Biochemical Pharmacology 82 (2011) 1320–1334

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Review

Enzymatic and non-enzymatic activities of SHIP-1 in signal transduction and cancer

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ARTICLE INFO

Article history:
Received 13 May 2011
Accepted 27 May 2011
Available online 13 June 2011

Keywords:
SHIP-1
Grb2
Shc
Dok-1/2
immune response

ABSTRACT

PI3K cascade is a central signaling pathway regulating cell proliferation, growth, differentiation, and survival. Tight regulation of the PI3K signaling pathway is necessary to avoid aberrant cell proliferation and cancer development. Together with SHIP-1, the inositol phosphatases PTEN and SHIP-2 are the gatekeepers of this pathway. In this review, we will focus on SHIP-1 functions. Negative regulation of immune cell activation by SHIP-1 is well characterized. Besides its catalytic activity, SHIP-1 also displays non-enzymatic activity playing role in several immune pathways. Indeed, SHIP-1 exhibits several domains that mediate protein-protein interaction. This review emphasizes the negative regulation of immune cell activation by SHIP-1 that is mediated by its protein-protein interaction.

Contents

Abbreviations: AA, amino acid; Ag, antigen; APC, antigen presenting cell; Btk, burton tyrosine kinase; DAG, diacylglycerol; Dok, downstream of kinase; Grb2, growth factor receptor-bound protein 2; IFN-β, interferon β; Ig, immunoglobulin; IKKα/ε, IκB kinaseα/ε; IL-1, interleukin 1; IP3, inositol triphosphate; IRAK, interleukin-1 receptor-associated kinase; IRF3, interferon regulatory factor 3; LAT, linker of activated T cells; MAPK, mitogen-activated protein kinase; MHC, major histocompatibility complex; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor κB; PI3K, phophoinositide 3 kinase; PIP3, phosphatidyl-inositol triphosphate; PKC, protein kinase C; PLCγ, phospholypase C gamma; PTEN, phosphatase and tensin homolog; RasGAP, Ras GTPase activating protein; Shc, src homology and collagen; SOS, Son of sevenless; TBK-1, tank binding kinase1; TLR, toll like receptor; TNF-α, tumor necrosis factor α; TRAF, TNF receptor-associated factor.

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doi:10.1016/j.bcp.2011.05.031

1. General introduction

Phosphoinositide 3 kinase (PI3K) cascade is a crucial signal transduction pathway that mediates many biological processes, such as cell proliferation, cell differentiation, chemotaxis, vesicle trafficking, cell cycle progression and cell survival. PI3K is activated by various cell type-dependent stimuli (*e.g.* growth factors, immune stimulation, cytokines...). PI3K activation results in phosphorylation of phosphatidylinositol biphosphate (PI-4,5-P2) to generate phosphatidylinositol triphosphate (PI-3,4,5-P3 or PIP3). This molecule is a crucial second messenger that enables recruitment of a wide range of proteins containing a pleckstrin homology (PH) domain. Among these, the best characterized is the serine/threonine kinase Akt which possesses a multitude of substrates implicated in various biological processes (reviewed in [1,2]) (Fig. 1). Among them: Akt is implicated in cell survival by inhibiting pro-apoptotic proteins such as Bax, Bad and caspase-9; it also activates MDM2, a E3 ligase that mediates ubiquitination and

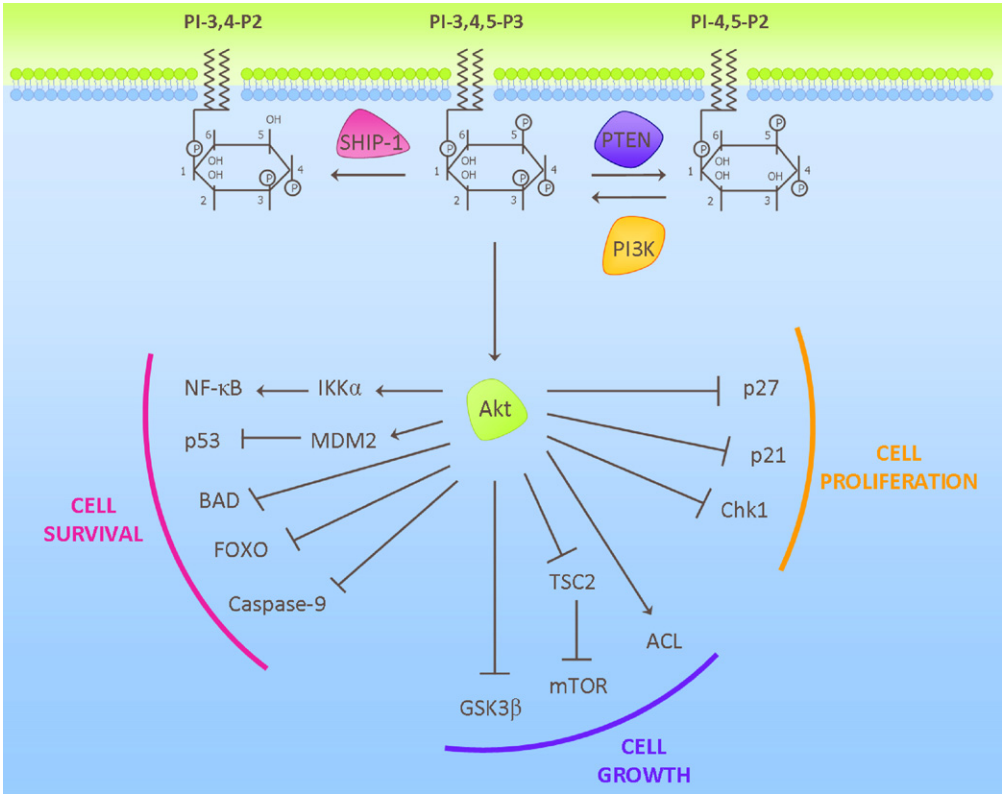

Fig. 1. PI3K–Akt axis. PIP3 is generated by PI3K. PTEN antagonizes PI3K and generates PI-4,5-P2 whereas SHIP-1 generates PI-3,4-P2. PIP3 is a docking site for Akt which phosphorylates a wide range of proteins and promotes cell growth, differentiation and survival.

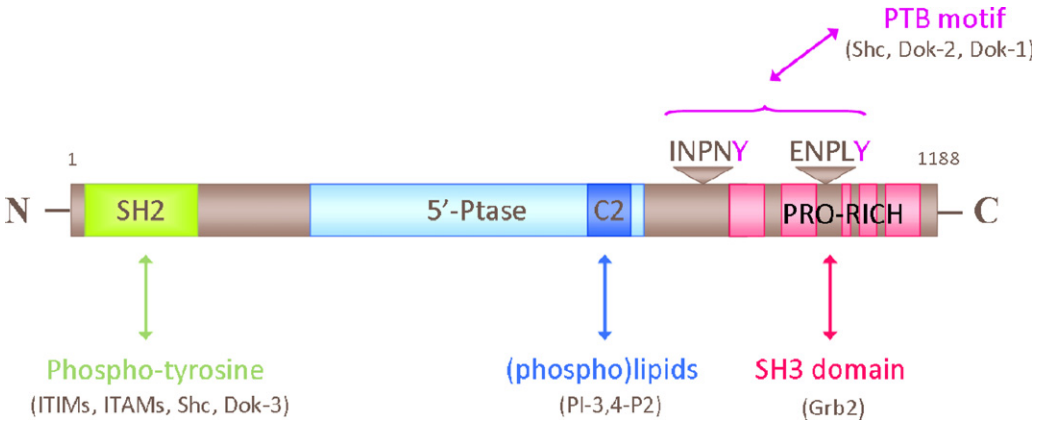

Fig. 2. Schematic representation of the SHIP-1 protein. The N-terminal part of the protein contains an SH2 domain, a central catalytic domain (5′-phosphatase), a C2 domain is located at the end of the catalytic region and the C-terminus encompasses a proline rich region (PRO-RICH) and two phosphorylable tyrosines (INPNY, ENPLY).

subsequent degradation of p53, thereby promoting cell cycle progression, and phosphorylates IKKα thereby promoting activation of the nuclear factor κB (NF-κB). Akt also promotes cell growth by inhibiting two inhibitors of mTOR, *i.e.* TSC2 and PRAS40, thereby stimulating protein synthesis. Akt also activates acid citrate lyase (ACL) that generates acetyl-CoA which in turn promotes fatty acids biosynthesis and ATP production (*via* the Krebs cycle). Finally, Akt promotes cell proliferation by inhibiting negative regulator of cell cycle p21, p27 and Chk1. Negative regulation of the PI3K pathway is achieved by inositol phosphatases such as PTEN that antagonizes PI3K and generates PI-4,5-P2 and SHIP-1 that dephosphorylates PIP3 to generate PI-3,4-P2 [3] (Fig. 1).

2. Structure and catalytic activity of SHIP-1

In the beginning of the nineties, different groups described a protein of 145 kDa that associated with Grb2 and Shc in different cell types upon stimulation with growth factors such as IL-3, steel factor, erythropoietin and G-CSF [4,5]. In 1996, this protein has been cloned and characterized as an inositol 5′-phosphatase [6–8] that the scientific community has decided to name SHIP-1 (SH2 containing inositol 5′phosphatase-1).

2.1. Structure

SHIP-1 is encoded by the *INPP5D* gene on chromosome 2 (location 2q37.1). The protein is composed of 1188 amino acids and possesses domains that mediate its interaction with other proteins on either side of its central catalytic region (Fig. 2). The amino-terminal part contains a Src homology 2 domain (SH2 domain) and the carboxy-terminus encompasses a proline rich region and two phosphorylable tyrosines. These different domains are respectively described in Sections 2.2, 2.3 and 2.4.

2.2. SH2 domain

The SH2 domain is located in the amino-terminal part of the protein. This domain is characterized by a highly conserved sequence (*i.e.* DGSFLSVR) and mediates interaction of SHIP-1 with tyrosine-phosphorylated proteins such as Shc [6–9], ITIM and ITAM motifs of membrane receptors [10–13] and Dok-3 [14].

2.3. Proline rich region

The carboxy-terminal part of SHIP-1 contains a proline rich region (PRR) which is composed of both type I (*i.e.* **PP**SQ**PP**LS**P**) and type II (*i.e.* **P**VK**P**SR, **PP**LS**P**KK, **PP**L**P**VK) proline rich motifs [6]. The proline rich region enables interaction of SHIP-1 with SH3-containing proteins like Grb2 [4,6,15]. Interestingly, the proline rich region also allows recruitment of SHIP-1 at the plasma membrane [16,17].

