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Allergen-Induced Airway Hyperresponsiveness Mediated by Cyclooxygenase Inhibition Is Not Dependent on 5-Lipoxygenase or IL-5, but Is IL-13 Dependent


Cyclooxygenase (COX) inhibition during allergic sensitization and allergen airway challenge results in augmented allergic inflammation. We hypothesized that this increase in allergic inflammation was dependent on increased generation of leukotrienes that results from COX inhibition, as leukotrienes are important proinflammatory mediators of allergic disease. To test this hypothesis, we allergically sensitized and challenged mice deficient in 5-lipoxygenase (5-LO). We found that 5-LO knockout mice that were treated with a COX inhibitor during allergic sensitization and challenge had significantly increased airway hyperresponsiveness (AHR) (p < 0.01) and airway eosinophilia (p < 0.01) compared with 5-LO knockout mice that were treated with vehicle. The proinflammatory cytokines have also been hypothesized to be critical regulators of airway inflammation and AHR. We found that the increase in airway eosinophilia seen with COX inhibition is dependent on IL-5, whereas the increase in AHR is not dependent on this cytokine. In contrast, the COX inhibition-mediated increase in AHR is dependent on IL-13, but airway eosinophilia is not. These results elucidate the pathways by which COX inhibition exerts a critical effect of the pulmonary allergen-induced inflammatory response and confirm that COX products are important regulators of allergic inflammation. The Journal of Immunology, 2005, 175: 8253–8259.

During allergic sensitization and allergen airway challenge, cyclooxygenase (COX) inhibition results in augmented allergic inflammation (1–4). This increase in the allergic phenotype occurs when mice are treated with a nonselective COX inhibitor or with pharmacologic agents that specifically inhibit COX-1 or COX-2 (1–4). This up-regulation of the allergen-induced pulmonary inflammatory response by COX inhibition is independent of signaling through the IL-4R or the STAT6 transcription factor (1). Additionally, COX inhibition results in augmented allergen-induced airway hyperresponsiveness (AHR) (2–4).

Currently, it is not known what factors regulate the heightened allergic phenotype that is driven by COX inhibition. Arachidonic acid can be metabolized not only through the COX enzymes, but also through the 5-lipoxygenase (5-LO) pathway, an important source of proinflammatory mediators (5). Leukotriene (LT)A₄ can be metabolized by either LTA₄ hydrolase into LTB₄, which is a potent neutrophil chemoattractant and a weaker eosinophil chemotactic agent, or by LTC₄ synthase into the cysteinyl LT (5). The cysteinyl LT have eosinophil chemotactic properties, are potent mucus secretagogues, and induce AHR (6). We have previously shown that COX inhibition in the murine model of allergic pulmonary inflammation results in increased cysteinyl LT levels (3). Recent studies suggest that signaling through the LTB₄ receptor is important in allergen-induced AHR (7, 8). Therefore, we hypothesized that the increase in LT resulting from COX inhibition mediates the augmented allergic phenotype in our model.

Cytokines are also important regulators of allergic inflammation and AHR. IL-5 is a critical factor in eosinophil development, differentiation, mobilization, activation, and survival (9–13). Animal models of allergic sensitization in which IL-5 has either been neutralized by Ab, or in which the gene producing IL-5 has been deleted, suggest that IL-5 is critical to the control of airway eosinophilia (14–16). The role of IL-5 in mediating AHR is less clear, as some investigators have found that IL-5 is necessary for AHR (16–19), whereas others have found that AHR can occur independently of IL-5 (20–22). Mouse studies examining the role of IL-13 suggest that it is necessary and sufficient to induce the allergic phenotype, including AHR (23–25). Experimental work in the murine model reveals that IL-13 acts on the resident cells in the airway to induce mucus secretion and AHR, rather than through recruitment of eosinophils to the lung or through type I-mediated hypersensitivity (25). Therefore, we also sought to determine whether the allergen-induced airway inflammation and AHR that occurs with COX inhibition was dependent on either IL-5 or IL-13.

