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Receptors and Signaling Mechanisms Required for Prostaglandin E2-Mediated Regulation of Mast Cell Degranulation and IL-6 Production


Mast cells are implicated in the pathogenesis of a broad spectrum of immunological disorders. These cells release inflammatory mediators in response to a number of stimuli, including IgE-Ag complexes. The degranulation of mast cells is modified by PGs. To begin to delineate the pathway(s) used by PGs to regulate mast cell function, we examined bone marrow-derived mast cells (BMMC) cultured from mice deficient in the EP1, EP2, EP3, and EP4 receptors for PGE2. Although BMMCs express all four of these PGE2 receptors, potentiation of Ag-stimulated degranulation and IL-6 cytokine production by PGE2 is dependent on the EP3 receptor. Consistent with the coupling of this receptor to Gs, PGE2 activation of the EP3 receptor leads to both inhibition of adenylate cyclase and increased intracellular Ca2+. The magnitude of increase in intracellular Ca2+ induced by EP3 activation is similar to that observed after activation of cells with IgE and Ag. Although PGE alone is not sufficient to initiate BMMC degranulation, stimulation of cells with PGE along with PMA induces degranulation. These actions are mediated by the EP3 receptor through signals involving Ca2+ mobilization and/or decreased cAMP levels. Accordingly, these studies identify PGE2/EP3 as a proinflammatory signaling pathway that promotes mast cell activation.

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Abbreviations used in this paper: LTB4, leukotriene B4; BMMC, bone marrow-derived mast cell; HSA, human serum albumin; EIA, enzyme immunoassay; [Ca2+]i, intracellular calcium concentration; fura 2-AM, fura 2-acetoxymethyl ester; [cAMP]i, intracellular cAMP level; PGE2, prostacyclin; LT, leukotriene; PKC, protein kinase C; CHO, Chinese hamster ovary.
The existence of multiple EP receptors coupled to different intracellular signals provides a molecular basis for the diverse physiological actions of PGE₂. However, the roles of the individual EP receptor subtypes in mediating specific actions of PGE₂ in various cell types and tissues, including mast cells, have not been well characterized. This is due in part to the lack of subtype-specific EP agonists and antagonists. Accordingly, using mouse lines deficient in the EP₁, EP₂, EP₃, or EP₄ receptors, we examined the modulation of mast cell functions and signaling by PGE₂. We find that the actions of PGE₂ in mast cells are mediated primarily through the EP₃ receptor.

Materials and Methods

Mice

The generation of mice deficient in EP₁, EP₂, EP₃, and EP₄ receptors has been previously reported (11–14). All mice used were at least 8 wk old and were bred and maintained in specific pathogen-free animal facilities at the University of North Carolina (Chapel Hill, NC), in accordance with the Institutional Animal Care and Use Committee guidelines.

Preparation of bone marrow-derived mast cells (BMMCs)

Bone marrow was isolated from the femurs of 2- to 3-month-old mice and placed in culture. Cells were grown for at least 4 wk in the presence of mouse IL-3-supplemented culture medium to select for pure populations of mast cells as described previously (15). Briefly, bone marrow-derived cells were cultured for 10 days in 1640 medium supplemented with 8% FCS, 8% mouse IL-3 culture supplement (Collaborative Biomedical Products, Bedford, MA), 20 mM HEPES, 4 mM t-glutaminate, 0.08 U/ml penicillin, 0.08 mg/ml streptomycin, 800 μM nonessential amino acids, 800 μM sodium pyruvate, 0.04 mg/ml gentamicin, and 92 μM 2-ME (BMMC culture medium). Cell cultures were maintained in a constant environment (humidified 37°C, 5% CO₂). To deplete adherent cells such as macrophages and monocytes from culture, cells in suspension were transferred to fresh dishes a few days after harvesting and then weekly at a concentration of 10⁶ to 10⁷ cells/ml. Cells cultured in this manner were examined visually after fixation with Carnoy’s fixative and toluidine blue staining. Although the percentage of mast cells of all cultures was not tested before each experiment, we have since examined populations of cells cultured from two different EP₂-c mice, two wild-type mice, and one culture derived from each of the following: an EP₁⁻/⁻, an EP₂⁻/⁻, and an EP₃⁻/⁻ mouse. Cultured cells were comprised of 98% toluidine blue positive staining granulated cells, regardless of the genotype.

