Dendritic-cell expression of Ship1 regulates Th2 immunity to helminth infection in mice

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In mouse models of infection with the gastrointestinal parasite Trichuris muris, appropriate dendritic-cell (DC) Ag sampling, migration, and presentation to T cells are necessary to mount a protective Th2-polarized adaptive immune response, which is needed to clear infection. SH2-containing inositol 5′-phosphatase 1 (SHIP-1) has been shown to be an important regulator of DC function in vitro through the negative regulation of the phosphoinositide 3-kinase (PI3K) pathway, but its role in vivo is relatively unexplored. In the current work, mice with a specific deletion of SHIP-1 in DCs (Ship1⁻/⁻ DC) were infected with the parasite T. muris. Ship1⁻/⁻ DC mice were susceptible to infection due to ineffective priming of Th2-polarized responses. This is likely due to an increased production of interleukin (IL-12p40) by SHIP-1-deficient DCs, as in vivo antibody blockade of IL-12p40 was able to facilitate the clearing of infection in Ship1⁻/⁻ DC mice. Our results describe a critical role for SHIP-1 in regulating the ability of DCs to efficiently prime Th2-type responses.

Keywords: Dendritic cell · PI3K · SHIP-1 · Th2 · Trichuris muris

Introduction

Immunity to the intestinal helminth parasite Trichuris muris is critically dependent on the formation of an appropriate adaptive immune response. If parasite infection leads to a type 2 immune response, characterized by T-helper type 2 (Th2) CD4⁺ T cells, the outcome is clearance of worms and “resistance” to infection. The production of the Th2 cytokines IL-4 and IL-13 serves to promote isotype class switching and subsequent production of IgE and IgG₁ by plasma cells, increase mucus production from goblet cells, and increase smooth muscle contractility to facilitate clearance of the infection [1–4]. Conversely, if infection leads to the formation of a T-helper type 1 (Th1) polarized immune response the end result is “susceptibility,” an inability to clear the parasite and a chronic infection [5, 6]. This is due to production of IL-12, IL-18, and IFN-γ, which are ineffective in eliminating the parasite and, in some cases, facilitate chronic infection.

While it is clear that a Th2 response is required for development of protective immunity, the events leading to the induction of a Th2 response are still under debate. Dendritic cells (DCs) are the predominant antigen-presenting cells (APC) of the immune system, responsible for detecting pathogen-associated molecular patterns (PAMPs) from invading species through expression of various pattern recognition receptors (PRRs). DCs rapidly infiltrate the intestine following Trichuris infection [7], and significantly more DCs are found in the intestines of resistant animals (Balb/c, C57Bl/6) compared with susceptible mice that fail to clear the infection (AKR), suggesting an important role for DCs in priming an appropriate adaptive response [8]. While these observations suggest an important role for DCs in facilitating immunity to
Trichuris infection, few studies have examined which DC subsets and functional molecules are required and how they guide the immune response to be protective or to facilitate chronic infection. Previously, we examined the response of mice lacking the DC-specific and T-cell-specific integrin, CD103 (integrin alpha E, Itgae), which facilitates the trafficking of these cells to mucosal epithelia and is expressed by a subset of T cells that regulate tolerance. Surprisingly, loss of CD103 from DCs (as well as CD103+ T cells in Itgae−/− mice) had no effect on the ability to clear the infection [9]. In summary, the role of DCs in resistance or susceptibility to Trichuris infection remains enigmatic.

The phosphoinositide 3-kinase (PI3K) pathway is involved in a number of cellular processes and its activity is typically associated with cellular survival, migration, and cytokine production. Negative regulation of the PI3K pathway is achieved by the SH2-containing inositol 5′-phosphatase 1 (SHIP-1, Inpp5d), which serves to hydrolyze the 5′ phosphate of phosphatidylinositol-3,4,5-trisphosphate (PI(3,4,5)P_3) generated by PI3K activity to produce phosphatidylinositol-3,4-bisphosphate (PI(3,4)P_2). SHIP-1 expression is restricted to the hematopoietic lineages, and SHIP-1-deficient mice (Ship1−/−) suffer a host of hematological disorders, including enhanced myelopoiesis, reduced lymphopoiesis, red-cell anemia, Th1-biased lymphocyte responses, lung consolidation, and a shortened lifespan [10–12]. While SHIP-1 is implicated with cellular survival, migration, and cytokine production, few studies have examined which DC subsets and functional molecules are required and how they guide the immune response to be protective or to facilitate chronic infection [9]. In summary, the role of DCs in resistance or susceptibility to Trichuris infection remains enigmatic.

