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Prostaglandin E₂ Produced by the Lung Augments the Effector Phase of Allergic Inflammation

Rachel J. Church,* Leigh A. Jania,† and Beverly H. Koller*,†,‡

Elevated PGE₂ is a hallmark of most inflammatory lesions. This lipid mediator can induce the cardinal signs of inflammation, and the beneficial actions of nonsteroidal anti-inflammatory drugs are attributed to inhibition of cyclooxygenase (COX)-1 and COX-2, enzymes essential in the biosynthesis of PGE₂ from arachidonic acid. However, both clinical studies and rodent models suggest that, in the asthmatic lung, PGE₂ acts to restrain the immune response and limit physiological change secondary to inflammation. To directly address the role of PGE₂ in the lung, we examined the development of disease in mice lacking microsomal PGE₂ synthase-1 (mPGES1), which converts COX-1/COX-2–derived PGH₂ to PGE₂. We show that mPGES1 determines PGE₂ levels in the naive lung and is required for increases in PGE₂ after OVA-induced allergy. Although loss of either COX-1 or COX-2 increases the disease severity, surprisingly, mPGES1⁻/⁻ mice show reduced inflammation. However, an increase in serum IgE is still observed in the mPGES1⁻/⁻ mice, suggesting that loss of PGE₂ does not impair induction of a Th2 response. Furthermore, mPGES1⁻/⁻ mice expressing a transgenic OVA-specific TCR are also protected, indicating that PGE₂ acts primarily after challenge with inhaled Ag. PGE₂ produced by the lung plays the critical role in this response, as loss of lung mPGES1 is sufficient to protect against disease. Together, this supports a model in which mPGES1-dependent PGE₂ produced by populations of cells native to the lung contributes to the effector phase of some allergic responses. The Journal of Immunology, 2012, 188: 000–000.
or COX-2, we confirm the role of this pathway in our model. We of asthma. First, using congenic mouse lines lacking either COX-1 II) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). 

cially two additional enzymes, cytosolic PGE2 synthase and mi-

PGES1, mPGES2), were thought to be capable of this enzymatic conversion, studies using mutant mouse lines carrying mutations in the genes for synthases mPges1, cPges/p23, or mPges2, respectively, have failed to support this in vivo function for any product other than mPGES1 (27–29). mPGES1 is expressed in many tissues and cell types of both humans and animals, including in the lung and leukocytes (5, 30, 31). Similar to COX-2, the expression of mPGES1 increases dramatically in response to inflammatory mediators, suggesting coupling with this enzyme; however, evidence demonstrates that mPGES1 can couple with both COX-1 and COX-2 to synthesize PGE2 (5, 29, 30, 32). Furthermore, studies using mice lacking PGES1 in models of pain nociception, rheumatoid arthritis, atherogenesis, and abdominal aortic aneurysm provide evidence that this synthase contributes to the pathogenesis of both acute and chronic inflammation (29, 33–35).

In this study, we elucidate the contribution of mPGES1-derived PGE2 in the development of allergic lung disease, a mouse model of asthma. First, using congenic mouse lines lacking either COX-1 or COX-2, we confirm the role of this pathway in our model. We then evaluate the role of PGE2 produced by mPGES1 expressed by resident airway cells and PGE2 released from recruited inflammatory cells in this allergic response.

Materials and Methods

Experimental animals

All experiments were conducted in accordance with standard guidelines as defined by the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee guidelines of the University of North Carolina at Chapel Hill. Experiments were carried out on age- and sex-matched mice between 8 and 12 wk of age. C57BL/6 (B6) (backcrossed 129S6/SvEv (129) filial generation (F)1 COX-2

crosomal PGE2 synthase-1 (mPGES1) (5). Although ini-

levels, lungs were flash frozen in liquid nitrogen, weighed, and stored at —80°C. Lung tissue was pulverized and homogenized in buffer containing 150 mM NaCl, 15 mM Tris-HCl, 1 mM CaCl2, and 1 mM MgCl2 supplemented with protease inhibitor (Roche). Values shown represent the total quantity of cytokine or mediator measured divided by tissue weight. Cytokines were determined by ELISA following manufacturer’s protocols: IL-13 (R&D Systems), IFN-γ (R&D Systems), IL-4 (R&D Systems), IL-17a (eBioscience). For quantification of PGE2, lung tissue was pul-

