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Prostaglandin E₂ Exerts Homeostatic Regulation of Pulmonary Vascular Remodeling in Allergic Airway Inflammation

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Abstract

Nonselective inhibition of PG synthesis augments inflammation in mouse models of airway disease, but the roles of individual PGs are not completely clarified. To investigate the role of PGE₂ in a mouse model of airway inflammation induced by a natural allergen, we used mice lacking the critical terminal synthetic enzyme, microsomal PGE₂ synthase (mPGES)-1. Mice lacking mPGES-1 (*ptges*^{-/-} mice) and wild-type C57BL/6 controls were challenged intranasally with low doses of an extract derived from the house dust mite *Dermatophagoides farinae* (Der f). The levels of PGE₂ in the bronchoalveolar lavage fluids of Der f-treated *ptges*^{-/-} mice were ~80% lower than the levels in wild-type controls. Der f-induced bronchovascular eosinophilia was modestly enhanced in the *ptges*^{-/-} mice. Both Der f-treated strains showed similar increases in serum IgE and IgG1, as well as comparable levels of Th1, Th2, and Th17 cytokine production by Der f-stimulated spleen cells. These findings indicated that mPGES-1-derived PGE₂ was not required for allergen sensitization or development of effector T cell responses. Unexpectedly, the numbers of vascular smooth muscle cells and the thickness of intrapulmonary vessels were both markedly increased in the Der f-treated *ptges*^{-/-} mice. These vascular changes were suppressed by the administration of the stable PGE₂ analog 16, 16-dimethyl PGE₂, or of selective agonists of the E-prostanoid (EP) 1, EP2, and EP3 receptors, respectively, for PGE₂. Thus, mPGES-1 and its product, PGE₂, protect the pulmonary vasculature from remodeling during allergen-induced pulmonary inflammation, and these effects may be mediated by more than one EP receptor.

Prostaglandins are bioactive lipid mediators that orchestrate a wide array of biologic responses in homeostasis, inflammation, and cancer (reviewed in 1). PGs form by cyclooxygenase (COX)³-mediated oxidation of arachidonic acid to PGH₂, which is converted to effector PGs by isoform-specific terminal synthases. PGE₂ is a ubiquitous PG generated by both hematopoietic (macrophages and dendritic cells) (2, 3) and nonhematopoietic (epithelial cells, fibroblasts, and smooth muscle) (4-6) cell types through conversion of PGH₂ by three specific terminal PGE₂ synthases (PGESs). A cytosolic PGES converts

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Disclosures

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PGH₂ to PGE₂ in transfected cells (7) but is not required for PGE₂ production in vivo (8). The other PGESs are membrane-bound enzymes, termed microsomal PGES (mPGES)-1 (9) and mPGES-2 (10). mPGES-1 accounts for the majority of basal PGE₂ synthesis in brain and spleen (11), and is also inducibly expressed along with COX-2 in many organs and cells. This inducible expression results in markedly increased PGE₂ production in inflammatory disease models (12). PGE₂ serves diverse (and often complex) functions. For example, PGE₂ directly mediates bone destruction in models of arthritis (13), enhances IL-23 production and suppresses IL-12 production by APCs (14), and facilitates the development of the proinflammatory IL-17-producing subset of Th17 cells in several models (14-18). However, the exogenous administration of PGE₂ also suppresses allergen-induced pulmonary inflammation in rat and mouse models of allergic pulmonary disease (19, 20). The ability of PGE₂ to either induce or suppress inflammation in different contexts may reflect the complexity of its receptor system. PGE₂ acts at four known, functionally divergent G protein-coupled receptors, termed E-prostanoid (EP) receptors (EP1, EP2, EP3, and EP4). Each EP receptor is broadly distributed to both hematopoietic and non-hematopoietic cell types, and each has a distinct signaling mechanism and set of actions, with the EP3 receptors existing as functionally diverse isoforms (21). The expression levels of some EP receptors are upregulated with cell activation (22), and many cell types coexpress two or more EP receptor types with potentially opposing or complementary actions (22, 23). Each of these factors contributes diversity and complexity to the actions of PGE₂.

