

PI3K γ Differentially Regulates Fc ϵ RI-Mediated Degranulation and Migration of Mast Cells by and toward Antigen

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Key Words

Phosphatidylinositol 3-kinase • Mast cell • Migration • Degranulation

Abstract

Phosphatidylinositol 3-kinase (PI3K) has been recognized as an important downstream effector of high-affinity receptor for IgE (Fc ϵ RI) signaling in mast cells, but little is known about the isoform specificity of PI3Ks on the Fc ϵ RI-mediated migration toward the antigen (Ag). In the present study, we explored the role of PI3K γ on mast cell migration. The treatment of bone marrow-derived mast cells (BMMCs) with a PI3K γ inhibitor, AS605240, significantly repressed Fc ϵ RI-induced degranulation and migration. The culture supernatants of wild-type mast cells stimulated with IgE and Ag attracted Fc ϵ RI $\beta^{-/-}$ mast cells which did not express Fc ϵ RI on their cell surface, indicating that the migration appears to be dependent on an autocrine/paracrine secretion of soluble factors from the mast cells. Adenosine, which is produced by mast cells, showed a strong activity to attract mast cells. Pertussis toxin (PTX) significantly inhibited the migration toward both the supernatant and adenosine. The supernatant from mast cells pretreated with wortmannin (Wort) and stimulated with IgE and Ag still exhibited the activity as chemoattractant, while the BMMCs pretreated with Wort did

not migrate toward the supernatant. Although PTX significantly reduced the activation of AKT/PKB and migration, PTX had no effects on degranulation. These results suggest that PI3K γ activation through PTX-sensitive G-protein-coupled receptor as a secondary response of Fc ϵ RI cross-linking regulates Fc ϵ RI-mediated mast cell migration toward the Ag, while simultaneously activated PI3K γ through a PTX-insensitive pathway might have an effect on degranulation.

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Introduction

Mast cells have a pivotal role in initiating allergic inflammation by releasing various mediators and cytokines induced by IgE-antigen (Ag) cross-linking of the high-affinity receptor for IgE (Fc ϵ RI). Many of the signaling molecules are involved in the activation of mast cells induced by the Fc ϵ RI cross-linking [1–3]. The critical role of phosphatidylinositol 3-kinase (PI3K) for the mast cell activation induced by Fc ϵ RI cross-linking has been well documented. In class IA PI3Ks, PI3K δ has been recognized as a downstream molecule of Fc ϵ RI signal-

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ing, with a crucial role in mast cell activation [4–7]. Class IB PI3K (PI3K γ) has been reported to amplify mast-cell function, while PI3K δ is considered to be not directly activated by Fc ϵ RI, it is instead triggered through secondary autocrine and paracrine loops [6, 8]. Previous studies reported reduction of Fc ϵ RI-induced degranulation upon genetic inactivation of p110 δ or p110 γ [4, 5, 9], suggesting that the simultaneous activation of PI3K δ and PI3K γ which are differentially activated after Fc ϵ RI cross-linking might regulate mast cell activation.

Mast cell accumulation at the site of allergic inflammation is thought to be causally related to the persistence of allergic inflammation, but the exact mechanism of mast cell migration to the inflamed site is still unclear. Several reports showed that in vitro, bone marrow-derived murine mast cells (BMMCs) sensitized with Ag-specific IgE migrated toward the Ag. A recent study reported that Fc ϵ RI-mediated mast cell migration involves autocrine/paracrine secretion of soluble factors produced by the activated mast cells. It was proposed that the initial phase of migration is triggered through the release of such factors from the mast cells at the point of contact with Ags, the following phase is directing the migration of mast cells through G-protein-coupled receptors (GPCRs) toward the site with higher concentrations of the soluble factors [8, 10, 11]. It has been proven that pharmacological inactivation of PI3Ks blocks Fc ϵ RI-mediated mast cell migration [8, 12], but it is still unknown at which step of the mast cell migration the PI3Ks were involved, and which subtype of PI3K was involved. In the present study, we explored the role of PI3K γ on the Fc ϵ RI-mediated mast cell migration.

Materials and Methods

Reagents

AS605240 inhibitor and sphingosine-1-phosphate (S1P) were purchased from Cayman Chemical (Ann Arbor, Mich., USA). Pertussis toxin (PTX) and adenosine were purchased from Sigma (St. Louis, Mo., USA). Anti-phospho-AKT/PKB or anti-AKT/PKB antibody and wortmannin (Wort) were purchased from Cell Signaling Technology (Beverly, Mass., USA). Leukotriene B₄ (LTB₄), macrophage inflammatory protein-1 α (MIP-1 α) and monocyte chemotactic protein 1 (MCP-1) were purchased from Calbiochem (San Diego, Calif., USA).

