

ORIGINAL ARTICLE

Augmentation of allergic inflammation in the airways of cyclooxygenase-2-deficient mice

JUNKO NAKATA,^{1,4} MITSUKO KONDO,^{1,4} JUN TAMAOKI,¹ TAKAKO TAKEMIYA,^{2,4} MICHIKO NOHARA,³
KANATO YAMAGATA⁴ AND ATSUSHI NAGAI¹

¹First Department of Medicine, ²First Department of Physiology, ³Second Department of Hygiene and Public Health, Tokyo Women's Medical University School of Medicine, Shinjuku, Tokyo, and ⁴Department of Neuropharmacology, Tokyo Metropolitan Institute for Neuroscience, Fuchu, Japan

Augmentation of allergic inflammation in the airways of cyclooxygenase-2-deficient mice

NAKATA J, KONDO M, TAMAOKI J, TAKEMIYA T, NOHARA M, YAMAGATA K, NAGAI A. *Respirology* 2005; 10: 149–156

Objective: Airway cyclooxygenase-2 (COX-2) is induced by cytokine-mediated inflammation such as occurs in asthma. However, the role of COX-2 in the pathophysiology of asthma is not fully understood.

Methods: Allergic inflammation, airway responsiveness to methacholine and mucous cell metaplasia after ovalbumin sensitization in the airways of COX-2 deficient (–/–) mice, COX-2 (+/+) mice and C57BL/6J mice treated with a selective COX-2 inhibitor, nimesulide were assessed. Histology, cell analysis, measurements of arachidonic acid metabolites and Th2 cytokine levels in bronchoalveolar lavage fluid (BALF), and measurement of serum IgE were performed.

Results: Eosinophil infiltration into the airway wall, and the number of eosinophils in BALF were greater in sensitized COX-2 (–/–) mice than in sensitized COX-2 (+/+) mice. The levels of cysteinyl leukotrienes (LTC₄/D₄/E₄), prostaglandin E₂ (PGE₂) and interleukin (IL)-13 as well as airway responsiveness did not differ in COX-2 (–/–) mice and COX-2 (+/+) mice. However, sensitized COX-2 (–/–) mice had higher LTC₄/D₄/E₄ and lower PGE₂ concentrations compared with non-sensitized COX-2 (–/–) mice. The number of PAS/alcan blue-positive airway epithelial cells and serum IgE were elevated in COX-2 (–/–) mice. Nimesulide-treated mice showed augmented eosinophilic inflammation, LTC₄/D₄/E₄ concentrations and mucous cell metaplasia.

Conclusion: These data indicate that COX-2 deficiency augments allergic inflammation and mucous cell metaplasia.

Key words: cysteinyl leukotrienes, eosinophils, IgE, mucous cell metaplasia, nimesulide, prostaglandin E₂.

INTRODUCTION

There is ample evidence that arachidonic acid metabolites including leukotrienes (LT) and prostanoids are involved in the pathogenesis of asthma; 5-Lipoxygenase (5-LO) products produce airway inflammation, bronchoconstriction, microvascular leakage and mucus secretion. Inhibitors of 5-LO and

antagonists of LT receptors prevent bronchoconstriction and airway inflammation and are thus, widely used for the treatment of asthma.^{1–3} In contrast, prostanoids possess both beneficial and adverse effects, and the source of prostanoids is different in various cell types. Mast cells release PGD₂ which induces bronchoconstriction and is involved in the immediate response of allergic asthma.⁴ Airway epithelial cells release PGE₂, which relaxes bronchial smooth muscle as an epithelial-derived relaxing factor.^{5,6}

Prostanoids are produced by cyclooxygenases that consist of two isoforms; one is a constitutive isoform, cyclooxygenase 1 (COX-1), and the other is an inducible isoform, cyclooxygenase 2 (COX-2).⁷ COX-1 is a housekeeping enzyme, and COX-2 is induced by cytokine-mediated inflammation and is inhibited by glucocorticoids. Recently, COX-2 mRNA was reported

Correspondence: Atsushi Nagai, First Department of Medicine, Tokyo Women's Medical University School of Medicine, 8-1 Kawado-cho, Shinjuku, Tokyo 162-8666, Japan. Email: anagai@chi.twmu.ac.jp

Received 8 March 2004; revised 2 September 2004; accepted for publication 6 September 2004.

to be down-regulated in nasal polyps of patients with aspirin-intolerant asthma.⁸ However, the role of COX-2 in the pathophysiology of asthma is not fully understood. Furthermore, COX-2 is constitutively expressed even in normal airway epithelium and resident inflammatory cells, but the role of COX-2 in normal airways also remains unclear.⁹

Gavett *et al.* reported that eosinophilic inflammation after ovalbumin (OVA) sensitization and challenge was augmented in both COX-1 (–/–) mice and COX-2 (–/–) mice compared with COX-2 (+/+) mice.¹⁰ However, lower PGE₂ levels and higher LT levels in bronchoalveolar lavage fluid (BALF) as well as airway hyperresponsiveness were found in COX-1 (–/–) mice but not in COX-2 (–/–) mice. Recently, Carey *et al.* reported that accentuated Th2 cytokines after OVA challenge were observed in COX-1 (–/–) mice but not COX-2 (–/–) mice.¹¹ In contrast, Peebles *et al.* reported that treatment with a COX-2 inhibitor as well as a COX-1 inhibitor increased airway hyperresponsiveness and interleukin (IL)-13 in lung supernatants in BALB/c mice.¹² In the present study, we aimed to elucidate the role of COX-2 in the allergic model by using COX-2 (–/–) mice and C57BL/6J mice treated with a COX-2 inhibitor. To simplify the experiment, we used a single antigen challenge after sensitization, whereas Gavett *et al.* used multiple antigen challenges.¹⁰ Furthermore, we assessed the relationship between mucous cell metaplasia and COX-2-deficiency, because arachidonic acid metabolites are associated not only with mucus secretion but also with mucous cell metaplasia, an important feature of asthma.^{13–15}