BALF collection and cell counts

Levels of cytokines and PGE2 were determined by immunoassay in BALF, lung tissue homogenate, and/or tissue culture supernatant. To determine cytokine production by stimulated splenocytes, cells were prepared as described above. Cells were cultured at a density of 1 × 10^6 cells/ml in the presence of 100 μg/ml OVA. After 72 h, supernatants were collected and stored at —80°C prior to evaluation by ELISA. To determine lung cytokine levels, lungs were flash frozen in liquid nitrogen, weighed, and stored at —80°C. Lung tissue was pulverized and homogenized in buffer containing 150 mM NaCl, 15 mM Tris-HCl, 1 mM CaCl2, and 1 mM MgCl2 supplemented with protease inhibitor (Roche). Values shown represent the total quantity of cytokine or mediator measured divided by tissue weight. Cytokines were determined by ELISA following manufacturer’s protocols: IL-13 (R&D Systems), IFN-γ (R&D Systems), IL-4 (R&D Systems), IL-17a (eBioscience). For quantification of PGE2, lung tissue was pul-

Bone marrow chimera generation

Recipient mice were exposed to 5 grays irradiation from a Cesium γ-ir-

radiator at 0 and 3 h. Femurs and tibias were collected from donor mice and flushed with cold PBS to isolate bone marrow. Bone marrow was introduced by tail vein injection into recipient mice immediately following the second round of radiation and after 8 wk animals were sensitized and challenged with OVA.

Statistical analysis

Statistical analysis was performed using Prism 4 (GraphPad). Comparisons of the means were made by F test, Student t test, or ANOVA followed by the Tukey-Kramer honestly significant difference post hoc test as necessary. Data are shown as mean ± SEM. Differences with p < 0.05 were considered statistically significant.

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Results

Allergic asthma in the COX-1 and COX-2–deficient mice

We first verified, using congenic mouse lines, the contribution of prostanoids to allergic lung disease induced by sensitization and challenge with OVA Ag. COX-1\(^{-/-}\) B6 congenic mice were generated by \(>10\) crosses between mice carrying a null allele at this locus and commercially purchased B6 mice. COX-2 mice survive poorly on most inbred genetic backgrounds, in part due to a patent ductus arteriosus (39, 40), and thus, most experiments assessing COX-2 function have used F2 mice, mice expected to carry a random assortment of 129- and B6-derived alleles. To circumvent this problem, we generated two lines of COX-2 mutant mice, the first line on the coisogenic 129 genetic background and the second congenic line on the B6 genetic background. The 129 COX-2\(^{-/-}\) females were intercrossed with B6\(^{+/+}\) males to generate congenic F1 progeny. The COX-2\(^{-/-}\) and COX-2\(^{+/+}\) littermates were used in the experiments presented in this study. Allergic airway disease was induced through sensitization by an i.p. injection of OVA emulsified in alum and challenged with repeated aerosols of Ag. Inflammation was assessed 24 h after the final Ag challenge.

As expected, our immunization protocol induced a robust cellular influx in B6 WT mice (COX-1\(^{+/+}\)) compared with saline-treated animals (Fig. 1A). The F1 OVA-treated controls (COX-2\(^{+/+}\)) displayed a similar pattern of increased cellularity. Surprisingly, the loss of either COX-1 or COX-2 had comparable impacts on recruitment of cells into the airways: in each line, the total number of cells recovered by BALF was about twice that observed in the genetically matched controls. The cellular infiltrate present in the airways following Ag challenge was marked by heightened levels of eosinophils, typical of Th2-type allergic responses (Fig. 1B). Characteristic of this type of response, IL-13 levels were elevated in the BALF of WT mice with allergic lung disease compared with saline controls (Fig. 1C). Loss of either COX-1 or COX-2 led to increased levels of this cytokine in the airways. Again, the magnitude of this increase, relative to the WT control, was surprisingly similar in the two lines. Elevated IgE levels were observed in both B6 COX-1\(^{+/+}\) and B6/129 F1 COX-2\(^{+/+}\) WT mice.
animals (Fig. 1D). However, unlike inflammatory disease in the lung, the loss of either the COX-1 or the COX-2 pathway significantly altered levels of this Ig isotype.

AHR following challenge with Mch, a potent airway constrictor, was quantified in intubated animals using a computer-controlled small-animal ventilator. This method allows for measurement of resistance in the central airway and tissue damping. We failed to observe AHR in response to Mch in mice with allergic lung disease. This was also true of the B6/129 F1 mice. Even in the COX-1 and COX-2 mice, which showed elevated levels of inflammation, no difference was observed in the response to methacholine in either parameter (Supplemental Fig. 1A, 1B).

