

Allergic lung responses are increased in prostaglandin H synthase-deficient mice

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To investigate the function of prostaglandin H synthase-1 and synthase-2 (PGHS-1 and PGHS-2) in the normal lung and in allergic lung responses, we examined allergen-induced pulmonary inflammation and airway hyperresponsiveness in wild-type mice and in *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice. Among non-immunized saline-exposed groups, we found no significant differences in lung function or histopathology, although PGE₂ was dramatically reduced in bronchoalveolar lavage (BAL) fluid from *PGHS-1*^{-/-} mice, relative to wild-type or *PGHS-2*^{-/-} mice. After ovalbumin sensitization and challenge, lung inflammatory indices (BAL cells, proteins, IgE, lung histopathology) were significantly greater in *PGHS-1*^{-/-} mice compared with *PGHS-2*^{-/-} mice, and both were far greater than in wild-type mice, as illustrated by the ratio of eosinophils in BAL fluid (8:5:1, respectively). Both allergic *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice exhibited decreased baseline respiratory system compliance, whereas only allergic *PGHS-1*^{-/-} mice showed increased baseline resistance and responsiveness to methacholine. Ovalbumin exposure caused a modest increase in lung PGHS-2 protein and a corresponding increase in BAL fluid PGE₂ in wild-type mice. We conclude that (a) PGHS-1 is the predominant enzyme that biosynthesizes PGE₂ in the normal mouse lung; (b) PGHS-1 and PGHS-2 products limit allergic lung inflammation and IgE secretion and promote normal lung function; and (c) airway inflammation can be dissociated from the development of airway hyperresponsiveness in *PGHS-2*^{-/-} mice.

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Introduction

Arachidonic acid, a polyunsaturated fatty acid esterified to cell membrane glycerophospholipids, is released in response to various stimuli and then oxidized by lipoxygenase (LO), cyclooxygenase, or cytochrome P-450 monooxygenase enzymes (1, 2). A wealth of data suggests that the leukotrienes (LTs), products of the arachidonate 5-LO pathway, are proinflammatory mediators that can reproduce many of the pulmonary manifestations of asthma including airway inflammation, bronchoconstriction, increased vascular permeability, and enhanced mucus secretion (1–3). Inhibitors of 5-LO or 5-LO-activating protein and antagonists of the LT receptors possess bronchodilatory/anti-inflammatory effects in the lung and have been used to treat patients with asthma (2, 4, 5). Moreover, recent studies show that 5-LO-deficient mice exhibit reduced allergen-induced airway eosinophilia and hyperresponsiveness compared with their wild-type counterparts (6).

The role of cyclooxygenase metabolites, or prostanoids, in allergic lung disease is less clear. Prostaglandin H synthase (PGHS), the first enzyme in this pathway, converts

arachidonic acid to PGG₂, then reduces it to PGH₂. Other enzymes subsequently convert PGH₂ to PGD₂, PGE₂, PGF_{2α}, prostacyclin (PGI₂), or thromboxane A₂ (TxA₂). Release of PGD₂ into the airways is an early event after allergen challenge in sensitized asthmatic patients (7), and both PGD₂ and PGF_{2α} cause bronchoconstriction in allergic asthmatic, but not normal, subjects (8, 9). Furthermore, pulmonary expression of PGHS appears to be increased during allergic inflammation in rodents and in human asthmatic subjects (10, 11). Together, these studies have led to the concept that PGHS-derived eicosanoids have detrimental effects in the lung after allergen exposure. However, PGE₂ and PGI₂ have bronchodilatory effects (9, 12). PGE₂ also blocks both the early and late asthmatic responses to allergen challenge in asthmatics (13) and inhibits leukotriene production (14) and IgE synthesis (15). Moreover, some patients with asthma develop airway inflammation and bronchoconstriction after ingestion/inhalation of salicylates or other nonsteroidal anti-inflammatory agents that are known to inhibit PGHS, and these effects are largely prevented by inhalation of PGE₂ (16, 17). Thus, PGHS-derived

eicosanoids may also have beneficial effects in the lung after allergen exposure.

Two distinct PGHS enzymes have been described in rodents and humans (18). PGHS-1 is constitutively expressed in a number of tissues, including the lung, and is believed to be a “housekeeping” enzyme that produces prostaglandins, which are required for maintenance of normal cell and organ function. In contrast, PGHS-2 is an inducible enzyme that is upregulated by cytokines and phorbol esters, highly expressed in inflamed tissues, and believed to produce prostaglandins involved in inflammatory processes (18). Importantly, the functional significance of these two PGHS enzymes in the lung under normal conditions and their relative importance in the pathogenesis of allergic lung disease remain unknown.

Recently, mice with disrupted *Pgbs-1* or *Pgbs-2* genes were generated using gene-targeting strategies (19–21), and the characteristics of the mice have been reviewed (22). Arachidonic acid-induced ear inflammation is reduced in homozygous PGHS-1-deficient (*PGHS-1^{-/-}*) mice, whereas homozygous *PGHS-2^{-/-}* mice had normal inflammatory responses to phorbol ester and arachidonic acid treatments (19, 20). To define the roles of these two PGHS enzymes and their bioactive eicosanoid products in normal lung physiology and to investigate their relative roles in the pathogenesis of allergen-induced lung inflammation and airway hyperresponsiveness, we used these mice in an established model of allergic airway disease in which animals are sensitized and challenged with ovalbumin antigen. The objectives of this study were to determine (a) whether there are any differences in airway function and histopathology among control nonallergic wild-type mice, *PGHS-1^{-/-}* mice, and *PGHS-2^{-/-}* mice; (b) whether PGHS products contribute to airway inflammatory responses after allergen challenge; (c) whether PGHS products contribute to baseline airway function or responsiveness to methacholine (Mch) in allergic mice; and (d) whether PGHS-1 or PGHS-2 deficiency affect lung lavage eicosanoid and IgE levels in nonallergic or allergic mice.

Methods

Materials. All chemicals and reagents were purchased from Sigma Chemical Company (St. Louis, Missouri, USA) unless otherwise specified.

Experimental animals. All animal studies were conducted in accordance with principles and procedures outlined in the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees at both the National Institute of Environmental Health Sciences (NIEHS) and the U.S. Environmental Protection Agency. Pathogen-free wild-type mice, *PGHS-1^{-/-}* mice, and *PGHS-2^{-/-}* mice were obtained from the breeding colony at NIEHS. They were housed under identical conditions and fed NIH 31 rodent chow (Agway, St. Mary, Ohio, USA) ad libitum. All mice were of a hybrid C57BL/6J × 129/Ola genetic background, intercrossed for 15–20 generations. Mice were genotyped using a combination of PCR and Southern blotting using DNA isolated from tail pieces as described (19, 20). Primers used to identify genotypes were as follows:

PGHS-1^{-/-} forward: 5′-GCAGCCTCTGTTCCACATACAC-3′; *PGHS-1^{+/+}* forward: 5′-AGGAGATGGCTGCTGAGTTGG-3′; both *PGHS-1* alleles reverse: 5′-AATCTGACTTTCTGAGTTGCC-3′; *PGHS-2^{-/-}* forward: 5′-ACGCGTCACCTTAATATGCG-3′; *PGHS-2^{+/+}* forward: 5′-ACACACTCTATCACTGGCACC-3′; both *PGHS-2* alleles reverse: 5′-ATCCCTTCACTAAATGCCCTC-3′. The PCR amplifications were performed in the presence of 10% dimethylsulfoxide, 2 mM MgCl₂, and AmpliTaq DNA polymerase on a Perkin-Elmer 9600 thermal cycler. After an initial 1-minute incubation at 94°C, samples were subjected to 30 cycles of 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds, and a final 7-minute incubation at 72°C. The PCR products were electrophoresed on 1.2% agarose gels containing ethidium bromide. The size of the PCR products are 646 bp for *PGHS-1^{-/-}*, 601 bp for *PGHS-1^{+/+}*, 905 bp for *PGHS-2^{-/-}*, and 760 bp for *PGHS-2^{+/+}*.

