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Prostaglandin I_2-IP Signaling Blocks Allergic Pulmonary Inflammation by Preventing Recruitment of $CD4^+$ Th2 Cells into the Airways in a Mouse Model of Asthma[1]

Zeina Jaffar,[2]* Maria E. Ferrini,* Mary C. Buford,* Garret A. FitzGerald,† and Kevan Roberts[2]*

PGI_2 plays a key role in limiting Th2-mediated airway inflammation. In studies to investigate the mechanism underlying such regulation, we found that the PGI_2 receptor, IP, is preferentially expressed by effector $CD4^+$ Th2 cells, when compared with Th1 cells. Adoptive transfer of DO11.10 Th2 cells pretreated with PGI_2 resulted in considerably attenuated pulmonary inflammation and airway hyperreactivity in BALB/c recipient mice in response to OVA inhalation. This suppression was independent of increased cAMP levels, because pretreatment of Th2 cells with dibutyryl cAMP before transfer had no effect on airway inflammation. Moreover, PGI_2 pretreatment of Th2 cells suppressed the ability of the cells to infiltrate the lungs but not the spleen. In vitro studies showed that PGI_2 did not affect IL-4 and IL-5 production or the level of IFN-γ by the T cells. However, the prostanoid strongly inhibited CCL17-induced chemotaxis of $CD4^+$ Th2 but not Th1 cells. The IP was implicated in this process since migration of wild-type Th2 cells in response to CCL17 was markedly reduced following treatment with PGI_2, whereas IP-deficient Th2 cells were unaffected and migrated effectively. Collectively, these experiments suggest that PGI_2, which is generated by endothelial cells during lung inflammatory response, serves to limit the influx of Th2 cells to the airways. Our results identify PGI_2-IP as an important pathway for inhibiting allergic pulmonary inflammation by controlling recruitment of $CD4^+$ Th2 cells into the inflammatory site. *The Journal of Immunology,* **2007, 179: 6193–6203.**

The prevalence of asthma has markedly increased worldwide in recent decades. The disease is characterized by chronic bronchial inflammation and airway hyperreactivity (AHR)[3] to a variety of environmental stimuli such as allergens. The hallmarks of allergic asthma include infiltration of the airways by $CD4^+$ Th2 cells and eosinophils, airway remodeling, and increased mucus secretion and serum IgE (1, 2). Th2 cytokines such as IL-4, IL-5, IL-9, and IL-13 play a key role in the asthmatic response and, consequently, it has been proposed that the disease is associated with dysregulated $CD4^+$ Th2 responses. In particular, IL-4 is critical in $CD4^+$ T cell commitment to a Th2 phenotype, and both IL-9 and IL-13 are important in mucus production and AHR, whereas IL-5 regulates eosinophil development, activation, and tissue recruitment (3–6). Thus, although Th2 cells are linked to atopy and asthma, Th1 cells, which are defined on the basis of their production of IFN-γ, have been linked to many chronic autoinflammatory disorders. With regard to regulation of allergic lung inflammation, IFN-γ has long been known to inhibit Ag-induced infiltration of T cells and eosinophils into the mouse airways (7). More recently, both naturally occurring $CD4^+CD25^+$ regulatory T cells (Tregs) and prostacyclin (PGI_2) have been shown to play important roles in suppressing allergic inflammation (8, 9).

PGI_2, a potent vasodilator and inhibitor of platelet aggregation, is a major product of cyclooxygenase (COX) system (10). In this process, arachidonic acid is liberated from membrane phospholipids by phospholipase A_2 and converted to PGH2 by COX. PGH2 is subsequently converted to PGI_2 by the isomerase PGI_2 synthase. PGI_2 is very labile and rapidly hydrolyzed at physiological pH to form the inactive 6-keto-$PGF_{1\alpha}$ (11). In vivo work has shown that the majority of injected PGI_2 disappears from the circulation within minutes (12). PGI_2 elicits its biological effects by binding to a cell surface G protein-coupled IP receptor (13). Activation of IP by an agonist leads to increased production of intracellular cAMP via stimulation of adenylate cyclase (13). Studies using IP-deficient mice ($IP^{-/-}$) provided insightful evidence that PGI_2-IP signaling in vivo is important in preventing thrombosis, inhibiting injury-induced vascular proliferation (14), modulating allergic airway responses (15), and mediating inflammatory swelling and pain (16).

Although IP expression by mouse medullary thymocytes has been reported (17), very little is known about the significance of PGI_2-IP signaling in T cell biology. Our previous work (9) has demonstrated that PGI_2 plays a key role in regulating Th2-mediated lung inflammatory responses. The precise mechanism of this regulation remained unclear. In contrast to PGD_2 and leukotriene

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[3] Abbreviations used in this paper: AHR, airway hyperreactivity; Treg, regulatory T cell; COX, cyclooxygenase; BAL, bronchoalveolar lavage; dbcAMP, dibutyryl cAMP; EPO, eosinophil peroxidase; LMC, lung mononuclear cell; Penh, enhanced pause; PGI_2, prostacyclin; PLN, peripheral lymph node cell; IP, PGI_2 receptor; EIA, enzyme immunoassay; PPAR, peroxisome proliferator-activated receptor; Penh, enhanced pause.

B_4, which induce T cell migration (18, 19), PGI_2 analogs have been demonstrated to inhibit both the recruitment of human granulocytes and fibroblasts at sites of inflammation and the adhesion of polymorphonuclear leukocytes to human vascular endothelial cells (20–22).

We have previously shown that PGI_2 production is increased during Th2 but not Th1-mediated pulmonary inflammation and plays a key role in regulating allergic responses. Our aim in this study was to determine the mechanism by which PGI_2 mediates regulation of effector Th2 cell responses both in vivo and in vitro. In this study, we show that the IP receptor is preferentially expressed by Th2 cells and by $CD4^+CD25^+$ regulatory T cells that were expanded in the presence of IL-4. In vivo studies demonstrate that adoptive transfer of DO11.10 Th2 cells that have been pretreated with PGI_2 significantly reduced airway inflammation in response to OVA aerosol. Moreover, the influx of the Th2 cells into the lungs was dramatically inhibited. PGI_2 did not affect cytokine production by T cells but was capable of inhibiting CCL17-induced chemotaxis of Th2 cells but not Th1 cells in vitro. IP was implicated in this process since the migration of wild-type effector Th2 cells to CCL17 was markedly reduced in the presence of PGI_2, whereas $IP^{-/-}$ Th2 cells migrated effectively. Together, these results suggest that PGI_2, generated by endothelial cells during the inflammatory response, serves to limit the influx of $CD4^+$ Th2 cells to the lungs. These experiments identify PGI_2-IP signaling as a potent immunoregulatory pathway for controlling effector Th2 cell recruitment into sites of inflammation, and should pave the way for further studies using this class of compounds for the treatment of asthma.

Materials and Methods

Animals

Animal studies were approved by the Montana Institutional Animal Care and Use Committee and performed according to the University of Montana and National Institutes of Health guidelines for animal use and care. C57BL/6 $IP^{-/-}$ mice were provided by Dr. G. FitzGerald (University of Pennsylvania, Philadelphia, PA). BALB/c, C57BL/6, DO11.10, and OT-II TCR-transgenic mice were obtained from The Jackson Laboratory. $IP^{-/-}$ and IL-4R$\alpha^{-/-}$ (The Jackson Laboratory) mice were bred and housed in microisolator cages under pathogen-free conditions (University of Montana, Missoula, MT).