Contribution of mPGES1 to PGE2 production in the naive and inflamed lung

The ability to catalyze the conversion of PGH2 to PGE2 has been assigned to three distinct enzymes: mPGES1, mPGES2, and cytosolic PGE2 synthase (5, 41, 42). However, studies using mutant mouse lines have shown in vivo alteration of PGE2 levels only in the mPGES1 mice (29, 43). We therefore first determined whether PGE2 levels are elevated in this model of allergic lung disease and, if so, whether this increase is dependent on the mPGES1 synthase. Allergic lung disease was induced in congenic mPGES1−/− mice and their B6 controls (mPGES1+/+), as described above. Lungs were harvested and the levels of PGE2 were determined by enzyme immunoassay (Fig. 2). PGE2 production could be detected in the lungs of healthy mice exposed to saline. This production was significantly reduced in the lungs obtained from mPGES1−/− mice, indicating that mPGES1 is active in the normal lung and contributes to the basal levels of PGE2 in this organ. PGE2 levels were substantially potentiated in the lungs of WT mice with allergic lung disease. This increase was entirely dependent on expression of the mPGES1 synthase, indicating that animals lacking this synthase provide an appropriate model for determining whether loss of this COX1/2 downstream pathway contributes to the increased disease observed in the COX1− and COX2−deficient mice.

Impact of PGE2 on allergic lung inflammation

PGE2 levels in the lung increase dramatically after allergic lung disease, and this increase is absent in both the COX1− and COX2−deficient mice (Supplemental Fig. 2), supporting the hypothesis that loss of this prostanoid could contribute to the increased disease observed in both of these mouse lines. To test this hypothesis, mice lacking mPGES1, and their congenic controls, were sensitized and challenged, as previously described. Twenty-four hours after the final challenge, the impact of mPGES1 synthase on inflammation of the airways, IgE production, and airway mechanics was assessed. Surprisingly, and contrary to our prediction based on analysis with the COX-1/COX-2−deficient mice, mice lacking mPGES1 had significantly less cellular infiltrate and associated eosinophilia in comparison with OVA-challenged WT animals (Fig. 3A, 3B). A significant decrease in IL-13 production was observed in the BALF of the mPGES1−/− mice compared with WT control animals (Fig. 3C). No difference in serum IgE levels was observed between mPGES1−/− mice and their genetic controls (Fig. 3D). IL-4 production is critical for IgE isotype switching (44); therefore, we characterized IL-4 concentrations present in lung homogenate following induction of allergy in this model (Fig. 3E). No difference was observed in the production of this Th2 cytokine between the inflamed lungs of WT and mPGES1−/− mice. To study this further, an additional experiment was carried out to examine proliferation and cytokine production by splenocytes from immunized and challenged mice. The proliferative response of the mPGES1−/− cells to Ag did not differ significantly from that of control cultures, nor was a significant difference observed in the production of IFN-γ or IL-17a. Similar to the BALF, a decrease in IL-13 levels was observed in these cultures, although in this case, the decreased production by mPGES1−/− cells did not achieve statistical significance (Supplemental Fig. 3).

Changes in airway mechanics were determined, as previously described. As was the case for the COX-1− and COX-2−deficient cohorts, inflammation and allergic airway disease induced using this immunization protocol did not result in AHR in any parameter, either in the WT animals or in the mPGES1−/− line (Supplemental Fig. 1C).

Effect of PGE2 on lung inflammation in mice carrying a transgenic OVA-specific TCR

The observation that IL-4 and IgE concentrations were unaffected by endogenous levels of PGE2 suggests that proinflammatory actions mediated by this prostanoid occur subsequent to the sensitization phase of Ag. To explore this, we examined the induction of IgE and the response of splenocytes to Ag in sensitized animals prior to challenge with aerosolized Ag (Supplemental Fig. 5). No difference was observed in serum IgE between WT and mPGES1−/− deficient mice. Splenocytes isolated from WT and mPGES1−/− animals a week after booster sensitization demonstrate similar proliferative responses, and no difference was observed in the production of IL-13, IFN-γ, or IL-17a between groups. Collectively, these results indicate that the reduced inflammation observed in the mPGES1−/− lungs is unlikely to reflect alterations in the sensitization of mice to Ag, but rather the response of exposure of the lungs in sensitized animals to Ag.

To explore this further, we determined whether loss of mPGES1 would alter the development of lung inflammation in OT-II mice. These mice carry a transgenic TCR specific to OVA (45), and thus, exposure of the airways to this Ag results in inflammation in nonsensitized animals. In this case, however, the prominent cell type in the BALF is the neutrophil, and thus, this response is thought to model nonatopic allergic lung disease (46). Mice lacking mPGES1 were crossed to congenic B6 mice carrying the OT-II transgene. As expected, mice lacking the transgene OT-II−, showed little inflammation in response to OVA challenge. In contrast, robust cell recruitment was observed in transgenic WT mice (Fig. 4A). Transgenic mPGES1−/− animals showed a significant attenuation in