METHODS

Mice

Mice that were homozygous mutants for COX-2 (COX-2 (–/–)) and wild-type mice (COX-2 (+/+)), aged

9–10 weeks and weighing 19–23 g, were used. The animal care and experimental protocol were reviewed and approved by the Institutional Animal Care and Use Committee of Tokyo Women's Medical University. Mice deficient in the COX-2 gene were generated from a 129/Sv × C57BL/6J cross followed by 10 backcrossings with C57BL/6J mice, and COX-2 (–/–) mice were obtained from Jackson Laboratories (Bar Harbor, ME).^{16,17} To ensure the genotype of the offspring, mating was generally performed between one male heterozygous (COX-2 (+/–)) mouse and one female COX-2 (+/–) mouse to obtain COX-2 (–/–) and COX-2 (+/+) offspring. The genotype of the mating pairs was confirmed by polymerase chain reaction on DNA extracted from mice tails. Animals were allowed free access to food and water in an animal room with a 12:12 h light:dark cycle. For the COX-2 inhibitor experiment, we separately purchased C57BL/6J mice having a close genetic background to the COX-2 (–/–) mice and COX-2 (+/+) mice.

Experimental protocols

The experimental protocol is shown in Fig. 1. In the OVA-sensitized group, mice were sensitized intraperitoneally with 10 µg OVA (Sigma-Aldrich, St. Louis, MO) and 0.2 mL aluminium hydroxide on day 0 and day 14. In the non-sensitized group, mice were injected with only aluminium hydroxide. On day 21, all mice were challenged with inhalation of OVA for 20 min. Five to 10 mice were placed in an acrylic box and exposed to aerosols of 5% OVA diluted in sterile saline, using an ultrasonic nebulizer (Pulmo-Sonic model 25, DeVilbiss Co., PA). On day 25, all mice were euthanized to assess inflammatory indices as detailed below. C57BL/6J mice were pretreated with the selective COX-2 inhibitor, nimesulide (10 mg/kg, Sigma),¹⁸ which was dissolved in carboxymethyl cellulose and administered orally via sonde coude 1 h before sensitization and challenge (Fig. 1).

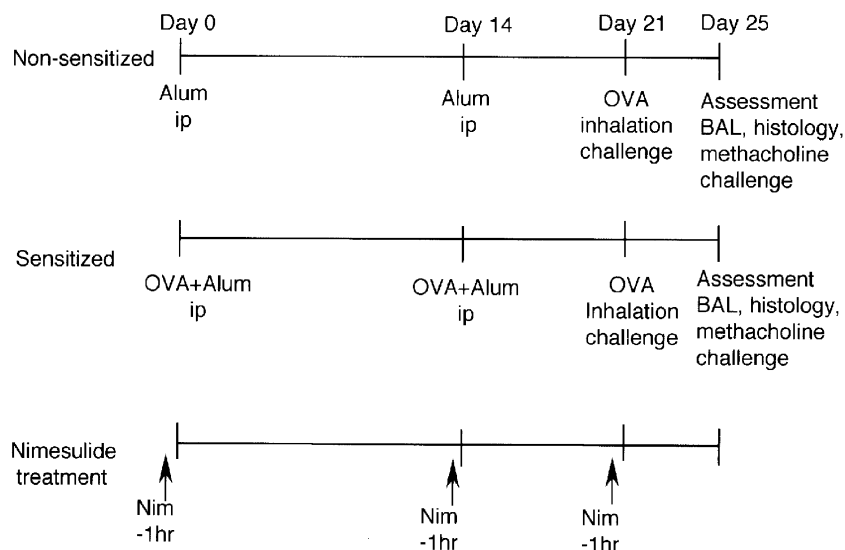


Figure 1 Experimental protocol. In ovalbumin (OVA)-sensitized groups, mice were sensitized intraperitoneally with 10 µg OVA and 0.2 mL aluminium hydroxide (Alum) on day 0 and day 14. In non-sensitized groups, mice were injected with only Alum. On day 21, all mice were challenged with inhalation of 50 mg/mL OVA for 20 min. On day 25, all mice were euthanized for assessment. Nimesulide (10 mg/kg) was given orally 1 h before sensitization and challenge.

Assessments of bronchoalveolar lavage fluid

Mice were anaesthetized with pentobarbiturate (50 mg/kg, ip) and tracheotomized using a custom-built cannula. The lungs were lavaged with three 1-mL aliquots of saline. BALF was centrifuged at 360 ×g for 10 min at 4°C, and the supernatants were stored at -80°C for the measurement of eicosanoids. Cells were resuspended in 1 mL saline and counted. Cytospin slides of BALF cells were prepared, stained with May-Grünwald-Giemsa, and a differential count was performed on at least 500 cells per sample. PGE₂ levels were determined by RIA (Amersham Bioscience, Piscataway, NJ), and cysteinyl LT (LTC₄/D₄/E₄) levels were determined by enzyme-linked immunosorbent assay (ELISA; Amersham Bioscience). IL-4 and IL-13 levels in BALF were measured by ELISA (Biosource International, CA) according to the manufacturer's protocol.

Lung histology

The lungs were fixed with 10% formalin and embedded in paraffin. Sections were stained with haematoxylin and eosin, or periodic acid-Schiff (PAS)/alcian blue. For the assessment of mucous cell metaplasia, the number of PAS/alcian blue-positive cells as percentages of total epithelial cells in all tangential bronchi in randomly selected sections were calculated.

Serum IgE

Serum IgE concentration was measured by ELISA (Yamasa Co. Choshi, Chiba, Japan). Values that were too low to be interpolated from the standard curve were assigned the lowest detectable value for the assay (250 ng/mL).

