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Immunology

The src homology 2-containing inositol phosphatase (SHIP) is the gatekeeper of mast cell degranulation

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ABSTRACT **To clarify the role that the src homology 2-containing inositol phosphatase (SHIP) plays in mast cell degranulation, the gene for SHIP was disrupted by homologous recombination in embryonic stem cells. Bone-marrow-derived mast cells from SHIP+/+, +/−, and −/− F_2 littermates were compared. SHIP−/− mast cells were found to be far more prone to degranulation, after the crosslinking of IgE preloaded cells, than SHIP+/− or +/+ cells. Intriguingly, IgE alone also stimulated massive degranulation in SHIP−/− but not in +/+ mast cells. This degranulation with IgE alone, which may be due to low levels of IgE aggregates, correlated with a higher and more sustained intracellular calcium level than that observed with SHIP+/+ cells and was dependent upon the entry of extracellular calcium. Immunoprecipitation studies revealed that the addition of IgE alone to normal mast cells stimulates multiple cascades, which are prevented from progressing to degranulation by SHIP. PI 3-kinase inhibitor studies suggested that IgE-induced activation of PI 3-kinase is upstream of the entry of extracellular calcium and that SHIP restricts this entry by hydrolyzing phosphatidylinositol 3,4,5-trisphosphate. These results show the critical role that SHIP plays in setting the threshold for degranulation and that SHIP directly modulates a "positive-acting" receptor.**

The src homology 2 (SH2)-containing inositol phosphatase (SHIP) is a recently cloned hemopoietic specific signaling intermediate (1–4) that becomes tyrosine-phosphorylated in response both to multiple cytokines and to B and T cell receptor engagement (5). It exists in several molecular mass forms, with the lower 135-, 125- and 110-kDa forms being generated from the 145-kDa full length protein *in vivo* by the cleavage of its proline rich COOH terminus (6). Although all forms become tyrosine-phosphorylated in response to cytokines, only the 145- and 135-kDa species bind the adaptor protein Shc, and only the 110-kDa form is associated with the cytoskeleton (6). The relative amounts of these different forms change with differentiation (7), and it remains to be determined if they carry out distinct functions within hemopoietic cells. Recently, SHIP has been implicated in the negative signaling pathways that abrogate activation in the cells of the immune system. Specifically, it has been shown to inhibit the activation of immune receptors in both mast cells and B cells by binding to the tyrosine-phosphorylated immunoreceptor tyrosine-based inhibition motif (ITIM) of the inhibitory co-receptor FcγRIIB and inhibiting the high affinity IgE receptor- (FcεR1) and the B cell receptor-induced influx of calcium, respectively (8, 9). However, its role in modulating the biological responses of activating receptors has not yet been determined. To gain further insight into the role that SHIP plays in regulating the responses elicited by these latter receptors, we generated a SHIP knockout mouse by homologous recombination in embryonic stem cells (10). Although these mice are viable and fertile, they suffer from progressive splenomegaly, massive myeloid infiltration of the lungs, wasting, and a shortened life span (10). Notably, granulocyte/macrophage progenitors from these mice are substantially more responsive to multiple cytokines (including interleukin-3, granulocyte/macrophage colony-stimulating factor, and Steel factor) than their wild-type littermates (10). We derived *m*ast *c*ells from the *b*one *m*arrow (BMMCs) of SHIP−/− mice and compared their IgE-mediated degranulation with BMMCs from SHIP+/+ and +/− mice. Our results not only demonstrate a vital role for SHIP in both setting the threshold for and limiting the degranulation process by hydrolyzing phosphatidylinositol 3,4,5-trisphosphate (PIP_3), but they also suggest that the sensitization of mast cells with IgE may be an active process.

MATERIALS AND METHODS

Mast Cell Isolation, FcεR1 Activation, and Degranulation Assay. Bone marrows from 4 to 8 week old SHIP+/+, +/−, and −/− littermates were plated in methylcellulose (Methocult M3234; Stem Cell Technologies, Paisley, Scotland) supplemented with 30 ng/ml murine interleukin-3, 50 ng/ml murine Steel factor, and 10 ng/ml human interleukin-6 for 10–14 days. They were then harvested and grown in suspension in Iscove's modified Dulbecco's medium (IMDM) containing 15% fetal calf serum (Stem Cell Technologies), 150 μM monothioglycerol (Sigma), and 30 ng/ml interleukin-3. For FcεR1 activation, cells were washed with IMDM, then starved for 2–4 h in IMDM, 10% fetal calf serum, and 150 μM monothioglycerol, and then resuspended in Tyrode's buffer (11). Then, if stimulating with IgE alone, cells were equilibrated to 37°C for 5 min before adding 10 μg/ml anti-dinitrophenyl (anti-DNP) IgE mAb SPE-7 (Sigma) for 15 min at 37°C. If stimulating with IgE + DNP, cells were incubated at 4°C with anti-DNP IgE for 1 h, washed twice with 23°C Tyrode's buffer, equilibrated in Tyrode's buffer to 37°C for 5 min, and then treated for 15 min with or without (base 1 h IgE) DNP-human serum albumin (DNP-HSA; Sigma). EGTA (5 mM) was added 1 min before the addition of IgE, when degranulation in response to IgE alone was measured, and 1 min before the addition of DNP-HSA, when degranulation in

