The Immunomodulatory Actions of Prostaglandin E₂ on Allergic Airway Responses in the Rat

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PGE_2 has been reported to inhibit allergen-induced airway responses in sensitized human subjects. The aim of this study was to investigate the mechanism of anti-inflammatory actions of PGE_2 in an animal model of allergic asthma. BN rats were sensitized to OVA using *Bordetella pertussis* as an adjuvant. One week later, an aerosol of OVA was administered. After a further week, animals were anesthetized with urethan, intubated, and subjected to measurements of pulmonary resistance \( (R_L) \) for a period of 8 h after OVA challenge. PGE_2 (1 and 3 \( \mu g \) in 100 \( \mu l \) of saline) was administered by insufflation intratracheally 30 min before OVA challenge. The early response was inhibited by PGE_2 (3 \( \mu g \)). The late response was inhibited by both PGE_2 (1 and 3 \( \mu g \)). Bronchoalveolar lavage fluid from OVA-challenged rats showed eosinophilia and an increase in the number of cells expressing cysteinyl-leukotrienes (cys-LTs) in BALB/c mice through inhibition of IFN-\( \gamma \). Since PGE_2 is anti-inflammatory and, in particular, may have inhibitory effects on T cells that are importantly involved in both LAR and AHR (4, 5).

The airway responses to allergen challenge are complex phenomena that involve mast cells, eosinophils, and T cells, in particular CD4^+ T cells of the Th2 phenotype (6). PGE_2 has been shown to have effects on all of these cell types in vitro, although some effects are likely to promote allergic inflammation, whereas others may act to suppress it. PGE_2 may induce IL-6 but suppress TNF-\( \alpha \) synthesis by mast cells (7). It also promotes histamine and GM-CSF release from mast cells (8). PGE_2 inhibits IgE production by human B cells (9), but it may also affect T cell help for Ab synthesis so as to favor IgE synthesis and allergic-type inflammation (10). A similar role in Th2 biasing of T cell responses has been shown in BALB/c mice through inhibition of IFN-\( \gamma \) (11).

The diverse biological effects of PGE_2 are attributable to the presence of four receptors (EP_1–4), of which two are positively coupled to adenylyl cyclase (EP_2 and EP_4) and two (EP_1 and EP_3) act by stimulating phosphoinositide-specific phospholipase C and inhibit adenylyl cyclase (reviewed in Ref. 12). It is difficult to predict the nature of the in vivo effects of PGE_2 on allergic airway responses because of the complexity of its cellular effects. We hypothesized, however, that PGE_2 would inhibit Th2 cytokine expression and cys-LT synthesis in the airways after allergen challenge and in so doing would inhibit allergic bronchoconstriction. The aim of this study was to examine the effects of PGE_2 on both allergen-induced early (EAR) and late bronchoconstriction and allergic inflammation in a well-characterized model of allergic asthma. To investigate the mechanism of these effects, we measured Th1 and Th2 cell markers, cys-LT levels in BALF, and the distribution of PGE_2 receptor subtypes in the lung.

**Materials and Methods**

**Animals and sensitization**

Male BN rats between 7 and 9 wk of age were purchased from Harlan Sprague Dawley U.K. (Blackthorn, U.K.) and maintained in a conventional animal facility at McGill University (Montreal, Canada). All rats were actively sensitized with a s.c. injection of 1 mg of OVA (grade V; Sigma-Aldrich, St. Louis, MO) precipitated in 4.28 mg of aluminum hydroxide gel (Anachemia Chemicals, Montreal, Canada) in 1 ml of normal saline. Simultaneously, 0.5 ml of *Bordetella pertussis* vaccine containing 6 \( \times 10^9 \) heat-killed bacilli/ml (Institut Armand Frappier, Laval-Des-Rapides, Canada) was injected i.p. as an adjuvant. A booster sensitization was performed at 7 days. Animals were anesthetized with pentobarbital (35 mg/kg i.p.), intubated, and exposed to an aerosol of 5% OVA (w/v) for 5 min.
For the evaluation of the effects of PGE\textsubscript{2} on the EAR, LAR, and airway inflammation, four groups of sensitized rats were studied. The first group was challenged with aerosolized OVA after administration of the vehicle saline (100 μl) intratracheally by insufflation. The second group was challenged with aerosolized OVA after an intratracheal insufflation of PGE\textsubscript{2} (1 μg). The third group was challenged with aerosolized OVA after an intratracheal insufflation of PGE\textsubscript{2} (3 μg), and the fourth group was challenged with aerosolized BSA after an intratracheal insufflation of saline (100 μl).

