

A Key Role for Prostaglandin I₂ in Limiting Lung Mucosal Th2, But Not Th1, Responses to Inhaled Allergen¹

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The cellular events that serve to regulate lung mucosal Th2 responses and limit allergic inflammatory reactions are unclear. Using the DO11.10 TCR transgenic mouse, we developed a model of T cell-mediated pulmonary inflammation and demonstrated that high levels of PGI₂ are produced in the airways following OVA inhalation. Selective inhibition of cyclooxygenase-2 in vivo specifically reduced PGI₂ synthesis and resulted in a marked increase in Th2-mediated, but not Th1-mediated, lung inflammation. The elevated Th2-mediated inflammatory response elicited by the cyclooxygenase-2 inhibitor was associated with enhanced airway hyperreactivity and was coincident with a marked increase in the levels of IL-4, IL-5, and IL-13 in the airways, but a reduction in IL-10 production. In keeping with these observations, we found that the mRNA for the PGI₂ receptor was expressed by Th2, but not Th1, cells, and transcripts for the PGI₂ receptor were induced by IL-4 and OVA peptide stimulation. Interestingly, treatment with PGI₂ or its stable analog, carbaprostacyclin, augmented IL-10 production by Th2 cells. Collectively, our findings reveal a key role for PGI₂ in differentially limiting Th2 responses, possibly by promoting production of the immunosuppressive cytokine IL-10 at the site of allergic lung inflammation. These results indicate an important role for prostanoids generated during inflammation in regulating mucosal T cell responses and highlight a potential risk in the use of cyclooxygenase-2-specific inhibitors by allergic asthmatics. *The Journal of Immunology*, 2002, 169: 5997–6004.

CD4⁺ Th lymphocytes differentiate into two distinct effector phenotypes, Th1 and Th2, defined by functional attributes that reflect their different cytokine profiles (1). Th1 cells produce IFN- γ and provide protection against intracellular pathogens and viruses. Th2 cells, characterized by their secretion of IL-4, IL-5, and IL-13, facilitate IgE production and eosinophilic inflammation, which contribute to the elimination of extracellular parasites and the pathogenesis of allergic disease such as asthma (2–4). Allergic asthma is a chronic inflammatory disorder in which a type 2 response by CD4⁺ T cells in the airways takes primacy in driving the inflammation (2–4). It has been suggested that such chronic inflammation arises from the dysfunction of events that normally lead to the resolution of the underlying T cell response. T cells at mucosal sites are subject to immune regulation, partly due to the actions of cyclooxygenase (COX)⁴-derived prostanoids (5) and cytokines such as IL-10 and TGF- β (6, 7). In addition, CD4⁺ CD25⁺ regulatory T cells that produce IL-10 have been shown to suppress mucosal immune responses (6, 8). The events that limit allergic Th2 responses in the lung are unclear, and conceivably there are distinct mechanisms that differentially regulate Th1 and Th2 effector functions. To this end we

have used a mouse model of T cell-mediated airway inflammation (9) to assess the role of prostanoids in influencing T cell responses in the lung mucosa following Ag inhalation.

Prostanoids are products of arachidonic acid metabolism synthesized via the cyclooxygenase (COX⁴) pathway. The two isoforms of this enzyme, COX-1 and COX-2, metabolize arachidonic acid to PGH₂, which is subsequently processed by specific enzymes to generate a series of products, most notably PGD₂, PGE₂, PGF_{2 α} , prostacyclin (PGI₂), and thromboxanes (Tx). COX-1 is constitutively expressed in most cell types, and is the predominant form present in the gastrointestinal tract, kidney, and platelets (10–12). COX-2 is an enzyme expressed at inflammatory sites by macrophages, neutrophils, and activated mesenchymal cells and is believed to produce prostanoids that contribute to inflammatory swelling, pain, and fever (10–12). Nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and indomethacin, are nonselective COX inhibitors and suppress these inflammatory processes. The inhibition of COX-1, however, underlies the gastrointestinal toxicity of NSAIDs in humans, and this has prompted the development of COX-2-selective inhibitors such as NS-398 and celecoxib, which circumvent such damaging effects (13). The spectrum of prostanoids elicited by COX-2 differs from that synthesized by COX-1. Specifically, COX-2 is thought to be important for the production of PGE₂ and PGI₂ (14). PGI₂ has anti-thrombotic effects in vivo and plays an important role in inflammation and pain perception (15). Despite these potent actions, the effects of PGI₂ appear to be very localized, since the prostanoid is highly unstable, with $t_{1/2}$ of ~ 30 s under physiological conditions (10).

Using DO11.10 mice, we have developed a model of T cell-mediated pulmonary inflammation to examine the role of PGs in influencing allergic airway responses. Our results show that selective inhibition of COX-2 in vivo specifically reduced PGI₂ levels in the lung and led to a significant elevation in local Th2 inflammatory responses to inhaled OVA. Consistent with this observation, we found that the mRNA for the PGI₂ receptor IP-R was expressed by Th2, but not Th1, cells, and transcripts for the IP-R were induced by IL-4 and OVA peptide stimulation. PGI₂ acted on

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⁴ Abbreviations used in this paper: COX, cyclooxygenase; AHR, airway hyperreactivity; BALF, bronchoalveolar lavage fluid; EPO, eosinophil peroxidase; INDO, indomethacin; IP-R, PGI₂ receptor; Penh, enhanced pause; NSAIDs, nonsteroidal anti-inflammatory drugs; PGI₂, prostacyclin; PLN, peripheral lymph node; Tx, thromboxane.

Th2 cells to elevate production of the anti-inflammatory cytokine, IL-10. Collectively, our findings demonstrate that PGI₂ plays an important role in preferentially limiting lung mucosal Th2 responses by promoting IL-10 production at the site of allergic inflammation.