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**Results**

DC-specific deletion of Ship1 in mice results in splenomegaly and expansion of circulating DCs

Constitutive deletion of Ship1 leads to a severe myeloproliferative disorder, resulting in lung consolidation and a shortened lifespan due to deregulated PI3K activity in multiple hematopoietic subpopulations [10, 11, 18]. This severe immune deregulation makes it difficult to examine the lineage-specific effects of SHIP-1 activity in vivo. By crossing Ship1+/F (WT) mice with Igaxcre (Cd11c-cre) transgenic mice we were able to generate mice with a specific deletion of Ship1 in DCs (Ship1ΔDC). Ship1ΔDC mice do not develop the spontaneous lung consolidation found in Ship1−/− mice [16], but do have enlarged spleens compared with WT (Ship1+/F) controls (Fig. 1A). Differential leukocyte analyses reveal a moderate, but significant, increase in the number of splenic DCs, as well as significantly increased numbers of both T and B cells in the spleens of Ship1ΔDC mice (Fig. 1B). Peripheral blood analyses of naive Ship1+/F mice and Ship1ΔDC mice reveal a significant increase in circulating CD11c+ DCs in Ship1ΔDC mice without any significant decrease in peripheral lymphocytes when compared with Ship1+/F mice (Fig. 1C and D). We also noticed this increase in peripheral DCs in other tissue sites, including the lung [16] and thymus (Supporting Information Fig. 1A). The increase in thymic DCs does not, however, influence thymocyte development, as we find equal frequencies of CD4 single-positive, CD8 single-positive, double-positive, and double-negative cells in naive Ship1+/F and Ship1ΔDC mice (Supporting Information Fig. 1B). There is, however, an increase in CD4+ T cells with an activated phenotype (CD4+CD44+CD62LlowCD69+) in the spleens of naive Ship1ΔDC mice (Supporting Information Fig. 1C), suggesting that in naive mice, Ship1-deficient DCs promote T-cell activation through increased number and frequency or altered activity.

**Ship1ΔDC mice are susceptible to T. muris infection**

Previously, we showed that Ship1 expression increases following helminth infection and that conventional Ship1 KO mice (cShip1−/−) and mice lacking Ship1 expression in the myeloid and neutrophil populations (Ship1ΔLysM) are susceptible to infection with the intestinal helminth T. muris [17]. Here we sought to examine the influence of DC expression of Ship1 in response to an intestinal infection with Trichuris muris. Ship1+/F and Ship1ΔDC mice were infected with 250 embryonated Trichuris eggs and worm burdens were examined 21 days post infection. Surprisingly, Ship1ΔDC mice failed to clear the infection (Fig. 2A) while, as expected, WT mice were able to expel worms by day 21. Although the intestines of naive Ship1+/F and Ship1ΔDC mice were indistinguishable histologically prior to infection, Trichuris-infected Ship1ΔDC mice displayed increased inflammatory cell infiltration, a reduced frequency of goblet cells, submucosal edema, and parasites attached to the epithelium in cecal tissue (Fig. 2B and C). Consistent with their susceptibility to infection, Ship1ΔDC mice had significantly reduced levels of serum IgE and Trichuris-specific IgG1 (Fig. 2D). From these results, we conclude that DC expression of Ship1 is required for clearance of Trichuris infection.