![FIGURE 2. PGE2 production by the naive and allergic mPGES1−/− lungs. PGE2 levels were measured in lung homogenate prepared from naive mice or after sensitization, and challenged with OVA. In the naive lung, concentrations of PGE2 were measured in naive mPGES1−/− mice relative to WT mice (p < 0.02). PGE2 levels are increased significantly in lungs collected from mice sensitized and challenged with OVA (p < 0.05). In contrast, no significant increase in PGE2 is observed in mPGES1−/− mice. PGE2 quantifications were made for two independent cohorts. Data represent one experiment. mPGES1+: +/− saline, n = 5; −/− saline, n = 5; +/+ OVA, n = 7; −/− OVA, n = 11.](http://www.jimmunol.org/content/123/8/4/F2.large.jpg)
cell infiltration compared with WT controls. This attenuation reflects significantly fewer granulocytes present in the BALF of WT animals. Although increased numbers of cells are also observed in BALF collected from mPGES1-/- animals, the total cell count was significantly reduced compared with similarly treated WT control animals (*p < 0.001). (B) The decrease in the cellularity of the BALF of the mPGES1-/- mice correlates with a significant decrease in the number of eosinophils in the BALF of these animals compared with the numbers present in the BALF from the control animals (*p < 0.001). (C) IL-13 levels in the BALF collected from OVA-sensitized and -challenged animals are significantly higher than those measured in saline-treated cohorts, both WT and mPGES1-/- animals; however, higher levels are observed in the samples collected from OVA-treated WT animals relative to levels in BALF from mPGES1-/- animals (*p < 0.05). (D) OVA sensitization and challenge results in an increase in total serum IgE of a similar magnitude in WT and mPGES1-/- animals. (E) IL-4 levels in whole-lung homogenates do not differ significantly between samples prepared from mPGES1-/- mice and controls. As expected, both groups showed levels elevated in comparison with samples prepared from saline treated cohorts. Experiments for (A)–(D) were conducted three times independently, and data from one experiment are shown. Data for (E) was generated once. For (A)–(D): mPGES1+/+ saline, n = 4; -/- saline, n = 3; +/- OVA, n = 10; -/- OVA, n = 11; for (E): mPGES1+/+ saline, n = 2; -/- saline, n = 3; +/- OVA, n = 5; -/- OVA, n = 5.

Individual contributions of lung and recruited inflammatory cells to PGE2-mediated allergic responses

As shown above, mPGES1 contributes to the PGE2 present in the healthy lung. To determine the relative contributions of mPGES1 produced by the lung and that produced by the recruited immune cells to this model of allergic lung disease, we studied the development of allergy in bone marrow chimeras. We first established that the contribution of mPGES1 to the allergic response was not altered in animals undergoing this experimental procedure. The difference in the OVA-induced cellularity of the BALF between WT mice irradiated and reconstituted with WT marrow (WT→WT) compared with that observed in mPGES1-/- animals irradiated and reconstituted with autologous marrow (knockout [KO]→KO) recapitulated the differences observed between these groups in previous experiments (Figs. 5 compared with 3A).

We next asked whether PGE2 produced by the immune cells recruited to the lung during allergic inflammation contributes to allergic airway disease. To address this, WT mice were irradiated and reconstituted with either WT (WT→WT) or mPGES1-/- (KO→WT) bone marrow. Reconstitution of mice with mPGES1-
PGE2 was from the lung itself. We evaluated whether mPGES1 contributed little to the inflammation in the lung, we next addressed mPGES1 significantly elevated cell counts relative to mPGES1 OT A. WT mice reconstituted with WT bone marrow (WT → WT). A significant decrease in BALF cellularity was observed, again primarily reflecting reduced recruitment of eosinophils (Fig. 7A, 7B). IL-13 production was also reduced in this group (Fig. 7C). These observations suggest that mPGES1 produced by cells in the lung, not recruited leukocytes, contributed to the development of allergic disease in response to OVA. Consistent with the studies reported above, IgE levels were not significantly affected by loss of lung mPGES1 (Fig. 7D).

**Discussion**

Previous studies have shown that in the absence of COX-1 or COX-2, Ag exposure results in more severe allergic lung disease (23). Using mice lacking mPGES1 synthase, we show that attenuation of PGE2 synthesis in the COX-1– and COX-2–deficient mice does not account for the increase in disease observed in these mouse lines. In fact, in this model, mPGES1-deficient mice showed reduced airway inflammation, indicating that PGE2 enhances this aspect of allergic disease.

The impact of the genetic composition of mouse lines on the development of various aspects of allergic lung disease has been well established (47–50). The majority of the early studies assigning roles for COX-1 and COX-2 metabolites in inflammatory responses were carried out using mice of mixed genetic background, thus the representation of B6 and 129 genes in the COX-deficient and control animals can be very different. We therefore first verified that the protection that COX-1 and COX-2 provided in this response could be observed when congenic animals were studied. Consistent with previous work, we report that both COX-1– and COX-2–deficient mice limit allergic inflammation in the lung. However, we show that both enzymes provided the mice with a similar level of protection. This differs from previous studies in which loss of COX-1 was reported to have a greater role than COX-2 both in production of PGE2 in the naive and inflamed lung as well as in limiting allergic inflammation (23). Because COX-1 and COX-2 have unique but overlapping patterns of expression and, depending on the cell type, can lead to the preferential production of a particular eicosanoid, this observation suggests that multiple PGs or PGs made by different cells types limit inflammation in this model.