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Abbreviations: BMMCs, bone marrow-derived mast cells; DNP, dinitrophenyl; DNP-HSA, dinitrophenyl-human serum albumin; ERK, extracellular signal-regulated kinase; FcεR1, high affinity IgE receptor; IP_4, inositol 1,3,4,5-tetrakisphosphate; ITAM, immunoreceptor tyrosine-based activation motif; MAPKs, mitogen-activated protein kinases; PIP_3, phosphatidylinositol 3,4,5-trisphosphate; SHIP, src homology 2-containing inositol phosphatase.
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response to IgE + DNP-HSA was measured. Degranulation induced by A23187 (0.5 μM; Sigma) was for 15 min at 37°C. The degree of degranulation was determined by measuring the release of β-hexosaminidase (12).

Calcium Measurements. SHIP+/+, +/−, and −/− BMMCs (5×10^6 cells per ml) were incubated with 2 μM fura-2/AM (Molecular Probes) in Tyrode's buffer at 23°C for 45 min. When stimulating with both IgE and DNP-HSA, the cells were incubated with IgE at the same time that they were loaded with fura-2/AM. The cells were then washed and resuspended in 1 ml of the same buffer at 5×10^5 cells per ml in a stirring cuvette. After stimulation by the addition of IgE anti-DNP (10 μg/ml) or of DNP-HSA (20 ng/ml; ±5 mM EGTA added 50 sec before stimulation), cytosolic calcium was measured by monitoring fluorescence intensity at 510 nm by exciting the sample with two different wavelengths (340 and 380 nm) with an MC200 monochromator from SLM–Aminco (Urbana, IL) with a 8100 V3.0 software program.

Immunoprecipitation and Immunoblotting. SHIP+/+, +/−, and −/− BMMCs were starved as above and incubated either at 37°C for 3 min with IgE anti-DNP (10 μg/ml) or at 4°C for 1 h with 10 μg/ml IgE followed by two washes and a 3-min incubation with 20 ng/ml DNP-HSA at 37°C. Cells were then washed with PBS, solubilized at 1.5×10^7 cells per ml with 0.5% Nonidet P-40 in 4°C phosphorylation solubilization buffer (13), and subjected to immunoprecipitation as indicated. Western blotting was carried out as described (13).

RESULTS AND DISCUSSION

Aggregation of the high affinity IgE receptor (FcεR1) on mast cells by multivalent allergens initiates a biochemical cascade that results in degranulation and the release of mediators of allergic reactions. This FcεR1 is composed of an α-, a β-, and two γ-subunits (14). The COOH-terminal cytoplasmic domains of the β- and γ-subunits contain immunoreceptor tyrosine-based activation motifs (ITAMs) that are critical for IgE-mediated activation (14). Because the SH2 domain of SHIP was shown recently to be capable of binding to these tyrosine-phosphorylated ITAMs (15, 16), we investigated the role of SHIP in FcεR1 activation by using primary mast cells derived from SHIP+/+, +/−, and −/− mice (10). BMMCs were prepared from 4- to 8-week F_2 littermates, and by 8 weeks in culture, greater than 99% of the cells in the 3 cultures were c-kit positive (with similar mean fluorescence; data not shown) and FcεR1 positive (with similar mean fluorescence) as assessed with a fluorescence-activated cell sorter (Becton Dickinson; Fig. 1*A*). Western analysis of these BMMCs with anti-SHIP antibodies revealed that the 145/135-kDa SHIP doublet (as well as the 125- and 110-kDa COOH-terminally truncated forms; ref. 6) was reduced in SHIP+/− and, as expected, absent in SHIP−/− cells (Fig. 1*B*). These findings suggest that SHIP is not essential for the differentiation of BMMCs.

We then compared the ability of these SHIP+/+, +/−, and −/− BMMCs to degranulate by first examining their response to the calcium ionophore A23187. Because calcium ionophores induce degranulation in the absence of FcεR1 crosslinking (reviewed in ref. 17), we used this property to determine whether the degranulation machinery was intact in SHIP−/− BMMCs. As shown in Fig. 2*A*, SHIP+/+, +/−, and −/− BMMCs were capable of a similar degree of degranulation in response to 0.5 μM A23187. We then primed the cells with an anti-DNP IgE and examined their degranulation after FcεR1 crosslinking with DNP-HSA. The SHIP+/+ cells consistently released ≈25% of their total granule contents, whereas the +/− cells released 40%, and the −/− cells released >80% (Fig. 2*A*). Even more remarkably, the SHIP−/− BMMCs released >80% of their granule contents when only IgE was added (Fig. 2*A*), suggesting that SHIP plays an important role in preventing inappropriate degranulation (i.e., in the absence of antigen). This was also observed with a monoclonal anti-erythropoietin IgE, indicating that this degranulation was independent of the source or specificity of the IgE. Similar results were obtained with SHIP+/+, +/−, and −/− mast cells derived from embryonic stem cells and day 14.5 fetal livers (data not shown).