Several PGs are generated concomitantly during inflammatory responses, and the precise contributions of individual PGs to the pathophysiology have not been fully clarified. PGD₂ and thromboxane (TX) are bronchoconstrictors potentially relevant to asthma (24, 25), and exogenous PGD₂ can amplify allergen-induced pulmonary eosinophilia in mice (26). However, global depletion of PGs with COX inhibitors (27) and knockouts of COX enzymes (28) substantially enhances eosinophilia, Th2 cytokine production, and airway hyper-responsiveness in mouse models of airway disease induced by allergen sensitization and challenge. PGE₂ may be one of the protective PGs in this response. Mice lacking EP3 receptors show exaggerated pulmonary inflammation and airway hyper-responsiveness in a model of allergic pulmonary inflammation induced by systemic sensitization with OVA, followed by airway challenge (19). In humans with allergic asthma, inhaled PGE₂ blocks the early- and late-phase responses to Ag challenge (29). Although COX inhibitors do not alter either lung function or clinical control of asthma in most humans, a subset of patients (~10%) has a variant (aspirin-exacerbated respiratory disease [AERD]) characterized by nasal polyposis and by episodes of airflow obstruction provoked by the administration of non-selective COX inhibitors. PGE₂ production in vitro is diminished in several cell and tissue types obtained from individuals with AERD compared with samples from controls (6, 30). Moreover, the administration of inhaled PGE₂ completely blocks aspirin-induced airflow obstruction in individuals with AERD (31). These findings suggest that PGE₂ modifies the severity and phenotypic features of asthma, and may play a dominantly suppressive role in the inflammation associated with this disease.

No studies to date have assessed the effect of a selective deficiency of endogenously generated PGE₂ (which would decrease signaling inputs from all EP receptors) in allergen-induced pulmonary inflammation. We hypothesized that mice lacking mPGES-1 would demonstrate exaggerated signatures of pulmonary inflammation and physiologic derangements when subjected to Ag challenge. In this study, using a model of pulmonary inflammation induced by the intranasal administration of an extract from the house dust mite, *Dermatophagoides farinae* (Der f), we demonstrate that the deficiency of PGE₂ modestly enhances bronchial inflammation and eosinophilia without altering allergen sensitization or the profile of cytokines generated by restimulated T cells. Unexpectedly,

however, we found that marked remodeling of the pulmonary vasculature occurred in the lungs of the Der f-challenged mice lacking mPGES-1. Administration of exogenous PGE₂ analogs concomitantly with the allergen, particularly agonists of the EP2 and EP3 receptors, reduced both inflammation and vascular remodeling. These observations suggest that although endogenously generated PGE₂ modestly suppresses allergen-induced inflammation, it acts at more than one EP receptor pathway to profoundly suppress remodeling of the pulmonary vasculature during allergen-induced pulmonary inflammation in this model.

Materials and Methods

Mice

Mice lacking mPGES-1 (*ptges*^{-/-} mice) were backcrossed to C57BL/6 mice for nine generations (2). Six-to 8-wk-old male *ptges*^{-/-} mice and age- and sex-matched C57BL/6 controls were purchased from Taconic Farms (Germantown, NY).

Reagents

Mice were treated with saline or Der f obtained from Greer Laboratories (XPB81D3A25; Lenoir, NC). The stable PGE₂ analog 16, 16-dimethyl PGE₂ was obtained from Cayman Chemical (Ann Arbor, MI). Selective agonists of the EP1 (D1-004), EP2 (AE1-259-01), EP3 (AE-248), and EP4 (AE-329) receptors were obtained from Ono Pharmaceuticals (Osaka, Japan). Each agonist is active in vivo (19), competes with PGE₂ at its target EP receptor in the low nanomolar concentration range, and is >100-fold selective for its respective target (21).

Immunization and challenge protocol

On days 0, 3, 7, 10, 14, and 17, mice were lightly anesthetized and treated intranasally with 3 µg Der f extract dissolved in 30 µl saline, or saline alone. In some experiments, 5 nmol 16, 16-dimethyl PGE₂ or the selective EP receptor agonists was administered concomitantly with the Der f. The solvent (2 µl DMSO) was evaporated with a speedvac and the agonists were resuspended in saline with Der f before each administration. Doses were chosen on the basis of those at which each agonist is known to block its target receptor with selectivity in vivo (19). Forty-eight hours after the last treatment, the mice were exsanguinated. The lungs were lavaged three times with 0.7 ml PBS/5 mM EDTA and excised. The left lower lobe was placed in fixative for pathology, whereas the remaining lobes were snap frozen and used for transcript analysis.

Restimulation of splenocytes

Spleens were collected and mechanically dispersed. Single-cell suspensions were made by passing the tissues through nylon mesh cell-strainers (BD Biosciences, San Jose, CA). RBCs were eliminated by lysis. The 2.5×10^5 lymph node cells and 4×10^6 splenocytes were seeded in 0.3 and 1 ml medium, respectively, and stimulated with 20 µg/ml Der f for 72 h. Cytokine release (IL-4, IL-5, IL-13, IL-17A, and IFN-γ) was assessed in the media with specific ELISA (eBiosciences, San Diego, CA).