Measurement of airway responsiveness

Airway responsiveness to methacholine (from 0.063 to 2%) was assessed by analysing enhanced pause (Penh) using a whole-body barometric plethysmo-

graphic chamber (Buxco, BioSystem XA, Troy, NY) as described previously.¹⁹ Penh was calculated as (expiratory time/relaxation time - 1) × (peak expiratory flow/peak inspiratory flow) according to the manufacturer's protocol.

Statistical analysis

Results are expressed as means ± SEM. Data were analysed by ANOVA with Fisher's least significant difference as a post-hoc analysis. Differences were considered significant if $P < 0.05$.

RESULTS

Bronchoalveolar lavage fluid cell analysis and lung histology

As shown in Table 1, the number of total cells in sensitized COX-2 (-/-) mice was significantly higher than that in sensitized COX-2 (+/+) mice ($P < 0.05$). The percentage of BALF eosinophils in sensitized COX-2 (-/-) mice was significantly higher than that in sensitized COX-2 (+/+) mice ($n = 6$, $P < 0.05$). Similarly, the percentage of eosinophils in BALF in sensitized nimesulide-treated mice was higher than that in sensitized COX-2 (+/+) mice ($n = 6$, $P < 0.05$). Histologically, there were few inflammatory cells in the airways of non-sensitized COX-2 (+/+) mice and non-sensitized COX-2 (-/-) mice (Fig. 2a,b). The lung tissue in sensitized COX-2 (-/-) mice showed pronounced inflammatory cells, especially eosinophils, in the submucosa of bronchi (Fig. 2d) when compared to sensitized COX-2 (+/+) mice (Fig. 2c). Sensitized, nimesulide-treated mice also showed an increase in eosinophilic inflammation in the submucosa (data not shown).

Mucous cell metaplasia

There were few PAS/alcian blue-positive mucous cells in non-sensitized COX-2 (+/+) mice (Fig. 3a).

Table 1 Total cell number and percentage of inflammatory cells in bronchoalveolar lavage fluid from cyclooxygenase (COX)-2 (+/+) mice, COX-2 (-/-) mice and nimesulide-treated mice

	COX-2 (+/+)	Non-sensitized COX-2 (-/-)	Nimesulide	COX-2 (+/+)	Sensitized COX-2 (-/-)	Nimesulide
Total cells ($\times 10^5$)	2.3 ± 0.4	9.9 ± 2.3	1.4 ± 0.2	7.6 ± 2.3	25.7 ± 11.2*	4.3 ± 2.2
Macrophages	94.5 ± 1.4	80.2 ± 2.0	82.0 ± 6.8	58.1 ± 8.7	17.3 ± 9.0*	39.1 ± 10.8
Eosinophils	0.9 ± 0.4	6.1 ± 1.5	8.9 ± 5.7	27.5 ± 6.2	69.8 ± 12.5*	52.4 ± 10.2*
Neutrophils	0.9 ± 0.1	6.1 ± 0.2*	1.4 ± 0.8	2.9 ± 0.9	3.2 ± 1.2	0.5 ± 0.3
Lymphocytes	3.6 ± 0.8	7.7 ± 2.3	0.5 ± 0.3	11.6 ± 2.3	9.7 ± 2.3	7.5 ± 2.4

$n = 6$. Data show mean ± SEM.

* $P < 0.05$ versus corresponding cyclooxygenase-2 (+/+) mice. COX, cyclooxygenase.