Preparation of polarized effector Th1 and Th2 cells

To drive T cell differentiation into Th1 or Th2 effector phenotype, peripheral lymph node cells (PLNs) from DO11.10 mice, depleted of $CD8^+$ T cells (by MACS beads; Miltenyi Biotec), were incubated (5×10^5/ml) for 4 days in the presence of $OVA_{323–339}$ peptide (1 μg/ml; Mimotopes), and either mouse IL-12 (1 ng/ml; R&D Systems) plus anti-IL-4 Ab (5 μg/ml, 11B11;American Type Culture Collection) for Th1 cells or IL-4 (2 ng/ml; R&D Systems) plus anti-IFN-γ Ab (5 μg/ml, R4-6A2; American Type Culture Collection) for Th2 cells. After 4 days of incubation, cells were restimulated as before, but this time in the presence of IL-2 (10 ng/ml; R&D Systems) for another 4 days. Cells were either untreated or pretreated with 2 or 10 μM PGI_2 (Cayman Chemical) by addition on days 4, 6, and 7 of differentiation (i.e., repeated administration for 3 days because PGI_2 is highly labile). After 8 days of incubation, polarized effector $CD4^+$ Th2 cells were adoptively transferred into BALB/c mice or used for in vitro studies. To generate $CD4^+$ Th2 cells from $IP^{-/-}$ mice, $CD8^+$ depleted PLNs were polarized as before using anti-CD3 (2 μg/ml) rather than OVA peptide. In some experiments, Th2 cells pretreated with dibutyryl cAMP (dbcAMP, 0.1 mM) for either 24 h or repeatedly for 3 days before adoptive transfer were also prepared to examine whether elevated cAMP levels influence airway inflammation. The concentrations of PGI_2 (2–10 μM) selected in our studies are based on the effectiveness of PGI_2 at inducing a maximum rise in cAMP by Th2 cells and are consistent with previously published work by our laboratory (9) and others studying immune-associated cells with similar culture conditions (23, 24).

Cytokine production and cell proliferation by Th2 cells

To examine cytokine production, 8-day polarized $CD4^+$ Th2 cells (5×10^5/ml) and Th2 cells pretreated with PGI_2 (10 μM; Cayman Chemical) were stimulated with $OVA_{323–339}$ peptide (1 μg/ml, in the presence of 10^6/ml Thy-1-depleted spleen cells pretreated with mitomycin C) or immobilized anti-CD3 (1 μg/ml, 2C11) for 24 h, and the supernatants were harvested for measurement of IL-4, IL-5, and IFN-γ by ELISA (BD Biosciences).

Similarly, the proliferative responses of Th2 cells and Th2 cells pretreated with PGI_2 (2×10^5 cells) were assessed following stimulation with OVA peptide (1 μg/ml) or immobilized anti-CD3 (1 μg/ml). Proliferation was determined after 48 h by [^{3}H]thymidine incorporation.

Adoptive transfer of DO11.10 and OT-II Th2 cells, OVA challenge, and AHR

Eight-day polarized DO11.10 or OT-II $CD4^+$ Th2 cells were injected i.v. into BALB/c or C57BL/6 mice, respectively (10^7cells/mouse). Mice (four to six per group) were then intranasally challenged by exposure to aerosolized solutions of OVA (0.5%, grade V; Sigma-Aldrich) for 20 min/day, over 7 consecutive days. Control mice were exposed to OVA aerosols but did not receive DO11.10 Th2 cells. AHR was measured on day 7 in response to methacholine inhalation by whole-body plethysmography (Buxco). Enhanced pause (Penh) was measured after each exposure, and values taken during a 3- min sequence were averaged. Mice were killed on day 8, and bronchoalveolar lavage (BAL) fluid was collected for analysis of the level of airway inflammation. Lung tissue was collected for histological analysis or dispersed by collagenase (type IV; Sigma-Aldrich) to prepare lung mononuclear cells (LMCs) for functional studies or determination of IP mRNA expression by RT-PCR and PGI_2 biosynthesis by enzyme immunoassay (EIA) as described below. In separate experiments, $CD4^+$ Th2 cells (10^7 cells/mice) were adoptively transferred into IL-4R$\alpha^{-/-}$ or $IP^{-/-}$ mice that were then exposed to aerosolized OVA.

To examine the effect of iloprost on the level of inflammation, 4-day polarized DO11.10 $CD4^+$ Th2 cells were transferred into BALB/c recipients (10^7cells/mouse) and either left untreated or injected with iloprost (5 μg/mouse i.p.) daily for the duration of aerosol challenge as before.

Purification of PLNs and LMCs

PLNs were obtained and purified by magnetic bead sorting using MidiMACS Separator beads (Miltenyi Biotec) to prepare $CD4^+$ T cells and $CD4^+CD25^+$ Tregs (from DO11.10 mice) or $CD8^+$ T cells and B cells (from BALB/c mice). Polarized $CD4^+$ Th1 and Th2 cells were prepared as described above. $CD4^+CD25^+$ Tregs were expanded in culture for 8 days under Th2-polarizing conditions (in the presence of IL-4, IFN-γ, and the continual presence of IL-2) and the percentage of expanded Tregs expressing Foxp3 was >86.3% (as enumerated by flow cytometry following intracellular staining using a Mouse Treg Flow kit; BioLegend). Real-time PCR was used to examine the expression of the IP mRNA by naive $CD4^+$ T cells, effector $CD4^+$ Th1 and Th2 cells, and expanded Tregs. Furthermore, IP mRNA expression by $CD8^+$ T cells and B cells activated (with 2 μg/ml anti-CD3 or 10 μg/ml LPS, respectively) in the presence or absence of IL-4) was determined.

Lung tissue, obtained from naive BALB/c mice or OVA-challenged recipients of DO11.10 Th2 cells, were dispersed using collagenase (25). The resultant LMCs were separated by magnetic bead sorting (Miltenyi Biotec) into $CD45^+$ and $CD45^-$ fractions, and $CD45^-$ cells were further separated into $CD31^+$ endothelial cells and $CD31^-$ fibroblasts according to the manufacturer's instructions. IP mRNA expression was determined by real-time PCR.

Real-time RT-PCR analysis of IP mRNA expression

Using the TRIzol technique, RNA was extracted from cells and total RNA (2 μg) was then reverse transcribed using Omniscript II (Qiagen) at 37°C for 1 h. The cDNA was amplified and quantified by real-time PCR to determine IP and GAPDH (housekeeping gene) expression as previously reported (9). Equal amounts of cDNA were used in triplicate and amplified with the TaqMan master mix according to the manufacturer's instructions (Applied Biosystems). Real-time detection of PCR products was performed using the PerkinElmer Applied Biosystems Prism 7700 Sequence Detection System. 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. Threshold cycle (C_T) was measured as the cycle number at which the reporter fluorescent emission increased above a threshold level. The amount of mRNA was expressed as fold difference relative to the amount obtained from unstimulated control cells. Amplification efficiencies were validated and normalized against GAPDH. For all samples,

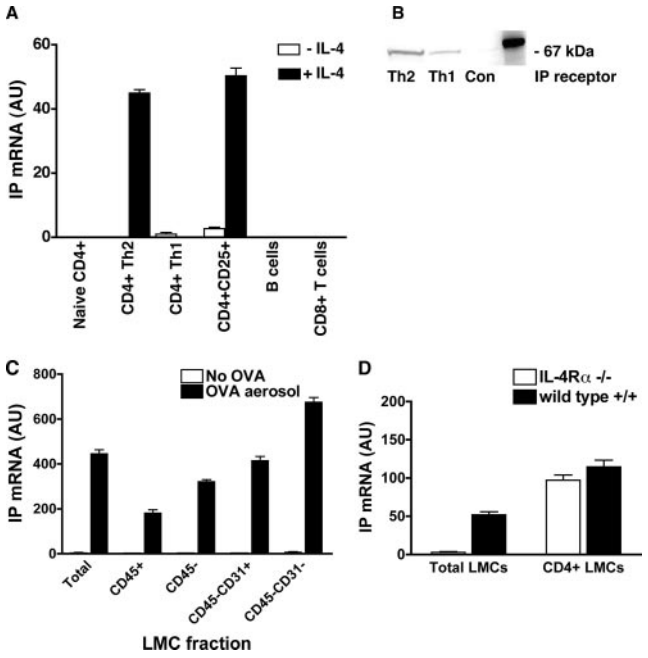

FIGURE 1. IP is expressed by $CD4^+$ Th2 cells and by lung stromal cells following allergic inflammation. *A,* Real-time RT-PCR expression of IP mRNA by naive $CD4^+$ T cells, effector $CD4^+$ Th1 and Th2 cells, $CD4^+CD25^+$ Tregs, $CD8^+$ T cells, and B cells stimulated in the presence or absence of IL-4. *B,* IP protein expression by $CD4^+$ Th2, Th1 cells, or naive unstimulated T cells using Western blot analysis. *C,* Expression of IP mRNA by LMC fractions from BALB/c mice that were injected with DO11.10 $CD4^+$ Th2 cells and exposed to OVA aerosols for 7 days, compared with control mice (no OVA aerosol). LMCs were prepared by collagenase dispersion of lung tissue and then separated by magnetic bead sorting into $CD45^+$ and $CD45^-$ cells. The $CD45^-$ cells were further purified into $CD45^-CD31^+$ and $CD45^-CD31^-$ cells. *D,* IP expression by total LMCs and lung $CD4^+$ T cells (separated by magnetic bead sorting) from OVA-challenged wild-type or IL-4R$\alpha^{-/-}$ mice. Results represent fold increase from unstimulated control or unchallenged samples. Data represent means ± SEM ($n = 3$). AU, Arbitrary units.

total RNA that was not reverse transcribed was also analyzed to determine genomic DNA contamination, which were found to be negligible.

