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Research Article

Mast cells stimulated by membrane-bound, but not soluble, steel factor are dependent on phospholipase C activation

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Abstract. The steel factor (SLF) and c-Kit growth factor/receptor pair are key molecules governing mast cell development and survival. SLF is expressed on stromal cells as a membrane-bound molecule (mSLF) which can be cleaved by proteases to release a soluble form (sSLF). We investigated the importance of phospholipase C (PLC) activation in mast cells stimulated by sSLF and mSLF. PLC antagonists U73122, neomycin sulfate and oleic acid inhibited mast cell thymidine incorporation stimulated by mSLF, but not by sSLF. These antagonists suppressed sSLF-induced Ca^{2+} transients but did not significantly interfere with c-Kit phosphorylation or PLC-γ2 recruitment. p85, the regulatory subunit of phosphatidylinositol 3-kinase (PI3-kinase), was found to be efficiently recruited to c-Kit following stimulation by sSLF or mSLF. However PKB/Akt, a kinase activated by PI3-kinase products, was phosphorylated following sSLF stimulation, but not with mSLF. Taken together, these studies demonstrate the importance of PLC activation by mSLF in supporting mast cells.

Key words. Mast cells; SL; c-Kit; calcium; Phospholipase C; PKB/Akt.

Mast cells are derived from hemopoietic stem cells in the bone marrow [1]. They exit the bone marrow in an immature state and mature in peripheral tissues [2]. Key molecules governing mast cell development and survival are the steel factor (SLF) and c-Kit growth factor/receptor pair [3]. This is evident from the fact that peripheral mast cells are almost completely absent in mice with severe but viable *W* or *Sl* alleles [4, 5] which have mutations in c-Kit and Sl loci, respectively. SLF is expressed primarily as a membrane-bound molecule (mSLF) which can be cleaved by proteases to release a soluble form (sSLF) [6]. Alternative splicing removes the proteolytic site resulting in a membrane-bound molecule [7]. In its soluble form, SLF can stimulate a wide variety of hemopoietic and non-hemopoietic progenitor cells [8]. It is particularly effective in synergy with cytokines [9].

c-Kit, like other receptor tyrosine kinases, activates a variety of signaling pathways including phosphatidylinositol 3-kinase (PI3-kinase), phospholipase (PLC)-γ, src family members and the ras pathway [10]. PI3-kinase activation has been implicated in a number of endpoints including receptor trafficking [11–13], chemotaxis [14–17], mast cell degranulation [18], adherence to fibronectin [19], differentiation [20] and promotion of cell survival [21, 22]. The ras pathway and Src family members, in conjunction with PI3-kinase have both been implicated in proliferative signals [10].

The majority of studies investigating signaling pathways activated by c-Kit have been performed with sSLF. However, there is evidence to suggest that the soluble and membrane-bound forms of SLF stimulate qualitatively different responses in c-Kit-positive cells [23–29]. Using

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the 32D murine myelomonocytic cell line model, we recently demonstrated that PLC-γ activation, but not PI3-kinase activation, was essential for mSLF to support c-Kit-positive 32D cells in vitro and in vivo [30]. In the current study, we extended these observations to determine the importance of PLC-γ and PI3-kinase activation in ex vivo mast cells stimulated by s- and m-SLF. We found that activation of PLC is essential for mSLF to support mast cells. Moreover, we observed that downstream elements of the PI3-kinase pathway displayed reduced activation by mSLF compared to sSLF.

Materials and methods

Mast cell culture

Bone marrow cells from 4- to 6-week-old C57Bl/6 mice (Jackson Labs, Bar Harbor, Maine) were removed and cultured in OPTI (Gibco Life Technologies, Burlington, Canada) supplemented with 5% fetal bovine serum (FBS; Gibco Life Technologies) and 4% conditioned medium from interleukin (IL)-3 producing WEHI-3 cells as described previously [31]. Cell cultures contained 55 µmol/l β-mercaptoethanol and antibiotics (both Sigma, Oakville, Canada). Cells were grown for 5 weeks prior to use.

mSLF culture system

SL/SL^4 and X9/D3 stromal cells were a gift from Dr. D. A. Williams (Indianapolis, Ind). SL/SL^4 cells are immortalized fibroblasts derived from Sl/Sl^4 mice and do not express any form of SLF. X9/D3 cells are SL/SL^4 cells that have been transfected with plasmid mixtures containing cDNAs of S117H SLF and a hygromycin-resistance gene. S117H SLF lacks the cleavage site required to produce sSLF. Therefore, these cells produce only the membrane-bound form of the ligand [27].

