The role of SHIP1 in macrophage programming and activation

M.J. Rauh1, L.M. Sly1, J. Kalesnikoff, M.R. Hughes, L.-P. Cao, V. Lam and G. Krystal2
The Terry Fox Laboratory, B.C. Cancer Agency, Vancouver, BC, Canada

Abstract
The SHIP1 (SH2-containing inositol-5′-phosphatase 1) acts as a negative regulator of proliferation, survival and end cell activation in haemopoietic cells. It does so, at least in part, by translocating to membranes after extracellular stimulation and hydrolysing the phosphoinositide 3-kinase-generated second messenger, PtdIns(3,4,5)P3 to PtdIns(3,4)P2. SHIP1−/− mice have, as a result, an increased number of neutrophils and monocyte/macrophages because their progenitors display enhanced survival and proliferation. These mice also suffer from osteoporosis because of an increased number of hyperactive osteoclasts and a significant neutrophil infiltration of the lungs. Interestingly, SHIP1−/− mice do not display endotoxin tolerance and we have found that lipopolysaccharide-induced endotoxin tolerance is contingent on up-regulating SHIP1, through the production of autocrine-acting transforming growth factor-β, in bone-marrow-derived macrophages and mast cells. Intriguingly, unlike bone-marrow-derived macrophages, SHIP1−/− peritoneal and alveolar macrophages produce 10-fold less NO than wild-type macrophages because these in vivo-generated macrophages have very high arginase I levels and this enzyme competes with inducible nitric oxide synthase for the substrate L-arginine. It is probable that, in the face of chronically increased PtdIns(3,4,5)P3 levels in their myeloid progenitors, SHIP1−/− mice display a skewed development away from M1 (killer) macrophages (which have high inducible nitric oxide synthase levels and produce NO to kill microorganisms and tumour cells), towards M2 (healing) macrophages (which have high arginase levels and produce ornithine to promote host-cell growth and collagen formation). This skewing probably occurs to avoid septic shock and suggests that the phosphoinositide 3-kinase pathway plays a critical role in programming macrophages.

Introduction
The PI3K (phosphoinositide 3-kinase) pathway plays a central role in regulating many biological events and the key second messenger within this pathway is PtdIns(3,4,5)P3 [1]. This membrane-associated phospholipid, which is present at low levels in resting cells, is rapidly synthesized from PtdIns(4,5)P2 by PI3K in response to a diverse array of extracellular stimuli and attracts PH (pleckstrin homology)-containing proteins to membranes to mediate its effects. To ensure that this pathway is kept in check, the tumour suppressor PTEN (phosphatase and tensin homologue deleted on chromosome 10; SH2 domain, Src homology 2 domain; SHIP1, phosphoinositide 3-kinase; PTB domain, phosphotyrosine-binding domain; PTEN, phosphatase and tensin homologue deleted on chromosome 10; SH2 domain; SH2P1, Src homology 2-containing inositol 5-phosphatase 1; TGF, transforming growth factor) acts as a negative regulator of proliferation, survival and end cell activation in haemopoietic cells. It does so, at least in part, by translocating to membranes after extracellular stimulation and hydrolysing the phosphoinositide 3-kinase-generated second messenger, PtdIns(3,4,5)P3 to PtdIns(3,4)P2. SHIP1−/− mice have, as a result, an increased number of neutrophils and monocyte/macrophages because their progenitors display enhanced survival and proliferation. These mice also suffer from osteoporosis because of an increased number of hyperactive osteoclasts and a significant neutrophil infiltration of the lungs. Interestingly, SHIP1−/− mice do not display endotoxin tolerance and we have found that lipopolysaccharide-induced endotoxin tolerance is contingent on up-regulating SHIP1, through the production of autocrine-acting transforming growth factor-β, in bone-marrow-derived macrophages and mast cells. Intriguingly, unlike bone-marrow-derived macrophages, SHIP1−/− peritoneal and alveolar macrophages produce 10-fold less NO than wild-type macrophages because these in vivo-generated macrophages have very high arginase I levels and this enzyme competes with inducible nitric oxide synthase for the substrate L-arginine. It is probable that, in the face of chronically increased PtdIns(3,4,5)P3 levels in their myeloid progenitors, SHIP1−/− mice display a skewed development away from M1 (killer) macrophages (which have high inducible nitric oxide synthase levels and produce NO to kill microorganisms and tumour cells), towards M2 (healing) macrophages (which have high arginase levels and produce ornithine to promote host-cell growth and collagen formation). This skewing probably occurs to avoid septic shock and suggests that the phosphoinositide 3-kinase pathway plays a critical role in programming macrophages.
Two models for SHIP1 recruitment to the plasma membrane

Left panel: IL-3 stimulates the Jak2-mediated tyrosine phosphorylation of the βγ subunit of the IL-3R at Tyr-577 (1). This attracts Shc through its PTB domain (2). Shc is then tyrosine-phosphorylated by Lyn or jak2, primarily at Tyr-239 and Tyr-317 (3) and this attracts SHIP1 through its SH2 domain (4) [21,22]. Right panel: the Ag-mediated co-clustering of the inhibitory FcγRIIB with the BCR stimulates the tyrosine phosphorylation of the ITIM within the FcγRIIB and this attracts SHIP1 through its SH2 domain (1). This leads to the phosphorylation of SHIP1 by Lyn (2) and this phosphorylation attracts Shc through its PTB domain (3). Shc in turn gets tyrosine-phosphorylated by Lyn (4), which enables Shc to compete with the FcγRIIB for SH2 domain of SHIP1 and wrest SHIP1 away from the plasma membrane (5) [23].