**DC expression of Ship1 facilitates Th2-dependent immunity following Trichuris infection**

We next sought to evaluate the immune response elicited in Trichuris-infected WT and Ship1ΔDC mice. We found that Ship1ΔDC
mice mounted an aberrant Th1 response following infection, as restimulation of the draining mesenteric lymph node (mLN) revealed increased frequency of IFN-γ-producing CD4⁺ T cells (Fig. 3A). In support of this Th1-bias in *Trichuris*-infected *Ship1ΔDC* mice, proximal colon RNA samples showed a significant reduction in the expression of the Th2-associated genes IL-4, IL-5, and IL-13 and the resistin-like molecule (Retnlb), and a significant increase in expression of the Th1-associated genes IFN-γ, IL-12b, and Tnf-a (Fig. 3B), as well as a significant increase in secreted IFN-γ and IL-12p40 from restimulated mLNs (Fig. 3C). While we found no difference in IFN-γ or TNFα levels in the serum of naïve *Ship1ΔDC* and WT mice, there was a significant increase in their levels in *Ship1ΔDC* mice following *Trichuris* infection (Supporting Information Fig. 2A). Interestingly, IL-12p40 levels were significantly increased in both naïve and *Trichuris*-infected *Ship1ΔDC* mice (Supporting Information Fig. 2A); however, there was no observed difference in levels of IL-12a, IL-17a, or IL-23a mRNA (Supporting Information Fig. 2B). Dendritic cells, and not macrophages, were the primary source of IL-12p40 in the draining mLN in both *Ship1ΔF* and *Ship1ΔDC* mice (Supporting Information Fig. 3), and *Trichuris*-infected *Ship1ΔDC* mice had both increased total numbers of IL-12p40⁺ DCs in the mLN and a higher expression of IL-12p40 per DC (Fig. 3D). Additionally, there was increased expression of the DC-associated integrin, *Itgax* (CD11c), in the colons of *Trichuris*-infected *Ship1ΔDC* mice (Fig. 3B). These data suggest the impaired expulsion of worms in *Ship1ΔDC* mice is due to

**Figure 1.** *Ship1ΔDC* mice have enlarged spleens and increased circulating DCs. (A, B) Spleens were isolated from naïve *Ship1ΔF* and *Ship1ΔDC* mice and (A) total and (B) differential leukocytes were analyzed by flow cytometry. (C, D) Peripheral blood from naïve *Ship1ΔF* and *Ship1ΔDC* mice was analyzed for (C) DC (CD11c⁺) and (D) lymphocyte (CD3⁺ and CD19⁺) frequencies by flow cytometry. Data are shown as means ± SEM of *n* = 3–6 mice per experiment representative of two independent experiments. *p* < 0.05 (Student’s *t*-test).

**Figure 2.** DC expression of *Ship1* is required for immunity to *Trichuris* infection. (A) *Ship1ΔF* (WT) and *Ship1ΔDC* mice were infected with 250 *T. muris* eggs. Worm burdens were determined microscopically from cecal contents 21 days post infection. (B) PAS-stained cecal section from naïve and *Trichuris*-infected *Ship1ΔF* and *Ship1ΔDC* mice. Original magnification 100×, scale bars = 100 μm. (C) Goblet cells were quantified from PAS-stained cecal sections. (D) Total serum IgE and *Trichuris*-specific IgG1 levels from naïve and *Trichuris*-infected mice were quantified by ELISA. Data are shown as means ± SEM of *n* = 4–5 per experiment and are representative of two independent experiments. *p* < 0.05 (Student’s *t*-test).
Figure 3. DC expression of Ship1 facilitates Th2 polarization following Trichuris infection. (A) Gating strategy and quantification for IL-13 and IFN-γ-expressing CD4+ T cells from restimulated mLNs of Trichuris-infected Ship1FF and Ship1ΔDC mice. (B) RNA was collected from the proximal colons of naive and Trichuris-infected mice. Quantitative RT-PCR was performed to determine expression of Il-4, Il-5, Il-13, Retnlb, Ifng, Tnfa, Il-12b, and Itgax. (C) Supernatants of αCD3/CD28 restimulated mLN cells were evaluated for secretion of IFN-γ and IL-12p40 by ELISA. (D) The frequency of total DCs (CD11c+MHC-II+), quantification of IL-12p40+ DCs, and geometric MFI of IL-12p40+ DCs in the mLNs of Trichuris-infected mice was quantified by flow cytometry. All data are shown as means ± SEM and are representative of two independent experiments (A, C, and D; n = 4–5 mice per experiment) or are pooled results from three independent experiments (B, n = 11–13). *p < 0.05 (Student’s t-test).
an aberrant production of IL-12p40 in Ship1ΔDC mice, through a combination of an expanded DC population and an increased per-cell production of IL-12p40, leading to a Th1-polarized adaptive immune response that, in turn, supports chronic infection.