2.4. Phosphorylable tyrosines

SHIP-1 carboxy-terminus encompasses two phosphorylable tyrosine motifs (*i.e.* INPN**Y** and ENPL**Y**). Upon phosphorylation, these motifs interact with proteins containing phosphotyrosine binding domain (PTB) such as Shc [7,18], Dok-1 and Dok-2 [19,20].

SHIP-1 is phosphorylated following activation of various membrane receptors, *e.g.* IL-3R, BCR, TCR, EPO-R, etc. [9,18,19,21]. The kinase Lyn, a member of the Src family, has been shown to interact with and to phosphorylate SHIP-1. However, it seems that Lyn is probably not the only Src family member implicated in SHIP-1 phosphorylation [22–24].

2.5. Catalytic activity

SHIP-1 displays a phosphatase activity and hydrolyses phosphatidylinositol triphosphate (PI-3,4,5-P3 or PIP3) on the D5 position of the inositol ring to generate phosphatidylinositol biphosphate (PI-3,4-P2). SHIP-1 has also been shown to dephosphorylate inositol tetraphosphate (PI-1,3,4,5-P4) *in vitro* [6–8].

Besides, it must be noted that tyrosine phosphorylation of SHIP-1 does not affect its catalytic activity [22]. However, many reports have demonstrated that plasma membrane location of SHIP-1 is crucial for its enzymatic activity [16,17,22]. Indeed, plasma membrane location does not affect catalytic activity *per se* but enables to bring SHIP-1 in close proximity of PI-3,4,5-P3 and therefore increases accessibility of SHIP-1 for its substrate. Plasma membrane location is mediated by the proline rich region and this domain is essential for catalytic activity *in vivo*. This hypothesis has been confirmed by demonstrating that SHIP-1 PRR deletion mutants are still able to dephosphorylate PIP3 *in vitro* but not *in vivo* [16,17].

Recently, it has been demonstrated that SHIP-1 is an allosteric enzyme [25]. Its enzymatic activity is modulated by the binding of specific effectors and increases through binding to its own product, *i.e.* PI-3,4-P2. Indeed, a C2 domain has been identified in the carboxy-terminal end of SHIP-1 phosphatase region. This C2 domain is well known to interact with phospholipids and mediates binding of SHIP-1 to PI-3,4-P2. Therefore, a SHIP-1 C2 deletion mutant (SHIP-1ΔC2) is still able to dephosphorylate PIP3 but its catalytic activity can not be increased *via* its binding to PI-3,4-P2 [25].

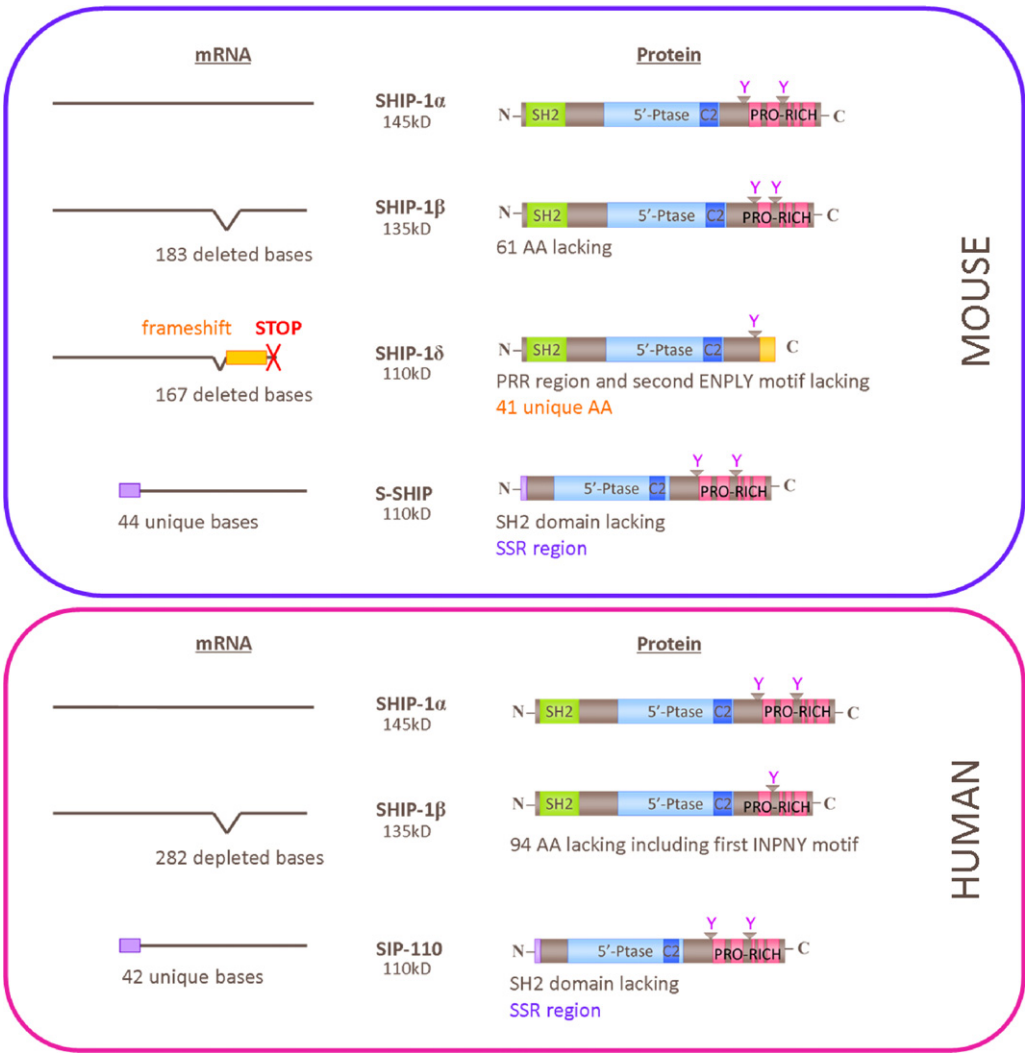

Fig. 3. Murine and human isoforms of SHIP-1. Murine isoforms of SHIP-1 are composed of SHIP-1α, of the two SHIP-1β and SHIP-1δ spliced variants and of the stem cell restricted isoform s-SHIP. Human isoforms of SHIP-1 include SHIP-1α, SHIP-1β and the stem cells restricted SIP-110 isoform. See text for details. SSR region: SHIP-1 stem cell restricted region.

2.6. Expression patterns

Northern blot and *in situ* hybridization have revealed that SHIP-1 is mainly expressed in hematopoietic cells but also in spermatids and testis [26]. SHIP-1 is expressed during early embryogenesis but only at sites of hematopoiesis such as yolk sac and liver of mouse embryos [26]. It is also well known that different SHIP-1 isoforms are expressed during hematopoietic cells maturation (see below in Section 3.1).

3. The SHIP-1 proteins tribe

Western blot analyses have revealed the existence of several SHIP-1 isoforms. The full length protein is composed of 1188 amino acids and weighs 145 kDa. At least two other SHIP-1 isoforms of 135 kDa and 110 kDa have been identified. These proteins are generated by alternative splicing, use of intracistronic promoter or C-terminal truncations. SHIP-1 has also a homolog called SHIP-2. The different SHIP proteins are described below (Sections 3.1, 3.2 and 3.3) (Fig. 3).

3.1. Splice variants and isoforms

Several SHIP-1 proteins have been detected by western and northern blotting. These spliced forms are named by a Greek letter: SHIP-1α is the 145 kDa protein previously described, SHIP-1β and SHIP-1δ are two splice variants of 135 and 110 kDa, respectively. Another isoform of 110 kDa has been identified and called s-SHIP in mice or SIP-110 in human (Fig. 3).

It has been demonstrated that the SHIP-1 isoforms are differentially expressed during hematopoietic cells maturation. Indeed, studies of SHIP-1 splice forms in human have been achieved in the ML-1 myeloblastic leukemia cell line. These cells behave like myeloid precursor cells and can be differentiated into monocytes upon treatment with TPA (12-O-tretradecanoyl-phorbol-13-acetate). Untreated precursor cells express an 110 kDa SHIP-1 protein, whereas the differentiated cells quickly express 135 and 145 kDa isoforms and loose expression of 110 kDa protein [27,28].

3.1.1. SHIP-1 isoform in mice

In mice, the SHIP-1β isoform is generated by mRNA splicing and the resulting protein lacks 61 amino acids (AA) encompassing several proline rich motifs (Fig. 3). Therefore, SHIP-1β lacks some interaction partners compared to SHIP-1α [28].

SHIP-1δ, an isoform of 110 kDa, has been discovered by Wolf et al. This isoform is also generated by alternative splicing resulting in an mRNA that lacks 167 bases. However, following the splice, the sequence continues in a different reading frame and produces 41 unique AA at the carboxy-terminus before reaching a stop codon

[29]. Therefore, SHIP-1δ retains the first INPNY motif observed in SHIP-1α isoform but the proline rich region and the second ENPLY motif are totally removed (Fig. 3).

Finally, Tu et al., have also identified a fourth SHIP-1 isoform in mice, called s-SHIP-1, which is only expressed in totipotent embryonic stem cells (ES) and hematopoietic stem cells (HSC). s-SHIP lacks the SH2 domain that is substituted in the mRNA sequence by a unique short sequence of 44 bases called SSR (SHIP stem cell region) [30]. This isoform is not produced by an alternative splicing but arises from transcription at an internal promoter located in the intron 5/6 region of the SHIP gene [30,31] (Fig. 3). s-SHIP and SHIP-1α expression are mutually exclusive. While SHIP-1α is only expressed in mature hematopoietic cells, s-SHIP expression is restricted to stem cells. Because s-SHIP lacks the SH2 domain, it can not interact with Shc, but still interacts with Grb2. Moreover, s-SHIP is mostly localized at the plasma membrane rather than in the cytoplasm. The author's hypothesis is that this membrane location enables s-SHIP to minimize the pool of PIP3, therefore contributing to maintain stem cells in a quiescent state [30]. Of note, stem cell-restricted s-SHIP expression is controversial because it has been recently shown by Nguyen et al. that s-SHIP is also expressed and plays a functional role in mature hematopoietic cells [32].

