

Accentuated T Helper Type 2 Airway Response after Allergen Challenge in Cyclooxygenase-1^{-/-} but Not Cyclooxygenase-2^{-/-} Mice

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Acute pharmacologic inhibition of cyclooxygenase (COX)-1 or -2 during allergen sensitization and exposure leads to enhanced T helper type 2 (Th2) airway responses. COX-1 and -2 play functionally distinct roles in lymphocyte development, and consequently, genetic deficiency of either enzyme, as opposed to acute pharmacologic inhibition, may modulate Th2-mediated allergic airway disease differently. An ovalbumin-induced mouse model of allergic airway disease was used. The immunophenotype of bronchoalveolar lavage lymphocytes was assessed by flow cytometry, bronchoalveolar lavage cytokines, and chemokines were measured by enzyme-linked immunosorbent assay, adhesion molecule expression was assessed by immunoblotting in combination with immunohistochemistry, and bronchoconstriction was assessed by whole body plethysmography. The airways of COX-1^{-/-} mice contained increased numbers of CD4⁺ and CD8⁺ T cells, exaggerated levels of the Th2 cytokines interleukin-4, -5, and -13, and increased levels of eotaxin and thymus- and activation-regulated chemokine. Allergen-induced bronchoconstriction was also increased in COX-1^{-/-} mice. Vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 levels were increased in lungs of both COX-1^{-/-} and COX-2^{-/-} mice relative to wild type. These data suggest that genetic deficiency of COX-1 but not COX-2 modulates T cell recruitment, Th2 cytokine secretion, and lung function in the allergic airway.

Keywords: cyclooxygenase; Th2 cytokine; ovalbumin

After ovalbumin (OVA) sensitization and exposure, lung inflammatory indices, as measured by bronchoalveolar lavage (BAL) cells, proteins, IgE, and histopathology, were significantly greater in cyclooxygenase (COX)-1^{-/-} and COX-2^{-/-} mice relative to wild-type (WT) mice. However, only allergic COX-1^{-/-} mice exhibited airway hyperresponsiveness (AHR) to methacholine (1). In contrast, Peebles and coworkers found that OVA-sensitized and -exposed mice treated with either a COX-1 or COX-2 selective inhibitor developed AHR relative to untreated mice (2).

COX-1 and COX-2 are known to play critical but functionally distinct roles in lymphocyte development (3), a cell type considered to be central in the orchestration of the asthmatic response. COX-1 is diffusely expressed in lymphoid cells in embryonic thymus, whereas COX-2 expression is confined to a subset of medullary stromal cells (3). COX-1 affects the transition from CD4⁻CD8⁻ to CD4⁺CD8⁺ cells. COX-2 plays

a role in the very early stages of thymocyte maturation in the differentiation of double positive cells into mature CD4⁺ thymocytes (3). Thus, absence of COX-1 or COX-2 during embryonic development may have long-term consequences in processes involving T cells, such as asthma. The lymphocyte, specifically the T helper type 2 (Th2) cell, is known to be a key player in asthma. We know that acute inhibition of COX-1 and/or COX-2 leads to an enhanced Th2 airway response in allergic mice (2), but the effect of genetic deficiency of COX-1 or COX-2 on Th2 airway responses has not been investigated.

The aim of the present study was to investigate the role of COX-1 and COX-2 in modulating Th2 responses in the allergic airway using COX-1^{-/-} and COX-2^{-/-} mice. We examined the effect of deficiency of COX-1 or COX-2 on lymphocyte subsets recruited into and cytokines elaborated by cells in the allergic airway. In addition, we determined airway levels of relevant chemokines and expression of adhesion molecules in these mice. The effect of COX-1 or COX-2 deficiency on allergen-induced bronchoconstriction was also examined. Some of the results of these studies have been previously reported in abstract form (4).

METHODS

Additional details on methods are provided in the online supplement.

Animals

Female, pathogen-free, 3- to 5-month-old COX-1^{-/-}, COX-2^{-/-}, and WT mice were of a hybrid C57BL/6J × 129/Ola genetic background intercrossed for > 20 generations and bred at NIH/NIEHS.

OVA Sensitization and Challenge

On Days 0 and 1, mice received an intraperitoneal injection of 20 µg of OVA (Grade V; Sigma, St. Louis, MO) emulsified in 0.2 ml aluminum hydroxide adjuvant (Alhydrogel; Accurate Chemical and Scientific Corp., Westbury, NY). On 5 consecutive days (Days 14–18), mice were challenged via the airways in a nose-only exposure chamber, for 30 minutes per day with 1% OVA in saline aerosol. Nonimmunized/saline-exposed mice were not used in this study, as we previously found no differences between COX-1^{-/-}, COX-2^{-/-}, and WT mice at baseline with respect to lung inflammatory indices, histopathology, or lung function.

Measurement of Bronchoconstriction

Airway obstruction at baseline and after OVA inhalation was assessed by barometric whole body plethysmography (Buxco, Troy, NY) using enhanced Pause as an index of airway obstruction.

