The role of SHIP1 in T-lymphocyte life and death

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Abstract

SHIP1 [SH2 (Src homology 2)-containing inositol phosphatase-1], an inositol 5-phosphatase expressed in haemopoietic cells, acts by hydrolysing the 5-phosphates from PtdIns(3,4,5)P₃ and Ins(1,3,4,5)P₄, thereby negatively regulating the PI3K (phosphoinositide 3-kinase) pathway. SHIP1 plays a major role in inhibiting proliferation of myeloid cells. As a result, SHIP1−/− mice have an increased number of neutrophils and monocytes/macrophages due to enhanced survival and proliferation of their progenitors. Although SHIP1 contributes to PtdIns(3,4,5)P₃ metabolism in T-lymphocytes, its exact role in this cell type is much less explored. Jurkat cells have recently emerged as an interesting tool to study SHIP1 function in T-cells because they do not express SHIP1 at the protein level, thereby allowing reintroduction experiments in a relatively easy-to-use system. Data obtained from SHIP1 reintroduction have revealed that SHIP1 not only acts as a negative player in T-cell lines proliferation, but also regulates critical pathways, such as NF-κB (nuclear factor κ-B) activation, and also appears to remarkably inhibit T-cell apoptosis. On the other hand, experiments using primary T-cells from SHIP1−/− mice have highlighted a new role for SHIP1 in regulatory T-cell development, but also emphasize that this protein is not required for T-cell proliferation. In support of these results, SHIP1−/− mice are lymphopenic, suggesting that SHIP1 function in T-cells differs from its role in the myeloid lineage.

Introduction

The PI3K (phosphoinositide 3-kinase) pathway regulates many important cellular functions, such as survival, proliferation, cell activation and migration [1–3]. Upon activation, PI3K generates PtdIns(3,4,5)P₃ from PtdIns(4,5)P₂. PtdIns(3,4,5)P₃ attracts PH domain (pleckstrin homology domain)-containing proteins to the plasma membrane, which allows their activation and subsequent signalling. PH domain-containing proteins include notably Akt (also called protein kinase B), a key messenger in cellular proliferation and apoptosis. Many important cellular functions, such as survival, proliferation, cell activation and migration [1–3]. Upon activation, PI3K generates PtdIns(3,4,5)P₃ from PtdIns(4,5)P₂. PtdIns(3,4,5)P₃ attracts PH domain (pleckstrin homology domain)-containing proteins to the plasma membrane, which allows their activation and subsequent signalling. PH domain-containing proteins include notably Akt (also called protein kinase B), a key messenger in cellular proliferation and apoptosis. Akt activation is reinforced by the demonstration that a dominant-negative mutation reducing catalytic activity of SHIP1 was found to be associated with acute myeloid leukaemia [12]. SHIP1 also acts at different levels in B-cell biology. SHIP1−/− mice exhibit a reduction in the number of pre-B and immature B-cells in the bone marrow, but an increased number of hypermature B-cells in the spleen. B-cells from SHIP1−/− mice are also hypersensitive to BCR (B-cell receptor) stimulation, but less sensitive to apoptosis [10,13–16]. These data reflect that the inhibitory signals mediated by SHIP1 have positive or negative impacts depending on the stage of B-cell development. The molecular mechanisms underlying SHIP1-mediated inhibition of B-cell proliferation are now well understood. Signalling pathways induced by BCR activation are inhibited by co-aggregation.
between BCR and FcγRIIB, a low-affinity Fc receptor. This co-aggregation occurs when IgG-containing immune complexes bind simultaneously the BCR and FcγRIIB and triggers tyrosine phosphorylation within the ITIM of the cytoplasmic tail of FcγRIIB [17]. This phosphorylation induces the recruitment of SHIP1 to the tyrosine-phosphorylated ITIM of FcγRIIB via its SH2 domain. SHIP1 then dephosphorylates PtdIns(3,4,5)3, leading to inhibition of PLCγ (phospholipase Cγ), Akt and Btk (Bruton’s tyrosine kinase), thereby inhibiting proliferation and calcium mobilization (for reviews, see [13,18–20]). SHIP1 also functions in FcγRIIB-mediated inhibitory signals upon FcεRI-induced degranulation in mast cells (for reviews, see [9,21–23]).