We saw no development of AHR in either the COX-1– or COX-2–deficient animals. This is not surprising given the genetic background of the mice. AHR is often absent in B6 mice (47, 48, 50). In previous studies, inflammation associated with loss of COX-1 but not COX-2 was reported to result in increased sensitivity to methacholine (23). It is possible that this difference reflected differences in the segregation of 129 and B6 alleles in the two populations. This would be expected, as the closure of the ductus arteriosus in mice lacking EP2 or COX-2 depends on the inheritance of a particular complement of 129 and B6 alleles (40, 51). In contrast, no such selective pressure would skew inheritance of alleles in the COX-1 population.

Early work suggested that PGE2 could play an important role in regulating the differentiation of mouse B lymphocytes to IgE-secreting cells (52). However, this role was not supported by the report that IgE levels were actually higher in the COX-1 and COX-2 Ag-treated animals (23). Our study did not observe this increase in the COX-1– and COX-2–deficient animals compared with their genetically matched controls and therefore does not support a role for PGE2 in switching B cells to IgE production. No
difference was noted in serum IgE levels among COX-1−/−, COX-2−/−, or mPGES1−/− and their control animals after induction of a Th2 response. However, direct comparison of the IgE response of COX-deficient animals reported in this study and those reported previously is difficult for a number of reasons. Not only do our studies use congenic mice, but also the cohort examined in this study was between 8 and 12 wk of age, whereas previous studies examined mice that ranged in age from 5–9 mo. In addition, these studies evaluated IgE levels in the BALF, whereas we examined serum IgE.

In patients with allergic asthma, inhaled PGE2 is reported to attenuate both the early- and late-phase response after exposure to Ag (12, 13, 15, 16). PGE2 has also been shown to limit inflammation in animal models of asthma (14, 17, 53). Given this, it seemed likely that the heightened inflammation observed in the COX-deficient mice reflected a loss of this protective prostanoid. Indeed, induction of allergic disease with OVA dramatically increased PGE2 levels in the lung, and this augmentation was not observed when COX-1, COX-2, or mPGES1 was absent, suggesting that both enzymes are capable of coupling with mPGES1

![Figure 6](http://www.jimmunol.org/)

**FIGURE 6.** Contribution of PGE2 from bone marrow-derived cell populations to OVA-induced lung inflammation. Lethally irradiated WT mice were reconstituted with either WT bone marrow (WT→WT) or mPGES1−/− bone marrow (KO→WT). (A) As expected, an increase in the cellularity of the BALF was observed in samples collected from the animals sensitized and challenged with OVA. No significant difference was measured in the total number of cells present in the BALF of the two groups (WT→WT versus KO→WT). (B) Morphological analysis of cell types present in BALF revealed elevated levels of eosinophils in both OVA-treated groups, and again, the numbers of these cells did not differ significantly between the animals that had received the WT versus the mPGES1−/− marrow. (C) No difference is observed in the level of IL-13 in the BALF of the two OVA-treated groups. (D) Total serum IgE concentrations are elevated to a similar degree in groups sensitized and challenged with OVA. This experiment was carried out twice, and data from one trial are shown. For WT→WT: saline, n = 3; OVA, n = 8; KO→WT: saline, n = 2; OVA, n = 8.

![Figure 7](http://www.jimmunol.org/)

**FIGURE 7.** Contribution of PGE2 produced by radiation-resistant lung populations to allergic inflammation. WT mice or mPGES1−/− mice exposed to lethal doses of radiation were reconstituted with WT bone marrow, (WT→WT) and (WT→KO), respectively. BALF was collected from OVA-sensitized and -challenged animals, and total cell numbers (A) and cell differentials (B) were determined. A decrease in both the total cell count and the number of eosinophils in the BALF was observed in samples from mPGES1−/− mice reconstituted with WT marrow compared with samples from similarly reconstituted and treated WT animals (WT→WT). *p < 0.01. (C) IL-13 levels are significantly higher in the BALF from OVA-sensitized and -challenged WT→WT mice compared with levels in BALF from similarly treated mPGES1−/− animals that received WT bone marrow (WT→KO). *p < 0.05. (D) Total serum IgE concentrations were elevated to similar levels following OVA sensitization and challenge in both WT→WT and WT→KO animals. This experiment was conducted twice, and data from one trial are shown. For WT→WT: saline, n = 4; OVA, n = 8; WT→KO: saline, n = 4; OVA, n = 7.
to promote prostanoid production during lung inflammation. However, unlike a genetic loss of COX enzymatic activity, a loss of mPGES1 did not result in heightened disease—in fact, quite the opposite; loss of this pathway attenuated the inflammatory response. Our results are consistent with a model in which the primary protective COX-dependent eicosanoid is prostacyclin, not PGE2. Mice lacking the 1-prostanoid receptor, specific for prostacyclin, were reported to have more severe allergic inflammation in the lung (54). Prostacyclin, but not PGE2, was also shown to protect against the development of fibrosis in the bleomycin model of idiopathic pulmonary fibrosis (55), suggesting that, at least in the rodent lung, this might be the most important anti-inflammatory prostanoid.

