

Selective downregulation of prostaglandin E₂-related pathways by the T_H2 cytokine IL-13

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Background: Levels of COX-2 and downstream products, such as prostaglandin (PG) E₂, are increased in inflammatory settings after stimulation by IL-1 β , LPS, and other innate factors. Although the T_H2 cytokines IL-4 and IL-13 have been reported to decrease COX-2 levels in some cell types, neither the effect of these cytokines on other PGE₂-related pathways nor their effect in primary human airway epithelial cells has been evaluated.

Objective: To determine the impact of IL-13 on PGE₂ pathways in primary human airway epithelial cells.

Methods: Because PGE₂ has anti-inflammatory, antifibrotic, and bronchodilating properties of relevance to asthma, the effect of IL-13 (10 ng/mL for 10 days) on PGE₂ pathway elements in first-passage air-liquid interface epithelial cells from 8 endobronchial brushings (5 asthmatic subjects and 3 healthy subjects) was evaluated. mRNA and protein levels for COX-1 and COX-2, membrane-bound PGE synthase 1, 15-PG dehydrogenase, and the receptors EP2 and EP4 were quantified by means of real-time PCR and Western blotting. PGE₂ levels in the supernatants were measured by means of enzyme immunoassay.

Results: IL-13 significantly inhibited the PGE₂ synthetic pathways COX-2 and PGE synthase 1 while upregulating the PGE₂ metabolizing enzyme 15-PG dehydrogenase. These enzymatic changes associated and correlated with decreased supernatant PGE₂ levels. Significant reductions in the mRNA for EP2 (but not EP4) were also observed. Changes in the PG pathway were both time and dose dependent (n = 3).

Conclusion: These data suggest that IL-13 induces systematic modulation of proteins related to the production, catabolism, and function of PGE₂, which might alter inflammatory and immune responses at the level of the epithelium and the submucosa below.

Clinical implications: Modulation of PGE₂ pathways by IL-13 might alter inflammatory and repair processes in asthma. (J Allergy Clin Immunol 2006;117:1446-54.)

Key words: IL-13, prostaglandin E₂, COX, asthma

Prostaglandin (PG) E₂ is a potent immunomodulatory arachidonic acid metabolite generated after upregulation of COX-2 in response to innate stimuli, such as IL-1 β or LPS. After the initial production of reactive-unstable intermediates by COX-2, further metabolism by PGE synthases leads to production of PGE₂. PGE₂ activates 4 receptor subtypes (EP1-EP4) present in differing amounts-ratios depending on the cell system. PGE₂ can then be rapidly degraded by the enzyme 15-PG dehydrogenase (PGDH).¹ Innate factors, such as IL-1 β , also suppress 15-PGDH, suggesting an overall upregulation of PGE₂ and its relevant enzymes in response to innate stimuli.²

In contrast to innate factors, elements of the adaptive-T_H2 immune system (IL-4 and IL-13) have been reported to significantly decrease synovocyte and monocyte COX-2 mRNA and protein levels *in vitro*.^{3,4} Epithelial cells also express large amounts of COX-2, but nothing has yet been reported regarding the effect of T_H2 cytokines on PG pathways in these cells. However, downregulation of COX-2 in human lung epithelial cells by T_H2 cytokines could have considerable functional implications in diseases such as asthma. In a murine model of asthma, downregulation of COX-2 by means of pharmacologic or genetic manipulation increased inflammatory and physiologic responses to ovalbumin challenge.^{5,6} Although the long-term effect of COX-2 inhibition in human asthma is not known, inhalation of PGE₂ in human asthma profoundly inhibits allergen challenge responses while also preventing exercise- and aspirin-induced asthma.⁷⁻⁹

On the basis of this background, we hypothesized that the T_H2 cytokine IL-13 would systematically downregulate PGE₂-related pathways in primary human airway epithelial cells in air-liquid interface (ALI) culture, mimicking what might be occurring *in vivo* in asthma. This study confirmed the effect of T_H2 cytokines on COX-2 mRNA and protein levels reported in other cells. We additionally evaluated the effect of IL-13 on the downstream synthetic enzyme PGE synthase 1 (microsomal PGE synthase, an inducible form), the PGE₂-metabolizing enzyme 15-PGDH, and PGE₂ levels, as well as 2 of the G protein-coupled receptors for PGE₂, EP2 and EP4. These were all measured at the mRNA and protein level in the primary human airway epithelial cell culture system.