Materials and Methods

Animals

DO11.10 mice (originally developed by Dr. D. Y. Loh, Howard Hughes Medical Institute, St. Louis, MO) were bred under aseptic conditions in a barrier facility at Southampton University. These animals were provided by Dr. E. Shevach (National Institutes of Health, Bethesda, MD). BALB/c mice were obtained from Harlan (Loughborough, U.K.).

Adoptive transfer of DO11.10 T cells and OVA challenge of recipient animals

DO11.10 Th1 or Th2 cells were adoptively transferred into BALB/c mice before exposure to OVA aerosols. To drive T cell differentiation into a Th1 or Th2 effector phenotype, peripheral lymph node (PLN) cells were incubated (5×10^5 /ml) in the presence of OVA_{323–339} peptide (1 μ g/ml), and either mouse IL-12 (1 ng/ml; R&D Systems, Abingdon, U.K.) and anti-IL-4 Ab (5 μ g/ml 11B11; American Type Culture Collection, Manassas, VA), or murine IL-4 (2 ng/ml; R&D Systems) and anti-IFN- γ Ab (5 μ g/ml; R4-6A2; American Type Culture Collection), respectively. After 4 days of culture, cells were restimulated as previously, but in the presence of IL-2 (100 U/ml; Cetus, Emeryville, CA). Cells were routinely depleted of both CD8⁺ T cells (using YTS169.4; Serotec, Oxford, U.K.) and APC (using anti-class II Ab M5/114; American Type Culture Collection) by panning before injection. On day 8, polarized effector CD4⁺ cells were injected i.v. into BALB/c mice (10⁷/mouse). Mice were intranasally challenged by exposure to aerosolized solutions of either PBS or OVA (grade V; Sigma, Poole, U.K.) for 20 min/day over 6 consecutive days using a Wright's nebulizer. Mice were killed on day 7, and bronchoalveolar lavage (BAL) fluid (BALF) was collected for analysis. Experimental groups comprised animals (four per group) treated daily (over the duration of aerosol challenge) with the COX-2-specific inhibitor NS-398 (10 mg/kg daily, i.p. injection every 8 h; Calbiochem, Nottingham, U.K.), the nonselective COX inhibitor indomethacin (INDO; a daily i.p. injection of 5 mg/kg; Sigma), and a control group not treated with NSAIDs (vehicle-treated only). Identical data were produced from control mice regardless of whether these animals were exposed to aerosolized PBS, not exposed to any aerosol, or treated with NSAIDs alone. Airway hyperreactivity (AHR) was measured in response to methacholine inhalation by whole-body plethysmography (Buxco Europe, Petersfield, U.K.). Animals were placed in chambers and exposed to aerosols of PBS (baseline), followed by increasing concentrations of methacholine. The enhanced pause (Penh) was measured after each 3-min exposure. Values were taken from the highest average over a 1-min period. For preparation of tissue for histopathologic analysis, lung tissue was fixed in formalin and embedded in paraffin. Tissue sections were prepared and stained with H&E.

Level of airway inflammation

BAL was performed by cannulating the trachea of each animal and washing the airways with 0.5 ml PBS to collect BALF. BALF from three or four animals were pooled, and eosinophil peroxidase (EPO) levels present in BAL cells or BALF (soluble EPO) were determined by colorimetric analysis as previously described (9). Cell differential percentages were determined by light microscopic evaluation of cytospin preparations and expressed as absolute cell numbers. Levels of IL-4, IL-5, IL-10, IL-13, and IFN- γ in the BALF were measured using sensitive commercially available ELISA kits (all from BioSource, Camarillo, CA; except IL-4 from R&D Systems), according to the manufacturer's instructions. To measure mucus production in the airways, the amount of mucin in the BAL fluid (from Th2 recipients exposed to OVA aerosols that were treated with NS-398 or from untreated controls) was determined by ELISA using the mucin-binding lectin jacalin (Calbiochem, Nottingham, U.K.) as described previously (16). Values were expressed as the percent increase in measurements obtained from untreated control groups.

Level of prostanoid synthesis in the BALF

Levels of PGE₂, PGD₂, TxA₂, and PGF_{2 α} in the BALF were measured by sensitive commercial enzyme immunoassay kits (all from Cayman Chemical, Ann Arbor, MI; except PGE₂ kits supplied by R&D Systems) according to the manufacturer's instructions. For determination of PGI₂ synthesis,

the concentration of the stable metabolite of prostacyclin, 6-keto-PGF_{1 α} , was measured using an immunoassay kit (R&D Systems).

In vitro effect of prostanoids on cytokine production by Th1 and Th2 cells

PLN cells were stimulated with OVA peptide (1 μ g/ml) and either IL-4 (2 ng/ml) or IL-12 (1 ng/ml) to polarize Th2 or Th1 cells, as described above. Either PGI₂ (5 μ M/ml daily; Cayman) or its stable analog carbaprostacyclin (0.5 μ M/ml; Cayman) was added to the cultures together with indomethacin (2 μ g/ml), which was used to inhibit all endogenous prostanoid biosynthesis. After 8 days cells were harvested and restimulated with anti-CD3 (2 μ g/ml) for 48 h, and the supernatant was analyzed for IL-4, IL-5, IL-10, and IFN- γ production by ELISA, as described previously (9). For IL-10 measurement JES5-2A5 (BD Pharmingen, San Diego, CA) was used as capture Ab, and biotinylated polyclonal anti-IL-10 Ab was used for detection (PeproTech, Rockyhill, NJ). Cells were routinely depleted of CD8⁺ (using YTS169.4) and class II⁺ cells (using M5/114) by panning before in vitro analysis. Flow cytometry was used to determine the purity of CD4⁺ T cells, which was >95%.