RNA analysis of EP receptor expression

Total RNA from cultured BMMCs was obtained using RNAzol B (TelTest, Friendswood, TX) according to the manufacturer's instructions. Total RNA (20 μg) was fractionated by gel electrophoresis under denaturing conditions (1.2% agarose/1% formaldehyde). The contents of the gel were then transferred for Northern blot analysis. Hybridization to [32P]cDNA probes specific to the EP¹-receptor (EP-β receptor or β-actin) was performed at 68°C for 1 h using a QuickHyb solution (Stratagene, La Jolla, CA). Washes (42°C, 15 min) were performed twice in 2x SSC/0.1% SDS and once in 0.2x SSC/0.1% SDS. RT-PCR analysis was conducted using the following primers: EP-1F (5’-GGTCCGCTGCTGCTGCAAACTG-3’) and EP-1R (5’-GGCAAGGACATATGGCGAGGTT-3’). The presence of EP₁ receptor isoforms was analyzed by RT-PCR analysis using isoform-specific primers as previously described (16).

Measurement of hexosaminidase release

BMMCs from wild-type and EP₁⁻/⁻, EP₂⁻/⁻, EP₃⁻/⁻, and EP₄⁻/⁻-deficient mice were loaded with murine DNP-specific IgE mAb (clone SPE-7 from Sigma-Aldrich, St. Louis, MO) overnight at a concentration of 100 ng/ml per million cells. They were washed twice with Siraginan buffer (119 mM NaCl, 5 mM KCl, 25 mM PIPES, 5.6 mM glucose, 1.0 mM CaCl₂, 0.4 mM MgCl₂, and 0.1% BSA, pH 7.2) to eliminate any excess Ab. BMMCs were then resuspended (5 × 10⁶ cells per 75 μl) in Siraginan buffer, transferred to 96-well microtiter plates, and preincubated for 15 min at 37°C. Cells were then exposed to 25 μl of prewarmed PGE₁, or PGE₂ (Cayman Chemicals, Ann Arbor, MI), PMA, or PGE₁, and PMA human serum albumin (HSA; Sigma-Aldrich). In some experiments, Ag was added immediately after PGE₁ or PGE₂ treatment, as noted in Fig. 2B. Plates were then incubated for 30 min (humidified 37°C, 5% CO₂) followed by centrifugation at 500 × g for 5 min (4°C). A 100-μl aliquot of the supernatant was then taken and the mast cell pellets with the remaining supernatants were lysed with 100 μl of 0.2% Triton X-100. The supernatant and an aliquot of the cell lysate were then transferred to a well containing 100 μl of 1 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide (Sigma-Aldrich) in citrate buffer (0.1 M citric acid, 0.1 M sodium citrate, pH 4.5). The reactions were then incubated for 1 h (humidified 37°C, 5% CO₂), then halted with the addition of 100 μl of 0.1 M Na₂CO₃/0.1 M NaHCO₃. The absorbance was read at 405 nm to measure hexosaminidase activity. Data are expressed as the percentage of released hexosaminidase relative to the total cellular hexosaminidase content.

Measurement of IL-6 production

As described above, BMMCs were loaded with IgE overnight. After a 30-min preincubation with 10 μM indomethacin to inhibit endogenous prostaglandin formation, cells were washed twice in BMMC culture medium. Cells were then resuspended at 5 × 10⁶ cells in 100 μl of fresh medium, plated in microtiter plates, and treated with Ag (50 ng/ml), PGE₁ (10 μM), or Ag and PGE₁ in a final volume of 200 μl. BMMCs were then incubated for 7 h (humidified 37°C, 5% CO₂). At the end of the incubation period, cells were centrifuged at 500 × g for 4 min (4°C) and supernatants were carefully transferred to new wells and aliquots were stored at −80°C until assayed. Samples were diluted 10-fold in BMMC culture medium to fit within the standard curve. IL-6 content was determined by enzyme immunoassay (EIA) (PerSeptive Biosystems, Framingham, MA) according to the manufacturer's protocol.

