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Prostaglandin E₂ Selectively Enhances the IgE-Mediated Production of IL-6 and Granulocyte-Macrophage Colony-Stimulating Factor by Mast Cells Through an EP₁/EP₃-Dependent Mechanism

Kaede Gomi,* Fu-Gang Zhu,* and Jean S. Marshall²*†

PGE₂ is an endogenously synthesized inflammatory mediator that is over-produced in chronic inflammatory disorders such as allergic asthma. In this study, we investigated the regulatory effects of PGE₂ on mast cell degranulation and the production of cytokines relevant to allergic disease. Murine bone marrow-derived mast cells (BMMC) were treated with PGE₂ alone or in the context of IgE-mediated activation. PGE₂ treatment alone specifically enhanced IL-6 production, and neither induced nor inhibited degranulation and the release of other mast cell cytokines, including IL-4, IL-10, IFN-γ, and GM-CSF. IgE/Ag-mediated activation of BMMC induced the secretion of IL-4, IL-6, and GM-CSF, and concurrent PGE₂ stimulation synergistically increased mast cell degranulation and IL-6 and GM-CSF, but not IL-4, production. A similar potentiation of degranulation and IL-6 production by PGE₂, in the context of IgE-directed activation, was observed in the well-established IL-3-dependent murine mast cell line, MC/9. RT-PCR analysis of unstimulated MC/9 cells revealed the expression of EP₁, EP₃, and EP₄ PGE receptor subtypes, including a novel splice variant of the EP₁ receptor. Pharmacological studies using PGE receptor subtype-selective analogs showed that the potentiation of IgE/Ag-induced degranulation and IL-6 production by PGE₂ is mediated through EP₁ and/or EP₃ receptors. Our results suggest that PGE₂ may profoundly alter the nature of the mast cell degranulation and cytokine responses at sites of allergic inflammation through an EP₁/EP₃-dependent mechanism. The Journal of Immunology, 2000, 165: 6545–6552.

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3 Abbreviations used in this paper: PMC, peritoneal mast cell; BMMC, bone marrow-derived mast cell; HSA, human serum albumin.
humans. We have focused on the effects of PGE2 on three cytokines, IL-4, IL-6, and GM-CSF, which are produced in physiologically relevant quantities during allergic disease and are enhanced in symptomatic asthma (25, 26). IL-4 was selected for study in view of its critical role in the development of type 2 immune responses and IgE class switch (1); IL-6, for its role in inducing the acute phase response and down-regulating inflammatory processes (27); and GM-CSF, for its involvement in the pathogenesis of allergic inflammation largely through its role as a development and survival factor for eosinophils (28). EP receptor expression and usage by MC/9 cells was also examined in this study.

Materials and Methods

Mice

C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME) were housed in sterility-filter hooded cages and provided food and water ad libitum. All experiments were approved by the Animal Research Ethics Boards of McMaster University (Hamilton, Ontario, Canada) and Dalhousie University (Halifax, Nova Scotia, Canada).

Mast cells

MC/9 cells (CRL 8306; American Type Culture Collection, Manassas, VA) were routinely grown in modified DME (Life Technologies, Burlington, Ontario, Canada) containing 36 mg/ml L-ascpartate, 0.1 mM nonessential amino acids, 50 μM 2-ME, 10% FCS, and 5 ng/ml rmIL-3 (PeproTech, Rocky Hill, NJ) at 37°C. 10% CO2. BMMC were generated from bone marrow of C57BL/6 mice. Briefly, mice were sacrificed, and intact femurs and tibias were removed. Sterile endotoxin-free medium was repeatedly flushed through the bone shaft using a needle and syringe, and the bone marrow cells were passed through a sterile wire screen to remove any bone fragments. The cell suspension was centrifuged at 320 × g for 20 min at 4°C, and cultured at a concentration of 0.5–1 × 105 nucleated cells/ml in RPMI 1640 (Life Technologies) supplemented with 10% FCS (Sigma-Aldrich Canada), 10 mM HEPES, and 50 μM 2-ME (BMMC medium). Nonadherent cells were transferred to fresh BMMC medium at least once a week. After 4–6 wk, mast cell purity of >95% was achieved as assessed by Acanth blue or Toluidine blue staining of fixed cytotoxic preparations.

