Prostaglandin E₂-induced interleukin-6 release by a human airway epithelial cell line

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Tavakoli, S., M. J. Cowan, T. Benfield, C. Logun, and J. H. Shelhamer. Prostaglandin E₂-induced interleukin-6 release by a human airway epithelial cell line. Am J Physiol Lung Cell Mol Physiol 280: L127–L133, 2001.—Human airway epithelial cell release of interleukin (IL)-6 in response to lipid mediators was studied in an airway cell line (BEAS-2B). Prostaglandin (PG) E₂ (10⁻⁷ M) treatment caused an increase in IL-6 release at 2, 4, 8, and 24 h. IL-6 release into the culture medium at 24 h was 3,396 ± 306 vs. 1,051 ± 154 pg/ml (PGE₂-treated cells vs. control cells). PGE₂ (10⁻⁷ to 10⁻¹⁰ M) induced a dose-related increase in IL-6 release at 24 h. PGF₂α (10⁻⁶ M) treatment caused a similar effect to that of PGE₂ (10⁻⁷ M). PGE₂ analogs with relative selectivity for PGE₂ receptor subtypes were studied. Sulprostone, a selective agonist for the EP-3 receptor subtype had no effect on IL-6 release. 11-Deoxy-16,16-dimethyl-PGE₂, an EP-2/4 agonist, and 17-phenyl trinor PGE₂, an agonist selective for the EP-1 > EP-3 receptor subtype (10⁻⁶ to 10⁻⁸ M), caused dose-dependent increases in IL-6 release. 8-Bromo-cAMP treatment resulted in dose-related increases in IL-6 release. RT-PCR of BEAS-2B cell mRNA demonstrated mRNA for EP-1, EP-2, and EP-4 receptors. After PGE₂ treatment, increases in IL-6 mRNA were noted at 4 and 18 h. Therefore, PGE₂ increases airway epithelial cell IL-6 production and release.

cytokines; eicosanoids; lung inflammation

THE AIRWAY EPITHELIUM is a first contact site for external stimuli such as infectious agents, exogenous inhaled antigens, and noxious agents. It may serve as a first line of defense against these stimuli by providing mucous secretion and mucus clearance to remove the exogenous materials, or it may initiate or amplify the local inflammatory reaction in the airway (21). In the setting of the airway inflammatory reaction, epithelial cells may function as both target and effector cells. As target cells, their defense functions such as mucociliary clearance, mucous secretion, and water and ion transport may be altered by a number of proinflammatory lipid or cytokine mediators. As effector cells, epithelial cells can produce a number of cytokine products that may directly or indirectly participate in the inflammatory cascade via the recruitment, activation, and alteration of the survival of inflammatory cells within the airway. Human airway epithelial cells also synthesize and release a variety of lipid mediators including, primarily, prostaglandin (PG) E₂, 15-hydroxyeicosatetraenoic acid (HETE), PGF₂α, and platelet-activating factor (PAF) (3, 13, 15, 35). In the setting of airway inflammation, these same cells are exposed to other lipid mediators produced locally by resident or recruited inflammatory cells. These mediators include leukotriene (LT) B₄, 5-HETE and LTD₄.

Airway epithelial cells produce a variety of cytokine products including α- and β-chemokines, colony-stimulating factors, lymphocyte chemoattractant factor, and pleiotropic cytokines such as interleukin (IL)-6, IL-11, tumor necrosis factor (TNF)-α, and IL-1 (23). Secretion of these cytokines by the airway epithelium may be a primary response to external stimuli, or it may serve as a secondary response to an inflammatory mediator. It is clear that some cytokines, such as TNF-α or IL-1, can stimulate epithelial cell cytokine production. Furthermore, lipid mediators such as PAF can stimulate subsequent cellular production of lipid mediators (38). It has been reported that murine alveolar macrophages respond to PAF, LTB₄, and PGE₂ with the production of IL-6 (37). However, less is known of the interaction of these networks of lipid and cytokine mediators in the airway epithelium. Therefore, we studied whether PGE₂ or other lipid mediators present in significant quantities in inflamed airways can modulate cytokine production by a human bronchial epithelial cell line.