Western immunoblot

Western blots were performed as previously described (26) using lysates prepared from DO11.10 Th1 and Th2 cells. A rabbit polyclonal anti-IP Ab (Cayman Chemical) was used to examine the expression of IP protein in these cells.

PGI_2 production

For determination of the spontaneous production (after 24 h) of PGI_2 by LMCs, levels of the stable metabolite, 6-keto-$PGF_{1\alpha}$ was measured using an EIA kit (Cayman Chemical).

Level of airway inflammation

BAL of four to six animals was pooled and eosinophil peroxidase (EPO) levels present in BAL cells were assessed by colorimetric analysis as described before (25). Cell differential percentages were determined by light microscopic evaluation of stained cytospin preparations and expressed as absolute cell numbers. Levels of cytokines IL-4, IL-5, IL-13, IFN-γ, and the chemokines TARC/CCL17 and eotaxin/CCL11 in the BAL were measured using ELISA kits (BioSource International, except IL-4 by R&D Systems).

Lung histology

Lung tissue was fixed in histochoice (Amresco) and embedded in paraffin using a Shandon Citadel tissue processor (Thermo Fisher Scientific). Microtome sections were cut at 5-μm thickness and stained with H&E using a Shandon Varistain 24-4 (Thermo Fisher Scientific).

Flow cytometry

Cells were stained and analyzed on a FACSAria (BD Biosciences) using FACSDiva software for performing three-color analysis to enumerate $CD4^+$ T cells (using GK1.5-allophycocyanin-Cy7) and OVA-specific T cells (KJ1-26-PE). The expression of CD54 (ICAM-1), CD31 (PECAM), ICAM-2 (from BD Biosciences), P-selectin glycoprotein ligand 1, and E-selectin ligand (R&D Systems) by untreated Th2 cells or Th2 cells pretreated with PGI_2 (10 μM) was determined.

Measurement of cAMP

Levels of cAMP in 8-day polarized $CD4^+$ Th1 or Th2 cells that were either untreated or treated with PGI_2 or dbcAMP were measured by EIA. Briefly, PGI_2 (10 μM), or dbcAMP (0.1 mM; Sigma-Aldrich) was added to Th1 or Th2 cells and incubated for 20 min or a different time course. Cell extraction for cAMP assay was performed by addition of 0.1 M HCl (1 ml/5 × 10^6 cells) and incubated at room temperature for 20 min. Supernatants were assayed using cAMP EIA kits (Cayman Chemical).

Chemotaxis

Polarized Th2 or Th1 cells (100 μl, 5 × 10^6/ml) in RPMI 1640 medium containing 2.5 mg/ml BSA (Sigma-Aldrich) were loaded into the top wells of a 5-μm Costar Transwell plate (24-well; Corning). CCL17/TARC (50 ng/ml; R&D Systems) was added to bottom wells of the chamber containing medium (600 μl) supplemented with BSA. PGI_2 (10, 1, or 0.1 μM; Cayman Chemical) or dbcAMP (0.1 mM; Sigma-Aldrich) was added to the cells to examine their effect on TARC-induced chemotaxis. The plate was incubated for 1 h at 37°C, and cells migrating to the bottom were counted by hemocytometer.

Statistical analysis

Comparisons were analyzed for statistical significance by the Mann-Whitney *U* test, with $p < 0.05$ being considered significant.

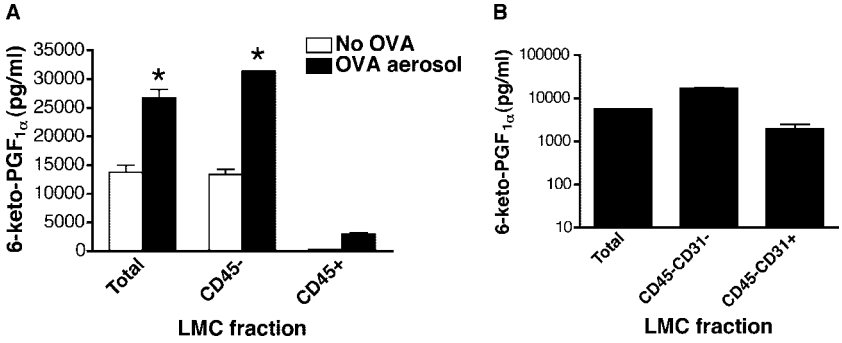

FIGURE 2. PGI_2 is predominantly produced by lung stromal cells from OVA-challenged mice. *A,* Production of PGI_2 by total LMCs, $CD45^-$, and $CD45^+$ LMC fractions. LMCs were obtained by collagenase dispersion of lung tissue from DO11.10 Th2 recipient mice that were exposed to OVA aerosols for 7 days. Control mice did not inhale OVA. LMCs were separated into different fractions by magnetic bead sorting and the spontaneous production of the stable PGI_2 metabolite 6-keto-$PGF_{1\alpha}$ was measured by EIA after 24 h. *B,* PGI_2 production by lung $CD45^-CD31^+$ endothelial cells and $CD45^-CD31^-$ fibroblasts from OVA-challenged mice. Data represent means ± SEM ($n = 3$). *, $p < 0.05$ compared with cells from unchallenged mice.