**Role of SHIP1 in T-lymphocytes**

**Data obtained from T-cell lines**

Although the role of SHIP1 has been intensely studied in myeloid and B-cells, its exact biological function in T-cells remains poorly understood. Pioneer studies have demonstrated a tyrosine phosphorylation of SHIP1 upon CD3 and CD28 stimulation, and redistribution of its catalytic activity at the plasma membrane [24,25], without highlighting a clear biological role of SHIP1 in the regulation of T-cell activation. SHIP1 was also reported to interact with the ξ chain of the TCR (T-cell receptor), at least in vitro [26]. An embryonic study has also demonstrated that FcγRIIB co-aggregation with the TCR results in tyrosine phosphorylation of SHIP1, indicating that, like in B-cells, SHIP1 would be responsible for FcγRIIB-elicited inhibitory signalling in T-cells [27]. Finally, SHIP1 was demonstrated to play a role in LFA-1 (lymphocyte function-associated antigen 1)-dependent adhesion [28].

The finding that the Jurkat T-cell line was defective in SHIP1 (and PTEN) expression [29–31] has opened up new possibilities for studying SHIP1’s biological role in T-cells by complementation experiments. Because of their deficiency in SHIP1 expression, Jurkat cells express a very high level of PtdIns(3,4,5)3 and a subsequent constitutive Akt activation compared with other leukaemic cell lines (such as CEM, MOLT-4 or HUT78) or human PBLs [32]. Restoration of SHIP1 activity in Jurkat cells was first carried out using a Tet-Off regulated system encoding the lipid phosphatase core of SHIP1 fused to the transmembrane region of rat CD2. Expression of this membrane-localized SHIP1 in Jurkat cells was sufficient to reduce Akt constitutive activity and the activation of downstream PI3K effector, constituting the first evidence that SHIP1 is capable of modulating important signalling pathways in T-cells [32]. Ectopic expression of the complete SHIP1 protein was also achieved in Jurkat cells by Horn et al. [33]. SHIP1-expressing Jurkat cells have reduced PtdIns(3,4,5)3 levels compared with the parental clone, and a subsequent diminution of Akt phosphorylation on Ser-473 and Thr-308. Phosphorylation of GSK-3β (glycogen synthase kinase 3β), an Akt substrate, is also down-regulated upon SHIP1 re-expression [33]. Importantly, expression of SHIP1 in Jurkat cells also reduces cellular proliferation, notably by affecting the G1 cell-cycle regulators Rb and p27Kip1 [33] and KLF2 (Krüppel-like factor 2), a factor implicated in T-cell quiescence [34]. Altogether, these reintroduction experiments have highlighted a role for SHIP1 in negatively regulating T-cell line proliferation. In support of this, down-regulation of SHIP1 expression has been described in ATLL (adult T-cell leukaemia) [35]. Using a lentiviral system, we have also expressed SHIP1 in Jurkat cells and have furthermore positioned this protein as a key adaptor in the activation of NF-κB (nuclear factor κB) upon oxidative stress. After exposure to H2O2, parental Jurkat cells activate NF-κB through phosphorylation of the inhibitor IκB (inhibitory κB) on Tyr-42, which triggers its calpain-mediated degradation and allows the freed NF-κB to migrate into the nucleus [36,37]. On the contrary, Jurkat cells expressing SHIP1 activate NF-κB through IKK (IkB kinase) activation and phosphorylation of the inhibitor IκB on Ser-32 and Ser-36 upon H2O2 stimulation. Cell lines that express SHIP1 naturally (i.e. CEM or the subclone Jurkat JR) also follow the same signalling pathway [38]. Importantly, we have also shown that Jurkat cells expressing SHIP1 are much more resistant to H2O2 and FasL-induced apoptosis than the parental cells [38]. This result may appear discrepant with previous data obtained with other cell types proposing that SHIP1 increases apoptosis by antagonising the anti-apoptotic function of PI3K. Indeed, a pro-apoptotic role of SHIP1 was described in several cell types, including B-, myeloid and erythroid cells [11,14,39–41]. On the contrary, some authors have reported that SHIP1 recruitment attenuates B-cell apoptosis induced by FcγRIIB independently of BCR co-ligation, and that failure to recruit SHIP1 increases the apoptotic phenotype [42,43]. Furthermore, it is worth noting that SHIP1-deficient mice are lymphopenic [10,11,44], suggesting that, depending on the cell type and the biological context, SHIP1 can exert either pro- or anti-apoptotic functions (Table 1).
Data obtained from primary T-cells