Cell Culture

C57BL/6J mice and Fc ϵ RI β -chain^{-/-} mice [13] were bred in the animal facility at Nihon University School of Medicine under specific pathogen-free conditions. All experiments were performed according to the guidelines of Nihon University. BMMCs were prepared from the femur of 4- to 8-week-old mice. Cells were

cultured in the RPMI 1640 (Sigma) supplemented with 10% FBS (Sigma), 4 ng/ml of murine recombinant IL-3, and 10% pokeweed mitogen-stimulated spleen-conditioned medium as a source of mast cell growth factors. BMMCs were used for experiments after 4–8 weeks of culture.

Migration Assay

BMMCs migration for 8 h was assayed using 24-well Transwell chambers (Corning Inc., Corning, N.Y., USA) separated by 5- μ m pore size polycarbonate filters. Under standard conditions, upper wells contained 1×10^6 cells/well sensitized with 1 μ g/ml IgE in 0.2 ml medium (RPMI 1640) supplemented with 1% bovine serum albumin (BSA), and lower wells contained 0.6 ml medium/well, or 10 ng/ml trinitrophenol (TNP)-BSA, or culture supernatant of wild-type (WT) BMMCs (1×10^6 /ml) stimulated with 1 μ g/ml IgE and 10 ng/ml Ag for 8 h.

β -Hexosaminidase Release Assay

Degranulation of mast cells was determined by β -hexosaminidase release as previously described [14]. BMMCs (2×10^5) were incubated with anti-TNP IgE (1 μ g/ml) in culture medium for >2 h. The sensitized cells were washed and stimulated with 1 or 10 ng/ml TNP-BSA at 37°C for 30 min. The cells were lysed with 0.1% Triton X-100. The supernatants and the total cell lysates were incubated with 1.3 mg/ml of *p*-nitrophenyl-*N*-acetyl- β -D-galactopyranoside (Sigma) in 0.1 M sodium citrate buffer (pH 4.5) at 37°C for 40 min. The reaction was terminated by the addition of 0.2 M glycine buffer (pH 11.0). The release of the product 4-*p*-nitrophenol was evaluated by optical absorbance at 415 nm.

Western Blotting

The stimulated cells were washed twice with ice-cold PBS and lysed on ice in Tris buffer saline containing 1% Nonidet P-40, 60 mM octyl- β -glucoside, 2 mM phenylmethylsulfonyl fluoride, 10 μ g/ml aprotinin, 2 μ g/ml leupeptin and pepstatin A, 50 mM NaF and 1 mM sodium orthovanadate, for 30 min. The cell lysates were centrifuged for 15 min at 14,000 g. The supernatants were then boiled in sample buffer, separated on 10% SDS-PAGE, and transferred onto Immobilon-P membrane (Millipore, Bedford, Mass., USA). The membrane was incubated with primary antibodies and the appropriate secondary horseradish peroxidase-conjugated antibodies. Signals were detected by enhanced chemiluminescence (GE Healthcare, Little Chalfont, Bucks., UK).

Statistical Analysis

The statistical significance was evaluated by using an analysis of variance (ANOVA). *p* values < 0.05 were considered significant. When statistical significance was reached, post-hoc tests (Fisher's protected least significant difference, and Scheffé's *F* test) were performed.

Results and Discussion

Pharmacological inhibition of PI3K γ activity by specific inhibitors, IC87114 and AS252424 was previously reported to repress Fc ϵ RI-induced degranulation [15, 16]. We tested a newly developed PI3K γ -specific inhibitor,