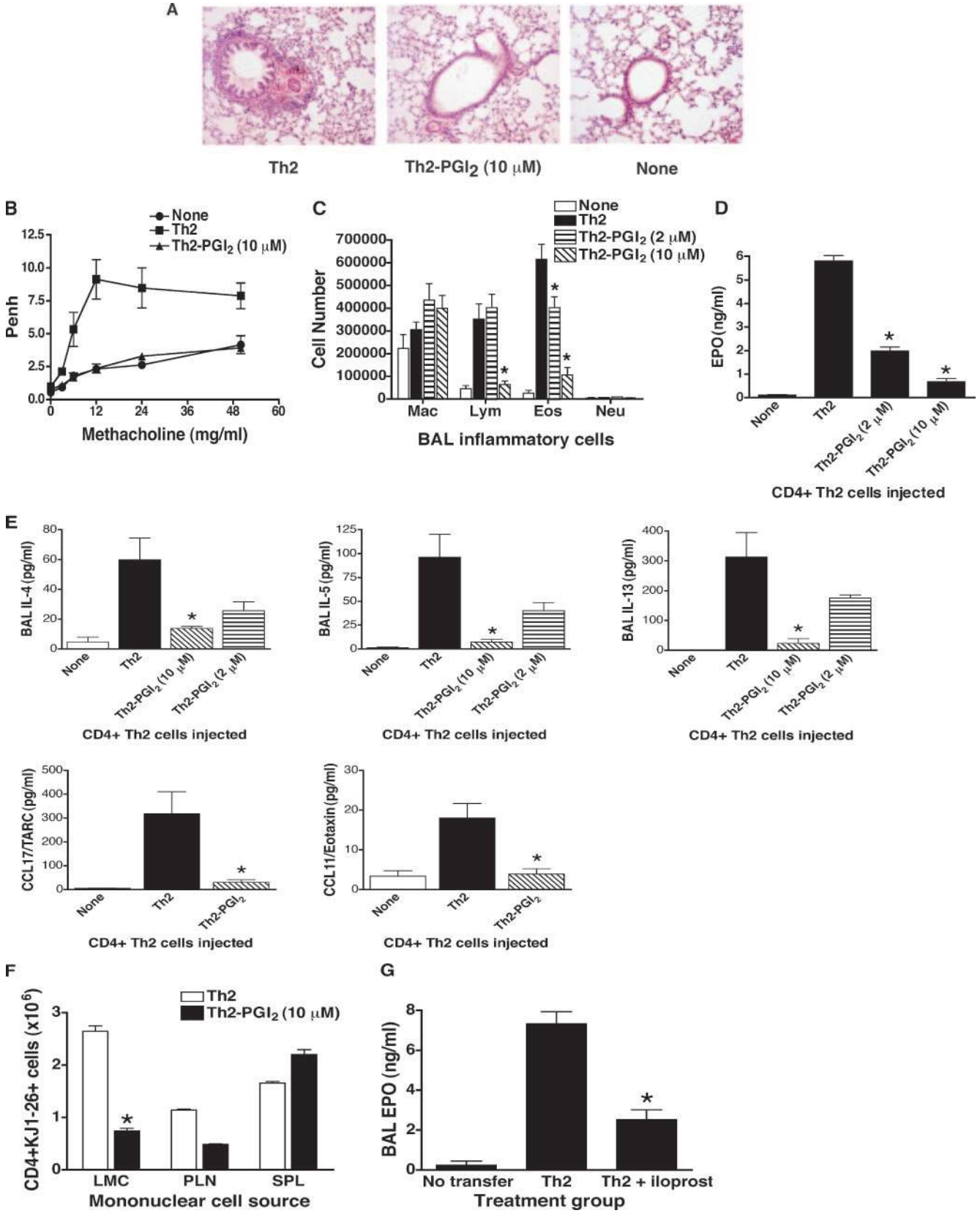

FIGURE 3. PGI$_2$ suppresses pulmonary inflammation and AHR. DO11.10 CD4$^+$ Th2 cells, either untreated (Th2) or pretreated with 2 or 10 μM PGI$_2$ (Th2-PGI$_2$), were injected into BALB/c recipient mice that were then exposed to OVA aerosols for 7 days. Control mice did not receive Th2 cells (None). *A,* Lung tissue histology stained with H&E (×25). Recipients of Th2 cells displayed peribronchial and perivascular eosinophilic inflammation that was markedly reduced in the Th2-PGI$_2$ group. *B,* Changes of Penh measurements in response to inhaled methacholine. Exaggerated increases in Penh following exposure to OVA aerosols indicate AHR. *C,* BAL fluid was collected and cell differential counts were determined by light microscopic evaluation of cytospin preparations. Results are expressed as absolute number of macrophages (Mac), lymphocytes (Lym), eosinophils (Eos), and neutrophils (Neu). *D,* The level of EPO activity in the BAL was determined by colorimetric analysis. *E,* Levels of Th2 cytokines and chemokines (TARC and eotaxin) present

Results

IP receptor is expressed by CD4$^+$ Th2 cells

To determine the role of PGI_2 in modulating immune responses, we initially assessed the expression of IP in various lymphocyte populations from PLNs. DO11.10 CD4$^+$ Th2 or Th1 cells were prepared as described in *Materials and Methods*. Th2 cells produced IL-4 (8.62 ± 1.21 ng/ml) and IL-5 (4.5 ± 0.26 ng/ml) but negligible IFN-γ (2.2 ± 0.1 ng/ml), while Th1 cells produced significant levels of IFN-γ (43.31 ± 6.8 ng/ml) but negligible IL-4 (0.72 ± 0.2 ng/ml) and IL-5 (0.21 ± 0.05 ng/ml) in response to stimulation with 1.0 μg/ml immobilized anti-CD3 (see also cytokine profile in Fig. 5). Resting CD4$^+$ T cells did not express IP mRNA. In contrast, transcripts for IP mRNA were expressed by effector CD4$^+$ Th2 cells and CD4$^+$CD25$^+$Foxp3$^+$ Tregs cultured in the presence of IL-4 and anti-IFN-γ (Fig. 1*A*). In contrast, CD4$^+$ Th1 cells, differentiated in the presence of anti-IL-4 and IL-12, yielded a weak signal. Both CD8$^+$ T cells and B cells, activated in the presence or absence of IL-4, were essentially devoid of IP mRNA. These data are key in demonstrating that the expression of IP mRNA is not simply a consequence of exposure to IL-4, but rather mirrors the Th2 polarization process (e.g., activated B cells are responsive to IL-4 and undergo rapid proliferation).

The IP mRNA expression profile was further confirmed by Western blot using a polyclonal anti-IP Ab, which showed increased expression of IP protein by Th2 cells compared with Th1 cells (Fig. 1*B*). It is difficult to determine what percentage of these cells express IP since no mAb to IP are available for use in flow cytometric analysis.

IP expression is increased in the lungs during allergic inflammation

The adoptive transfer of OVA-specific Th2 cells into naive mice to model allergic asthma has proven a powerful technique to delineate underlying mechanisms and to identify factors involved in limiting the development of allergen-induced inflammation and AHR (8, 9). Using this model, we investigated the expression of IP mRNA in stromal and inflammatory cells present in the lung tissue of mice with OVA-induced airway inflammation. RNA was isolated from total LMCs from OVA-challenged mice or control animals. RNA was also isolated from LMCs separated by magnetic bead sorting into CD45$^+$ and CD45$^-$ fractions or further separated into CD45$^-$CD31$^+$ and CD45$^-$CD31$^-$ cells. Total LMCs from control mice expressed negligible amounts of IP mRNA. In contrast, IP expression was significantly up-regulated in LMCs of OVA-challenged animals, being highly expressed by both CD45$^+$ and CD45$^-$ fractions (Fig. 1*C*). Interestingly, IP was substantially expressed by lung stromal CD45$^-$ cells that consisted of CD45$^-$CD31$^+$ endothelial cells and CD45$^-$CD31$^-$ fibroblasts (Fig. 1*C*).

To examine the role of IL-4 in promoting IP expression by LMCs, OVA-specific CD4$^+$ Th2 cells were adoptively transferred into IL-4Rα$^{-/-}$ or wild-type mice that were then exposed to OVA aerosols for 7 days. RNA was isolated from total LMCs or mononuclear cells separated by magnetic bead sorting into CD4$^+$ T cells. Interestingly, expression of IP mRNA in the lungs was dependent on the IL-4 response since there was little expression by LMCs from IL-4Rα$^{-/-}$ mice, with notable exception of the purified CD4$^+$ T cells (Fig. 1*D*). This is presumably a consequence of IP expression by the injected CD4$^+$ DO11.10 Th2 cells following recruitment and expansion in the lungs.

PGI_2 is predominantly produced by lung stromal cells during allergic inflammation

We have previously shown that PGI_2 levels in the BAL fluid of mice were markedly increased following OVA inhalation (9). In the present study, we examined the cellular source of PGI_2 in the lung tissue of OVA-challenged mice. Production of the stable PGI_2 metabolite 6-keto-$PGF_{1\alpha}$ was significantly increased by LMCs of challenged animals compared with control mice, being predominantly produced by CD45$^-$ cells. Conversely, CD45$^+$ LMCs (consisting of T cells and APCs) produced small amounts of the metabolite of PGI_2 when compared with the CD45$^-$ stromal cells (Fig. 2*A*). Further separation of CD45$^-$ lung stromal cells revealed that both CD45$^-$CD31$^+$ endothelial cells and CD45$^-$CD31$^-$ fibroblasts produce high levels of PGI_2 (Fig. 2*B*). It is important to note that unlike LMCs, neither effector CD4$^+$ Th1 nor Th2 cells produced any PGI_2 in response to in vitro stimulation with anti-CD3 (data not shown). In summary, during allergic inflammation elicited in response to OVA aerosol, PGI_2 production was markedly elevated in the lungs and the predominant cellular source appears to be CD45$^-$ lung stromal cells.