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TABLE I. Subject characteristics for studies of the effects of IL-13 (10 ng/mL) on PG

Subject	Subject group	Age (y)	Sex	FEV ₁ (%)	Corticosteroids
1	Normal	28	Female	110	None
2	Normal	32	Male	126	None
3	Normal	33	Male	88	None
4	Mild asthma	23	Male	103	None
5	Moderate asthma	41	Female	96	FP, 250 µg twice daily
6	Severe asthma	54	Male	51	FP, 500 µg twice daily
7	Severe asthma	41	Female	51	10 mg of methylprednisolone/FP, 500 µg twice daily
8	Severe asthma	45	Male	64	15 mg of prednisone/FP, 500 µg twice daily

FP, Fluticasone propionate.

Abbreviations used

ALI: Air-liquid interface
GAPDH: Glyceraldehyde-3-phosphate dehydrogenase
IL-4Rα: IL-4 receptor α
PG: Prostaglandin
15-PGDH: 15-PG dehydrogenase
STAT: Signal transducer and activator of transcription

METHODS

Bronchoscopy protocol with epithelial cell brushing

Bronchoscopy with endobronchial epithelial brushing was performed on 8 subjects (healthy control subjects = 3, patients with mild-to-moderate asthma = 2, patients with severe asthma = 3). Epithelial brushings from an additional 3 healthy subjects were similarly obtained for separate dose-response and time-course studies. Asthmatic subjects all met the American Thoracic Society criteria for asthma (Table I). Subjects with mild-to-moderate asthma had an FEV₁ of greater than 80% of predicted value while taking β-agonists alone or with low-dose inhaled corticosteroids, without a history of prior urgent health care or oral corticosteroid use. Subjects with severe asthma were patients referred to National Jewish for severe corticosteroid-dependent asthma treated with continuous oral corticosteroids or frequent bursts, with a history of frequent hospitalizations, emergency department visits, or both; evidence for ongoing severe airflow limitation; and oral or high-dose inhaled corticosteroid use.¹⁰ None of the subjects currently smoked or had a history of smoking more than 5 pack-years. Healthy control subjects had no history of respiratory disease, viral illness, or tobacco use and were taking no medications.

Bronchial brushings were performed as previously described with a standard, sterile, single-sheathed nylon cytology brush (Olympus BC9C-26101; Olympus, Tokyo, Japan).¹¹ A total of 8 brushings were performed in different subsegments visible through the bronchoscope. The protocol was approved by the institutional review board, and all subjects provided informed consent.

Primary bronchial epithelial cell ALI culture

The effect of IL-13 on the expression and activation of PGE₂-related pathways was evaluated in cultured human primary bronchial epithelial cells. Freshly harvested epithelial cells were placed directly into 10 mL of ice-cold PBS, centrifuged, washed, and resuspended in

1 mL of serum-free, hormonally supplemented bronchial epithelial growth medium (Clonetics, San Diego, Calif) containing 50 µg/mL gentamicin and 50 µg/mL amphotericin. After counting the cells, the cell concentration was brought to 3×10^4 /mL. A total of 9×10^4 cells were seeded into 60-mm tissue-culture dishes coated with rat-tail type I collagen (BD Discovery Labware, Bedford, Mass). Cells were cultured at 37°C in a 5% CO₂ environment. When the epithelial cells reached 70% to 80% confluence, they were dissociated with trypsin-EDTA and passed onto collagen-coated polyester Transwell inserts of 12 mm in diameter (pore size, 0.4 µm) at 4×10^4 cells/cm². After a week in the immersed culture condition, epithelial cells reached 100% confluence and were shifted to an ALI condition by removing all but 50 µL of the apical medium. From day 0 of ALI, cells were stimulated in triplicate every 48 hours with addition of IL-13 (10 ng/mL) or PBS (as a negative control) into the lower chamber. In the majority of studies, ALI conditions were maintained for 10 days because previous studies have demonstrated that 10 ng/mL IL-13 for 10 days is required for the mucociliary differentiation, a hallmark of IL-13 stimulation.¹¹ For dose-response studies, cells were treated every 48 hours with increasing doses of IL-13 (0.5, 1, 5, 10, and 20 ng/mL) for a total of 10 days. For time-course studies, cells were treated with IL-13 (10 ng/mL) for 1, 4, 7, 9, and 10 days. Cells were then collected in TRIzol for mRNA determination by means of real-time RT-PCR or collected in Western lysis buffer for measurement of PGE₂ pathway-related proteins. Upper and lower chamber supernatants were collected for PGE₂ levels.

