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SHIP1 Is a Repressor of Mast Cell Hyperplasia, Cytokine Production, and Allergic Inflammation In Vivo

D. James Haddon,* Frann Antignano,2† Michael R. Hughes,2* Marie-Renée Blanchet,* Lori Zbytnuik,* Gerald Krystal,3 and Kelly M. McNagny3*

SHIP1 inhibits immune receptor signaling through hydrolysis of the PI3K product phosphatidylinositol 3,4,5-trisphosphate, forming phosphatidylinositol 3,4-bisphosphate. In mast cells, SHIP1 represses FcεRI- and cytokine-mediated activation in vitro, but little is known regarding the function of SHIP1 in mast cells in vivo or the susceptibility of Ship1−/− mice to mast cell-associated diseases. In this study, we found that Ship1−/− mice have systemic mast cell hyperplasia, increased serum levels of IL-6, TNF, and IL-5, and heightened anaphylactic response. Further, by reconstituting mast cell-deficient mice with Ship1+/+ or Ship1−/− mast cells, we found that the above defects were due to loss of SHIP1 in mast cells. Additionally, we found that mice reconstituted with Ship1−/− mast cells suffered worse allergic asthma pathology than those reconstituted with Ship1+/+ mast cells. In summary, our data show that SHIP1 represses allergic inflammation and mast cell hyperplasia in vivo and exerts these effects specifically in mast cells. The Journal of Immunology, 2009, 183: 228–236.

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D.J.H. designed and performed research, analyzed and interpreted data, and wrote the manuscript. F.A. and M.R.H. designed and performed research, analyzed and interpreted data, and edited the manuscript. M.-R.B. and I.Z. performed research. G.K. and K.M.M. analyzed and interpreted data and edited the manuscript.

2 F.A. and M.R.H. contributed equally to this study.

3 Address correspondence and reprint requests to Dr. Kelly M. McNagny, The Biomedical Research Centre, 2222 Health Sciences Mall, Vancouver, British Columbia, V6T 1Z3 Canada. E-mail address: kelly@brc.ubc.ca

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†The Biomedical Research Centre and †Terry Fox Laboratory, University of British Columbia, Vancouver, British Columbia, Canada
susceptibility to anaphylaxis. Furthermore, by reconstituting mast cell-deficient Kit<sup>W<sub>wsh/dw-sh</sub></sup> mice with Ship1<sup>+/+</sup> or Ship1<sup>−/−</sup>-BMMCs, we established that loss of SHIP1 in the mast cell lineage was responsible for these defects (with the exception of increased IL-5). In addition, we found that mice reconstituted with Ship1<sup>−/−</sup>-mast cells suffered worse allergic asthma pathology than those reconstituted with Ship1<sup>+/+</sup> mast cells. In summary, our data show that SHIP1 is a repressor of allergic inflammation and mast cell hyperplasia in vivo and that it exerts these effects specifically in mast cells.

**Materials and Methods**

**Mice**

All mice were housed in specific pathogen-free mouse facilities at The Biomedical Research Centre or the British Columbia Cancer Research Centre, with approval from the University of British Columbia Animal Care Committee and according to the Canadian Council on Animal Care guidelines. C57BL/6 (B6) and B6.Cg-Ki<sup>nhs/dw</sup>-2H1NhrJaeBsm1 (Kit<sup>W<sub>sh/dw-sh</sub></sup>) mouse strains were acquired from The Jackson Laboratory, and each were maintained as homozygous colonies by sibling breeding. B6-congenic Ship1<sup>−/−</sup> mice were a kind gift from Dr. F. R. Jirik (University of Calgary, Calgary, Alberta, Canada) and were backcrossed at least 12 times. F<sub>2</sub>-Ship1<sup>−/−</sup> mice (F<sub>2</sub>-Ship1<sup>−/−</sup>) were maintained on a B<sub>6</sub> × 129/Sv mixed background. All mice used were at least 4 wk old and controls were littermates or were age- and sex-matched from the same colony.