Materials and Methods

Mice

Pathogen-free 8- to 10-wk-old female mice were used in all experiments. The 5-LO knockout (KO) mice and their wild-type (WT) control mice were on a 129 genetic background and were purchased from The Jackson Laboratory and housed in specific pathogen-free conditions under conditions approved by the Institutional Animal Care and Use Committee at Vanderbilt University School of Medicine. Mice were housed five per cage in microisolator cages located in a屏障ed facility. The Journal of Immunology, 2005, 175: 8253–8259.
Lung tissues were ground as previously described (4), and the levels of IL-5 and IL-13 in the lung tissue homogenates were measured in the 129 WT and 5-LO KO mice using ELISA kits following the manufacturer’s instructions. Differential counts were based on counts of 100 cells using standard morphologic criteria to classify the cells as eosinophils, lymphocytes, or other mononuclear leukocytes (alveolar macrophages and monocytes).

**Measurement of cytokine proteins**

Lung tissues were ground as previously described (4), and the levels of IL-5 and IL-13 in the lung tissue homogenates were measured in the 129 WT and 5-LO KO mice using ELISA kits following the manufacturer’s instructions (R&D Systems). The experiments using 5-LO KO and 129 mice were performed more recently and the newer fluorescent-labeled microsphere bead technology was used in these experiments.
lower than those in 5-LO KO mock (p < 0.01) and 5-LO KO OVA (p < 0.05) groups.

The augmented IL-5 and IL-13 production resulting from COX inhibition is dependent on 5-LO

Previously, we found that the levels of IL-5 in ground lung supernatants peaked on day 16 of our protocol; therefore, we measured cytokines on this day. We found that IL-5 (767 ± 218 vs 200 ± 126 pg/ml; p < 0.01) and IL-13 (449 ± 105 vs 167 ± 61 pg/ml; p < 0.01) concentrations were greater in the lung supernatants of the WT OVA-indo group compared with the WT OVA group (Fig. 3). However, there was only a trend for an increase in IL-5 (276 ± 123 vs 63 ± 8 pg/ml; p = 0.06) and IL-13 (225 ± 67 vs 100 ± 5 pg/ml; p = 0.1) in the 5-LO KO OVA-indo group compared with the WT OVA group (Fig. 3). This suggests that the increase in Th2 cytokines that occurs with COX inhibition during the development of allergic inflammation is dependent on 5-LO.

Elevated IgE levels resulting from COX inhibition is dependent on 5-LO

Because the WT OVA-indo and 5-LO KO OVA-indo mice had greater numbers of inflammatory cells in the BAL fluid compared with the rest of the mice in their respective groups, we sought to determine whether this might be a result of an effect of COX inhibition on serum IgE levels (Fig. 4). We found that the WT OVA-indo mice had higher serum concentrations of IgE compared with WT OVA mice (30.3 ± 2.7 vs 13.7 ± 1.9 μg/ml, respectively; p < 0.01). However, there was no difference in the serum IgE levels between the 5-LO KO OVA-indo and 5-LO KO OVA mice as there were undetectable serum IgE levels in all the 5-LO KO OVA mice and undetectable levels in four of the eight 5-LO KO OVA mice. We also measured OVA-specific IgE levels in the serum of the mice and the results are reported as the OD450 as stated in Materials and Methods. The WT OVA-indo mice had significantly greater serum concentrations of OVA-specific IgE compared with WT OVA mice (304 ± 26 vs 91 ± 15 OD450; n = 6 in each group; p < 0.0001). OVA-specific IgE levels were undetectable in the 5-LO OVA-indo and 5-LO OVA mice. Therefore, this suggests that the increase in serum IgE levels seen with COX inhibition is dependent on the presence of 5-LO.

COX inhibition mediated increase in BAL eosinophils is dependent on IL-5, but not IL-13

IL-5 is a critical cytokine in eosinophil growth, differentiation, and survival; however, IL-13 has also been reported to be a factor in eosinophil recruitment to the airways (23). We therefore wanted to determine whether the increased airway eosinophilia seen with COX inhibition during the development of allergic pulmonary inflammation was mediated by IL-5 or IL-13. In these experiments, we used WT, IL-5 KO, and IL-13 KO mice, all on a C57BL/6 background. We found that WT OVA-indo mice had increased...
FIGURE 4. Total IgE levels in serum harvested from WT and 5-LO KO mice at day 18 (n = 8 for each group). Data are representative of three separate experiments. * p < 0.01 compared with all other groups.