**Neutralization of IL-12p40 in Trichuris-infected Ship1ΔDC mice promotes resistance**

Previous reports of Trichuris infection in Ship1−/− mice have implicated deregulated IL-12 production as a factor leading to susceptibility to infection, although a cellular source of IL-12 was not determined. We have found here that Ship1ΔDC mice have an increase in DC number as well as in their production of IL-12p40 prior to any external stimulation (Supporting Information Fig. 2A), and sought to examine whether neutralizing IL-12p40 with an antibody would facilitate clearance of helminth infection in Ship1ΔDC mice. Neutralization of IL-12p40 enhanced expulsion of Trichuris in Ship1ΔDC mice as demonstrated by reduced worm burdens at day 21 when compared with mice that received an isotype-matched control antibody (rat Ig, Fig. 4A). Histological examination revealed reduced inflammatory cell infiltration, an increase in the frequency of goblet cells, and a clearance of the parasites in Ship1ΔDC mice treated with neutralizing antibodies to IL-12p40 (Fig. 4B and C). Supporting this enhanced parasite clearance, Ship1ΔDC mice treated with neutralizing antibodies to IL-12p40 also had increased serum levels of Trichuris-specific IgG1, although there was no significant increase in total serum IgE levels (Fig. 4D).

Antibody neutralization of IL-12p40 also led to a reduction in the Th1 response including reduced IFN-γ-producing CD4+ T cells in the mLNs, IFN-γ secretion from restimulated mLN cells, and IFN-γ expression in colon tissue (Fig. 5A–C). In addition, IL-12p40 neutralization was accompanied by an increased expression of the Th2-associated genes Il-4, Il-5, Il-13 (Fig. 5C). From these data, we conclude that Ship1 is involved in the negative regulation of IL-12p40 release by DCs and neutralization of IL-12p40 in Ship1ΔDC mice facilitates resistance to Trichuris infection by establishing the appropriate Th2 response.

**Discussion**

Here, we demonstrate that the specific loss of SHP-1 from DCs leads to splenomegaly and increased circulating DCs. DC-specific SHP-1 deletion also results in enhanced production of IL-12p40, promoting an adaptive Th1 response and chronic infection with Trichuris. Blockade of IL-12p40 in Ship1ΔDC mice with a neutralizing antibody restored the formation of a protective Th2 response and clearance of the infection. This highlights the important role DCs play in shaping the adaptive immune response to Trichuris, and reveals how a subtle perturbation in the normal dampening of PI3K activity can dramatically alter the development of anti-helminth immune responses.

While the initiation of a normal type 1 immune response is well established, with DCs acting as the dominant APC to naïve CD4+ T cells and producing IL-12 to promote Th1 polarization, the steps involved in the initiation of type 2 responses are still under debate. This is, in part, due to the fact that DCs do not produce large amounts of IL-4, a key factor required for promoting naïve CD4+ T-cell polarization and maturation into a Th2 cell. DCs clearly have an important role in immunity to helminth infection; in elegant experiments where CD11c+ cells were inducibly depleted using a diphtheria toxin-dependent ablation system, protective Th2 responses were severely impaired [19, 20]. Our results are consistent with this observation and show that deregulated PI3K signaling activity (through the deletion of Ship1 specifically in DCs, but not in other leukocyte populations) results in increased production of IL-12 and a Th1-biased immune skewing that impairs immunity to Trichuris infection.

Although these results suggest a pivotal role for DCs in helminth immunity, it is also likely that they require the assistance of other innate leukocyte populations in order to prime Th2 responses. Indeed, experiments aimed at examining the need for MHC class II expression by distinct subsets of APCs have shown that expression of MHC-II by DCs alone, and not by other leukocyte populations, is insufficient to confer resistance to chronic Trichuris infection [21]. This suggests that other MHC class II positive innate populations (such as mast cells [22], basophils [23, 24], eosinophils [25, 26], ILC2s [27], macrophages, and neutrophils) are required to support DCs in the formation of a protective Th2 response.