Measurement of Abs and bronchoalveolar lavage fluid mediators

Serum levels of mouse IgE, IgG1, and IgG2a were measured by ELISA using mouse Ab kits (BD Biosciences). The lipid fraction of the bronchoalveolar lavage (BAL) fluid was purified with C18 Sep-Pack columns (Waters, Milford, MA). PGE₂ and cys-LT levels were measured by ELISA in accordance with the manufacturer's protocol (Cayman Chemical and

Amersham Biosciences [Piscataway, NJ], respectively). Vascular endothelial growth factor-A levels were measured by ELISA (BD Biosciences).

Quantitation of PGs by gas chromatography-mass spectrometry in inflamed lung

Lung tissue from Der f-challenged mice (~100 mg) was weighed and ground manually with a mortar and pestle and incubated for 30 min in the presence of 50 μ M arachidonic acid. The ground tissue then was added to 5 ml ice-cold methanol containing 1.0 ng each of the following internal standards: [2 H $_4$]-15-F $_{2t}$ -isoprostane ([2 H $_4$]-8-iso-PGF $_{2a}$), [2 H $_4$]-PGD $_2$, [2 H $_4$]-PGE $_2$, [2 H $_3$]-11-dehydro-TXB $_2$ (11-dehydro-TXB $_2$), and [2 H $_4$]-6-keto-PGF $_{1\alpha}$ (all purchased from Cayman Chemicals). The lipids were extracted and separated from the solid particulates by centrifugation. The liquid layer was transferred to another tube and the methanol was dried under a stream of nitrogen. The residue was reconstituted in 10 ml 0.01M hydrochloric acid and applied to a C-18 Sep-Pak column (Waters) was prewashed with 5 ml methanol and 5 ml H $_2$ O (pH 3.0). The amounts of PGs were quantitated by gas chromatography-mass spectrometry (GC-MS), as described previously (32). For each assay, the precision was $\pm 5\%$, and the accuracy for each assay was 95%.

Histology

The left lungs were fixed for at least 8 h in 4% paraformaldehyde and embedded in glycolmethacrylate. The 2.5 mm-thick glycolmethacrylate sections were stained with H&E for general morphologic examination. For histological study of the mucus-secreting cells of the airway epithelia (goblet cells), lung sections were stained with Periodic acid-Schiff (PAS). Congo red staining was used to highlight eosinophil infiltrates.

The extent of cellular infiltration in the bronchovascular bundles (BVB) was evaluated by a pathologist without knowledge of the particular mouse strain or procedure and was categorized arbitrarily as: grade 0, no inflammation; grade 1, mild inflammation; grade 2, moderate inflammation; and grade 3, severe inflammation. For each mouse, 15 BVB were evaluated.

The stained goblet cells were enumerated in at least four independent BVB from the lung sections obtained for each animal in the different experimental groups. The length of the basal lamina of the corresponding bronchus was measured by Image J (National Institutes of Health image analysis software [<http://rsbweb.nih.gov/ij>]). Only the comparable large-caliber, preterminal bronchi (diameter 200–220 μ m) were examined, because minimal changes occur in terminal bronchioles. The data were expressed as the average of goblet cell counts stained in each bronchus in each section per millimeter basal lamina.

In separate experiments, mouse tissues were fixed in 10% neutral buffered formalin for 24 h and embedded in paraffin blocks. For immunohistochemical analysis of proliferating cell nuclear Ag (PCNA) and smooth muscle actin (SMA), tissue sections were deparaffinized and hydrated. Endogenous peroxidase was inhibited by incubation with freshly prepared 3% H $_2$ O $_2$ with 0.1% sodium azide. SMA mAb (ready to use, clone 1A4, Covance, Princeton, NJ) or rabbit polyclonal PCNA Ab (1:50, clone FL-261, Santa Cruz Biotechnology, Santa Cruz, CA) was applied to the sections, and incubations were carried out for 1 h at room temperature. Ab binding was visualized with a LSAB+ System-AP kit (DakoCytomation, Carpinteria, CA) for SMA, and an ABC staining system (Santa Cruz Biotechnology) for PCNA. Slides were counterstained with H&E, dehydrated, and mounted.

For Immunofluorescence to detect both SMA and von Willebrand factor (vWF), mouse lung tissues were embedded in OCT and cut into 5- μ m-thick sections. Frozen sections were air dried and fixed in acetone for 1 min. Sections were incubated with vWF Ab (polyclonal rabbit Ab, 1:400, Dako) at 37°C for 1.5 h. Then the mixture of secondary Ab to PCNA

(Alexa Fluor 568 goat anti-rabbit IgG, 1:1000, Invitrogen, Carlsbad, CA) and FITC-conjugated SMA Ab (1:1000, Clone 1A4, Sigma-Aldrich, St. Louis, MO) was applied to the sections, and the sections were incubated at 37°C for 2 h. Sections were washed thoroughly with PBS and mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA) with the nuclear stain DAPI.