3.1.2. SHIP-1 isoform in humans

In addition to SHIP-1α, only two other isoforms of SHIP-1 have been described in humans. Like in mice, a SHIP-1β isoform of 135 kDa is observed in human cells. This protein is the result of an alternative splicing and lacks several proline rich motifs and also the first INPNY motif (Fig. 3). Therefore protein interaction partners of SHIP-1β should greatly differ compared with SHIP-1α [28]. Expression of SHIP-1α and SHIP-1β has been observed *in vivo* in human primary PBMC (peripheral blood mononuclear cells), although SHIP-1β expression is dramatically weaker than that of SHIP-1α [28].

Kavanaugh et al. have also identified a protein of 110 kDa called SIP-110 which is the human form of s-SHIP [8]. SIP-110 protein lacks the SH2 domain and its mRNA also encompasses a unique sequence of 42 bases called SSR region [30] (Fig. 3).

3.2. C-terminal truncation

In addition to numerous splice variants, Damen et al. have observed that different SHIP-1 isoforms (*i.e.*, proteins of 135, 125 and 110 kDa) result from C-terminal truncations of the 145 kDa full length protein. Calpain is probably the protease involved in the cleavage of SHIP-1 [33]. These authors speculate that this mechanism plays a role, at least in part, in the generation of SHIP-1 isoforms that have been observed during hematopoietic cells maturation [33]. However another study reports that these C-terminal truncations of SHIP-1 are generated *in vitro* during cell lysis [34]. By inhibiting protease activity with boiling SDS lysis buffer, Horn et al. have showed that only the 145 and the 135 kDa forms of SHIP-1, corresponding to SHIP-1α and SHIP-1β respectively, can be observed in various hematopoietic cells from murine and human origin. Indeed the 125 and 110 kDa isoforms are not observed *in vivo*. Therefore the data of Horn et al. do not support a physiological role for the 125 and 110 kDa C-terminal truncated proteins.

3.3. The SHIP-1 homolog: SHIP-2

In 1997, another SH2-containing inositol 5-phosphatase, closely related to SHIP-1, was cloned and called SHIP-2. This protein contains an SH2 domain in the N-terminal part (54% of identity with SHIP-1 SH2 domain) and a central catalytic domain (64% of identity with SHIP-1 catalytic domain) [35]. The C-terminus encompasses a unique phosphorylable motif (*i.e.* NNPA**Y**), a SAM domain [36] and a proline rich region, which is quite different from that of SHIP-1 [35]. Like its homolog, SHIP-2 dephosphorylates PI-3,4,5-P3 into PI-3,4-P2 *in vitro* and *in vivo* [35,37,38].

In contrast with SHIP-1, SHIP-2 is expressed in a wide range of tissues and more abundantly in heart, liver, skeletal muscles, and brain [35].

SHIP-2 is well characterized as a negative regulator of insulin-induced glycogen synthesis. Upon binding with insulin, the insulin receptor and the insulin receptor substrate (IRS) are tyrosine phosphorylated. Once phosphorylated, IRS interacts with the p85 subunit of PI3K and promotes PIP3-dependent Akt activation. Akt has mainly two effects on the glycogen synthesis. On one hand, Akt promotes the translocation of the glucose transporter, GLUT4, to the plasma membrane thereby promoting glucose uptake. On the other hand, Akt inhibits glycogen synthase inhibitor, GSK3β, and therefore increases glycogen synthesis. By reducing the pool of PIP3, SHIP-2 decreases Akt activation, glycogen synthesis [39] and glucose uptake [37]. SHIP-2 is therefore considered as a crucial negative regulator of glucose homeostasis. These observations have been confirmed in transgenic mice over-expressing SHIP-2. These mice exhibit a reduction of Akt phosphorylation in liver, adipose tissue and skeletal muscles. As a result, they develop insulin insensitivity and their whole-body glucose homeostasis is impaired [38].

Of note, two different SHIP-2 KO mice have been generated and differ greatly from each other. The first one exhibits a severe phenotype with death observed within 3 days after birth [40]. The SHIP-2 KO newborns exhibit serious hypoglycaemia due to an increased sensitivity to insulin. Studies of heterozygous animals have demonstrated that plasma membrane GLUT4 level and glycogen synthesis are both significantly higher in SHIP-$2^{+/-}$ animals compared to their WT counterparts [40]. The SHIP-2 KO phenotype leads to conclude to a major role of SHIP-2 in negative regulation of glucose homeostasis. However, the authors realized that another gene, *phox2a*, has also been depleted in their SHIP-2 KO mouse model [41]. Therefore, it remains to be resolved whether SHIP-2 KO phenotype is caused by SHIP-2 depletion or by inactivation of phox2a. The second SHIP-2 KO mouse model has been generated in 2005 [42]. Importantly, in contrast with the first SHIP-2 KO mice, these mice survive to adult stage but they fail to gain a normal weight compared to their WT littermates. Moreover, serum analyses reveal that the KO mice exhibit a decrease of cholesterol, triglycerides and nonesterified free fatty acids on normal diet compared to their WT counterparts. Strikingly, these SHIP-2 KO mice do not become obese on a high fat diet indicating a role for SHIP-2 in obesity resistance. Surprisingly, insulin signaling is not affected by SHIP-2 deficiency in the basal state. However, after insulin administration an increased Akt activation is observed in the liver and skeletal muscles of SHIP-2 KO mice compared to WT animals [42]. Discrepancies between both SHIP-2 KO mice render the characterization of SHIP-2 functions complicated and further studies would clarify the precise role of SHIP-2 in glucose homeostasis.

4. SHIP-1 KO mice phenotype

SHIP-1 KO mice were generated in 1998 [43,44]. There are viable and fertile but their life span is reduced. At 4 weeks of age, these mice exhibit enourmously enlarged spleen and lymph nodes [44]. These mice develop progressive myeloid hyperplasia, especially caused by granulocytes and macrophages/monocytes populations, leading to splenomegaly, lymphadenopathy and invasion of many vital organs such as lungs, heart, kidneys, skeletal muscles,... [43,44]. Myeloid infiltration in the lungs leads to respiratory failure and to a dramatic decrease of life span with death observed as early as at 4 weeks of age and only 40% of animal surviving until 14 weeks [43]. The myeloid hyper-proliferation is caused by the combination of two factors. First, the myeloid cells and their precursors are more sensitive to growth factors (*e.g.* IL-3, GM-CSF)[43] and second, these cells exhibit

a decreased sensitivity to pro-apoptotic stimuli (*e.g.* CD95L, cycloheximide, sorbitol) [44]. Growth factors sensitivity and apoptosis escape are caused by robust and constitutive Akt activation [43,44]. The phenotype of SHIP-1 KO mice clearly shows that SHIP-1, by modulating PIP3 level, is a key negative regulator of cytokine-induced myeloid cells proliferation.

In the lymphoid compartment, it has been observed that development of SHIP-1 KO B cells is altered. Pre- and immature B cells are decreased in bone marrow of SHIP-1 KO mice compared to their WT counterparts [43]. Surprisingly, the number of mature B cells in lymph nodes and spleen is increased compared to WT mice [45]. It is speculated that SHIP-1 deficiency leads to B cells survival enhancement which could explain the increase of peripheral mature B cells albeit the fact that there is a reduction of pre- and immature B cells in bone marrow. In accordance with the increase of mature B cells, elevated serum Ig levels are observed in SHIP-1 KO mice. Indeed, the SHIP-1 KO B cells are unresponsive to immune terminaison signals and therefore SHIP-1 KO mice exhibit an enhanced response to specific antigen stimulation [45]. These results strongly support that SHIP-1 plays a role in both development and immune function of B cells.

SHIP-1 deficiency is also associated with defect in NK cells. SHIP-1 KO mice exhibit an alteration of NK receptor subset. Natural cytotoxicity of NK cells is mediated by two subtypes of receptors. On one hand "activating receptors" interact with ligands on cell surface of infected or tumor cells. These receptors mediate recognition of non-self antigen and deliver positive signal for killing. On the other hand, "inhibitory receptors" (*e.g.* CD94, Ly49 or KIR) mediate interaction with MHC class I molecules. These receptors recognize self antigen and prevent cytotoxicity against normal self cells. Therefore disruption of NK cells receptors repertoire can compromise NK cytolytic functions. SHIP-1 deficiency leads to over-representation of inhibitory NK receptors such as Ly49A [46,47]. As a result, NK cytotoxicity is reduced in SHIP-1-deficient mice and they exhibit reduced graft rejection [47].

In contrast with the hyper-proliferation of myeloid and B cells, the number of T cells and their precursors is reduced in KO mice [43,48,49]. Indeed, SHIP-1 KO T cells are more sensitive to pro-apoptotic stimuli such as CD95L [50]. Moreover, in SHIP-1 KO mice, peripheral T cells are mostly constitutively active and behave like regulatory T cells (T_{reg}) [48,49]. Indeed, SHIP-$1^{-/-}$ T cells express specific T_{reg} markers like Foxp3, CD4 and CD25 [48,49,51] and can suppress allogenic T cell response *in vitro* [48,49]. These results indicate that SHIP-1 is a negative regulator of T_{reg} differentiation. Strinklinly, SHIP-1 KO mice exhibit a dramatic decrease of Th17 cells concomitent with an increase of T_{reg} differentiation. It is supposed that SHIP-1 plays a key role in regulating CD4 differentiation *via* its ability to promote Th17 differentiation and to limit T_{reg} development [51].

To elucidate the role of SHIP-1 in T cells, Tarasenko et al. have recently generated a mouse with T cell-specific knock-down of SHIP-1 [52]. In contrast with previous reports, they have not observed any decrease in T cell number either in thymus or in lymph nodes. They have demonstrated that SHIP-1 T cell-specific deficiency is not associated with increased T_{reg} differentiation or with abnormal T cells differentiation. To explain this apparent discrepancy with results previously obtained, they suggest that the inflammatory environment in SHIP-1 null mice is responsible for the observed increased level of activated T cells and elevated number of regulatory T cells [52]. However, they have noticed a reduction of $CD4^+$ Th2 cells concomitantly with an increase of $CD4^+$ Th1 cells. Therefore, these mice exhibit a decreased humoral immune response suggesting that SHIP-1 plays a key role in shaping Th2 response [52].