Measurement of airway responses to Ag challenge

Two weeks after sensitization, animals were anesthetized with urethane (1.25 g/kg i.p.) for measurements of allergen-induced airway response. Animals were intubated endotracheally with polyethylene tubing (PE240; Commercial Plastics, Montreal, Canada) and placed on a heating pad to maintain a rectal temperature of 36°C. Airflow was measured by placing the tip of the endotracheal tube inside a Plexiglas box (~250 ml). A pneumotachograph (Fleisch No. 0; Bionetics, Montreal, Canada) coupled to a differential transducer (PX 170-14DV; Omega Engineering, Stamford, CN) was connected to the other end of the box to measure airflow. A water-filled catheter connected to a pressure transducer (Transpac II; Sorensen, Abbott, IL) was advanced into the lower end of the esophagus to measure changes in transpulmonary pressure. Pulmonary resistance (R\textsubscript{L}) was determined by multiple linear regression from transpulmonary pressure and airflow using commercial software (RHT Infodat, Montreal, Canada) (13). Animals were challenged for 5 min with an aerosol of either OVA or BSA (5% w/v). A disposable nebulizer (Hudson model 1400; Hudson, Temecula, CA) was used with an output of 0.15 ml/min. R\textsubscript{L} was measured every 5 min for 30 min after challenge and subsequently at 15-min intervals for a total period of 8 h. The EAR was defined as the maximal value of R\textsubscript{L} expressed as percent baseline R\textsubscript{L} in the first 30 min after challenge. The LAR was calculated as the area under the curve of R\textsubscript{L} against time (cm H\textsubscript{2}O ∙ ml\textsuperscript{-1} ∙ s) from 3 to 8 h after challenge, after correction of R\textsubscript{L} for the baseline value. Animals were then sacrificed for bronchoalveolar lavage (BAL).

Bronchoalveolar lavage

BAL was performed 8 h after challenge with five instillations of 5 ml of saline. The first 5-ml aliquot was spun, and the supernatant was used for analysis of cys-LTs. Approximately 22 ml of fluid were recovered with each BAL, and the volume did not differ significantly among treatment groups. The total cell count and cell viability were estimated using a hemacytometer and trypan blue stain. Slides were prepared using a Cytospin model II (Shandon, Pittsburgh, PA). The differential cell count was assessed with May-Grunwald-Giemsa staining, eosinophil counts by immunocytochemistry, and IL-4, IL-5, and IFN-γ mRNA by in situ hybridization.

Immunocytochemistry and in situ hybridization for eosinophils and T cell cytokines

Cytospin slides were prepared on poly-L-lysine-coated glass slides, fixed in 4% paraformaldehyde, and washed with PBS before processing. BAL cells were immunostained with an Ab, BMK13 mAb (kindly provided by Dr. R. Moqbel (University of Alberta, Edmonton, Canada), directed against major basic protein (MBP) using the alkaline phosphatase anti-alkaline phosphatase method. MBP-positive cells were counted by an investigator blinded to group status. A minimum of 500 BAL cells was counted, and the percentage of cells expressing MBP immunoactivity was evaluated.

In situ hybridization was performed as previously described (14) on cytopsins from rats in experimental groups 1, 3, and 4. Antibody and sense riboprobes were prepared from cDNAs coding for rat IL-4, IL-5, and IFN-γ mRNA. cDNAs were first inserted into a PGEM vector and linearized with appropriate enzymes. In vitro transcription was conducted in the presence of [\textsuperscript{35}S]-UTP and the T7 or SP6 RNA polymerases. After perreamplification and prehybridization steps, the preparations were incubated with antisense or sense probes (10^6 cpm/unit). Posthybridization washing was performed in decreasing concentrations of standard saline citrate at 40°C. Unhybridized single-strand RNA was removed by RNase A (20 mg/ml). After dehybridization, the slides were immersed in NBT2 emulsion and exposed for 10 days. The autoradiographs were developed in Kodak D-19, fixed, and counterstained with hematoxylin. Slides were coded, and positive cells were counted blindly. For negative controls, cytopsins were hybridized with sense probes or pretreated with RNase before the application of probes.