We cannot rule out the possibility that the lack of a protective role for PGE2 in this study is specific to this particular model and immunization protocol. A recent study examining the function of mPGES1 in a house dust mite Ag (Der f)-induced allergic model reported that PGE2 limited vascular changes associated with chronic exposure to Ag, whereas decreased PGE2 had no significant impact on total recruitment of inflammatory cells to the lungs after Der f challenge (56). However, the vascular remodeling that this study showed was enhanced in the mPGES1<sup>−/−</sup> mice is associated with chronic models of asthma and is not apparent in the acute model used in our study, preventing extension of this finding to this model of allergic lung disease. In contrast to our findings, decreased PGE2 had no significant impact on recruitment of inflammatory cells to the lungs after Der f challenge. Again, this difference might reflect different roles for PGE2 in an acute allergic response, such as that induced by OVA and adjuvant versus a chronic model established by inhalation of a complex Ag with intrinsic ability to activate the innate immune response. Alternatively, it could reflect the fact that the mPGES1<sup>−/−</sup> animals were compared with purchased WT B6 mice, whereas both the mPGES1<sup>−/−</sup> and WT mice used in our studies were bred in the same facility, as studies have highlighted the importance of environmental factors, including the microbiome, in molding the immune response (57–60), and it is possible that some phenotypes reflect such differences in addition to the genetic lesion under study.

Both our findings and the phenotype of mPGES1<sup>−/−</sup> animals in the Der f allergic model do not support early reports of heightened inflammation in EP<sub>2</sub><sup>−/−</sup> mice sensitized and challenged with OVA (26). The reason for this discrepancy is not apparent; however, we have been unable to reproduce this finding using B6 congenic EP<sub>3</sub><sup>−/−</sup> mice (M. Nguyen and B.H. Koller, unpublished observations). Furthermore, previous work in our laboratory has indicated that PGE2, through the EP<sub>3</sub> receptor, can promote inflammation by augmenting IgE-mediated mast cell degranulation, and, in some circumstances, PGE2 alone is sufficient to mediate this response in rodents (61, 62).

Much of the support for the hypothesis that PGE2 plays a protective role in the lung, limiting inflammation, comes from studies in which exposure of mice to Ag is accompanied by inhalation of PGE2, its stable analog, or a PGE2 receptor preferring antagonist and agonist (17, 53, 63). In vitro studies have reinforced this hypothesis, with studies such as those which have shown PGE2 to be effective in limiting migration of eosinophils and increasing production of IL-10 by dendritic cells and naive T cells (22, 63, 64). However, extrapolating findings from either or both of these types of studies to develop models that predict the contribution of PGE2 to inflammatory responses in vivo has proven difficult. Some of this difficulty is related to the fact that very few of the pathways attributed to PGE2 through pharmacological studies with inhaled PGE2 or PGE2 receptor preferring agonists/antagonists are supported by evaluation of mice lacking specific PGE2 receptors or a combination of receptors. In some cases, the discrepancies may reflect the effective dose and specificity of the reagents used. For example, early studies assigning anticoagulatory properties to PGE2 were later shown to reflect the ability of PGE2 at concentrations used in these studies to activate the prostacyclin receptor (65). Thus, it is possible that some of the protective actions of inhaled PGE2 and EP receptor agonist are incorrectly assigned to the PGE2 pathway. Carrying out these experiments in mice lacking the I-prostanoid receptor and EP receptors should resolve many of these issues. In some cases, inconsistency among results obtained using the various approaches might simply reflect the fact that loss of a PGE2 receptor may have far less consequence for the organism than stimulation of the same pathway due to compensatory pathways active in vivo. For example, stimulation of naive T cells with PGE2 in vitro can inhibit production of a proinflammatory cytokine, such as IFN-γ (64), but in vivo, the absence of PGE2 does not necessarily lead to altered expression of this cytokine following stimulation (56), emphasizing the point that many other inflammatory mediators, distinct from PGE2, can activate the same downstream pathways to upregulate responses. Inhaled PGE2 through the EP<sub>2</sub> receptor limits airway constriction to methacholine (11). However, in mice with inflamed airways, the dose-response curve is not shifted to the left in mice lacking EP<sub>2</sub> (J.M. Hartney and B.H. Koller, unpublished observations), suggesting that in the inflamed airway, other pathways available are capable of regulating airway tone.