B-cells are activated [6]. SHIP1 is also present in mature granulocytes, monocyte/macrophages, mast cells and platelets [3,7–9]. In addition, there are two alternate splice forms of SHIP1 and their levels also change during haemopoiesis [10,11].

In addition to full-length SHIP1 and its two splice forms, a 104 kDa sSHIP has recently been identified. This sSHIP is the only form of SHIP1 expressed in embryonic stem (ES) cells [12] and is co-expressed with full-length SHIP1 in haemopoietic stem cells [12]. In both cases, it disappears with differentiation. sSHIP mRNA is transcribed from a promoter within the intron between exons 5 and 6 of the SHIP1 gene and so the protein lacks an SH2 domain and is thus neither tyrosine-phosphorylated nor associated with Shc after stimulation [12]. Related to this, SHIP1 knockout mice have been generated to date by deleting the first exon and so sSHIP is still expressed in these mice [4]. In fact, the expression of sSHIP is higher and more prolonged in SHIP1−/− when compared with SHIP1+/− ES cells when they are induced to differentiate [4]. It is thus probable that the SHIP1−/− mouse phenotype would be more severe if both sSHIP and full-length SHIP1 were deleted. As it is, full-length SHIP1−/− mice are viable but have a shortened lifespan, overproduce granulocytes and macrophages and suffer from progressive splenomegaly, extramedullary haemopoiesis, massive myeloid infiltration of the lungs [13], perturbed natural killer (NK) cell development [14] and severe osteoporosis (due to an increased number of Paget-like, hyper-resorptive osteoclasts) [15]. Since many of these phenotypic characteristics are shared with PTEN−/− mice [16], it is probable that it is the higher levels of PtdIns(3,4,5)P3 in SHIP1−/− mice that are primarily responsible for its phenotype.

Since SHIP1’s 5-phosphatase activity does not appear to change after extracellular stimulation or subsequent tyrosine phosphorylation (reviewed in [2]), it is supposed that SHIP1 mediates its inhibitory effects by translocating to sites of synthesis of PtdIns(3,4,5)P3 [and perhaps Ins(1,3,4,5)P5]. In support of this, green fluorescent protein-tagged SHIP1 has been shown to translocate to the plasma membrane in response to stimulation [8,17]. In terms of how SHIP1 translocates to the plasma membrane, it is probable that different mechanisms are employed, depending on the stimulus and cell type [18–24]. For example, it may utilize its own SH2 domain to take it to the FcγRIIB, MAFA (mast cell-function-associated antigen) or FceRI in mast cells or use Shc’s PTB domain to take it to the IL-3R in mast cells or Shc’s SH2 domain to take it to CD16 in NK cells [24] (see Figure 1). Interestingly, SHIP1 has also been shown to bind to the cytoskeleton. For example, thrombin stimulation of human platelets causes the tyrosine phosphorylation and translocation of SHIP1 to the actin cytoskeleton, perhaps through actin-binding filamins [8,25].

As far as SHIP1’s biological roles are concerned, it appears to limit mast cell activation and adhesion to fibronectin (in large part by restraining extracellular calcium entry and activation of NF-κB (nuclear factor κ B) and certain protein kinase C isofoms [26,27]), proliferation, chemotaxis and activation of B-cells (reviewed in [2]), thrombin- or collagen-induced activation and fibrinogen-induced spreading of platelets [3,9,28,29], neutrophil survival [30], monocyte/macrophage phagocytosis [8] and early erythroid (BFU-E) colony formation [31]. In addition, an inactivating mutation in the catalytic domain of SHIP1 has recently been found within the blast cells of a patient with acute myelogenous leukaemia [32], suggesting, perhaps, that SHIP1 normally acts as a tumour suppressor in haemopoietic progenitors.

While most SHIP1-induced effects are probably mediated by its hydrolysis of PtdIns(3,4,5)P3, SHIP1 may also hydrolyse Ins(1,3,4,5)P5 in some cell types [33] and could thus affect the levels of the higher inositol polyphosphates like IP6, which has been shown to play an essential role in transporting mRNA out of the nucleus for translation [34], and IP7, which has been shown to compete with PtdIns(3,4,5)P3 for PH-containing proteins [35].

Interestingly, there is also evidence that PtdIns(3,4)P2 may act as a second messenger in some cells by attracting PH-containing proteins, such as Bam32 and TAPP2 [36,37]. If so, one might expect a SHIP1 knockout [with its increased PtdIns(3,4,5)P3 and decreased PtdIns(3,4)P2] [38] to be qualitatively different from a PTEN knockout [with its increased PtdIns(3,4,5)P3 and PtdIns(3,4)P2].