**Cell culture**

Bone marrow cells were flushed from the femurs of 6-wk-old Ship1<sup>+/+</sup> and Ship1<sup>−/−</sup> mice and plated in IMDM supplemented with 15% FCS (StemCell Technologies), 150 μM monothioglycerol (Sigma-Aldrich), 100 U/ml penicillin, 100 μg/ml streptomycin (Sigma-Aldrich), and 30 ng/ml recombinant mouse IL-3 (R&D Systems). Cells were maintained at 37°C in a 5% CO2 humidified atmosphere, selected for nonadherence, and considered to be BMSCs after 6 wk in culture as verified by Wright-Giemsa staining and flow cytometry (data not shown).

**Reconstitution of mast cell-deficient Kit<sup>W<sub>wsh/dw-sh</sub></sup> mice**

Kit<sup>W<sub>wsh/dw-sh</sub></sup> mice were injected with 1 × 10<sup>7</sup> Ship1<sup>+/+</sup> or Ship1<sup>−/−</sup>-BMSCs in 200 μl of HBSS via the tail vein (designated Ship1<sup>+/−</sup>-BMCC and Ship1<sup>−/−</sup>-BMCC mice). After 12 wk the mice were bled for serum collection, subjected to passive systemic anaphylaxis, or sensitized for allergic asthma and then euthanized via CO2 inhalation. After euthanizing the mice, serum (50 μl) was collected from the saphenous vein and assayed using BD mouse inflammation and mouse Th1/Th2 cytometric bead array kits (BD Biosciences). Serum (50 μl) was also used to determine the concentration, parenchymal infiltration, and epithelial damage (maximum score of the following parameters: perivascular infiltration, peribronchial infiltration, parenchymal infiltration, and epithelial damage (maximum score of 16).

**Flow cytometry**

Samples were blocked with anti-CD16/32 in 10% goat serum and then stained with anti-c-kit-PE (BD Pharmingen), anti-CD45.2-biotin (eBioscience), or anti-Fc<sub>γRII-biotin</sub> (eBioscience) followed by streptavidin-FITC (BD Pharmingen) or anti-CD45.2-PE (eBioscience). Latex beads (10 μm; Invitrogen) were added to HBSS with 0.1% BSA before peritoneal washes and to BAL to calculate total cell recovery via flow cytometry. All flow cytometry experiments were performed on a BD FACSCalibur with CellQuest software and postcollection analyses were performed with FlowJo (Tree Star).

**Statistics**

The unpaired t-test (two-tailed) was used for analysis of tissue mast cell numbers, asthma BAL cellular infiltrate, BAL differentials, lung clinical scores, serum cytokine levels, and passive systemic anaphylaxis (PSA) assays. A Kruskal-Wallis test was used for the mesentry of BMCC-reconstituted mice (due to all 0 values in the Ship1<sup>−/−</sup>-condition). A log-rank (Mantel-Cox) test was used to compare the PSA survival rates. Separate allergic asthma experiments were normalized based on their OVA-treated B6 levels before analysis. All of the above tests were performed using GraphPad Prism 5 or Microsoft Excel X software. Missing samples from some tissues used in the enumeration of mast cells in Ship1<sup>−/−</sup> and BMCC-reconstituted mice were due to errors during sample processing.

