Lysophosphatidylserine Enhances Exogenous Type II Phospholipase A₂-Induced Activation of Rat Serosal Mast Cells

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We have previously shown that exogenous type II phospholipase A₂ (PLA₂) alone elicits degranulation of mast cells, including rat serosal mast cells (SMC) and mouse bone marrow-derived mast cells (BMMC). Here we report that lysophosphatidylserine (lysoPS), a co-factor for activation of rodent SMC in response to some tyrosine kinase-coupled agonists, enhanced type II PLA₂-elicted histamine release from rat SMC. In contrast, mouse BMMC was insensitive to lysoPS. Our findings demonstrate a novel route for activation of SMC in that type II PLA₂ can act as a direct activator of SMC with enhancement by lysoPS, which is generated from membrane phosphatidylserine possibly by the action of the same enzyme.

Key words: type II phospholipase A₂; lysophosphatidylserine; mast cell; degranulation

The involvement of type II secretory phospholipase A₂ (PLA₂) in inflammatory reaction has been documented in recent years.¹ This enzyme is often detected at various inflamed sites,² and is released from a variety of cells that are stimulated with proinflammatory stimuli in vitro,³ and exacerbates inflammation when injected in vivo.⁴ Type II PLA₂ increases eicosanoid generation by various cell types under appropriate conditions.⁵ Furthermore, type II PLA₂ is likely involved in regulation of the degranulation of mast cells,⁶,⁷ which play a central role in allergic states and are implicated in a wide variety of chronic inflammatory processes.⁷

Serine phospholipids, among which lysophosphatidylserine (lysoPS) is the most active, are potent stimulators of rodent serosal mast cells (SMC), a connective tissue mast cell (CTMC) surrogate.⁸ Although serine phospholipids alone have no appreciable effect, they greatly enhance degranulation and prostaglandin D₂ generation by rat SMC sensitized with IgE and stimulated with antigen.⁹ They also augment exocytosis of rodent SMC stimulated with several cytokines coupled to tyrosine kinases, such as nerve growth factor,¹⁰ c-kit ligand,¹¹ and interleukin-3.¹² Mouse SMC can be activated by lysoPS alone in vitro and in vivo,¹³ revealing a species specificity. Whereas mouse bone marrow-derived mast cells (BMMC), a progenitor population of in vitro-derived mature mast cells, are insensitive to lysoPS, coculture of BMMC with 3T3 fibroblasts results in differentiation toward more CTMC-like mast cells, thereby leading to an acquired lysoPS sensitivity.¹⁴ On the other hand, SMC activation by calcium ionophore or G-protein-coupled polycationic agonists (compound 48/80, substance P, and bradykinin, etc.) is not potentiated by lysoPS,⁹ revealing ligand specificity. Because the effect of lysoPS is stereo-specific in that lysoPS with (d)-serine has much less activity than its natural form with (l)-serine and no other related phospholipids elicit similar effect to lysoPS, it is postulated that there may be a lysoPS-specific recognition site in CTMC.⁸

Here we report the effect of lysoPS on the activation of mast cells exposed to type II PLA₂. LysoPS, which is postulated to be generated by type II PLA₂ at inflamed sites, potentiates type II PLA₂-mediated SMC activation.

MATERIALS AND METHODS

Preparation of Mast Cells SMC were isolated from Wistar rats (Nippon Bio-supply Center) as described previously.⁸ Briefly, rats (male, 300—400 g) were anesthetized with ether and then exsanguinated. Hank’s balanced salt solution (Nissui) containing 0.1% (w/v) bovine serum albumin (BSA) was injected into the peritoneal cavity. Peritoneal fluid was harvested after abdominal massaging and the cell suspension so obtained was layerd over 38% BSA in Hank’s balanced salt solution and then centrifugated. The pellet was washed and suspended at 1 × 10⁶ cells/ml in 10 mM Tris—HCl (pH 7.4) supplemented with 150 mM NaCl, 3.7 mM KCl, 1 mM CaCl₂, 0.1% (w/v) glucose, and 0.5% (w/v) gelatin (Sigma) (TG buffer). CTMC of >98% purity and >95% viability were obtained and used in the present study. A mouse IL-3-dependent BMMC line, MC-MKM, was maintained in 50% enriched medium (Dulbecco’s modified Eagle’s medium (Nissui) containing 10% fetal calf serum, 2 mM glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 50 µg/ml gentamycin and 50 µM 2-mercaptoethanol) and 50% WEHI-3B (Japanese Cancer Research Resources Bank)-conditioned medium (WEHI-CM) as a source of IL-3, as described previously.¹⁵

Activation of Mast Cells Type II PLA₂ was purified to near homogeneity from rat platelets by immuno-affinity chromatography as described previously.¹⁶ LysoPS (Avanti) was dissolved in chroloform/methanol=1/1 (v/v) at 1 mM. A 10-µl portion of lysoPS solution was transferred to a glass test-tube and the solvent was evaporated under N₂. The lipid film was suspended in 100 µl distilled water, vortexed for 15 s and sonicated for 3 min in a bath-type sonicator. Then the lysoPS suspension (adjusted to 10⁻⁴ M) was diluted to 10⁻⁶—10⁻⁸ M with TG buffer. Mast cells (1 × 10⁶ cells/ml) were challenged with type II PLA₂ and/or lysoPS in TG buffer. After incubation at 37 °C for 10 min, the reaction was terminated by adding cold EDTA (final 5 mM) to the cell suspension, followed by immediate centrifugation at 750 × g for 3 min at 4 °C.