Real-time RT-PCR

Using the TRIzol technique (9), RNA was extracted from purified CD4⁺ T cells obtained from 8-day polarized Th1 and Th2 cells or from PLN cells before (naïve) and after culture for 3 days with type 2 cytokines (2 ng/ml; except IL-10, 10 ng/ml) or specific Abs (5 μ g/ml 11B11 (anti-IL-4) or 5 μ g/ml anti-IL-13). Total RNA (2 μ g) was then reverse transcribed using Omniscript II (Qiagen, Crawley, U.K.) at 37°C for 1 h using oligo(dT)₁₅ as a primer, and the cDNA was PCR amplified and quantified using the TaqMan technique. Real-time detection of PCR was performed using the Perkin-Elmer AB1 PRISM 7700 Sequence Detection System (PE Applied Biosystems, Warrington, U.K.). The expressions of IP-R (using forward primer, 5'-TTTCCGAAAGGCTGTCTTCC-3'; reverse primer, 5'-ATCCCATGGACAGAACGG-3'; and FAM-TAMRA probe, 5'-ACGCCTCAAGTTCTGG TTGTGTTGCC-3'), GAPDH (housekeeping gene), IL-4, IL-5, and IFN- γ were determined in this way (for sequences, see Ref. 17). Equal amounts of cDNA were used in triplicate and amplified with the TaqMan master mix according to the manufacturer's instructions (PE Applied Biosystems). Thermal cycling conditions were 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of two-step PCR consisting of 15 s at 95°C and 1 min at 60°C. The threshold cycle was measured as the cycle number at which the reporter fluorescent emission increased above the threshold level. The amount of mRNA was expressed as the fold difference relative to the amount obtained from unstimulated control cells. Amplification efficiencies were validated and normalized against GAPDH. For all samples, total RNA that was not reverse transcribed was also analyzed to determine genomic DNA contamination, which was negligible.

Statistical analysis

Cell number and cytokine and prostanoid levels in the BALF of Th1 and Th2 recipient mice before and after Ag inhalation or treatment with COX inhibitors were compared using the Wilcoxon test for paired data or the Mann-Whitney test. Values of $p < 0.05$ were accepted as statistically significant.

Results

Inhibition of COX-2 reduces PGI₂ synthesis and augments lung Th2 inflammation

We have used a murine model of pulmonary inflammation to investigate whether prostanoids influence lung mucosal Th2 responses. DO11.10 CD4⁺ Th1 or Th2 effector cells, which constitutively express a TCR specific for an OVA_{323–339} peptide, were adoptively transferred (10⁷ cells/mouse) into BALB/c mice, which were then exposed to aerosolized OVA for 6 consecutive days. Histological examination of lungs from Th2 recipient mice exposed to OVA aerosols displayed marked peribronchiolar and perivascular eosinophilic inflammation (Fig. 1A). Treatment of mice with the COX-2-selective inhibitor, NS-398, resulted in a dramatic increase in pulmonary eosinophilic inflammation. Analysis of BALF also revealed that following Ag inhalation, recipients of Th2 cells developed pronounced inflammation in the airways, with a marked infiltration by eosinophils and lymphocytes (Fig. 1B). However, no infiltration was observed in control mice not