**Results**

**Ship1<sup>−/−</sup> mice have mast cell hyperplasia in multiple tissues**

We suspected that the chronic inflammatory phenotype of Ship1<sup>−/−</sup> mice could be due in part to increased tissue mast cell numbers. With this in mind, we enumerated the tissue mast cells of Ship1<sup>−/−</sup> and Ship1<sup>+/+</sup> mice and found significantly higher numbers in the spleen, lung, ileum, mesentery, and back skin of Ship1<sup>−/−</sup> mice (Fig. 1A). We also found that Ship1<sup>−/−</sup> mice had higher numbers of colon mast cells, although the counts were more variable than those for other tissues assessed. Similarly, Ship1<sup>−/−</sup> mice had over two times more peritoneal mast cells than wild-type controls (Fig. 1B). The frequency of peritoneal mast cells was similar between Ship1<sup>−/−</sup> and wild-type mice, reflecting a greater number of total peritoneal cells in Ship1<sup>−/−</sup> mice than in Ship1<sup>+/+</sup> mice (6.8, SD 2.8 vs 2.0, SD 0.13 cells (×10<sup>5</sup>); p < 0.0114). Thus, Ship1<sup>−/−</sup> mice have mast cell hyperplasia in the spleen, lungs, colon, ileum, mesentery, peritoneal cavity, and back skin.

**Mast cell hyperplasia in Ship1<sup>−/−</sup> mice is mast cell autonomous**

Because SHIP1 is expressed in many hematopoietic cell types, it was unclear whether the mast cell hyperplasia we observed in ubiquitous Ship1<sup>−/−</sup> mice was mast cell autonomous. To answer this question, we i.v. reconstituted Kit<sup>W<sub>wsh/dw-sh</sub></sup> mast cell-deficient mice with Ship1<sup>+/+</sup> or Ship1<sup>−/−</sup>-BMCCs (designated Ship1<sup>+/−</sup>-BMCC and Ship1<sup>−/−</sup>-BMCC mice) and, following 12 wk of engraftment, found mast cell hyperplasia in the spleen, lung, colon, ileum, mesentery, and back skin of Ship1<sup>−/−</sup>-BMCC mice (Fig. 2A). Likewise, Ship1<sup>−/−</sup>-BMCC mice also had a greater number and frequency (0.92, SD 0.52% vs 0.10, SD 0.13%; p < 0.0015) of peritoneal mast cells than Ship1<sup>+/−</sup>-BMCC controls (Fig. 2B).
KitW-sh/W-sh mice were mast cell deficient in all tissues assessed, and the mast cell numbers in the tissues of B6 and KitW-sh/W-sh mice reconstituted with wild-type mast cells were similar to those of previous reports (17). Thus, the mast cell hyperplasia observed in Ship1/H11002 mice is a mast cell autonomous defect.

Ship1/H11002 mice have mast cell-dependent systemic inflammation

Because Ship1/H11002 mast cells exhibit enhanced degranulation and cytokine secretion in response to FcεRI stimulation and react abnormally to c-kit stimulation in vitro, we investigated the levels of inflammatory cytokines in the serum of adult Ship1/H11002 mice (6, 10, 11). Ship1/H11002 mouse serum contained significantly increased levels of IL-5, IL-6, and TNF (and a small increase in CCL2) compared with Ship1/H11001 controls (Fig. 3A). To determine whether Ship1/H11002 mast cells were responsible for the increased levels of inflammatory serum cytokines observed in Ship1/H11002 mice, we evaluated the serum of Ship1/H11002-BMMC-reconstituted mice. We detected significantly higher levels of IL-6 and TNF in the serum of Ship1/H11002-BMMC mice, whereas the levels of IL-5 and CCL2 were only marginally increased compared with those of controls (Fig. 3B). We concluded that Ship1/H11002 mast cells induce increased serum levels of the inflammatory cytokines IL-6 and TNF in vivo.