Experimental design. Male and female mice, 5–9 months old (median 6.7 months), weighing 20–35 g were used. Within each experimental group, the sex ratio was approximately equal, and the ages of the groups did not differ significantly. This age range was used because of the time necessary to breed sufficient numbers of mice and to genotype the animals. On day 1, wild-type, *PGHS-1^{-/-}*, and *PGHS-2^{-/-}* mice ($n = 13, 13, 11$, respectively) were sensitized intraperitoneally with 20 μg of ovalbumin (Grade V; Sigma Chemical Co.) in 0.2 mL aluminum hydroxide adjuvant (Alhydrogel; Accurate Chemical & Scientific Corp., Westbury, New York, USA). Control wild-type, *PGHS-1^{-/-}*, and *PGHS-2^{-/-}* mice ($n = 8, 8, 7$, respectively) received Alhydrogel only. On 6 consecutive days (days 15–20), sensitized mice were challenged for 30 minutes per day with 1% ovalbumin in saline aerosol. Control mice were exposed to saline aerosol only. One day after the last aerosol exposure (day 21), airway function was measured, and the mice were sacrificed to assess inflammatory indices as detailed below.

Airway reactivity measurements. Mice were anesthetized with urethane (1.5 g/kg) and tracheotomized using a custom-built cannula (0.89-mm inner diameter, 1.27-mm outer diameter.) with ports for inspiration, expiration, and monitoring of airflow and pressure, similar to a design described previously (23). Animals were ventilated with a constant flow of air regulated by a mass-flow controller (FC-260; Tylan Manufacturing, Carson, California, USA). Respiratory solenoids (NVZ 110-6GZ-MS; SMC Pneumatics, Indianapolis, Indiana, USA) controlled inspiration (45% of breathing cycle) and allowed passive expiration. Breathing frequency ($50 \times \text{kg}^{-0.25}/\text{min}^{-1}$) and tidal volume (7.5 mL/kg) were determined by animal body weight. Skeletal muscle movement was blocked by administration of succinylcholine chloride (15 mg/kg, intraperitoneally). Body temperature was maintained at 37°C using an isothermal heating pad, and heart rate and waveform were monitored (SRA-200; Micro-Med, Louisville, Kentucky, USA). A single-point pressure transducer connected to 1 port of the tracheal cannula and a pneumotachograph (SCXL004DN; SenSym, Milpitas, California, USA) connected to 2 ports closer to the point of insertion in the trachea provided pressure and flow signals,

respectively. A custom software program (Mouse Reactivity System; U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA) was used to control ventilation and calculate respiratory indices. Flow was integrated to obtain volume and maximum airway opening pressure, total respiratory system compliance (C_T), resistance (R_T), and resistance at 70% tidal volume were calculated for each breath cycle. Compliance and resistance were calculated using the constant-flow inflation method single-compartment model (24). All values were averaged over 6-second intervals and stored in data files. After establishing a stable baseline, mice were challenged by intravenous infusion of doubled doses of Mch in PBS (25, 50, 100, 200 $\mu\text{g}/\text{kg}$; 0.1 $\mu\text{g}/\mu\text{L}$; 1-second delivery) every 2 minutes using a syringe pump (model 200; KD Scientific, Boston, Massachusetts, USA) for precise timing and delivery of the cholinergic agonist. To standardize lung volumes before the first dose and 1 minute after each dose, the expiratory port was occluded until airway pressure reached ~ 30 cm H_2O . Resistance of the tracheal cannula (0.25 cm $\text{H}_2\text{O}/\text{mL}$ per second; $\sim 25\%$ of baseline R_T) was subtracted from measured values of R_T , and responses to each dose were calculated as (peak R_T - baseline R_T) or (baseline C_T - lowest C_T) using the baseline value immediately before each dose.

Bronchoalveolar lavage and analysis of bronchoalveolar lavage fluid cells, proteins, and arachidonic acid metabolites. After measurement of airway function, lungs were lavaged with five 1-mL aliquots of HBSS. Approximately 90% of the total injected volume was consistently recovered. The bronchoalveolar lavage (BAL) fluid was placed on ice and centrifuged at 360 g for 12 minutes at 4°C. Aliquots of BAL fluid supernatants were kept at 4°C for biochemical analysis (see below) or stored at -80°C for IgE or eicosanoid analyses. Cells were resuspended in 1.0 mL of HBSS and counted (Coulter Electronics Ltd., Hialeah, Florida, USA). Slides of BAL fluid cells were prepared (Cytospin 3; Shandon, Pittsburgh, Pennsylvania, USA), stained with Leuko-Stat (Fisher Scientific Co., Pittsburgh, Pennsylvania, USA), and at least 500 cells per sample were differentiated using conventional morphological criteria for macrophages/monocytes, lymphocytes, neutrophils, and eosinophils. Slides were number coded and cells differentiated in a blinded fashion. Assays for total protein, microalbumin, lactate dehydrogenase (LDH) and *N*-acetyl- β -D-glucosaminidase (NAG) were modified for use on the Cobas Fara II centrifugal spectrophotometer (Hoffman LaRoche Inc., Nutley, New Jersey, USA) as described previously (25). PGE_2 and LTB_4 levels were determined by RIA using kits supplied by Amersham Life Sciences Inc. (Arlington Heights, Illinois, USA) according to the manufacturer's instructions. $\text{LTC}_4/\text{LTD}_4/\text{LTE}_4$ levels were determined by enzyme immunoassay (Amersham Life Sciences). For the LTB_4 and cysteinyl LT analyses, BAL samples were purified using Amprep C2 ethyl columns (Amersham Life Sciences), according to the manufacturer's instructions. After BAL, the left lung was frozen in liquid nitrogen, and the remaining lung was fixed as described below.

Protein immunoblotting. Microsomal fractions were prepared from frozen mouse lungs by differential centrifu-

gation at 4°C as described (26), resuspended in 50 mM Tris-Cl (pH 7.4), 1 mM dithiothreitol, 1 mM EDTA, and 20% (vol/vol) glycerol, and stored at -80°C. Goat anti-mouse PGHS-1 (Santa Cruz Biotechnology Inc., Santa Cruz, California, USA) and rabbit anti-mouse PGHS-2 (Cayman Chemical Co., Ann Arbor, Michigan, USA) were specific for their respective PGHS isoforms and used according to the manufacturers' instructions. Recombinant PGHS-1 and PGHS-2 protein standards were prepared as described by Chulada et al. (27). For immunoblotting, proteins were resolved by electrophoresis in 10% SDS (wt/vol) polyacrylamide gels (80 \times 80 \times 1 mm) (Novex, San Diego, California, USA) and transferred electrophoretically to nitrocellulose membranes. Membranes were immunoblotted using the primary antibodies, goat anti-rabbit or rabbit anti-goat IgG conjugated to horseradish peroxidase (Bio-Rad Laboratories, Richmond, California, USA), and the ECL Western Blotting Detection System (Amersham International, Buckinghamshire, United Kingdom). Autoradiographs were scanned using a ChemImager 4000 Low Light Imaging System (Alpha Innotech Corp., San Leandro, California, USA).

Isolation of total RNA and Northern analysis. Total RNA was prepared from frozen lungs using TRIreagent (Molecular Research Center Inc., Cincinnati, Ohio, USA) according to the manufacturer's instructions. Northern blots were performed using either a 1.7-kb mouse PGHS-1 cDNA probe (Oxford Biomedical, Oxford, Michigan, USA) or a 2.1-kb mouse PGHS-2 cDNA probe (20) as described previously (19, 20).

Lung histopathology. Lungs were perfusion fixed at 20 cm H_2O in 10% neutral buffered formalin, processed routinely, and embedded in paraffin. Serial sections (5-6 μm) were stained with either hematoxylin and eosin, alcian blue/periodic acid-Schiff, or mucicarmine. A semiquantitative histopathologic scoring system was developed based upon the presence and abundance of the following: (a) lymphoid aggregates (0, absent; 1, identifiable at low magnification around fewer than 50% of vessels and bronchi; 2, identifiable around more than 50% of vessels and bronchi; 3, identifiable around more than 50% of vessels and bronchi with acute inflammation and extension to involve small vessels); (b) large, eosinophilic alveolar macrophages (0, absent; 1, present in fewer than 25% of alveolar spaces; 2, present in 25-75% of alveolar spaces; 3, present in more than 75% of alveoli, often in tightly packed clusters); (c) multinucleated giant cells (0, absent; 1, occasionally identified while examining slides at medium magnification [$\times 10$ objective] and not generally visible at low power [$\times 2$ or $\times 4$ objective]; 2, present and numerous, easily identified at lower magnification); (d) large eosinophilic intracytoplasmic inclusions (crystals) in macrophages (0, absent; 1, identified in a few cells upon examination at high-power magnification [$\times 20$ objective]; 2, present and numerous, sometimes large and extracellular; 3, extremely numerous with many lying free within alveolar spaces); (e) alveolar consolidation with mononuclear and polymorphonuclear leukocytes or mixed inflammation (0, absent; 1, consolidation in less than 25% of alveolar spaces; 2, consolidation in more than 25% of alveoli); (f) eosinophils (0, absent; 0.5, a few

identified within inflammatory exudates at high magnification; 1, present in multiple fields but never more than 10% of cells identified); and (g) goblet cells (0, absent; 1, present in fewer than 50% of the airways; 2, abundant in the majority of airways). A total inflammatory score (range 0–16), taken as the sum of the individual scores, was determined by an investigator blinded to genotype and treatment group assignment. Intraobserver variability was determined by reexamination of 20 randomly selected slides for all 7 parameters at a later time. The same value was assigned 80% of the time. In the remaining 20%, the second score differed from the first by 1 grade. The total inflammatory score was highly correlated with other measures of inflammation including total BAL fluid inflammatory cells ($r^2 = 0.723$, $P < 0.00001$).