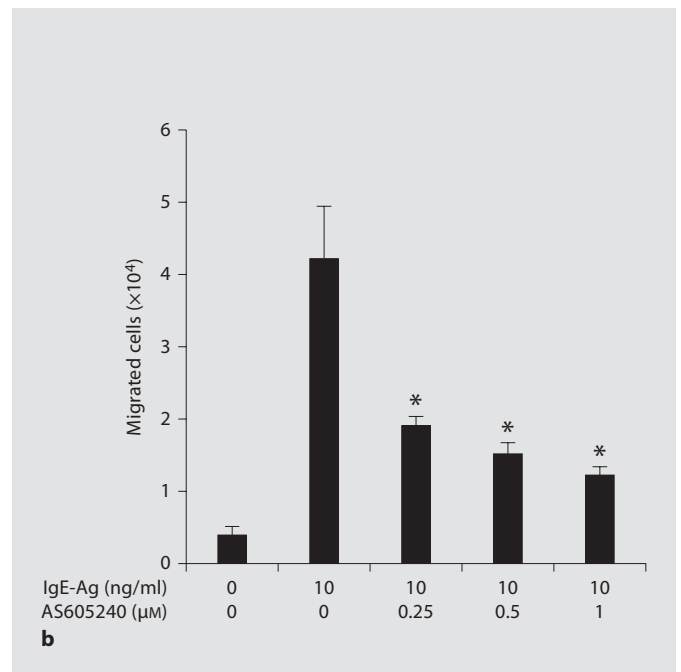
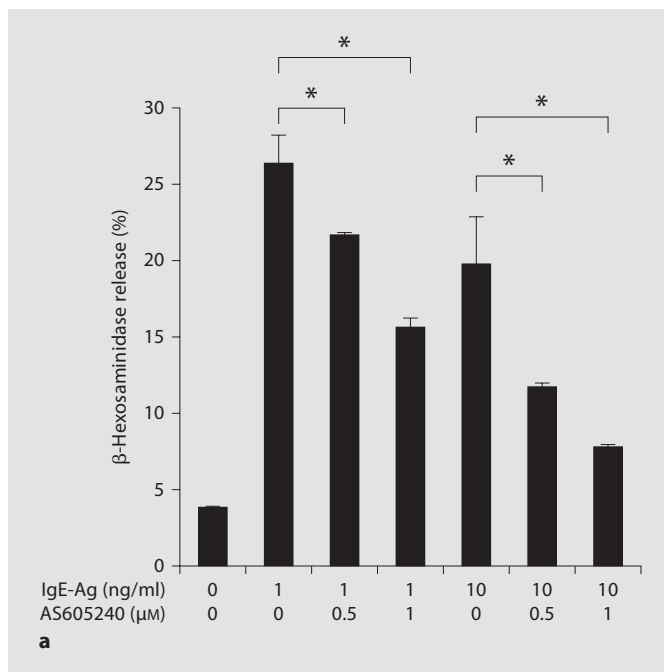


Fig. 1. Effects of PI3K γ inhibitor on degranulation and migration of mast cells. **a** BMMCs were sensitized with anti-TNP IgE and preincubated with or without AS605240. The cells were incubated with TNP-BSA for 30 min. Degranulation was monitored by β -hexosaminidase activity released into culture supernatants. Data are means $\pm$ SD of three samples. **b** Anti-TNP IgE-sensitized

BMMCs were seeded in the upper wells of Transwell, and the medium containing TNP-BSA were put into the lower wells. The migrated cells in the lower wells were counted after 8 h. Data are expressed as a number of migrated cells. Data are means $\pm$ SD of three samples. * $p < 0.05$.

AS605240, to confirm the role of PI3K γ on Fc ϵ RI-mediated mast cell activation. Consistent with the previous report, the treatment of BMMCs with AS605240 was found to significantly inhibit Fc ϵ RI-induced degranulation in a dose-dependent manner at both high- and low-dose stimulation (fig. 1a). We next tested the effect of AS605240 on Fc ϵ RI-mediated mast cell migration toward the Ag, and found that the treatment of BMMCs with AS605240 inhibited the migration in a dose-dependent manner (fig. 1b). These results indicate that PI3K γ activation is crucial for producing a substantial response to Ag in mast cells.

Fc ϵ RI-mediated migration has been proven to be mediated by an autocrine/paracrine secretion of soluble factors [8]. We examined which stage of Fc ϵ RI-induced production of chemoattractants or chemotaxis is dependent on PI3K γ . We put the Fc ϵ RI $\beta^{-/-}$ mast cells which did not express Fc ϵ RI on their cell surface in the upper wells and the supernatants from WT mast cells activated by IgE and Ag for 1 h in the lower wells, and measured the migration of mast cells from the upper well to the lower well. The supernatants attracted Fc ϵ RI $\beta^{-/-}$ mast cells (fig. 2a),

indicating that the supernatant contained chemoattractants for mast cells. The number of mast cells migrated toward the supernatant appeared to be at a comparable level to that toward Ag as previously reported [8]. The supernatant from mast cells pretreated with 100 nM Wort also had a chemotactic activity, indicating that PI3Ks were not involved in the early production and release of chemoattractants induced by Fc ϵ RI cross-linking (fig. 2b), while the same pretreatment of mast cells in the upper wells significantly inhibited their migration (fig. 2c).