Reagents and antibodies

Recombinant murine sSLF was produced as described previously [32] and assessed for purity by SDS-PAGE, Western blot and bioassay on c-Kit-positive cells by [^{3}H]-thymidine incorporation. Neomycin sulfate, oleic acid and U73122 were purchased from Calbiochem (La Jolla, Calif.). ITS liquid media supplement was purchased from Sigma.

Immunoprecipitation and Western blotting were performed with antibodies used at concentrations recommended by suppliers. Rabbit anti-murine c-Kit (immunoprecipitation) was obtained from Upstate Biotechnology (Lake Placid, N. Y.). Rat anti-murine c-Kit (Western blot) was prepared from R62 hybridoma cells. Supernatants were used at a 1:5 dilution. Anti-phospho PKB/Akt (phospho-Ser473-specific) and anti-p85 were obtained from BD PharMingen (Mississauga, Canada). Anti-phosphotyrosine was obtained from Cell Signaling Technology (Mississauga, Canada). Rabbit anti-PLC-γ2 (immunoprecipitation) and murine anti-PLC-γ2 (Western blot) were obtained from Santa Cruz Biotechnology (Santa Cruz, Calif.). All secondary horseradish peroxidase-conjugated antibodies were used at 1:2500 and were obtained from Sigma.

Stimulation assays

For bioassays with sSLF, mast cells were washed three times in RPMI + 0.5% FBS, starved of growth factor for about 5 h, and then plated in the same medium with sSLF (50 or 150 ng/ml) at a density of 5×10^5 cells per well in 96-well flat-bottom plates. For Sl/Sl^4 or X9/D3 co-culture assays, the adherent cells were treated with mitomycin C (1.8 µg/ml) for 2 h at 37 °C, washed three times with PBS, trypsinized, counted and then plated at a concentration of 1×10^4 cells/well in 96-well plates that had been pre-coated with 0.1% gelatin (Sigma). The cells were allowed to adhere for 4–6 h. Mast cells were washed three times in RPMI + 0.5% FBS and starved of growth factor for 5 h, and then added to the monolayers at a density of 5×10^5 cells per well. PLC antagonists, when used, were pre-incubated with the mast cells for 20–60 min. In all cases, after 18 h of stimulation, 1 µCi of [^{3}H]-thymidine was added to each well for 6 h. Cells were then harvested and incorporated radioactivity was determined by scintillation counting (Top Count, Packard, Perkin Elmer, Boston Mass). Results were analyzed for statistical significance using two-way ANOVA with Bonferroni's post-tests. Assays were performed at least twice with similar results.

Spectrofluorimetry

$[Ca^{2+}]_c$ measurements were performed by flow cytometry. Bone marrow-derived mast cells (BMMCs) (5×10^5 cells/ml) were growth factor deprived for ~ 2 h in Tyrode's buffer [HEPES (10 mM), NaCl (100 mM), KCl (5 mM), $CaCl_2$ (1.4 mM), $MgCl_2$ (1 mM), glucose (5.6 mM), bovine serum albumin (BSA) (0.1%)]. Subsequently, cells were incubated in loading buffer (30 min; 5 µM indo-1 AM, 0.03% pluronic F-127 in Tyrode's buffer), washed (twice) and incubated (longer than 15 min, 4 °C) to allow for the complete removal and/or conversion of indo-1 AM to Ca^{2+}-sensitive indo-1. Measurements were performed using a laser tuned to 338 nm while monitoring emissions at 405 and 450 nm. The concentration of intracellular free Ca^{2+} was calculated according to the following formula [33]:

$$[Ca^{2+}]_i = K_d \times (F_{min}/F_{max}) \times (R - R_{min})/(R_{max} - R),$$

where R is the ratio of the fluorescence intensities measured at 405 and 450 nm during the experiments and F is the fluorescence intensity measured at 450 nm. R_{min}, R_{max}, F_{min} and F_{max} were determined from in situ calibration of unlysed cells using 4 µM ionomycin in the absence (R_{min}

and F_{min}; 10 mM EGTA) and presence (R_{max} and F_{max}) of Ca^{2+}. K_d (250 nM) is the dissociation constant for indo-1 at 37 °C. R_{min}, R_{max}, F_{min} and F_{max} varied depending upon settings and were determined at the beginning of each experimental procedure. All measurements were repeated at least twice with similar results.