The total smooth muscle cell (SMC) numbers and the thickness of the arteriolar medial muscular layer (tunica media) were quantitated by Image J on SMA-stained sections. Digital pictures with comparable arterioles were taken from each slide, and 3–5 arterioles of each slide were quantified. “Comparable arterioles” in this assessment means subpleural medium-sized arterioles with diameters of 30–50 μm (33), where the vascular remodeling was found in Der f-treated *ptges*^{-/-} mice. The readouts were presented as the number of SMCs per 100 μm basement membrane, and as the mean thickness of the medial arteriolar walls in three to five arterioles from each section.

Results

Generation of PGE₂ during challenge of mice with Der f

To determine the role of mPGES-1 in the generation of PGE₂ in the lung during intrapulmonary sensitization and challenge with Der f, male *ptges*^{-/-} mice and age-matched wild-type (WT) C57BL/6 controls received intranasal doses of Der f twice weekly for 3 wk. Two low doses of the extract were tested to define the optimal range for inducing a biologic response. BAL fluid collected from Der f-treated WT mice 48 h after the last challenge contained higher levels of PGE₂ than those present in samples from saline-treated WT mice (Fig. 1A). There were no significant increases in PGE₂ levels in the BAL fluid collected from the challenged *ptges*^{-/-} mice. To determine whether the absence of mPGES-1 changed the levels of any other PGs potentially relevant to pulmonary inflammation, lungs from the Ag-challenged mice were mechanically dispersed and incubated for 30 min in the presence of arachidonic acid (50 μM), and the supernatants were analyzed by GC-MS. As was the case for the ELISA analysis of BAL fluid, levels of PGE₂ detected by GC-MS in the supernatants from the lung cells of the *ptges*^{-/-} mice were decreased by ~70% relative to the WT controls. In contrast, the two strains showed no differences in the levels of PGF₂ α , 6-keto-PGF₁ α , PGD₂, or TXB₂ (Fig. 1B). For both the WT and the *ptges*^{-/-} mice, the levels of cysteinyl leukotrienes in the BAL fluid were similar and near the lower limit of detection for the ELISA assay (5–10 pg/ml, not shown).

Der f-mediated immune responses in PGE₂-deficient mice

To determine whether the absence of mPGES-1 altered sensitization to the Der f Ag, serum was collected from the mice for the measurement of total IgE and IgG1. When compared with the saline-treated control animals, Der f-treated mice of both genotypes showed comparably increased levels of total IgE and IgG1 (Fig. 2A). To determine whether the absence of mPGES-1 altered the pattern of cytokine generation during a recall response, single-cell suspensions were prepared from the spleens of both saline- and Der f-treated mice. The spleen cells were stimulated with Der f *ex vivo* for 72 h; and supernatants were assayed for the presence of IL-4, IL-5 and IL-13 (as indicators of a Th2 response), IFN- γ (as an index of a Th1 response), and IL-17A (as an indicator of a Th17 response). Spleen cells from both strains generated all of these cytokines when stimulated with Der f, with the exception of IL-4, which was below the limit of detection (not shown). There were no significant differences between strains in the levels of any cytokine (Fig. 2B).

Bronchial inflammatory responses of Der f-treated PGE₂-deficient mice

To determine the role of PGE₂ in the control of bronchial inflammation provoked by Der f, BAL fluids were analyzed for cell count and differentials. Treatment with Der f increased the total yield of cells in the BAL fluid in both strains, with the numbers of eosinophils, neutrophils, and lymphocytes all increasing relative to the respective cell numbers in saline-treated controls. There were no significant differences between the WT and *ptges*^{-/-} mice in total cell counts (Fig. 3A). The percentages of eosinophils were moderately but significantly greater in the BAL fluids from the *ptges*^{-/-} mice than from the WT controls (Fig. 3B). Neutrophil numbers and percentages tended to be greater in the WT controls (Fig. 3C), but the differences were not significant. Histologic examination of the lungs revealed the accumulation of eosinophils, neutrophils, and lymphoid aggregates around the BVB of the Der f-treated mice based on H&E staining (Fig. 4A). Congo red staining revealed prominent eosinophilia in the BVB of both genotypes (as shown for one sample, Fig. 4B). The Der f-treated mice also exhibited metaplasia of the goblet cells based on staining with PAS (Fig. 4C). Both the extent of inflammation and goblet cell metaplasia were modestly increased in the Der f-treated *ptges*^{-/-} mice compared with the WT controls (Fig. 4D). The intranasal administration of 16, 16-dimethyl PGE₂, a stable analog of PGE₂, significantly decreased both bronchovascular (BV) cell infiltration and goblet cell metaplasia in the *ptges*^{-/-} mice but not in the WT controls (Fig. 4D; as shown for individual mice, Fig. 4A-C). The 16, 16-dimethyl PGE₂ treatment of the *ptges*^{-/-} mice also significantly suppressed total BAL fluid cellularity, eosinophils, and neutrophils (Fig. 3A-C).