\textbf{PGE\textsubscript{2} induces allergic airway responses}

We examined the distribution of EPR mRNA in the lungs of two sensitized and two naive rats. Fourteen days after sensitization, the lungs were removed, fixed, and sectioned in 4-μm-thick slices on silane-coated slides. Hybridization was performed using digoxigenin-labeled RNA probes. The probes were constructed using PCR amplification and cloning into the PCR-II vector (Invitrogen, San Diego, CA). The cRNA probe for the EP receptor mRNA was obtained from a 226-bp fragment from 715 to 941 of the eEP\textsubscript{γ} coding sequence. For the EP\textsubscript{1} receptor, we used a 192-bp cRNA probe hybridizing to a segment corresponding to positions 683–875. For the EP\textsubscript{2} receptor, we used a 242-bp cRNA probe hybridizing to positions 765–1007. This particular probe hybridizes with all EP\textsubscript{2} splice variants. Finally, for the EP\textsubscript{3} receptor, we used a 321-bp cRNA probe hybridizing to positions 1121–1133. The oligonucleotides were linealized, and digoxigenin-labeled cRNA sense and antisense probes were synthesized using the DIG-RNA labeling kit from Boehringer Mannheim (Laval, Canada). For in situ hybridization, a standard protocol was used as previously described (15). Detection of the digoxigenin-labeled cRNA probe was performed using HRP-linked Abs (Boehringer Mannheim) and diaminobenzidine substrate (Pierce, Rockford, IL). The tissues were counterstained with hematoxylin (Fisher, Nepean, Canada).

Analysis of BAL cys-LTs

The levels of cys-LTs in BALF were measured using an enzyme immunoassay following the manufacturer’s instructions (Cayman Chemical, Ann Arbor, MI). The antisera is reported to have cross-reactivity for leukotrienes (LT) C\textsubscript{4} (100%), D\textsubscript{4} (100%), and E\textsubscript{4} (67%). Methanol was added to each BALF sample (500 μl), and the precipitated protein was removed by centrifugation. A solid phase extraction cartridge (Cayman Chemical) was used to extract cys-LTs that were eluted with HPLC grade hexane. The samples were then dried by vacuum centrifugation and reconstituted in enzyme immunoassay buffer. Samples were placed in 96-well plates in duplicate and incubated for 18 h at room temperature with acetylicholines- terase tracer and cys-LT antisera. Ellman’s reagent was then added and developed for 60–90 min. The plates were read at 405 nm. A standard curve was constructed using concentrations of LTD\textsubscript{2} ranging from 7.8 to 1000 pg/ml. Results were not corrected for extraction efficiency.

\textbf{Statistical analysis}

Comparisons among several means were performed by ANOVA, and post hoc testing was done with a Tukey test or a Fisher least significant difference test. Values of \( p < 0.05 \) were considered significant.

\textbf{Results}

\textbf{Effects of PGE\textsubscript{2} on early and late airway responses to OVA challenge}

Sensitized rats undergoing challenge with OVA showed rapid albeit minor increases in R\textsubscript{L} (Fig. 1). The increase in R\textsubscript{L} was sustained throughout most of the 8-h period of observation after challenge. There were further superimposed peaks in R\textsubscript{L} (Fig. 1). The increase in R\textsubscript{L} after OVA challenge was significantly inhibited by PGE\textsubscript{2} (1 μg) pretreatment compared with the OVA-sensitized and OVA-challenged controls (\( p < 0.01 \)). The LAR after OVA challenge was statistically significantly inhibited by PGE\textsubscript{2}, 1 μg (\( p = 0.02 \)) and 3 μg (\( p = 0.002 \)), and was different from the BSA-challenged controls (\( p = 0.004 \); Fig. 3).