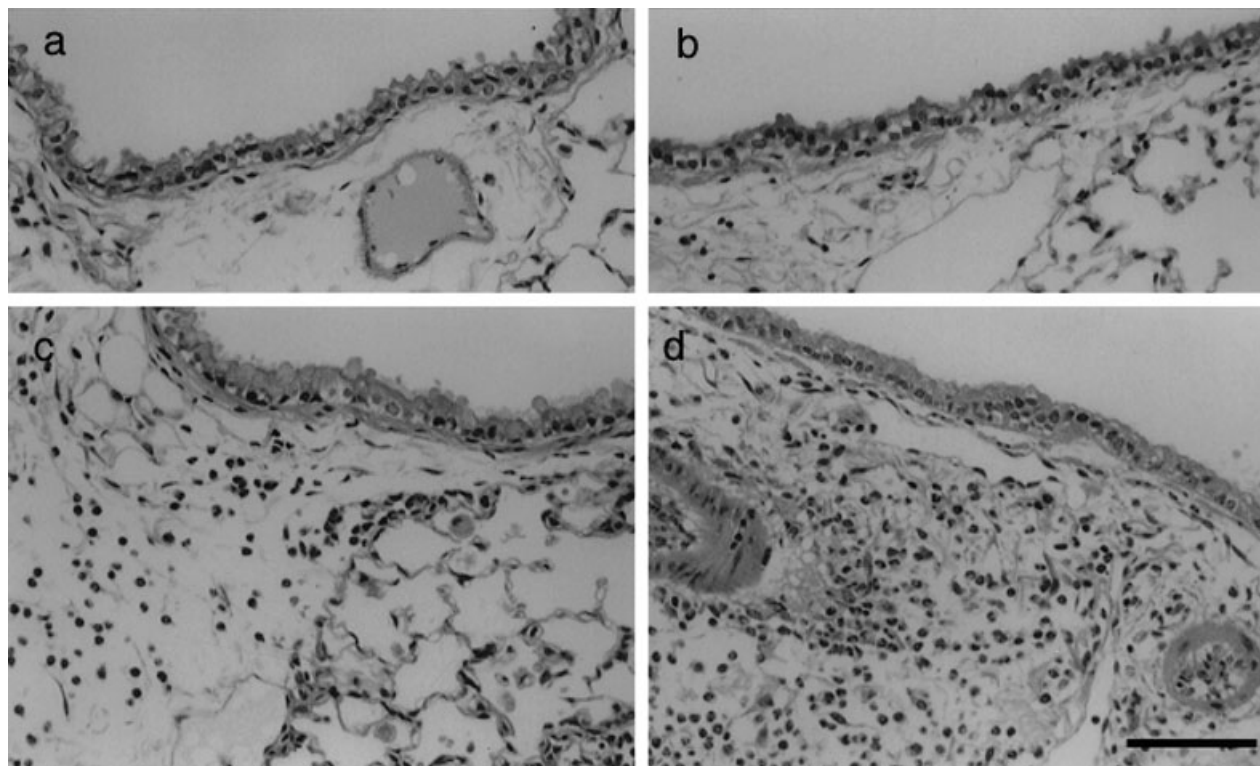


Figure 2 Light micrographs of bronchi stained with haematoxylin and eosin. (a) Non-sensitized cyclooxygenase (COX)-2 (+/+) mice, (b) non-sensitized COX-2 (-/-) mice, (c) sensitized COX-2 (+/+) mice, and (d) sensitized COX-2 (-/-) mice. Intense eosinophilic infiltration is observed in (d) sensitized COX-2 (-/-) mice compared with (c) sensitized COX-2 (+/+) mice. Non-sensitized mice did not show eosinophilic infiltration (a, b). Scale bar = 100 μ m.

However, in non-sensitized COX-2 (-/-) mice, a moderate increase in mucous cells was noted (Fig. 3b). As shown in Fig. 4, the number of mucous cells in non-sensitized COX-2 (-/-) mice was similar to that in sensitized COX-2 (+/+) mice. In non-sensitized, nimesulide-treated mice, mucous cell metaplasia was also noted, and the number of mucous cells was significantly higher than in non-sensitized COX-2 (+/+) mice ($n=6$, $P<0.05$). Sensitized COX-2 (-/-) mice showed remarkable mucous cell metaplasia (Fig. 3d), but the number of mucous cells was not significantly different in sensitized mice in the three groups (Figs 3c,d and 4).

Arachidonic acid metabolites in bronchoalveolar lavage fluid

LTC₄/D₄/E₄ and PGE₂ concentrations in BALF supernatants are shown in Fig. 5. PGE₂ concentrations in non-sensitized mice or sensitized mice were not significantly different in the three groups. In contrast, LTC₄/D₄/E₄ concentrations in non-sensitized and sensitized nimesulide-treated mice were significantly higher than those in corresponding COX-2 (+/+) mice ($n=6$, $P<0.05$). Next, we compared PGE₂ and LTC₄/D₄/E₄ concentrations in non-sensitized mice and sensitized mice. LTC₄/D₄/E₄ concentrations were significantly higher and PGE₂

concentrations were significantly lower in sensitized COX-2 (-/-) mice than in non-sensitized COX-2 (-/-) mice ($n=6$, $P<0.05$). However, these concentrations were not significantly different between sensitized COX-2 (+/+) mice and non-sensitized COX-2 (+/+) mice, or between sensitized nimesulide-treated mice and non-sensitized nimesulide-treated mice.

Interleukin-4 and interleukin-13 concentrations in bronchoalveolar lavage fluid

IL-4 and IL-13 concentrations in BALF supernatants are shown in Fig. 6. IL-4 and IL-13 concentrations in sensitized mice were not significantly different in the three groups ($n=4$). However, IL-4 concentrations in non-sensitized COX-2 (-/-) mice were higher than those in non-sensitized COX-2 (+/+) mice ($n=4$, $P<0.05$). IL-13 concentrations in sensitized mice were higher than those in non-sensitized mice ($n=4$, $P<0.05$).

Serum IgE

As shown in Fig. 7, serum IgE concentrations in non-sensitized COX-2 (-/-) mice were significantly higher than those in non-sensitized COX-2 (+/+) and