Measurement of total IgE. Total IgE in BAL fluid supernatant was measured by sandwich ELISA, using rat anti-mouse IgE (clone R35-72), purified mouse IgE standards (clone IgE-3), biotinylated rat anti-mouse IgE (clone R35-118) and other reagents from PharMingen (San Diego, California, USA) according to the manufacturer's instructions. Plates were developed using streptavidin-alkaline phosphatase (Kirkegaard and Perry Laboratories Inc., Gaithersburg, Maryland, USA) and *p*-nitrophenylphosphate disodium, and read at 405 nm on a Thermomax plate reader (Molecular Devices, Menlo

Park, California, USA). Concentrations of IgE were calculated from a quadratic equation fit of the standard curve. Values that were too low to be interpolated from the standard curve were assigned the lowest detectable standard value of the assay (6.25 ng/mL).

Statistical analysis. All values are expressed as mean \pm SE. Data were analyzed by ANOVA using SYSTAT software (SYSTAT Inc., Evanston, Illinois, USA). When *F* values indicated that a significant difference was present, Fisher's LSD test for multiple comparisons was used. Values were considered significantly different if *P* was less than 0.05.

Results

BAL fluid inflammatory cells are increased in PGHS-deficient mice after allergen exposure. There were no significant differences in the numbers or types of BAL fluid cells between nonimmunized wild-type, *PGHS-1*^{-/-}, and *PGHS-2*^{-/-} mice (Table 1). After allergen exposure, the total cell number in immunized wild-type mice increased 11-fold, and the majority of these cells were eosinophils (66%) (Table 1). Compared with allergic wild-type mice, the total number of cells in allergic *PGHS-1*^{-/-} mice was 6-fold greater and the number of eosinophils was 8-fold greater ($P < 0.001$). Numbers of lymphocytes and neutrophils, but not macrophages, were also significantly

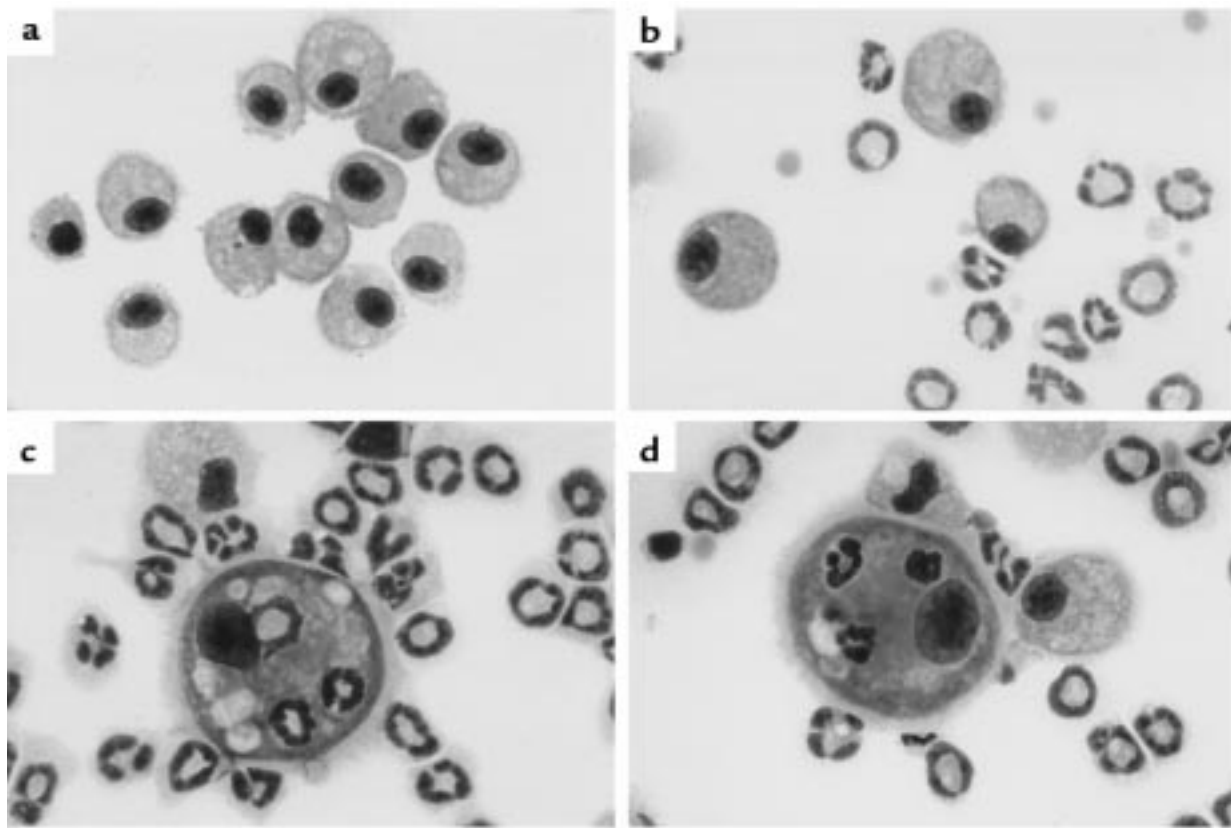


Figure 1

Stained cytopins of BAL fluid cells showing alveolar macrophage morphology in wild-type and *PGHS*-deficient mice. Wild-type, *PGHS-1*^{-/-}, and *PGHS-2*^{-/-} mice were administered adjuvant only and exposed to saline aerosol or sensitized with ovalbumin in adjuvant and challenged with ovalbumin aerosol. BAL cells were collected 1 day after final challenge. (a) Nonimmunized wild-type mice. (b) Allergic wild-type mice. (c) Allergic *PGHS-1*^{-/-} mice. (d) Allergic *PGHS-2*^{-/-} mice. $\times 100$.

Table 1Numbers (10⁶) and percentages of inflammatory cells in BAL fluid from nonimmunized and allergic wild-type, *PGHS-1*^{-/-}, and *PGHS-2*^{-/-} mice^A

Cell type	Saline			OVA		
	Wild-type (8)	<i>PGHS-1</i> ^{-/-} (8)	<i>PGHS-2</i> ^{-/-} (7)	Wild-type (13)	<i>PGHS-1</i> ^{-/-} (13)	<i>PGHS-2</i> ^{-/-} (10)
Total cells	0.111 ± 0.018	0.098 ± 0.010	0.086 ± 0.012	1.179 ± 0.367	7.640 ± 0.864 ^{B,C}	4.881 ± 1.132 ^{B,D}
Macrophages	0.108 ± 0.018 (97)	0.092 ± 0.010 (94)	0.084 ± 0.013 (98)	0.288 ± 0.083 (24)	0.383 ± 0.068 ^B (5)	0.275 ± 0.065 (6)
Eosinophils	0.001 ± 0.001 (1)	0.002 ± 0.001 (2)	0.000 ± 0.000 (0)	0.777 ± 0.255 (66)	6.456 ± 0.791 ^{B,C} (85)	4.216 ± 1.028 ^{B,D} (86)
Neutrophils	0.001 ± 0.000 (1)	0.001 ± 0.001 (1)	0.000 ± 0.000 (0)	0.033 ± 0.014 (3)	0.085 ± 0.027 ^{B,C} (1)	0.033 ± 0.009 (1)
Lymphocytes	0.002 ± 0.000 (1)	0.003 ± 0.001 (3)	0.001 ± 0.000 (1)	0.081 ± 0.023 (7)	0.716 ± 0.075 ^{B,C} (9)	0.356 ± 0.073 ^{B,D} (7)