BAL

After assessment of bronchoconstriction, BAL was performed. A differential count was determined on BAL fluid cells, and the remaining BAL fluid was aliquoted and frozen for cytokine and chemokine analyses.

Fluorescence-activated Cell Sorter Analysis of BAL Lymphocytes

Expression of CD3, CD4, CD8, and B220 cell surface markers on BAL lymphocytes was examined using flow cytometry. All antibodies were

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obtained from PharMingen (San Diego, CA). Data were collected using an LSR flow cytometer (Becton Dickinson, San Jose, CA) and analyzed using CellQuest software (Becton Dickinson).

Quantitation of Interleukin-4, Interleukin-5, Interleukin-13, Eotaxin, and Thymus- and Activation-regulated Chemokine in BAL Fluid

Levels of interleukin (IL)-4, IL-5, IL-13, IFN- γ , eotaxin and TARC (thymus- and activation-regulated chemokine) in BAL fluid were measured with the following commercially available ELISA kits according to manufacturer's instructions: IL-4 (Biosource International, Camarillo, CA); IL-5 (PharMingen); IL-13, IFN- γ , eotaxin, and TARC (R&D systems, Minneapolis, MN).

Immunoblotting

Whole-lung lysates were analyzed by Western blotting for vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 expression using goat anti-mouse VCAM-1 or ICAM-1 (Santa Cruz Biotechnology, Santa Cruz, CA).

Immunohistochemistry

Paraformaldehyde-fixed, paraffin-embedded lung sections were stained with goat anti-VCAM-1 or goat anti-ICAM-1 (Santa Cruz Biotechnology). Intensity of staining was assessed by a pathologist blinded to genotype, using a semiquantitative scoring system.

Statistical Analysis of Data

All values are expressed as means $\pm$ SEM. Immunohistochemical data were analyzed by the nonparametric test Kruskal-Wallis, and *post hoc* comparisons of means were performed with the Tukey test. All other data were analyzed by ANOVA. 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 $p < 0.05$. All tests were performed using Systat software (SYSTAT Inc., Evanston, IL).

RESULTS

BAL Fluid Inflammatory Cells

Analysis of BAL cells revealed significantly increased total cells in airways of allergic COX-1 $^{-/-}$ ($p < 0.05$) and COX-2 $^{-/-}$ ($p < 0.05$) mice compared with WT mice (Figure 1). Analysis of the specific cell types revealed significantly increased eosinophils ($p < 0.05$) and lymphocytes ($p < 0.05$) in both COX-1 $^{-/-}$ and COX-2 $^{-/-}$ mice compared with WT mice. There were no significant

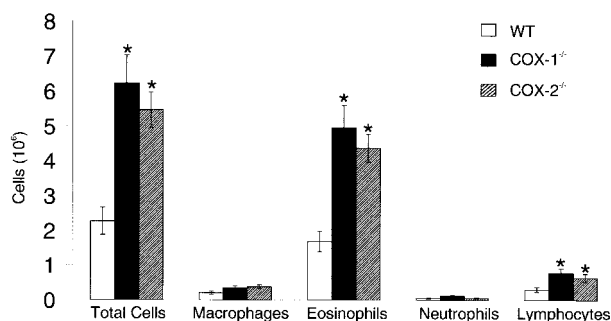


Figure 1. Total bronchoalveolar lavage (BAL) cell count and differential in allergic wild-type (WT), COX-1 $^{-/-}$, and COX-2 $^{-/-}$ mice. Mice were sensitized with ovalbumin (OVA) emulsified in aluminum hydroxide adjuvant on Days 0 and 1. Mice were challenged via the airways for 30 minutes per day with 1% ovalbumin in saline aerosol on Days 14–18. Twenty-four hours after the last OVA-aerosol challenge, mice were killed, and differential analysis was performed on BAL fluid cells stained with Wright-Giemsa. Data are presented as the mean $\pm$ SEM ($n = 15$ –16 per genotype). * $p < 0.05$ versus WT mice.

differences between COX-1 $^{-/-}$ and COX-2 $^{-/-}$ in these parameters (Figure 1). There was no significant difference in numbers of macrophages and neutrophils between the genotypes. These data demonstrate that both COX-1 and COX-2 products limit the numbers of inflammatory cells in the allergic mouse airway.

Immunophenotype of BAL Lymphocytes

To examine the effect of COX-1 and COX-2 deficiency on the subsets of lymphocytes recruited to the allergic airway, fluorescence-activated cell sorter analysis was performed on BAL cells from allergic COX-1 $^{-/-}$, COX-2 $^{-/-}$, and WT mice. The cells in the lymphocyte gate positive for the specific lymphocyte markers are presented in Figure 2. In each experiment, BAL cells were pooled from two mice. Analysis was repeated in 7–10 independent experiments per marker, and the results were averaged. There were significantly greater numbers of CD3 $^{+}$, CD3 $^{+}$ CD4 $^{+}$, and CD3 $^{+}$ CD8 $^{+}$ cells in the airways of COX-1 $^{-/-}$ mice compared with WT. The airways of COX-1 $^{-/-}$ mice also contained significantly higher numbers of CD3 $^{+}$ CD8 $^{+}$ cells compared with the airways of COX-2 $^{-/-}$ mice. The airways of COX-1 $^{-/-}$ mice tended to have greater numbers of CD3 $^{+}$ and CD3 $^{+}$ CD4 $^{+}$ cells compared with COX-2 $^{-/-}$ mice; however, these changes were not statistically significant. There was no significant difference between the genotypes with respect to total numbers of B220 $^{+}$ cells in the airways. These results show that products of COX-1 play an important role in modulating the recruitment of T cell subsets, but not B cells, to the allergic airway.