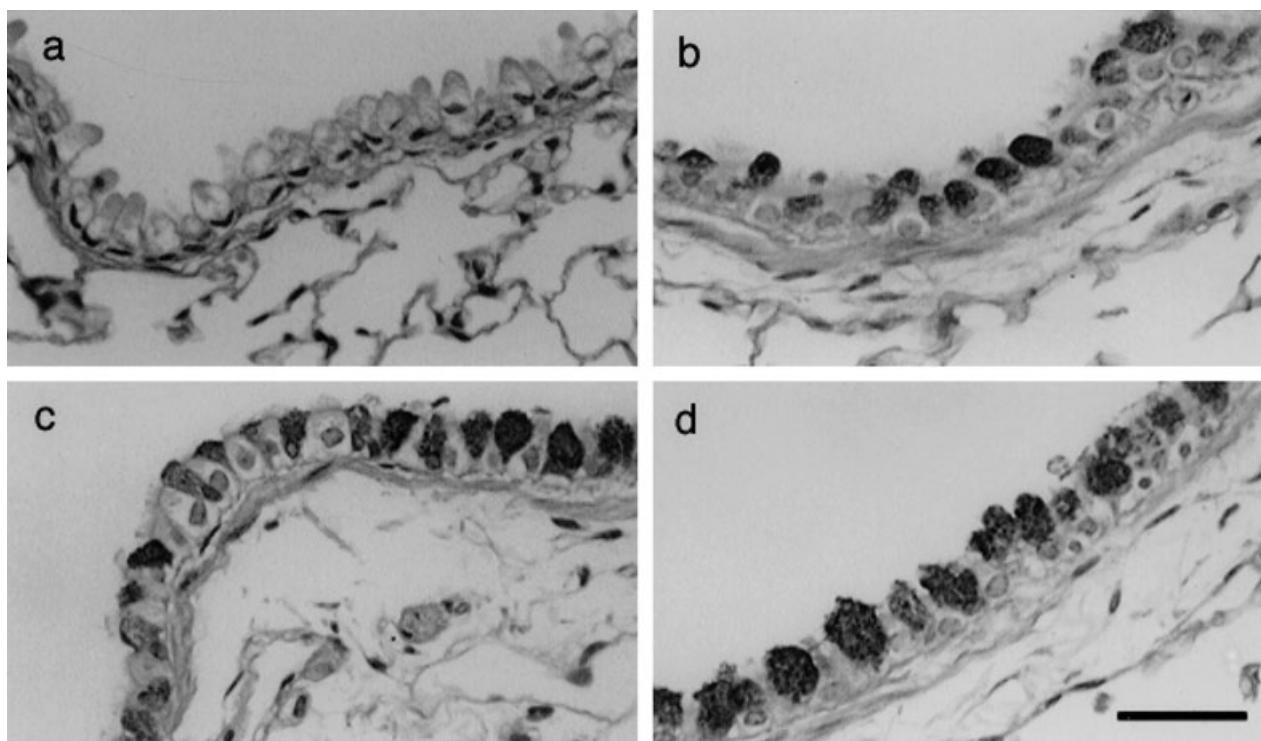


Figure 3 Light micrographs of bronchi stained with periodic acid-Schiff/alcian blue. (a) Non-sensitized cyclooxygenase (COX)-2 (+/+) mice, (b) non-sensitized COX-2 (-/-) mice, (c) sensitized COX-2 (+/+) mice, and (d) sensitized COX-2 (-/-) mice. Mucous cell metaplasia in (d) sensitized COX-2 (-/-) mice is more prominent than in (c) sensitized COX-2 (+/+) mice. Moderate mucous cell metaplasia is observed in (b) non-sensitized COX-2 (-/-) mice, whereas no mucous cell metaplasia is observed in (a) non-sensitized COX-2 (+/+) mice. Scale bar = 50 μ m.

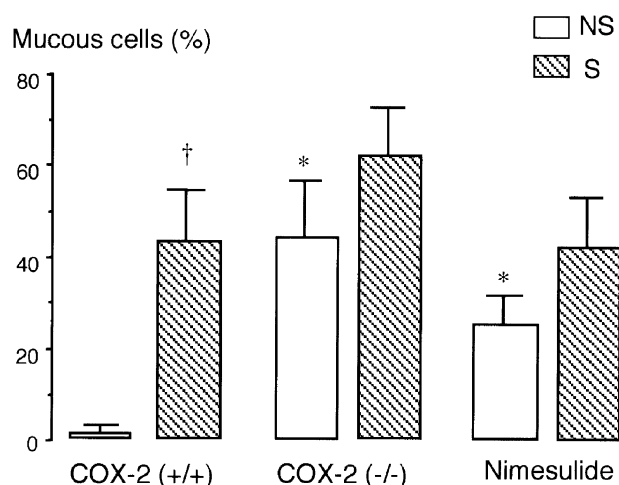


Figure 4 Percentage of mucous cells. The percentage of mucous cells was not significantly different in sensitized mice in the three groups. It was higher in sensitized cyclooxygenase (COX)-2 (+/+) mice than in non-sensitized COX-2 (+/+) mice. Non-sensitized COX-2 (-/-) mice and nimesulide-treated mice show more mucous cells than non-sensitized COX-2 (+/+) mice ($P < 0.05$). Open bar, non-sensitized group (NS); hatched bar, sensitized group (S). Data are presented as mean $\pm$ SEM, $n = 6$ for each group. * $P < 0.05$ versus corresponding COX-2 (+/+) mice. [†] $P < 0.01$ versus non-sensitized COX-2 (+/+) mice.

nimesulide-treated mice ($n = 6$, $P < 0.05$). IgE concentrations in sensitized COX-2 (-/-) mice were also high, but the differences were not statistically significant. IgE concentrations in nimesulide-treated mice were not different from those in COX-2 (+/+) mice.

Airway responsiveness

Penh values after methacholine challenge in sensitized mice are shown in Fig. 8. Penh values were not significantly different in the three groups ($n = 7-8$).

DISCUSSION

In this study, we have demonstrated that eosinophilic airway inflammation induced by OVA sensitization was augmented in COX-2 (-/-) mice and mice treated with a COX-2 inhibitor. The percentage of eosinophils in BALF was similar between COX-2 (-/-) mice and mice treated with a COX-2 inhibitor. However, the total number of cells increased in COX-2 (-/-) mice but not in the mice treated with a COX-2 inhibitor. This difference might be explained by the genetic defect in COX-2 (-/-) mice compared with acute inhibition of COX-2 occurring only at the time of allergen sensitization and challenge in mice treated with the