\textbf{Effects of PGE\textsubscript{2} on BALF cell counts}

The total cell counts were significantly higher in the OVA-challenged rats with or without PGE\textsubscript{2} (3 μg) pretreatment compared with the BSA-challenged rats (\( p = 0.04 \); Fig. 4). The differential cell counts obtained using the May-Grunwald-Giemsa stain did not
show any statistically significant differences. Although granulocyte numbers tended to be lower in the PGE2-pretreated rats, these differences were not significant. No attempt was made to distinguish between neutrophils and eosinophils using May-Grunwald-Giemsa staining because of previous observations that this stain tends to lead to an underestimate of eosinophil numbers in OVA-challenged BN rats (16). For this reason, eosinophils were analyzed using immunochemical staining instead. There was a substantial eosinophilia after OVA challenge (p < 0.001). After 3μg PGE2 pretreatment, there was a substantial reduction in eosinophil numbers to 2% of BAL cells (p < 0.001; Fig. 5). This latter value was not different from eosinophil numbers in the BALF of the BSA-challenged rats.

Effects of PGE2 pretreatment on IL-4, IL-5, and IFN-γ mRNA-positive cells in BALF

Cytokine expression in BAL cells from OVA- or BSA-challenged and saline-pretreated rats was compared with OVA-challenged and PGE2-pretreated animals. Only rats pretreated with PGE2 3μg intratracheally were studied because this dose seemed to produce a maximal inhibitory effect on the LAR. Approximately 10% of BAL cells of OVA-challenged rats were positive for IL-5 mRNA by in situ hybridization (Fig. 6A). This number was reduced to 3% by PGE2 (p < 0.001) which was comparable with the BSA-challenged rats (2%; p = NS). IL-4 mRNA-positive cells were ~8% of the BAL cells in OVA-challenged rats (Fig. 6B), and there was a small but significant reduction in this number to ~6% after PGE2 pretreatment (p = 0.012). About 3% of BAL cells were IL-4 positive in the BSA-challenged group, which was significantly less than in the PGE2-pretreated group (p = 0.003) and in the OVA-challenged group (p < 0.001). IFN-γ expression was very low in PGE2-pretreated rats.
saline-pretreated and OVA-challenged rats and was higher in BSA-challenged controls ($p < 0.012$; Fig. 6C). PGE$_2$-treated animals had intermediate numbers of IFN-γ cells that were not significantly different from either of the other groups.

**Effects of PGE$_2$ on cys-LT levels in BALF**
There was a significant difference in BALF levels of cys-LTS among the OVA-challenged/saline-pretreated, OVA-challenged/PGE$_2$-pretreated and BSA-challenged groups of rats ($p = 0.02$ by ANOVA). The increase in cys-LTs in the BALF of OVA-challenged rats was significantly higher than in rats after BSA challenge ($p = 0.047$; Fig. 7). Animals treated with PGE$_2$ before challenge had substantial and significant inhibition of cys-LTs ($p = 0.007$ compared with OVA-challenged and saline-pretreated rats).

**Pulmonary EP receptor distribution**
The distribution of EP receptors was examined by in situ hybridization. There were no obvious differences between naive and sensitized animals. The most abundant receptor mRNA found was that of the EP4 subtype (Fig. 8). There was weak staining for the EP3 receptor on the alveolar epithelium. There was no detectable mRNA for any of the EP receptors on airway smooth muscle cells.

**Discussion**
The results of this study demonstrate the ability of PGE$_2$ to modulate the magnitude of allergic bronchoconstriction and inflammation in an animal model. The reduction in Th2 cell cytokines that was observed suggests the possibility that an inhibitory effect on the CD4$^+$ T cells was responsible for the reduction in airway inflammation. There was also a potent inhibition of cys-LT synthesis as evidenced by total inhibition of the allergen-induced increase in these mediators in BALF. The cys-LTs are of particular importance in mediating the LAR (17–19), and the reduction of their synthesis by PGE$_2$ could account for the observed reduction of the LAR. The EAR was also significantly inhibited by PGE$_2$. The predominant EP receptor expressed in the lungs was EP4. None of the EP receptors was detected by in situ hybridization on the airway smooth muscle.