Because SHIP was shown recently to inhibit FcεR1 activation in mast cells by binding to the phosphorylated tyrosine-based inhibition motif of the inhibitory coreceptor FcγRIIB and inhibiting calcium influx (8, 9), we investigated the relationship between the presence of SHIP and the entry of extracellular calcium triggered by the activation of the FcεR1 alone. As shown in Fig. 2*B*, when SHIP+/+, +/−, and −/− BMMCs were loaded with fura-2/AM, sensitized with anti-DNP IgE, and stimulated with DNP-HSA, the peak of intracellular calcium was highest in the SHIP−/−, intermediate in the SHIP+/−, and lowest in the +/+ cells, consistent with their ability to degranulate. More intriguingly, when cells were treated with IgE alone, all three cell types responded with an increase in intracellular calcium (Fig. 2*C*); SHIP−/− BMMCs responded with the highest influx of calcium, and SHIP+/+ responded with the lowest. Moreover, whereas the cytosolic calcium concentration returned slowly to baseline levels in SHIP+/+ and +/− BMMCs, it actually increased in a biphasic fashion in SHIP−/− cells when they were stimulated with IgE alone. In all cases, the addition of EGTA both prevented degranulation (Fig. 2*A*) and markedly reduced the increase in intracellular calcium to a level that was indistinguishable in +/+, +/−, and −/− mast cells (shown for SHIP −/− cells; Fig. 2 *B* and *C*). This effect of EGTA suggested that the increase in intracellular calcium in response to either IgE

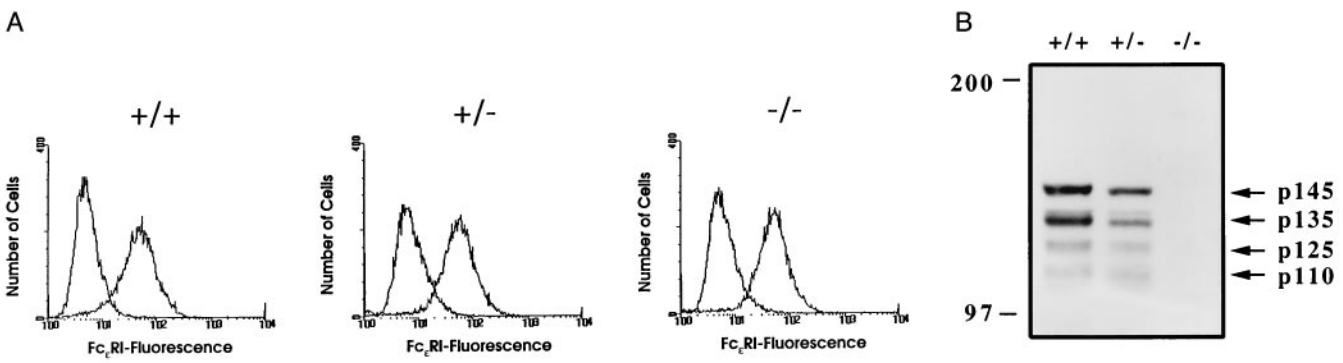

FIG. 1. (*A*) Cell surface expression of the FcεR1 in BMMCs from SHIP+/+, +/−, and −/− mice as assessed by a fluorescence-activated cell sorter with fluorescein isothiocyanate-labeled IgE (anti-erythropoietin 26). Background staining was with propidium iodide. No fluorescein isothiocyanate-labeled IgE staining was observed with FcεR1-negative cells. (*B*) Anti-SHIP (anti-N + anti-M; ref. 6) immunoblots of total cell lysates from SHIP +/+, +/−, and −/− BMMCs.