Mast cell activation with various stimulating agents

Mast cells were resuspended in experimental medium consisting of RPMI 1640 (Canadian Life Technologies), 10% FCS, 1% penicillin/streptomycin, 50 μM 2-ME, and normal human lung fibroblast- or murine monocyte macrophage J774 cell line-conditioned medium as a source of IL-6. B-9 cells were washed, resuspended at 5 × 104 cells/ml in B-9 medium, and incubated with standards and samples for 3 days at 37°C. Then, 10 μl/well 0.5 mM MTII (Sigma-Aldrich Canada) was added, and, after 40 min at 37°C, Triton-HCl was added and the plates were stored for 18–24 h in the dark. The optical densities of the resulting reaction product were determined at 570 nm. IL-6 concentrations were reported as U/ml of bioactivity, where 1 U equals ~0.45 pg of IL-6. The sensitivity of the B-9 assay has been determined to be 10 U/ml. None of the reagents used in this study, including PGE2, at the highest concentration used in this study (1 μM), altered B-9 cell growth under these conditions. Moreover, other mast cell-derived cytokines, including TNF-α, GM-CSF, and IL-4, do not cause proliferation of B-9 cells under these conditions (18).

ELISAs

Murine IL-4 and IL-10 were assayed using ELISA kits purchased from R&D Systems (Minneapolis, MN). IL-5 and IL-12 ELISA kits were obtained from Amersham Life Science (ON, Canada) and Genzyme Diagnostics (Cambridge, MA), respectively. GM-CSF was assayed using ELISA kits purchased and used from both R&D Systems and Amersham Life Science. Cyclic AMP was measured by enzyme immunoassay purchased from Amersham Pharmacia Biotech (Quebec, Canada).

Murine IFN-γ was measured by an “in-house” sandwich ELISA with all incubations performed at room temperature. Briefly, Maxisorp ELISA plates (Nunc/Inter Med, ON, Canada) were coated for 18–24 h at 4°C with 50 μl/well of 2 μg/ml anti-mouse IFN-γ capture Ab (BD Pharmingen, ON, Canada) diluted in either borate-buffered saline (pH 8.3) or freshly prepared 0.1 M bicarbonate solution in distilled water. The wells were aspirated, and incubated for 1 h with 100 μl/well blocking solution (10 mg BSA/ml PBS, pH 7.4). The blocking solution was decanted, and the wells were washed four times with PBS (pH 7.4) containing 0.05% Tween 20. Wells were aspirated after the final wash to ensure complete removal of liquid. Standards and samples were added to the plate at 50 μl/well and incubated between 1.5 and 2 h. The wells were washed as described above, and secondary biotinylated anti-mouse IFN-γ Ab (BD Pharmingen) at 0.5 μg/ml in blocking solution was added at 50 μl/well. After 1 h, the wells were washed and 50 μl/well of streptavidin-peroxidase reagent (Can-adian Life Technologies) prepared in blocking solution in 50 μl/well, and color development was allowed for up to 1 h. The colored product was read at 492 nm.

β-Hexosaminidase release assay

Briefly, 1 × 104 BMMC or MC/9 cells per ml were incubated for 15 min at 37°C in HEPESTyrosines buffer (137 mM Na, 5.6 mM glucose, 2.7 mM KCl, 0.5 mM NaH2PO4, 1 mM CaCl2, 10 mM HEPES, 0.1% BSA, pH 7.3, 0.45 pg of IL-6. The sensitivity of the B-9 assay has been determined to be 10 U/ml. None of the reagents used in this study, including PGE2, at the highest concentration used in this study (1 μM), altered B-9 cell growth under these conditions. Moreover, other mast cell-derived cytokines, including TNF-α, GM-CSF, and IL-4, do not cause proliferation of B-9 cells under these conditions (18).

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β-Hexosaminidase release assay

Briefly, 1 × 104 BMMC or MC/9 cells per ml were incubated for 15 min at 37°C in HEPESTyrosines buffer (137 mM Na, 5.6 mM glucose, 2.7 mM KCl, 0.5 mM NaH2PO4, 1 mM CaCl2, 10 mM HEPES, 0.1% BSA, pH 7.3,
PGE₂ induces enhancement of IL-6 production by BMMC