^ALungs were lavaged as described in Methods. Numbers in parentheses below group name indicate *n* of animals in that group. Data show mean ± SE and average percentage of total cells (in parentheses below means). ^B*P* < 0.01 vs. corresponding saline-exposed control group. ^C*P* < 0.05 vs. allergic wild-type and allergic *PGHS-2*^{-/-} groups. ^D*P* < 0.001 vs. allergic wild-type group.

increased in allergic *PGHS-1*^{-/-} vs. allergic wild-type mice. Interestingly, allergic *PGHS-1*^{-/-} BAL fluid macrophages displayed morphologic characteristics consistent with cellular activation (including increased size, vacuolated cytoplasm, and evidence for phagocytosis of amorphous debris and cells), whereas allergic wild-type BAL fluid macrophages did not (Figure 1). BAL fluid from allergic *PGHS-2*^{-/-} mice contained 4-fold more total cells than allergic wild-type mice (*P* < 0.001) but significantly fewer than allergic *PGHS-1*^{-/-} mice (*P* < 0.005) (Table 1). As before, the increased number of BAL fluid cells in the *PGHS-2*^{-/-} animals was largely due to increased numbers of eosinophils (86% of total cells, *P* < 0.001 vs. wild-type mice). As with allergic *PGHS-1*^{-/-} macrophages, allergic *PGHS-2*^{-/-} macrophages appeared activated (Figure 1). These data show that after immunization and allergen exposure (a) *PGHS*-deficient mice have increased airway inflammatory cell influx (primarily eosinophils and lymphocytes) compared with wild-type mice; (b) the magnitude of inflammatory cell influx is greater in *PGHS-1*^{-/-} vs. *PGHS-2*^{-/-} mice; and (c) alveolar macrophages from *PGHS*-deficient mice display a morphology that is consistent with cellular activation.

PGHS-deficient mice have histopathologic evidence for increased lung inflammation after allergen exposure. Lungs from non-immunized wild-type, *PGHS-1*^{-/-}, and *PGHS-2*^{-/-} mice were histologically normal (Figure 2, a and e, shown only for

wild-type mice). After allergen exposure, immunized wild-type mice exhibited mild inflammation confined mostly to perivascular/peribronchial regions, characterized by the presence of lymphoid aggregates, focal areas of consolidation with mononuclear and polymorphonuclear leukocytes, occasional large eosinophilic alveolar macrophages, and rare multinucleated giant cells (Figure 2, b and f). In contrast, lungs from allergic *PGHS-1*^{-/-} mice had severe inflammation that not only involved perivascular/peribronchial regions but also extended into the airspace (Figure 2, c and g). Lymphoid aggregates were identifiable around most blood vessels and bronchi and often involved the smallest vessels. Airway epithelium was thickened, alveoli were packed with clusters of large eosinophilic macrophages and eosinophils, multinucleated giant cells were numerous (Figure 2i), and eosinophilic crystals were present within the cytoplasm of macrophages and sometimes in the extracellular space (Figure 2j). Ultrastructurally, the crystals were electron dense and were usually surrounded by a single membrane. Their morphology was distinctly different from the typical hexagonal appearance of Charcot-Leyden crystals. There was no evidence for airway remodeling or changes in smooth muscle mass in allergic wild-type or *PGHS*-deficient mice, probably because of the relatively short time course of allergen exposure. Lungs from allergic *PGHS-2*^{-/-} mice exhibited inflammation that was less intense than

Table 2Lung inflammatory scores in nonimmunized and allergic wild-type, *PGHS-1*^{-/-}, and *PGHS-2*^{-/-} mice^A

Lesion	Saline			OVA		
	Wild-type (8)	<i>PGHS-1</i> ^{-/-} (8)	<i>PGHS-2</i> ^{-/-} (7)	Wild-type (13)	<i>PGHS-1</i> ^{-/-} (13)	<i>PGHS-2</i> ^{-/-} (11)
Lymphoid aggregates	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	1.1 ± 0.1	2.8 ± 0.1	2.3 ± 0.2
Eosinophilic macrophages	0.1 ± 0.1	0.3 ± 0.2	0.0 ± 0.0	0.8 ± 0.2	2.8 ± 0.1	1.7 ± 0.2
Multinucleated giant cells	0.1 ± 0.1	0.3 ± 0.2	0.0 ± 0.0	0.7 ± 0.2	1.9 ± 0.1	1.5 ± 0.2
Crystals in macrophages	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	1.6 ± 0.1	0.8 ± 0.2
Alveolar consolidation	0.3 ± 0.2	0.3 ± 0.2	0.0 ± 0.0	0.9 ± 0.1	1.8 ± 0.1	1.2 ± 0.1
Eosinophils	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.6 ± 0.1	0.3 ± 0.1
Goblet cells	0.1 ± 0.1	0.4 ± 0.2	0.0 ± 0.0	1.3 ± 0.2	2.0 ± 0.0	1.7 ± 0.1
Total Score	1.0 ± 0.5	1.9 ± 0.8	0.4 ± 0.2	5.1 ± 0.6 ^B	13.6 ± 0.4 ^{C,D}	9.5 ± 1.0 ^C

^AHistopathological scoring system was based on 7 independent variables as described in detail in Methods. Data show mean ± SE. Numbers in parentheses below group name indicate number of animals in that group. ^B*P* < 0.005 vs. all nonimmunized control mice. ^C*P* < 0.001 vs. allergic wild-type and all nonimmunized mice. ^D*P* < 0.001 vs. allergic *PGHS-2*^{-/-} mice.