GPCR agonists including adenosine, LTB₄, and several chemokines have been shown to induce the mast cell migration [8, 9, 11, 17]. Fc ϵ RI aggregation has been shown to lead to a rapid release of these soluble factors [6, 8]. In figure 3a, we demonstrated that preincubation of mast cells with PTX, which uncouples GPCR from its Gi/o and in turn reduces the affinity of GPCR toward its agonist, partially inhibits the migration to the culture supernatant of stimulated BMMCs with IgE and Ag, suggesting that the migration is almost totally dependent on PTX-sensitive GPCR (fig. 3a).

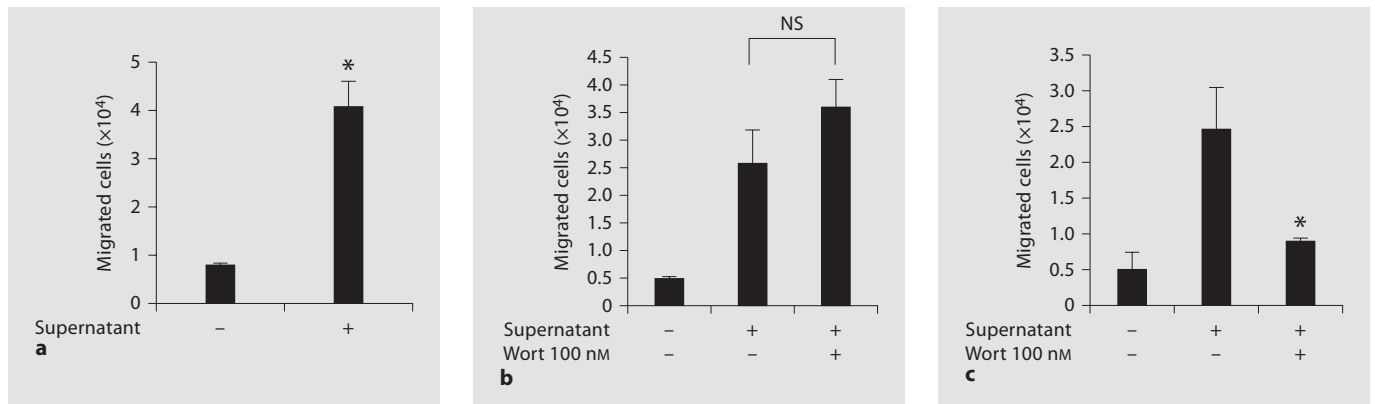


Fig. 2. Effects of PI3K inhibitor on migration of mast cells. **a** Fc ϵ RI $\beta^{-/-}$ BMMCs were seeded in the upper wells of Transwell, and the supernatants from WT BMMCs incubated with IgE and Ag were put in the lower wells. **b** Fc ϵ RI $\beta^{-/-}$ BMMCs were seeded in the upper wells, and the supernatants from BMMCs, which were pretreated with or without Wort for 1 h and incubated with IgE and Ag for 1 h, were put in the lower wells. **c** Fc ϵ RI $\beta^{-/-}$ BMMCs

pretreated with Wort for 1 h were seeded in the upper wells, and the supernatants from WT BMMCs incubated with IgE and Ag for 1 h were put into the lower wells. In each assay, the migrated cells in the lower wells were counted after 8 h. Data are expressed as a number of migrated cells. Data are means $\pm$ SD of three samples. * $p < 0.05$. NS = Not significant.