Immunoprecipitations and Western blotting

For immunoprecipitation studies, mast cells were starved for 6 h in RPMI + ITS liquid media supplement + 0.1% BSA (hereafter referred to as ITS-RPMI). Cells were spun down and resuspended at a concentration of 2×10^7 cells/ml. A 2x solution of SLF ± inhibitors in ITS-RPMI pre-heated to 37 °C was added to an equal volume of cells and incubated for the indicated times in a 37 °C water bath. After incubation (5 min for sSLF, 30 min for co-culture; these times were found to be optimal for their respective stimuli), samples were removed and placed on ice. Cells were washed in ice-cold PBS twice and lysed by freezing and thawing in lysis buffer [500 µl; 50 mM Tris (pH 7.0), 1% NP-40, 50 mM EDTA, protease inhibitor cocktail (Roche, Laval, Canada), phosphatase inhibitor cocktail (Sigma), 200 µM sodium orthovanadate, 20 mM NaF and 1 mM PMSF]. Cell lysates were pelleted in a microcentrifuge at 10,000 rpm for 20 min at 4 °C. The supernatant was recovered and 50 µl of a 50% protein A slurry (AmershamPharmacia Biotech, Baie D'Urfe, Canada) that was pre-coated for 1 h anti-c-kit (5 µl/sample) was added. The samples were incubated for 2 h at 4 °C with rotation and were then washed three times: once with lysis buffer, once with PBS and, finally, with Tris (50 mM, pH 6.8). After the final wash, the beads were resuspended in gel-loading buffer and boiled for 5 min. Samples were resolved on a polyacrylamide gel (6% gel). The proteins were transferred to nitrocellulose and blocked in Tris-buffered saline + 0.1% Tween-20 (TBST) containing 1% gelatin (Bio-Rad, Mississauga, Canada). The membrane was incubated with primary antibody overnight, washed thee times and incubated with a secondary antibody conjugated to horseradish peroxidase for 1 h at room temperature. The membrane was washed three times and then visualized by chemiluminescence (NEN Life Science Products, Guelph, Canada). All Western blots were performed twice with similar results.

For experiments requiring stimulation of BMMCs by mSLF, X9/D3 fibroblasts expressing mSLF [27] were plated in six-well plates at 1×10^6 cells/well and allowed to adhere overnight. BMMCs (10^7 cells per 5 ml per well) were added to the fibroblasts, the plates were centrifuged at 1250 rpm for 2 min and then incubated at 37 °C for the required time. Following stimulation, the plates were placed immediately on ice. Medium was gently removed and lysis buffer was added directly to the wells. Immunoprecipitation or Western blotting were carried out as described above.

Results

PLC antagonists inhibit mast cells stimulated by mSLF but not sSLF

To investigate the importance of PLC-γ activation for mast cells stimulated by SLF, we determined the effect of PLC antagonists on mast cells stimulated by soluble or membrane-bound SLF. The concentration of sSLF used was at the mid-point of its dose response curve. Stimulation by mSLF was performed by co-culture with mSLF-expressing X9/D3 cells as described in Materials and methods. Control experiments demonstrated that SLF-negative Sl/Sl[4] cells did not stimulate mast cells above background, confirming that stimulation by X9/D3 cells was due to mSLF (not shown). As shown in figure 1, neomycin sulfate, U73122 and oleic acid all inhibited mast cell [^{3}H]-thymidine incorporation stimulated by mSLF in a dose-dependent manner. Furthermore, these antagonists were effective at concentrations previously observed to inhibit PLC activity [34–40]. In contrast, the antagonists had minimal effects on [^{3}H]-thymidine incorporation stimulated by sSLF.

PLC antagonists suppress sSLF-stimulated Ca^{2+} transients

PLC-γ activation stimulates the release of Ca^{2+} from intracellular stores followed by influx through store-operated Ca^{2+} channels [41]. To further investigate the effects of the PLC antagonists on c-Kit signaling, we determined their effect on Ca^{2+} transients stimulated by sSLF. As shown in figure 2, pre-incubation of mast cells with neomycin, U73122 or oleic acid inhibited the Ca^{2+} signal stimulated by sSLF to varying degrees. Importantly, the ability of the antagonists to inhibit the Ca^{2+} signal (see insets) corresponded with their ability to inhibit mast cells stimulated by mSLF. For example, U73122, which was the most potent inhibitor of Ca^{2+} signals, also exhibited the greatest degree of mast cell inhibition. Furthermore, oleic acid displayed a biphasic response for inhibition for both Ca^{2+} mobilization and mast cell stimulation. Inhibition of Ca^{2+} transients by neomycin was less well correlated with inhibition of thymidine incorporation. However, this may have been due to differences in time scale of the two assays (minutes vs 18 h). These observations therefore support the conclusion that PLC inhibition, as measured by inhibition of Ca^{2+} signals, correlates with mast cell inhibition when stimulated by mSLF.