Ship1/H11002 mice are hyperresponsive to passive systemic anaphylaxis

Because Ship1/H11002 mice possess mast cell hyperplasia in multiple tissues and Ship1/H11002 mast cells exhibit increased degranulation in response to IgE crosslinking in vitro, we suspected that Ship1/H11002 mice would be more susceptible to anaphylaxis (6). To test this hypothesis, we subjected Ship1/H11002 mice and littermate controls to PSA by sensitizing them with anti-DNP IgE, challenging them with DNP-HSA the next day, and monitoring their rectal temperature for 90 min (Fig. 4). Interestingly, the body temperature of Ship1/H11002 mice (before challenge) was on average 1°C lower than that of wild-type controls. This may reflect low-level degranulation induced by the IgE sensitization, as Ship1/H11002 mast cells aberrantly degranulate in response to IgE without crosslinking Ag in vitro (6). More importantly, we found that Ship1/H11002 mice suffered increased mortality, more severe hypothermia (at t = 20 min; 5°C, SD 0.6°C vs 3°C, SD 0.7°C; p < 0.0001), and no body temperature recovery compared with wild-type controls (Figs. 4, A and B).

It was unclear whether the increased severity of anaphylaxis suffered by Ship1/H11002 mice was due to increased mast cell activation and mast cell numbers or to the reactivity of other cell

**FIGURE 1.** Mast cell hyperplasia in multiple tissues of Ship1/H11002 mice. A, Toluidine blue histological staining revealed that Ship1/H11002 mice (n = 11) had significantly higher numbers of metachromatic tissue mast cells than Ship1/H11001 controls (n = 10). B, Ship1/H11002 mice (n = 10) had a higher total number (graph) but similar frequency (gate statistics) of SSChigh/c-kithigh peritoneal wash mast cells compared to Ship1/H11001 mice (n = 9), as determined by flow cytometry. Data represent pooled results from two independent experiments.
types to the mediators released during PSA challenge (reviewed in Ref. 21). Therefore we subjected reconstituted Ship1<sup>-/-</sup>-BMMC mice to PSA and found that they also suffered a much larger temperature drop than Ship1<sup>+/+</sup>-BMMC mice (at t = 35; 7°C, SD 2.2°C vs 1°C, SD 2.5°C; p = 0.0037) (Fig. 4C). Notably, unlike ubiquitous Ship1<sup>-/-</sup> mice, Ship1<sup>-/-</sup>-BMMC mice exhibited body temperature recovery 40 min postchallenge. Although Kit<sup>W-sh/W-sh</sup> mice were resistant to PSA, B6 mice suffered a similar temperature drop as that of Ship1<sup>-/-</sup>-BMMC mice (Fig. 4D). In summary, we find that the increased severity of anaphylaxis experienced by Ship1<sup>-/-</sup> mice is largely mast cell autonomous.
Allergic airway inflammation is greater in Ship1−/− mice than in Ship1−/−-BMMC controls

Naive Ship1−/− mice have allergic asthma-like symptoms, including severe airway inflammation, mucus hyperproduction, and symptoms of airway remodeling (14). To determine the role of Ship1−/− mast cells in allergic asthma pathology, we induced an acute form of the disease in Ship1−/−-BMMC- and Ship1−/−-BMMC-reconstituted mice (Fig. 5A). We found that asthmatic Ship1−/−-BMMC mice had over two times more infiltrating cells in their airways than Ship1−/−-BMMC mice, including increased eosinophils, which are the primary effector cells in asthma (Figs. 5, B and C). Perivascular, peribronchial, and parenchymal infiltration, as well as epithelial damage, were most prominent in the lungs of asthmatic Ship1−/−-BMMC mice, and the clinical scores of asthmatic Ship1−/−-BMMC lung sections were significantly higher than those of Ship1−/−-BMMC mice (Fig. 5, D and E). KitW-sh/W-sh mice suffered comparable symptoms as those of B6 mice, similar to the findings of a study published after we completed our asthma course that also used an OVA-induced (with alum adjuvant) model of allergic asthma (see Fig. E1 in Ref. 20). Ship1−/−-BMMC mice had slightly reduced number of infiltrating BAL cells compared with KitW-sh/W-sh mice, highlighting the importance of wild-type reconstituted controls. We concluded that

FIGURE 3. Increased inflammatory cytokine levels in the serum of Ship1−/− mice and mice reconstituted with Ship1−/− mast cells. A, Cytometric bead arrays of mouse cytokines were used to assay the serum of both F2-Ship1−/− (n = 7) and F2-Ship−/− (n = 7) mice. Data represent pooled results from two independent experiments. B, Cytometric bead arrays were also used to assay the serum of B6 (n = 4), Ship1−/−-BMMC (n = 8), Ship1−/−-BMMC (n = 8), and KitW-sh/W-sh (n = 6) mice. Data represent pooled results from two independent experiments.