**SHIP1 is required for endotoxin tolerance**

LPS (also known as endotoxin) is a major glycolipid in the outer membrane of Gram-negative bacteria that potentially...
triggers inflammation by stimulating immune system cells to produce various pro-inflammatory molecules, including pro-inflammatory cytokines and NO [39]. It does so by binding to a soluble LBP (LPS-binding protein) and this LPS−LBP complex binds CD14 on the cell surface. CD14 then presents the LPS−LBP complex to the MD−2−TLR4 complex (where TLR4 stands for Toll-like receptor 4) and this triggers the dimerization of TLR4. After dimerization, the cytoplasmic tail of TLR4 recruits several intracellular adaptor proteins to initiate signalling cascades that lead to pro-inflammatory cytokine and iNOS (inducible nitric oxide synthase) (and thus NO) synthesis (reviewed in [40]).

Although these LPS-induced pro-inflammatory molecules are indispensable for counteracting the growth of bacteria, overproduction can lead to endotoxin shock [41]. However, if animals or isolated macrophages are first exposed to a low, non-lethal dose of LPS, this induces a 2−3−week state of cell refractoriness to a second, high LPS exposure, such that far less pro-inflammatory cytokine and NO are produced after the second exposure and the animals survive. This phenomenon, referred to as endotoxin tolerance [41], is not simply the result of a global shutdown of LPS-induced events since normal or even increased levels of anti-inflammatory genes are produced by LPS-stimulated, tolerated cells [41]. This induction of tolerance is supposed to protect the host from cellular damage and, thus, probably represents an adaptation to a persistent bacterial infection. The mechanisms underlying this phenomenon have not as yet been fully elucidated. Relevant to this, we have found that SHIP1−/− BMmΦs (bone-marrow-derived macrophages) and BMMCs (bone-marrow mast cells) do not display endotoxin tolerance [40]. Moreover, an initial LPS treatment of wild-type BMmΦs or BMMCs increases the level of SHIP1 approx. 10-fold and this increase appears to be critical for the refractoriness to subsequent LPS stimulation. Interestingly, we also found that this increase in SHIP1 protein is mediated by the LPS-induced production of autocrine-acting TGFβ (transforming growth factor-β) [40], which is reminiscent of previous studies showing that TGFβ and activin elicit their inhibitory effects on haemopoietic cells, in part, by significantly up-regulating SHIP1 [33].

Our results also add to the controversy surrounding the role of the PI3K pathway in LPS-induced pro-inflammatory mediator production and in endotoxin tolerance since the literature is split on whether this pathway is a positive or negative regulator of these events [42]. Our results with SHIP1−/− BMmΦs and BMMCs are consistent with this pathway being a positive regulator of LPS-stimulated events and that its down-regulation is essential for endotoxin tolerance.

Although we propose that SHIP1 plays a crucial role in promoting endotoxin tolerance (see Figure 2), it obviously does not do so in isolation but acts in concert with many other negative regulators. These include IRAK-M, SOCS1, SOCS3, Twist 1 and 2, MyD88s, the p50 subunit of NF-κB and osteopontin (reviewed in [40]). However, these other molecules cannot invoke tolerance in the absence of SHIP1, suggesting, perhaps, that the increased PtdIns(3,4,5)P3 levels, which occur in the absence of SHIP1, raise the threshold required for hyperresponsiveness invoked by these other negative regulators.

**The role of SHIP1 in macrophage programming**

Macrophages can be subdivided into ‘killer’, classically activated, M1 macrophages and ‘healer’, alternately activated, ‘M2’ macrophages [43]. The M1 macrophages are characterized by having high iNOS levels and producing large amounts of NO to combat bacterial and viral infections and to destroy tumour cells [44,45]. The M2 macrophages, on the other hand, which are characterized by high arginase levels (which sequesters L-arginine away from iNOS), are supposed to play an important role in ‘cleaning up’ after an infectious agent is destroyed by phagocytosing cellular debris and stimulating host-cell proliferation and collagen synthesis. Given our findings that the PI3K pathway appears to play a positive role in pro-inflammatory mediator production from BMmΦs, we were surprised to find that in vivo-derived SHIP1−/− peritoneal and alveolar macrophages produce 10-fold less NO than wild-type macrophages. Subsequent studies revealed that this was because the SHIP1−/− in vivo-derived macrophages, unlike the wild-type macrophages, had an M2 phenotype (M.J. Rauh and G. Krystal, unpublished work). We thus hypothesized that in the face of chronic activation of the PI3K pathway and, thus, hyperactivation of mast cell and macrophage responses, SHIP1−/− peritoneal and alveolar macrophages mount a constitutive anti-inflammatory phenotype to avoid septic shock by reducing NO production.
Figure 3 | A model suggesting that chronically increased PtdIns(3,4,5)P3 levels skew monocytic progenitors towards M-2 ‘healer’ macrophages

Bone marrow from SHIP1−/− mice when cultured in the presence of M-CSF and mouse serum yield M2-like macrophages displaying constitutively high levels of arginase 1, whereas SHIP1+/+ bone marrow under the same conditions yield M1-like macrophages.

References


Received 21 June 2004