PLC antagonists do not prevent c-Kit phosphorylation or PLC-γ recruitment to c-Kit

To investigate the specificity of these compounds, we determined their effects on c-Kit and PLC-γ phosphorylation. Mast cells were stimulated with sSLF or co-incubated with SL/SL[4] or X9/D3 cells and subjected to immunoprecipitation with anti-c-Kit antibodies. Five- and

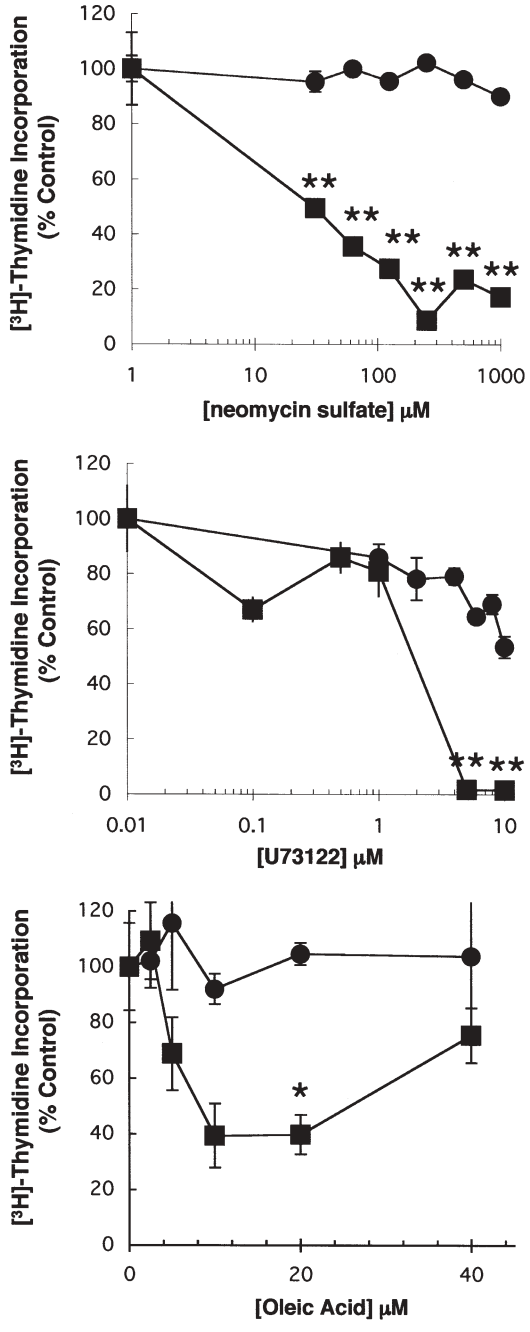

Figure 1. PLC-γ antagonists specifically inhibit BMMCs stimulated by mSLF. BMMCs were pre-incubated with the PLC antagonists for 20 min, then incubated with either sSLF (circles; 150 ng/ml) or mSLF-expressing X9/D3 cells (squares) as described in Materials and methods. The cells were incubated for 18 h followed by 6 h with [^{3}H]-thymidine. Incorporated radioactivity was determined by scintillation counting. After subtracting background counts, the results are presented as the percentage of control in the absence of an antagonist. * $p < 0.05$, ** $p < 0.01$.

30-min stimulations were used for sSLF and mSLF, respectively, since preliminary experiments had established that these times were optimal for c-Kit phosphorylation (data not shown). Immunoprecipitates were then analyzed by Western blotting for anti-phosphotyrosine. As shown in figure 3, stimulation of mast cells with X9/D3, but not SL/SL^4 stromal cells stimulated c-kit phosphorylation to levels similar to sSLF (in this experiment, 50 ng/ml was used). Moreover, pre-incubation with neomycin, oleic acid or U73122 did not affect the intensity of c-Kit phosphorylation in response to mSLF in relation to the total c-Kit immunoprecipitated.