Measurement of LTβ production

As described above, BMMCs were loaded with IgE overnight. BMMCs were washed twice and resuspended in fresh culture medium, and 5 × 10⁶ cells in 100 μl of medium were transferred to 96-well tissue culture plates, treated with Ag (50 ng/ml), PGE₁ (10 μM), or Ag and PGE₁, and incubated at 37°C for 20 min. After the incubation period, cells were centrifuged at 500 × g for 5 min (4°C). Supernatants were carefully transferred to new wells and stored at −80°C until assayed by EIA according to the manufacturer’s protocol (Cayman Chemicals).

Measurement of intracellular calcium concentrations ([Ca²⁺]ᵢ)

BMMCs were loaded with murine anti-DNP IgE by overnight incubation with the mAb as described above. Cells were incubated in the presence of 10 μM indomethacin (Sigma-Aldrich) for 30 min and then loaded in calcium buffer (135 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 5.6 mM glucose, 10 mM HEPES, 0.1% BSA, and 10 μM indomethacin, pH 7.4). After washing, cells were resuspended in calcium buffer (10² cells/ml) and loaded with 10 μM fura 2-acetoxymethyl ester (fura 2-AM; Molecular Probes, Eugene, OR) for 45 min (humidified 37°C, 5% CO₂). Cells were then washed in calcium buffer and transferred to a cuvette. Fluxes in intracellular free [Ca²⁺]ᵢ in response to the indicated treatments of Ag and PGE₂ were recorded with a luminescence spectrometer (LS50B; PerkinElmer, Wellesley, MA) at excitation wavelengths of 340/380 nm and an emission wavelength of 510 nm. The fluorescence ratio signal was calibrated by adding 1 μM ionomycin (Sigma-Aldrich) and the addition of 5 mM EGTA to determine the maximal and minimal fluorescence, respectively. The cytosolic Ca²⁺ concentrations were calculated using pCa of fura 2 for Ca²⁺ of 224 nM according to the method previously reported (17). Stock solutions of PGE₂ and indomethacin were prepared in 100% ethanol. Fura 2-AM and ionomycin were dissolved in DMSO.

Measurement of intracellular cAMP levels ([cAMP]ᵢ)

BMMCs were washed twice with Tyrode’s solution (137 mM NaCl, 2.7 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂, 0.2 mM NaH₂PO₄, 12 mM NaHCO₃, 5.5 mM glucose) and resuspended at 2 × 10⁶ cells per 200 μl of solution. Cells were then transferred to a sample vial and incubated at 37°C with stirring for 1 min and then treated with PGE₂, PGE₂, prostacyclin (PGL₁), or vehicle control for 1 min. Each treatment was conducted on three different cell samples. Purification and EIA analysis of [cAMP] were then conducted according to the manufacturer’s protocol (Amersham Pharmacia, Piscataway, NJ). The triplicate data points were then used to calculate baseline levels of [cAMP] and the change in [cAMP], with results expressed as the percent of the control baseline level.
Results

EP receptors expressed by BMMCs

BMMCs were cultured from EP1-deficient (EP1−/−), EP2-deficient (EP2−/−), EP3-deficient (EP3−/−), EP4-deficient (EP4−/−), and wild-type control mice. Total RNA was prepared from these cells and the level of expression of the PG receptors was examined by Northern analysis. The EP3 receptor was easily detected in control BMMCs (Fig. 1, left). EP2 mRNA could not be detected in either the normal or the mutant cell lines by Northern analysis of total RNA; however, RT-PCR analysis of the RNA indicated low levels of the EP2 receptor transcript in BMMCs (data not shown). Expression of the EP3 receptor was easily detected in wild-type BMMCs but not in the cells derived from the EP3-deficient animals (Fig. 1, center). To determine which EP3 receptor splice variants are expressed by BMMCs, RT-PCR analysis was conducted using primer sets specific for each of the isoforms. After gel electrophoresis and ethidium bromide staining, PCR products corresponding to EP3α and EP3β isoforms were easily detected (data not shown). However, expression of the EP3γ isoform was not observed. In contrast, all three EP3 isoforms were easily detected on similar analysis of RNA prepared from mouse kidney. By Northern analysis, high levels of EP4 expression were easily detected in the BMMCs (Fig. 1, right).