Finally, David Curtis's team has generated a mouse model (SHIP-$1^{Ie20/Ie20}$) where expression of both SHIP-1 and s-SHIP is altered due to a point mutation in the catalytic domain of SHIP-1 (I641T) [32]. This mutation results in reduction of SHIP-1 as well as s-SHIP expression and in a severe impairment of their catalytic activity. In contrast with KO mice, where only intron 1 is targeted and where s-SHIP, arising from transcriptional initiation at an internal promoter, is still expressed [43], both SHIP-1 and s-SHIP are altered in SHIP-$1^{Ie20/Ie20}$ mice. Indeed, These mice develop myeloid infiltration more rapidly than SHIP-1 KO mice. As a result, SHIP-$1^{Ie20/Ie20}$ mice exhibit a more severe phenotype. Most importantly, an early lethality is observed with no mice surviving longer than 7 weeks. The death is due, as in KO mice, to myeloid infiltration of the lungs leading to respiratory failure. In contrast with SHIP-$1^{-/-}$ mice, SHIP-$1^{Ie20/Ie20}$ mice also develop important alteration of B and T cells development. Surprisingly, the authors have observed expression of s-SHIP in mature hematopoietic cells at levels comparable to embryonic stem cells. This observation does not correlate with previous reports showing that s-SHIP expression is stem cell-restricted. Their hypothesis is that the more severe phenotype observed in SHIP-$1^{Ie20/Ie20}$ is due to alteration of the catalytic activity and of the expression both SHIP-1 and s-SHIP. In this regard, the phenotype of SHIP-$1^{Ie20/Ie20}$ mice can be rescued by crossing SHIP-$1^{Ie20/Ie20}$ with SHIP-$1^{-/-}$ mice. Indeed, heterozygous SHIP-$1^{-/Ie20}$ mice have a similar survival to SHIP-$1^{-/-}$ mice and development of myeloid hyper-proliferation is observed at the same rate than in their KO littermates. This is the first evidence for a functional role of s-SHIP in adult hematopoiesis [32].

5. miR-155, a SHIP-1 repressor

miR-155 is a miRNA produced by transcription of the *BIC* gene. It has been first identified because it is over-expressed in diffuse large B cell lymphoma [53]. Transgenic mice over-expressing miR-155 in the B cell compartment rapidly exhibit a splenomegaly due to pre-B cells extensive proliferation. These mice develop lymphoblastic leukemia closely related to diffuse large B cell lymphoma observed in humans. It is therefore admitted that miR-155 mediates initiation and/or development of that type of cancer [54].

It has been demonstrated that SHIP-1 is a primary target of miR-155. Indeed, miR-155 recognizes a specific sequence in 3′UTR sequence of SHIP-1 mRNA [55–57]. Moreover, phenotype of mice over-expressing miR-155 is closely related to SHIP-1 KO mice. In order to compare both phenotypes, O'Connell et al. have generated hematopoietic stem cells stably expressing miR-155 or a SHIP-1 shRNA. They have grafted these cells in irradiated mice and have observed that both mice strains exhibit the same phenotype. They develop a dramatic myeloid hyper-proliferation leading to splenomegaly. These results indicate that over-expression of miR-155 has the same effect than SHIP-1 deficiency [56]. Moreover, in a murine model that over-expresses miR-155 in B cell lineage, it has been shown that SHIP-1 expression is down-modulated [55]. In this case, miR-155 targets not only SHIP-1 but also CEBPβ, two negative regulators of IL-6 pathway. Repression of these proteins blocks B cell differentiation and promotes their proliferation *via* robust Akt activation. This process could be at the origin of B lymphoblastic leukemia observed in these mice [55].

Direct repression of SHIP-1 by miR-155 has also been demonstrated in B cells from patient with diffuse large B cell lymphoma. In this type of cancer, over-expression of miR-155 is due to an enhanced autocrine production of TNF-α and can be reduced by use of TNF-α antagonists. This treatment restores SHIP-1 expression and greatly reduces cell proliferation [57]. Moreover, it has also been described that miR-155 is over-expressed in cells from patients with acute myeloid leukemia (AML) [58]. Strikingly, it has been shown that SHIP-1 expression is strongly reduced in this type of cancer [59]. However, a direct link between SHIP-1 down-modulation and miR-155 over-expression in this case has never been demonstrated.

6. SHIP-1 interaction partners and biological relevance of these interactions

As it has been previously described, SHIP-1 possesses several interaction domains that mediate its binding to other proteins. Therefore biological functions of SHIP-1 differ according to proteins that it interacts with. In Sections 6.1–6.5, we will focus on interaction partners of SHIP-1 in various cell types and on the biological significance of these interactions.

6.1. SHIP-1 in B cells

6.1.1. SHIP-1 interacts with ITIM motifs of FcγRIIB and reduces BCR activation

B cells are crucial components of adaptive immunity. Activation of B cells by recognition of specific antigen *via* their B cell receptor (BCR) leads to monoclonal expansion and production of antibodies directed against the corresponding antigen.

Mechanistically, activation of B cells starts by BCR crosslinking with a specific antigen, resulting in phosphorylation of BCR ITAM motifs and activation of Syk, a tyrosine kinase. Subsequently, Syk induces phosphorylation of CD19, which triggers recruitment and activation of PI3K that produces PIP3 [60]. Two important PH-containing proteins are recruited to PIP3: Akt, which promotes cell growth, survival and differentiation, and Btk. This kinase is a key regulator of B cells because it activates PLCγ2, which causes PI-4,5-P2 cleavage, thereby generating the soluble effector inositol triphosphate (IP3) and diacylglycerol (DAG). DAG enables recruitment and activation of PKC whereas IP3 mediates intracellular Ca^{2+} influx. These two pathways lead to activation of transcription factors such as NF-κB and NFAT which are required for proper B cells activation (review in [61]) Fig. 4A.

Negative regulation of BCR activation is achieved by the Fc receptor, *i.e.* FcγRIIB. This receptor is a low affinity receptor interacting with immunoglobulin at the end of immune response when the concentration of soluble Ig is important. Activation of FcγRIIB leads to its co-aggregation with BCR which in turn leads to inhibition of B cells activation (Fig. 4B). SHIP-1 is a crucial intermediate in this process. Mechanistically, upon BCR and FcγRIIB co-aggregation, Fc receptor is phosphorylated on ITIM motifs and interacts with the SH2 domain of SHIP-1 [62,63]. SHIP-1 is therefore recruited to plasma membrane and can dephosphorylate PIP3, thereby inhibiting recruitment of Akt and Btk [64] (Fig. 4B). Therefore, it has been described that, in SHIP-1 KO B cells, co-ligation of BCR and FcγRIIB does not reduce Akt activation or Ca^{2+} influx [11].

6.1.2. SHIP-1 interacts with Shc and decreases Ras activation following BCR and FcγRIIB co-aggregation

BCR crosslinking by antigen not only causes PI3K pathway activation but also triggers Ras-mediated MAPK activation. In this pathway, activation of BCR leads to the phosphorylation of the adaptator protein Shc. Following its phosphorylation, Shc forms a complex with Grb2 and SOS, a GDP exchange factor. Interaction with Shc brings SOS to the plasma membrane where it can mediate exchange of GDP by GTP on the GTPase Ras. Ras activation triggers a phosphorylation cascade that activates MAPK and participates in B cells activation and proliferation (reviewed in [9]) (Fig. 4A).

Negative regulation of this pathway is also achieved by co-ligation of BCR and FcγRIIB (Fig. 4B). Upon co-aggregation of both receptors, SHIP-1 is recruited to FcγRIIB ITIM motifs *via* its SH2 domain and is tyrosine phosphorylated, thereby enabling interaction between its proline rich region and the SH2 domain of Shc [62]. The SH2 domain of SHIP-1 is subsequently released from the FcγRIIB and interacts with phosphotyrosines of Shc and therefore competes with Grb2 for binding to phospho-Shc [65]. As a result, the interaction between SHIP-1 and Shc displaces complex formation between Shc, Grb2 and SOS and impairs Ras activation [62,65]. In this regard, in SHIP-1-deficient B cells, Erk activation cannot be decreased upon co-ligation of BCR and FcγRIIB [11]. Strikingly, in this pathway, catalytic activity of SHIP-1 is not required.

6.1.3. SHIP-1 interacts with Dok-3 and inhibits JNK activation

Upon co-aggregation of FcγRIIB and BCR, SHIP-1 interacts with Dok-3. This leads to a ternary complex formation between SHIP-1, Dok-3 and Grb2. Grb2 seems to stabilize complex formation since it has been described that Grb2 deficiency reduces interaction between SHIP-1 and Dok-3 upon co-ligation of FcγRIIB and BCR. Moreover SHIP-1–Dok-3–Grb2 complex formation is important for