MATERIALS AND METHODS

Cell culture. BEAS-2B cells, a human bronchial epithelial cell line transformed by an adenovirus 12-SV40 hybrid virus, were supplied by J. E. Lechner (National Cancer Institute, National Institutes of Health, Bethesda, MD) (19). The cells were cultured in the serum-free, hormonally defined culture medium LHC-8 (Biofluids, Rockville, MD) and grown on 175-cm² tissue culture flasks (Falcon, Becton Dickinson, Oxford, CA) that were coated with a thin layer of type I rat tail collagen (Collaborative Research, Bedford, MA). For passage, cells were detached from the collagen film with 0.02% trypsin, 1% polyvinylpyrrolidone, and 0.02% ethylene glycol bis (E-PET, Biofluids) that was subsequently neutralized with

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soybean trypsin inhibitor (Biofluids). Replicate cultures were made by passing cells into either 6-well, 35-mm plates precoated with a thin layer of type I rat tail collagen (Collaborative Research) for secretion studies or into 175-cm² tissue culture flasks coated with a thin layer of type I rat tail collagen for gene expression studies. Experiments were performed when the cells were near confluence.

Reagents. PGE₂, PGF₂α, 5-HETE, LTB₄, LTD₄, and PAF were purchased from Calbiochem (San Diego, CA). 17-Phenyltrinitor PGE₂, a selective EP-1 > EP-3 receptor agonist (2), 11-deoxy,16,16-dimethyl-PGE₂, a relatively selective EP-2 receptor agonist (16, 28, 31), and sulprostone, a selective EP-3 receptor agonist were purchased from Cayman Chemical (Ann Arbor, MI). 8-Bromo-cAMP was purchased from Sigma (St. Louis, Mo).

Experimental design. At the start of each experiment, medium (LHC-8) was replaced by fresh medium. For IL-6 time-course experiments, PGE₂ (10⁻⁷ M), 5-HETE (10⁻⁷ M), LTB₄ (10⁻⁷ M), LTD₄ (10⁻⁷ M), or PAF (10⁻⁷ M) was added to cell cultures in six-well plates, and supernatants were collected 2, 4, 8, and 24 h later. For subsequent dose-response experiments, PGE₂ was added in concentrations ranging from 10⁻⁷ to 10⁻¹₀ M, and supernatants were collected at 24 h. Similar designs were used for dose-response experiments with PGE₂ receptor-specific agonists, with doses ranging from 10⁻⁶ to 10⁻⁹ M. DNA was measured with benzimidazole (Hoechst 33258, Jansen Chimica, Geel, Belgium) (18), and cytotoxicity was determined by lactate dehydrogenase (LDH) assay (Sigma).

ELISA for secreted IL-6. Immuno-reactive levels of IL-6 in culture medium were determined with a sandwich-type ELISA (R&D Systems, Minneapolis, MN). The microtiter plates were coated with specific murine monoclonal antibodies directed against human IL-6. One hundred microliters of cell-free culture medium diluted 1:20 were plated in duplicate for the IL-6 assay. The assay was developed by the addition of horseradish peroxidase-linked goat polyclonal antibody directed against human IL-6.

Ribonuclease protection assay. Total cellular RNA was extracted from BEAS-2B cells in 175-cm² flasks with TRIzol (RNA Century Marker template set; Ambion) were employed to determine the size of protected fragments. Both the IL-6 and GAPDH probe concentrations used were in excess of 0.001 for each time point; Fig. 2). Maximal cumulative increases in IL-6 secretion were detected at 24 h. Concentration-dependent increases in PGE₂-induced IL-6 secretion were studied at 24 h after stimulation with PGE₂ (10⁻⁷ to 10⁻¹⁰ M; P < 0.001 by ANOVA; Fig. 3). In a separate experiment, the effect of PGE₂ at 10⁻⁹ M
was tested. PGE$_2$ at $10^{-6}$ M resulted in an IL-6 concentration of $3,345 \pm 331$ pg/ml at 24 h vs. $1,129 \pm 52$ pg/ml for control cultures ($n = 12; P < 0.001$), an effect not greater than the effect of PGE$_2$ at $10^{-7}$ M. Therefore, treatment with PGE$_2$ at $10^{-7}$ M appeared to induce a peak effect. No evidence of PGE$_2$-mediated toxicity was apparent by LDH determination when compared with control cultures at 24 h. In addition, no differences in cell number were present between PGE$_2$-stimulated and control cultures at 24 h as determined by cellular DNA quantification.