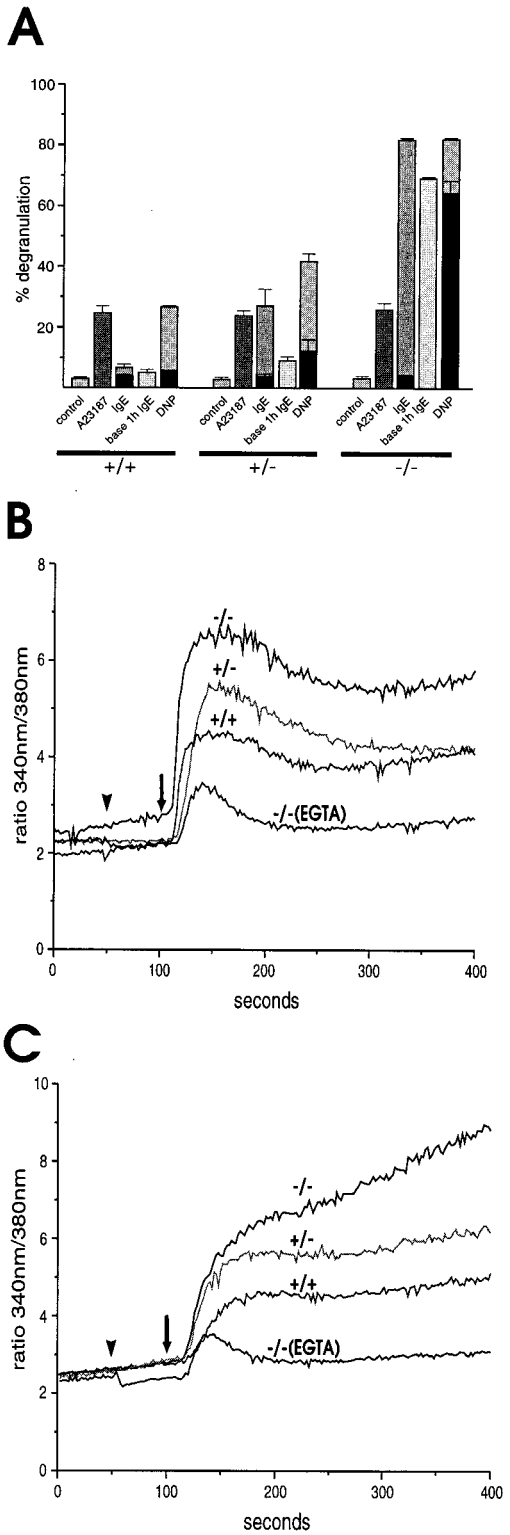

FIG. 2. (*A*) Degranulation of SHIP+/+, +/−, and −/− BMMCs in response to the calcium ionophore A23187 or to anti-DNP IgE with or without DNP-HSA. Control values are those obtained with cells incubated for 15 min at 37°C in the absence of both IgE and DNP-HSA. The base 1-h IgE values are the controls for the IgE + DNP-HSA (in figure, see DNP). The black bars are the values obtained when 5 mM EGTA was added 1 min prior to IgE or DNP stimulation. Each bar is the mean of quadruplicates ±SD, and similar results were obtained in four separate experiments with three independently isolated lines of each genotype. (*B*) Intracellular Ca^{2+} measurements in SHIP+/+, +/−, and −/− BMMCs in response to anti-DNP IgE + DNP-HSA with or without EGTA. (*C*) Intracellular Ca^{2+} measurements in SHIP+/+, +/−, and −/− BMMCs in response to anti-DNP IgE alone with or without EGTA. The arrow indicates the time when DNP-HSA (*B*) or IgE (*C*) was added. The arrowhead depicts the time of EGTA (5 mM) addition. The Ca^{2+} profiles of +/+ and +/− BMMCs obtained in the presence of EGTA (not shown) are similar to those shown with the −/− cells. Identical results were obtained in four separate experiments.

alone or IgE + DNP-HSA was made predominantly from the extracellular medium and that most likely SHIP was acting downstream of the initial release of intracellular calcium stores. Taken together, these data reveal that SHIP serves a dual role in mast cell degranulation; it not only inhibits the influx of extracellular calcium after the recruitment of the inhibitory FcγRIIB but also modulates the entry of calcium triggered by the activating FcεR1 in the absence of the coclustering of the inhibitory receptors.

Although current dogma states that the crosslinking of the IgE-preloaded FcεR1 by a multivalent antigen is the essential first step in triggering the signaling cascades that lead to degranulation, both the influx of calcium in SHIP+/+, +/−, and −/− BMMCs and the massive degranulation in SHIP−/− BMMCs with IgE alone suggested that substantial intracellular signaling occurs during the IgE-preloading step (i.e. in the absence of antigen). Interestingly, BioSep SEC S3000 (Phenomenex, Belmont, CA) HPLC fractionation of the anti-DNP IgE and the anti-erythropoietin IgE (which separates monomeric IgE from dimers and larger aggregates on the basis of size) suggested that this degranulation may be occurring with monomeric IgE (data not shown). This was suggested further by rechromatography of the monomeric anti-DNP IgE, after its incubation at 37°C for 1 h in Tyrode's buffer. However, although no detectable aggregates were observed after this incubation period, at this time we cannot rule out that a very low level of aggregation of IgE—either while in solution (because our lower limit of detection of IgE aggregates after HPLC is 0.5% of the total protein eluted) or after binding to FcεR1 on the cell surface—is responsible for the observed IgE-induced degranulation of SHIP−/− BMMCs or the IgE-induced tyrosine phosphorylations in the +/+ BMMCs (see below). Regardless, during this sensitization step, our degranulation results suggested that SHIP may be acting as a "gatekeeper," preventing the degranulation signal from progressing unless overwhelmed by the massive signaling initiated by crosslinking agents.