Th2 Cytokines Are Elevated in COX-1 $^{-/-}$ Airways

To evaluate the role of COX-1 and COX-2 in the release of Th1/Th2 cytokines into the allergic airway, BAL fluid levels of IL-4, IL-5, IL-13, and IFN- γ were measured. In COX-1 $^{-/-}$ mice, there was an approximately twofold increase in IL-4 ($p < 0.05$) compared with levels in COX-2 $^{-/-}$ and WT mice (Figure 3A), an approximately sixfold increase in IL-5 ($p < 0.05$) compared with COX-2 $^{-/-}$ and WT mice (Figure 3B), and an approximately ninefold increase in IL-13 ($p < 0.05$) compared with COX-2 $^{-/-}$ and WT mice (Figure 3C). There was no significant difference in the levels of IL-4, IL-5, and IL-13 between COX-2 $^{-/-}$ and WT mice. Levels of IFN- γ were below the limits of detection in all three genotypes (data not shown). These results demonstrate that the Th2 cytokines IL-4, IL-5, and IL-13 are produced in an exaggerated fashion in OVA-sensitized and -exposed COX-1 $^{-/-}$ mice.

Chemokines Are Elevated in COX-1 $^{-/-}$ Airways

Chemokines are important regulators of cell recruitment to the allergic airway. To determine whether the increased cell recruitment in the allergic COX-1 $^{-/-}$ and COX-2 $^{-/-}$ mice might be

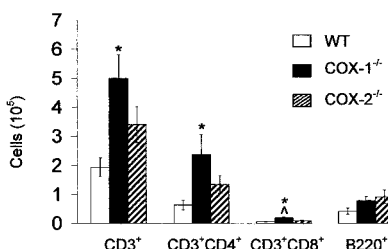


Figure 2. Cells recovered from BAL fluid of WT, COX-1 $^{-/-}$, and COX-2 $^{-/-}$ mice on Day 19 were stained for surface expression of CD3, CD4, CD8, and B220 antigens and analyzed by flow cytometry. Data represent the product of the total BAL count, the percentage of lymphocytes on differential, and the percentage of CD3 $^{+}$, CD3 $^{+}$ CD4 $^{+}$, CD3 $^{+}$ CD8 $^{+}$, and B220 $^{+}$ in the lymphocyte gate. Data are presented as the mean $\pm$ SEM of 7–10 experiments per group. In each experiment, BAL cells were pooled from two mice. * $p < 0.05$ versus WT mice; $\Delta p < 0.05$ versus COX-2 $^{-/-}$ mice.

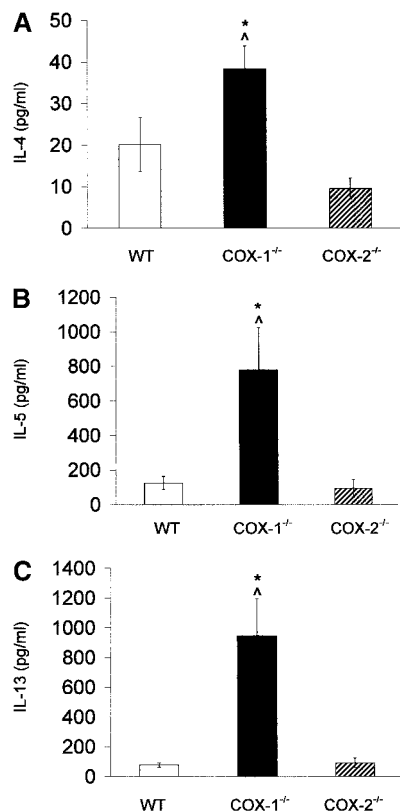


Figure 3. Th2 cytokine levels in BAL fluid from WT, COX-1^{-/-}, and COX-2^{-/-} mice. On Day 19, BAL fluids were collected from allergic mice. Interleukin (IL)-4 (A) IL-5 (B), and IL-13 (C) levels were determined by ELISA. Each value is mean $\pm$ SEM (n = 10 per genotype). *p < 0.05 versus WT mice; ^p < 0.05 versus COX-2^{-/-} mice.

mediated through upregulation of chemokines, we measured BAL fluid levels of eotaxin (a hallmark eosinophil chemoattractant) and TARC (a lymphocyte-directed chemokine that specifically chemoattracts Th2 cells). There was an approximately threefold increase ($p < 0.05$) in eotaxin in BAL fluid from COX-1^{-/-} mice compared with levels in COX-2^{-/-} and WT mice (Figure 4A). TARC was also elevated approximately twofold in BAL fluid from the COX-1^{-/-} mice ($p < 0.05$) compared with COX-2^{-/-} and WT mice (Figure 4B). Thus, the enhanced inflammatory and Th2 cytokine response in the COX-1^{-/-} mice is associated with the increased elaboration of these eosinophil and Th2 cell regulating chemokines.