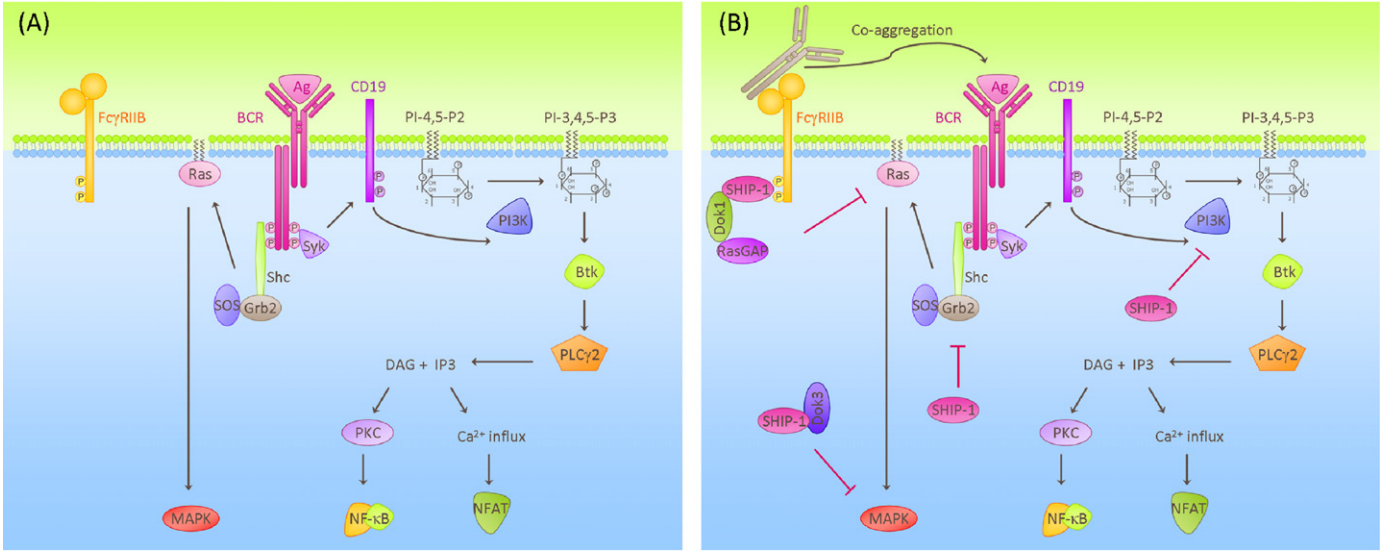

Fig. 4. SHIP-1 negatively regulates BCR signaling. (A) BCR activation and signaling cascades. Antigen-induced BCR activation leads on one hand to phosphorylation CD19 by Syk, to activation of PI3K pathway and to the recruitment of Btk that triggers PLCγ2 activation, thereby inducing activation of the transcription factor NFAT and NF-κB. On the other hand, BCR activation leads to recruitment of Shc, Grb2 and SOS complex that promotes Ras-induced MAPK activation. (Ag = antigen). (B) SHIP-1 inhibits BCR signaling. MAPK inhibition is achieved by SHIP-1 and Dok-3 complex. SHIP-1 inhibits Ras activation by displacing Grb2 and SOS from their interaction with Shc or by recruiting Dok1 and RasGAP to FcγRIIB at the plasma membrane. SHIP-1 also decreases PIP3 pool, thereby down-modulating PLCγ2 activation.

Ca^{2+} influx inhibition after co-aggregation of FcγRIIB and BCR. Indeed, in Grb2 positive B cells, co-aggregation of both receptors dramatically compromises Ca^{2+} influx whereas Ca^{2+} mobilization is moderately reduced in Grb2-deficient cells [66]. In agreement with this report, it has been described that Dok-3 deficiency leads to a decrease of SHIP-1 activation and an enhancement of MAPK, NF-κB and JNK activation [67].

Interaction between SHIP-1 and Dok-3 has also been reported upon BCR activation and in absence of FcγRIIB activation [14]. Interaction between SHIP-1 and Dok-3 reduces JNK activation. The precise mechanism of JNK inhibition is not yet elucidated but requires SHIP-1 catalytic activity [14].

Interestingly, JNK activation is often associated with induction of apoptosis [68]. In the present model, interaction between Dok-3 and SHIP-1, by reducing JNK activation probably promotes cell survival. In this regard, it has been observed that upon co-aggregation of BCR and FcγRIIB, SHIP-1 decreases B cells activation but promotes cell survival. Indeed, in SHIP-1 negative B cells, co-ligation of BCR and FcγRIIB leads to robust JNK activation and induction of apoptosis [12].

6.1.4. SHIP-1 interacts with Dok-1 and decreases MAPK activation

Negative regulation of MAPK is mediated by SHIP-1 and Dok-1 (Fig. 4B). Indeed, upon co-ligation of FcγRIIB and BCR, SHIP-1 is recruited to ITIM motifs of this Fc receptor and is tyrosine phosphorylated. This phosphorylation enables interaction of SHIP-1 with the Dok-1 PTB domain. The latter is also subsequently phosphorylated and can recruit RasGAP, a Ras GTPase activating protein that catalyses hydrolysis of GTP by Ras, thereby promoting GDP-binding to Ras and inactivation of Ras pathway. Here, SHIP-1, as well as Dok-1, act as carrier proteins allowing recruitment of RasGAP to the plasma membrane. Indeed, the recruitment of RasGAP to plasma membrane is sufficient to decrease MAPK activation [20].

6.2. SHIP-1 in T cells

T cells are key components of adaptive immune response. Activation of T cells is achieved by interaction between T cell receptor (TCR) with foreign peptide linked to an MHC molecule which is expressed on cell surface of antigen presenting cells (APC). Activation of T cells results in IL-2 secretion, clonal expansion and differentiation. Mechanistically, TCR is a membrane receptor composed of two chains α and β and of the CD3 complex which transduces signal from plasma membrane to cytosolic targets. Interaction of TCR with a peptide linked to an MHC molecule leads to the phosphorylation of ITAM motifs of CD3 chain and to the recruitment of tyrosine kinases such as Zap70. The latter phosphorylates the transmembrane adaptator LAT which on one hand promotes PI3K-induced Akt activation and on the other hand recruits and activates PLCγ1 [61]. As in B cells, PLCγ1 mediates production of IP3 and DAG by cleaving PI-4,5-P2, thereby triggering Ca^{2+} influx and PKC activation. As a result, NFAT and NF-κB transcription factors are activated (Fig. 5A). Of note, activation of PI3K can also be achieved by CD28 co-receptor. This membrane receptor interacts with B7 molecules present on APC surface and transduces signal that cooperates with TCR signaling to properly activate T cells [61].

6.2.1. SHIP-1 interacts with LAT and Dok-1/2 and decreases T cells activation

SHIP-1 is implicated in negative regulation of T cells activation. Following TCR engagement, Dok-1 and Dok-2 are rapidly phosphorylated on tyrosine and interact with SHIP-1 which is constitutively associated with Grb2 *via* its proline rich region [19]. The ternary complex Grb2–SHIP-1–Dok-1/2 is recruited to LAT, probably *via* Grb2 (LAT possesses Grb2-binding motifs). Recruitment of this complex to LAT inhibits Akt and PLCγ1 activation (Fig. 5B). As a result, Dok-1 and Dok-2 silencing by siRNA induces

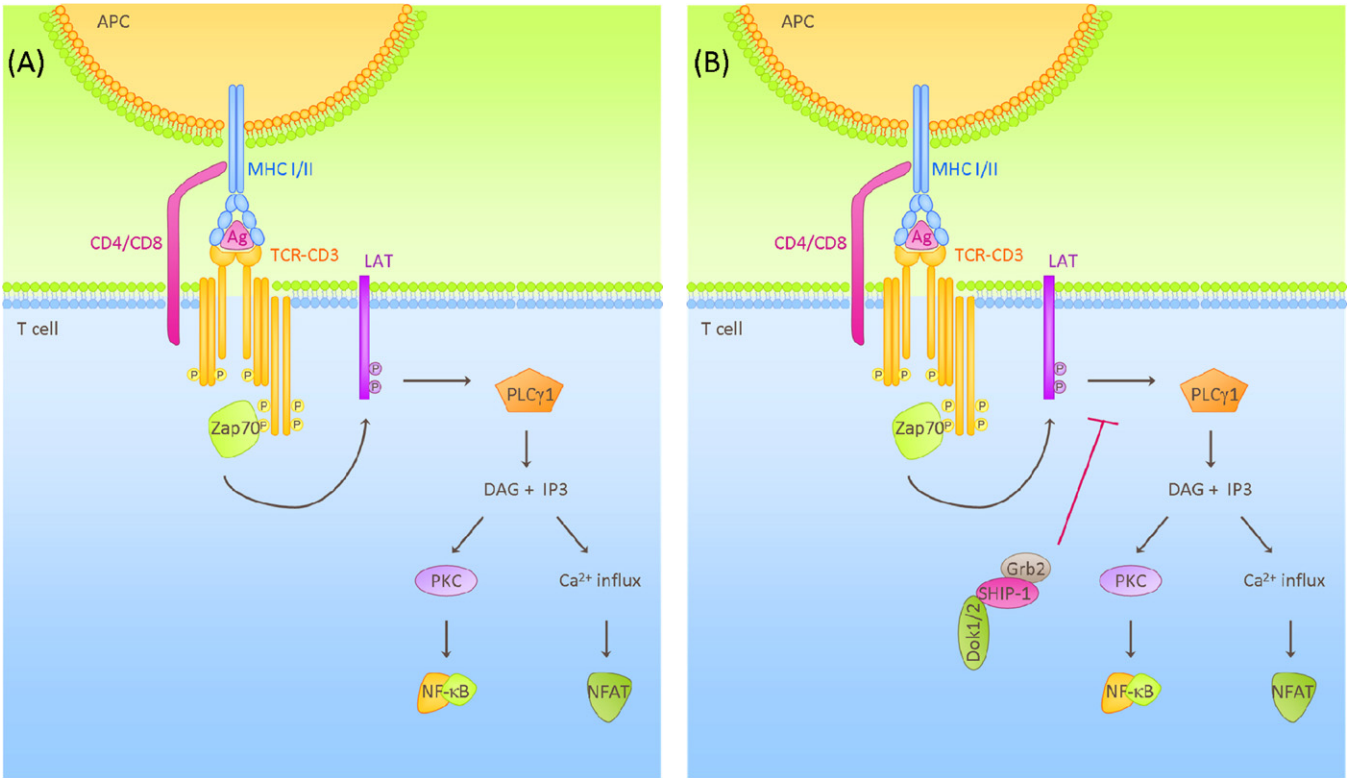

Fig. 5. Negative regulation of TCR activation by SHIP-1 deduced from *in vitro* observations. (A) TCR activation. TCR is activated by crosslinking of an antigen (Ag) presented by MHC molecule expressed on antigen presenting cells (APC). TCR triggering leads to phosphorylation of LAT, recruitment of PLCγ1 and subsequent activation of the transcription factors NFAT and NF-κB. (B) SHIP-1 decreases TCR activation. SHIP-1, Grb2 and Dok-1/2 complex competes with PLCγ2 for binding to LAT.

increased IL-2 production and prolonged Akt activation. It is speculated that in this process SHIP-1 and Dok-1/-2 act as signal gatekeepers controlling TCR activation threshold [19]. Therefore, these data suggest that SHIP-1-deficient T cells should be hyper-responsive to TCR stimulation. However, this hypothesis is not confirmed by *in vivo* analyses demonstrating that T cells from SHIP-1 KO mice are indeed poorly sensitive to TCR triggering. Indeed, weak production of cytokines and decreased proliferation are observed upon TCR stimulation in KO T cells compared to WT cells [49]. However, Tarasenko et al. have recently generated mice with T-cell specific deletion of SHIP-1; in contrast with germline SHIP-1 KO mice, they have observed that T-cell specific deletion of SHIP-1 do not alter sensitivity to TCR signaling. Furthermore, PI3K signaling cascade does not seem to be increased or prolonged in T cells form these mice and antigen-driven expansion of T cells is not affected either [52]. Taken together, these *in vivo* analyses, even if they are not in agreement with each other, do not support a functional role of SHIP-1 in TCR regulation.