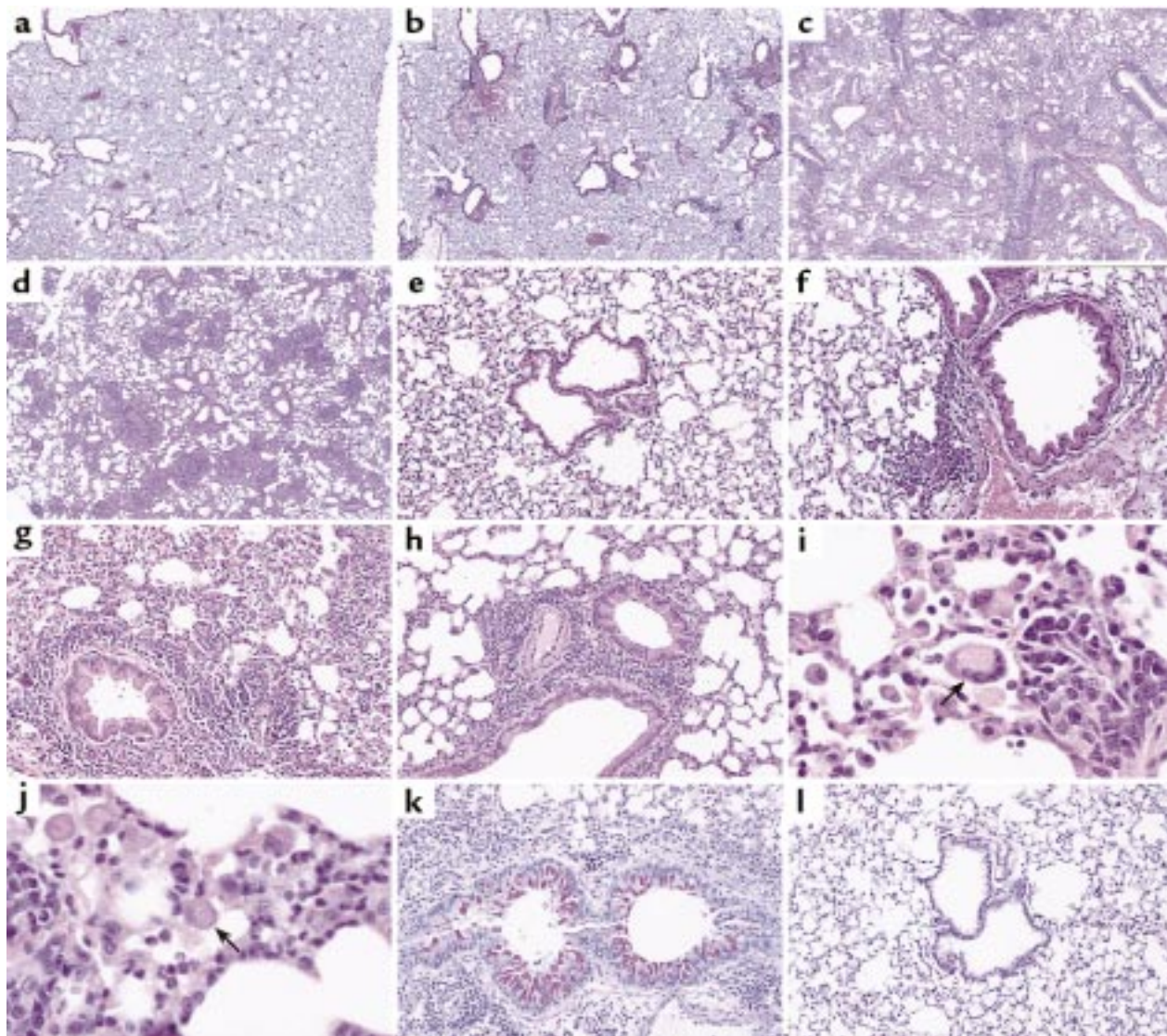


Figure 2

(a–l) Lung histopathology showing increased inflammation in PGHS-deficient mice compared with wild-type mice. (a, e, and l) Nonimmunized wild-type mice. (b and f) Allergic wild-type mice. (c, g, i, j, and k) Allergic *PGHS-1*^{-/-} mice. Arrow in i indicates multinucleated giant cell. Arrow in j indicates eosinophilic crystals within macrophage. (d and h) Allergic *PGHS-2*^{-/-} mice. All sections were stained with hematoxylin and eosin except k and l, which were stained with alcian blue/periodic acid-Schiff. ×10 (a–d), ×40 (e–h, k, and l), and ×80 (i and j).

allergic *PGHS-1*^{-/-} mice, but more severe than allergic wild-type mice (Figure 2, d and h). Compared with allergic *PGHS-1*^{-/-} mice, the inflammation observed in allergic *PGHS-2*^{-/-} mice was more focal and confined mainly to interstitial regions surrounding vessels and bronchi. In addition, allergic *PGHS-2*^{-/-} mice showed loss of fine alveolar septae in comparison with allergic wild-type mice (Figure 2f), and remaining septae were thickened. These alterations in parenchymal alveolar structure were comparable to those observed in allergic *PGHS-1*^{-/-} mice. Airway mucus production, as determined by staining with alcian blue/periodic acid-Schiff and mucicarmine stains, was more pronounced in allergic *PGHS-1*^{-/-} (Figure 2k) and *PGHS-2*^{-/-} mice than in allergic wild-type or nonimmunized mice of any genotype (Figure 2l).

A semiquantitative, histopathologic lung scoring system was used to evaluate the degree of inflammation in

the different experimental groups. Nonimmunized wild-type mice had a total lung inflammatory score of 1.0 ± 0.5 units, which was not significantly different from that of nonimmunized *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice (Table 2). After allergen challenge, immunized wild-type mice had a total lung inflammatory score of 5.1 ± 0.6 units, which was significantly greater than nonimmunized mice of any genotype ($P < 0.005$). In contrast, allergic *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice exhibited significantly more lung inflammation (total inflammatory scores 13.6 ± 0.4 and 9.5 ± 1.0 units, respectively; $P < 0.001$ vs. wild-type mice). The degree of lung inflammation in allergic *PGHS-1*^{-/-} mice was significantly greater than that in allergic *PGHS-2*^{-/-} mice ($P < 0.001$). Furthermore, the scores for each of the individual histologic variables except goblet cell metaplasia was significantly higher in allergic *PGHS-1*^{-/-} mice than in allergic *PGHS-2*^{-/-} mice (P

< 0.05; Table 2). Taken together, these data confirm that allergic PGHS-deficient mice have significantly increased lung inflammation compared with allergic wild-type mice and that *PGHS-1*^{-/-} mice exhibit the most profound histopathologic alterations.

PGHS-deficient mice exhibit increased alveolar epithelial permeability, lysosomal enzyme release, and cytotoxicity after allergen exposure. To determine if the increased lung inflammation present in *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice after allergen exposure was accompanied by changes in alveolar epithelial permeability, BAL fluid total protein and microalbumin were measured (Figure 3). Protein levels in nonimmunized wild-type, *PGHS-1*^{-/-}, and *PGHS-2*^{-/-} mice were not significantly different (48 ± 10, 64 ± 4, and 63 ± 4 mg/mL, respectively). BAL fluid protein levels in allergic *PGHS-1*^{-/-} mice were 4-fold higher than in allergic wild-type mice (491 ± 31 vs. 114 ± 16 mg/mL; *P* < 0.001) suggesting that increased epithelial permeability accompanied the inflammatory process. Allergic *PGHS-2*^{-/-} mice also had higher BAL fluid protein (311 ± 56 mg/mL; *P* < 0.001 vs. immunized wild-type mice), although these levels were significantly less than in *PGHS-1*^{-/-} mice (*P* < 0.001). Analysis of BAL fluid microalbumin gave similar results (data not shown).

To explore whether the allergen-induced inflammation in the *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice was accompanied by increased lysosomal enzyme release, we measured BAL fluid NAG, an established marker of alveolar macrophage activation (28). Compared with allergic wild-type mice, allergic *PGHS-1*^{-/-} mice exhibited a 3-fold increase in BAL fluid NAG (*P* < 0.001 vs. allergic wild-type mice), and allergic *PGHS-2*^{-/-} mice had a 2-fold increase in BAL fluid NAG (*P* < 0.005 vs. allergic wild-type mice; *P* < 0.01 vs. allergic *PGHS-1*^{-/-} mice) (Figure 3b). BAL LDH was measured as an index of cellular toxicity. There were no significant differences in this variable between allergic wild-type mice and nonallergic mice of any genotype (Figure 3c). In contrast, allergic *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice had 2- to 3-fold higher BAL fluid LDH levels than did allergic wild-type mice (*P* < 0.005). These results suggest that the increased inflammation in PGHS-deficient mice is associated with increased alveolar epithelial permeability, release of lysosomal enzymes from activated macrophages, and cellular toxicity.

Both allergic PGHS-1^{-/-} and *PGHS-2*^{-/-} mice exhibit decreased baseline respiratory system compliance, whereas only allergic *PGHS-1*^{-/-} mice exhibit increased respiratory system resistance and reactivity to Mch. Baseline total respiratory system resistance (*R*_T) was not significantly different among nonimmunized wild-type, *PGHS-1*^{-/-}, and *PGHS-2*^{-/-} mice (Figure 4a). In contrast, allergic *PGHS-1*^{-/-} mice exhibited significantly increased baseline *R*_T, ranging from 47% greater before the first dose (25 μg/kg) of Mch to 80% greater before the last dose (200 μg/kg), in comparison with all other groups. In addition, Mch induced significantly greater increases in *R*_T in allergic *PGHS-1*^{-/-} mice compared with all other groups, except at 200 μg/kg Mch, where the increase was not significantly different from that in allergic wild-type mice. Allergic wild-type mice were hyperresponsive compared with nonimmunized wild-type mice after dosing with 50 μg/kg Mch. Relative to baseline resistance [(Mch response - base-

line)/baseline], allergic wild-type and *PGHS-1*^{-/-} mice were equally hyperresponsive compared with their respective nonallergic controls at the first 3 doses of Mch (data not shown). Interestingly, there were no differences between nonallergic and allergic *PGHS-2*^{-/-} mice with respect to baseline *R*_T or Mch-induced increases in *R*_T.

In contrast with *R*_T, baseline respiratory system compliance (*C*_T) was significantly reduced in both allergic *PGHS-1*^{-/-} and *PGHS-2*^{-/-} groups compared with allergic wild-type mice and all nonimmunized groups (Figure 4b). There was no significant difference in baseline *C*_T between allergic *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice, and the reduction in compliance averaged 35% for both groups in comparison with the other 4 groups. Mch infusion decreased *C*_T to a significantly greater degree in allergic