FIGURE 4. Hyperresponsive anaphylaxis in Ship1−/− mice and in mice reconstituted with Ship1−/− mast cells. To induce passive systemic anaphylaxis, Ship1−/− (n = 10, red line) and Ship1−/− (n = 9, blue line) mice were sensitized with anti-DNP IgE and challenged the following day with DNP-HSA. Increased mortality (A) and hypothermia (B), measured rectally, were observed in Ship1−/− mice. Anaphylaxis was induced and monitored in Ship1−/−-BMMC (n = 8, red line) and Ship1−/−-BMMC (n = 8, blue line) mice (C), as well as B6 (n = 5, black line) and KitW-sh/W-sh (n = 5, gray line) controls (D) as described above. Data for C and D were measured concurrently but separated for clarity. Data in each panel represent pooled results from two separate experiments. (+, p < 0.05; **, p < 0.01; ***, p < 0.001). Bars represent SE.
FIGURE 5. Increased allergic asthma pathology in mice reconstituted with Ship1−/− mast cells. A, Asthma was induced in B6 (n = 10), Ship1−/−-BMMC (n = 13), Ship1+/+ -BMMC (n = 10), and KitW-sh/W-sh (n = 11) mice via two i.p. sensitizations (with alum adjuvant; days 1 and 7) and five intranasal (i.n.) challenges (days 21, 22, 23, 26, and 27) of OVA before sacrificing (Sac.) the mice (day 28). B, Inflammation was assessed by enumerating the cellular infiltrate in the BAL using flow cytometry. C, Differentials were performed with a modified Wright-Giemsa stain; B6 (n = 7), Ship1−/−-BMMC (n = 9), Ship1+/+ -BMMC (n = 6), and KitW-sh/W-sh (n = 7). BAL data represent pooled results from three independent experiments, and differentials from two independent experiments. D, Selected representative micrographs of H&E-stained lungs from PBS- or OVA-treated Ship1−/−-BMMC-reconstituted mice and controls (bars = 100 μm). E, Clinical scores were assigned to each lung based on the following parameters: perivascular infiltration, peribronchial infiltration, parenchymal infiltration, and epithelial damage (maximum score of 16). Data represent pooled results from three independent experiments. Each circle represents a mouse.
Ship1<sup>−/−</sup>-BMMC mice suffered worse allergic asthma pathology than Ship1<sup>+/+</sup>-BMMC mice.

Discussion

Our results show the following: 1) Ship1<sup>−/−</sup> mice have systemic mast cell hyperplasia, increased serum levels of the inflammatory cytokines IL-6, TNF, and IL-5, and increased susceptibility to anaphylaxis; 2) the above phenotypes are mast cell autonomous (with the exception of increased serum IL-5); and 3) mice reconstituted with Ship1<sup>−/−</sup> mast cells suffered worse allergic asthma pathology than Ship1<sup>+/+</sup> controls. Previous studies have focused on the function of SHIP1 in mast cells in vitro. In this study we demonstrate that SHIP1 is also a repressor of mast cells in vivo and that the loss of SHIP1 increases the symptoms of two mast cell-associated diseases. Furthermore, we demonstrate a cell-autonomous role for SHIP1 in mast cell homeostasis.