These data indicate that the production of the anti-inflammatory PGI_2 is augmented in endothelial cells and fibroblasts during airway inflammation and suggest involvement of this prostanoid in the regulation of the inflammatory response.

PGI_2 suppresses pulmonary inflammation and AHR

Because IP is preferentially expressed by Th2 cells, we examined how PGI_2 influences Th2 responses in vivo. It has been reported that the majority of injected PGI_2 disappears rapidly from the circulation because it hydrolyzes to form the inactive compound 6-keto-$PGF_{1\alpha}$ (12) and stable analogs of PGI_2 may cross-react with other prostanoid receptors (13). To circumvent the issue of short serum half-life, we pretreated effector DO11.10 CD4$^+$ Th2 cells with 10 μM PGI_2 (Th2-PGI_2). Th2-PGI_2 cells or untreated Th2 cells were then adoptively transferred into BALB/c recipients to model allergic asthma. Following exposure to OVA aerosols for 7 days, a pronounced peribronchial and perivascular eosinophilic inflammation was observed in the lung parenchyma of recipients of untreated Th2 cells (Fig. 3*A*). Moreover, these animals developed an increase in AHR (Fig. 3*B*) and airway inflammation characterized by infiltration of eosinophils and T cells into the BAL (Fig. 3*C*). In contrast, recipients of Th2-PGI_2 cells (10 μM) had a marked reduction in peribronchial and perivascular inflammation, significantly attenuated AHR, and a pronounced inhibition in the level of eosinophil and lymphocyte infiltration into the BAL in response to OVA aerosols (Fig. 3, *A–C*). Consistently, there was a marked decrease in EPO levels in the BAL (Fig. 3*D*). Treatment of Th2 cells with a lower dose of PGI_2 (2 μM) was less effective, resulting in a moderate reduction in airway eosinophils and EPO levels, but did not cause a significant decrease in the number of

in the BAL were measured by ELISA. *F,* The number of CD4$^+$KJ1-26$^+$ cells present in LMCs, PLNs, or spleen (SPL) cells following OVA challenge was determined by FACS analysis. *G,* Effect of in vivo treatment with the stable PGI_2 analog iloprost (5-μg i.p. injections daily for the duration of OVA aerosol challenge) on level of pulmonary eosinophilia elicited by adoptively transferred CD4$^+$ Th2 cells. Following OVA inhalation for 7 days, EPO levels were determined in the BAL by colorimetric analysis ($n = 4$). Data represent means ± SEM from three to six separate experiments. ∗, $p < 0.05$ compared with recipients of untreated Th2 cells.

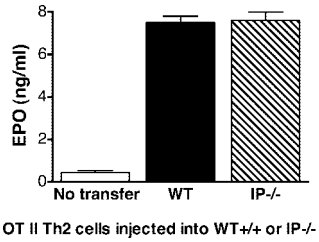

FIGURE 4. Airway inflammation in IP$^{-/-}$ mice following adoptive transfer of OT-II Th2 cells. OT-II CD4$^+$ Th2 cells were injected into either C57BL/6 or IP$^{-/-}$ recipient mice that were then exposed to OVA aerosols for 7 days. EPO levels in the BAL from wild-type (WT$^{+/+}$) and IP$^{-/-}$ recipients were determined by colorimetric analysis. Data represent means ± SEM (n = 3) and are representative of three separate experiments.

lymphocytes in the BAL (Fig. 3, *C* and *D*). Moreover, PGI$_2$ pretreatment (10 μM) significantly inhibited the levels of IL-4, IL-5, and IL-13 and the amounts of chemokines CCL17 and CCL11 present in the BAL following OVA challenge, when compared with levels in the untreated Th2 group (Fig. 3*E*). FACS analysis revealed that pretreatment with PGI$_2$ resulted in a significant decrease in the number of KJ1-26$^+$ T cells present in LMCs following OVA inhalation, but the numbers in the spleen or PLNs of these mice were essentially unaffected (Fig. 3*F*).

The suppressive effects of PGI$_2$ on lung inflammatory responses appear to be transient because the unresponsiveness displayed by Th2 cells pretreated with PGI$_2$ was reversed when mice were rested for a period of 2 wk after transfer of the cells (data not shown). Moreover, treating mice with the stable PGI$_2$ analog iloprost markedly attenuated the level of pulmonary eosinophilia elicited by adoptively transferred CD4$^+$ Th2 cells (Fig. 3*G*).

In summary, PGI$_2$ attenuates airway Th2-mediated inflammation, possibly by limiting CD4$^+$ Th2 cell recruitment to the lungs.

Airway inflammation in IP$^{-/-}$ mice injected with OT-II Th2 cells

It has been previously demonstrated that IP$^{-/-}$ mice developed augmented allergic lung inflammation (15), thus implying that PGI$_2$ plays a key role in regulating airway inflammatory responses. In this study, we determined whether this regulation was a consequence of PGI$_2$ acting on either effector CD4$^+$ Th2 cells or lung stromal cells (endothelial cells and fibroblasts). To this end, OT-II CD4$^+$ Th2 cells were injected into either C57BL/6 recipients or IP$^{-/-}$ mice and the animals were then exposed to OVA aerosols for 7 days. The data show that both wild-type (+/+) and IP$^{-/-}$ mice had a similar eosinophilic inflammatory response in the airways (Fig. 4). Because the level of inflammation elicited by IP-bearing CD4$^+$ OT-II cells was similar irrespective of whether the host expressed IP or not, this strongly suggests that the anti-inflammatory effects of PGI$_2$ arise primarily as a consequence of its action on CD4$^+$ Th2 cells.

PGI$_2$ does not affect proliferative responses, cytokine or adhesion molecule expression by Th2 cells

We examined whether the suppressive effects on airway inflammation was a consequence of PGI$_2$ influencing the polarization of