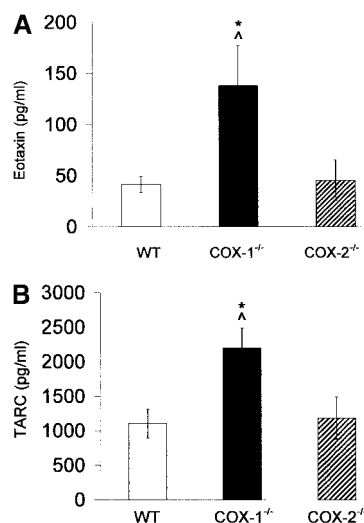


Figure 4. Chemokine levels in BAL fluid from WT, COX-1^{-/-}, and COX-2^{-/-} mice. On Day 19, BAL fluid was collected from allergic mice. Eotaxin (A) and TARC (B) levels were determined by ELISA. Each value is mean $\pm$ SEM (n = 10 per genotype). *p < 0.05 versus WT mice; ^p < 0.05 versus COX-2^{-/-} mice.

Adhesion Molecule Expression

We examined expression of ICAM-1 and VCAM-1, as both of these adhesion molecules have been implicated in eosinophil extravasation from blood vessels into the airways (5). As shown in Figure 5, there was upregulation of VCAM-1 in the lungs of both COX-1^{-/-} and COX-2^{-/-} mice compared with WT mice as determined by immunoblotting. When VCAM-1 expression was analyzed by immunohistochemistry, total staining was increased in lungs of both COX-1^{-/-} and COX-2^{-/-} mice compared with WT mice (Figure 6 and Table 1). Specifically, staining intensity was increased in the lymphatic endothelium and alveolar capillary endothelium of lungs from both COX-1^{-/-} and COX-2^{-/-} mice compared with WT mice (Table 1). Staining intensity in the visceral pleural mesothelium of lungs from COX-1^{-/-} mice was also upregulated compared with WT mice. Arteriovenous endothelial staining was not significantly different between the genotypes. Likewise, ICAM-1 expression was increased in lungs of COX-1^{-/-} and COX-2^{-/-} mice relative to WT mice by immunoblotting (Figure 5), although results tended to be more variable than for VCAM-1. When assessed by immunohistochemistry, there was a significant increase in staining intensity in alveolar walls of COX-1^{-/-} and COX-2^{-/-} mice compared with WT mice (Figure 6 and Table 2). This difference in staining intensity was not as dramatic as the difference seen in VCAM-1 staining between the COX-deficient and WT mice. The ICAM-1 staining of alveolar walls was smooth, linear, and apparently concentrated on the outer surface of the alveolar walls, suggestive of localization to alveolar pneumocytes. These results indicate that deficiency in either COX-1 or COX-2 results in increased expression of VCAM-1 and ICAM-1 in the lung after allergen challenge.

Allergen-induced Bronchoconstriction

Previously, our laboratory demonstrated increased responsiveness to methacholine in the allergic COX-1^{-/-} mice. We sought to determine whether deficiency in either COX enzyme increases allergen-induced bronchoconstriction. Enhanced pause was measured on Day 13 before the mice received OVA aerosol, and again on Day 19, 24 hours after the last OVA aerosol. The percentage increase of enhanced pause on Day 19 was 39.3 $\pm$ 6.1% for COX-1^{-/-} mice, 24.2 $\pm$ 5.0% for COX-2^{-/-} mice, and 20.5 $\pm$ 4.3% for WT mice (Figure 7). Thus, allergen induced significantly more bronchoconstriction in the COX-1^{-/-} mice compared with both

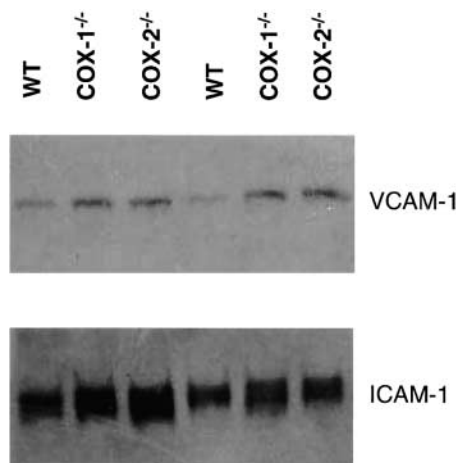


Figure 5. VCAM-1 and ICAM-1 expression in lungs from allergic WT, COX-1^{-/-}, and COX-2^{-/-} mice as determined by immunoblotting. Representative blots are shown from two mice of each genotype.