To assess the effects of PGE₂ activation alone on mast cell cytokine production, BMMC were activated with various doses of PGE₂ for up to 24 h, and supernatants were assayed for cytokines of interest. As previously demonstrated in rat PMCs (18), IL-6 production in BMMC was enhanced by PGE₂ in a dose-dependent manner (baseline IL-6 production of 21.6 ± 5.5 U/ml was increased to 595 ± 72 U/ml (p < 0.001) and 280 ± 77 U/ml (p < 0.01) following stimulation with PGE₂ at 10⁻⁸ M and 10⁻⁶ M, respectively (n = 8)). In contrast, PGE₂ lacked any significant effect on the production of IL-4, IL-5, IL-10, IFN-γ, and GM-CSF (data not shown), whereas BMMC were capable of producing each of these cytokines in response to FcεRI cross-linking alone (IL-4, IL-5, GM-CSF) (32–34), IL-3 treatment (IL-10) (35), or IL-12 treatment (IFN-γ) (36).

PGE₂ synergistically increases IL-6 and GM-CSF responses in the context of IgE-mediated activation

Mast cells are known to be activated via cross-linking of their surface FcεRI by specific allergen. To examine the regulatory effects of PGE₂ in the context of IgE-mediated activation, BMMC were passively sensitized with anti-DNP IgE or anti-TNP IgE for 18–30 h, and subsequently incubated with respective Ag, DNP-HSA, or TNP-BSA (at 10 ng/ml), in the presence or absence of PGE₂. IgE-mediated activation increased the BMMC production of IL-6, GM-CSF, and IL-4 over that of media-treated controls (Fig. 1A–C). Costimulation of IgE-sensitized mast cells with DNP-HSA and PGE₂ resulted in increased IL-6 and GM-CSF production over IgE-mediated activation alone (p < 0.01 for IL-6; p < 0.001 for GM-CSF) (Fig. 1A and B). IgE-mediated IL-4 production, in contrast, was not enhanced by PGE₂, and at higher concentrations, PGE₂ (≥10 nM) had suppressive effects on IL-4 production (p < 0.01) (Fig. 1C). We also investigated the modulation of IL-6 production by PGE₂ in an IL-3-dependent murine mast cell line, MC/9. IL-6 production by MC/9 cells was also potentiated by PGE₂ in the context of IgE-mediated activation; however, PGE₂ alone failed to consistently induce IL-6 production by a range of PGE₂ doses (10⁻⁸, 10⁻⁷, 10⁻⁶ M) (data not shown).

Time course of PGE₂ effects on cytokine production

Kinetic studies were performed investigating IL-6 and GM-CSF release in response to PGE₂ and IgE-mediated activation of BMMC. IgE-mediated IL-6 and GM-CSF production, which was minimal or absent at 1 h, was readily detected by 6 h, and cytokine levels were maintained up to the 24-h time point (Table I). PGE₂-mediated potentiation of IL-6 and GM-CSF production in IgE/Ag-activated cells was readily apparent by 6 h poststimulation. IgE-mediated activation also induced significant IL-4 release by 6 h (98.7 ± 11.4 pg/ml; p < 0.001 with respect to the media control value of 11.3 ± 4.7 pg/ml; n = 2), and such secretion was not modulated at this time point by PGE₂ (102 ± 4 pg/ml for concurrent IgE/Ag and PGE₂ treatment; n = 3).

PGE₂ induces potentiation of mast cell degranulation

To examine the effects of PGE₂ on mast cell degranulation, BMMC and MC/9 cells were activated for 20 min with PGE₂ alone or in combination with IgE/Ag-activation, and the degree of β-hexosaminidase release was measured as a marker of degranulation. PGE₂ activation alone did not induce β-hexosaminidase release in supernatant or in pellet samples, however, PGE₂ alone failed to consistently induce IL-6 production over IgE-mediated activation alone (p < 0.01 for IL-6; p < 0.001 for GM-CSF) (Fig. 1A and B). IgE-mediated IL-4 production, in contrast, was not enhanced by PGE₂, and at higher concentrations, PGE₂ (≥10 nM) had suppressive effects on IL-4 production (p < 0.01) (Fig. 1C). We also investigated the modulation of IL-6 production by PGE₂ in an IL-3-dependent murine mast cell line, MC/9. IL-6 production by MC/9 cells was also potentiated by PGE₂ in the context of IgE-mediated activation; however, PGE₂ alone failed to consistently induce IL-6 production by a range of PGE₂ doses (10⁻⁸, 10⁻⁷, 10⁻⁶ M) (data not shown).