The effect of PGF$_{2\alpha}$ on IL-6 production was also studied. PGF$_{2\alpha}$ in a log higher concentration ($10^{-6}$ M) also stimulated cellular IL-6 production over 2–24 h (Fig. 4A). Figure 4B presents the dose effect of PGF$_{2\alpha}$ on IL-6 production from BEAS-2B cells. This effect was dose dependent but at higher concentrations than required for PGE$_2$ stimulation.

PGE$_2$ analogs with some PGE$_2$ receptor subtype specificity were used to determine their effect on IL-6 secretion by epithelial cells. Sulprostone, an EP-3 > EP-1 receptor subtype agonist, in concentrations as high as $10^{-6}$ M had no effect on IL-6 production. An agonist with relative specificity for the EP-2/4 receptor subtypes, 11-deoxy-16,16-dimethyl-PGE$_2$, stimulated IL-6 production in a dose-dependent manner, whereas 17-phenyl trinor PGE$_2$, an EP-1 receptor subtype agonist, also stimulated IL-6 production in a dose-dependent manner at concentrations ranging from $10^{-6}$ to $10^{-8}$ M (Fig. 5).
Effect of 8-bromo-cAMP on IL-6 release. Ligand binding and activation of the EP-2 or EP-4 receptor subtypes resulted in the activation of adenylate cyclase and an increase in intracellular cAMP. Therefore, 8-bromo-cAMP was added to cells to determine if this cAMP analog was capable of inducing changes in IL-6 production. As demonstrated in Fig. 6, the addition of 8-bromo-cAMP to cell cultures induced dose-related increases in IL-6 release, suggesting that the PGE₂ induction of IL-6 release was, at least in part, mediated via EP-2/4 receptor activation.

Detection of PGE₂ receptor subtype mRNAs. Because a PGE₂ receptor agonist with some specificity for the EP-1 receptor and an agonist with some specificity for the EP-2 receptor caused an increase in epithelial cell release of IL-6, studies were performed to determine whether these cells expressed mRNA for these receptors. RT-PCR was performed on total cellular RNA from BEAS-2B cells and, as controls, on total cellular RNA from HeLa cells and RNAs from lung and kidney. As can be seen in Fig. 7, RT-PCR with primers specific for mRNA for the EP-1 receptor subtype revealed an appropriately sized band of RNA from BEAS-2B cells, from lung tissue, and from kidney tissue (Fig. 7A). Similarly, RT-PCR with primers specific for the EP-2 receptor subtype produced an appropriately sized band of RNA from BEAS-2B cells and from lung and kidney but not from HeLa cells (Fig. 7B). In addition, RT-PCR with primers specific for the EP-4 receptor subtype resulted in the production of an appropriately sized band (Fig. 7C). Figure 7D presents the results of RT-PCR for β-actin and demonstrates undegraded mRNA from BEAS-2B cells and, as controls, on total cellular RNA from HeLa cells and RNAs from lung and kidney.
in all samples. Therefore, BEAS-2B cells express mRNA for the EP-1, the EP-2, and the EP-4 receptor subtypes.

Effect of PGE_2 on IL-6 mRNA levels. PGE_2 treatment of these airway epithelial cells resulted in an increase in IL-6 production over a prolonged period of time. Therefore, experiments were performed to determine whether the PGE_2 treatment resulted in an increase in the cellular steady-state levels of IL-6 mRNA during this time. BEAS-2B cells were stimulated with PGE_2 (10^{-7} M) for 4 or 16 h. Total cellular RNA was extracted, and RPAs were performed. As demonstrated in Fig. 8, PGE_2 treatment increased the steady-state levels of IL-6 mRNA at both times, suggesting that PGE_2 modulates IL-6 gene expression and therefore augments IL-6 protein synthesis in the BEAS-2B cells.

Therefore, PGE_2 and, to a lesser degree, PGF_2 stimulation of airway epithelial cells result in an increase in IL-6 synthesis and release by these cells.