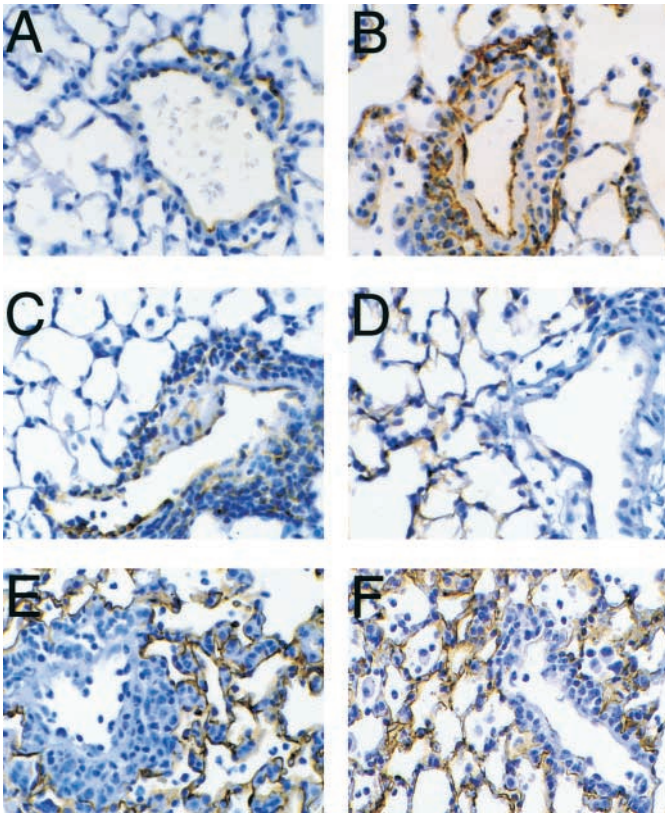


Figure 6. Immunohistochemical detection of VCAM-1 and ICAM-1 in the allergic lungs of WT, COX-1^{-/-}, and COX-2^{-/-} mice. Sections shown are representative of staining observed in lungs from each genotype. Expression of VCAM-1 was significantly increased in the lungs of COX-1^{-/-} (B) and COX-2^{-/-} (C) mice compared with WT (A) mice, specifically, in the lymphatic endothelium and alveolar capillary endothelium. Expression of ICAM-1 in the alveolar walls was significantly increased in the lungs of COX-1^{-/-} (E) and COX-2^{-/-} (F) mice compared with WT (D) mice.

COX-2^{-/-} and to WT mice ($p < 0.05$) with no significant difference between COX-2^{-/-} and WT mice. These findings indicate that the increases in T cells, Th2 cytokines, and chemokines in COX-1^{-/-} mice are associated with enhanced allergen-induced bronchoconstriction.

DISCUSSION

A murine model of allergic airway disease was used to determine whether deficiency in COX-1 or COX-2 alters lymphocyte recruitment, cytokine secretion, chemokine production, adhesion mole-

TABLE 2. SEMIQUANTITATIVE ANALYSIS OF INTERCELLULAR ADHESION MOLECULE-1 STAINING IN LUNGS OF WILD-TYPE, COX-1^{-/-}, AND COX-2^{-/-} MICE*

Region of staining	Wild-Type	COX-1 ^{-/-}	COX-2 ^{-/-}
Alveolar wall	2.7 ± 0.3	3.8 ± 0.1†	3.6 ± 0.2†

* Scoring system based on the intensity of staining in the alveolar wall (scale of 1–4).

† $p < 0.05$ versus wild-type mice; $n = 10$ per group.

cule expression, and bronchoconstriction in the allergic mouse airway. After systemic immunization and repeated airway challenges with OVA, the airways of the COX-1^{-/-} mice contained increased numbers of CD4⁺ and CD8⁺ T cells. COX-1^{-/-} mice also had an exaggerated Th2 airway cytokine response, and the chemoattractants eotaxin and TARC were markedly elevated in the airways of these mice. Correspondingly, allergen-induced bronchoconstriction was increased in the COX-1^{-/-} mice. VCAM-1 and ICAM-1 expression were upregulated in lungs of both COX-1^{-/-} and COX-2^{-/-} mice compared with WT. These studies show that COX-1 but not COX-2 is important in the regulation of Th2-mediated allergic airway inflammation and bronchoconstriction.