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Figure 1. PGE₂ effects on IgE-activated mast cells. BMMC passively sensitized with anti-DNP IgE were incubated with DNP-HSA alone or in the presence of various doses of PGE₂. BMMC incubated with medium alone were used as controls. Following 24 h of incubation, supernatants were harvested and assayed for IL-6 (A), GM-CSF (B), and IL-4 (C) content. Bars represent mean values ± SEM. ***p < 0.001 compared with media controls. ###p < 0.01; ####p < 0.001 compared with IgE-mediated activation alone. n.d., not detected in the assay (limit of detection for the GM-CSF and IL-4 ELISAs were 1 and 2 pg/ml, respectively).
release by BMMC (Fig. 2A) or MC/9 cells (Fig. 2B). IgE-mediated activation induced significant β-hexosaminidase release by both mast cell populations, and concurrent stimulation with PGE₂ consistently enhanced this release by at least 30% (Fig. 2).

Effects of PGE₂ on mast cell degranulation and IL-6 production are unlikely to be mediated by the second messenger, cAMP

Our findings of enhanced degranulation induced by PGE₂ in the context of IgE-mediated activation are in contrast to the inhibitory effects of this prostanoid on mast cell degranulation reported when mast cells were preincubated with PGE₂ before addition of other mast cell stimuli (19–21). In the latter studies, intracellular cAMP was implicated as the second messenger mediating the inhibitory effects. To investigate whether cAMP played a critical role in PGE₂-mediated enhancement of degranulation and IL-6 production in IgE/Ag-activated mast cells, BMMC and MC/9 were stimulated with cAMP-elevating agents. In contrast to the stimulatory effects observed with PGE₂, forskolin, a direct activator of adenylate cyclase, inhibited IgE-mediated β-hexosaminidase release in both BMMC and MC/9 cells (Fig. 2), and failed to potentiate IL-6 production in IgE/Ag-activated MC/9 cells (Fig. 3). Two additional cAMP-elevating agents, β-isoproterenol and the phosphodiesterase inhibitor, pentoxifylline, also failed to potentiate IL-6 production in the context of IgE-mediated activation (Fig. 3).

Involvement of EP₁/EP₃ receptors in the potentiation of β-hexosaminidase and IL-6 production in IgE/Ag-activated mast cells

PGE₂ acts by interacting with one of four receptor subtypes designated EP₁, EP₂, EP₃, and EP₄ (22). To examine whether PGE₂ receptor agonists could modulate IgE/Ag-induced β-hexosaminidase release and IL-6 production, MC/9 cells were stimulated with a panel of synthetic agonists that demonstrate preferential binding of one or more EP subtypes. The EP₁ agonist, 17-phenyl-α-trinor-PGE₂, and the EP₁/EP₃ selective agonist, sulprostone, potentiated β-hexosaminidase release (Fig. 4) and IL-6 production (Fig. 5 and Table II) by IgE/Ag-activated mast cells. PGE₁, a PGE₂ homologue which binds with comparable affinity as PGE₂ to EP₂, EP₃, and EP₄ yet more weakly to EP₁, strongly potentiated β-hexosaminidase release by MC/9 cells. However, PGE₁ induced IL-6 production to a substantially lower degree than PGE₂ (Fig. 5 and Table II). The EP₂/EP₃-selective agonist, PGE₃ alcohol, failed to enhance β-hexosaminidase release (Fig. 4) and IL-6 production (Fig. 5 and Table II) above IgE-mediated activation alone. These data implicate the involvement of the EP₁ and/or EP₃ receptors in β-hexosaminidase release and IL-6 production. Involvement of EP₃ in mediating β-hexosaminidase release was further suggested

### Table I. Kinetics of cytokine production by BMMC in response to IgE/Ag and PGE₂

<table>
<thead>
<tr>
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<th>IL-6 (U/ml)</th>
<th>GM-CSF (pg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>6 h</td>
</tr>
<tr>
<td>Media</td>
<td>12.7 ± 1.3*</td>
<td>78.0 ± 15.5</td>
</tr>
<tr>
<td>PGE₂</td>
<td>10.0 ± 0.0</td>
<td>63.3 ± 9.3</td>
</tr>
<tr>
<td>IgE/DNP</td>
<td>12.7 ± 1.3</td>
<td>643 ± 20*</td>
</tr>
<tr>
<td>IgE/DNP + PGE₂</td>
<td>19.0 ± 2.5</td>
<td>1110 ± 283***</td>
</tr>
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</table>

* Cells were passively sensitized with anti-DNP IgE, and incubated with DNP-HSA (10 ng/ml) in the presence or absence of PGE₂ (1 μM). Supernatants were harvested at different times and assayed for IL-6 and GM-CSF. Figures represent mean values ± SEM. *, Denotes p < 0.05; ***, denotes p < 0.001 compared with media controls; ###, denotes p < 0.001 compared with IgE-mediated activation alone. ND, Not detectable in the assay (limit of detection for the GM-CSF ELISA was 1 pg/ml).