Vascular smooth muscle remodeling in Der f-treated mice

To determine whether the lack of mPGES-1-derived PGE₂ resulted in remodeling of the airway smooth muscle, lung sections were stained for α -SMA, a marker of bronchial and vascular SMCs. In both strains, the extent of muscularization of both large and small bronchi was comparable and was unchanged by Der f treatment (Fig. 5A). Many of the vascular structures in the Der f-treated *ptges*^{-/-} mice, however, showed markedly reduced luminal diameter and apparent hyperplasia of the vascular wall (Fig. 5A, arrows). Compared with the Der f-treated WT controls and with saline-treated animals, the Der f-treated *ptges*^{-/-} mice exhibited increased numbers of SMA⁺ SMCs in the small vessels, as well as significantly increased thickness of the SMA-staining layer (Fig. 5B). Neither strain showed substantial staining of the SMA⁺ cells for PCNA, although spleen tissue and human tonsillar tissue (used as positive controls) exhibited robust staining localized to lymphoid cells (Fig. 5C). Staining of frozen sections with Abs to vWF showed prominent endothelial cells in the lesions (Fig. 5D). However, the numbers of vWF⁺ cells in the lesions of the Der f-treated *ptges*^{-/-} mice were not different from the numbers in the saline-treated controls.

To determine whether the vascular smooth muscle hyperplasia observed in the *ptges*^{-/-} mice was due to a loss of PGE₂, we delivered 16, 16-dimethyl PGE₂ intranasally to cohorts of mice concomitantly with Der f. Both the thickness of the smooth muscle layer and the numbers of SMA⁺ cells in the *ptges*^{-/-} mice were significantly decreased by intranasal treatment with 16, 16-dimethyl PGE₂ (Fig. 5B; as shown for one representative experiment, Fig. 5A). Neither parameter was altered by the administration of 16, 16-dimethyl PGE₂ to the WT mice.

Effect of selective EP receptor agonists on BV inflammation and remodeling

To determine which EP receptor(s) contributed to the vascular pathology, we delivered selective agonists of the EP1 (D001), EP2 (AE1-259-01), EP3 (AE-248), and EP4 (AE-329) receptors intranasally to Der f-treated mice. Treatment of the mice with either the EP2 or EP3 receptor-selective agonists modestly but significantly reduced Der f-induced inflammation and goblet cell metaplasia (Fig. 6B; as shown for one mouse on PAS stain in each agonist

group, Fig. 6A), essentially eliminating the window of difference between the WT and *ptges*^{-/-} strains. Both agonists substantially prevented the vascular changes (Fig. 6D, as shown for individual mice, Fig. 6C), especially the EP2 agonist. In contrast, the EP1 receptorselective agonist decreased the vascular remodeling (Fig. 6D), and suppressed the goblet cell metaplasia with no significant effect on inflammation (Fig. 6A, 6B). The EP4 receptor-selective agonist AE-329 had no effect on any parameter. None of the EP receptorselective analogs altered inflammation or goblet cell metaplasia in the WT mice (not shown).

Measurement of mediators of vascular homeostasis

To determine whether the absence of PGE₂ altered the expression of molecules implicated in the development of vascular remodeling, we compared Der f-treated *ptges*^{-/-} mice with WT controls for lung expression of transcripts encoding products known to regulate pulmonary vascular remodeling and hypertension. There were no significant differences between the lungs of the two strains in expression of CCL2 (an endothelial-derived chemokine linked to pulmonary vascular smooth muscle hyperplasia) (34), bone morphogenic protein-II (a negative regulator of vascular proliferation) (35), an essential growth factor platelet-derived growth factor (PDGF), and ApoE, a signature of peroxisome proliferator-activated receptor γ -mediated transcriptional events recently implicated in protection from vascular remodeling in the absence of bone morphogenic protein-II (35). Levels of vascular endothelial growth factor-A were measured in BAL fluids, and CCL2 levels were monitored in the BAL fluid and serum as well. There were no differences between the two strains in any of these parameters (not shown).