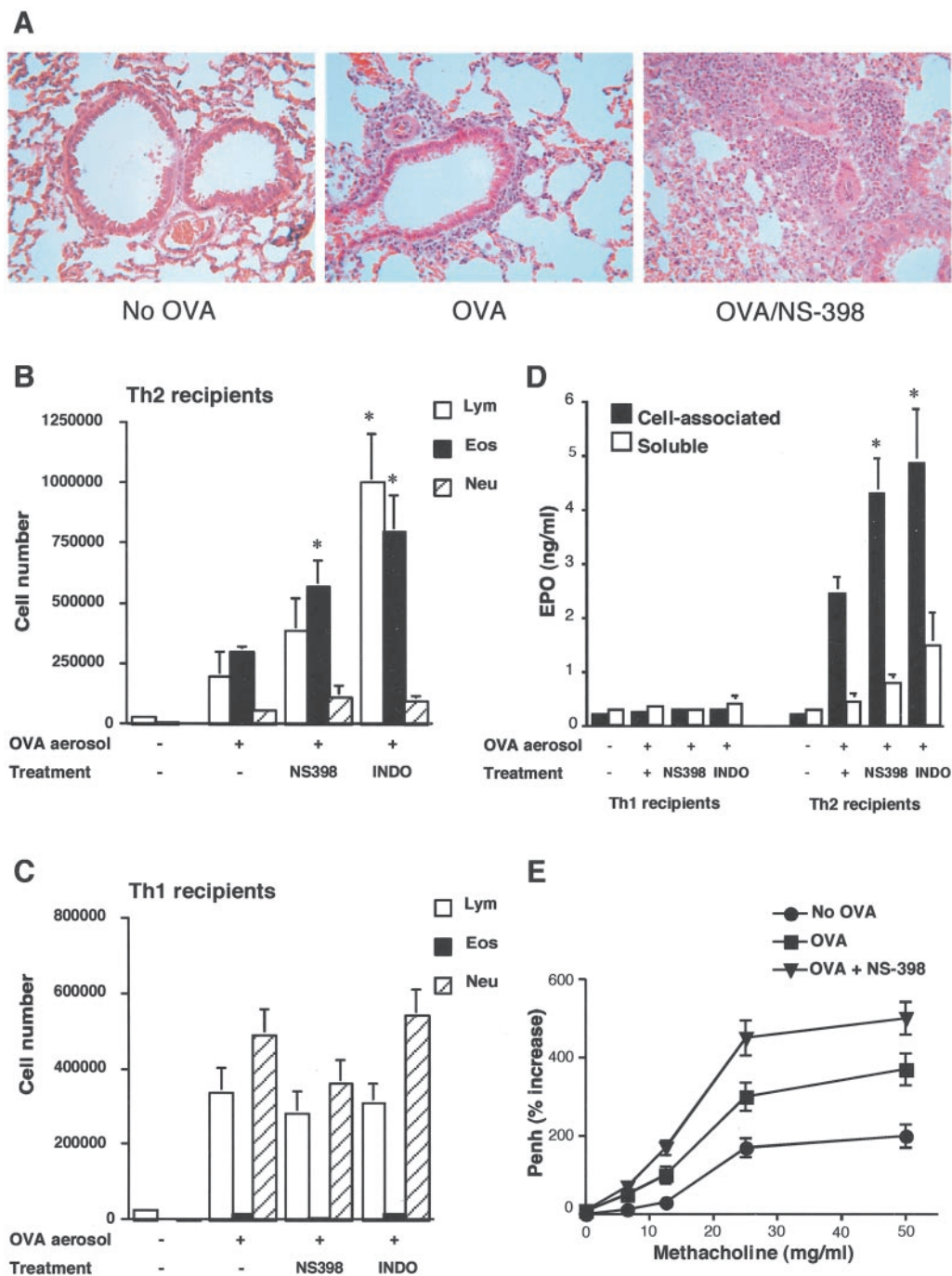


FIGURE 1. Airway inflammation following OVA inhalation and treatment with NS-398. DO11.10 Th2 or Th1 cells were injected into BALB/c recipient mice, which were then exposed to OVA aerosols for 6 consecutive days. Control mice were not exposed to aerosolized OVA and were not treated with NS-398 or indomethacin (INDO). **A**, Lungs were prepared for histology and were stained with H&E ($\times 25$ magnification). Th2 recipient mice displayed peribronchiolar and perivascular eosinophilic inflammation following challenge with OVA, which was markedly increased after treatment with NS-398. BALF cell differential counts in Th2 (**B**) or Th1 (**C**) recipients were determined by light microscopic evaluation of cytospin preparations, and the results are expressed as absolute number of lymphocytes (Lym), eosinophils (Eos), and neutrophils (Neu). **D**, The level of cell-associated and soluble EPO activity in the BALF of Th2 recipients was determined by colorimetric analysis. **E**, Measurement of changes in Penh in response to inhaled methacholine in Th2 recipient mice. Exaggerated increases in Penh following exposure to OVA aerosols indicate AHR. The data shown are the mean $\pm$ SEM from six separate experiments. *, $p < 0.05$ compared with OVA-challenged groups not treated with NSAIDs.

exposed to OVA aerosols. Treatment of recipient mice with NS-398 or indomethacin (the latter of which blocks both COX-1 and COX-2) markedly augmented the intensity of the eosinophilia (Fig. 1B). In contrast, Th1 recipients developed an airway neutrophilia that was not significantly affected by treatment with NS-398 or indomethacin (Fig. 1C). A striking increase in the total number

of eosinophils was reflected by elevated levels of cell-associated EPO activity in the BALF of NS-398- and indomethacin-treated animals (Fig. 1D). Soluble EPO activity was also increased in the BALF from treated mice, suggesting that not only does COX inhibition augment eosinophil numbers in the airways, but it may also facilitate their activation and subsequent release of mediators.

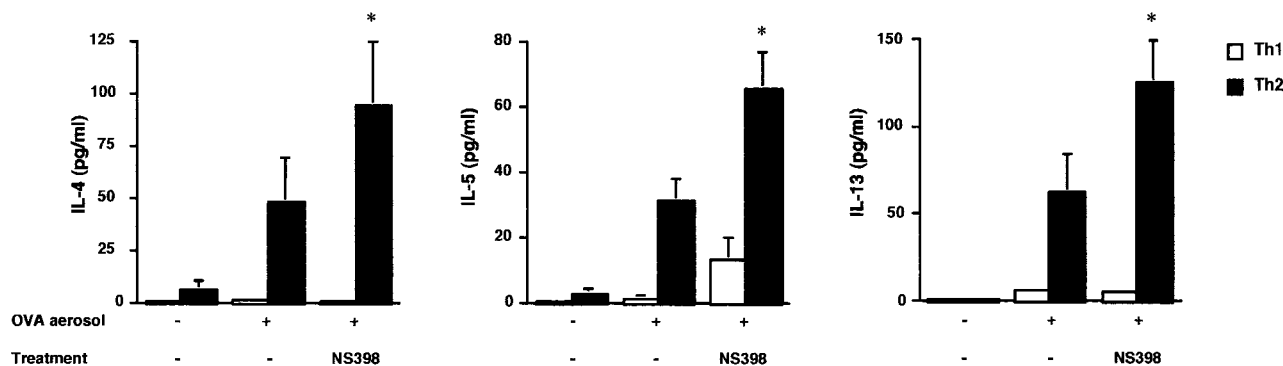


FIGURE 2. Treatment of Th2 recipient mice with NS-398 augments type 2 cytokine production in the airways following OVA inhalation. BALF levels of IL-4, IL-5, and IL-13 from DO11.10 Th1 or Th2 recipient mice were determined by ELISA. Control mice were not exposed to aerosolized OVA and were not treated with NS-398. The data shown are the mean $\pm$ SEM from five separate experiments. *, $p < 0.05$ compared with OVA-challenged groups not treated with NS-398.

In addition, NS-398 treatment of Th2 recipients resulted in an increase in the amount of mucus present in the BALF (41% rise compared with mucus production by untreated mice). The number of macrophages in the BALF of Th1 and Th2 recipients was unchanged following OVA inhalation or treatment with the inhibitors (data not shown). Exposure of Th2 recipients to OVA aerosols resulted in AHR, as measured by exaggerated increases in Penh in response to inhaled methacholine. Coincident with the increase in inflammation, NS-398 treatment of Th2 recipients resulted in enhanced AHR (Fig. 1E).