Not only were we unable to assign a protective role to PGE2, but also our studies indicate a novel role for PGE2: in some allergic responses, PGE2 acts as a proinflammatory mediator, enhancing inflammation in the lung. To further define this proinflammatory action of PGE2, we generated bone marrow chimeras, animals in which either the lung or the recruited immune cells were deficient in the enzyme. The results from studies with these animals indicated that PGE2 produced by the lung, rather than from the recruited immune cells, contributed to the inflammatory response. Furthermore, PGE2 does not alter the development of Ag-specific T and B cell populations, but rather plays a role either in the expansion of these populations after challenge or in the recruitment of the cells to the lung. This interpretation was supported by study of mPGES1-deficient animals carrying an OVA-specific transgene. Loss of PGE2 synthesis limited the development of inflammation when these animals were challenged with Ag, implicating PGE2 in the effector phase of this response in the lung. The lack of a role for PGE2 in the sensitizing phase of the allergic response correlates well with the studies of these mice in the Der f allergic model (56). No difference was observed in the repertoire of T cells elicited by this Ag.

We cannot yet identify precise mechanisms by which PGE2 contributes to the inflammatory response in the lung. As discussed above, PGE2 can augment mast cell degranulation in vitro and in vivo, and this action is mediated through the EP<sub>2</sub> receptor (61, 62), suggesting that perhaps PGE2 augments inflammation by increasing the release of mediators from these cells. However, the immunization protocol used in this study is not mast cell dependent (66), making it unlikely that this effector cell contributes substantially to the inflammatory response. PGE2 can also increase vascular permeability and thus increase vascular leakage and formation of inflammatory exudates (67). For example, instillation of PGE2 was reported to increase migration of neutrophils into airways in response to complement exposure (68). This response was attributed to vascular changes, as it was attenuated by treatment with a vasoconstrictor. PGE2 has been reported to influence
many aspects of epithelial cell physiology, including chemokine and cytokine profiles, release of mucins, ion transport, and ciliary beat (69–73). For instance, PGE2 can stimulate the release of IL-6 from many cell types (61, 69, 74, 75) and IL-6 can contribute to inflammation in some allergenic models (75, 76). Additional experiments will be required to define precisely the circumstances and the mechanism by which inhibition of PGE2 limits disease in this allergic lung.

In summary, our studies show that loss of mPGES1, the primary enzyme required for production of PGE2 from COX-1 and COX-2 metabolites, is not required for Th2 polarization following sensitization of mice to OVA. However, although PGE2 has largely been considered protective, playing a role in limiting the inflammatory response during the effector phase to inhaled allergens, we show that under some circumstances, this is not the case. Acute inflammation in response to OVA is attenuated in mice with decreased levels of PGE2, both in mice carrying an OVA-specific transgene and in mice sensitized by exposure to Ag in the presence of adjuvant. These findings emphasize the complexity of the role for this prostanoid in immune responses and underscore the challenges of targeting PGE2 and its receptors in the treatment of lung diseases.

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Disclosures

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References


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Figure S1
Supplemental Figure 1. Measurements of airway mechanics in COX-1 -/-, COX-2 -/-, and mPGES1 -/- mice

Airway mechanics were assessed in COX-1 -/- mice and their congenic controls (A) COX-2 -/- mice and their congenic controls (B) and mPGES1 -/- mice and their controls (C). Anesthetized and intubated mice, attached to a computer-controlled small-animal ventilator, were exposed to increasing concentrations of aerosolized Mch and central airway resistance (Rn) and tissue damping (G) were assessed. No significant differences were observed between any groups at any concentration for either parameter. (AHR measurements were made on two independent cohorts for COX-1, COX-2, and mPGES1. COX-1 data representative of 2 independent experiments: +/- saline n=7, -/- saline n=8, +/- OVA n=15, -/- OVA n=13; for COX-2: +/- saline n=5, -/- saline n=4, +/- OVA n=11, -/- OVA n=12; for mPGES1: +/- saline n=4, -/- saline n=4, +/- OVA n=9, -/- OVA n=11 )
**Supplemental Figure 2. Production of PGE$_2$ in the lungs of COX-1 and COX-2 deficient animals**