Effect of PGs on mast cell degranulation

Degranulation of BMMCs was monitored by measuring the release of hexosaminidase into the culture supernatant. PGE2 and PGE4 bind with equal affinity to the four known EP receptors. However, PGE1 has a higher affinity than PGE2 for the IP prostacyclin receptor (18). Incubation of mast cells with either PGE1 or PGE2 alone had no effect on hexosaminidase release by wild-type mast cells (Fig. 2). By contrast, in the presence of IgE-Ag receptor complexes, PGE1 and PGE2 significantly augmented release of hexosaminidase in a dose-dependent manner, even at very low doses of 1 × 10^-8 M and across the range of Ag concentrations that were tested (Fig. 2A). No measurable difference in the relative effectiveness of PGE1 and PGE2 was observed. To reconcile the differing results of previous studies, which have found that PGs can inhibit or potentiate mast cell mediator release, it has been suggested that the effect might depend on the timing of the addition of this lipid mediator, relative to exposure of the cells to Ag (19). Therefore, we examined the ability of PGE1 and PGE2 to modulate degranulation of BMMCs when added either immediately before Ag or 20 min before stimulation with Ag. As can be seen in Fig. 2, under both experimental conditions, exposure to either PGE1 or PGE2 enhanced hexosaminidase release by BMMCs in response to Ag in a dose-dependent manner.
mice also responded in a similar manner to control populations when exposed to both Ag and PGE1 (Fig. 3D). In stark contrast, the enhanced response to IgE-Ag after PGE1 treatment was completely abrogated in EP3-deficient BMMCs (Fig. 3C). These results demonstrate that the enhancement of FcεRI-mediated degranulation by PGs is mediated by EP3 receptors.

Effects of PGs on cytokine and leukotriene release by mast cells

Activation of mast cells also stimulates cytokine release. Thus, we next determined whether PGs could modify release of cytokines and whether this effect was also mediated by the EP3 receptor. As described above, mast cells were incubated with IgE and then treated with Ag alone or Ag plus PGE1. Seven hours later, the supernatant was harvested and IL-6 levels were determined. After Ag stimulation, high levels of IL-6 were released from mast cells (Fig. 4) and this IL-6 release was significantly enhanced in the wild-type mast cells by the treatment with PGE1. In contrast, PGE1 failed to enhance the release of IL-6 in the EP3-deficient mast cells. Similar to wild-type control BMMCs, IL-6 release by EP2- and EP4-deficient BMMCs was enhanced upon exposure to PGE1 (Fig. 4). Along with IL-6, leukotriene (LT) production is another important event associated with mast cell activation. Incubation of wild-type mast cells with PGE1 alone did not increase LT release. In contrast, LT release was significantly stimulated after treatment of mast cells with IgE-Ag. In contrast to its effects on IL-6, PGE1 did not further augment LT release in BMMCs exposed to IgE and Ag.
**EP**₃ receptor signaling in BMMCs

Based on our finding that mast cells express multiple EP₁ receptor isoforms and the diverse signaling pathways used by these isoforms, we explored EP₁ receptor signaling in BMMCs by comparing [cAMP], of wild-type and EP₁-deficient cells after incubation with PGE₁ and PGE₂. Treatment of wild-type BMMCs with PGE₁ significantly increased [cAMP]. In contrast, exposure of the cells to PGE₂ did not significantly alter levels of cAMP in wild-type cells (Fig. 5A). Because PGE₁, but not PGE₂, may also activate the Gₛₐ-coupled IP receptor, we examined the effect of PGI₂ on BMMCs. IP receptor activation in mast cells causes a marked stimulation of cAMP levels (Fig. 5A). To obtain maximum stimulation of adenylate cyclase we used PGE₁ in subsequent experiments to determine the impact of loss of the EP₁ receptor on [cAMP]. Stimulation of cAMP levels by PGE₁ was markedly enhanced in EP₁⁺⁻ compared with wild-type BMMCs, suggesting that EP₁ receptors couple to Gₛₐ proteins in BMMCs (Fig. 5B).