Measurements of IL-4, IL-5, and IL-13 in the BALF revealed that the levels of cytokines were markedly augmented in Th2 recipients, but not Th1 recipients, following OVA inhalation. Fur-

thermore, consistent with the increase in eosinophilic inflammation, NS-398 treatment significantly elevated the production of these cytokines in the airways of Th2 recipients (Fig. 2).

We subsequently assessed the effects of NS-398 and indomethacin on prostanoid biosynthesis in the airways. BALF from Th1 and Th2 recipients, before Ag inhalation, contained significant baseline levels of PGE₂, PGF_{2 α} , PGD₂ (Fig. 3), and TxB₂, but negligible amounts of the stable metabolite of PGI₂, 6-keto-PGF_{1 α} (Fig. 3). The levels of these prostanoids, particularly the PGI₂ metabolite, were markedly increased following OVA inhalation. Treatment of Th1 and Th2 recipient mice with NS-398 inhibited production of the metabolite of PGI₂ (54.2 and 52.9% inhibition, respectively), but did not affect the levels of other prostanoids (Fig.

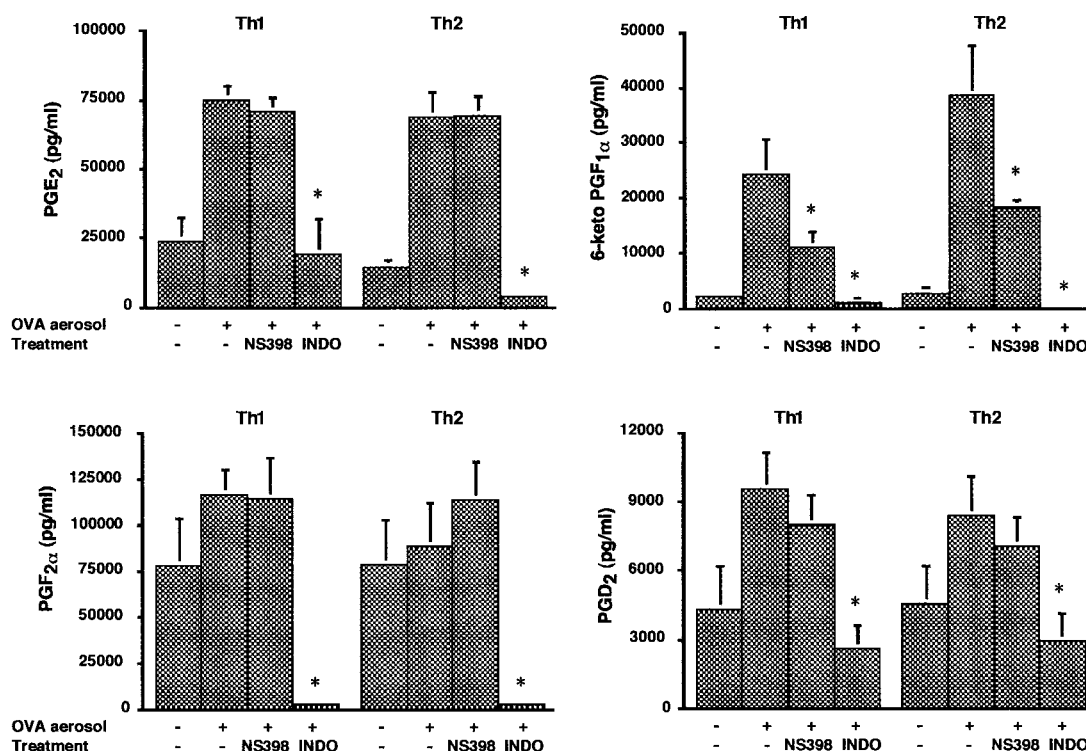


FIGURE 3. Prostanoid synthesis in the airways following OVA inhalation and treatment with NS-398 or indomethacin. DO11.10 Th1 or Th2 recipient mice were exposed to OVA aerosols for 6 consecutive days and were either untreated or injected daily with NS-398 or indomethacin (INDO). Control mice were not exposed to aerosolized OVA and were not treated with NSAIDs. BALF prostanoid levels were determined by EIA. NS-398 treatment specifically resulted in 52.9% inhibition of levels of the stable PGI₂ metabolite, 6-keto-PGF_{1 α} , in the BALF of Th2 recipient mice. The data shown are the mean $\pm$ SEM from six separate experiments. *, $p < 0.05$ compared with OVA-challenged groups not treated with NSAIDs.

3). However, indomethacin (which irreversibly inactivates both COX-1 and COX-2) blocked the biosynthesis of all prostanoids examined. These results demonstrate that in the lung, NS-398 treatment selectively inhibits PGI₂ synthesis, in contrast to indomethacin, which blocked all prostanoid production. Consequently, the increase in airway eosinophilia and Th2 cytokines induced by NS-398 treatment was associated with a reduction in PGI₂ synthesis.

PGI₂ receptor is expressed on Th2 cells and serves to promote IL-10 production

Since a reduction of PGI₂ production is associated with an increase in lung Th2 inflammation, but does not influence Th1 responses, we next determined whether there was differential expression of IP-R on Th1 vs Th2 cells. Using real-time RT-PCR, the expression of IP-R mRNA was found to be restricted to CD4⁺ Th2, but not Th1, cells (Fig. 4A). IP-R mRNA was not expressed by naive (unstimulated) CD4⁺ T cells purified from PLN cells or by whole PLN cells that had not been polarized. In contrast, the P815 (mastocytoma) cell line, which was analyzed for comparison, constitutively expressed this receptor (Fig. 4A). Both polarized Th1 and Th2 cells expressed mRNA for the appropriate cytokines (Th1 cells expressed IFN- γ , whereas Th2 cells expressed IL-4 and IL-5 mRNA), confirming their effector phenotype (Fig. 4B). To identify whether the expression of the receptor was regulated by cytokines, PLN cells from DO11.10 mice were stimulated for 3 days with OVA peptide in the presence of Th2 cytokines IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. IP-R mRNA expression by CD4⁺ T cells was evoked in the presence of IL-4 only (Fig. 4C). IL-4 was unique in this respect, as although IL-13 marginally increased this expression, the effect was lost on the inclusion of a neutralizing Ab to IL-4. Moreover, basal expression of the IP-R mRNA was lost when anti-IL-4 (Fig. 4C), but not anti-IL-13, Ab was added to the cultures.