DISCUSSION

The airway epithelium has the ability to synthesize or release a variety of inflammatory mediators such as metabolites of arachidonic acid, PAF, and cytokines as well as products that may downregulate the inflammatory response, including eicosanoid metabolites, cytokine receptor antagonists such as IL-1 receptor antagonist, and solubilized cytokine receptors such as soluble TNF receptor (20, 22). These inflammatory mediators can participate in modulating the local inflammatory process in neighboring cells and tissues. Lipid mediators present in the airway may be produced by resident airway cells and by inflammatory cells resident in or recruited to the lung. Lipid mediators such as PAF, LTB_4, and PGE_2 may regulate cytokine expression in inflammatory cells such as alveolar macrophages and peripheral blood mononuclear cells (33, 34, 37).

IL-6 is a multifunctional cytokine important in the production of acute-phase proteins, the immune response to viruses and bacteria, immunoglobulin production, the differentiation of T cells, and, perhaps, for tissue regeneration as well as in a variety of endocrine effects (10, 29, 32). In the airway and alveolar spaces, IL-6 is produced by alveolar macrophages and airway epithelial cells (11). In experimental animals, intratracheal administration of IL-6 induces an infiltration of neutrophils into the interstitial space and into the alveolar space (11). Increased IL-6 has been demonstrated in bronchoalveolar lavage fluid from patients
with asthma (25). Furthermore, increased IL-6 production has been reported in airway epithelial cells from patients with asthma (24) and from alveolar macrophages harvested in the setting of the late asthmatic reaction (9).

In this study, we report that PGE₂ treatment of a human bronchial epithelial cell line increased the cellular production of IL-6 over a 2- to 24-h period and that this effect is associated with an increase in steady-state levels of IL-6 mRNA. This effect was not produced by treatment of cells with other lipid mediators such as PAF, LTB₄, and LTD₄. Although PGE₂ may have anti-inflammatory properties including suppression of cellular production of IL-1, inhibition of leukocyte migration, inhibition of superoxide and LT release from polymorphonuclear leukocytes, and inhibition of T lymphocyte proliferation (8), PGE₂ may have important proinflammatory effects as well. These may include vasodilator properties and an increase in vascular permeability in synergy with bradykinin and LTs. In an experimental animal model, carrageenan-induced tissue inflammation, edema, and hyperalgesia were prevented by the administration of an anti-PGE₂ antibody, and this treatment also reduced tissue levels of IL-6, which suggests that PGE₂ can contribute to local inflammatory responses that have been initiated by exogenous stimuli or inflammatory cell products (12, 30). Furthermore, PGE₂ has been reported to stimulate IL-6 release from murine macrophages, murine and rat osteoblasts (17, 26), and human lymphocytes (HSB-2 cells) and astrocytoma cells (7, 40). The IL-6 produced by BEAS-2B cells after PGE₂ stimulation is roughly similar to stimulated IL-6 production by other cells: 500 pg·mL⁻¹·3 h⁻¹ from rat osteoblasts (26), 400–600 pg·mL⁻¹·24 h⁻¹ from mouse osteoblasts (17), and 200–1,000 pg·mL⁻¹·12 h⁻¹ from human lung fibroblasts stimulated by transforming growth factor-β (6).

PGE receptors are pharmacologically and molecularly characterized into at least four subtypes (28). Each subtype may have distinct signal transduction pathways. The EP-1 receptor signals through activation of phospholipase C. The EP-2 receptor signals via activation of adenylyl cyclase and increases in cAMP as does the subtype designated EP-4 (1, 14, 27, 28). Activation of the EP-3 subtype signals via a reduction in cAMP (4, 28, 36). The results of the EP receptor agonist studies and the effect of 8-bromo-cAMP on IL-6 release suggest that the effect of PGE₂ on IL-6 release from BEAS-2B cells may be mediated, at least in part, through an EP-2/4 receptor linked to adenylyl cyclase.

Given the ability of cytokines, including IL-6, to activate arachidonate metabolism in some tissues (5, 21), these findings suggest that there is a complex regulatory network that may be significant in the modulation of inflammatory responses in the airway mucosa and lumen. These pathways may have an important role in the pathogenesis of inflammatory airway disorders.

REFERENCES