The influence of PI3K activity in regulating IL-12 production from DCs is controversial, and could be due to differing effects and expression patterns of the various PI3K isoforms. PI3K exists as heterodimers containing a regulatory and a catalytic subunit that are grouped into three different classes: class I, class II, and class III. The most widely studied are the class IA PI3Ks, which consist of three different catalytic subunits (p110α, p110β, and p110δ) and five distinct regulatory subunits (p85α, p55α, p50δ, p85β, and p55γ). Consistent with the results presented here, siRNAs targeting p110α and p110β in human APCs in response to LPS stimulation, p110β expression was found to positively correlate with JNK activity and IL-12 production, suggesting enhanced PI3K activity could, indeed, support increased production of IL-12 [28]. Surprisingly, however, others have shown that deletion of the regulatory subunit p85α in Pik3r1−/− mice (p85α−/−) or pharmacological inhibition of PI3K activity using wortmannin leads to increased production of IL-12, suggesting a negative regulatory role for PI3Ks in IL-12 production [29].

Previously, we reported that Ship1ΔLysM mice, which lack Ship1 expression in macrophages and neutrophils, also displayed heightened IL-12 production in resting mice and Trichuris-infected mice [17]. While this could be due to production of IL-12p40 by macrophage populations in the intestine, we were unable to detect appreciable levels of IL-12p40 in macrophages in the mLNs of Ship1ΔLysM mice, with DCs remaining the dominant producers of IL-12p40 (Supporting Information Fig. 4A and B). We currently cannot rule out the possibility that part of the
observed phenotype observed in Ship1ΔDC mice is due to production of IL-12p40 by CD11c+ macrophage populations in the intestine that are likely to be deficient for SHIP-1 as well. However, we were unable to detect appreciable levels of IL-12p40 in Ship1-deficient macrophage populations in other tissues of Ship1ΔDC mice (Supporting Information Fig. 4B). Ship1ΔLysM mice develop splenomegaly similar to Ship1ΔDC (Supporting Information Fig. 4C), and the ensuing expansion of DCs in this compartment could explain the heightened levels of IL-12p40 and resulting susceptibility to Trichuris infection.

Figure 4. Neutralization of IL-12 in Trichuris-infected Ship1ΔDC mice facilitates immunity to infection. (A) Day 21 worm counts from Ship1ΔDC mice treated i.p. with antibodies to IL-12p40 or an isotype control (Rat Ig). (B) PAS-stained cecal sections from Trichuris-infected mice. Original magnification 200×, scale bars = 50 µm. (C) Goblet cells were quantified from PAS-stained cecal sections. (D) Trichuris-specific IgG1 and (E) total IgE levels from infected mice were quantified by ELISA. Data are shown as means ± SEM of 4–5 mice from a single experiment. *p < 0.05 (Student’s t-test).

Figure 5. Neutralization of IL-12 in Trichuris-infected Ship1ΔDC mice promotes Th2 polarization. (A) Intracellular IL-13 and IFN-γ expression in CD4+ T cells isolated from the mLNs was measured by flow cytometry. (B) Secreted IFN-γ collected in the supernatant of αCD3/CD28 restimulated mLN cells was quantified by ELISA. (C) RNA was collected from proximal colons of Trichuris-infected mice. Expression of Ifng, Il-4, Il-5, and Il-13 was determined by quantitative RT-PCR. Data are shown as means ± SEM of n = 4–5 mice from a single experiment. *p < 0.05 (Student’s t-test).
In summary, we show that selective deletion of Ship1 expression in DCs (Ship1ΔDC) renders mice susceptible to infection with Trichuris. This is the result of increased production of IL-12p40 in Ship1ΔDC mice, leading to a reduced Th2 response and instead a Th1-dominated response featuring high levels of IFN-γ and a chronic infection. Neutralization of IL-12p40 in Ship1ΔDC mice enhanced the formation of a Th2 response and facilitated resistance to Trichuris infection.

Materials and methods

Mice

Inpp5dΔ/Δ (Ship1Δ/Δ) mice were described previously and provided by W. Kerr [30]. Itgax-Cre (B6.Cg-Tg(Itgax-cre)1-1Reiz/J) and B6.129P2-Lyz2tm1(cre)Ifo/J mice were obtained from the Jackson Laboratory (Bar Harbor, ME, USA). Animals were bred and maintained in a specific pathogen-free (SPF) environment at the Biomedical Research Centre and all animal work was approved by the Animal Care Committee of UBC.