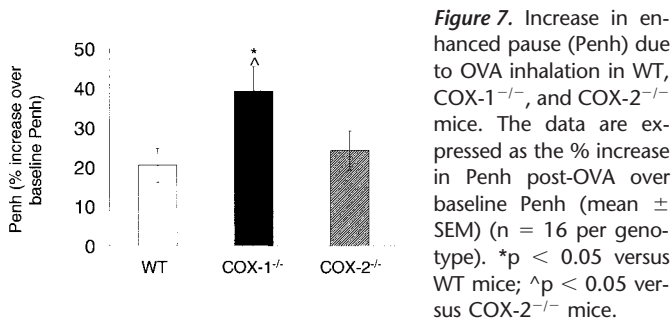
The importance of T cells in asthma lies in the cytokines they produce. CD4⁺ T lymphocytes can differentiate toward a T helper type 1 (Th1) or Th2 phenotype. Th2 cells are thought to orchestrate the airway hyperreactivity and inflammation characteristic of asthma (6, 7). Th2 cells secrete cytokines such as IL-4, -5, and -13. The contribution of COX products to Th2-mediated inflammation remains controversial. Some reports suggest that COX products augment Th2 inflammation. For example, prostaglandin (PG)_{E2} can inhibit production of the Type 1-associated cytokines IL-2 and IFN- γ in murine and human CD4⁺ T cells and thus may favor a Type 2-like cytokine response (8). In contrast, other reports suggest that COX products inhibit Th2 inflammation. For example, PGE₂ inhibits Th2 cytokine secretion and IgE production by B cells (9, 10). Treatment of spleen cells from BALB/c mice infected with *Leishmania mexicana* with indomethacin, a nonspecific COX inhibitor, upregulates IL-12 production and polarizes the immune response toward a Th1 type (11). Relatively little is known about the role of prostaglandins in Th2-driven allergic airway disease. Recently, Fujitana and coworkers demonstrated that overproduction of PGD₂ results in increased Th2 cytokines and eotaxin, with a concomitant increase in eosinophils and lymphocytes in the allergic airway (12). Peebles and coworkers found that nonselective COX inhibition during allergen sensitization increased production of the Th2 cytokines IL-5 and IL-13 (13). More recently they showed that OVA-sensitized mice treated with either a selective COX-1 inhibitor or a selective COX-2 inhibitor had significantly greater airway responsiveness

TABLE 1. SEMIQUANTITATIVE ANALYSIS OF VASCULAR CELL ADHESION MOLECULE-1 STAINING IN LUNGS OF WILD-TYPE, COX-1^{-/-}, AND COX-2^{-/-} MICE*

Region of Staining	Wild-Type	COX-1 ^{-/-}	COX-2 ^{-/-}
Arteriovenous endothelium, central vessels (1–4)	2.3 ± 0.3	3.2 ± 0.2	2.8 ± 0.2
Arteriovenous endothelium, peripheral vessels (1–2)	1.6 ± 0.2	2.0 ± 0.0	1.9 ± 0.1
Lymphatic endothelium (1–4)	2.2 ± 0.3	3.5 ± 0.2†	3.2 ± 0.2†
Alveolar capillary endothelium (1–4)	1.0 ± 0.2	3.5 ± 0.3†	2.9 ± 0.3†
Visceral pleural mesothelium (1–3)	0.8 ± 0.2	2.3 ± 0.3†	1.5 ± 0.4
Total score	7.9 ± 0.9	14.5 ± 0.8†	12.2 ± 1.1†

* Scoring system based on the intensity of staining in the different regions of the lung. The scales for each region are indicated in parentheses.

† $p < 0.05$ versus wild-type mice; $n = 10$ per group.



and higher levels of IL-13 in lung supernatants than untreated mice that were OVA-sensitized (2).

Numbers of CD4⁺ and CD8⁺ T cells and levels IL-4, -5, and -13 were elevated in the airways of COX-1^{-/-} mice. The increased Th2 cytokines found in COX-1^{-/-} airways might be a result of increased numbers of CD4⁺ T cells secreting these cytokines or a greater proportion of the CD4⁺ cells in COX-1^{-/-} airways being Th2 as opposed to Th1 cells. Depending on the conditions of stimulation, CD8⁺ T cells can produce Th1-like or Th2-like cytokines, so that a distinction in Tc1 (c for cytotoxic) and Tc2 cells was proposed for this subset (14). CD8⁺ T cells are increased in the airways of COX-1^{-/-} mice, and elaboration of "Th2" cytokines by these cells may have contributed to the increased Th2 cytokine levels found in the airways of the COX-1^{-/-} mice. However, we do not discount the possibility that other cell types, such as mast cells or basophils, may also contribute to the production of Th2 cytokines in this model. The Th2 response in the COX-1^{-/-} mice was associated with increased eosinophilia and AHR as previously reported in these mice (1). These findings are in good agreement with prior reports indicating that elevated Th2 cytokines play a pivotal role in the development of eosinophilia and AHR. IL-5 is an important Th2 cytokine involved in the maturation, recruitment, and survival of eosinophils. The finding that COX-2^{-/-} mice had similar IL-5 levels to WT mice was surprising given that COX-2^{-/-} mice had increased eosinophils compared with WT mice. This suggests that different and/or additional mechanisms of eosinophil recruitment are active in the COX-2^{-/-} mice. IL-4 stimulates B cell production of allergen-specific IgE. The increased IL-4 found in COX-1^{-/-} mice correlates with previous findings of increased BAL fluid IgE in allergic COX-1^{-/-} mice (1). However, BAL fluid IgE levels were also increased in allergic COX-2^{-/-} mice that do not have elevated IL-4 levels. Thus, it is possible that an increase in IL-4 is not responsible for the increased BAL fluid IgE levels in COX-1^{-/-} mice. The increased BAL fluid IgE may simply be a reflection of increased alveolar epithelial permeability, resulting in increased transudation of serum IgE from the vasculature into the airway, as a consequence of increased airway inflammation in both allergic COX-1^{-/-} and COX-2^{-/-} mice.