We next examined the effect of PGE₁ and PGE₂ on calcium signaling in mast cells. As shown, the addition of PGE₁ alone to BMMCs causes a brisk increase in [Ca²⁺], (Fig. 6A, left). Moreover, the magnitude of the change in [Ca²⁺], induced by PGE₂ is similar to that elicited by Ag treatment (Fig. 6B). This increase in [Ca²⁺], in response to PGE₂ is completely dependent on expression of the EP₁ receptor because PGE₁ had no effect on [Ca²⁺], in EP₁-deficient BMMCs (Fig. 6A, right). We next examined whether PGE₂ could enhance IgE-Ag-induced Ca²⁺ mobilization in wild-type cells, this increase in [Ca²⁺], is substantially enhanced after treatment of cells with Ag alone. Treatment of the wild-type cells with PGE₂ resulted in an easily measurable increase in [Ca²⁺], No change in [Ca²⁺], was observed in the EP₁⁺⁻ cells. The loading of these cells with fura-2 was confirmed by observation of an increase in [Ca²⁺], upon exposure to ionomycin. B: Changes in [Ca²⁺], in IgE-loaded EP₁⁺⁻ and wild-type BMMCs were monitored after addition of Ag or Ag and PGE₂. As expected, treatment of IgE-loaded BMMCs with Ag resulted in a rapid increase in [Ca²⁺], This change was observed in both the EP₁⁺⁻ and wild-type BMMCs. Addition of PGE₂ together with Ag resulted in an enhancement of this response in wild-type BMMCs. In contrast, no enhanced response was observed in the EP₁⁻⁻ cells. C, To further demonstrate the ability of PGE₂ to enhance Ca²⁺ mobilization observed after Ag activation, IgE-loaded wild-type cells were treated with Ag alone followed 1 min later by addition of PGE₂. Treatment with PGE₂ resulted in a further increase in [Ca²⁺], over that observed upon treatment with Ag alone. Data shown are representative of three independent experiments.
was increased with increasing concentrations of PMA. This response was mediated for 30 min at 37°C with various concentrations of PMA in the presence or absence of 10 μM PGE1. Degranulation of the BMMCs was assessed by measuring the release of hexosaminidase into the supernatant. As expected, treatment of mast cells with PMA up to 100 ng/ml did not result in a significant increase in hexosaminidase release in either wild-type or EP3R−/− cells. However, in the presence of PGE1, release of hexosaminidase was increased with increasing concentrations of PMA. This response was dependent on expression of the EP3 receptor, because no increase in hexosaminidase release was observed in the EP3R−/− BMMCs treated with both PGE1 and PMA. Data shown are representative of three independent experiments.

was not observed in the EP3R-deficient mast cells (data not shown). Both PGE1 and PGE2 had similar effects on [Ca2+], (data not shown).

In the presence of PMA, PGE1 stimulates mast cell degranulation

Although PGE alone stimulates [Ca2+]i flux to a similar degree as IgE-Ag, it fails to effect a mediator release. This suggests that additional signals are required to trigger mast cell degranulation. Similarly, whereas activation of protein kinase C (PKC) modulates mast cell degranulation, treatment of mast cells with PKC-activating PMA alone does not result in mast cell degranulation. Thus, we next determined whether PGE1 and PMA could act synergistically to induce degranulation. Consistent with previous studies, treatment of wild-type or EP3R−/− BMMCs with PMA alone did not alter hexosaminidase release (Fig. 7) or [Ca2+]i (data not shown). However, in wild-type BMMCs treated with PMA, PGE1 caused a dose-dependent increase in BMMC degranulation. These actions of PGE1 are mediated through the EP1 receptor, because mediator release was not enhanced in EP3R-deficient mast cells treated with PMA and PGE1.

Discussion

BMMCs have provided an important means for examining the receptors and intracellular pathways that regulate mast cell function. These cell populations, in combination with the increasing availability of mice deficient in receptors implicated in mast cell biology, provide an important method for dissecting the requirements for mast cell degranulation and production of inflammatory mediators. Using this approach, we show that PGE2 can potentiate mast cell degranulation and cytokine production and that these actions are mediated primarily by the EP3 receptor. Furthermore, we show that EP3 receptor activation is associated with both increases in [Ca2+]i and inhibition of adenylate cyclase activity.

Expression analysis and pharmacological studies have indicated that mast cells express a number of prostanoid receptors, including several PGE2 receptors and the Gαs-coupled prostacyclin receptor. Pharmacological studies are consistent with the expression of EP3 and EP4 on IL-3-dependent mast cell lines and on rat peritoneal mast cells (20–22). Expression analysis of MC9 mouse mast cell line revealed the expression of EP1, EP3, and EP4 (19). We show here that BMMCs express high levels of the Gaαs-coupled EP4 receptor. Although we could not detect expression of the EP2 receptor by Northern analysis, RT-PCR studies indicate that mast cells do express this receptor. This finding is consistent with the cloning of the mouse EP2 receptor from a mouse mastocytoma cell line. The relatively low expression of the EP2 receptor in comparison to EP3 in virtually all tissues that coexpress these two receptors has been noted previously (22). In addition to these Gaαs-coupled receptors, we show that BMMCs express high levels of the EP1 receptor, including both the EP1α and EP1β isoforms. Consistent with previous reports, we detected high levels of EP1 receptor expression. Activation of this receptor has been shown to result in an increase in [Ca2+]i.