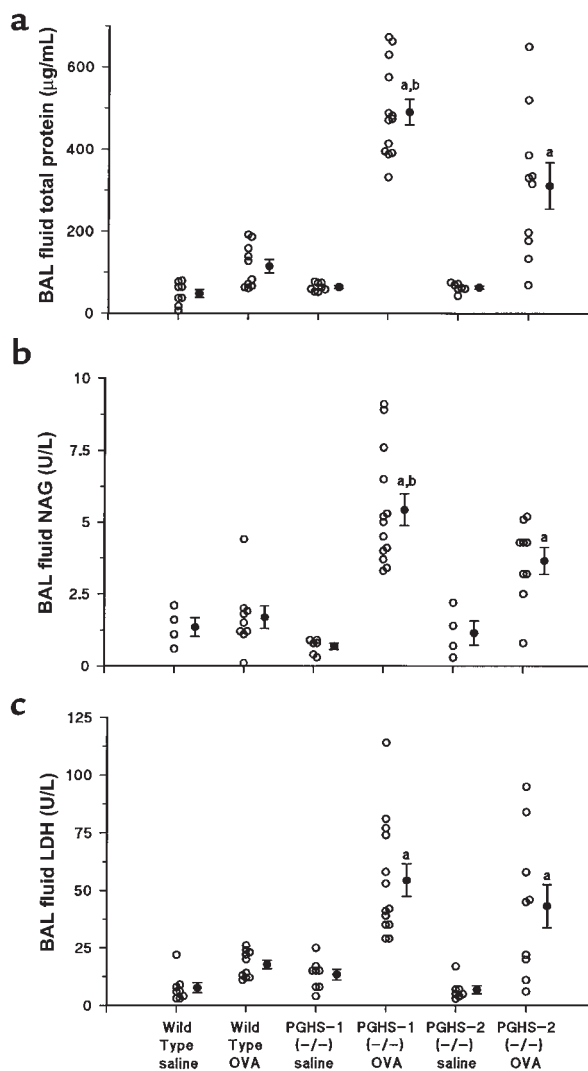
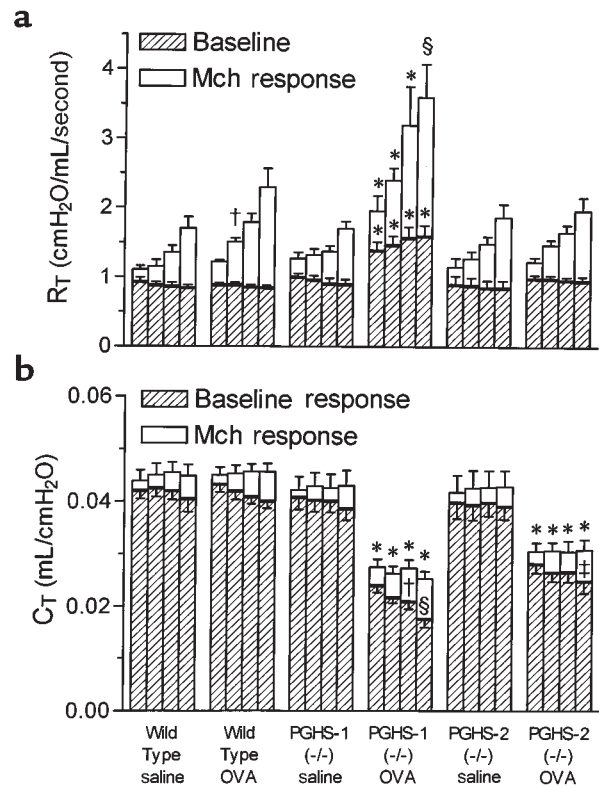


Figure 3 BAL fluid proteins are increased in allergic PGHS-deficient mice compared with wild-type mice. Wild-type, *PGHS-1*^{-/-}, and *PGHS-2*^{-/-} mice were divided into treatment groups and BAL fluid total protein, NAG, and LDH were determined as described in Methods. Each open circle represents results from 1 animal. Filled circles and error bars show mean ± SE. (a) BAL fluid total protein. (b) BAL fluid NAG. (c) BAL fluid LDH. ^a*P* < 0.01 vs. all nonimmunized (saline) and wild-type ovalbumin (OVA) groups. ^b*P* < 0.01 vs. *PGHS-2*^{-/-} OVA.

Figure 4

Airway responsiveness to methacholine in PGHS-deficient and wild-type mice. Wild-type, *PGHS-1*^{-/-}, and *PGHS-2*^{-/-} mice were divided into treatment groups as described in Figure 1. Airway responses to intravenous Mch were measured 1 day after final aerosol exposure, as described in Methods. Bars represent mean ± SE of 7–13 mice per group. (a) R_T before dosing with Mch and at peak of response to 25, 50, 100, and 200 μg/kg Mch (left to right for each group). For each dose, significant differences among groups were determined for associated baselines (shown above hatched bars) and increases in R_T due to Mch (shown above open bars). **P* < 0.05 vs. all other groups. †*P* < 0.05 vs. wild-type saline. §*P* < 0.05 vs. all groups except wild-type OVA. (b) Total respiratory system-compliance (C_T), measured simultaneously with R_T, before dosing with Mch and at nadir of response to Mch. For each dose, significant differences among groups were determined for associated baselines (hatched bars + open bars; shown above open bars) and decreases in C_T due to Mch (shown within open bars). **P* < 0.05 vs. wild-type saline, wild-type OVA, *PGHS-1*^{-/-} saline, and *PGHS-2*^{-/-} saline. †*P* < 0.05 vs. *PGHS-1*^{-/-} saline and *PGHS-2*^{-/-} OVA. §*P* < 0.05 vs. wild-type OVA and *PGHS-1*^{-/-} saline. ‡*P* < 0.05 vs. *PGHS-2*^{-/-} saline.



PGHS-1^{-/-} and *PGHS-2*^{-/-} mice compared with nonimmunized *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice, respectively, at higher doses of Mch. At these doses, the Mch-induced decreases in C_T in allergic *PGHS-1*^{-/-} mice were greater than in allergic wild-type or allergic *PGHS-2*^{-/-} mice, although the overall change was not great.

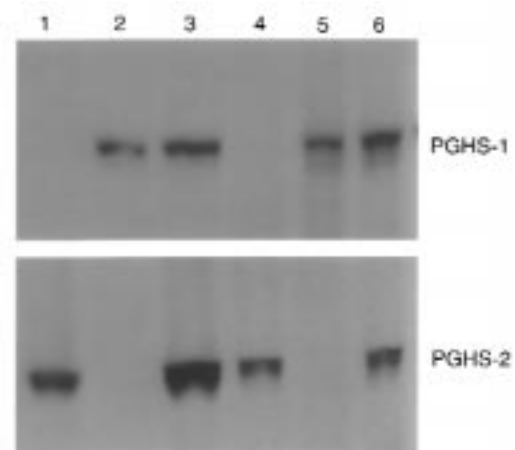
PGHS-1 is the major enzyme that biosynthesizes PGE₂ in mouse airways under basal conditions, whereas lung *PGHS-2* protein is upregulated after allergen exposure. Protein immunoblotting with antibodies specific for *PGHS-1* and *PGHS-2* revealed that nonimmunized wild-type mice constitutively expressed both *PGHS-1* and *PGHS-2* proteins in the lung, whereas *PGHS-1*^{-/-} mice expressed only *PGHS-2* protein and *PGHS-2*^{-/-} mice expressed only *PGHS-1* protein (Figure 5). Furthermore, the levels of

expression of *PGHS-1* and *PGHS-2* in *PGHS-2*^{-/-} and *PGHS-1*^{-/-} lungs, respectively, were comparable to those in wild-type lungs, indicating that *PGHS-1* and *PGHS-2* proteins are not coordinately regulated in vivo.

To determine which of the two *PGHS* isoforms was primarily responsible for PGE₂ biosynthesis in mouse airways under basal conditions, PGE₂ levels were measured in BAL fluid from nonimmunized wild-type, *PGHS-1*^{-/-}, and *PGHS-2*^{-/-} mice. BAL fluid obtained from nonimmunized wild-type and *PGHS-2*^{-/-} mice contained equivalent amounts of PGE₂ (206 ± 42 and 201 ± 38 pg/mL, respectively) (Figure 6a). In contrast, BAL fluid obtained from nonimmunized *PGHS-1*^{-/-} mice contained significantly less PGE₂ (56 ± 7 pg/mL; *P* < 0.05 vs. wild-type and *PGHS-2*^{-/-} mice) indicating that *PGHS-1* is

Figure 5

PGHS-1 and *PGHS-2* expression in nonimmunized and allergic wild-type, *PGHS-1*^{-/-}, and *PGHS-2*^{-/-} mice. Wild-type, *PGHS-1*^{-/-}, and *PGHS-2*^{-/-} mice were divided into treatment groups, and the abundance of *PGHS-1* and *PGHS-2* protein in the lungs was determined by Western blotting using immunospecific antibodies as described in Methods. Results are representative of experiments with 18 different animals (3 mice in each of the 6 groups). Lane 1, *PGHS-1*^{-/-} mice, allergic; lane 2, *PGHS-2*^{-/-} mice, allergic; lane 3, wild type mice, allergic; lane 4, *PGHS-1*^{-/-} mice, nonimmunized; lane 5, *PGHS-2*^{-/-} mice, nonimmunized; lane 6, wild-type mice, nonimmunized.



the major enzyme responsible for basal production of PGE₂ in mouse airways. After allergen exposure, PGE₂ levels in immunized wild-type mice (383 ± 55 pg/mL) were significantly increased relative to all nonallergic groups and allergic *PGHS-1*^{-/-} mice (*P* < 0.005), but not relative to allergic *PGHS-2*^{-/-} mice. Accompanying this increase in BAL fluid PGE₂ was a corresponding increase in lung PGHS-2, but not PGHS-1 immunoreactive protein (Figure 5). Lung PGHS-2 protein was also increased, albeit to a lesser extent, in allergic *PGHS-1*^{-/-} mice compared with nonimmunized *PGHS-1*^{-/-} mice, but BAL fluid PGE₂ levels were not significantly different (Figures 5 and 6a). The increase in PGHS-2 protein was not accompanied by a corresponding increase in PGHS-2 mRNA (data not shown), suggesting that these allergen-induced changes likely occur at the posttranscriptional level. These data show that PGHS-1 is the predominant enzyme that biosynthesizes PGE₂ in mouse lung under basal conditions and that lung PGHS-2 protein is upregulated after allergen exposure.