PGE$_2$ levels were assessed in lung homogenate obtained from naïve or OVA sensitized and challenged COX-1 -/- (A) or COX-2 -/- (B) mice and their congenic controls. **A.** The level of PGE$_2$ measured in the lungs of naïve COX-1 -/- mice is reduced compared to congenic wildtype controls (*p<0.05). Following challenge with antigen in sensitized animals, wildtype mice have substantially elevated concentrations of lung PGE$_2$ (# p<0.01). No PGE$_2$ augmentation is observed in the COX-1 -/- inflamed lung. **B.** Naïve COX-2 -/- animals and their congenic controls have similar levels of PGE$_2$ measured in lung homogenate. Wildtype animals sensitized and challenged with OVA experience a significant enhancement of lung PGE$_2$ compared to saline treated controls (* p<0.05). No elevation of PGE$_2$ is measured in the homogenate of inflamed lungs obtained from COX-2 -/- mice. (PGE$_2$ measurements in the lungs of COX-deficient mice were made once. For COX-1: +/- saline n=3, -/- saline n=3, +/- OVA n=5, -/- OVA n=6; For COX-2: +/- saline n=3, -/- saline n=3, +/- OVA n=4, -/- OVA n=6).
**Figure S3**

**A.**

- **Sen**
  - mPGES1 +/+ vs mPGES1 -/-. 
  - OVA proliferation vs OVA concentration.

- **Sen/Chal**
  - mPGES1 +/+ vs mPGES1 -/-. 
  - OVA proliferation vs OVA concentration.

**B.**

- **IL-13 (pg/ml)**
  - Saline vs OVA.

**C.**

- **IFN-\gamma (pg/ml)**
  - Saline vs OVA.

**D.**

- **IL-17a (pg/ml)**
  - Saline vs OVA.

**E.**

- **Total Serum IgE (ng/ml)**
  - Saline vs OVA.
**Supplemental Figure 3: Ex vivo mPGES1 splenocyte responses** Wildtype and mPGES1 -/- animals were sensitized and/or challenged with OVA antigen as described. Briefly, animals were sensitized with OVA in alum on days 0 and 14. On days 21-25 some mice were challenged with OVA and spleens were collected 24 hours later. To examine the cellular response of mice receiving sensitizing OVA only, spleens from some animals were harvested on day 21 prior to challenge.  

A. Splenocytes from sensitized and sensitized/challenged animals were plated in the presence of increasing concentrations of OVA. Following a 72 hour incubation, proliferation was assessed using WST-1 reagent. No significant difference in proliferation was observed between mPGES1-deficient animals and wildtype controls in either sensitized or sensitized/challenged mice.  

B-D Splenocytes were plated in media containing 100 μg/ml OVA. Following a 72 hour incubation, cell supernatants were collected and IL-13 (B), IFN-γ (C), and IL-17a (D) cytokine levels were measured. OVA restimulation resulted in elevated cytokine levels from the splenocytes of both sensitized only and challenged animals. While a reduction was observed in IL-13 levels measured from mPGES1 -/- sensitized/challenged mice compared to wildtype controls, this difference failed to reach statistical significance. No other differences in supernatant cytokine production were observed for any experimental groups.  

(E) Sensitization with antigen resulted in elevated serum IgE in wildtype and mPGES1 -/- animals. This augmentation did not differ significantly between groups. (IgE measurements in sensitized only animals and all ex vivo splenocyte experiments were conducted twice. Data from one trial is shown. For sensitized only A: mPGES1: +/- OVA n=5, mPGES1 -/- n=7; For sensitized/challenged A: mPGES1 +/- n=4, mPGES1 -/- n=4; For sensitized only B and D: mPGES1: +/- saline n=6, -/- saline n=6, +/- OVA n=5, -/- OVA n=7; For sensitized only C: mPGES1: +/- saline n=4, -/- saline n=4, +/- OVA n=4, -/- OVA n=4; For
sensitized/challenged B-D: mPGES1: +/- saline n=4, +/- saline n=4, +/- OVA n=9, +/- OVA n=7;
For sensitized only E: mPGES1: +/- saline n=4, +/- saline n=4, +/- OVA n=6, +/- OVA n=7.)
PGE$_2$ Mediated Lung Inflammation
Supplemental Figure 4. Granulocyte Cell Analysis in OT-II transgenic mPGES1 mice

mPGES1 +/+ and -/- animals carrying an OVA-specific TCR transgene, along with transgene negative controls, were aerosolized for 5 consecutive days with OVA. 24 hours after the final challenge, BALF was collected. Total cell counts were made by hemacytometer analysis and granulocyte content was determined by cytospin preparation followed by staining with Fast Green. Wildtype mice harboring a copy of the transgene show a dramatic elevation in BALF granulocyte content, compared to transgene negative controls. This augmentation was significantly reduced in mPGES1 -/- mice (* p<0.05). (This experiment was repeated 2 times. Data shown represents one trial. mPGES1 +/- n=4, mPGES1 -/- n=5, OT-II/ mPGES1 +/- n=16, OT-II/mPGES1 -/- n=9)
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