IL-13 has a number of biological effects similar to those of IL-4, such as the regulation of isotype class switching in B cells to IgE synthesis. The increased BAL IgE found in COX-1^{-/-} mice compared with COX-2^{-/-} and WT mice (1) may also be due to increased levels of IL-13. Recently, IL-13 has been shown to be a potent regulator of bronchoconstriction and AHR. Blockade of endogenous levels of IL-13 in sensitized mice by administration of a soluble form of the IL-13 R α 2 chain (which binds only IL-13) reverses AHR and pulmonary mucus cell hyperplasia (15, 16). Acute administration of IL-13 itself was sufficient to induce AHR (15, 16). Mice with a targeted deletion of IL-13 failed to develop allergen-induced AHR and AHR was restored with administration of recombinant IL-13 (17). Venkayya and colleagues recently

showed that administration of Th2 cell-conditioned medium, recombinant IL-4 or recombinant IL-13 to the airways of naive mice rapidly induced AHR (18). In the present study, only allergic COX-1^{-/-} mice exhibit increased bronchoconstriction after OVA inhalation, and Gavett and colleagues previously showed that only allergic COX-1^{-/-} mice are hyperresponsive to methacholine (1). The dramatically increased airway levels of IL-13 detected in the COX-1^{-/-} mice may explain why OVA-induced bronchoconstriction and AHR are only observed with COX-1 deficiency.

The eosinophil is thought to be an important mediator of bronchoconstriction, and thus it is noteworthy that despite increased eosinophilia in the allergic COX-2^{-/-} mice relative to WT mice, there is no increase in bronchoconstriction in COX-2^{-/-} mice relative to WT mice. The asthmagenic phenotype in the COX-1^{-/-} mice is reminiscent of recent findings in aspirin-sensitive asthma in humans. This syndrome seems to be triggered by inhibition of COX-1, and selective COX-2 inhibitors are well tolerated by patients with aspirin-sensitive asthma (19). It is tempting to speculate that some fundamental difference in the relative contributions of the COX isoforms to airway disease lies at the root of both.

The migration of leukocytes into tissue depends on the expression of specific chemokines during the progression of inflammation. Many studies have demonstrated that chemokines orchestrate the recruitment and/or activation of eosinophils and lymphocytes in the allergic airway. Eotaxin and TARC have been shown to contribute to this type of response. Eotaxin is well established as a potent eosinophil chemoattractant (20, 21). In addition, it has been shown that eotaxin is a chemoattractant for Th2 cells. Th2 cells express the eotaxin receptor CCR3, and eotaxin induces chemotactic migration of Th2 cells (22). TARC, a more recently discovered chemokine, is reported to play a crucial role in recruitment of Th2 cells into the lung (23). *In vitro* studies have shown that TARC induces selective migration of lymphocytes, especially of the Th2 phenotype (24). Evidence for an *in vivo* role of TARC in allergic inflammation derives from a study showing that pretreatment of sensitized mice with anti-TARC antibody attenuated OVA-induced airway eosinophilia and diminished the degree of AHR with a concomitant decrease in Th2 cytokine levels (23). Moreover, TARC is constitutively expressed and upregulated in allergic inflammation. Sekiya and coworkers showed that the bronchial epithelium of subjects with asthma expressed higher levels of TARC compared with that of normal subjects (25). Recently, it has been shown that patients with asthma exposed to a relevant allergen release large quantities of TARC in BAL fluid (26). The present study demonstrates for the first time that deficiency in COX-1 results in increased airway elaboration of these two important chemokines after OVA challenge. The accentuated Th2 response in the COX-1^{-/-} mice may be a consequence of increased recruitment of Th2 cells to the airway as a result of increased levels of these Th2 cell-attracting chemokines. Eotaxin and IL-5 have been reported to synergize in regulating eosinophil responses (27, 28), and this may be relevant to the present study as both IL-5 and eotaxin are elevated in the COX-1^{-/-} mice. However, it is possible that other undefined mechanisms are responsible for the increased eosinophilia in the COX-1^{-/-} mice. Th2 cytokines, in particular IL-13, are potent inducers of eotaxin production by lung stromal cells (29, 30). Less is known about the induction of TARC, but as with eotaxin, production can be induced by Th2 cytokines (31). Thus, it is possible that the elevated levels of eotaxin and possibly TARC found in the COX-1^{-/-} mice are due to heightened production of Th2 cytokines in these mice. However, the possibility that COX-1 products have the ability to directly affect the elaboration of these chemokines cannot be ruled out.