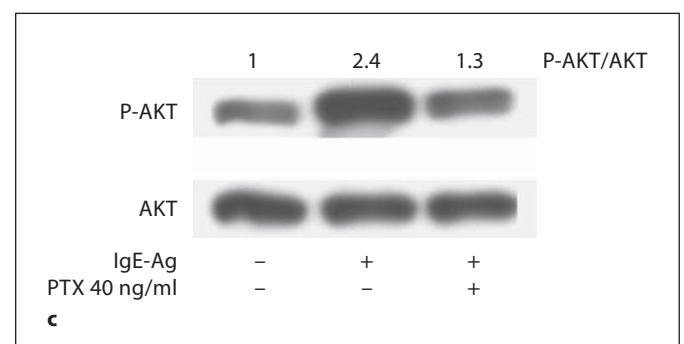
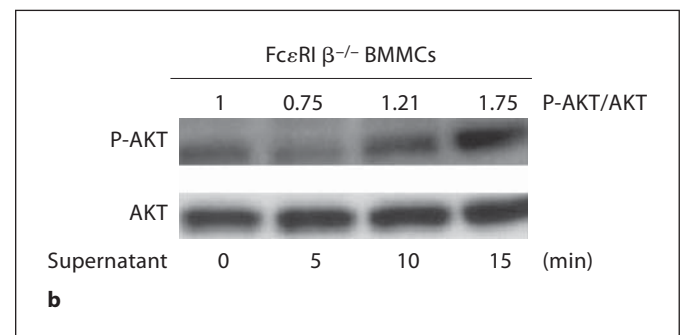
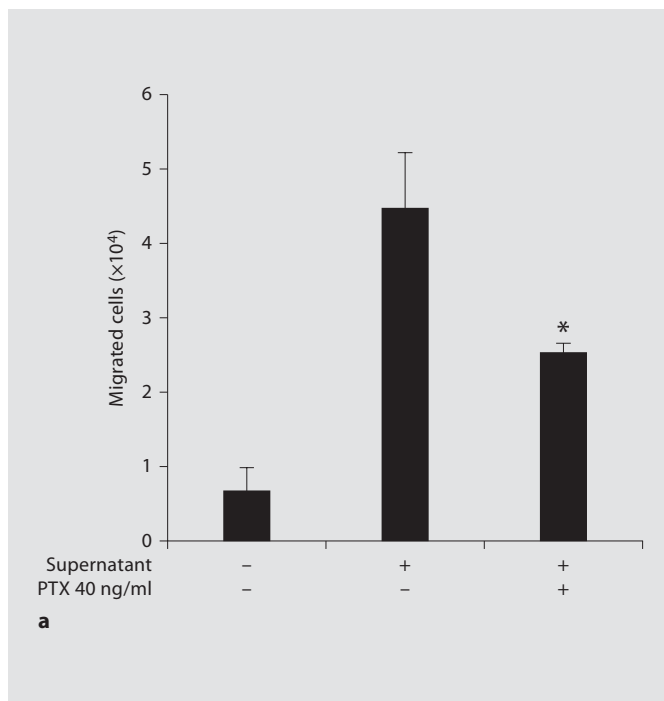


Fig. 3. Effects of PTX on migration and AKT/PKB activation of mast cells. **a** Fc ϵ RI $\beta^{-/-}$ BMMCs pretreated with PTX were seeded in the upper wells, and the supernatants from WT BMMCs incubated with IgE and Ag for 1 h were put into the lower wells. The migrated cells in the lower wells were counted after 8 h. Data are expressed as a number of migrated cells. Data are means $\pm$ SD of three samples. * $p < 0.05$. **b** BMMCs were stimulated with the su-

pernatant for 5, 10, and 15 min. **c** PTX-treated BMMCs were stimulated with IgE and Ag for 3 min. Phosphorylated AKT/PKB and total AKT/PKB were detected by Western blotting. The experiment shown is a representative of two independent experiments. The band intensity was measured by NIH Image J software. The ratio of the intensity of phosphorylated AKT/PKB to total AKT/PKB was calculated.