Mast cell hyperplasia is observed in chronic inflammatory processes, fibrotic disorders, wound healing, and neoplastic tissue transformation, but little is known about normal mast cell homeostasis (reviewed in Ref. 22). Our results demonstrate that SHIP1 is a cell-autonomous repressor of mast cell hyperplasia and a key player in mast cell homeostasis. Ship1<sup>−/−</sup> mice have increased numbers of granulocyte-macrophage progenitors in their bone marrow that exhibit enhanced sensitivity to multiple cytokines (7). In our studies, because the reconstituted BMMCs were composed exclusively of mast cells and their committed precursors and the numbers of mast cells observed in the tissues of Ship1<sup>−/−</sup>-BMMC mice were comparable to or greater than those in ubiquitous Ship1<sup>−/−</sup> mice, we concluded that the mast cell hyperplasia observed in Ship1<sup>−/−</sup> mice is not due to the increased number or sensitivity of granulocyte-macrophage progenitors. Furthermore, we found that the loss of SHIP1 specifically in mast cells was not sufficient to induce the excessive granulocyte and macrophage numbers previously observed in the blood, spleen, and lungs of Ship1<sup>−/−</sup> mice (data not shown) (7).

Interestingly, we found that Ship1<sup>−/−</sup> mice have elevated serum levels of the inflammatory cytokines IL-5, IL-6, and TNF compared with those of Ship1<sup>+/+</sup> controls. This is in agreement with a previous report that Ship1<sup>−/−</sup> mice have increased levels of serum IL-6 (8). We demonstrated that the increased levels of IL-6 and TNF were due to loss of SHIP1 specifically in mast cells. Because Ship1<sup>−/−</sup> mast cells produce more IL-6 and TNF than wild-type mast cells upon FceRI-mediated stimulation in vitro, we suspect that the increased serum levels of these cytokines arose directly from Ship1<sup>−/−</sup> mast cells via stimulation with endogenous IgE (10). Although the high level of IL-6 in the serum of Ship1<sup>−/−</sup> mice was previously attributed to macrophages, in this study we demonstrate that Ship1<sup>−/−</sup> mast cells also contribute to the increased IL-6 levels in vivo (8). Furthermore, the increased IL-6 was previously proposed to inhibit B cells and enhance myeloid cell development, but we found the frequency of B cells and myeloid cells in the bone marrow, peripheral blood, and spleen of Ship1<sup>−/−</sup>-BMMC-reconstituted mice were similar to that in controls (data not shown) (23). Thus, our data suggest that increased serum IL-6 is not sufficient to cause the hematopoietic lineage skewing observed in Ship1<sup>−/−</sup> mice, and a local increase of IL-6 in the bone marrow may be required.

Our studies also suggest that the systemic mast cell hyperplasia observed in Ship1<sup>−/−</sup> mice is due to the high levels of IL-6 and TNF produced by Ship1<sup>−/−</sup> mast cells. TNF is required for mast cell development and stimulates mast cell colony formation in vitro (24, 25). In addition, IL-6 promotes mast cell development from mixed cultures, likely via a secondary mediator such as PGE (24). We suspect that the systemic mast cell hyperplasia observed in Ship1<sup>−/−</sup> mice is also due to the hypersensitivity of Ship1<sup>−/−</sup> mast cells to stimulation with SCF or IgE (without crosslinking Ag) (6, 11). SCF and IgE induce mast cell proliferation and survival, respectively, and endogenous levels of these factors may be capable of sustaining higher levels of hypersensitive Ship1<sup>−/−</sup> mast cells in vivo (26, 27).

We also found that Ship1<sup>−/−</sup> mice suffer considerably more severe anaphylaxis than controls. Although mast cells are the primary effector cells in the PSA model of anaphylaxis, other cell types also play a role in its pathology. For example, IL-4 or IL-13 treatment increases the severity of anaphylaxis by enhancing the responsiveness of target cells to the vasoactive mediators released during anaphylaxis (reviewed in Ref. 21). We found that Ship1<sup>−/−</sup>-BMMC mice also suffered more severe anaphylaxis than controls, suggesting that this effect is largely mast cell autonomous. We suspect that the increased severity of anaphylaxis observed in Ship1<sup>−/−</sup> (and Ship1<sup>−/−</sup>-BMMC) mice is due to their systemic mast cell hyperplasia and the enhanced degranulation of the Ship1<sup>−/−</sup> mast cells, but we have not ruled out the possibility that the increased levels of IL-6 and TNF in their serum could have also contributed to this effect (6).