To test this hypothesis, we explored tyrosine phosphorylation events elicited by IgE alone in SHIP+/+, +/−, and −/− BMMCs. As shown in Fig. 3*A*, the β-subunit of the FcεR1 (as well as the γ-subunit; data not shown) is indeed tyrosine-phosphorylated in all three cell types in response to IgE alone. We consistently found that the β-subunit was more phosphorylated in response to IgE in the SHIP−/− BMMCs. This increased phosphorylation might reflect the fact that the tyrosine phosphatase, SHP-1, which associates constitutively with the FcεR1 (18), is modulated dramatically by anionic phospholipids (ref. 19; see below). Moreover, in +/+ and +/− cells, SHIP is tyrosine-phosphorylated constitutively, and its phosphorylation level increases with the addition of IgE (Fig. 3*B*). Intriguingly, immunoprecipitating Shc from these cells revealed that its tyrosine phosphorylation also occurs with IgE stimulation alone, but its phosphorylation is markedly reduced in SHIP−/− BMMCs (Fig. 3*C*). This suggests that Shc needs to bind to SHIP to become tyrosine-phosphorylated after FcεR1 activation by IgE. The same result was obtained after the antigen-crosslinking of IgE-preloaded cells (data not shown). When SHIP+/+ cells were incubated with IgE alone for 1 h and then crosslinked with DNP-HSA for 3 min, the level of Shc tyrosine phosphorylation was similar to that obtained with 3 min of stimulation with IgE alone (Fig. 3*C*, lanes 2 and 8). Moreover, after incubating for 1 h with IgE alone, Shc was