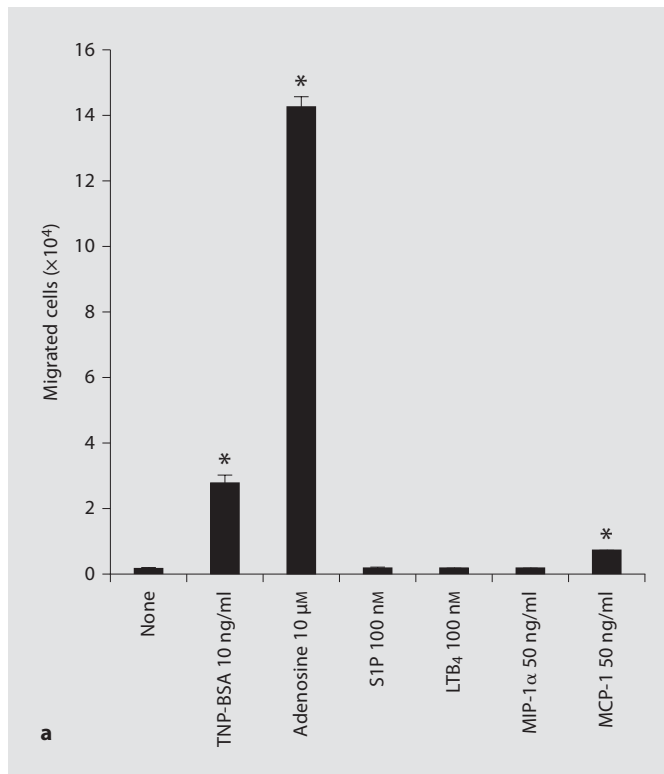
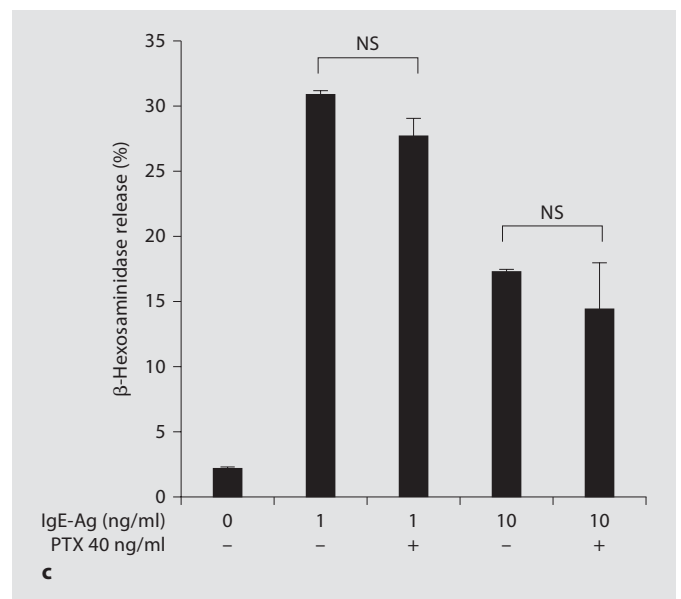
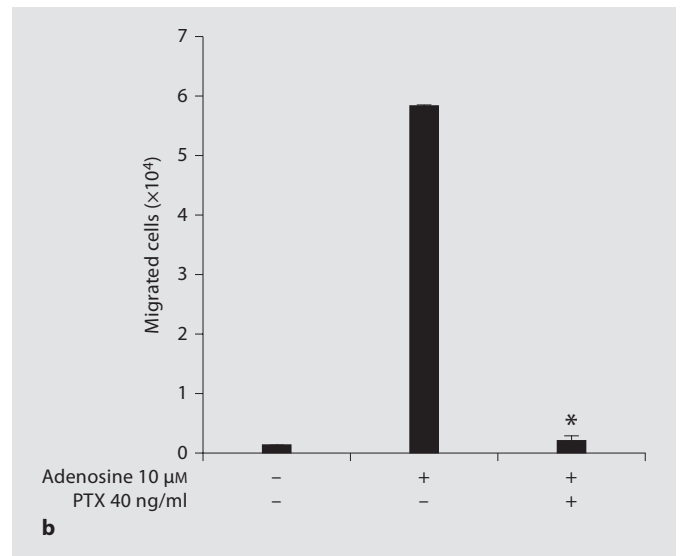


Fig. 4. Involvement of PTX-sensitive GPCRs in FcεRI-mediated migration, but not in degranulation. **a** BMMCs were seeded in the upper wells of Transwell and adenosine, S1P, LTB₄, MIP-1α or MCP-1 were put in the lower wells. The migrated cells in the lower wells were counted after 8 h. **b** PTX-pretreated BMMCs were seeded in the upper wells and the medium containing adenosine was put in the lower wells. The migrated cells in the lower wells were counted. Data are expressed as a number of migrated cells. Data are means ± SD of three samples. * $p < 0.05$. **c** BMMCs were sensitized with anti-TNP IgE and were preincubated with or without PTX, and then stimulated with TNP-BSA. Degranulation was monitored by β-hexosaminidase activity released into the culture supernatants. NS = Not significant.



Transient class IA and subsequent class IB activation of PI3K by a receptor tyrosine kinase and by a GPCR, respectively, has also been reported in FcεRI-stimulated mast cells [4–7, 18]. To test whether soluble factors in the supernatant from FcεRI-stimulated mast cells can lead to PI3K activation, we examined the phosphorylation of AKT/PKB as a cellular marker of PI3K activation. We demonstrated that BMMCs incubated with the supernatant had AKT/PKB phosphorylation (fig. 3b), presumably through the activation of GPCR/PI3Kγ pathway, as the pretreatment of the cells with PTX blocked the phosphorylation of AKT/PKB (fig. 3c).

Genetic inactivation of p110δ or p110γ has been reported to inhibit the FcεRI-mediated degranulation of mast cells [4, 5, 9]. PI3Kδ has been shown to be directly activated as a downstream molecule of FcεRI signaling and be essential for the degranulation of mast cells. PI3Kγ activation mediated by autocrine signals through GPCRs has been shown to be required for amplifying degranulation of mast cells [9]. IgE- and Ag-mediated mast cell activation induces the release of adenosine, which activates PI3Kγ, through the A(3) adenosine receptor expressed on the cell surface [6, 19, 20], and leads to a transient increase of PI-3,4,5-P₃ levels that can initiate a sustained calcium