FIGURE 5. Total cells, macrophages (macs), eosinophils (eos), and lymphocytes (lymphs) in the BAL fluid on day 18 from allergically sensitized and nonsensitized WT, IL-5 KO, and IL-13 KO mice either treated with the nonselective COX inhibitor indomethacin (indo) or vehicle in the drinking water. Statistical significance is indicated by * p < 0.05 compared with the respective OVA, mock, and mock-indo groups for that particular strain and †, p < 0.05 compared with the respective mock and mock-indo groups for that particular strain.

These results show, that despite an increase in BAL cell influx in the IL-13 KO OVA-indo mice, the allergen-induced increase in AHR with COX inhibition was IL-13 dependent.

We also calculated the PC200 of methacholine for all the WT, IL-5 KO, and IL-13 KO groups. For the C57BL/6 WT mice, the PC200 values, reported as micrograms of methacholine per gram of mouse, were 405 ± 6 for WT mock, 347 ± 28 for WT mock-indo, 244 ± 39 for WT OVA, and 179 ± 51 for WT OVA-indo. The PC200 values for the WT OVA-indo and WT OVA groups were each lower than either the WT mock or WT mock-indo (p < 0.05), but were not different from each other. For the IL-5 KO mice, the PC200 values were 387 ± 24 for the IL-5 KO-mock, 287 ± 58 for the IL-5 KO-indo, 281 ± 44 for IL-5 KO OVA, and 160 ± 37 for IL-5 KO OVA-indo. The IL-5 KO OVA-indo group had a significantly lower PC200 compared with both the IL-5 KO OVA and IL-5 KO mock groups (p < 0.05), but there were no differences between the other groups. For the IL-13 KO groups, the PC200 value was greater than 411 µg/kg for all mice, except for two of eight in the IL-13 KO OVA-indo group. There was no statistical difference in PC200 between the IL-13 KO groups.

The increased AHR seen with COX inhibition is dependent on IL-13, but not IL-5

To determine whether the mechanism that led to an increase in AHR seen with COX inhibition was driven by a cytokine that has been reported to be important in AHR, we again used WT, IL-5 KO, and IL-13 KO mice, all on a C57BL/6 background. We found that there was no difference in AHR between WT OVA, WT mock-indo, and WT mock mice; however, the WT OVA-indo group had significantly greater AHR (p < 0.05) compared with the other three groups (Fig. 6a). We also found similar results in the IL-5 KO mice. There was no difference in AHR between the IL-5 KO OVA, mock-indo IL-5 KO OVA, and mock IL-5 KO OVA mice; however, the IL-5 KO OVA-indo group had significantly greater AHR (p < 0.05) compared with the other three groups (Fig. 6b). In contrast, we found there were no differences in AHR among the IL-13 KO OVA-indo mice, IL-13 KO OVA mice, IL-13 KO mock-indo mice, and IL-13 KO mock mice (Fig. 6c).

COX inhibition increases IL-13 in WT and IL-5 KO mice, and IL-5 in IL-13 KO mice, all on a C57BL/6 background

As AHR was increased in the WT OVA-indo and IL-5 KO OVA-indo mice compared with these strains’ respective OVA groups, yet AHR was not increased in the IL-13 KO OVA-indo group, we sought to determine whether IL-13 levels in the lung were increased in the WT OVA-indo and IL-5 KO OVA-indo mice. Cytokine proteins were measured in the ground lung supernatants on day 16 (Fig. 7). IL-5 was not detectable in the lung supernatants of IL-5 KO mice, nor was IL-13 detectable in the lung supernatants of the IL-13 KO mice. However, in WT mice, there was a trend (p = 0.1) for an increase in IL-5 in the OVA-indo mice (36.4 ± 15 pg/ml) compared with the OVA mice (<15.6 pg/ml, limit of detection). IL-13 levels in the lung supernatants of the IL-5 KO OVA-indo group were 174 ± 46 pg/ml compared with undetectable (<31.2 pg/ml) in the IL-5 KO OVA group (p < 0.05). In WT mice, the OVA-indo group had significantly greater levels of IL-13 in the lung supernatants compared with the WT OVA group (110 ± 17 vs 28.4 pg/ml; p < 0.05). Levels of IL-13 were not detected in the lung supernatants of either nonsensitized IL-5 KO mice or nonsensitized WT mice, whether treated with indomethacin or not. There was significantly greater IL-5 levels in the lungen of the IL-13 KO OVA-indo group compared with the IL-13 KO OVA mice (171 ± 28 vs <15.6 pg/ml, limit of detection; p = 0.001).
Discussion