Increased LT biosynthesis occurs in allergic PGHS-1^{-/-} but not *PGHS-2*^{-/-} mice. To determine if increased metabolism along the 5-LO pathway occurs in allergic PGHS-deficient mice, we measured levels of LTB₄ and the cysteinyl leukotrienes in BAL fluid. LTB₄ levels were increased in BAL fluid from allergic *PGHS-1*^{-/-} mice compared with allergic wild-type and *PGHS-2*^{-/-} mice and nonimmunized mice of any genotype (Figure 6b). Cysteinyl leukotrienes were also increased in BAL fluid from allergic *PGHS-1*^{-/-} mice compared with allergic *PGHS-2*^{-/-} and wild-type mice (21.4 ± 2.9, 8.2 ± 3.1, and 7.1 ± 2.9 pg/mL, respectively; *P* < 0.05). These results suggest that increased metabolism along the 5-LO pathway could contribute, in part, to the observed differences in pathology, inflammation, and lung function in allergen-challenged *PGHS-1*^{-/-} mice.

Total IgE levels in BAL fluid are increased in both PGHS-1^{-/-} and *PGHS-2*^{-/-} mice, but not wild-type mice, after allergen challenge. Total BAL fluid IgE levels were minimal in all nonimmunized groups of mice (Figure 7). There was no significant increase in IgE in allergic wild-type mice in comparison with nonimmunized mice. In contrast, there were significant increases in total IgE in both *PGHS-1*^{-/-} and *PGHS-2*^{-/-} allergic groups in comparison with nonimmunized groups and allergic wild-type mice (*P* < 0.001). The difference between allergic *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice was significant (*P* < 0.01).

Discussion

The purpose of this study was to define the relative functional roles of the two PGHS enzymes and their eicosanoid products in the lung under normal conditions and to investigate their involvement in the pathogenesis of allergen-induced lung inflammation and airway hyperresponsiveness. Mice with disrupted *Pgbs-1* or *Pgbs-2* genes were used to circumvent potential problems related to lack of isoform specificity of available PGHS inhibitors and possible effects of these agents on unrelated pathways (18, 29). Under basal conditions, both *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice were indistinguishable from wild-type mice with respect to various lung histologic, functional, and biochemical variables, although

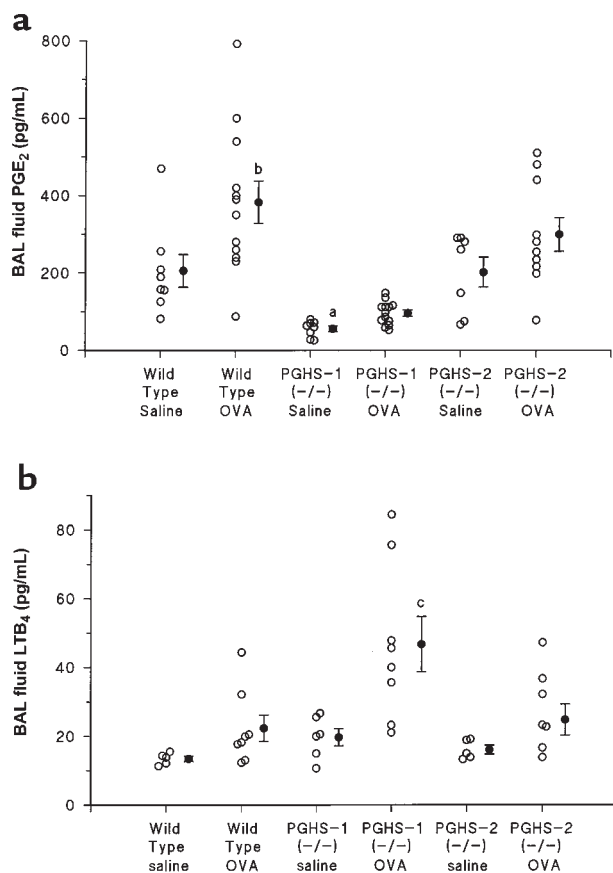


Figure 6

BAL fluid eicosanoid levels in PGHS-deficient and wild-type mice. BAL fluid PGE₂ (a) and LTB₄ (b) were determined by RIA in wild-type, *PGHS-1*^{-/-}, and *PGHS-2*^{-/-} mice (groups described in Figure 1) as described in Methods. Each open circle represents results from 1 animal. Filled circles and error bars show mean ± SE. ^a*P* < 0.05 vs. wild-type groups and *PGHS-2*^{-/-} saline groups. ^b*P* < 0.005 vs. saline-exposed wild-type and OVA-exposed *PGHS-1*^{-/-} groups. ^c*P* < 0.005 vs. all other groups.

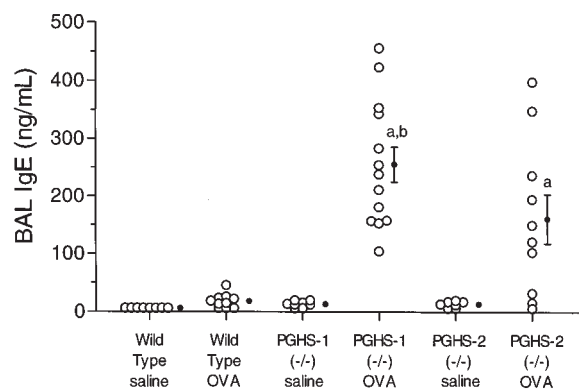


Figure 7

Total IgE levels in BAL fluid of PGHS-deficient and wild-type mice. Total IgE levels in BAL fluid from nonimmunized and allergic wild-type, *PGHS-1*^{-/-}, and *PGHS-2*^{-/-} mice were analyzed as described in Methods. Each open circle represents results from animal. Filled circles and error bars show mean ± SE. ^a*P* < 0.001 vs. all saline control groups and wild-type OVA. ^b*P* < 0.01 vs. *PGHS-2*^{-/-} OVA.

PGHS-1^{-/-} mice had markedly reduced BAL fluid PGE₂. Thus, the constitutive “housekeeping” PGHS isoform responsible for the majority of PGE₂ biosynthesis within the lung was apparently not required to maintain normal lung cell and organ function under ordinary conditions. After allergen exposure, lung inflammation, airway hyperresponsiveness, and indices of lung injury were significantly greater in immunized *PGHS-1*^{-/-} mice compared with wild-type mice. Thus, PGHS-1 products have mainly protective effects in the allergic mouse lung. Immunized *PGHS-2*^{-/-} mice also showed greater lung inflammation after allergen exposure, suggesting that PGHS-2-derived eicosanoids are also primarily protective. The increased inflammatory response of allergic *PGHS-2*^{-/-} mice is compatible with recent data showing an acquired deficiency of PGHS-2 expression in another proinflammatory lung condition, human idiopathic pulmonary fibrosis (30). Interestingly, the increased airway inflammation in allergic *PGHS-2*^{-/-} mice was not associated with airway hyperresponsiveness to Mch. Although airway inflammation is frequently correlated with airway hyperresponsiveness, and treatment of inflammation often improves this condition, this correlation has not been observed in some studies of animals and humans (4, 31).