influx and degranulation [6]. Adenosine has been recognized as an important chemoattractant for mast cells [8]. In the present study, we demonstrated that adenosine has a strong chemotactic activity for BMMC, and pretreatment with PTX inhibited the adenosine-induced migration of BMMC (fig. 4a, b). These results suggest that FcεRI-mediated mast cell migration would be caused largely by PI3Kγ activation through PTX-sensitive GPCR receptors. In the present study, PTX showed the partial inhibitory effect on the migration of mast cells, although we could not exclude the possibility of PTX-insensitive receptors involved in the mast cell migration, our results demonstrated that FcεRI-mediated mast cell migration would be largely caused by PI3Kγ activation through PTX-sensitive GPCR. Furthermore, we showed that adenosine had a strong activity to attract mast cells compared with other GPCR ligands in the range of physiological concentrations, and a previous report demonstrated that adenosine deaminase, and an adenosine A3 receptor (ADA3R) inhibitor, MRS 1523, inhibited Ag-induced migration [8]. Therefore, we considered that adenosine would be one of the important factors for mast cell migration. Previous reports showed that activation of ADA3R coupled to PTX-sensitive G proteins by exogenous adenosine could potentiate Ag-induced degranulation of mast cells [21–24]. Therefore, we assumed that ADAR-mediated PI3Kγ activation might concurrently enhance both migration and degranulation. We examined the inhibitory effect of PTX on FcεRI stimulation-induced degranulation of mast cells in our experimental model. Contrary to our expectation, we found that the pretreatment of mast cells with PTX showed no effects on the degranulation (fig. 4c), suggesting that the activation of

PTX-sensitive GPCRs such as ADAR activation is independent of the degranulation. In this study, we could not identify the mechanism by which the PI3Kγ potentiates the FcεRI-induced degranulation. Further study is required to determine this mechanism.

In conclusion, we demonstrated that the PI3K activation by PTX-sensitive GPCRs signaling had a pivotal role in the migration of mast cells, and did not have a significant role in the production of chemoattractants from mast cells. The activation of PI3Kγ, which might also be activated by FcεRI cross-linking through a different pathway from that of PTX-sensitive GPCR, augmented degranulation. We suggest that PI3Kγ might be differentially activated upon FcεRI cross-linking, and that the simultaneously and separately activated PI3Kγ signalings might regulate degranulation and migration of mast cells. Our results presented herein give an insight into the mechanism of mast cell migration in allergic inflammation. Because PI3Kγ is involved in both degranulation and migration by Ags, selective inhibition of PI3Kγ offers a possibility to treat allergy.

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Disclosure Statement

The authors declare that no financial or other conflict of interest exists in relation to the content of the article.

References

- Bradding P, Walls AF, Holgate ST: The role of the mast cell in the pathophysiology of asthma. *J Allergy Clin Immunol* 2006;117:1277–1284.
- Marshall JS: Mast-cell responses to pathogens. *Nat Rev Immunol* 2004;4:787–799.
- Rivera J, Gilfillan AM: Molecular regulation of mast cell activation. *J Allergy Clin Immunol* 2006;117:1214–1226.
- Ali K, Bilancio A, Thomas M, Pearce W, Gilfillan AM, Tkaczyk C, Kuehn N, Gray A, Giddings J, Peskett E, Fox R, Bruce I, Walker C, Sawyer C, Okkenhaug K, Finan P, Vanhaesebroeck B: Essential role for the p110δ phosphoinositide 3-kinase in the allergic response. *Nature* 2004;431:1007–1011.
- Ali K, Camps M, Pearce WP, Ji H, Ruckle T, Kuehn N, Pasquali C, Chabert C, Rommel C, Vanhaesebroeck B: Isoform-specific functions of phosphoinositide 3-kinases: p110δ but not p110γ promotes optimal allergic responses in vivo. *J Immunol* 2008;180:2538–2544.
- Gilfillan AM, Tkaczyk C: Integrated signaling pathways for mast-cell activation. *Nat Rev Immunol* 2006;6:218–230.
- Rommel C, Camps M, Ji H: PI3Kδ and PI3Kγ: partners in crime in inflammation in rheumatoid arthritis and beyond? *Nat Rev Immunol* 2007;7:191–201.
- Kitaura J, Kinoshita T, Matsumoto M, Chung S, Kawakami Y, Leitges M, Wu D, Lowell CA, Kawakami T: IgE- and IgE+Ag-mediated mast cell migration in an autocrine/paracrine fashion. *Blood* 2005;105:3222–3229.
- Laffargue M, Calvez R, Finan P, Trifilieff A, Barbier M, Altruda F, Hirsch E, Wymann MP: Phosphoinositide 3-kinase γ is an essential amplifier of mast cell function. *Immunity* 2002;16:441–451.