In our previous studies, we found that COX inhibition up-regulated the allergic phenotype in a mouse model of allergen-induced inflammation (1, 3, 4). Others have found that deficiency of COX-1, but not COX-2 deficiency, results in increased Th2 cytokine production, AHR, and LT generation (28, 29). COX inhibition enhances the production of LT, presumably by decreasing PGE2, a mediator that negatively regulates LT synthesis (30, 31). An alternative theory is that COX inhibition prevents prostanoid formation and that the increased arachidonic acid substrate is therefore “shunted” toward LT synthesis. However, there is experimental evidence suggesting that shunting does not occur (32). Murine models reveal that LT are important regulators of pulmonary allergic inflammation. Mice lacking a functioning 5-LO enzyme had significantly decreased airway eosinophilia, serum IgE levels, and AHR compared with WT mice (33). Specific inhibitors of 5-LO and 5-LO-activating protein decreased airway and lung eosinophilia and airway mucus expression (34). Therefore, an important question to be answered was whether the increase in allergic inflammation that occurs with COX inhibition seen in our model is a result of an increase in LT generation. We found that the COX inhibition-induced increase in allergic inflammation and AHR was not solely dependent upon LT expression, as the 5-LO KO OVA-indo mice had significantly increased airway inflammation and AHR compared with the increase for the 5-LO KO OVA group. We reported AHR by two methods: 1) dose-response curves of lung resistance to increasing doses of methacholine and 2) the PC200. The changes in AHR as measured by the methacholine dose-response curve and the PC200 were not entirely consistent. This is a reflection of the fact that the differences between groups observed in the methacholine dose-response curves occurred at higher doses of methacholine. Both the methacholine dose-response curves and PC200 values are measures of AHR and one is not necessarily more valid than the other. In this report, we use the differences in the methacholine dose-response curves to define AHR.

The increases in airway eosinophilia and AHR in the 5-LO KO OVA-indo mice were not as great as was seen in the WT OVA-indo mice, thus not ruling out the possibility that LT may be playing some role in the COX inhibition-mediated enhancement of allergic inflammation. Lung IL-5 and IL-13 expression was significantly increased in the WT OVA-indo mice compared with the WT OVA group, and although lung IL-5 and IL-13 expression was 3- and 2-fold greater in the 5-LO KO OVA-indo mice compared with the 5-LO KO OVA group, these differences were not statistically significant, suggesting that regulation of these cytokines may be partially dependent on 5-LO expression. There was no difference in total serum IgE levels in the 5-LO KO OVA-indo and 5-LO KO OVA mice, whereas there was a significant increase in the WT OVA-indo group compared with the WT OVA mice, suggesting that the COX inhibition-mediated increase in serum IgE is dependent on the expression of 5-LO.

Because the augmentation of allergic inflammation by COX inhibition is not solely dependent on increased LT synthesis, this suggests that a COX product restrains allergic inflammation independently of an effect on LT expression. Murine models suggest that several prostanoids may regulate allergic inflammation. Mice lacking the PGE2 receptor EP3 had increased allergic inflammation compared with either WT mice or mice lacking each of the other three EP receptors (35), suggesting that signaling through
EP3 down-regulates allergic inflammation. Mice that are deficient in the PGD2 receptor IP had enhanced allergic inflammation, both in acute and chronic models of allergen-induced pulmonary disease (36, 37), suggesting that PGD2 also suppresses the allergen-induced inflammatory response. In contrast, in in vivo models, there is the suggestion that PGD2 up-regulates the allergen-induced inflammatory response. Mice lacking the PGD2 receptor DP had diminished pulmonary allergic inflammation (38), whereas mice that overexpress PGD2 synthase had augmented an augmented allergic phenotype (39). Studies using either a thromboxane synthase inhibitor or a thromboxane receptor antagonist decreased allergic inflammation (40), suggesting that this mediator also enhances allergic inflammation in the lung. Given that the overall effect of inhibiting COX increases allergic inflammation, it is likely that the prostanooids that restrain allergic inflammation have greater activity than those that increase the allergic phenotype.