Peripheral blood and tissue leukocyte isolation

Peripheral blood was sampled by saphenous vein and collected into microvette EDTA-coated tubes (Sarstedt, Montreal, Canada). Mice were euthanized via CO₂ exposure and spleens and thymi were passed through a 70 μm cell strainer to obtain a single-cell suspension. Red blood cells were lysed in peripheral blood and splenocyte samples using ammonium chloride buffer (150 mM NH₄Cl, 1 mM KHCO₃, pH 7.3).

T. muris infection

Mice were infected on day 0 with 225–250 embryonated eggs and parasite burdens were quantified on day 21 post infection. T. muris excretory–secretory antigens and eggs were collected as described previously [31].

Histology

Cecal tissues were fixed overnight in 10% buffered formalin and paraffin-embedded. A total of 5-μm-thick tissue sections were stained with periodic acid–Schiff (PAS) for analysis.

Immune response

mLNs were excised and passed through a 70 μm cell strainer to generate a single-cell suspension. mLN cells (4 × 10⁶/mL) were cultured for 72 h in media containing 1 μg/mL each of antibodies against CD3 (145-2C11) and CD28 (37.51; eBioscience, San Diego, CA). Cytokine production from cell-free supernatant and serum samples was quantified by ELISA using commercially available antibodies (eBioscience) or with the mouse inflammation cytometric bead array (BD Biosciences, San Jose, CA, USA). For intracellular staining, mLN cells were restimulated with 50 ng/mL of PMA and 750 ng/mL of ionomycin (Sigma, St. Louis, MO, USA) in the presence of Brefeldin A (eBioscience) for 4 h prior to analysis by flow cytometry. Total serum IgE was quantified by ELISA (BD Biosciences). Trichuris-specific serum IgG’s were determined using Trichuris antigen coated ELISA plates (5 μg/mL overnight in carbonate buffer) and HRP-conjugated mouse IgG1 antibodies followed by TMB (BD).

Flow cytometry

To block nonspecific antibody binding, samples were first incubated in staining buffer containing 10% goat serum and 5 μg/mL of anti-CD16/32 (clone 2.4G2, UBC AbLab). Fluorescently conjugated antibodies to CD3, CD4, CD8, CD11b, CD11c, CD44, CD62L, CD69, Gr1, IL-12p40, IL-13, and IFN-γ (eBioscience); CD11c, CD19, and CD45 (in-house, UBC AbLab), and F4/80 (Life Technologies, Carlsbad, CA, USA) were used for flow cytometry. Dead cells were excluded using eFluor fixable viability dyes and intracellular staining was performed using the intracellular fixation and permeabilization buffer set (eBioscience). Samples were acquired on a BD LSRII and data analysis was performed using FlowJo software (TreeStar, San Carlos, CA, USA).

RNA isolation and qPCR

Proximal colon tissue samples were homogenized with Trizol and RNA was reverse transcribed using a cDNA synthesis kit (Life Technologies). qPCR was performed with Sybr green (KAPA Biosystems, Woburn, MA, USA) using gene-specific primer pairs (Table 1). Values are expressed relative to Actb.

In vivo antibody treatment

Isotype and IL-12p40 (clone C17.8) were purchased from Bio-X-Cell (West Lebanon, NH, USA). Mice received 1 mg of antibody i.p. every 4 days starting from day 4 to 20 post infection.

Statistics

Results are shown as mean ± SEM. Statistical significance was determined by the Student’s t-test (p < 0.05).
Table 1. Primer list. Forward and reverse primer pairs for gene-specific detection by quantitative RT-PCR. Primers were used at a final concentration of 200 nM

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Acknowledgments: We thank Rupi Dhesi and Les Rollins for core support, Andy Johnson and the UBC Flow facility, and Michael Williams and the UBC AbLab. This work was supported by the AllerGen Network Centre of Excellence (K.M.M. and M.J.G.) and Canadian Institute of Health Research (K.M.M., MOP-137142). M.J.G. was supported by an AllerGen CAIDATI training award, and F.A. is the recipient of a CIHR/Canadian Association of Gastroenterology/Crohn’s and Colitis Foundation of Canada postdoctoral fellowship.

Conflict of interest: The authors declare no financial or commercial conflict of interest.

References


Abbreviations: PI3K: phosphoinositide 3-kinase SHIP-1: SH2-containing inositol 5′-phosphatase 1

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Received: 4/3/2015
Revised: 15/9/2015
Accepted: 15/10/2015
Accepted article online: 31/10/2015