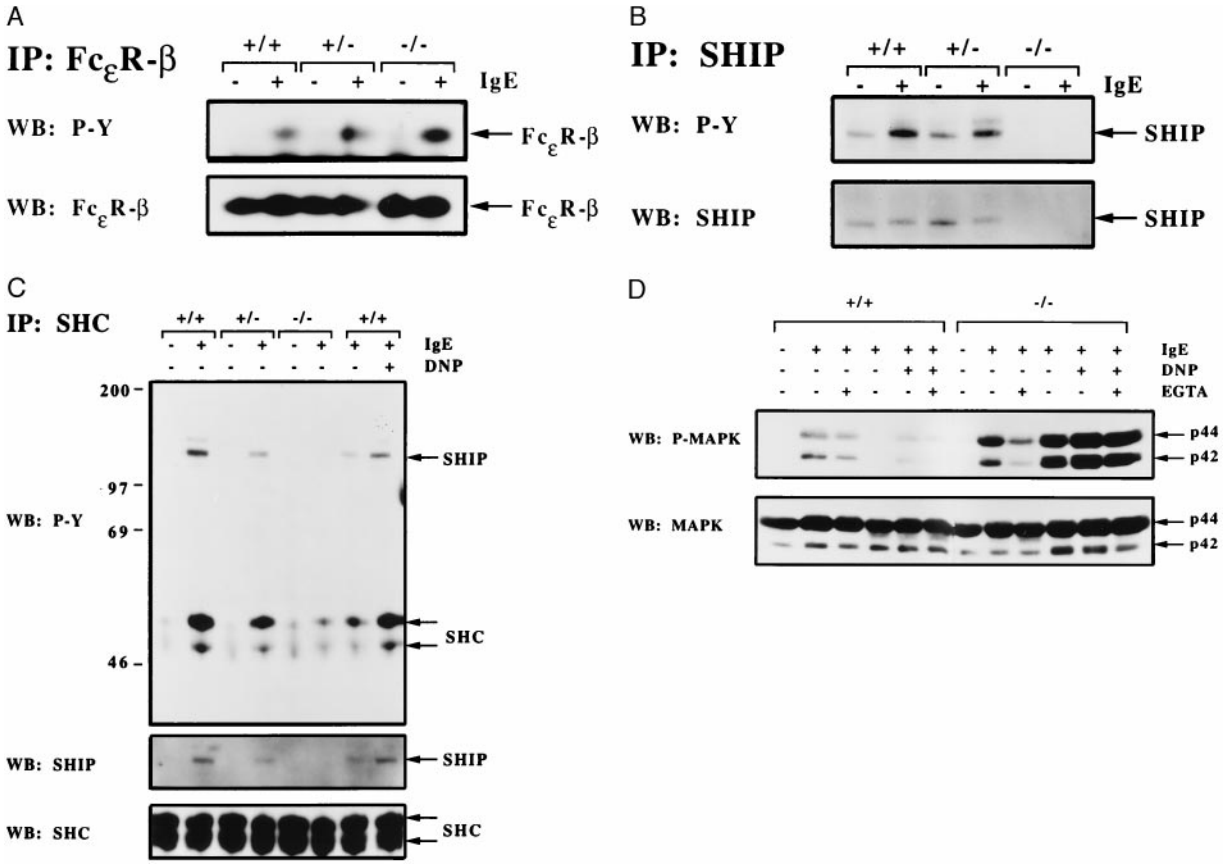

FIG. 3. Tyrosine phosphorylations following IgE sensitization. SHIP+/+, +/−, and −/− BMMCs were starved for 2–4 h and then incubated either at 37°C for 3 min with 10 μg/ml anti-DNP IgE or at 4°C for 1 h with 10 μg/ml IgE followed by two washes and a 3 min incubation with 20 ng/ml DNP-HSA at 37°C. Cells were then washed, solubilized, and subjected to immunoprecipitation (IP) with anti-FcεR1 β-subunit (*A*), anti-SHIP (*B;* anti-N + anti-M; ref. 6), or anti-Shc antibodies (*C*; Transduction Laboratories, Lexington, KY). Western blotting was carried out with 4G10 (Upstate Biotechnology, Lake Placid, NY) as described (13). The blots were reprobed with the immunoprecipitating antibody to show equal loading. (*D*) Total cell lysates from SHIP+/+ and −/− BMMCs with or without EGTA were incubated for 1 min before the 3-min stimulation with IgE or DNP-HSA and then subjected to Western analysis with anti-phospho-MAPK (New England BioLabs) and reprobed with anti-ERK 1-CT (Upstate Biotechnology) to confirm equal loading. The last three lanes of both the +/+ and −/− samples are total cell lysates from cells treated for 1 h with IgE at 4°C, shifted up to 37°C and treated with or without EGTA for 1 min before stimulating for 3 min with DNP-HSA.

still tyrosine-phosphorylated above control levels (i.e. when IgE was not added; Fig. 3*C*, lanes 1 and 7). This finding might explain previous RBL-2H3 results (in which IgE-preloaded cells were used as negative controls) that suggest that Shc is tyrosine-phosphorylated constitutively in these cells (20).

The mitogen-activated protein kinases (MAPKs), extracellular signal-regulated kinase (ERK) 1 and 2, are also tyrosine-phosphorylated by IgE alone in SHIP+/+ and −/− BMMCs, but in SHIP −/− cells, this phosphorylation is greater and prolonged dramatically (Fig. 3*D*). This increased activation of ERK 1 and 2 is interesting for several reasons. First, the fact that ERK activation is dependent upon Syk kinase activity (21) suggests that IgE alone is also sufficient to activate Syk. Second, ERK activation leads to the activation of phospholipase A_2 and the subsequent synthesis of arachidonic acid-derived metabolites (22). Thus, the substantially prolonged ERK activation in SHIP−/− BMMCs suggests that SHIP may play a role in regulating the synthesis of these inflammatory mediators as well. Third, this prolonged activation of ERK in SHIP−/− cells occurs under conditions where the tyrosine phosphorylation of Shc is reduced markedly (Fig. 3*C*), suggesting that Shc-dependent activation of the Ras pathway may not play a significant role in the IgE-mediated activation of MAPK in BMMCs. Interestingly, EGTA significantly reduced IgE-induced ERK 1 and 2 phosphorylation, especially in SHIP−/− cells (Fig. 3*D*), suggesting that the entry of extracellular calcium occurs upstream of MAPK activation.

Because the 5-phosphatase activity of SHIP has been shown recently to be required for its ability to reduce calcium entry during FcγRIIB-mediated inhibition (9), we investigated whether it was SHIP's ability to hydrolyze PIP_3 or inositol 1,3,4,5-tetrakisphosphate (IP_4) that was important in restricting IgE-mediated extracellular calcium entry by testing the effects of PI 3-kinase inhibitors. In preliminary experiments, we established that both wortmannin and LY294002 completely inhibited degranulation in SHIP−/− cells induced by IgE alone (Fig. 4*A*). This is consistent with results obtained with IgE-crosslinked RBL-2H3 cells (23). We then explored the effects of these inhibitors on the IgE-mediated influx of extracellular calcium. LY294002 dose-response studies revealed that as little as 10 μM LY294002 reduced the influx of extracellular calcium into SHIP−/− cells to the levels observed in IgE-stimulated SHIP+/+ BMMCs (Fig. 4*B*). On the other hand, LY294002, even at 100 μM, had far less effect on the IgE-stimulated influx of calcium in SHIP+/+ cells (Fig. 4*B*). Similar results were obtained with wortmannin (data not shown). These results suggest that during the incubation of SHIP+/+ BMMCs with IgE, the activation of PI 3-kinase and the generation of PIP_3 play a less significant role in mediating the entry of extracellular calcium, because SHIP effectively hydrolyzes this phospholipid to PI-3,4-P_2. Thus, degranulation in the absence of antigen is prevented. However, in the absence of SHIP, IgE alone can increase PIP_3 to levels that augment the entry of extracellular calcium sufficiently for degranulation to occur.