Although these antagonists did not significantly affect c-Kit phosphorylation levels, they may affect the ability of c-Kit to recruit and activate PLC-γ2. To determine if any of the PLC antagonists affect phosphorylation of PLC-γ2, a similar experiment was performed using PLC-γ2-specific immunoprecipitating antibodes. This anti-serum was used since PLC-γ2 is hemopoietic specific and could therefore be distinguished from the stromal cell-derived form. As shown in figure 4, stimulation of mast cells with X9/D3 stromal cells stimulated PLC-γ2 phosphorylation to levels similar to sSLF (in this experiment, 150 ng/ml sSLF was used to generate sufficient PLC-γ2 phosphorylation). In addition, pre-incubations with neomycin or oleic acid did not affect the intensity of PLC-γ2 phosphorylation in response to mSLF in relation to the total PLC-γ2 immunoprecipitated. U73122 did slightly reduce the level of PLC-γ2 phosphorylation by approximately 30% compared to unstimulated cells. These observations therefore suggest that these reagents do not act at the level of c-Kit phosphorylation, nor do they interfere significantly with its interaction with PLC-γ.

p85 is recruited to c-Kit stimulated by mSLF

Previously, we demonstrated an apparent redundancy between PLC-γ and PI3-kinase in 32D-Kit cells stimulated by sSLF [30]. The inability of PI3-kinase activation to compensate for loss of PLC-γ activation when stimulated by mSLF suggests that the PI3-kinase pathway may not be fully activated. We therefore investigated activation of the PI3-kinase pathway in cells stimulated by mSLF. Mast cells stimulated for 15, 30 or 60 min by mSLF or for 5 min with sSLF (a pre-determined maximal stimulatory time point for sSLF) as a positive control were collected, and lysates were subjected to immunoprecipitation with anti-c-Kit antibodies. The immunoprecipitates were analyzed by Western blotting with anti-sera specific for the p85 regulatory subunit of PI3-kinase. The extended time course with mSLF was to ensure that potentially slower recruitment due to slower activation by mSLF would be observed. As shown in figure 5, p85 association with c-Kit could be detected following co-incubation with sSLF or X9/D3 cells, but not SL/SL^4 cells. Furthermore, the association appeared to persist for at least 60 min following