We show that treatment of BMMCs with PGE1 or PGE2 alone does not induce degranulation of BMMCs. However, addition of PGE1 to BMMCs stimulated with IgE and Ag potentiates degranulation of these cells. This is consistent with the findings reported by Leal-Berumen et al. (23), using rat peritoneal mast cells, and with results obtained using BMMCs and IL-3-dependent mast cell lines (19, 20). Our results contrast sharply, however, with other studies that showed that PGE2 could inhibit the release of mediators from both rat peritoneal mast cells (24, 25) and human lung mast cells (26, 27). It is likely that at least some of these differences reflect the use of different mast cell populations in these studies. As discussed below, these observations suggest that the impact of PGE2 on mast cell function may be dependent on the maturity, tissue type, and perhaps activation state of the cell.

The studies reported here also show that PGE1 can enhance the production of IL-6 by IgE-Ag-stimulated mast cells. Unlike previous reports, we failed to observe an increase in production of IL-6 by cells stimulated by PGE alone. Stimulation of rat peritoneal mast cells with PGE1 or PGE2 was reported to induce a significant increase in the release of IL-6 from 3 to 18 h after treatment (23). In similar studies using BMMCs, no significant increase in IL-6 release was observed at early time points on comparison of medium-treated and PGE1-treated BMMCs (19). However, in these same studies IL-6 production in response to PGE1 alone was noted at later time points. We did not observe substantial release of IL-6 in the response to PGE1 or PGE2 alone, even at these later time points (data not shown).

The enhancement of Ag-stimulated degranulation of BMMCs by PGE1 and PGE2 is perhaps surprising in light of our demonstration that these cells express both the EP2 and the EP3 receptors and of the established coupling of these receptors to stimulatory G proteins in all cell types examined to date. Agents that stimulate an increase in [cAMP], have been shown to inhibit mast cell degranulation (28), and an increase in [cAMP], due to PGE1 addition is believed to precede the inhibition of histamine release from rat peritoneal mast cells in response to stimulation of IgE receptors (27). There are a number of possible explanations for the observed lack of inhibition of BMMC degranulation after treatment with PGE1 and the similarity of the response of EP2−/− or EP3−/− mast cells compared with wild-type cells. It is possible, for instance, that although these cells express very high levels of EP3 receptor mRNA, this does not correspond to high numbers of EP3 receptors at the cell surface of BMMCs. Alternatively, the coupling of the EP3 receptors to adenylate cyclase might be inefficient in these immature mast cells, or rapid desensitization of this receptor in BMMCs might reduce their impact on cell physiolog. Alteration of the EP2/EP3 inhibitory pathways as mast cells mature after migration to various tissues might alter the response of mast...
cells to PGE2, resulting in a primarily inhibitory function for this lipid mediator. This later interpretation provides an explanation for the inhibitory response to PGE2 observed in a number of studies that used mature tissue mast cells.

The observed enhancement of Ag-mediated BMMC degranulation upon treatment with PGE1 or PGE2 is consistent with the activation of the EP3 and/or the EP1 receptor, because both of these receptors have been shown to mediate increases in [Ca2+]i, and this pathway is critical to the degranulation of mast cells. Using EP3 receptor-deficient BMMCs, we show that the PGE2-mediated enhancement of mast cell degranulation is entirely due to activation of this receptor. Similarly, the ability of PGE to enhance IL-6 production is abolished in EP3-deficient mast cells. These results are consistent with earlier pharmacological studies that implicated either the EP3 or the EP1 receptor in the activation of mast cells and/or mast cell lines (19, 20). When EP3 receptor-deficient mice were tested directly, they exhibited a degranulation response that was sensitive to PG potentiation. Together with the complete absence of a PGE response in the EP3-deficient mast cells, this suggests that the EP1 receptor is not likely involved in this response in BMMCs.