### Table II. EP₁/EP₃-selective agonist agonists on cytokine production by BMMC

<table>
<thead>
<tr>
<th></th>
<th>IL-6 (U/ml)</th>
<th>GM-CSF (pg/ml)</th>
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<tbody>
<tr>
<td>Media</td>
<td>12.7 ± 1.3</td>
<td>ND</td>
</tr>
<tr>
<td>PGE₂</td>
<td>10.0 ± 0.0</td>
<td>ND</td>
</tr>
<tr>
<td>IgE/DNP</td>
<td>12.7 ± 1.3</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>IgE/DNP + PGE₂</td>
<td>19.0 ± 2.5</td>
<td>1.1 ± 0.1</td>
</tr>
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</table>

* Cells were passively sensitized with anti-DNP IgE, and incubated with DNP-HSA (10 ng/ml) in the presence or absence of PGE₂ (1 μM). Supernatants were harvested at different times and assayed for IL-6 and GM-CSF. Figures represent mean values ± SEM. *, Denotes p < 0.05; ***, denotes p < 0.001 compared with media controls; ###, denotes p < 0.001 compared with IgE-mediated activation alone. ND, Not detectable in the assay (limit of detection for the GM-CSF ELISA was 1 pg/ml).

### Figure 2. β-Hexosaminidase release by BMMC and MC/9 in response to PGE₂ and IgE-mediated activation. BMMC (A) and MC/9 (B) were previously sensitized with anti-TNP IgE and incubated with TNP-BSA (10 ng/ml) in the presence or absence of PGE₂ (1 μM) for 15 min at 37°C. BMMC incubated in buffer alone served as a control for spontaneous β-hexosaminidase release. Bars represent mean values ± SEM. ***, p < 0.001 compared with media controls. ###, p < 0.001 compared with IgE-mediated activation alone. A23, A23187 (calcium ionophore; 1 μM); Forsk, Forskolin (10 μM). Data shown are representative of at least three independent experiments.

### Figure 3. Effect of cAMP-elevating agents on IL-6 production by IgE/Ag-activated mast cells. MC/9 cells were passively sensitized with anti-TNP IgE and subsequently incubated for 24 h with TNP-BSA (10 ng/ml) alone or in the presence of β-isoproterenol (10 μM), forskolin (10 μM), or pentoxifylline (1 mg/ml). MC/9 cells incubated with PGE₂ (1 μM) and TNP-BSA (10 ng/ml) served as controls. Bars represent mean % change (±SEM) in IL-6 response with respect to IgE-mediated activation from at least two independent experiments.
We used RT-PCR to determine which PGE receptor subtypes were expressed by MC/9 cells. Quiescent MC/9 cells expressed EP<sub>1</sub>, EP<sub>3</sub>, and EP<sub>4</sub> receptors (Fig. 6A). However, MC/9 cells failed to express mRNA encoding EP<sub>2</sub> in three independent RNA preparations, whereas a signal for EP<sub>2</sub> of the expected size (401 bp) was observed in murine uterus (data not shown). For the EP<sub>2</sub> receptor, in addition to a weak signal for the expected PCR product (336 bp), a more intense band corresponding to a larger amplicon at ~750 bp was observed (Fig. 6A), and the latter PCR product may represent a splice variant similar to that described in the rat (37).

To rule out the possibility of genomic contamination, RNA preparations were treated with DNase I to degrade any contaminating genomic DNA, and then subjected to PCR with or without prior reverse transcription. No PCR products were obtained for any of the EP receptors including EP<sub>1</sub> when reverse transcription was not performed (Fig. 6A). Nesting primers were employed to amplify the EP<sub>1</sub> receptor signal, and two PCR products of expected sizes (249 bp and 668 bp) were obtained (Fig. 6B). Subsequent sequence analysis indicated that the putative EP<sub>1</sub>-variant receptor contained an intron positioned within the sixth transmembrane domain (data not shown), and, hence, in the rat, the EP<sub>1</sub>-variant receptor arose from the failure to use a splice site located within this domain (37).