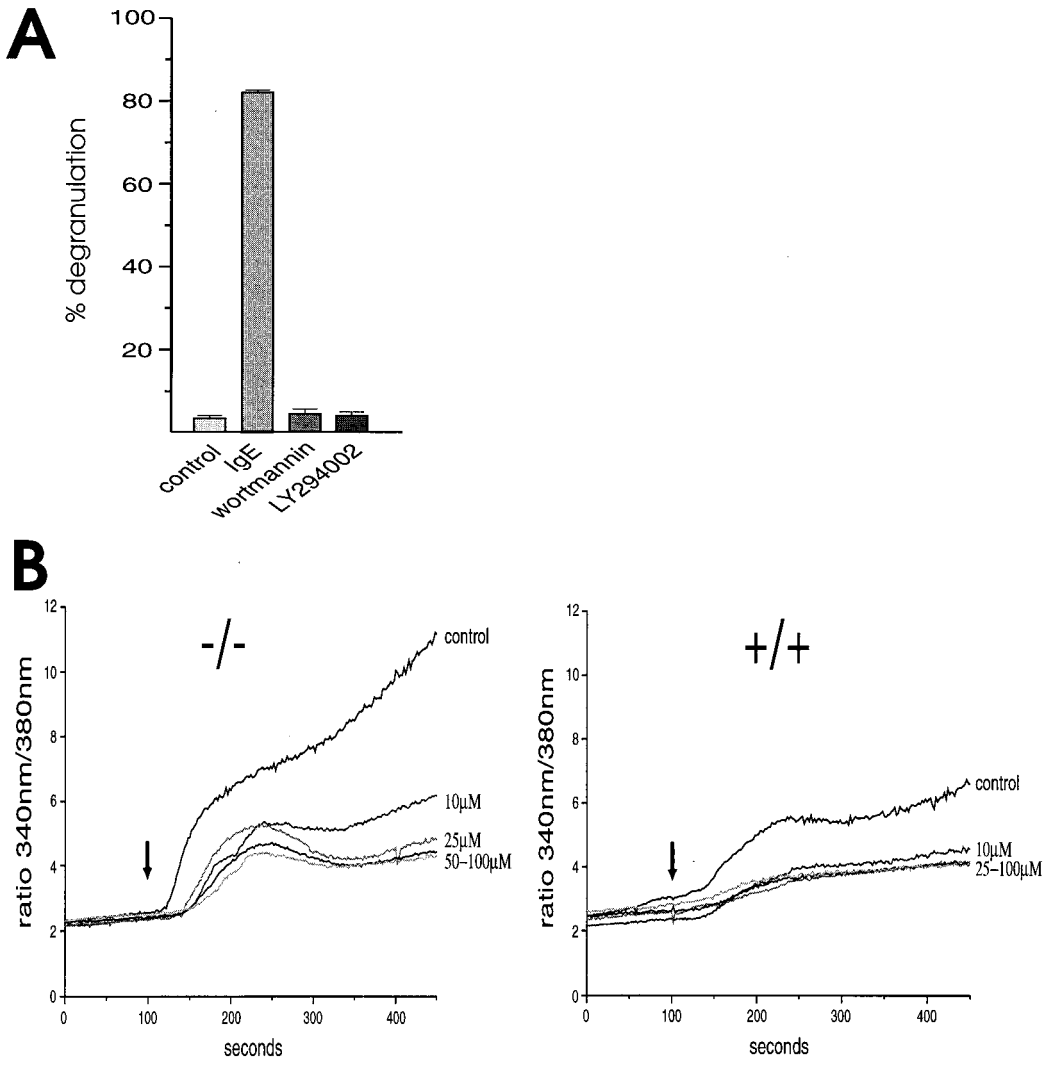

FIG. 4. Wortmannin and LY294002 inhibit IgE-induced degranulation and calcium influx. (*A*) Wortmannin at 25 nM or LY294002 at 25 μM was added to SHIP$-/-$ BMMCs 25 min before the addition of IgE, and degranulation was measured as described in *Materials and Methods*. (*B*) LY294002, at the concentrations indicated, was added 25 min before the addition of 5 μg/ml anti-DNP IgE (indicated by the arrow) to SHIP$-/-$ (*Left*) or SHIP$+/+$ (*Right*) BMMCs.