To examine the function of the IP-R on Th2 cells, PLN from DO11.10 mice were differentiated into Th1 or Th2 effector cells by stimulation with OVA peptide and the appropriate cytokines in the presence of PGI₂ or its stable analog, carbaprostacyclin. Cells were harvested on day 8 and restimulated with anti-CD3 for 48 h, and the supernatants were analyzed for the production of cytokines. Both PGI₂ and its stable analog promoted production of the anti-inflammatory cytokine IL-10 by Th2 cells, but not by Th1 cells (Fig. 5). In contrast, the prostanoid and its analog did not affect the levels of IL-4 and IL-5 produced by Th2 cells or of IFN- γ by Th1 cells (Fig. 5). Indeed, in the absence of indomethacin in the culture, Th2 cells produced high levels of IL-10 compared with cells cultured in the presence of indomethacin, which inhibits PGI₂ production (data not shown). This implies that endogenous PGI₂ synthesis plays an important role in elevating IL-10 production by Th2 cells.

To address whether the inhibition of COX-2 in vivo influenced IL-10 production at the site of Th2-mediated inflammation, we examined BALF IL-10 levels in Th2 recipient mice that been treated with NS-398. Treatment with the COX-2 inhibitor increased the levels of IL-4, IL-5, and IL-13 in the airways (see Fig. 2), which is consistent with the elevated inflammatory response. In marked contrast, the amount of IL-10 present in the BALF was markedly reduced following treatment with NS-398 (Fig. 6). The reciprocal relationship between IL-10 levels and the Th2 cytokines in the airways is consistent with the possibility that the actions of the COX-2-specific inhibitor are mediated at least in part by modulating IL-10 production. Only negligible amounts of IL-10 were present in the BALF from mice that received Th1 cells.

Collectively, these results suggest that the expression of the IP-R by Th2 cells and the ability of PGI₂ to augment IL-10 production provide a mechanism by which this prostanoid may exert an immunomodulatory function.

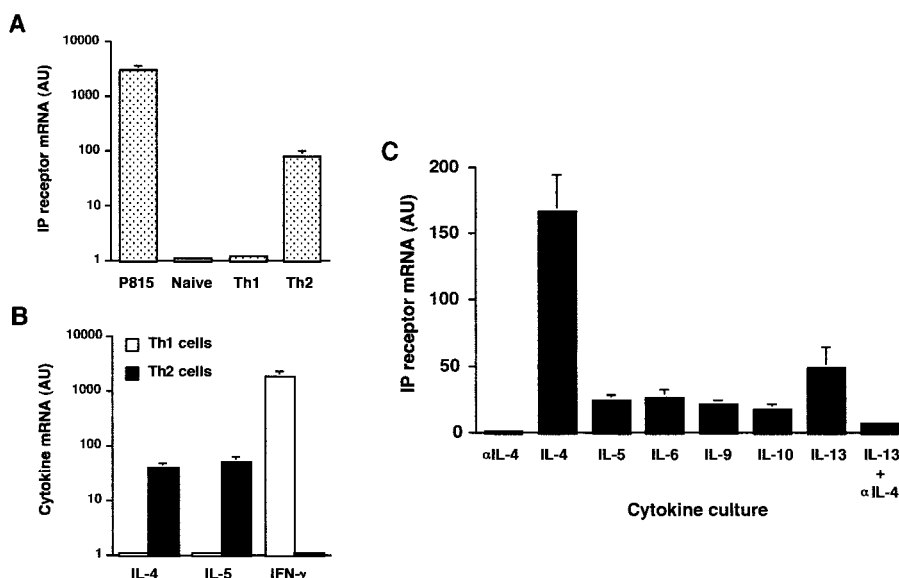
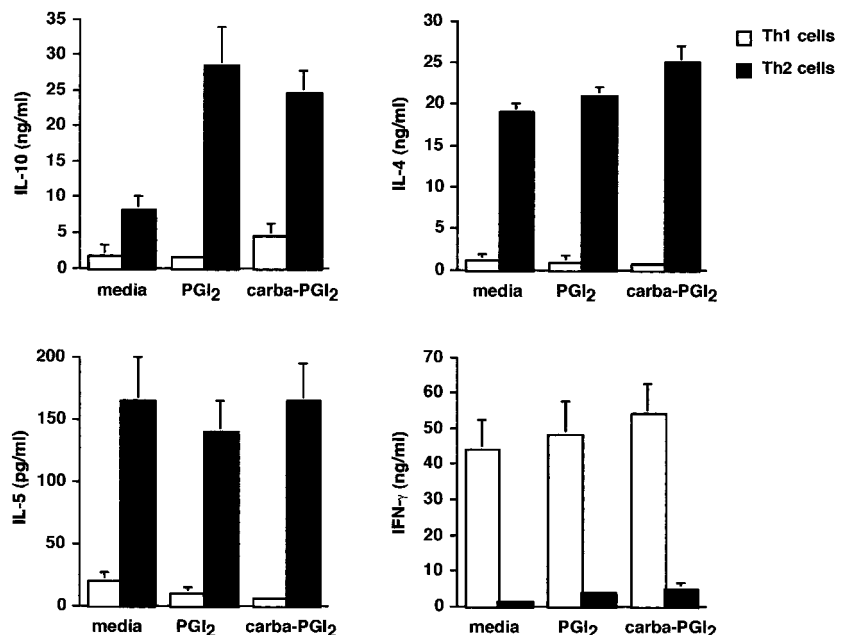