Quantitative real-time RT-PCR for COX-1, COX-2, PGE synthase 1, 15-PGDH, and EP2 and EP4 receptors

PG pathway-related mRNA expression in epithelial cells was determined by means of reverse transcription, followed by real-time quantitative PCR. Total RNA was extracted from epithelial cells by using TRIzol reagent. Reverse transcription was performed as described previously.¹² The PGE₂-related pathway primers and probes, labeled with 5'-reporter dye 6-carboxy fluorescein and 3'-quencher dye 6-carboxy N, N, N',N'-tetramethyl-rhodamine, were all purchased from Applied Biosystems (Assays on Demand, Foster City, Calif; catalog nos: COX-1, Hs00277289_s1; COX-2, Hs00153133; 15-PGDH, Hs00168359; PGE synthase 1, Hs00610420; EP2, Hs00168754; EP4, Hs00168761_m1). EP1 and EP3 mRNA were not evaluated because mRNA for these receptors was not detected on a preliminary gene array (data not shown).

VIC-labeled ready-for-use human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers and probe were obtained from Applied Biosystems (Genbank accession no. NM-002046; part no.

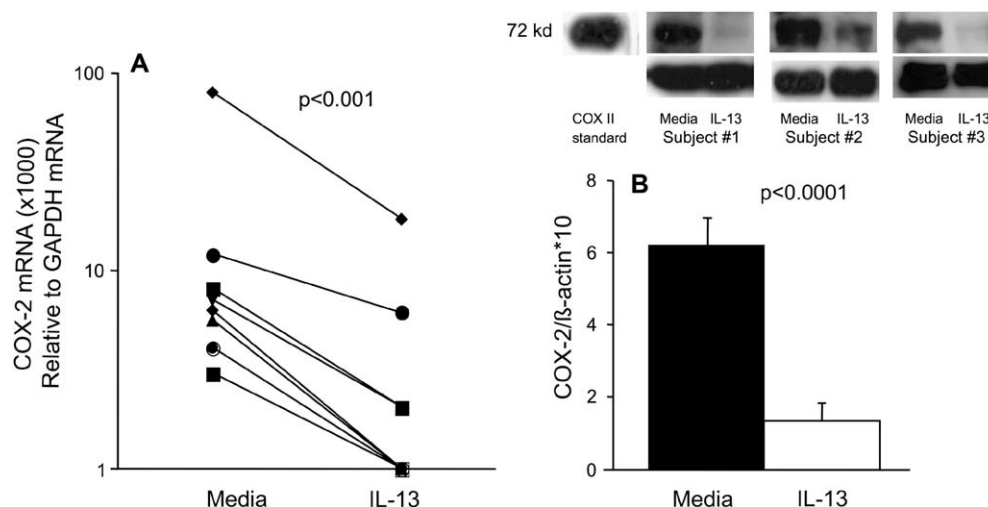


FIG 1. A, IL-13 (10 ng/mL for 10 days) significantly decreases COX-2 mRNA levels ($P < .001$) compared with levels seen in cells stimulated with media alone. B, Similarly, COX-2 protein levels are also decreased compared with media control levels ($P < .0001$). Representative Western blots are shown above the graph.

4310884E). Real-time PCR was performed on the ABI Prism 7700 sequence detection system (Applied Biosystems). The 25- μ L PCR reaction contained 25 ng of cDNA template, 900 nM of primers, and 200 nM of probe. GAPDH was evaluated by using the same PCR protocol as for the PGE₂-related pathway elements. The specificity of PCR for both PGE₂ pathways and GAPDH was verified by no signal in no template controls and no amplification in epithelial cell RNA samples. All PGE₂ pathway mRNAs of interest were indexed to GAPDH by using the following formula: $1/(2^{CT}) \times 100\%$.

Western blots for PGE₂-related pathways

Cells cultured with IL-13 (10 ng/mL) or media alone were lysed, and 25 μ g of protein per sample resolved on 10% SDS-PAGE for COX-1 and COX-2, 15-PGDH, and EP2 and EP4. Because the molecular weight of PGE synthase 1 is 14 kd, a 15% gel was used for this enzyme. The separated proteins were transferred and immunoblotted with COX-1 (1:300) and COX-2 antibodies (1:1000), 15-PGDH antibody (1:250), PGE synthase 1 antibody (1:1000), or EP2 (1:400) and EP4 antibodies (1:1000; all from Cayman Chemical, Ann Arbor, Mich). Horseradish peroxidase-conjugated secondary antibody (F[ab']₂) was added (1:5000), and the membrane was developed with ECL Western blot detection reagents and exposed to ECL Hyperfilm. The membrane was stripped and reprobed with β -actin antibody (1:1000; Sigma, St Louis, Mo) to ensure equal loading. Densitometry was performed on the blot by using the Scion Image analyzer and indexed to β -actin for analysis.