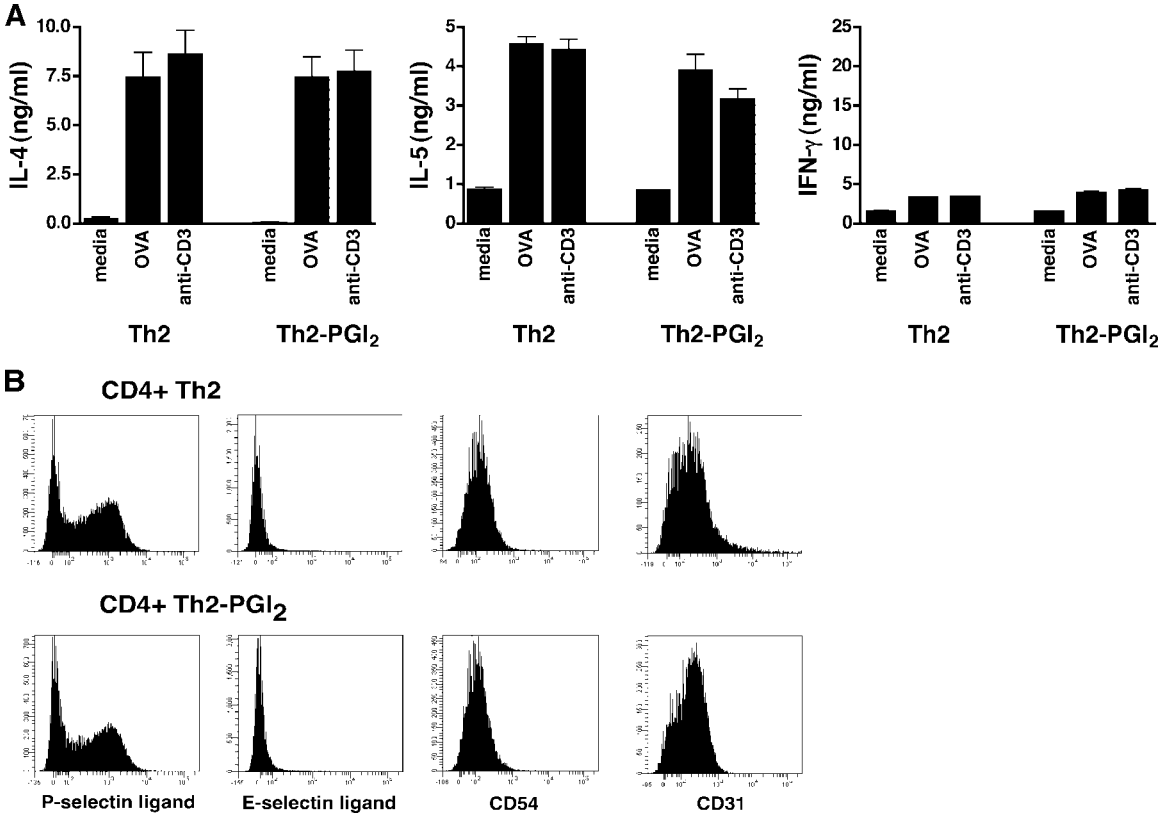

FIGURE 5. PGI$_2$ does not affect cytokine or adhesion molecule expression by Th2 cells in vitro. *A,* IL-4, IL-5, and IFN-γ production by untreated CD4$^+$ Th2 cells compared with Th2 cells pretreated with PGI$_2$ (Th2-PGI$_2$, 10 μM) was determined by ELISA following restimulation of the polarized cells with OVA$_{323–339}$ peptide (1 μg/ml) or anti-CD3 (1 μg/ml) for 24 h. *B,* FACS analysis of the expression of P-selectin ligand (glycoprotein ligand 1), E-selectin ligand, CD54 (ICAM-1), and CD31 (PECAM) by Th2-PGI$_2$ cells compared with untreated Th2 cells. Data represent means ± SEM (n = 3) and are representative of three separate experiments.

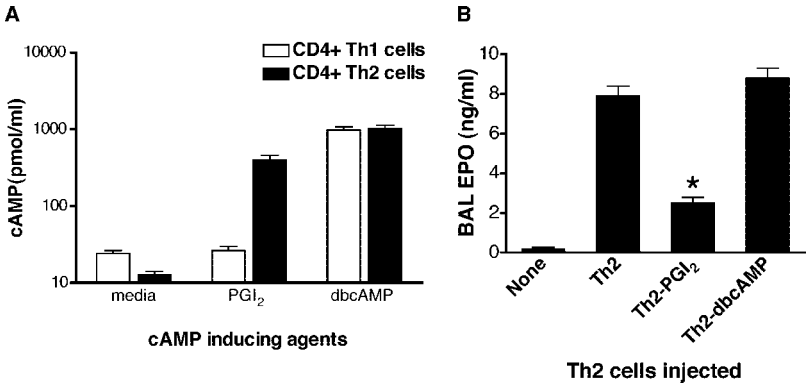

FIGURE 6. Role of cAMP in the suppression of pulmonary inflammation. *A,* Levels of cAMP in Th1 and Th2 cells pretreated with PGI_2 (Th2-PGI_2, 10 μM) or dbcAMP (Th2-dbcAMP, 0.1 mM) for 15 min were determined by EIA and compared with levels from untreated Th1 and Th2 cells (medium control). *B,* Role of cAMP in airway eosinophilic inflammation. Levels of EPO in BAL from recipients of Th2-PGI_2 (10 μM), Th2-dbcAMP (0.1 mM), or untreated Th2 cells were determined by colorimetric analysis. Data represent means ± SEM ($n = 3$) and are representative of four separate experiments. *, $p < 0.05$ compared with untreated Th2 group.

Th2 cells by inhibiting either Th2 cytokine production or cell proliferation in vitro. Th2 cells stimulated with OVA peptide or anti-CD3 produced IL-4 and IL-5 but negligible amounts of IFN-γ. PGI_2 pretreatment of Th2 cells (Th2-PGI_2) did not affect their IL-4 and IL-5 production or their level of IFN-γ, demonstrating that the cells retained their Th2 profile (Fig. 5*A*). Similarly, PGI_2 pretreatment had no effect on the Th2 cell yields or proliferative responses as assessed by [^{3}H]thymidine incorporation (data not shown).

In addition, we examined whether PGI_2 pretreatment of Th2 cells affected their expression of adhesion molecules important in their migration to the lungs. FACS analysis revealed that the expression of CD54/ICAM-1 and CD31/PECAM by Th2 cells was essentially unaffected by PGI_2 (10 μM) treatment. Similarly, PGI_2 did not affect the expression of P-selectin ligand by the Th2 cells (Fig. 5*B*). Th2 cells did not express E-selectin ligand (Fig. 5*B*) or ICAM-2 (data not shown) and this profile was not changed by pretreatment with PGI_2. Moreover, the ability of CD4$^+$ Th2 cells to bind to fibronectin was not affected by PGI_2 (data not shown). In total, these results imply that the failure of Th2 cells to infiltrate the airways following pretreatment with PGI_2 was neither a consequence of the prostanoid modulating the production of cytokines nor the expression of integrins/addressins by the Th2 cells.

Role of cAMP in the suppression of Th2-mediated pulmonary inflammation

Given that prostanoids can modulate cAMP levels (27), we assessed whether the suppression of Th2-mediated lung inflammation by PGI_2 was a consequence of elevated cAMP levels. A 30-fold increase in cAMP level was detected in the Th2 cells treated with PGI_2 compared with untreated Th2 cells (Fig. 6*A*). In contrast, PGI_2 did not induce any increase in cAMP by Th1 cells. These data support the notion that Th2 cells express functional IP

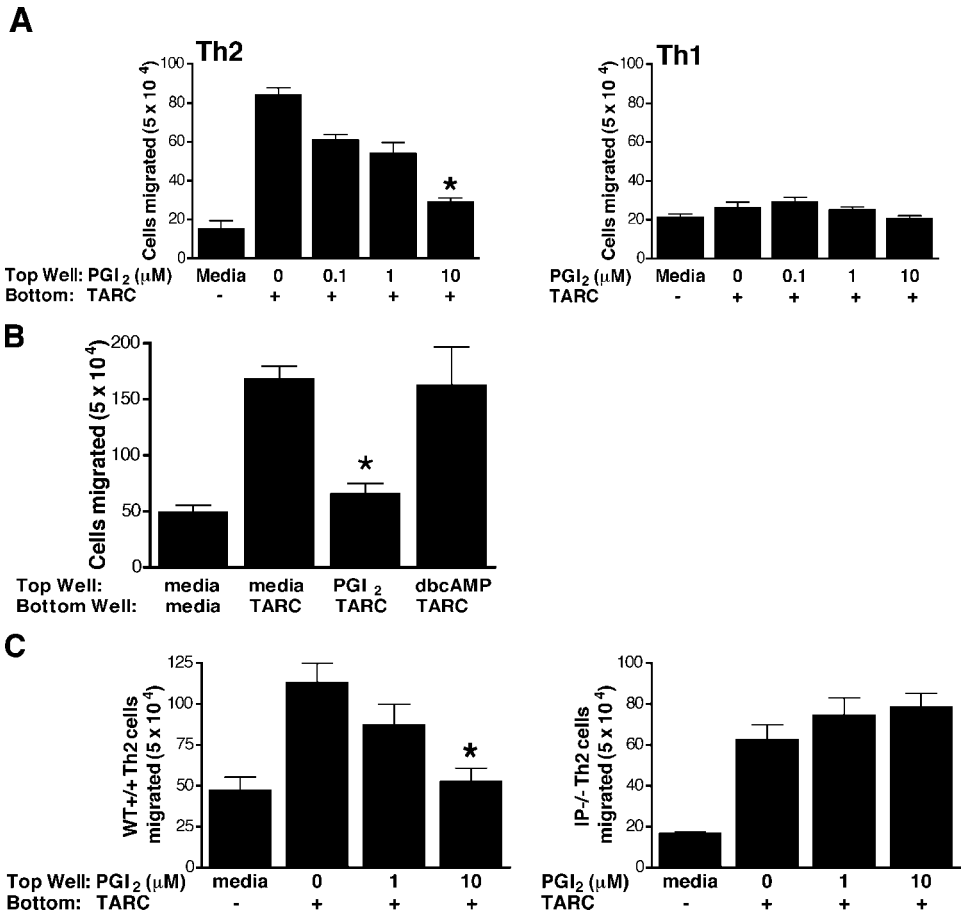

FIGURE 7. PGI_2 inhibited chemotaxis of Th2 cells that was independent on raised cAMP levels. *A,* Effect of various concentrations of PGI_2 on TARC/CCL17-induced chemotaxis of effector CD4$^+$ Th2 cells or Th1 cells in vitro. *B,* Effect of PGI_2 and dbcAMP treatment on TARC-induced chemotaxis by Th2 cells. *C,* Effect of PGI_2 on TARC-induced chemotaxis by Th2 cells derived from IP$^{-/-}$ or wild-type (WT$^{+/+}$) mice. Data represent means ± SEM ($n = 3$) and are representative of four separate experiments. *, $p < 0.05$ compared with chemotaxis of untreated Th2 cells.

receptors, whereas Th1 cells lack these receptors. As expected, dbcAMP (a cell-permeable cAMP analog that increases cellular cAMP) induced a sharp (50- to 100-fold) increase in the level of cAMP by both Th2 and Th1 cells (Fig. 6*A*). Interestingly, addition of PGI_2 and dbcAMP induced a rapid rise (within 1 min) in cAMP in the Th2 cells that remained elevated after 24 h (data not shown).