FIGURE 4. Expression of IP-R mRNA in CD4⁺ Th2, but not Th1, cells: induction by IL-4. **A**, Expression of IP-R mRNA by naive T cells (unpolarized) and fully polarized Th1 or Th2 cells. P815 cells, which constitutively express IP-R, were analyzed for comparison. **B**, Expression of cytokine mRNA by Th1 or Th2 cells. RNA was extracted from CD4⁺ T cells or P815 cells and subjected to real-time quantitative RT-PCR (TaqMan). Amplification efficiencies were validated and normalized against GAPDH (housekeeping gene) values. Results are expressed as the fold difference relative to values obtained from control (nonstimulated) cells. **C**, Effect of type 2 cytokines on the expression of the IP-R mRNA. Lymph node cells were cultured for 3 days with OVA peptide and Th2 cytokine (IL-4, IL-5, IL-6, IL-9, IL-10, or IL-13) or anti-IL-4 Ab. RNA was extracted from purified CD4⁺ T cells and subjected to real-time RT-PCR analysis. The results are expressed as the fold difference relative to values from cells unstimulated with cytokines (but cultured with anti-IL-4). The data shown are the mean $\pm$ SEM ($n = 3$) and are representative of three separate experiments. AU, arbitrary units.

FIGURE 5. PGI₂ promotes IL-10 production by Th2, but not Th1, cells. Th1 or Th2 cells were polarized for 8 days in the presence of OVA peptide and either IL-12 or IL-4, respectively. PGI₂ (5 μ M/ml) or its stable analog carbaprostacyclin (carba-PGI₂; 0.5 μ M/ml) was added to the cultures. After 8 days cells were harvested and restimulated with anti-CD3 for 48 h. Supernatants from Th1 and Th2 cells were analyzed for the production of IL-10, IL-4, IL-5, and IFN- γ by ELISA. The data shown are the mean $\pm$ SEM ($n = 3$) and are representative of three separate experiments.



Discussion

CD4⁺ T cells producing the type 2 cytokines IL-4, IL-5, and IL-13 are thought to play a major role in driving mucosal inflammation in asthma. Conceivably, such chronic inflammation arises from the dysfunction of events that normally lead to resolution of the underlying T cell responses. Typically, T cells at mucosal sites are subject to severe immune regulation, in part due to the actions of TGF- β , IL-10, and prostanoids produced locally (5–7). Whether the nature of such regulatory mechanisms changes following the onset of an inflammatory response remains unclear. It is possible that distinct mechanisms may serve to differentially regulate Th1 and Th2 effector function. In addition, a role for IL-12 and IFN- γ has been proposed in limiting pulmonary Th2 responses (18). However, IL-12 is most effective during the early stages of T cell polarization, since polarized Th2 effector cells do not express the β_2 -chain of the IL-12R. We propose that specific mechanisms exist by which COX-2-derived mediators, generated during allergic inflammation, inhibit the responses of CD4⁺ Th2 cells.

During an inflammatory response multiple prostanoids are generated from the oxidative metabolism of arachidonic acid by COX-1 and COX-2 that act on a range of cell types (11, 12). It has been previously proposed that COX-2 plays an important role in

limiting inflammatory processes (5, 19). The inhibition of COX-2 is known to augment proliferative responses in the intestine to dietary Ag (5). This may result from COX-2 facilitating the synthesis of anti-inflammatory cyclopentenone PGs, which are thought to play a role in the resolution of inflammation (19).

Using a mouse model of pulmonary inflammation, we have shown that following OVA inhalation, animals that received Th2 DO11.10 cells developed pronounced airway inflammation with infiltration of eosinophils and lymphocytes, whereas recipients of Th1 cells developed pulmonary neutrophilia. Treatment with NS-398, a COX-2-selective inhibitor, markedly augmented the intensity of the eosinophilia and AHR in the Th2 recipient mice, but did not affect the level of inflammation in the Th1 recipients. Consistently, IL-4, IL-5, and IL-13 production was elevated in the airways of Th2 recipients following treatment with a COX-2 inhibitor. We found that the BALF of resting animals contained significant amounts of prostanoids, including PGF_{2 α} , PGE₂, PGD₂, and TxB₂, which are thought to play an important role in maintaining homeostasis. Before OVA inhalation the BALF contained negligible levels of the stable metabolite of PGI₂. However, the concentration of this prostanoid was markedly elevated in Th1 and Th2 recipient mice following Ag inhalation. The inhibition of COX-2 by NS-398 treatment resulted in a selective reduction in PGI₂ synthesis in the airways. Although the levels of other prostanoids were also increased following challenge, their synthesis was not affected by the COX-2 inhibitor. This implies that COX-1 is responsible for the majority of PGs present in the BALF, which is in agreement with previous observations using COX-1- and COX-2-deficient mice (20). Moreover, the selective inhibition of PGI₂ synthesis by NS-398 in the lung is in accordance with recent reports that treatment of healthy individuals with a COX-2-specific inhibitor resulted in marked reduction of systemic prostacyclin biosynthesis (21) and that the production of PGI₂ by activated macrophages is COX-2 dependent (14).