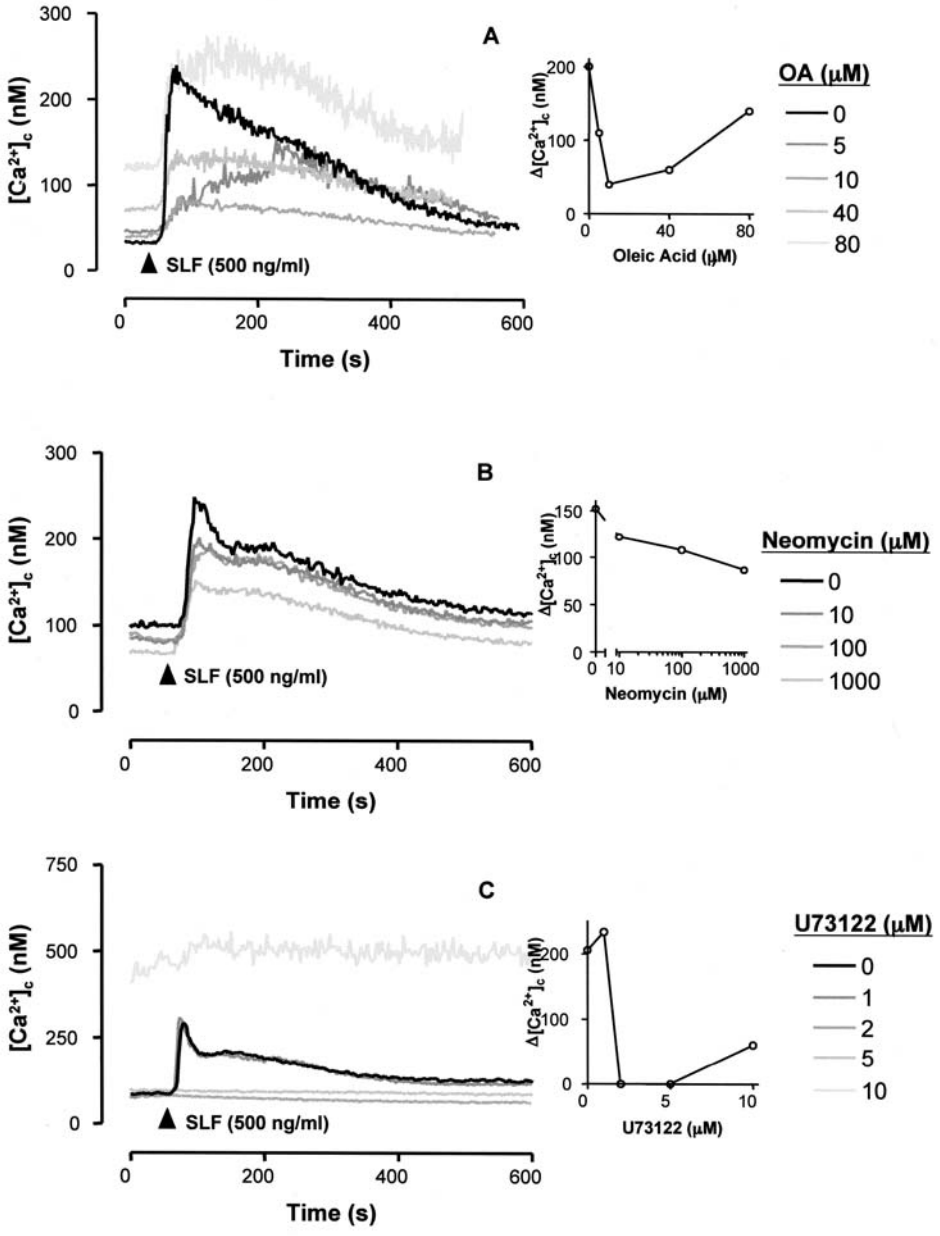

Figure 2. PLC antagonists inhibit Ca^{2+} transients stimulated by sSLF. (*A*, *C*) BMMCs were incubated in the absence or presence of oleic acid (OA; 5–80 μM) or U73122 (1–10 μM) for 2 h prior to loading with indo-1 at 37 °C in Tyrode's buffer. Cells were then loaded with Indo-1 AM for 30 min (in the presence of the inhibitors), washed twice (10 min each) and then stimulated with SLF (500 ng/ml) while being analyzed by flow cytometry. (*B*) Indo-1-loaded BMMCs were exposed to neomycin (0–1000 μM) for 30 min prior to stimulation with SLF (500 ng/ml) and analysis with flow cytometry. Separate calibrations were performed for each loading method. (*A–C*) Insets: decrease in SLF-induced maximal Ca^{2+} change due to PLC antagonists.

initiation of the co-incubation. This observation therefore suggests that mSLF can stimulate recruitment of PI3-kinase to c-Kit.

Impaired PKB/Akt phosphorylation by mSLF but not sSLF

To further investigate activation of the PI3-kinase pathway, we determined the phosphorylation status of PKB/Akt, a kinase that is phosphorylated and activated by PI3-kinase products [42]. To distinguish between PKB/Akt phosphorylation events in mast cells and X9/D3 cells, we pre-incubated the X9/D3 cells with the PI3-kinase inhibitor wortmannin. As shown in figure 6 A, pre-incubation of X9/D3 cells with wortmannin (100 nM) did not affect the ability of sSLF to stimulate PKB/Akt phosphorylation in co-cultures. Mast cells were then either exposed to sSLF or co-incubated with wortmannin-pre-treated X9/D3 cells for varying periods. Lysates were then

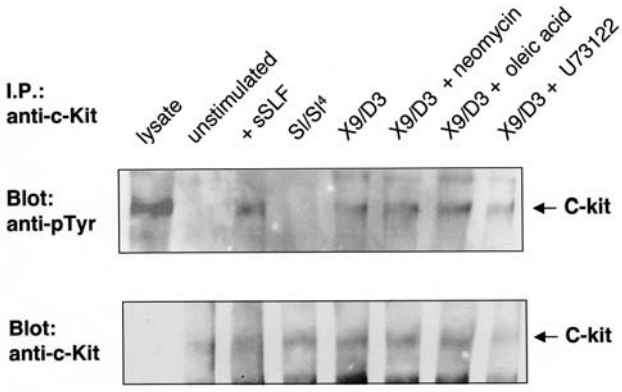

Figure 3. c-Kit autophosphorylation stimulated by mSLF is unaffected by PLC antagonists. BMMCs were co-incubated with either mSLF-negative Sl/Sl^4 as a negative control or mSLF-positive X9/D3 cells for 30 min with 500 µM neomycin sulfate, 5 µM oleic acid or 5 µM U73122. Lysates were immunoprecipitated with anti-c-Kit antibodies and analyzed by Western blotting with anti-phosphotyrosine. The blots were stripped and reprobed with the anti-c-Kit antibodies. The lanes marked 'lysate' refer to whole-cell lysates obtained from cells stimulated with sSLF.