**Discussion**

Elevated numbers of mast cells and evidence of mast cell activation are observed in a variety of inflammatory disorders, including asthma (38), rheumatoid arthritis (39), and inflammatory bowel disease (40). However, the full role of mast cells in the pathogenesis of such inflammatory disorders is largely unexplored. Mast cells are storehouses of preformed mediators including histamine...
and proteases, and are potent sources of a number of proinflammatory cytokines and chemokines. Levels of the lipid mediator, PGE₂, are also elevated in the context of many inflammatory conditions (41), and PGE₂ has been demonstrated to possess potent immunomodulatory actions and to shift the immune response toward a type 2 response through inhibition of type 1 cytokine production and either enhancing or having no effect on the production type 2 cytokines (1). Consequently, PGE₂ may support the induction and chronicity of certain types of inflammation.

Our current data show that PGE₂ alone selectively modulates cytokine production by murine mast cells, BMMC and MC/9, both of which are considered models of mucosal mast cells. In otherwise unactivated BMMC, PGE₂ enhanced IL-6 production and failed to alter the production of many other cytokines, including IL-4, IL-5, IL-10, and GM-CSF, that are known to be produced by mast cells under alternate stimulation conditions. However, PGE₂ displayed a broader range of potent effects on cytokine production when used in conjunction with IgE/Ag stimulation. IgE-mediated activation alone induced significant release of IL-4, IL-6, and GM-CSF, and further addition of PGE₂ led to a synergistic increase in the production of both IL-6 and GM-CSF, but not IL-4, suggesting selectivity in the ability of PGE₂ to interact with FceRI-mediated cytokine induction.

The potentiation of IL-6 release by PGE₂ in the context of IgE-mediated activation was unlikely to be the result of increased secretion of stored cytokines as detectable levels of IL-6 were not observed in the cell pellets of unstimulated BMMC or PGE₂-stimulated BMMC, moreover, in IgE/Ag-activated BMMC, where low levels of IL-6 were recovered from cell pellets, concurrent PGE₂ treatment slightly increased these levels rather than decreasing them as one would expect if PGE₂ was acting by facilitating the release of stored cytokine (data not shown).

Originally described as a proinflammatory cytokine, there is growing evidence that IL-6 exerts important anti-inflammatory actions both in vivo and in vitro (27). For instance, endotoxemia-induced circulating levels of proinflammatory cytokines TNF-α, MIP-2, IFN-γ, and GM-CSF were higher in IL-6 gene knockout mice than in wild-type littermates (42), and, in humans, recombinant IL-6 administration up-regulated production of antagonists for the proinflammatory cytokines, IL-1 and TNF-α (43). Moreover, PGE₂ was recently reported to induce production of the anti-inflammatory agent, α₁-acid glycoprotein, in rat alveolar macrophages costimulated with dexamethasone (44). This acute phase protein possesses antiinflammatory properties and inhibits neutrophil activation, among other anti-inflammatory effects that serve to reduce existing inflammation. In light of these data, the observed potentiation of IL-6 production by PGE₂ during IgE-mediated activation of mast cells may have in vivo significance by potentially facilitating the resolution of inflammation induced by earlier release of histamine and other proinflammatory mast cell-derived mediators.

GM-CSF is a potent growth factor for granulocytes and macrophages, and induces the differentiation of neutrophils, eosinophils, and macrophages from myeloid progenitor cells (28). GM-CSF also maintains the viability and enhances the activity of mature eosinophils and neutrophils. Our data indicate that GM-CSF production by mast cells is increased in the presence of PGE₂ and IgE-mediated activation, and such increased levels of secreted GM-CSF may partly explain the selective retention of granulocytes observed at sites of mast cell activation and PGE₂ production in chronic inflammation.