Because PGE$_2$ has been shown to induce bronchodilation in mice through interaction with EP$_2$ receptors (20), it is possible that a direct effect on airway smooth muscle cells could have contributed to the inhibitory response to this PG. However, the paucity of

**FIGURE 5.** Effects of PGE$_2$ on OVA-induced BAL eosinophilia. Eosinophil counts were determined using immunostaining of cytopsins with BMK13 mAb and the alkaline phosphatase anti-alkaline phosphatase technique. There was a significant increase in eosinophil numbers in OVA/OVA rats, and there was a significant inhibition of eosinophilia by PGE$_2$.  

**FIGURE 6.** Effects of PGE$_2$ on OVA-induced T cell cytokine expression in BALF cells. IL-4 mRNA expression (A) assessed by in situ hybridization was increased in OVA/OVA animals compared with OVA/BSA animals and was significantly reduced by PGE$_2$. IL-5 expression (B) was also significantly elevated after OVA challenge and inhibited by PGE$_2$. IFN-γ expression (C) was lower in OVA-sensitized and OVA-challenged animals than in BSA-challenged rats. IFN-γ expression was intermediate in PGE$_2$-pretreated animals and was not significantly different from the other groups. Post hoc comparisons are shown by square brackets. +ve, Positive.  

**FIGURE 7.** Effects of PGE$_2$ on cys-LTs in BALF following OVA challenge. The cys-LTS were assayed by ELISA. There was a significant increase in levels after OVA challenge of OVA sensitized rats ($p = 0.047$ compared with the OVA/BSA group) that was significantly reduced in PGE$_2$-treated animals ($p = 0.007$ compared with the OVA/OVA group).
expression of EP receptors on airway smooth muscle cells in the BN rat would suggest that the allergic airway responses may not have been mediated primarily by a direct effect of PGE2 on airway smooth muscle. However, such an effect is not excluded by the current study because the inhibition of the EAR that was observed may have been the result of either inhibition of airway smooth muscle contraction or inhibition of mast cell degranulation. The extent to which direct actions of PGE2 on airway smooth muscle account for any of its salutary effects on allergic airway responses in the current study or in any of the published studies is unclear. There are several studies of the effects of PGE2 on the mast cell. It has been reported to both enhance IgE-dependent mast cell degranulation (histamine release) in vitro (7, 8) and inhibit Ag-induced histamine release (21). Although PGE2 has been shown to reduce the early response in human subjects, it appears to do so predominantly through an effect on PGD2 synthesis (3). In this latter study, the effect of PGE2 on cys-LTs was not significant, suggesting a differential effect on the inhibition of these two arachidonate-derived mediators synthesized by the mast cell. The inhibition of the late response in the current study can also be accounted for by inhibition of mediator release.

In vitro experiments have provided substantial evidence of the potential for PGE2 to have anti-inflammatory and immunomodulatory actions. PGE2 has been shown to alter mast cell cytokine synthesis; it induces IL-6 (7, 8) and GM-CSF (8), whereas it suppresses TNF-α synthesis (7). Several other targets of the actions of PGE2 warrant mention, in particular B and T lymphocytes. Our data using in situ hybridization indicate that T cell cytokine expression was reduced by PGE2, indicating an effect on T cell function. Because T cell activation is presumably caused by presenta-
tion of Ag to T cells in the airway wall, then PGE2 could exert effects on the T cell through actions either on the T cell itself or on APCs. Mitogen-stimulated CD4+ T cells have been shown to undergo a change in phenotype in vitro on exposure to PGE2 with a reduction in IFN-γ and an increase in IL-5 expression (10). Differences in the sensitivity of APCs to the inhibitory properties of PGE2 have also been invoked as an explanation for Th2 biasing in BALB/c mice (11). Our results indicate that the net result of the complex actions of PGE2 in vivo is a selective inhibition of Th2 cytokines whereas IFN-γ, representative of Th1 cytokines, is unchanged.