In the present study the increase in airway Th2, but not Th1, inflammation following treatment with the COX-2 inhibitor was invariably associated with a reduction in PGI₂ synthesis. This suggests that COX-2-specific generation of PGI₂ may form a mechanism by which inflammatory processes in the lung limit the underlying Th2, but not Th1, responses. Consistent with this observation, the PGI₂ receptor mRNA was found to be expressed

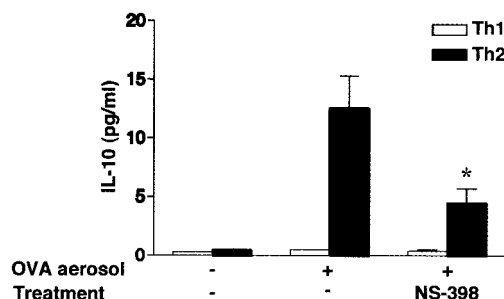


FIGURE 6. Effect of NS-398 treatment on IL-10 production in the airways. IL-10 levels in the BALF of Th1 or Th2 recipient mice were determined by ELISA. Control mice were not exposed to aerosolized OVA and were not treated with NS-398. The data shown are the mean $\pm$ SEM from five separate experiments. *, $p < 0.05$ compared with OVA-challenged groups not treated with NS-398.

by CD4⁺ Th2, but not by Th1 cells or unstimulated T cells. Moreover, IP-R mRNA was induced in these T cells by IL-4 following OVA stimulation. No other Th2 cytokine was effective at inducing IP-R mRNA transcripts. The rapid induction of the IP-R mRNA by IL-4 suggests that this is a direct effect of the cytokine, rather than a consequence of the Th2 polarization process. These observations extend the functional attributes of IL-4 and highlight this cytokine not only as essential for the differentiation of CD4⁺ Th2 cells, but also in promoting a mechanism that limits the progression of allergic inflammation. Although it is well documented that the IP-R is abundantly expressed on platelets and medullary thymocytes (22, 23), this is the first demonstration that activated T cells express this receptor. Collectively these findings suggest that PGI₂ plays an important immunoregulatory function by limiting lung mucosal Th2, but not Th1, responses.

We found that in vitro PGI₂ evoked a marked increase in the production of IL-10 by Th2 cells, but did not affect IL-4 and IL-5 levels or the production of IFN- γ by Th1 cells. Conversely, treatment of mice with the COX-2-specific inhibitor reduced IL-10 production in the airways of Th2 recipients. It is likely that the Th2 cells were the source of IL-10, as this cytokine was not detected in the BALF of Th1 recipients. Since the expression of IL-10 is increased by agents that raise cAMP levels (24), it is possible that this is the mode by which the IP-R mediates its anti-inflammatory effects. In this context both PGI₂ and PGE₂ have been shown to elevate IL-10 production by murine peritoneal macrophages (25). IL-10 is known to exert multiple immunosuppressive effects, including inhibition of eosinophil migration, decreased CD80 expression and IL-5 production, and the consequent reduction in pulmonary eosinophilic inflammation (26, 27). As such, PGI₂ provides a negative feedback mechanism that limits the severity of the Th2-mediated inflammatory response. This form of immune regulation may act solely via IL-10 or in combination with a direct effect of PGI₂ on Th2 function in vivo, for example, by inhibiting T cell chemotaxis. Certainly, other prostanoids, such as PGE₂, have been shown to exert multiple immunomodulatory effects on T cells, including inhibition of T cell proliferation, chemotaxis, and Fas-mediated apoptosis (28–30). The source of PGI₂ in the lungs of OVA-challenged animals is unclear; however, endothelial cells and macrophages are known to produce large amounts of the prostanoid (31, 32). Since PGI₂ is highly labile, its immunomodulatory action is likely to be restricted to the site of inflammation.

PGE₂ synthesis at tissue sites is thought to favor the development of Th2 responses by inhibiting IL-12 production by dendritic cells (33). Our data extend this observation, and we propose that local prostanoid production strongly influences the subsequent progression of the T cell response (i.e., whether a Th1 or Th2 response is favored). Although we found that PGI₂ is produced during both Th1- and Th2-mediated lung inflammation, the IL-4-dependent nature of the IP-R expression on T cells implies that the immunomodulatory action of this prostanoid is limited to a Th2 inflammatory response. PGI₂, by acting on Th2 cells activated in the presence of IL-4, would serve to limit the Th2 response. It is possible that such regulatory mechanisms are not restricted to allergic inflammation. For example, infection with the filarial helminth *Brugia malayi* typically elicits a potent Th2-type host immune response. This response is followed by the development of a profound T cell hyporesponsiveness whose onset is dependent on IL-4 generated by the host (34). Interestingly, the filarial parasite is also capable of using arachidonic acid to generate both PGI₂ and PGE₂ (35). Conceivably, the immune evasion displayed by this parasite may involve the cooperation of IL-4 and COX-derived prostanoids to elicit immune regulation.

COX-2-specific inhibitors are a new class of drugs that have the same anti-inflammatory and analgesic properties as aspirin and reduce inflammation without affecting the housekeeping function of the COX-1 products (11). In 10–15% of patients with asthma the ingestion of aspirin precipitates a life-threatening exacerbation of the disease. Such aspirin intolerance is associated with the release of cysteinyl leukotrienes that mediate the bronchial obstruction (36). It has been proposed that drugs that selectively inhibit COX-2, such as celecoxib and rofecoxib, may be used safely by aspirin-intolerant asthmatics (37); however, the effect of administering these drugs to other cohorts of asthmatics has not been examined. Moreover, it has recently been reported that treatment with celecoxib has been associated with the onset of fatal allergic vasculitis (38). Our data imply that during chronic allergic inflammation in the lung, inhibition of COX-2 increases the inflammatory reaction by ablating the immunoregulatory effects of PGI₂. These findings thus highlight a potential risk in the use of COX-specific inhibitors by allergic asthmatics. Alternatively, a defect in this novel form of immune regulation may contribute to the chronic inflammation evident in this disease.

In summary, our observations reveal a key role for PGI₂ in regulating allergic responses in the lung mucosa, possibly by acting on Th2 cells to promote IL-10 production. This study provides important insight into the regulatory processes that limit the severity of Th2-mediated inflammatory reactions. There is increasing evidence that PGs are important modulators of immunity (30), and thus a better understanding of the multifarious activities of these mediators is crucial for the design of novel approaches aimed at immune intervention/modulation.

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