Cytokines are also very important mediators of allergic inflammation. We previously found that the increase in the pulmonary airway eosinophilia modulated by COX inhibition was independent of signaling through either the IL-4Rα or STAT6 (1). However, the pathway by which COX inhibition increases airway eosinophilia is unknown. IL-5 is a critical factor in eosinophil development, differentiation, mobilization, activation, and survival (9–13). In vitro, IL-5 primes eosinophils to respond to eotaxin and the ability of eotaxin to mediate eosinophil chemotaxis is augmented by increased levels of IL-5 (41). We found that the increase in airway eosinophilia was dependent on IL-5 expression. In WT OVA-indo mice without a C57BL/6 background, there was a significant increase in airway inflammation and eosinophilia compared with WT OVA mice, which was absent in IL-5 KO OVA-indo mice. We found that the COX inhibition driven increase in airway eosinophils was not dependent on IL-13, as IL-13 KO OVA-indo mice had significantly greater airway inflammation compared with IL-13 KO OVA mice and was comparable to the inflammation seen in WT OVA-indo mice. This finding would be consistent with the report that IL-13 induced eosinophil recruitment into the lung by an IL-5 and eotaxin-dependent mechanism (42).

Although we found that the increase in airway eosinophilia resulting from COX inhibition was dependent on IL-5, we found that the increase in allergen-induced AHR mediated by COX inhibition was not dependent on this cytokine, but instead was dependent on IL-13. There is considerable debate over the necessity for eosinophils in the pathogenesis of airway reactivity (43–45). Although some studies in mice have shown that IL-5 is essential for the development of AHR (16–19), others have not (21, 22, 46). In our experiments, we found that the WT OVA-indo group had significantly higher AHR compared with the WT OVA, WT mock-indo, and WT mock groups. There was a small increase in the AHR of the WT OVA group compared with the WT mock group, but this increase was statistically insignificant. This diminished AHR response in the WT OVA group compared with the nonsensitized control may reflect a strain influence on the development of AHR, as C57BL/6 mice have diminished airway reactivity as compared with BALB/c mice (47, 48). The IL-5 KO OVA-indo group had significantly greater airway reactivity compared with the IL-5 KO OVA group and the two IL-5 KO nonsensitized groups, and these three groups were not statistically significantly different from each other. These results show that COX inhibition during allergic sensitization has profound effects on airway physiology in WT and IL-5 KO mice that are on a C57BL/6 background. Our results further show that IL-13 is responsible for the increased AHR in both the WT and IL-5 KO OVA-indo groups, as lung expression of IL-13 was significantly increased in these two groups compared with their respective OVA groups and IL-13 KO OVA-indo mice did not have significantly greater AHR compared with either IL-13 KO OVA mice or either of the two nonsensitized groups.

These findings are consistent with several studies examining the role of IL-13 on airway inflammation and pulmonary physiology. In a mouse model, anti-IL-13 Ab treatment significantly reduced AHR while having no effect on eosinophilia (49). In this study, combined administration of anti-IL-5 and anti-eotaxin Abs before allergen challenge inhibited airway eosinophilia, but did not alter AHR (49). Intratracheal administration of rIL-13 induced AHR from 6 to 24 h after administration, and this increase in AHR was dissociated from airway eosinophilia. These investigators also found that the IL-13-mediated increase in AHR was independent of IL-5 and eotaxin (50). Additionally, adoptive transfer of Th2 polarized cells from IL-13 KO mice that make high levels of IL-4 and IL-5 led to airway inflammation, yet failed to induce AHR (51), and this induction of AHR was dependent on signaling through IL-4Rα (52). Taken together, these studies show that IL-13 critically regulates AHR, without much effect on airway inflammation.

In summary, we found that LT are not necessary for the increase in the allergen-induced inflammatory response and AHR that results from COX inhibition during the development of allergic inflammation. We also found that the increase in airway eosinophilia seen with COX inhibition is dependent on IL-5 whereas the increase in AHR is not dependent on this cytokine. In contrast, the COX inhibition-mediated increase in AHR is dependent on IL-13, but airway eosinophilia is not. These results confirm that COX products are important regulators of allergic inflammation.

Disclosures

The authors have no financial conflict of interest.

References


