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

Anders Lundequist,† Samridhi N. Nallamshetty,† Wei Xing,† Chunli Feng,† Tanya M. Laidlaw,‡† Satoshi Uematsu,§ Shizuo Akira,§ and Joshua A. Boyce*,†,‡

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. The Journal of Immunology, 2010, 184: 433–441.

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)-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 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 nonhematopoietic 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 pathophysiologic changes in response have not been fully clarified. PGD2 and thromboxane (TX) are bronchoprotectors potentially relevant to asthma (24, 25), and exogenous PGE2 can amplify allergen-induced pulmonary eosinophilia in mice (26). However, global depletion of PGs with COX inhibitors (27) and knockouts of COX enzymes

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Abbreviations used in this paper: AERD, aspirin-exacerbated respiratory disease; BAL, bronchoalveolar lavage; BV, bronchovascular; BVB, BV bundles; COX, cyclooxygenase; Der f, extract of Dermatophagoides farinae; EP, E-prostanoid; GC-MS, gas chromatography-mass spectrometry; mPGES, microsomal PGE2 synthase; PAS, Periodic acid-Schiff; PCNA, proliferating cell nuclear Ag; PGES, PGE2 synthase; SMC, smooth muscle cell; TX, thromboxane; vWF, Von Willebrand factor; WT, wild-type.

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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 (pges−/− mice) were backcrossed to C57BL/6 mice for nine generations (2). Six- to 8-wk-old male pges−/− 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 (XPB8131A25; Lenoir, NC). The stable PGE2 analog 16, 16-dimethyl PGE2 was obtained from Cayman Chemicals (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 diluted 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 × 10⁶ lymph node cells and 4 × 10⁶ 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-17α, 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 IgG1, 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-Pak columns (Waters, Milford, MA). PGE2 and cys-LT levels were measured by ELISA in accordance with the manufacturer’s protocol (Cayman Chemical and Amesham 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 μM arachidonic acid. The ground tissue then was added to 5 ml ice-cold methanol containing 1.0 mg/ml each of the following internal standards: [3H]-H2O, [3H]-15-F2-isoprostane ([3H]-8-iso-PGJ2), [3H]-PGD2, [3H]-PGE2, [3H]-11-dehydro-TXB2 (11-dehydro-TXB2), and [3H]-H2O-keto-PGF1α (all purchased from Cayman Chemicals). The lipids were extracted and separated from the solid particles 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, Milford, MA). The lipids were extracted and separated from the solid particles 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, Milford, MA). The lipids were extracted and separated from the solid particles 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, Milford, MA). The lipids were extracted and separated from the solid particles 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, Milford, MA). The lipids were extracted and separated from the solid particles 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, Milford, MA). The lipids were extracted and separated from the solid particles 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, Milford, MA). The lipids were extracted and separated from the solid particles 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, Milford, MA). The lipids were extracted and separated from the solid particles 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, Milford, MA). The lipids were extracted and separated from the solid particles 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, Milford, MA). The lipids were extracted and separated from the solid particles 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, Milford, MA). The lipids were extracted and separated from the solid particles 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 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, Carpenteria, 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-μm-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 compareable arterioles were taken from each slide, and 3–5 arterioles of each slide were quantitated. “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 altered 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 decreased by ∼70% relative to the WT controls. In contrast, the two strains showed no differences in the levels of PGF2α, 6-keto-PGF2α, PGD2, or TXB2 (Fig. 1B). For both the WT and the ptges−/− mice, the levels of cysteinyloleukotrienes 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
expressed as total cell numbers (a percentage (SEM of 30–40 animals per group in 8–10 experiments.

To determine whether the lack of mPGES-1–derived PGE2 resulted in remodeling of the airway smooth muscle, lung sections were stained for vWF, 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 WVF 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.

\( ptges^{--/} \) 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 \( \alpha \)-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 WVF 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.

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**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 receptor-selective 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 receptor-selective 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 (PGDF), 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 non-hematopoietic 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 μg and 3 μg 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 μg 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 Der f and the effects of the indicated EP receptor agonists. A. 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 ×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.

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 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).

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 ×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.

The Journal of Immunology 439
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.

Disclosures

The authors have no financial conflicts of interest.

References