As described above, generation of SHIP1-deficient mice has considerably increased our understanding of its biological role. These mice have notably revealed that SHIP1 does not function similarly in the lymphoid to in the myeloid lineage. Indeed, whereas SHIP1-KO (knockout) mice have more myeloid cells than wild-type mice, they exhibit a reduction in the T-cell population [10,11]. An elegant study carried out by Rothman’s group [44] recently addressed SHIP1’s role in primary mouse T-cells. These authors have demonstrated that, as in B-cells, SHIP1 appears to play a biphasic role in T-cell physiology. First, SHIP1, together with the adaptor protein Dok-1, plays a key role in T-cell development, since mice deficient in both SHIP1 and Dok-1 exhibit an important reduction in the percentage of CD4+ and CD8+ double-positive cells, together with an increased number of CD4+ and CD8– double-negative cells [44]. On the other hand, there is a reduced number of T-cells in the spleen from SHIP1-KO mice, due to a severe reduction of CD8+ T-cells, suggesting that SHIP1 is important in maintaining the CD4+/CD8+ ratio in the periphery. But the most intriguing feature of CD4+ T-cells from SHIP1-KO mice is that they are phenotypically and functionally Tregs (regulatory T-cells) [44]. Indeed, splenic T-cells from SHIP1-KO mice are activated, but do not respond to TCR stimulation or produce cytokines in response to PMA stimulation. They exhibit furthermore Treg markers [such as high TGFβ (transforming growth factor β) production and expression of the transcription factor Foxp3] and are able to suppress IL-2 (interleukin 2) production by CD4+ CD25+ T-cells upon CD3 stimulation. T-cells from mice deficient in both SHIP1 and Dok-1 share the same phenotype, highlighting the crucial role of the adaptor Dok-1 for SHIP1 activity [44]. An analogous co-operation of Dok-1 with SHIP1 has already been demonstrated in FcyRIIB signalling in B-cells, where SHIP1 serves as an adaptor protein to recruit Dok-1 to the receptor, which in turn inhibits the Ras/MAPK (mitogen-activated protein kinase) pathway [45].

Altogether, these data prompt us to conclude that SHIP1 functions differently in T-cells from in myeloid or B-cells for at least three reasons. (i) SHIP1 does not seem to inhibit T-cell proliferation (at least primary T-cells), but instead its absence creates anergy to TCR stimulation. (ii) Whereas SHIP1 is clearly a pro-apoptotic protein in myeloid and, under certain conditions, in B-cells, it appears to play an anti-apoptotic role in T-cell lines. (iii) Moreover, the fact that T-cell-specific loss of PTEN produces some opposite phenotypes to SHIP1 KO (i.e. T-cell hyperproliferation) suggests that the signalling role of SHIP1 in T-cells is not restricted to its lipid phosphatase activity with respect to PtdIns(3,4,5)3, the common substrate of the two enzymes [46]. The mechanisms underlying these opposite roles are currently under investigation in our laboratory.

Note added in proof (received 29 January 2007)

Recently, SHIP1 was reported to be involved in a multimolecular complex together with Dok-1 and -2, Grb-2 (growth-factor-receptor-bound protein 2) and LAT’ (linker for activation of T-cells). This complex, rapidly formed upon TCR stimulation, participates in the attenuation of the TCR signalling pathway [47].

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