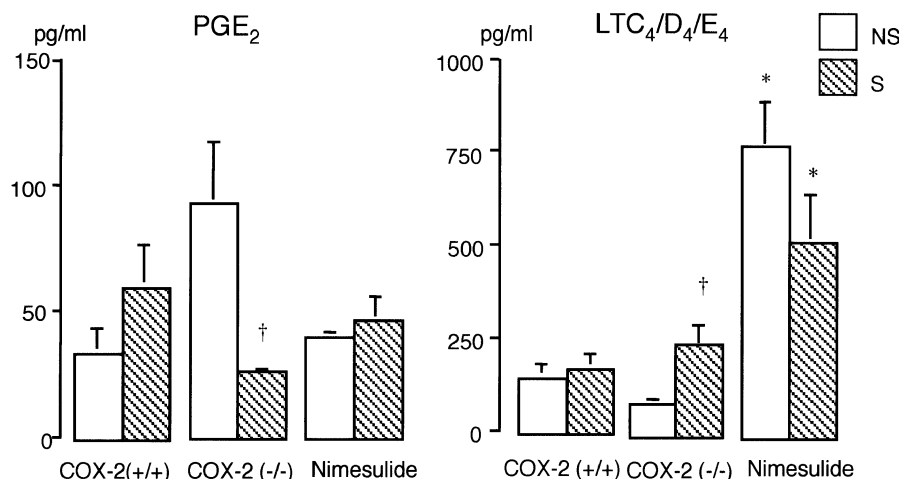


Figure 5 Concentrations of PGE₂ and cysteinyl leukotrienes in bronchoalveolar lavage fluid. PGE₂ concentrations in non-sensitized mice or sensitized mice are not significantly different in the three groups. LTC₄/D₄/E₄ concentrations in non-sensitized and sensitized nimesulide-treated mice were higher than those in corresponding cyclooxygenase (COX)-2 (+/+) mice ($P < 0.05$). LTC₄/D₄/E₄ concentrations were higher and PGE₂ concentrations were lower in sensitized COX-2 (-/-) mice than in non-sensitized COX-2 (-/-) mice ($P < 0.05$). Open bar, non-sensitized group (NS); hatched bar, sensitized group (S). Data are presented as mean $\pm$ SEM, $n = 6$. * $P < 0.05$ versus corresponding COX-2 (+/+) mice. ** $P < 0.05$ versus non-sensitized mice.

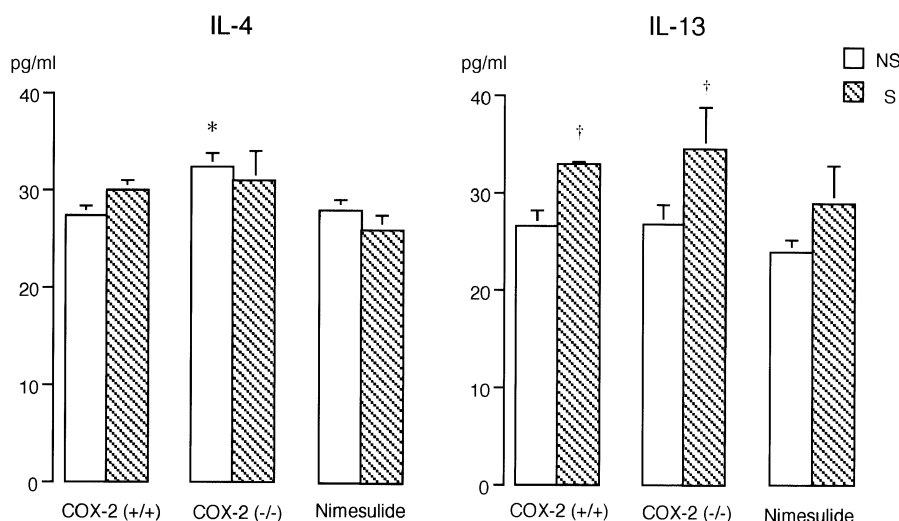


Figure 6 Concentrations of interleukin (IL)-4 and IL-13 in bronchoalveolar lavage fluid. IL-4 and IL-13 concentrations in sensitized mice were not significantly different in the three groups. However, IL-4 concentrations in non-sensitized cyclooxygenase (COX)-2 (-/-) mice were higher than those in non-sensitized COX-2 (+/+) mice ($P < 0.05$). IL-13 concentrations in sensitized mice were higher than those in non-sensitized mice ($P < 0.05$). Open bar, non-sensitized group (NS); hatched bar, sensitized group (S). Data are presented as mean $\pm$ SEM, $n = 4$. * $P < 0.05$ versus corresponding COX-2 (+/+) mice. † $P < 0.05$ versus non-sensitized mice.

COX-2 inhibitor. Various chemoattractants for eosinophils such as LTB₄, IL-5 and eotaxin are involved in the pathophysiology of asthma. Gavett *et al.* demonstrated that after sensitization and challenge, LTB₄, as well as cysteinyl LT in BALF, were increased in COX-1 (-/-) mice but not in COX-2 (-/-) mice relative to COX-2 (+/+) mice.¹⁰ A more recent study showed that IL-5 and eotaxin levels in BALF after sensitization and challenge were increased in COX-1 (-/-) mice but not

in COX-2 (-/-) mice, although COX-2 (-/-) mice had increased eosinophils, with similar levels to those seen in COX-1 (-/-) mice.¹¹ The investigators speculated that different and/or additional mechanisms of eosinophil recruitment are active in COX-2 (-/-) mice. Our data demonstrated that LT predominated over PGE₂ in both COX-2 (-/-) mice and mice treated with a COX-2 inhibitor. It is quite feasible that these changes in arachidonic acid metabolism may be

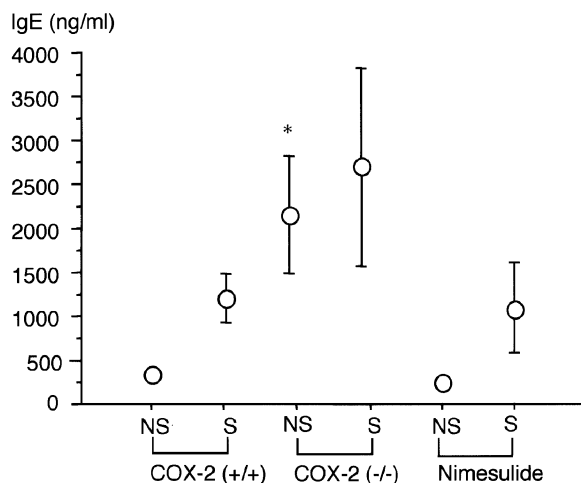


Figure 7 Serum IgE concentrations. IgE concentrations in non-sensitized cyclooxygenase (COX)-2 (-/-) mice were significantly higher than those in non-sensitized COX-2 (+/+) and nimesulide-treated mice ($P < 0.05$). IgE concentrations in sensitized COX-2 (-/-) mice were also high, but differences were not statistically significant. IgE concentrations in nimesulide-treated mice were not different from those in COX-2 (+/+) mice. Data are presented as mean $\pm$ SEM, $n = 6$. * $P < 0.05$ versus corresponding COX-2 (+/+) mice.