Previous studies examining the effects of PGE₂ on mast cell degranulation have led to conflicting findings. Several groups have reported an inhibitory effect of PGE₂ and PGE₁ on histamine release. Kaliner and Austen (20) demonstrated that PGE₁ (1 μM) inhibited histamine release by rat mast cells in response to FcεRI cross-linking, and a similar inhibitory effect on degranulation was observed in human lung mast cells preincubated with PGE₂ (>1 μM) for 5 min before FcεRI cross-linking with anti-IgE (21). Hogaboam et al. (19) reported that PGE₂ treatment inhibited histamine release in rat PMCs activated with calcium ionophore, A23187; however, PGE₂ was without effect on IgE-mediated histamine release by rat PMCs under the experimental conditions employed by this group. In contrast, PGE₂ has also been shown to potentiate histamine release by mast cells. Nishigaki et al. (12) reported that PGE₂ potentiated ionomycin-mediated degranulation in the murine mast cell line, BNU-2c13, and our group has previously demonstrated that although PGE₂ alone neither induced nor inhibited spontaneous histamine release by rat PMCs, PGE₂ enhanced such release from mast cells concurrently activated with anti-IgE (18). Here, we further demonstrate PGE₂-mediated potentiation of degranulation in two different mast cell populations, BMMC and the IL-3-dependent mast cell line, MC/9. As observed in rat PMCs, PGE₂ treatment alone did not induce degranulation in either mast cell population yet strongly enhanced β-hexosaminidase release induced by IgE/Ag-activation.

The opposing stimulatory and inhibitory actions described for PGE₂ in the context of mast cell degranulation may reflect differences in the timing of PGE₂ treatment relative to the administration of other stimuli, and to possible differences in EP receptor subtype expression by the mast cell populations. In studies describing an inhibitory effect for PGE₂ on histamine release, mast cells were preincubated with PGE₂ for ≥5 min before the addition of the other stimuli (19, 21); whereas, in experiments where PGE₂ potentiated mast cell degranulation, concurrent activation with PGE₂ and the secretagogue was employed (12, 18, and this study). Cyclic AMP has been implicated as the second messenger mediating PGE₂-directed inhibition of degranulation (21, 45, 46). Conversely, increased Ca²⁺ rather than cAMP was implicated in a study where degranulation was potentiated by PGE₂ (12), and these observations are not surprising considering the absolute requirement for increased intracellular Ca²⁺ in the induction of mast cell degranulation (47). The role of cAMP in mediating degranulation is less clear. Biphasic increases in cAMP are observed in IgE-mediated degranulation; however a causal link between increased CAMP and histamine release has not been established. Here, we have shown that CAMP-elevating agents, forskolin, pentoxifylline, and β-isoproterenol, fail to reproduce the enhancing effects of PGE₂ on both β-hexosaminidase release and IL-6 production, suggesting that the observed effects of PGE₂ are mediated by a CAMP-independent mechanism.

PGE₂ exerts its effects on target cells by interacting with specific G protein-coupled receptors, of which there are four subtypes (EP₁, EP₂, EP₃, and EP₄). EP₁ coupling elevates intracellular Ca²⁺ levels; signaling through EP₂ and EP₃ results in the activation of adenylate cyclase and subsequent increases in intracellular cAMP; and signaling through EP₃ is generally associated with diminished levels of intracellular cAMP although a number of splice variants of this receptor coupled to different G proteins have been described (22). Using RT-PCR, we have demonstrated that MC/9 cells express EP₁, EP₂, and EP₄, but not EP₂ receptors. The presence of EP₁ and EP₄ receptors has been reported for the mucosal type mast cells BNU-2c13 (12) and P815 (11), respectively. EP₁ and EP₄ receptors are ubiquitously expressed in tissues (51) and have been identified on murine macrophage-like cell line, RAW 264.7 cells (29), primary and transformed murine B lymphocytes (48, 49), and human HSB.2 early T cells (50). EP₁ expression is somewhat more limited, and is most abundantly expressed in the kidney (51) where
it is restricted to the collecting duct and regulates natriuretic actions of PGE2 (52). Using primers specific for EP1, we observed two bands, a minor band of 336 bp corresponding to the expected PCR product and a stronger band of ~750 bp. Thus far, a splice variant for EP1 receptors (EP1-v) has only been described in the rat and arises from failure to use a potential splice site located in the sixth transmembrane domain (37). In contrast to the EP1 receptor, EP1-v is devoid of a carboxyl terminus and lacks signaling capacity. Experiments where CHO cells were cotransfected with EP1 and EP1-v showed that although the variant receptor alone was not coupled to Ca\(^{2+}\) mobilization, it inhibited Ca\(^{2+}\) mobilization mediated by EP1 (37) and hence, may serve as a sink for the EP1 receptor (53). The larger EP1 PCR product observed in this study is of the predicted size for a splice variant analogous to that observed in the rat, and did not arise from genomic DNA contamination in RNA samples. Sequence analysis confirmed that it contained the second intron as would be expected in the absence of splicing events occurring in the sixth transmembrane domain during processing of primary RNA transcripts.