To further examine the mechanism by which activation of the EP3 receptor can modify mast cell function, we have examined the impact of loss of EP3 receptor expression on cAMP accumulation and on alterations in [Ca2+]i. Transfection of both the EP3α and EP3β receptors into Chinese hamster ovary (CHO) cells has been reported to inhibit forskolin-induced cAMP accumulation (29). We show here that treatment of BMMCs with PGE1 leads to an increase in [Ca2+]i and that, consistent with the activation of Gαs, this accumulation of [cAMP]i is enhanced in the EP3 receptor-deficient cells.

In addition to their ability to inhibit cAMP accumulation, both the EP3α and the EP3β isoforms have been shown to stimulate an increase in [Ca2+]i upon transfection into CHO cells. This increase in [Ca2+]i, was pertussis toxin-sensitive, consistent with the coupling of both of these isoforms to Gαs proteins (30). We report that treatment of BMMCs with PGE1 or PGE2 resulted in a rapid increase in [Ca2+]i. Furthermore, we show that this response is completely dependent on the expression of the EP3 receptor. Therefore, it is likely that in BMMCs, similar to the CHO cells, the EP3α and the EP3β are coupled to Gαs proteins, whose Gαs subunits can mediate increases in [Ca2+]i, by activation of phospholipase C. It is likely that this in turn is responsible for the potentiation of mast cell degranulation observed on treatment with PGE2. However, we cannot rule out the possibility that PGE/EP3 contribute to this response, at least in part, by inhibition of adenylate cyclase activity and [cAMP]i accumulation. This seems less likely given the observation that the loss of EP4 and EP2 receptors had no effect on mast cell degranulation.

There are a number of possible explanations for the inability of PGE to initiate mast cell degranulation, despite the substantial increase in [Ca2+]i. First, it is possible that subtle differences in the magnitude, duration, and/or the stores of Ca2+ released lead to the differential effect on mast cell activation. Alternatively, it is possible that although activation of the EP3 receptor leads to changes in Ca2+ sufficient for mast cell degranulation, activation of additional pathways is necessary to bring about these physiological changes. It has been suggested that activation of PKC plays an important role in mast cell degranulation. To determine whether the failure of mast cells to degranulate in response to PGE2 alone is related to its inability to activate the PKC pathway, we examined mast cell degranulation in EP3-deficient and wild-type BMMCs treated with PMA alone and PMA together with PGE1. PMA treatment of BMMCs did not result in any changes in [Ca2+]i. The ability of EP3 receptor activation to act synergistically with PMA to bring about degranulation of mast cells suggests that PKC activity can contribute to mast cell activation and that the failure of PGE2 to activate this pathway prevents PGE2 alone from initiating mast cell degranulation in these cells.

We cannot formally rule out the possibility that other cell types might contribute to the observed differences between the responses of the BMMCs derived from the various EP-deficient mouse lines. Because the number of mast cells present was not determined for every preparation of cells (only representative cultures of each genotype) used in the reported studies, we cannot rule out the possibility that differences in the number of mast cells or presence of contaminating populations contribute to the observed difference in the response of wild-type and EP3−/− populations to PGE2. However, the fact that the baseline response to IgE and Ag was similar in all of the EP-deficient cells (only the ability of PGE to modulate this response was altered in the EP3−/− deficient cells) suggests that loss of these receptors does not alter the development and/or growth of BMMCs under these culture conditions.

The studies reported here, together with those of other investigators, begin to develop a model that unifies the often “opposing” actions of prostanoids on mast cell physiology. The effect of PGE2 is determined by both the expression profile of the various prostanoid receptors and by the activity of the intracellular pathways to which they are coupled. It is easy to envision a model in which this contributes to the ability of mast cells to modulate their responses in both a temporal and spatial manner. For example, early during an inflammatory process, PGE2 produced upon local tissue damage leads to heightened production of cytokines necessary for the recruitment of inflammatory cells. This could lead to altered expression or activity of the various EP receptors on the mast cells, with a shift from the Gαs- to the Gαi-linked receptors and restoration of homeostasis as the inflammation is resolved. It is also possible that different EP receptor pathways dominate in the various mast cell populations. This would contribute to the adaptation of mast cells to the particular organ in which it is located. The generation of mouse lines with deficiencies in a single or combination of receptors and the study of these animals in model systems in which a role for mast cells has been defined should help us answer these questions in the future.

References