6.2.2. SHIP-1 controls PIP3 pool upon CD3/CD28 triggering

Upon co-stimulation of CD3 and CD28 receptors, SHIP-1 is tyrosine phosphorylated and localizes at the plasma membrane [69]. The docking site of SHIP-1 at plasma membrane is not defined, although an interaction between CD3 ITAM motifs and SHIP-1 has been observed *in vitro* [13]. The enzymatic activity of SHIP-1 also increases after CD28 co-ligation, but this observation was carried out *in vitro*. The authors speculate that, as the interaction between SHIP-1 and FcγRIIB in B cells, SHIP-1 recruitment to plasma membrane after CD28 engagement controls PIP3 activation threshold [69]. These data indicate that SHIP-1 could potentially regulate T cells development and activation. However, *in vivo* studies with SHIP-1 KO mice clearly rule out this hypothesis since T cells from SHIP-1 KO mice develop normally despite the fact that thymocytes as well as mature T cells number is reduced. However, ratios of CD4/CD8 negative, single-positive and double-positive T cells are normal in SHIP-1 KO mice [49]. Moreover, in mice with T-cell specific knock-out of SHIP-1, SHIP-1 deficiency does not altered PI3K pathway upon stimulation of CD28 and CD3 [52].

6.3. SHIP-1 in NK cells

NK cells are cytotoxic lymphoid cells containing cytolytic granules that mediate rejection of virally infected and tumor cells. These cells can also produce immunoregulatory cytokines such as IFN-γ and TNF-α. NK cells express a wide range of receptors that can detect abnormalities on target cells, *e.g.* MHC low or MHC-negative cells, and induce their apoptosis. NK cells also possess Fc receptors (*e.g.* FcγRIIIA) that recognize antibodies coated on infected or tumor cells and promote elimination of these target cells. This process is called antibody-dependent cell-mediated cytotoxicity.

6.3.1. SHIP-1 interacts with Shc and regulates CD16-mediated NK cells cytotoxicity

SHIP-1 is a negative regulator of CD16-mediated cytotoxicity in NK cells [70,71]. CD16, also called FcγRIIIA, is a Fc-receptor that can bind to Fc chain of IgG linked to cell-associated antigens. Upon activation, CD16 translocates to lipids rafts, is phosphorylated and induces PIP3-mediated PLCγ2 activation. This leads to PLCγ2-induced IP3 formation and to an increase of intracellular Ca^{2+} influx. Enhancement of calcium concentration is required for cytolytic granules reorientation and exocytosis (Fig. 6A).

Down-modulation of CD16 pathway is achieved by recruitment of Shc to phosphorylated CD16 ζ chain. Shc is subsequently phosphorylated and recruits SHIP-1 *via* binding of SHIP-1 SH2

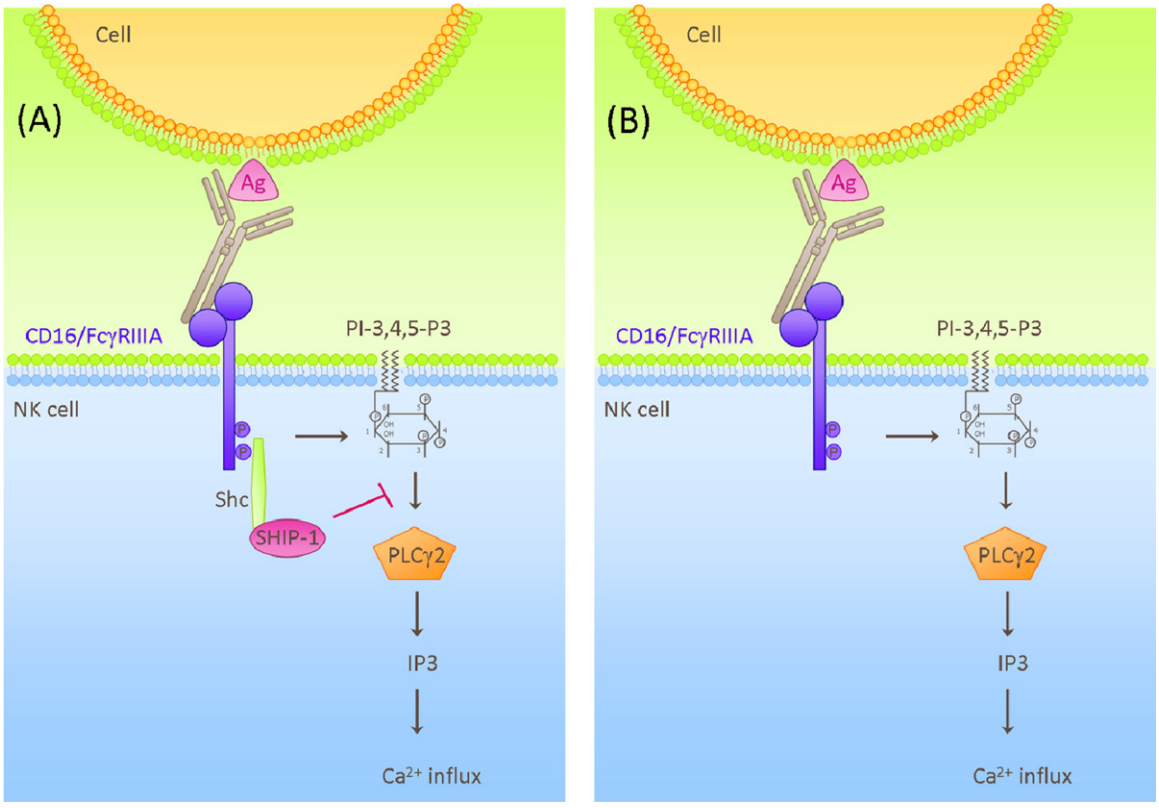

Fig. 6. Negative regulation of CD16-mediated cytotoxicity. (A) CD16/FcγRIIIA pathway. CD16 is activated by binding to Ig linked to cell-associated antigen. CD16 is subsequently phosphorylated and induces PIP3-mediated PLCγ2 activation. This leads to generation of IP3 and to an increase of intracellular calcium influx. (Ag: antigen) (B) Down-modulation of CD16 signaling pathway by SHIP-1. Shc binds to CD16 receptor and recruits SHIP-1 which reduces PLCγ2 activation by hydrolysing PIP3.

domain to phosphotyrosines of Shc (Fig. 6B). Recruitment of SHIP-1 to CD16 leads to a decrease of Ca^{2+} influx and to a global reduction of NK cells cytotoxicity [70]. Strikingly, it has been demonstrated that catalytic activity of SHIP-1 is necessary for negative regulation of CD16-induced cytotoxicity in primary human NK cells [71,72]. These results indicate that SHIP-1 decreases CD16-induced PLCγ2 activation through PIP3 hydrolysis [71] (Fig. 6B). Furthermore, SHIP-1 deficiency also leads to enhanced cytokines production upon CD16 crosslinking. Indeed, SHIP-1 KO NK cells produce much more IFN-γ after contact with IgG-coated tumor cells than WT cells, indicating that SHIP-1 plays a role in the regulation of CD16-mediated cytokine production [72].

6.4. SHIP-1 in mast cells

Mast cells are granulocytic cells characterized by cytoplasmic granules containing various mediators including cytokines, chemokines, proteolytic enzymes and vasoactive mediators. Degranulation of mast cells is caused by various stimuli, the best characterized is the crosslinking of their high affinity Fc receptor, *i.e.* FcεRI, by IgE. Because mast cells release vasodilator and anticoagulant molecules such as heparin and histamine upon degranulation, they are well described for their role in allergic reactions [73].

Mechanistically, FcεRI is activated by binding to IgE. This leads to the phosphorylation of ITAM motifs of the Fc receptor and the recruitment and activation of the Src kinase Syk, which in turn phosphorylates numerous substrates giving rise to PI3K pathway activation, intracellular Ca^{2+} influx and degranulation. On the other hand, activation of FcεRI also leads to production of cytokines such as TNF-α and IL-6. Cytokines production is mediated by Ras-dependent MAPK activation. This pathway requires phosphorylation of the membrane co-receptor LAT and recruitment of Shc, Grb2 and SOS, which activate Ras pathway in a mechanism closely related to BCR-induced Ras activation in B cells.

SHIP-1 is described as a negative regulator of mast cells degranulation [43]. Indeed, SHIP-1 KO mast cells are more sensitive to degranulation upon FcεRI crosslinking by either IgE alone or antigen-bound IgE than their WT littermates [74]. In KO mast cells, FcεRI-induced Ca^{2+} influx is more important than in WT cells, leading to more intense and faster release of histamine granules. Furthermore, SHIP-$1^{-/-}$ mast cells also produce much more inflammatory cytokines such as TNF-α, IL-4, IL-6, IL-13 in response to IgE-stimulation compared to their WT counterparts [75]. Increase of cytokine production is due to alteration of PIP3-induced activation of Akt, PKC, NF-κB and the MAPKs p38 and JNK [75].

6.4.1. SHIP-1 interacts directly with FcεRI under supra-optimal IgE stimulation

Mechanistically, Gimborn et al. suggest that, upon supra-optimal IgE stimulation, FcεRI is tyrosine phosphorylated on its ITAM motifs and can recruit SHIP-1 *via* its SH2 domain, therefore SHIP-1 is brought in close proximity of its substrate and could hydrolyse PIP3, thereby down-modulating PIP3-dependent pathway such as Akt activation [76]. According to this hypothesis, it has been demonstrated that SHIP-1 interacts with ITAM motifs of FcεRI *in vitro* [13]. Gimborn et al. have also observed that SHIP-1 interacts *via* its proline rich region with F-actin in peripheral submembranal areas in unstimulated as well as in IgE-stimulated mast cells [76]. Moreover, actin cytoskeleton is known to be implicated in negative regulation of mast cell degranulation. Therefore recruitment of SHIP-1 to FcεRI could also bring actin cytoskeleton to the Fc receptor thereby decreasing mast cells activation. Indeed, treatment of mast cells with actin depolymerising drugs, such as latrinculin B, decreases SHIP-1 phosphorylation and increases mast cell degranulation upon supra-optimal IgE stimulation compared to untreated cells, suggesting a functional role for SHIP-1 and F-actin interaction [76].