To identify the EP receptors mediating PGE\(_2\)-directed potentiation of degranulation and IL-6 production, MC/9 cells were stimulated with EP subtype-selective agonists in the presence of IgE/Ag-activation. Both the EP2 agonist, 17-phenyl-\(\omega\)-trinor-PGE\(_2\), and the EP2/EP3 selective agonist, sulprostone, potentiated \(\beta\)-hexosaminidase release and IL-6 production in IgE/Ag-activated mast cells. Misoprostol, an EP2/EP3 selective agonist also enhanced IgE-mediated degranulation. Such potentiation of degranulation or IL-6 production was not observed with the EP2/EP3-selective agonist, PGE1 alcohol. PGE1, a structural homologue of PGE2, that binds EP1 with weaker affinity than PGE2 and binds with comparable affinity to EP2, EP3, and EP4, enhanced IgE-mediated degranulation to a similar degree as PGE2, but did not potentiate IL-6 production. Taken together, these findings strongly suggest the involvement of both EP1 and/or EP3 receptors in PGE\(_2\)-directed potentiation of degranulation and IL-6 production by IgE/Ag-activated mast cells.

The importance of EP1 and/or EP3 receptors in regulating mast cell function is intriguing in view of the fact that EP2 and EP4 receptors have generally been associated with immunomodulatory modulation. For instance, TNF-\(\alpha\) inhibition in human blood monocytes (54), B cell differentiation to IgE-secreting plasma cells (48), and IL-8 production by human colonic epithelial cells (55) have all been reported to be mediated by PGE2 via EP2 and/or EP4 receptors. Moreover, in the human HSB-2 early T cell line, PGE2 induced IL-6 production via EP2/EP4 receptors, and costimulation with Con A further enhanced IL-6 levels by up-regulating EP2 receptor expression and down-regulating that of EP2 and EP3 (50). Interestingly, a study by Kozawa and colleagues (56) investigating PGE2-induced IL-6 synthesis in the murine osteoblast-like cell line, MC3T3, reported that both EP1 and EP3 receptors contributed to the production of IL-6. These data implicate the involvement of second messengers, Ca\(^{2+}\) and cAMP in IL-6 induction by osteoblasts, and a similar role for these two second messengers may be involved in IL-6 production by mast cells as rat PMC IL-6 production is both highly calcium dependent and is induced by the cAMP-elevating agent, cholina toxin (47). In this study, EP1 and/or EP3 appear to play a substantial role in mast cell IL-6 production. Although activation of EP1 receptors is generally associated with diminished intracellular cAMP levels, an isoform in the mouse has been shown, at higher agonist concentrations, to stimulate adenylate cyclase and increase intracellular cAMP levels (22). Coupling through EP3 has also been linked with elevated Ca\(^{2+}\) in the murine mast cell line, Bnu-2c13 (12). Hence, stimulation of mast cells with PGE2 alone may, through coupling to EP1/EP3 receptors, elevate intracellular Ca\(^{2+}\) and/or cAMP to levels exceeding the threshold required for IL-6 production, and it is possible that concurrent activation with PGE2 and IgE/Ag results in synergism of such initial responses leading to potentiation of IL-6 production.

Taken overall, our results suggest a more complex role for PGE2 in the modulation of allergic inflammation and disease than has been previously recognized. We have demonstrated that PGE2 modulates IL-6 production in otherwise unstimulated BMMC with no change in the production of many other cytokines or in the induction of mast cell degranulation. However, in the context of IgE-mediated activation, PGE2 enhances preformed mediator release and selectively up-regulates the production of IL-6 and GM-CSF, and these effects likely occur through coupling to EP3 and/or EP4 receptors. The residence of mast cells in the skin and mucosal linings positions them among our first line of defense against environmental insults, irritants, and pathogens. Mast cell mediators induce PGE2 production by neighboring tissue cells (57, 58), and newly secreted PGE2 may act to modulate cytokine production by mast cells and alter localized inflammatory reactions in an autocrine and paracrine manner. Understanding the mechanisms by which PGE2 modulates cytokine production will undoubtedly be of prime importance if we are to harness the beneficial effects of prostanoids and related molecules in the treatment of inflammatory disease.

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References