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The amount of histamine released into the supernatant was determined by radioenzymatic assay using $[^3H]$methyl-S-adenosyl-L-methionine (Du Pont NEN) and a crude preparation of rat kidney histamine methyltransferase.$^{17}$ The percentage release of histamine was calculated by dividing the amount in each supernatant by that in sonicated cells (Branson Sonifier, 20 pulses, setting 4, 50% pulse cycle).

RESULTS

Rat SMC were treated with various concentrations of type II PLA$_2$ in combination with $10^{-6}$ M lysoPS, and the histamine released into the supernatant was measured (Fig. 1). Simultaneous treatment of the cells with type II PLA$_2$ and lysoPS led to the release of more histamine than from those treated with PLA$_2$ alone, although the response varied among cells from each individual rat. LysoPS alone did not elicit appreciable mediator release (data not shown).$^{8,9}$ In a particular case, about a 17% release of histamine was seen with 10 µg/ml PLA$_2$ + $10^{-6}$ M lysoPS, whereas there was no substantial release at the same dose of PLA$_2$ in the absence of lysoPS (Fig. 1, rat C). The enhancement of type II PLA$_2$-induced histamine release by lysoPS was examined as a function of lysoPS concentration (Fig. 2). Histamine release elicited by 10 µg/ml PLA$_2$ was enhanced by lysoPS in a dose-related fashion from $10^{-8}$ to $10^{-6}$ M: concentrations almost comparable with that required for the induction of mast cell activation by IgE and antigen.$^{8,9}$ We could not assess the effect of higher concentrations of lysoPS because it tended to cause cell lysis at around $10^{-5}$ M.

Type II PLA$_2$ alone elicited histamine release from mouse BMMC ($15 \pm 2\%$, $n = 2$) at 40 µg/ml. Addition of $10^{-6}$ M lysoPS did not promote type II PLA$_2$-mediated histamine release from BMMC ($13 \pm 2\%$, $n = 2$). LysoPS did not promote histamine release when BMMC were treated with suboptimal concentrations of type II PLA$_2$ (data not shown).

DISCUSSION

Mast cells play a central role in allergic inflammation by releasing granule-associated amines and newly generated lipid mediators upon IgE-dependent activation. Although activation of mast cells through an IgE-independent pathway has been also implicated in acute and chronic inflammatory diseases and in the maintenance of host homeostasis, factors that affect activation of mast cells in non-allergic microenvironments are not fully understood. Because of the significant correlation of type II PLA$_2$ with inflammation,$^{11}$ it seems important to determine the effect of this putative ‘pro-inflammatory’
enzyme on mediator release from mast cells. We previously reported that type II PLA₂ promoted generation of prostaglandin D₂ by rat SMC that had been primed with suboptimal concentrations of IgE and antigen.⁵⁰ We also found that type II PLA₂ alone directly induced degranulation of rat SMC as well as mouse BMMC to release histamine at concentrations comparable with those detected at sites of severe systemic inflammation.⁶⁰ In the present study, we have shown that lysoPS, a lipid mediator, potentiates type II PLA₂-mediated degranulation of rat SMC. That rat SMC release histamine when exposed to pathophysiological concentrations of type II PLA₂ in the presence of lysoPS indicates a novel IgE-independent route for mediator release by SMC.

Serine phospholipids, particularly lysoPS, are potent enhancers but not direct stimulators of rat SMC to release their granule contents induced by several stimuli, such as Fe₉RI crosslinking,⁵⁹ nerve growth factor,¹⁰ and c-KIT ligand,¹¹ known as activators of cellular tyrosine kinases. SMC activation induced by calcium ionophore or by G-protein-coupled polymeric compounds is not affected by lysoPS.⁸⁹ We have now shown that type II PLA₂ can be classified into lysoPS-sensitive secretagogues; histamine release elicited by type II PLA₂ was markedly potentiated by lysoPS. Since type II PLA₂-mediated histamine release is suppressed by tyrosine kinase inhibitors,⁶¹ lysoPS might generally potentiate certain steps common to the tyrosine kinase-dependent transmembrane signaling pathway leading to SMC activation. LysoPS is likely to be generated through hydrolysis of PS by PLA₂.¹⁰ Because type II PLA₂ is often detected in abundance in environments surrounding SMC,¹¹,²² particularly at inflamed sites where type II PLA₂ has the ability to produce lysoPS from membrane debris of infiltrating leukocytes,¹² it might act on SMC not only as a secretagogue but also as a regulator of the generation of a co-factor, lysoPS. As opposed to the effect on SMC, lysoPS did not show an appreciable effect on type II PLA₂-mediated histamine release from mouse BMMC, a progenitor population of mature mast cells. This is consistent with previous observations that lysoPS promoted IgE-dependent mediator release from rodent SMC but not from BMMC and that BMMC, differentiated into CTMC-like phenotypes following coculture with 3T3 fibroblasts, acquired lysoPS sensitivity.¹⁶

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REFERENCES