6.4.2. SHIP-1 interacts with LAT and p66Shc and down-modulates FcεRI signaling

As it has been observed in T cells, SHIP-1 can be recruited to LAT in mast cells after FcεRI stimulation *via* interaction of phosphorylable motif of LAT with SHIP SH2 domain [77]. Recruitment of SHIP-1 to LAT is stabilized by its interaction with p66Shc [78]. The ternary complex promotes SHIP-1 plasma membrane location where SHIP-1 can dephosphorylate PIP3 and reduce Akt activation. As a result, a great similarity is observed between SHIP-1 KO and p66Shc KO mast cells, both exhibiting enhanced degranulation and cytokine production following FcεRI engagement [78].

6.4.3. SHIP-1 interacts with Dok-1 and decreases FcεRI-induced MAPK activation

Down-modulation of FcεRI-induced mast cells activation can be achieved by co-aggregation of FcεRI with the low affinity receptor, *i.e.* FcγRIIB, in a mechanism closely related to co-aggregation of BCR and FcγRIIB in B cells. Co-ligation of both receptors also results in tyrosine phosphorylation of FcγRIIB ITIM motifs which recruit SHIP-1 *via* its SH2 domain. Once recruited, SHIP-1 is tyrosine phosphorylated and interacts with the PTB domain of Dok-1 which is subsequently phosphorylated and enables recruitment of RasGAP. The latter catalyses hydrolysis of GTP bound to Ras, thereby promoting GDP-binding to Ras and inactivation of Ras pathway. Here, SHIP-1, as well as Dok-1, act as adaptators enabling recruitment of RasGAP to the plasma membrane [79].

6.5. SHIP-1 in monocytes/macrophages

6.5.1. SHIP-1 is degraded during IL-4 stimulation thereby promoting skewing toward M2 phenotype

Macrophages can be subdivided in two classes: the M1 and M2 types. The M1 macrophages are implicated in the first inflammatory response where they eradicate tumor and microbial infected cells. M1 macrophages are characterized by high secretion of inflammatory cytokines such as TNF-α, IL-1 and IL-6 and are efficient producers of reactive oxygen species (ROS) and nitric oxide (NO) [80]. M2 macrophages are implicated in inflammatory response termination and in parasites and extracellular pathogens elimination. These cells are hypo-responsive to inflammatory cytokines and act as scavengers, thereby promoting tissue repair, regeneration and remodelling [80].

In primary macrophage, IL-4 stimulation results in SHIP-1 phosphorylation and subsequent proteasomal degradation. Mechanistically, following IL-4 stimulation, SHIP-1 is tyrosine phosphorylated by one or several members of Src kinase family [24]. This phosphorylation triggers its polyubiquitination which is probably mediated by the c-Cbl or Cbl-b E3 ligases. Indeed, both proteins can be immunoprecipitated with SHIP-1. Poly-ubiquitinated SHIP-1 is in turn degraded by the proteasome [24]. Strikingly, it has been demonstrated that SHIP-1 deficiency leads to increased macrophage M2 skewing [81,82]. As a result, SHIP-1 KO bone marrow-derived macrophages (BMDM) constitutively express markers of M2 subtype (*e.g.* high expression levels of arginase-1 and chitinase like Ym1) [81]. Moreover, skewing toward M2 phenotype is caused, at least in part, by generation of high levels of intracellular PIP3 [81] which can be observed when SHIP-1 is absent. These data suggest that SHIP-1 degradation by IL-4 is perhaps required to induce M2 differentiation of activated macrophages. Strikingly, this hypothesis has been recently confirmed. The Sly's group has demonstrated that SHIP-1 degradation is a crucial step for M2 skewing [83]. Indeed, over-expression of SHIP-1 or inhibition of its degradation decreases

expression of specific markers of M2 subtype (especially arginase-1 expression) upon IL-4 treatment, while SHIP-1 silencing clearly enhances expression of these markers [83]. Collectively these data indicate that SHIP-1 potentially controls M1/M2 skewing.

6.5.2. SHIP-1 is essential for development of endotoxin tolerance

Role of SHIP-1 in endotoxin tolerance is well described in the case of LPS-induced tolerance. Endotoxin tolerance is a phenomenon of desensitization of immune cells. In this process, tolerized macrophages produce less pro-inflammatory cytokines and nitric oxide in response to second challenge with LPS. Endotoxin tolerance is thought to protect host from cell damages caused by hyper-activation of innate immune cells. Indeed, over-production of pro-inflammatory cytokines can result in sepsis syndrome which can in turn lead to death. Therefore endotoxin tolerance represents an adaptation to chronic or persistent microbial infection (reviewed in [84]).

Mechanistically, LPS-induced TLR-4 activation triggers two different pathways. On one hand, TLR-4 activation induces recruitment of TIRAP and MyD-88 and subsequent activation of IRAK-1 and -4, which in turn induce activation of NF-κB and MAPK. This leads to the production of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α (Fig. 7A). On the other hand, activated TLR-4 also recruits TIRAM and TRIF and triggers activation of a complex containing TBK-1 and IKKε which leads to activation of the transcription factor IRF3 and subsequent transcription of IFN-β (Fig. 7A) [85]. These processes are in part controlled by PI3K since PI3K activation is required for proper NF-κB activation and for IFN-β secretion after TLR-4 triggering [3].

First evidence for a role of SHIP-1 in endotoxin tolerance has been described when Krystal's group has observed that SHIP-1 KO BMDM are hyper-responsive to LPS stimulation compared to their WT littermates. Furthermore, SHIP-1 KO mice cannot induce endotoxin tolerance [86]. These data define a negative role for SHIP-1 in TLR-4 signaling *in vitro* and *in vivo*.

Basically, negative regulation of TLR-4 is mediated by TRL-4-dependent TGF-β secretion. This cytokine stimulates transcription of various inhibitors of TLR-4 pathway, among them SHIP-1 expression [86–88] *via* activation of the transcription factor SMAD4 [87]. Up-regulation of SHIP-1 decreases PI3K activation and subsequently TLR-4-induced cytokines production (Fig. 7B). Indeed, production of IL-6, IFN-β, TNF-α and IL-1 is increased in SHIP-1 KO BMDM [86] and in SHIP-1 RNAi transfected macrophages [88].

Furthermore, An et al. have also observed that SHIP-1 deficiency increases MyD-88 and TLR-4 interaction indicating that SHIP-1 somehow inhibits this complex formation. However, the precise mechanism is not yet identified [88] (Fig. 7B).

Strikingly, it has been described that SHIP-1 reduces IFN-β production not only after TLR-4 [89] but also after TLR-3 stimulation [90], indicating that SHIP-1 is probably implicated in negative regulation of several TLR pathways. TLR-3 is an endosome-located receptor which is activated by binding of single- and double-stranded RNA. Activation of TLR-3 leads to recruitment of TRIF, IKKε and TBK-1. This complex mediates activation of IRF3 and IFN-β transcription [85] (Fig. 7A). Gabhann et al. have demonstrated that SHIP-1 reduces TBK-1 activation and therefore decreases IFN-β production. Indeed, in SHIP-1 KO macrophages, TBK-1 is constitutively associated with TRIF and TRAF-3 and signaling of TLR-3 is increased [90]. As in the case of TRL-4 and MyD-88 association, SHIP-1 destabilizes complex formation between TBK-1 and TRIF–TRAF-3 (Fig. 7B). The precise mechanism is not elucidated but it has been shown that catalytic activity of SHIP-1 is required for dissociation of this complex [90].

6.5.3. SHIP-1 interacts with Lyn and negatively regulates M-CSF macrophages activation

Macrophage colony stimulating-factor (M-CSF) is a cytokine promoting proliferation, differentiation and cell survival of monocytes, macrophages, and bone marrow progenitor cells. M-CSF receptor signaling is mediated by PI3K-induced Akt activation. Negative regulation of M-CSF is achieved by Lyn and SHIP-1. Indeed, M-CSF induces phosphorylation of Lyn which can subsequently recruit SHIP-1 *via* its SH2 domain. This interaction relocalizes SHIP-1 to the cell membrane where it dephosphorylates PIP3 and minimizes M-CSF-dependent Akt activation. In this regard, SHIP-1 KO as well as Lyn KO macrophages exhibit an acute and longer Akt activation compared to WT cells [23].

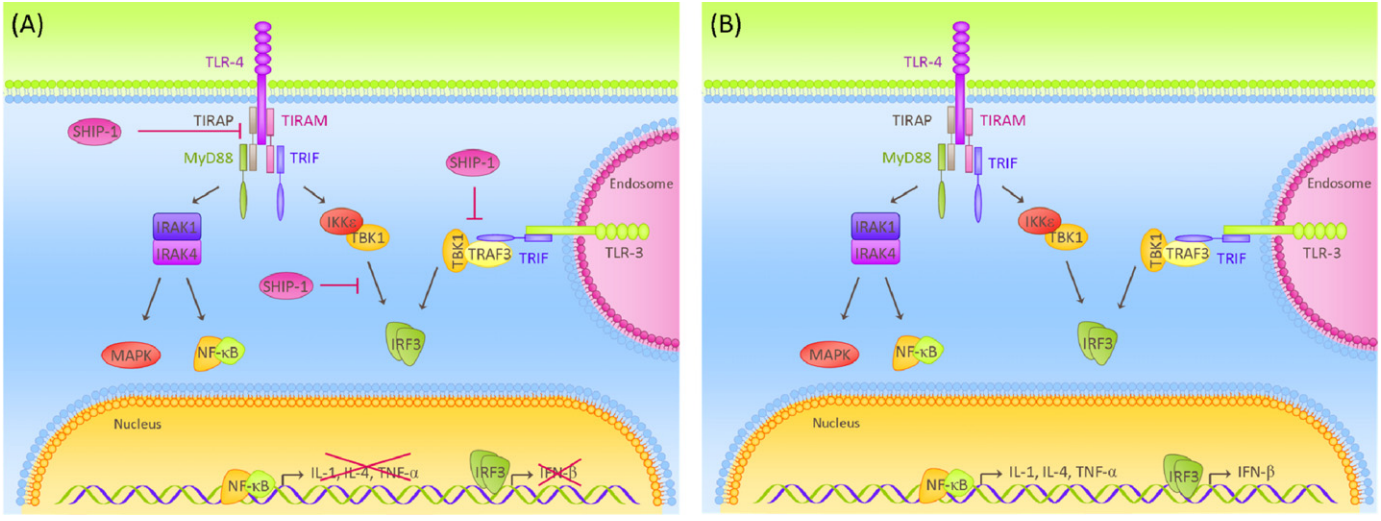

Fig. 7. Mechanism of immune tolerance induced by SHIP-1. (A) TLR-4 and TLR-3 signaling pathways. On one hand, TLR-4 recruits TIRAP and MyD-88 and activates IRAK1/4 which in turn leads to the activation of the transcription factors MAPK and NF-κB. These transcription factors induce production of pro-inflammatory cytokines such as IL-6 and TNF-α. On the other hand, TLR-4 also recruits TIRAM and TRIF and activates IKKε and TBK-1 complex thereby inducing IRF3 activation and IFN-β production. TLR-3 is located in endosomal compartment and triggers recruitment of TRIF, TRAF-3 and TBK-1 thereby also activating IRF3. (B) SHIP-1 interferes with TLR signaling. SHIP-1 decreases association between TLR-4 and MyD-88, and association of TBK-1 with the complex composed of TLR-3, TRAF-3 and TRIF. SHIP-1 down-modulates PIP3 pool thereby inhibiting TLR-4-induced cytokines production.