Discussion

PGE₂ is not only ubiquitous and generated at baseline in all organs (11), but its production increases in numerous inflammatory conditions. Because exogenous PGE₂ is bronchoprotective when administered exogenously to experimental rodents (19, 20) or to humans with asthma (29, 31), we sought to address the physiologic role of endogenous PGE₂ in allergen-induced pulmonary inflammation. In this study, we determined that mPGES-1 is the dominant enzyme required to generate PGE₂ in Der f-induced pulmonary inflammation; the lack of mPGES-1 modestly potentiates BV inflammation in this model; and the deficiency of mPGES-1-derived PGE₂ does not alter the magnitude or profile of T cell-derived cytokines generated during a recall response, or substantially change Ab generation. Most importantly, the study uncovers a function for endogenous PGE₂ in maintaining the homeostasis of the pulmonary vasculature by limiting the extent of arterial smooth muscle hyperplasia in response to peribronchial and perivascular inflammation. Furthermore, this homeostatic function likely reflects the effects of several EP receptor subtypes, possibly reflecting more than one target cell.

Induced expression of mPGES-1 and PGE₂ production are characteristic of multiple hematopoietic (4, 21) and nonhematopoietic cells (2) involved in pulmonary inflammation. BAL fluids from humans with atopic asthma contain higher levels of PGE₂ (as well as several other COX products) than do BAL fluids from nonasthmatic control subjects (36), suggesting that asthmatic inflammation is associated with COX activity and ongoing PGE₂ synthesis. To determine the physiologic role of mPGES-1-derived PGE₂ in allergic inflammation, we used a model of pulmonary inflammation induced by the intranasal administration of Der f, a clinically important allergen to which patients with asthma are often sensitized. The endogenous adjuvants of the extract directly activate cells of the innate immune system, induce inflammation, and break tolerance to the Der f protein Ags (37). Because we anticipated that PGE₂ would function primarily as an inhibitor of inflammation in this model, we titrated the dose and frequency of Der f administration so as to elicit both

PGE₂ generation and mild-to-moderate inflammation in WT animals. We found that both 1 mg and 3 mg doses delivered twice weekly were sufficient to induce intrapulmonary generation of PGE₂ in these mice, the vast majority of which was abrogated by the absence of mPGES-1 (Fig. 1). Thus, administration of a natural allergen at doses far lower and less frequent than those used to elicit chronic inflammation in a previous study (38) elicits mPGES-1–derived PGE₂ generation. Because 1 mg Der f was insufficient to elicit cellular infiltration in the airways of the majority of the animals but was sufficient to elicit mPGES-1–dependent production of PGE₂, it appears that the inducible mPGES-1 system of the lung requires a very low threshold of stimulation to induce its function, consistent with a protective role. PGs other than PGE₂ can suppress allergic pulmonary inflammation (39, 40), and the absence of mPGES-1 resulted in compensatory overproduction of PGI₂ in a mouse model of cardiovascular disease (41). However, the lack of mPGES-1 did not potentiate the synthesis of PGI₂ (reflected by concentrations of 6-keto-PGF₁α [or any other PG] detected by GC-MS in lung supernatants) (Fig. 1B) in our study. Thus, compensatory changes in the production of other PGs are unlikely to account for phenotypic features of Der f-treated *ptges*^{−/−} mice.

The potential immunologic functions of PGE include actions on dendritic cells (17) and naive T cells (15) to promote Th17-type immune responses. PGE₂ can also suppress (17) or stimulate (22) Th1-type immunity, can favor Th2-type responses (42), or can block T cell proliferation and IL-5 production (43). Most of these reported effects were invoked in experiments with exogenously applied PGE₂, and/or using mice selectively deficient in individual EP receptors. In our study, mPGES-1 deficiency did not substantially alter parameters of the immune response to Der f (Fig. 2) or change BAL fluid cellularity (Fig. 3), suggesting that inducible PGE₂ is not required to induce, suppress, or amplify Th1, Th2, or Th17 cytokine production, or to facilitate Ab production in this integrated model of airway disease. Nevertheless, the potentiation of BV cellular infiltration (Fig. 4A, 4B, 4D) and goblet cell metaplasia (Fig. 4C) in *ptges*^{−/−} mice support an anti-inflammatory role for PGE₂ in our model. The administration of the stable PGE₂ analog 16, 16-dimethyl PGE₂ reduced the histologic indices of tissue inflammation only in the *ptges*^{−/−} mice, suggesting that the impact of homeostatic influence of endogenous PGE₂-EP receptor signaling on inflammation was already maximal in WT Der f-treated mice. The suppression of Der f-induced inflammation by the EP3 receptor selective agonist AE-248 (Fig. 6) is consistent with a previous report in a model induced by sensitization and challenge with OVA (19). This effect was attributed to EP3 receptor-dependent suppression of chemokine generation by epithelial cells in the previous study. The efficacy of the EP2 receptor-selective agonist (AE1-259-01) observed in our study was not evident in the previous OVA-induced model (19). It is possible that these discrepancies are due to the fact that AE1-259-01 was administered i.p. in the previous study, rather than topically as in our study. As multiple hematopoietic cells express EP2 receptors, the suppressive effects of AE1-259-01 in our study could reflect the known EP2 receptor-dependent suppression of mediator generation by mast cells (23), chemotaxis of eosinophils (44), and cytokine production by mature T cells (43). The contribution from EP2 receptors was suggested in a recent study in which systemic administration of PGE₂, but not of the EP1/EP3 receptor-selective agonist sulprostone, suppressed inflammation and indices of mast cell activation in WT mice treated with dust mite allergens (45). Thus, the element of control over pulmonary inflammation by endogenous PGE₂, at least in this model, appears to reflect complementary inputs from at least two EP receptors. The relevant cellular targets and the possibility that EP2/EP3 receptor interactions play a role remain to be determined.