PGE2 has effects that are of interest in the consideration of this substance as a therapeutic agent for allergic asthma. It inhibits IL-4-induced production of IgE by human B cells (9). There is contrary evidence indicating that PGE2 may also affect T cell help for Ab synthesis so as to favor IgE synthesis and allergic type inflammation. PGE2 has been shown to suppress the Th1 type cytokines IL-2 and IFN-γ cells and to stimulate Th2 cells (10, 22). A similar role in Th2 biasing of T cell responses has been shown in BALB/c mice through inhibition of IFN-γ (11). This effect seems to be in part mediated by an inhibition of IL-12p70 heterodimer production by Ag APCs to PGE2 in this mouse strain (11). Other reports have shown that murine B cells may respond to PGE2 analogs with an enhancement of IL-4-induced IgE production (23).

PGE2 inhibited airway eosinophilia by ~80%. There are several potential mechanisms for this observation. IL-5 is a central cytokine involved in the induction of eosinophilia by allergen challenge (24–26) interacting with other protein chemoattractants such as eotaxin (27). Ab-neutralizing experiments on models of allergic

**FIGURE 8.** Distribution of EP4 receptors by in situ hybridization for mRNA expression. There was endothelial expression of the EP4 receptor (top left). There was also expression of the EP4 receptor subtype on alveolar epithelium (top right) and inflammatory cells (bottom left). There was bronchial epithelial expression of the EP4 receptor, but no expression was detected on airway smooth muscle (bottom right). Arrows indicate positive staining in cell types referred to in the four panels.
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as asthma as well as knockout mice show a clear dependence of eosinophilia on IL-5 (26, 28). It is quite plausible that the inhibition of IL-5 by PGE₂ is responsible, at least in part, for the reduction of eosinophilia. It is also possible that PGE₂-induced inhibition of PGD₂ release by mast cells could have contributed to the inhibitory effect on eosinophil infiltration as PGD₂ has been shown to be a potent chemottractant for these cells (29). Cys-LTs were also strikingly reduced in BALF and, although not potently chemotactic, they have also been shown to be involved in the induction of eosinophilia by OVA challenge in sensitized mice (30) and rats (31). This finding provides another potential mechanism for the reduction in eosinophilia. Eosinophilia perhaps could be reduced also by effects of PGE₂ on the eosinophil itself. Interestingly, cycooxygenase-deficient mice have exaggerated airway inflammation after sensitization and challenge, indicating that cyclooxygenase products produced endogenously are in sufficient concentrations to modulate allergic airway responses (32). Indomethacin treatment has similar effects in mice (33).

The results of the current experiments clearly indicate sensitivity to PGE₂ of the cells synthesizing cys-LTs during the late response. The inhibition of cys-LTs in the BAL fluid of OVA-challenged animals in the current experiments was substantial. The observed inhibition exceeded the effects of a single administration of either topical or systemic corticosteroids on cys-LTs in the rat (34, 35). The mechanism by which cys-LTs were inhibited is not known. Although unproven, eosinophils are considered to be a probable source of cys-LTs in human asthmatic subjects. In rodents, however, eosinophils do not synthesize significant amounts of cys-LTs (36, 37). Mast cells or macrophages are alternative sources (37), but to date their implication in cys-LT synthesis in vivo in rats has not been confirmed. Inhibition by PGE₂ of the synthesis of LTB₄, another product of 5-lipoxygenase, by polymorphonuclear leukocytes has also been reported (38). If this occurred in the current experiment, it may have contributed to the reduction in BAL eosinophilia, that has been shown to be inhibited by antagonists of LTB₄ in this animal model (39).

In conclusion, PGE₂ is a potent inhibitor of allergic airway responses in the BN rat model of allergic asthma. The effects are likely exerted at several sites, but the current study clearly shows effects on T cell cytokine expression and on the control of eosinophilia. The synthesis of cys-LTs is also inhibited by PGE₂. These findings support the notion that PGE₂ could be a useful anti-inflammatory treatment for asthma. However PGE₂ has complex immunomodulatory properties in vitro so that elucidation of the pertinence of these effects in vivo would be important preceding the application of this molecule as a therapeutic agent.

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References