Taken together, our results open up the possibility that the binding of IgE alone to the FcεR1 on normal primary mast cells activates an associated Src family member (predominantly Lyn; ref. 12), most likely via CD45 (24), to tyrosine phosphorylate the β- and γ-ITAMs (Fig. 5). SHIP is then attracted via its SH2 domain (1) to the COOH-terminal ITAM of the β-(15) and/or the γ-chain (16), becomes phosphorylated at one or both of its NPXY motifs (1), and then attracts Shc, via its phosphotyrosine binding domain, to be phosphorylated. Wortmannin's inhibition of the entry of extracellular calcium suggests that PI 3-kinase is also translocated to the plasma membrane and activated early in this process, but exactly how this process occurs remains to be determined. PI 3-kinase activity in antiphosphotyrosine immunoprecipitates from IgE-stimulated SHIP$+/+$ and $-/-$ BMMCs is identical (data not shown). Syk is attracted to the γ-ITAM (21), becomes phosphorylated by Lyn, and stimulates the tyrosine phosphorylation of phospholipase C-γ1 and γ2 (25) and the initial release of intracellular calcium via IP_3. This emptying of intracellular calcium triggers the entry of extracellular calcium, and this entry is augmented substantially by the generation of PIP_3 mediated by PI 3-kinase. Two recent, elegant studies involving the overexpression of various cDNAs in B cell lines suggest that this increase in PIP_3 may attract the Btk/Tec family tyrosine kinase, Btk (which has been shown to be activated in response to FcεR1 crosslinking in BMMCs; ref. 26), to the plasma membrane for activation, subsequent phosphorylation/activation of PLC-γ2, further generation of IP_3, and sustained emptying of intracellular stores of calcium (27, 28). Moreover, this sustained emptying appears to be requisite for the entry of store-operated calcium from the extracellular medium (27, 28). SHIP prevents intracellular calcium levels from reaching the critical threshold levels required for the synthesis of arachidonic acid metabolites (via MAPK) and degranulation by hydrolyzing PIP_3. LY294002 (and wortmannin) reduces intracellular calcium levels in IgE-treated SHIP$-/-$ cells to that seen in $+/+$ cells, consistent with SHIP's hydrolysis of PIP_3, and not IP_4 (which could prevent activation of the plasma membrane bound calcium channel activator, $GAP1^{IP4BP}$; ref. 29) being the critical event in preventing IgE alone from triggering sufficient calcium entry for degranulation. However, our studies do not completely rule out the contribution of IP_4 to the IgE-mediated influx of calcium.

In summary, our results suggest that IgE alone is capable of triggering substantial signaling in normal mast cells *in vitro*. Although we cannot rule out that this signaling is due to a low level of IgE aggregation, it is nonetheless conceivable that monomeric IgE triggers signaling by causing a conformational change in the multispanning β-subunit (in a fashion analogous to that proposed for seven-spanner receptors). Intriguingly,

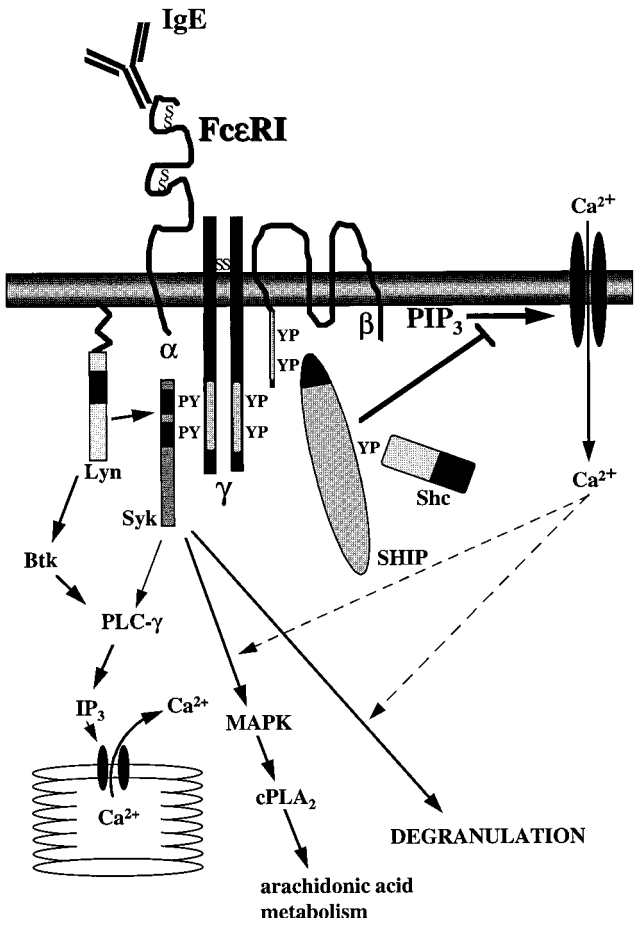

FIG. 5. Model of FcεR1 activation by IgE alone in normal primary mast cells. See text for details. SH2 domains are shown in black. YP and PY, phosphotyrosine; $cPLA_2$, cytosolic phospholipase A_2.

this signaling by IgE alone may also occur *in vivo* (especially under conditions where levels of serum IgE are elevated markedly, such as during allergic attacks or after specific infections) and serve to prime the cells for subsequent activation by multivalent antigens. Related to this, several reports have shown recently that IgE alone, both *in vivo* and *in vitro*, dramatically increases FcεR1 expression on the surface of mast cells and basophils (30–32). Moreover, Yamaguchi *et al.* (31) showed that FcεR1 expression on peritoneal mast cells from IgE−/− mice is reduced dramatically compared with FcεR1 expression on cells from normal mice. These results are consistent with the capacity of circulating IgE alone to initiate signaling *in vivo*, perhaps via a low level of aggregation.

Importantly, our results also reveal a vital role for SHIP in both setting the threshold for and limiting the degranulation process. This role raises the possibility that naturally occurring mutations in SHIP could contribute to specific hyperallergic conditions in humans.

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