The most surprising effect of the loss of mPGES-1–derived PGE₂ was the profound degree of vascular remodeling that occurred in response to treatment with Der f (Fig. 5A, 5B). Vascular remodeling as a feature of allergen-induced pulmonary inflammation in mice was

first reported in a model using OVA sensitization, followed by repetitive inhalation challenge of “Th2-biased” BALB/c mice for 7 consecutive days (46). Similar findings were reported with BALB/c mice in a model using a protocol of long-term, repetitive Der f administration (47). The latter study used ~8-fold higher doses of Der f than ours, as well as more frequent administration (5 times a week for 7 wk). In both of these studies, remodeling occurred in both large and small vessels, and the lesions included both proliferating endothelial cells and proliferating SMCs. A more recent study showed vascular remodeling in systemically sensitized C57BL/6 mice in response to chronic administration of OVA or of an extract from *Aspergillus fumigatus*, which reflected proliferation by SMCs but not endothelial cells (48). The latter study demonstrated that the vascular remodeling required CD4⁺ T cells, was partly blocked by an antagonist of IL-13, and was attenuated in a congenic strain lacking IL-4. None of these studies implicated lipid mediators in the pathophysiology of the remodeling. Intriguingly, IL-4 and IL-13 both downregulate the expression of COX-2 and mPGES-1 in bronchial epithelial cells (4). Similar to these investigators, we did not find any increment in right ventricular pressures induced by allergen challenge of either strain under normoxic conditions (data not shown).

Our results support a crucial function of PGE₂ in maintaining the homeostasis of Ag-driven vascular remodeling in a context where the stimulus is too low to elicit maximal vascular changes in WT controls. Although the increment in vascular cells and vessel wall thickness was restricted to smooth muscle in our study, there was no evidence for increased proliferation based on PCNA staining (Fig. 5C). We suspect that the ability of PGE₂ to modulate the smooth muscle response to inflammation in this short-term model relates both to upstream effects on the inflammatory response itself, as well as to direct effects on myofibroblast or fibrocyte recruitment or SMC turnover rates. The lack of obvious proliferation of the SMCs may reflect the short-term and low-dose nature of the stimulus used in this model, relative to previous studies in WT mice using more robust challenge protocols (46-48).

We have demonstrated a critical role for inducible mPGES-1 and its product, PGE₂, in maintaining vascular homeostasis in pulmonary inflammation induced by the clinically relevant dust mite allergen. This requirement reflects not only the known input through EP3 receptors, but also a previously unrecognized contribution from EP2 receptors. Our findings suggest that appropriate EP receptor-selective agonists may ameliorate the vasculopathy associated with pulmonary inflammation, such as occurs in pulmonary hypertension and certain diffuse lung disorders.

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Abbreviations used in this paper

AERD	aspirin-exacerbated respiratory disease
BAL	bronchoalveolar lavage
BV	bronchovascular
BVB	BV bundles

COX	cyclo-oxygenase
Der f	extract of <i>Dermatophagoides farinae</i>
EP	E-prostanoid
GC-MS	gas chromatography-mass spectrometry
mPGES	microsomal PGE ₂ synthetase
PAS	Periodic acid-Schiff
PCNA	proliferating cell nuclear Ag
PGES	PGE ₂ synthase
SMA	smooth muscle actin
SMC	smooth muscle cell
TX	thromboxane
vWF	Von Willebrand factor
WT	wild-type

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FIGURE 1.