In vivo experiments demonstrated that adoptive transfer of effector Th2 cells pretreated with 0.1 mM dbcAMP (Th2-dbcAMP) for either 3 days repeatedly (data not shown) or 24 h had no effect on the airway eosinophilic inflammation in response to OVA aerosols when compared with pretreatment with 10 μM PGI_2 (Th2-PGI_2) which induced a marked reduction in inflammation (Fig. 6*B*). Collectively, these results indicate that the observed suppression of allergic inflammation evoked by PGI_2 pretreatment was independent of increased cAMP levels by the effector Th2 cells.

PGI_2 inhibited chemotaxis of Th2 cells but not Th1 cells

Given that PGI_2 dramatically reduced the number of OVA-specific T cells in the airways of challenged mice, we examined whether PGI_2 can regulate the chemotaxis of effector Th2 cells. Our observations demonstrate that PGI_2 (which did not itself induce T cell chemotaxis) was capable of inhibiting CCL17/TARC-induced chemotaxis of $CD4^+$ Th2 cells in vitro in a dose-dependent manner (Fig. 7*A*). Moreover, the prostanoid did not affect traffic of Th1 cells, which migrated very weakly to CCL17. This is consistent with previous data showing lack of IP expression by Th1 cells. In contrast to PGI_2 treatment, addition of dbcAMP did not cause a reduction in CCL17-induced chemotaxis of Th2 cells (Fig. 7*B*). In keeping with these findings, PGI_2 did not evoke a rise in cAMP level in Th1 cells, whereas dbcAMP induced a significant increase in cAMP by the cells (see Fig. 6*A*). These results thus suggest that PGI_2 inhibits Th2, but not Th1, cell migration which is independent on increased cellular cAMP. Importantly, these observations further imply that PGI_2 is acting via IP receptors that are predominately expressed by Th2 cells.

To determine the precise role of IP in regulating chemotaxis, Th2 cells were prepared using $IP^{-/-}$ mice. Fig. 7*C* shows that following addition of PGI_2, migration of wild-type Th2 cells in response to CCL17 was markedly reduced, whereas Th2 cells lacking the IP receptor migrated effectively. These results strongly implicate the IP receptor in the inhibition of Th2 cell migration by PGI_2.

Discussion

PGs are lipid mediators generated from arachidonic acid by the action of COX-1 and COX-2 enzymes and specific isomerases. It is well established that PGs play an important homeostatic function in the lung, particularly as regulators of cell proliferation, differentiation, and apoptosis (28). High levels of PG production are evident in the normal lung, with typically large amounts of PGE_2 and $PGF_{1\alpha}$ found in the BAL (9). Interestingly, PGI_2 biosynthesis in the lung differed from other prostanoids in this respect, since its production was coincident with the onset of a Th2-mediated pulmonary inflammatory reaction and was highly dependent on COX-2 (9). PGI_2 was initially discovered as a novel lipid mediator produced by vascular tissue that both inhibits platelet aggregation and is a potent vasodilator (29, 30). Consequently, this mediator regulates vascular tone and reduces pulmonary and systemic blood pressure (31). Several cells express COX-2 and prostacyclin synthase enzymes that are required for PGI_2 production, and these include endothelial cells (32), human follicular dendritic cells (33), thymic nurse cells (epithelial origin) (34), and human fibroblasts (35).

Mouse IP mRNA expression has been found in neurons, megakaryocytes, and smooth muscle cells of the aorta, coronary arteries, and pulmonary arteries (17). In addition, the receptor is highly expressed in mature thymocytes and splenic lymphocytes (17). The biological significance of PGI_2 comes from studies using $IP^{-/-}$ which confirmed that PGI_2-IP signaling in vivo is important in preventing thrombosis, inhibiting injury-induced vascular proliferation (14), regulating allergic airway responses (15), and mediating inflammatory swelling and pain (16). Endogenously produced PGI_2 can also act intracellularly via the nuclear peroxisome proliferator-activated receptor (PPAR) $\beta\delta$ (36, 37). Particularly, PGI_2 has been shown to activate constitutively expressed PPAR$\beta\delta$ in a kidney cell line to induce apoptosis (38), in uterine stroma to mediate embryo implantation (39), and in colorectal cancer (40). Interestingly, there is evidence that PPAR$\beta\delta$-mediated responses can also be dependent on cAMP signaling (41).

Studies detailing the immunomodulatory effects of PGI_2 are lacking. Our observation that IP is expressed by T cells and augmented by IL-4 formed the basis of our present study, which focuses on the mechanisms by which PGI_2 regulates Th2 immune responses and subsequent allergic lung inflammation. Our data demonstrate that although $CD4^+$ T cell do not produce PGI_2, IP receptor is preferentially expressed by $CD4^+$ effector Th2 cells, with little expression found by both naive $CD4^+$ T cells and $CD4^+$ Th1 cells. Expanded $CD4^+CD25^+Foxp3^+$ Tregs, but not activated B cells and $CD8^+$ T cells, cultured in the presence of IL-4 did express IP mRNA. The biological significance of IP receptor signaling on Tregs remains to be resolved.

To develop a better understanding of the anti-inflammatory effects of PGI_2, the cellular provenance of PGI_2 and its receptor expression during Th2-mediated pulmonary inflammation was determined. It was found that during allergic inflammation the predominant cellular source of the prostanoid in the lung was $CD45^-CD31^+$ endothelial cells and $CD45^-CD31^-$ fibroblasts. Since IL-4 was shown to be effective at inducing IP expression by $CD4^+$ T cells, it was important to determine whether IP expression was also up-regulated on structural elements in the inflamed lungs. IP mRNA expression was found to be increased in the lungs following allergic pulmonary inflammation and IP expression was not restricted to $CD4^+$ T cells since, although it was highly expressed by leukocytes ($CD45^+$), it was also expressed by stromal cells (consisting of $CD45^-CD31^+$ endothelial cells and $CD45^-CD31^-$ fibroblasts). A role for IL-4 in augmenting IP expression was evident since the adoptive transfer of $CD4^+$ Th2 cells into IL4R$\alpha^{-/-}$ mice resulted in a markedly reduced level of IP expression in LMCs. These findings suggest that during allergic inflammation PGI_2 could conceivably exert an effect at both the level of $CD4^+$ T cells and lung stromal cells. In this regard, the adoptive transfer of OT-II Th2 cells into $IP^{-/-}$ recipient mice resulted in similar levels of airway inflammation to that found in wild-type recipients. This observation would strongly suggest that the anti-inflammatory effects of PGI_2 arise primarily as a consequence of its action on $CD4^+$ Th2 cells.