7. SHIP-1: dual role in cancer and therapy

7.1. Diseases linked to SHIP-1

Considering the negative regulatory role of SHIP-1 in the hematopoietic lineage, mutations and loss of SHIP-1 expression are frequently observed in cancer cells. One good example is the cells from acute myeloid leukemia (AML) patients, where SHIP-1 expression is often down-regulated [59]. In several cases, a punctual mutation replacing a valine at position 684 by a glutamic acid has also been observed. This mutation, located near the conserved phosphatase domain, decreases SHIP-1 catalytic activity, providing growth advantage and apoptosis resistance for these cancer cells [59,91]. SHIP-1 is also truncated, or totally absent, in T cells from patients with T acute lymphoblastic leukemia (T-ALL) [92]. These SHIP-1 truncations seem to be caused by extensive alternative splicing [92].

Considering that SHIP-1 inhibition confers advantage to cancer cells, it has been described that oncogenic proteins induce SHIP-1 down-regulation. Effectively, the proto-oncogene BCR/ABL induces SHIP-1 down-modulation [24,93]. BCR/ABL is a fusion protein resulting of a chromosomal translocation that is implicated in the development of chronic myelogenous leukemia (CML) [94]. Importantly, in cells from CML patients, expression of SHIP-1 is weak and sometimes totally undetectable [93]. Mechanistically, BCR/ABL-dependent down-modulation of SHIP-1 requires an intact tyrosine kinase activity of BCR/ABL [24,93]. Indeed, SHIP-1 is phosphorylated either directly by BCR/ABL or indirectly by a BCR/ABL-activated Src kinase and is subsequently targeted for polyubiquitination probably mediated by the E3 ligase c-Cbl. Polyubiquitinated SHIP-1 is subsequently degraded by the proteasome [24]. Another proto-oncogene known to down-regulate SHIP-1 is Tax, a viral protein implicated in adult T cell leukemia/lymphoma (ATLL) [95]. This type of cancer is due to HTLV-1 infection. In this case, down-modulation of SHIP-1 is mediated at the transcriptional level by Tax-induced NF-κB activation [96].

It has also been demonstrated that SHIP-1 loss promotes leukemogenesis in a Friend virus-infected mouse model [97]. Friend virus induces development of erythroleukemia *via* activation of Fli-1, a member of the Ets transcription factor family. One of the Fli-1 targets is SHIP-1 that possesses two binding domains for Ets factor in its promoter. Fli-1 acts as a transcriptional repressor of SHIP-1 and reduces dramatically its expression in these cancer cells. Lack of SHIP-1 does not lead *per se* to tumor development, indeed WT mice as well as SHIP-1 KO mice develop erythroleukemia at same rate after Friend virus infection, but SHIP-1 deficiency accelerates tumor progression [97].

7.2. Therapy linked to SHIP-1

7.2.1. Activation of SHIP-1 and multiple myeloma treatment

Considering the negative regulatory role of SHIP-1 in myeloid cells, compounds able to increase SHIP-1 activity could be of great interest for therapeutic treatments. Kennah et al. have identified a class of molecules which bind to the C2 domain of SHIP-1 and increase its catalytic activity. These molecules have been successfully used for treatment of multiple myeloma cells line *in vitro* [98]. Cells treated with these molecules exhibit reduced Akt phosphorylation, decreased cell proliferation and enhanced apoptosis. Moreover, these compounds act in synergy with other molecules already used in cancer therapy such as dexamethasone [98]. Given to their specific action on myeloid cells, SHIP-1 activators could be useful for treatment of myeloma.

7.2.2. Inhibition of SHIP-1 and bone marrow transplantation

Graft-versus-host disease (GVHD) is a common complication arising after bone marrow (BM) transplantation. In the initial phase, donor T cells recognize recipient antigen-presenting-cells (APC) and engage a first immune response. In the second step, donor APCs present exogenously acquired recipient antigens *via* major histocompatibility complex (MHC) to donor T cells, leading to massive activation of T cells and to a broad attack against host tissues [99].

It has been demonstrated that SHIP-1-deficient mice express more myeloid suppressor cells that their WT counterparts [100]. Myeloid suppressor cells (MySC) are a type of immunoregulatory cells that can repress allogeneic T cells response. Considering that MySC are well known to control GVHD during allogeneic BM transplantation and that SHIP-1 KO mice over-produce this type of cells, Kerr's group has studied the impact of SHIP-1 deficiency on normal adult physiology. They have developed a mice model where both alleles of SHIP-1 are flanked with LoxP site and where Cre recombinase is expressed under the control of Mx1 promoter (MxCreSHIP$^{flox/flox}$ mice). As a result, treatment of these mice with polyI:C causes production of IFN-α and IFN-β which induce Cre and in turn shut off SHIP-1 expression [101]. In adult mice, SHIP-1 depletion leads to increased production of MySC, as it has been observed in SHIP-1 KO mice. The authors have therefore tested survival of WT versus MxCreSHIP$^{flox/flox}$ mice after fully mismatched BM transplantation. In this experiment BM donors are on H2b background and recipients are on H2d background, both types are therefore MHC incompatible. In this model, SHIP-1 deficiency is induced in recipients 7 days before BM engraftment. Before transplant, WT as well as MxCreSHIP$^{flox/flox}$ mice are first irradiated and are subsequently grafted with bone marrow cells from donor mice. The authors have observed a 2-fold enhancement of survival of SHIP-1-deficient mice compared to their WT littermates. Interestingly, It has been observed that MxCreSHIP$^{flox/flox}$ mice recover more rapidly a normal weight after engraftment and exhibit reduced manifestation of GVHD at 3–4 weeks post-tranplant compared to WT mice. Strikingly, reversible SHIP-1 inhibition during 1 week does not lead to the myeloproliferative disorder that has been observed in SHIP-1 KO mice [101].

In order to develop therapeutic treatments based on SHIP-1 inhibition, Brooks et al. have screened chemical compounds to find SHIP-1 inhibitors [102]. By this method, they have identified 3α-aminocholestane (3AC) as a potent inhibitor of SHIP-1 enzymatic activity. As in the MxCreSHIP$^{flox/flox}$ mouse model, the authors have observed that transient treatment with 3AC increases production of MySC in mice and impairs their splenocytes ability to induce allogeneic T cells response. They have also demonstrated that treatment of mice with 3AC boosts their granulocytes production and enhances blood cell recovery after irradiation compared to untreated mice. All of these data indicate a potent therapeutic role for inhibitors of SHIP-1. First, reversible and transient inhibition of SHIP-1 abrogates GVHD in allogeneic bone marrow transplantation and could therefore potentially inhibit T cells responses that mediated graft rejection in solid organ transplantation [101]. Second, SHIP-1 inhibition, through enhancing recovery of blood cell number after irradiation, could potentially promote blood cells production in patients with myelodysplastic syndrome or in patients recovering from myelosuppressive infection [102].

To our knowledge, no drug has been generated to prevent interaction between SHIP-1 and one of its partners. This could be explained by the fact that SH2 domain and proline rich region are highly conserved sequences and that inhibitors of such domains could also abrogate interactions of other SH2 or PRR-containing proteins.

8. Concluding remarks

SHIP-1 has been first identified and characterized as an inositol phosphatase. However, the interaction properties of SHIP-1 regulate its enzymatic as well as non enzymatic activities. In most cases, interaction partners of SHIP-1 bring it at the plasma membrane where it hydrolyses PIP3, thereby down-modulating PH-containing proteins such as Akt and Btk. Moreover, SHIP-1 can also function independently of its catalytic activity. A good example is the down-modulation of Ras pathway in B cells. In this cascade, SHIP-1 acts as a carrier protein, enabling recruitment of RasGAP at the plasma membrane, thereby down-regulating Ras activation.

Some properties of SHIP-1 are still not elucidated and need further investigations. Indeed, SHIP-1 KO T cells are more sensitive to apoptosis. How does SHIP-1 play this anti-apoptotic role in T cells? KO NK cells present an alteration of NK receptors repertoire. How does SHIP-1 influence NK receptors expression? KO myeloid cells develop a myelosupressive activity. How does SHIP-1 repress this type of differentiation? Answers to these questions could maybe be elucidated by finding new interaction partners of SHIP-1 that are implicated in theses processes and could therefore provide some clues to better understand SHIP-1 activities.

Inhibitors and activators of SHIP-1 are attractive for medical therapy development. However, complete abrogation of SHIP-1 catalytic activity is hazardous since loss of SHIP-1 enzymatic activity is correlated with development of myeloproliferative disorders. Nevertheless, specific inhibitors able to displace some interactions between SHIP-1 and its partners could be very useful to down-modulate specific pathways without affecting other ones.

Acknowledgements

Financial supports were provided by the "Fond national de la recherche scientifique" (FNRS) and by "Télévie". CC is a PhD student supported by the Télévie, GG and JP are post-doctoral researcher and research director from the FNRS, respectively. We are grateful to Xavier Rambout for valuable correction and discussion.

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