- 10 Ishizuka T, Okajima F, Ishiwara M, Iizuka K, Ichimonji I, Kawata T, Tsukagoshi H, Dobashi K, Nakazawa T, Mori M: Sensitized mast cells migrate toward the antigen: a response regulated by p38 mitogen-activated protein kinase and Rho-associated coiled-coil-forming protein kinase. *J Immunol* 2001;167:2298–2304.
- 11 Taub D, Dastyh J, Inamura N, Upton J, Kelvin D, Metcalfe D, Oppenheim J: Bone marrow-derived murine mast cells migrate, but do not degranulate, in response to chemokines. *J Immunol* 1995;154:2393–2402.
- 12 Sawada J, Itakura A, Tanaka A, Furusaka T, Matsuda H: Nerve growth factor functions as a chemoattractant for mast cells through both mitogen-activated protein kinase and phosphatidylinositol 3-kinase signaling pathways. *Blood* 2000;95:2052–2058.
- 13 Hiraoka S, Furumoto Y, Koseki H, Takagaki Y, Taniguchi M, Okumura K, Ra C: Fc receptor β subunit is required for full activation of mast cells through Fc receptor engagement. *Int Immunol* 1999;11:199–207.
- 14 Furumoto Y, Hiraoka S, Kawamoto K, Masaki S, Kitamura T, Okumura K, Ra C: Polymorphisms in Fc ϵ RI β chain do not affect IgE-mediated mast cell activation. *Biochem Biophys Res Commun* 2000;273:765–771.
- 15 Bilancio A, Okkenhaug K, Camps M, Emery JL, Ruckle T, Rommel C, Vanhaesebroeck B: Key role of the p110 δ isoform of PI3K in B-cell antigen and IL-4 receptor signaling: comparative analysis of genetic and pharmacologic interference with p110 δ function in B cells. *Blood* 2006;107:642–650.
- 16 Pomel V, Klicic J, Covini D, Church DD, Shaw JP, Roulin K, Burgat-Charvillon F, Valognes D, Camps M, Chabert C, Gillieron C, Francon B, Perrin D, Leroy D, Gretener D, Nichols A, Vitte PA, Carboni S, Rommel C, Schwarz MK, Ruckle T: Furan-2-ylmethylene thiazolidinediones as novel, potent, and selective inhibitors of phosphoinositide 3-kinase γ . *J Med Chem* 2006;49:3857–3871.
- 17 McCloskey MA, Fan Y, Luther S: Chemotaxis of rat mast cells toward adenine nucleotides. *J Immunol* 1999;163:970–977.
- 18 Nashed BF, Zhang T, Al-Alwan M, Srinivasan G, Halayko AJ, Okkenhaug K, Vanhaesebroeck B, Hayglass KT, Marshall AJ: Role of the phosphoinositide 3-kinase p110 δ in generation of type 2 cytokine responses and allergic airway inflammation. *Eur J Immunol* 2007;37:416–424.
- 19 Vanhaesebroeck B, Ali K, Bilancio A, Geering B, Foukas LC: Signalling by PI3K isoforms: insights from gene-targeted mice. *Trends Biochem Sci* 2005;30:194–204.
- 20 Wymann MP, Bjorklof K, Calvez R, Finan P, Thomast M, Trifilieff A, Barbier M, Altruda F, Hirsch E, Laffargue M: Phosphoinositide 3-kinase γ : a key modulator in inflammation and allergy. *Biochem Soc Trans* 2003;31:275–280.
- 21 Hua X, Chason KD, Fredholm BB, Deshpande DA, Penn RB, Tilley SL: Adenosine induces airway hyperresponsiveness through activation of A3 receptors on mast cells. *J Allergy Clin Immunol* 2008;122:107–113.
- 22 Nunomura S, Yoshimaru T, Ra C: Na-Tosyl-Phe chloromethyl ketone prevents granule movement and mast cell synergistic degranulation elicited by costimulation of antigen and adenosine. *Life Sci* 2008;83:242–249.
- 23 Tilley SL, Wagoner VA, Salvatore CA, Jacobson MA, Koller BH: Adenosine and inosine increase cutaneous vasopermeability by activating A(3) receptors on mast cells. *J Clin Invest* 2000;105:361–367.
- 24 Zhong H, Shlykov SG, Molina JG, Sanborn BM, Jacobson MA, Tilley SL, Blackburn MR: Activation of murine lung mast cells by the adenosine A3 receptor. *J Immunol* 2003;171:338–345.