We found decreased baseline compliance in both allergic *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice, compared with allergic wild-type mice. In contrast, only allergic *PGHS-1*^{-/-} mice showed increased baseline respiratory system resistance and hyperresponsiveness to Mch challenge. However, relative to baseline resistance values, wild-type and *PGHS-1*^{-/-} allergic groups were equally hyperresponsive to Mch, because baseline values were significantly increased only in allergic *PGHS-1*^{-/-} mice. This result indicates that the sensitivity of the 2 groups to nonspecific contractile agonists was equivalent. Thus, allergic wild-type mice displayed the expected hyperresponsiveness phenotype using this criteria, but relative to the overall greater resistance in allergic *PGHS-1*^{-/-} mice, these changes were largely insignificant. These differences in responsiveness appear to be consistent with the observed pathology in that extensive airway and interstitial inflammatory infiltrates were observed in allergic *PGHS-1*^{-/-} mice, whereas only focal interstitial infiltrates were observed in allergic *PGHS-2*^{-/-} mice. Increased airway resistance and hyperresponsiveness are generally associated with alterations in airway structure and function. Decreased compliance is typically associated with changes in alveolar parenchymal structure, although structure is less important than smooth muscle tone and surfactant function in determining lung compliance. Therefore, after allergen challenge, it appears that both PGHS-1 and PGHS-2 products are involved in maintaining lung parenchymal structure and compliance, whereas only PGHS-1 products limit changes in airway inflammation, resistance, and responsiveness.

The leukotrienes, products of the 5-LO pathway, are generally considered to be proinflammatory mediators that have detrimental effects in the lung and are thought to be critical for the development of airway hyperresponsiveness after allergen exposure (1–6). Indeed, recent studies with 5-LO-deficient mice have shown dramatic reductions in airway responsiveness and degree of inflammation after oval-

bumin sensitization and exposure in comparison with wild-type mice (6). In contrast, PGHS-derived eicosanoids have been reported to have both detrimental and beneficial effects in the airway, thus their role in the development of allergic lung disease is more complex. For example, PGD₂, PGF_{2α}, and TxA₂ cause bronchoconstriction and are thought to be important in the development of airway hyperresponsiveness in animals and humans (8, 9, 32–34). On the other hand, PGE₂ is a bronchodilator, blocks the early and late asthmatic responses to allergen challenge, and prevents the bronchoconstriction that accompanies nonsteroidal anti-inflammatory agent ingestion in aspirin-sensitive asthmatics (9, 13, 15–17). Prostaglandins of the E-series and their analogs have also been shown to inhibit recruitment of inflammatory cells into the lung, decrease their survival, and inhibit their activation (14, 35–37). Thus, whereas the mechanisms for these effects are unknown, the data presented here that shows accentuation of allergen-induced lung inflammation in both *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice supports the contention that overall, cyclooxygenase products are critical for maintenance of normal lung function and limitation of pulmonary inflammation after allergen exposure.

Disruption of the *Pghs-1* and *Pghs-2* genes might lead to increased cellular arachidonic acid availability and subsequent shunting of the limited substrate down alternative eicosanoid metabolism pathways. Hence, one hypothesis to explain the increased inflammation observed in the PGHS-deficient mice might involve increased production of proinflammatory mediators such as LTB₄ and the cysteinyl leukotrienes (LTC₄/LTD₄/LTE₄). Recent studies with *5-LO*^{-/-} mice have produced conflicting results as to whether this shunting phenomenon actually occurs. While Goulet et al. have reported enhanced release of PGE₂ and thromboxane B₂ in peritoneal macrophages from *5-LO*^{-/-} mice in vitro (38), Funk does not find evidence for such shunting (39). We found no significant differences in BAL fluid LTB₄ levels between nonimmunized PGHS-deficient and wild-type mice; however, LTB₄ and the cysteinyl leukotrienes were significantly increased in BAL fluid from allergic *PGHS-1*^{-/-} mice compared with allergic wild-type and *PGHS-2*^{-/-} mice. These results suggest that increased 5-LO metabolism may be one mechanism whereby allergic lung responses are accentuated in the *PGHS-1*^{-/-} mice.

We found that wild-type mice constitutively express both PGHS-1 and PGHS-2 proteins in the lung. Demoly et al. have recently reported PGHS-1 and PGHS-2 immunoreactivity in bronchial epithelium of normal and asthmatic humans (40). These results are distinctly different than recent in vitro data, which demonstrate that the dominant isoform in cultured human lung epithelial cells is PGHS-2 (41, 42). We also observed that expression of PGHS-1 and PGHS-2 in nonimmunized *PGHS-2*^{-/-} and *PGHS-1*^{-/-} mouse lungs, respectively, were comparable to expression in nonimmunized wild-type mouse lungs. Thus, there did not appear to be a compensatory upregulation of the alternate PGHS isoform in either *PGHS-1*^{-/-} or *PGHS-2*^{-/-} mice. In contrast, it was recently reported that PGHS-1 and PGHS-2 proteins are coordinately regulated in vitro in immortalized lung cells derived from *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice (43).

These *in vivo/in vitro* differences in expression and regulation of PGHS isoforms indicate caution when interpreting the results of studies with cultured cells.

PGHS-2 protein was constitutively expressed in the lung, but did not appear to contribute significantly to basal PGE₂ generation. Indeed, nonimmunized *PGHS-2*^{-/-} mice had essentially normal BAL fluid PGE₂ levels. PGE₂ levels in allergic wild-type and *PGHS-2*^{-/-} mice were not significantly different, suggesting that PGHS-1 is also primarily responsible for PGE₂ production after allergen challenge. Multiple factors may influence BAL PGE₂ levels after allergen challenge, including changes in PGHS-2 protein expression, changes in PGHS-1 or PGHS-2 activity, and/or alterations in substrate availability. We observed increased lung PGHS-2 protein expression in allergen-exposed wild-type and *PGHS-1*^{-/-} mice. Interestingly, allergen induced PGHS-2 protein more in wild-type than in *PGHS-1*^{-/-} mice. This suggests the possibility that PGE₂ or other PGHS-1-derived eicosanoids may be necessary for optimal induction of PGHS-2 to occur after allergen challenge. Niki et al. have recently reported increased expression of PGHS-2 in an air pouch model of allergic inflammation (10). Others have reported increased PGHS-2 expression in inflamed tissues and after treatment with proinflammatory cytokines, lipopolysaccharide, and phorbol esters (18, 19, 41–43).

It should be noted that we designed our experimental protocol to examine differences between wild-type and PGHS-deficient mice in the late asthmatic response. Animals were sacrificed 1 day after the last of repeated exposures to ovalbumin, at a time when recruitment and activation of inflammatory cells had occurred and bronchial hyperresponsiveness to Mch was present. We did not examine the early changes that occur after allergen challenge, including mast-cell degranulation and initial bronchoconstriction. However, levels of total BAL fluid IgE in allergic *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice were significantly greater compared with allergic wild-type mice, indicating that the former groups would respond to an allergen challenge with heightened immediate responses. The failure of the wild-type mice to produce increased IgE after allergen challenge may simply reflect a lower level of IgE synthesis compared with PGHS-deficient mice. Another possibility is that the level of inflammation in the lungs of PGHS-deficient mice is sufficient to alter alveolar epithelial permeability and allow significantly increased transudation of serum IgE from the vasculature compared with allergic wild-type mice. Further work will be necessary to determine if differences in airway inflammation and lung function also exist between PGHS-deficient and wild-type mice in the early asthmatic response.

In summary, we observed no differences between control wild-type, *PGHS-1*^{-/-}, and *PGHS-2*^{-/-} mice with respect to baseline respiratory system resistance and compliance or lung histopathology, despite the fact that the *PGHS-1*^{-/-} mice had markedly reduced BAL fluid PGE₂. After ovalbumin sensitization and exposure, *PGHS-1*^{-/-} mice exhibited significantly increased lung inflammation and airway hyperresponsiveness compared with wild-type mice. The allergen-induced lung inflammatory response was also more pronounced in

PGHS-2^{-/-} mice compared with wild-type mice, but this was not accompanied by an increase in airway responsiveness. Based upon these findings, we conclude that (a) PGHS-1 is the predominant enzyme that biosynthesizes PGE₂ in normal mouse lung; (b) PGHS-1 and PGHS-2 products have mainly protective effects in the lung after allergen exposure; and (c) there is a dissociation between the presence of airway inflammation and the development of airway hyperresponsiveness in *PGHS-2*^{-/-} mice. Additional studies of *PGHS-1*^{-/-} and *PGHS-2*^{-/-} mice will likely provide further insights into the pathogenesis and mechanisms of allergic lung disease and asthma in humans.

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