Notwithstanding the importance of chemokines in localization of leukocytes to inflamed tissue, they are only partially responsible

for the preferential recruitment of specific leukocyte subsets during inflammation. Cellular adhesion, mediated through specific adhesion molecules, is the primary event in cell recruitment to sites of inflammation. Animal studies using knockout mice or adhesion molecule-blocking reagents suggest that both ICAM-1 and VCAM-1 are critically important for both eosinophil and T cell recruitment to the lung after allergen exposure (32–34). Increased VCAM-1 and ICAM-1 levels have been detected in bronchial biopsies from subjects with asthma compared with control subjects (35). We detected increased VCAM-1 and ICAM-1 expression in the lungs of both COX-1^{-/-} and COX-2^{-/-} mice after allergen exposure. Increased expression of these adhesion molecules in the lungs of allergic COX-1^{-/-} and COX-2^{-/-} mice is likely responsible for, or at least contributes to, the increased airway inflammation observed in these mice. The mechanism of VCAM-1 and ICAM-1 upregulation in the COX-deficient mice is unknown but several possibilities exist. VCAM-1 and ICAM-1 can be preferentially upregulated by Th2 cytokines (36–38). VCAM-1 levels are upregulated in allergic COX-1^{-/-} mice and COX-2^{-/-} mice to a similar extent compared with WT mice. However, Th2 cytokines are only elevated in COX-1^{-/-} mice; therefore, it is likely that an alternative and/or additional mechanism of adhesion molecule upregulation is operative in the COX-2^{-/-} mice. This could perhaps be mediated by differences between COX-1^{-/-} and COX-2^{-/-} mice in the levels of certain prostaglandins and/or leukotrienes in the airway. A reduction in COX products, or conversely an increase in lipoxygenase products, may lead to an increase in VCAM-1 or ICAM-1 expression. Several COX products are known to influence expression of VCAM-1 and ICAM-1. For example, treatment with iloprost, a stable analog of prostacyclin, reduces expression of VCAM-1 (39). PGE₁ can also reduce VCAM-1 expression (40, 41), PGE₂ can reduce ICAM-1 expression (42) and lipoxygenase products have been shown to induce the expression of adhesion molecules (43).

Previous data from our laboratory indicate that the eicosanoid profiles between WT, COX-1^{-/-}, and COX-2^{-/-} mice differ, but that these differences vary depending on the stimulus. At baseline, BAL fluid PGE₂ levels are significantly reduced in COX-1^{-/-} mice relative to both WT and COX-2^{-/-} mice (1). After OVA sensitization and exposure, PGE₂ is significantly reduced and leukotrienes (LTB₄ and cysteinyl leukotrienes) are significantly increased in the BAL fluid of COX-1^{-/-} mice relative to WT and COX-2^{-/-} mice. After LPS exposure, both COX-1^{-/-} and COX-2^{-/-} mice have significantly reduced BAL fluid PGE₂ levels relative to WT mice; however, LTB₄ levels are not different between the genotypes (44). After vanadium pentoxide exposure, BAL fluid PGE₂ levels in COX-1^{-/-} mice increase 25-fold, whereas levels in COX-2^{-/-} are unchanged (45). In contrast, there are no differences between the genotypes in BAL fluid levels of PGD₂ and LTB₄ after vanadium pentoxide exposure. Future studies will determine levels of other eicosanoids in mouse BAL fluid (including PGF_{2α}, PGI₂, TXA₂, PGJ₂, lipoxins, P450 eicosanoids, etc.) and also attempt to rescue the COX-1-null phenotype with individual eicosanoid mediators.

The enhanced Th2 response and AHR found only in the COX-1^{-/-} but not the COX-2^{-/-} mice contrasts with recent findings by Peebles and coworkers, who reported that selective pharmacologic inhibition of COX-1 or COX-2 leads to increased lung levels of IL-13 and the development of AHR in allergic mice. There may be several reasons for these differences. First, the timing of cytokine measurements are different. Indeed, Peebles and colleagues showed that IL-13 levels vary depending on the day of sampling. Second, Peebles and coworkers used BALB/c mice, whereas the COX-deficient mice are on a hybrid C57BL/6Jx129/Ola background. It is well known that differences exist between strains of mice regarding their cytokine response and the develop-

ment of AHR after allergic sensitization (46). Finally, COX-1 or COX-2 were inhibited only at the time of sensitization in the Peebles study, whereas the mice used in our study were genetically deficient in either COX-1 or COX-2 from conception. COX-1 and COX-2 products are crucial factors in the development of T lymphocytes (3) and thus the lack of either enzyme during development may have affected the development of T lymphocytes and other facets of the immune system involved in the allergic response. From a pathophysiologic perspective, both experimental approaches complement each other. The inhibitor work allows for examination of acute effects of COX inhibition on lung function, but depends on the specificity of the inhibitors that may affect other pathways. The knockout mice allow for examination of chronic effects (including effects on the immune system development) on lung function, but may be associated with secondary or compensatory changes in expression of other genes.

In summary, these studies demonstrate for the first time that genetic deficiency in COX-1 but not COX-2 promotes the asthmatic phenotype by increasing the flux of CD4⁺ and CD8⁺ T cells to the airway and by eliciting an augmented Th2 cytokine and enhanced chemokine response in the allergic airway.

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