Effect of Der f challenge on PG generation by pulmonary tissues of *ptges*^{-/-} mice and WT C57BL/6 controls. Mice were treated intranasally twice weekly for 3 wk with the indicated doses of Der f. Forty-eight hours after the last challenge, the mice were euthanized and BAL fluid was collected. Lungs were mechanically dispersed and incubated in the presence of arachidonic acid. *A*, PGE₂ levels in BAL fluids (detected by ELISA) after the lipid fractions were purified. Data are the mean ± SD from four to six mice per group. The experiment was performed three times. *B*, GC-MS analysis of all PGs generated by lung homogenates from the indicated mouse strains after 30 min of incubation in the presence of arachidonic acid (50 μM). The supernatants were processed for GC-MS as detailed in *Materials and Methods*. Results depicted are mean ± SD from five mice/group.

FIGURE 2.

Parameters of the immune response to Der f in *ptges*^{-/-} mice and WT C57BL/6 controls. *A*, Total IgE (*top*) and IgG1 (*bottom*) in serum from mice treated with saline or Der f were measured by ELISA. Results are the mean $\pm$ SD from 10–12 mice/group in three separate experiments. *B*, Cytokine generation by restimulated spleen cells. Spleens were harvested from mice 48 h after the final Der f challenge. The cells were mechanically dispersed and passed through nylon mesh strainers before being stimulated for 72 h with Der f (20 μ g/ml) in medium. The content of cytokines was determined by ELISA. Results are the mean $\pm$ SEM from 8–10 mice per group from two separate experiments.

FIGURE 3.

Accumulation of leukocytes in the BAL fluid from *ptges*^{-/-} mice and WT C57BL/6 controls. The lungs of the saline- or Der f-treated mice were lavaged three times with 0.7 ml PBS/5 mM EDTA. Slides were prepared by cytocentrifugation and stained with Difquick. *A*, Total cell counts. *B*, Eosinophils expressed as total cell numbers (*left*) and as a percentage (*right*). *C*, Neutrophils expressed as total cell numbers (*left*) and as a percentage (*right*). The results are the mean $\pm$ SEM of 30–40 animals per group in 8–10 experiments.

FIGURE 4.

Histologic analysis of pulmonary inflammation in Der f-treated mice and effect of 16, 16-dimethyl PGE₂. *A*, Images of H&E-stained lungs from the indicated groups of mice (original magnification $\times 200$). *B*, Perivascular cellular infiltrates showing eosinophils stained with Congo red dye (original magnification $\times 630$). *C*, PAS-stained lung showing goblet cells (original magnification $\times 200$). Results in (*A–C*) are from single mice. *D*, Quantitative analysis of Der f-induced BV inflammation (*left*) and goblet cell metaplasia (*right*) and the effect of 16, 16-dimethyl PGE₂. Results are the mean $\pm$ SEM from 32–47 mice/group from 6–10 independent experiments.

FIGURE 5.

Histologic and immunohistochemical analysis of smooth muscle hyperplasia occurring in response to Der f. **A**, Representative lung sections from mice of the indicated genotypes treated with saline or Der f. Sections were stained with an Ab against α -SMA to visualize bronchial (open arrows) or vascular (closed arrows) smooth muscle. Original magnification $\times 200$. **B**, Quantitative analysis of vascular SMC numbers (*left*) and thickness (*right*). The effect of 16, 16-dimethyl PGE₂ is shown. Results are the mean $\pm$ SEM from 30 mice per group. **C**, Double immunostaining with Abs against α -SMA (*red*) and PCNA (*brown*). Results are from an individual mouse representative of five. Original magnification $\times 200$ (*left*), $\times 630$ (*right*). Stains of spleen (*left*) and human tonsil (*right*) are shown as controls. Original magnification $\times 200$. **D**, Immunofluorescence of frozen lung sections showing double staining for SMA (*green*) and vWF (*red*) as markers of smooth muscle and endothelium, respectively. Representative stains of lungs from saline (*left*) and Der f-treated (*right*) ptges^{-/-} mice are shown. Original magnification $\times 630$.

FIGURE 6.

Effects of treatment with selective EP receptor agonists on the histologic responses of *ptges*^{-/-} mice to Der f. **A**, PAS stains showing goblet cell metaplasia in response to Der f and the effects of the indicated EP receptor agonists. **B**, Quantitative analysis of inflammation (*top*, as assessed by hematoxylin-azure-2-eosin stains of BVB) and goblet cells (*bottom*), showing the effects of the agonists for the indicated receptors. **C**, Hematoxylin-azure-2-eosin stains showing BVB of the same mice as in (**A**). Original magnification $\times 200$ for both **A** and **C**. **D**, Quantitative analysis of vascular remodeling in *ptges*^{-/-} mice treated with agonists for the indicated receptors. SMC numbers (*top*) and thickness of the smooth muscle layer (*bottom*) are shown. Results are the mean of 11–25 mice/group from at least three experiments.