Because PGI_2 production and its receptor are up-regulated during Th2-mediated pulmonary inflammation, we investigated the possibility that one action of PGI_2 was to exert an immunomodulatory effect in vivo by acting directly on $CD4^+$ Th2 cells. PGI_2 is highly unstable, with a half-life of ~30 s under physiological conditions, and is rapidly hydrolyzed into its stable metabolite 6-keto-$PGF_{1\alpha}$ (10–12). To circumvent the issue of short serum half-life, we pretreated DO11.10 $CD4^+$ Th2 cells with PGI_2 before adoptive transfer into BALB/c recipient mice. Following OVA challenge, animals that received these Th2-PGI_2 cells had a marked reduction in peribronchial and perivascular inflammation, AHR, and the

level of both T cell and eosinophil infiltration in the airways, when compared with recipients of untreated Th2 cells. Moreover, the amounts of IL-4, IL-5, and IL-13, and chemokines CCL17 and CCL11 present in the BAL, were significantly inhibited in recipients of Th2-PGI_2 cells compared with the Th2 group. PGI_2 pretreatment did not influence the polarization of Th2 cells in vitro, since cytokine production and cell proliferation was unaffected. Moreover, treating Th2 recipients with daily i.p. injections of iloprost significantly reduced the airway inflammation, possibly by inhibiting the recruitment of Th2 cells into the lungs. Iloprost is a stable analog of PGI_2 and although mainly used clinically for treatment of pulmonary arterial hypertension (42), recent evidence suggests that iloprost exhibits anti-inflammatory effects by signaling via IP (43, 44). Interestingly, the suppressive effects of PGI_2 on the Th2-dependent lung inflammatory response appear to be transient, since mice rested for a period of 2 wk after transfer of Th2-PGI_2 cells developed a strong airway eosinophilic inflammation to OVA challenge that was comparable to that of the Th2 group. This suggests that the action of PGI_2 on Th2 cells is short-lived and is not deleterious to cell survival in vivo or affects recall response of the cells.

Given that prostanoids can inhibit cellular responses by modulating cAMP levels (27), we assessed whether the suppression of Th2-mediated lung inflammation was a result of PGI_2 signaling via IP to raise cellular cAMP and subsequently inhibiting chemotaxis of $CD4^+$ Th2 cells. Our data show that PGI_2 induced a significant rise in cAMP levels in Th2 but not Th1 cells. This observation supports the notion that only Th2 cells express functional IP and that exogenous PGI_2 acted via the IP receptor alone. As expected, dbcAMP markedly raised cAMP in both Th2 and Th1 cells. Moreover, in vivo experiments demonstrated that pretreatment of effector Th2 cells with dbcAMP had no effect on the airway eosinophilic inflammation they elicited following transfer, whereas PGI_2 pretreatment suppressed the inflammatory response. Collectively, the data suggest that these anti-inflammatory effects of PGI_2 were independent of elevated cAMP. It is readily assumed that cAMP mediates the major biological activities of PGI_2 and its analogs since these agents increase cAMP in many cell types. However, cAMP-independent responses elicited by PGI_2-IP signaling have been reported by other investigators (45, 46). Additionally, cloned IP receptors can couple to multiple G protein pathways, suggesting other second messengers could mediate the action of IP agonists (47). Consequently, it is possible that a similar non-cAMP-mediated event is involved in the regulation of Th2 responses by PGI_2-IP signaling. In this context, increased cAMP levels were demonstrated not to inhibit production of Th2 cytokines (48).

Although pretreatment with PGI_2 did not affect Th2 cell polarization, it is possible that PGI_2 reduced the ability of DO11.10 Th2 cells to infiltrate the lungs in response to OVA inhalation. Quantification of clonotype-positive T cells in tissues from challenged animals revealed that the number of $CD4^+KJ1\text{-}26^+$ Th2 cells present in the lungs, but not spleen or lymph nodes, was significantly reduced in recipients of Th2-PGI_2 cells compared with that of the Th2 group. Thus, PGI_2 pretreatment of Th2 cells appears to modulate the migration of effector Th2 cells into the lungs but not spleen or lymph nodes. This may arise as a consequence of PGI_2 inhibiting the Th2 cell response to chemokines or the expression of adhesion molecules important in the migration of Th2 cells to the lungs. In this context, selectins and α_4 integrins have been implicated in T cell recruitment during pulmonary immune responses (49). Our observations revealed that the expression of ICAM-1 and PECAM by Th2 cells was not affected by PGI_2 pretreatment nor the ability of Th2 cells to bind the vascular addressin P-selectin or fibronectin. In summary, PGI_2 appears to inhibit traffic of Th2 cells into the lungs but this is unlikely to be a consequence of the prostanoid modulating the expression of addressin ligands and/or the affinity of integrins.

The selective recruitment of leukocytes into an inflammatory site is largely dependent on the spectrum of chemokines/chemokine receptors expressed at the inflammatory site. Studies in asthma have shown that CCL11/eotaxin and CCL5/RANTES are potent chemoattractants for eosinophils, whereas CCL17/TARC plays a key role in the recruitment of Th2 cells into the site of allergic inflammation (50–52). CCL17 is a CC chemokine that binds specifically to CCR4 and/or CCR8 to induce chemotaxis of $CD4^+$ Th2 cells (53–55). Our data led us to examine whether PGI_2 regulates Th2 cell migration in vitro. We found that PGI_2 did not itself induce T cell chemotaxis; however, the prostanoid was capable of inhibiting CCL17-induced chemotaxis of effector $CD4^+$ Th2 cells, and this action was independent on increased levels of cAMP. Moreover, the prostanoid did not affect traffic of Th1 cells, which migrated weakly to CCL17. This is consistent with previous data showing lack of IP expression by Th1 cells. Collectively, these results imply that the failure of Th2 cells to infiltrate the airways following pretreatment with PGI_2 appears to be a consequence of the prostanoid blocking their chemotaxis. To confirm whether IP was involved in this process, Th2 cells were prepared from $IP^{-/-}$ $CD4^+$ T cells. Our results show that migration of wild-type effector Th2 cells in response to CCL17 was markedly reduced following addition of PGI_2, whereas Th2 cells lacking IP migrated effectively to the chemokine. These results strongly implicate the IP receptor in the inhibition of Th2 cell chemotaxis by PGI_2. The data presented here are consistent with the notion that PGI_2 suppresses allergic airway inflammation by inhibiting a key process in the asthmatic response, i.e., CCL17-induced recruitment of $CD4^+$ Th2 into the lungs. We have previously suggested that the anti-inflammatory properties of PGI_2 may be a consequence of promoting IL-10 production by $CD4^+$ Th2 cells, which limits the development of the Th2 response (9). Thus, the possibility that PGI_2 also acts by abrogating CCL17-induced migration of fully differentiated effector Th2 cells provides a complimentary mechanism by which this mediator suppresses the T cell response at the site of allergic inflammation.

PGI_2 did not inhibit cell movement per se and did not reduce the binding of biotinylated TARC to $CD4^+$ Th2 cells (our unpublished observations). Our findings are consistent with the hypothesis that exposure of $CD4^+$ Th2 cells to PGI_2 blocks CCR4 and/or CCR8 signaling. The opposing actions of CCL17/CCR4 and PGI_2 are also evident in human platelets with the former promoting (56) and the latter inhibiting (30) platelet aggregation. In this context, CCL17/TARC and IP receptors are G-linked receptors. Examples of chemokine receptor cross-talk include desensitization of CCR1 by the IL-8 receptors CXCR1 and CXCR2 (57, 58), and a role for protein kinase Cε in such cross-desensitization has been proposed (59).

Collectively, our results suggest that PGI_2, which is typically generated by endothelial cells during the asthmatic inflammatory response, acts by signaling via the IP receptor to limit the influx of $CD4^+$ Th2 cells into the airways. Given that PGI_2 is known to inhibit the interaction of platelets with vascular endothelium (60), it seems likely that prostacyclin serves to fine-tune the proinflammatory effects of chemokines with respect to inflammatory cell recruitment from the blood. PGI_2-IP signaling may uncouple or desensitize CCL17 receptors on $CD4^+$ Th2 cells in the blood to prevent recruitment to the lungs from the circulation. In support of these findings, it has recently been

proposed that PGI_2 is involved in the control of T cell numbers in the germinal center (33).

In summary, these results identify the PGI_2-IP system as an important pathway for regulating recruitment of Th2 cells into the lungs and should pave the way for further studies using this class of compounds for the treatment of allergic asthma.

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Disclosures

The authors have no financial conflict of interest.

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