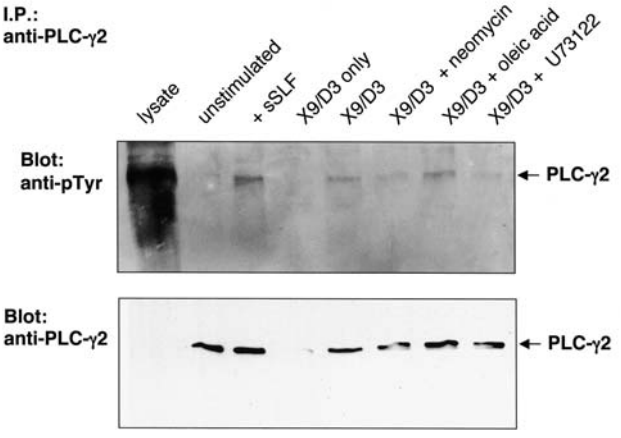

Figure 4. Effect of PLC antagonists on PLC-γ2 phosphorylation. BMMCs were co-incubated with mSLF-positive X9/D3 cells for 30 min with 500 µM neomycin sulfate, 5 µM oleic acid or 5 µM U73122. Lysates were immunoprecipitated with anti-PLC-γ2 antibodies and analyzed by Western blotting with anti-phosphotyrosine. The blots were stripped and reprobed with the anti-PLC-γ2 antibodies. The lanes marked 'lysate' refer to whole cell lysates obtained from cells stimulated with sSLF.

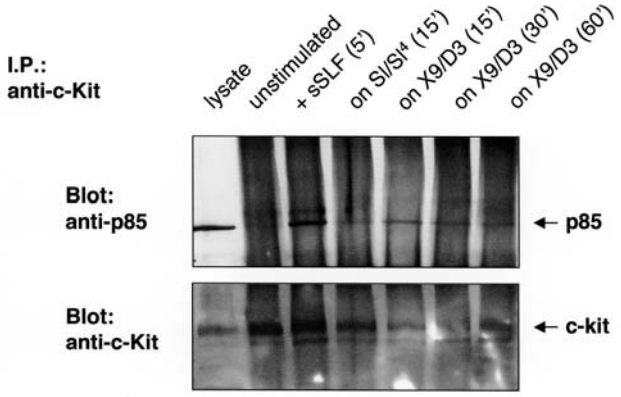

Figure 5. p85 co-immunoprecipitates with c-Kit following stimulation with mSLF. BMMCs were co-incubated with either mSLF-negative Sl/Sl^4 or mSLF-positive X9/D3 cells for the indicated times. Lysates were immunoprecipitated with anti-c-Kit antibodies and analyzed by Western blotting with p85-specific anti-sera. The blots were stripped and reprobed with the anti-c-Kit antibodies.

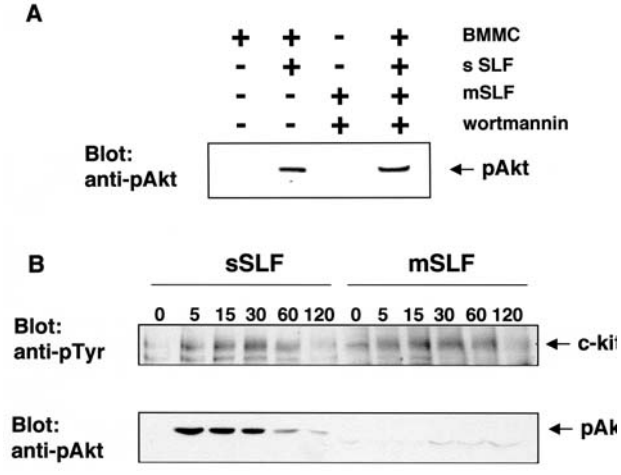

Figure 6. PKB/Akt phosphorylation stimulated by sSLF but not mSLF. (*A*) X9/D3s were pre-treated with wortmannin, washed, co-incubated with BMMCs and sSLF was added. Wortmannin pre-treatment of fibroblasts did not affect PKB/Akt phosphorylation in BMMCs. (*B*) BMMCs were stimulated with either sSLF or mSLF, collected, lysed and analyzed by Western blotting with anti-phosphotyrosine or anti-phospho-PKB/Akt. Note that the faint band observable in the mSLF-stimulated phosphoAkt lanes is also present in the unstimulated sample and originates from the X9/D3 cells.