In our studies, we also found that Ship1<sup>−/−</sup>-BMMC mice suffered worse allergic asthma pathology than Ship1<sup>+/+</sup>-BMMC mice in an acute, OVA-induced (with alum adjuvant) model of allergic asthma (28). Because Kit<sup>W-sh/W-sh</sup> mice also developed symptoms of asthma, it made it difficult to determine to what extent Ship1<sup>−/−</sup> mast cells were proinflammatory or whether they instead lacked the anti-inflammatory activity present in Ship1<sup>+/+</sup> mast cells. Indeed, asthmatic Ship1<sup>+/+</sup>-BMMC mice had lower numbers of infiltrating cells and clinical scores than Kit<sup>W-sh/W-sh</sup> controls, suggesting an anti-inflammatory role for mast cells in this model. With this in mind, we believe that Ship1<sup>−/−</sup> mast cells also contributed to inflammation for the following reasons. First, mast cells amplify allergic asthma symptoms in a similar model of asthma (without alum adjuvant) (29). Second, mast cells are hyperplastic in the lungs of asthmatic mice, and the pre-existing mast cell hyperplasia of the lungs in Ship1<sup>−/−</sup>-BMMC mice would likely predispose them to heightened allergic asthma pathology (30). Third, IL-6 and TNF, inflammatory cytokines associated with increased asthma pathology, are elevated in the serum of Ship1<sup>−/−</sup>-BMMC mice (20, 31). Finally, Ship1<sup>−/−</sup> mast cells intensified the severity of anaphylaxis, another mast cell-dependent inflammatory disease model. The fact that Ship1<sup>−/−</sup>-BMMC mice exhibit greater allergic asthma pathology than Ship1<sup>+/+</sup>-BMMC mice strongly suggests a mast cell-autonomous role for SHIP1 in allergic asthma response.

The PI3K pathway is involved in cytokine production and the amplification of FceRI-mediated degranulation in mast cells in vitro and enhances anaphylaxis in vivo (reviewed in Ref. 32). In mast cells, FceRI and c-kit receptors activate heterodimeric class IA PI3Ks, whereas G protein-coupled receptors, including the adenosine A3 receptor, activate class IB PI3Ks. SHIP1 negatively regulates PI3K activation via its phosphatase activity and can also act as a signaling adaptor (33). Other investigators have reported that the loss of the p85α subunit of class IA PI3K causes selective loss of gastrointestinal mast cells but does not affect mast cell degranulation in vitro or PSA in vivo (34). Loss of the p110δ subunit of PI3K (class IA) results in reductions in numbers of some dermal mast cells, degranulation, cutaneous anaphylaxis, and TNF and IL-6 production (35). PI3Kγ<sup>−/−</sup> (class IB)-cultured mast cells, in contrast, have reduced degranulation, and PI3Kγ<sup>−/−</sup> mice experience less severe PSA (36).
The PI3K inhibitor LY294002 has also been reported to reduce asthma symptoms in vivo (37). In combination with our results, the studies outlined above indicate that SHIP1 may repress p85α subunit-dependent PI3K activity (class IA) during the formation or maintenance of gastrointestinal mast cells, p110δ-dependent PI3K activity (class IA) in the formation or maintenance of some dermal mast cells and TNF and IL-6 production, and both PI3K activity in degranulation and anaphylaxis. This suggests the breadth of SHIP1 activity compared with the PI3K activity in degranulation and anaphylaxis. This suggests and both PI3K

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Disclosures

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