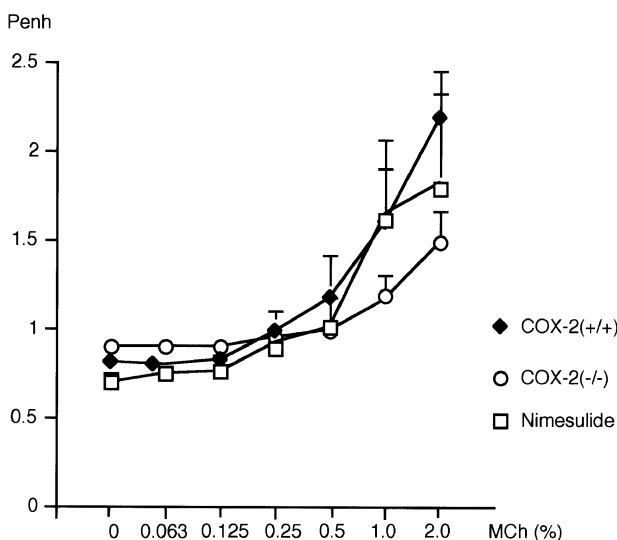


Figure 8 Airway responsiveness to methacholine. Penh was measured with gradually increasing doses of methacholine (0.063–2%) in sensitized mice. All mice demonstrated increased Penh, and there were no significant differences in the three groups. Data are presented as mean $\pm$ SEM ($n = 7$ –8).

associated with the augmented eosinophilic inflammation that was observed.

In the present study, nimesulide-treated mice showed higher BALF LTC₄/D₄/E₄ concentrations as compared with COX-2 (+/+) mice. This suggests that acute COX-2 inhibition may stimulate the 5-LO path-

way. However, LT concentrations in sensitized COX-2 (-/-) mice were not different from those in sensitized COX-2 (+/+) mice. Furthermore, PGE₂ concentrations were not different in sensitized mice in the three groups. Therefore, we asked whether arachidonic acid metabolism was changed by sensitization, and attempted to compare PGE₂ and LTC₄/D₄/E₄ concentrations in sensitized mice with those in non-sensitized mice. As shown in Fig. 5, LTC₄/D₄/E₄ concentrations were higher, whereas PGE₂ concentrations were lower in sensitized COX-2 (-/-) mice than in non-sensitized COX-2 (-/-) mice. Inhibition of COX could increase cysteinyl LT by shunting arachidonate into the 5-LO pathway in allergic models.²⁰ Because COX-2 up-regulation is not possible after antigen challenge in COX-2 (-/-) mice, the observed increase in LT and decrease in PGE₂ might result from shunting of arachidonate into the 5-LO pathway. This concept has been proposed for a long time to explain aspirin-intolerant asthma, and this asthma phenotype is characterized by COX-1 inhibitor-induced asthma attacks. Recent evidence shows that patients with aspirin-intolerant asthma have decreased activity of COX-2 and lower production of PGE₂ in the upper airway and peripheral blood cells.²¹ However, the mechanism of aspirin-intolerant asthma remains unclear. Although extrapolation of murine results to the human system is hazardous, intrinsic COX-2 deficiency might induce severe asthma attacks triggered by inhibition of COX-1 that is a critical pathway for PGE₂ production.

In spite of augmented eosinophilic airway inflammation in sensitized COX-2 (-/-) mice and COX-2 inhibitor-treated mice, we could not demonstrate augmentation of airway responsiveness to methacholine or increased IL-13 concentrations in BALF. As IL-13 is a central mediator of airway hyperresponsiveness,²² this may explain our data. These data are similar to previous reports that COX-2 (-/-) mice do not show any augmentation of methacholine responsiveness and IL-13 levels in BALF, despite COX-2 (-/-) mice having increased eosinophils similar to that seen in COX-1 (-/-) mice.^{10,11} In contrast, Peebles *et al.* reported that treatment with a COX-2 inhibitor as well as a COX-1 inhibitor increased airway hyperresponsiveness and IL-13 levels in lung supernatants in BALB/c mice that had the genetic characteristics of a Th2 dominant response.^{12,23} In the present study, we used C57BL/6J mice, and the different results might reflect differences in genetic background. Thus, airway responsiveness and cytokine production may be profoundly affected by the strain of mice used.²³

Mucous cell metaplasia is another prominent feature of asthma. Our data showed that mucous cell metaplasia was induced by OVA sensitization. Interestingly, moderate mucous cell metaplasia was observed in COX-2 (-/-) mice and nimesulide-treated mice even without sensitization (Figs 3b and 4). COX-2 is constitutively expressed in normal airway epithelium and resident inflammatory cells,⁹ and as such constitutive COX-2 expression may be protecting against mucous cell metaplasia or mucin production. It is known that cysteinyl LT are involved in mucous cell metaplasia as well as mucus secretion in

asthma.^{14,15} In our experiment, an increase in BALF LT was observed in non-sensitized, nimesulide-treated mice, suggesting that elevated LT may induce mucous cell metaplasia. However, cysteinyl LT levels were not increased in non-sensitized COX-2 (-/-) mice relative to COX-2 (+/+) mice. Our data showed that serum IgE level as well as mucous metaplasia were increased in non-sensitized COX-2 (-/-) mice. The lack of COX-2 during development is reported to affect T cell differentiation and the immune system in COX-2 (-/-) mice.^{24,25} In fact, our data showed higher IL-4 concentrations in BALF in non-sensitized COX-2 (-/-) mice compared with those in non-sensitized COX-2 (+/+) mice. An increase in IL-4 through development might induce mucous cell metaplasia and IgE synthesis in COX-2 (-/-) mice even without sensitization.