analyzed by Western blotting with phosphotyrosine-specific antibodies or anti-sera specific for phosphorylated PKB/Akt (Ser473). The amount of sSLF used was at approximately the mid-point of its dose response curve and chosen to match in intensity of signal with mSLF stimulation. As shown in figure 6B, both sSLF and mSLF stimulated c-Kit tyrosine phosphorylation. Although the kinetics of activation were somewhat different, both signals were equally diminished in intensity by 120 min. We observed that while sSLF was very efficient in inducing PKB/Akt phosphorylation, mSLF was strongly impaired in its ability to generate phosphorylated PKB/Akt. Taken together, these results suggest that although mSLF is able to stimulate PI3-kinase recruitment to c-Kit, downstream phosphorylation of PKB/Akt is blocked.

Discussion

In this study, we investigated the role of PLC-γ activation in mast cell stimulation by SLF. We observed that PLC antagonists inhibited mast cells supported by mSLF but not

sSLF. Furthermore, we showed that although PI3-kinase was fully recruited to c-Kit stimulated by mSLF, downstream phosphorylation of PKB/Akt was impaired compared to that by sSLF. Taken together, these results demonstrate the importance of PLC-γ activation for mast cells stimulated by mSLF.

Three different PLC antagonists were able to inhibit mast cell stimulation by mSLF. U73122 is a small-molecule inhibitor with specificity for PLC [36, 37, 43]. Neomycin sulfate is thought to act by sequestering PLC substrates in the plasma membrane through ionic interactions [44]. Inhibition of PLC activity by oleic acid has been investigated in other cell types. Gamberucci and co-workers [45] have proposed that the effects of the fatty acid appear to be dependent on the ratio of fatty acid to cells rather than the absolute fatty acid concentration. Richieri and Kleinfeld [38] studied the inhibitory effects of oleic acid in cytotoxic T cell signalin. After 2 min, they observed an overall decrease in total inositol phosphate metabolites after stimulating T cells with concanavalin A in the presence of oleic acid. However, the levels of total inositol phosphate metabolites rises rose 15 min and the authors suggested that the inhibitory effect of free fatty acids is independent of phosphatidylinositol turnover. Nevertheless, the observation that although all three antagonists operate via different mechanisms to inhibit Ca^{2+} signaling, they all inhibit mSLF survival signals, but not sSLF survival signals, strongly supports the conclusion that mast cells stimulated by mSLF are dependent on PLC-γ activation.

Previously, we showed that with sSLF, PI3-kinase or PLC-γ activation were functionally 'redundant' in that activation of either pathway was sufficient to provide a mitogenic stimulus [30]. However, with mSLF, PI3-kinase activation by Kit appears dispensible, whereas PLC-γ is essential. These observations therefore raised the question as to the extent of PI3-kinase activation by mSLF. In this study, we observed that the p85 regulatory subunit of PI3-kinase was efficiently recruited to c-Kit following activation by mSLF. However, PKB/Akt, a downstream effector of PI3-kinase, was not phosphorylated following mSLF stimulation. This impaired phosphorylation of PKB/Akt potentially explains the requirement for PLC-γ activation following stimulation by mSLF. Our results, however, do not explain why PKB/Akt fails to get phosphorylated. mSLF may stimulate less receptor internalization than sSLF resulting in less association with downstream signaling elements. Internalization has been identified as an important component of signaling by other receptors, paticularly the insulin receptor [46, 47] and the nerve growth factor receptor [48]. Of interest this context is that we previously identified important roles for PI3-kinase and Ca^{2+} influx in mediating internalization of c-Kit stimulated by sSLF [13]. The role of receptor trafficking in regulating c-Kit function remains to be fully investigated.

Previously, Blume-Jensen et al. [49] showed that mice expressing c-Kit receptors mutated in the PI3-kinase recruitment site maintained normal hematopoiesis. In addition, Fukao et al. [50] showed that mice deficient in the p85α regulatory subunit of PI3-kinase had undetectable levels of gastrointestinal mast cells and severely reduced levels of peritoneal mast cells, yet maintained dermal mast cells, which are supported by mSLF, at levels only slightly below wild type [50]. These observations are consistent with our results indicating that PI3-kinase plays a lesser role in stimulation of c-Kit-expressing cells by mSLF. In conclusion, our studies provide new insight into the roles of PLC-γ and PI3-kinase in mSLF-dependent cells and identify PLC-γ as a potential target for topical anti-inflammatory or anti-allergic treatments.

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