initially found to limit signaling from the CD16 recep- 
tor and thus decrease the ADCC response (Galandrini et al., 2002). 
Human NK cells are able to be dissected into two populations: 
CD56−CD16− and CD56+CD16− NK cells that produce cytokines at 
high level and CD56−CD16− NK cells that produce cytokines 
inefficiently but instead have greater cytolytic activity. SHIP1 is 
expressed at a lower level in the CD56−CD16− NK subset potential- 
ly providing a molecular basis for their com- 
paratively high cytokine production (Trotta et al., 2005). The same 
group correlated this effect with the presence of MiR-155 and pro- 
posed MiR-155 as a regulator of SHIP1 expression and thus, a 
regulator of NK cell activity. They were also able to show that 
NK cells from mice deficient for the MiR-155 precursor, Bic, were 
not able to produce IFNγ as efficiently as NK cells from wild type 
mice when co-stimulated with IL-12 and IL-18 or with IL-12 and 
anti-CD16 antibody. While these results could be due to MiR-155 
regulation of SHIP1, the impact of MiR155 on other key IP signal- 
ard proteins such as PTEN (Yamanaka et al., 2009) and the PI3K 
subunit PTEN needs to be rigorously excluded before this MiR155 
SHIP1 circuit in NK cells is confirmed. As discussed above PI3K 
enzymes are important in NK cell chemotaxis. SHIP1 has been 
shown to be important in the chemotaxis of other types of leuko-
cytes (Kim et al., 1999; Nishio et al., 2007). Thus we believe it 
would be interesting to analyze the importance of SHIP1 on NK 
cell chemotaxis.

INPP4
Inositol polyphosphate 4-phosphatase (INPP4) catalyzes the 
removal of the D-4 phosphate from PI(3,4,5)P3 to form PI(3,4)P2. 
There are two different isozymes of INPP4: INPP4α and INPP4β 
with α and β splice variants of both. INPP4 has recently been 
shown to function as a tumor suppressor indicating that both 
PI(3,4,5)P3 and PI(3,4)P2 can give positive growth signals and that 
SHIP1 in some instances could act as a proto-oncogene (Brooks et 
al., 2010; Fühler et al., 2012). Similar to the way that SHIP1 is 
expressed largely in hematopoietic lineages, in B, NK, and mast 
cells only INPP4α mRNA is highly expressed with potentially 
very low levels of INPP4α also being expressed. Interestingly, 
INPP4B has been shown to have a prominent role in osteoclast 
function where SHIP1 is also known to inhibit OC:resorptive 
behavior ex vivo (Ferron et al., 2011). Thus further studies of 
INPP4B in lymphocytes, including NK cells, seems merited.

CONCLUSION
While there is some controversy in specifics, there is overwhelm- 
ing evidence to show that the inositol phospholipid signaling 

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pathway plays a prominent role in the regulation of NK cell development and function. The PI3K pathway has a clear role in the regulation of actin skeleton rearrangement, the formation of the NK immune synapse, cytokine production, and cytolytic competency. In summary, the data discussed above, PI(4,5)P2 is important for cytolytic competency while PI(3,4,5)P3 is important for cytokine production and thus play important roles in both NK cell effector functions in several contexts. Given that PI3KPSK is required for immune synapse formation and PI3K isoforms are required for cytokines these properties warrant investigation in the context of SHIP deletion. Lastly, as mentioned above, both INPP4 and Pten are important regulators in other hematopoietic cell types, and thus their role in NK cell biology should be examined.

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Conflict of Interest Statement: William G. Kerr has patents pending and issued concerning the analysis and targeting of SHIP1 in disease. Matthew Gumbleton has no conflicts to disclose.

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