In conclusion, COX-2 (-/-) mice and mice treated with a COX-2 inhibitor demonstrated augmented eosinophilic inflammation and mucous cell metaplasia. These results suggest that COX-2 may play a protective role in allergic inflammation and mucous cell metaplasia.

ACKNOWLEDGEMENTS

The authors wish to thank Yoshimi Sugimura (Tokyo Women's Medical University School of Medicine) and Masayuki Shino (Osaka, Japan) for their technical assistance and Ono Pharmaceutical Co. for measurement of cysteinyl LT.

REFERENCES

- Wenzel SE. Arachidonic acid metabolites: mediators of inflammation in asthma. *Pharmacotherapy* 1997; **17**: 3S-12S.
- Henderson WR, Lewis DB, Albert RK *et al.* The importance of leukotrienes in airway inflammation in a mouse model of asthma. *J. Exp. Med.* 1996; **184**: 1483-94.
- Drazen JM, Israel E, O'Byrne PM. Treatment of asthma with drugs modifying the leukotriene pathway. *N. Engl. J. Med.* 1999; **340**: 197-206.
- Murray JJ, Tonnel AB, Brash AR *et al.* Release of prostaglandin D₂ into human airways during acute antigen challenge. *N. Engl. J. Med.* 1986; **315**: 800-4.
- Pavord ID, Wong CS, Williams J, Tattersfield AE. Effect of inhaled prostaglandin E₂ on allergen-induced asthma. *Am. Rev. Respir. Dis.* 1993; **148**: 87-90.
- Prie S, Cadieux A, Sirois P. Removal of guinea pig bronchial and tracheal epithelium potentiates the contractions to leukotrienes and histamine. *Eicosanoids* 1990; **3**: 29-37.
- Smith WL, Dewitt DL. Prostaglandin endoperoxide H synthases-1 and -2. *Adv. Immunol.* 1996; **62**: 167-215.
- Picado C, Fernandez-Morata JC, Juan M *et al.* Cyclooxygenase-2 mRNA is downexpressed in nasal polyps from aspirin-sensitive asthmatics. *Am. J. Respir. Crit. Care Med.* 1999; **160**: 291-6.
- Watkins DN, Peroni DJ, Lenzo JC, Knight DA, Garlepp MJ, Thompson PJ. Expression and localization of COX-2 in human airways and cultured airway epithelial cells. *Eur. Respir. J.* 1999; **13**: 999-1007.
- Gavett SH, Madison SL, Chulada PC *et al.* Allergic lung responses are increased in prostaglandin H synthase-deficient mice. *J. Clin. Invest.* 1999; **104**: 721-32.
- Carey MA, Germolec DR, Bradbury JA *et al.* Accentuated T helper type 2 airway response after allergen challenge in cyclooxygenase-1-/- but not cyclooxygenase-2-/- mice. *Am. J. Respir. Crit. Care Med.* 2003; **167**: 1509-15.
- Peebles RS, Hashimoto K, Morrow JD *et al.* Selective cyclooxygenase-1 and -2 inhibitors each increase allergic inflammation and airway hyperresponsiveness in mice. *Am. J. Respir. Crit. Care Med.* 2002; **165**: 1154-60.
- Kim YD, Kwon EJ, Park DW, Song SY, Yoon SK, Baek SH. Interleukin-1 β induces MUC2 and MUC5AC synthesis through cyclooxygenase-2 in NCI-H292 cells. *Mol. Pharmacol.* 2002; **62**: 1112-18.
- Liu YC, Khawaja AM, Rogers DF. Effects of the cysteinyl leukotriene receptor antagonists pranlukast and zafirlukast on tracheal mucus secretion in ovalbumin-sensitized guinea-pigs in vitro. *Br. J. Pharmacol.* 1998; **124**: 563-71.
- Vargaftig BB, Singer M. Leukotrienes mediate murine bronchopulmonary hyperreactivity, inflammation, and part of mucosal metaplasia and tissue injury induced by recombinant murine interleukin-13. *Am. J. Respir. Cell Mol. Biol.* 2003; **28**: 410-19.
- Dinchuk JE, Car BD, Focht RJ *et al.* Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. *Nature* 1995; **378**: 406-9.
- Morham SG, Langenbach R, Loftin CD *et al.* Prostaglandin synthase 2 gene disruption causes severe renal pathology in the mouse. *Cell* 1995; **83**: 473-82.
- Range SP, Pang L, Holland E, Knox AJ. Selectivity of cyclo-oxygenase inhibitors in human pulmonary epithelial and smooth muscle cells. *Eur. Respir. J.* 2000; **15**: 751-6.
- Hamelmann E, Schwarze J, Takeda K *et al.* Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am. J. Respir. Crit. Care Med.* 1997; **156**: 766-75.
- Dworski R, Sheller JR, Wickersham NE *et al.* Allergen-stimulated release of mediators into sheep bronchoalveolar lavage fluid. Effect of cyclooxygenase inhibition. *Am. Rev. Respir. Dis.* 1989; **139**: 46-51.
- Picado C. Aspirin-intolerant asthma: role of cyclooxygenase enzymes. *Allergy* 2002; **57** (Suppl. 72): 58-60.
- Wills-Karp M, Luyimbazi J, Xu X *et al.* Interleukin-13: central mediator of allergic asthma. *Science* 1998; **282**: 2258-61.
- Drazen JM, Arm JP, Austen KF. Sorting out the cytokines of asthma. *J. Exp. Med.* 1996; **183**: 1-5.
- Rocca B, Spain LM, Pure E, Langenbach R, Patrono C, FitzGerald GA. Distinct roles of prostaglandin H synthases 1 and 2 in T-cell development. *J. Clin. Invest.* 1999; **103**: 1469-77.
- Rocca B, Spain LM, Ciabattini G, Patrono C, FitzGerald GA. Differential expression and regulation of cyclooxygenase isozymes in thymic stromal cells. *J. Immunol.* 1999; **162**: 4589-97.