ELISAs for PGE₂

PGE₂ in the upper and lower supernatants was quantified by means of enzyme immunoassay by using reagents purchased from Cayman Chemical and performed by ELISA Tech (Aurora, Colo). Samples were analyzed without any purification, and the sensitivity of the assay was less than 15 pg/mL.

Statistics

Variables were checked for normality of distribution. 15-PGDH mRNA data were not normally distributed and were log transformed for analysis before being converted back to the linear scale for presentation. Paired t tests were performed to compare the values for

media alone versus IL-13 (10 ng/mL) stimulation over the 10-day period. The study was not designed to compare responses in subject groups because of small sample size and overall consistency in response. However, exploratory analyses were performed in which the 2 subjects with mild or moderate asthma were combined with the 3 subjects with severe asthma and compared with the 3 healthy control subjects to determine whether large differences in the asthmatic versus the normal response existed. The groups were compared at baseline with unpaired t tests. Changes in response to IL-13 were compared by means of the paired t test. Pearson correlation analysis was performed to evaluate relationships between enzymes and mediators corrected for multiple comparisons. For all comparisons, P values of less than .05 were considered significant.

RESULTS

Epithelial cell cultures from 8 subjects were available for initial analysis (Table I). Because of the small sample size, the asthmatic subjects were grouped together for comparison with the healthy control subjects. There were no differences at baseline in any PG-related pathway element between the groups, perhaps on the basis of the 1-month *ex vivo* culture system. Similarly, all pathway elements responded to IL-13 without difference across groups, except for 15-PGDH protein (see specific section for details).

PGE₂ synthetic enzymes (COX-1, COX-2, and PGE synthase 1)

After 10 days in ALI culture, COX-1 mRNA was low, and levels did not differ significantly compared with those after IL-13 stimulation (data not shown). COX-1 protein was also only minimally detectable and was not affected by IL-13. In contrast, COX-2 mRNA was present in the cells with media alone, likely because of low-level endotoxin exposure over the 1-month culture period and was markedly suppressed (approximately 80%) in all

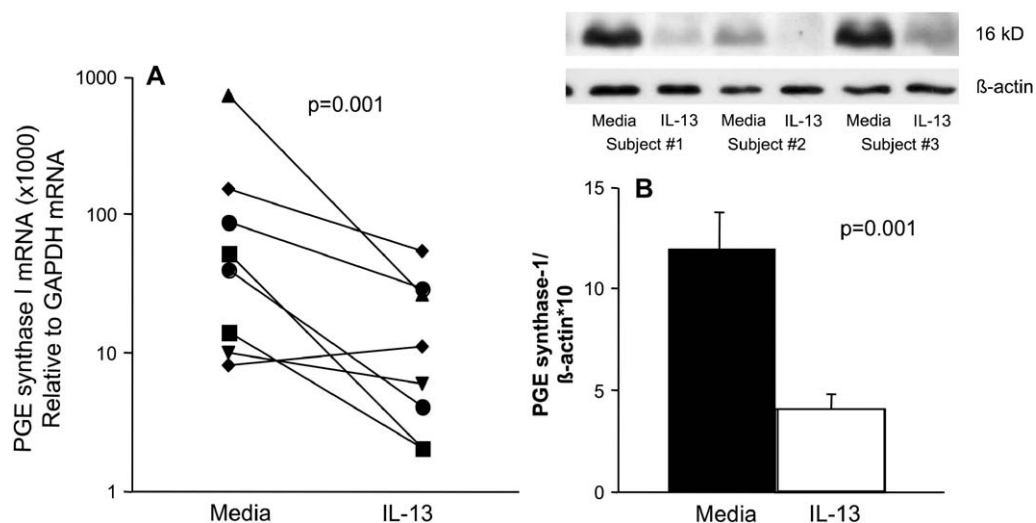


FIG 2. A, IL-13 (10 ng/mL) significantly decreases PGE synthase 1 mRNA levels ($P < .01$) compared with levels in cells stimulated with media alone. B, Similarly, PGE synthase 1 protein levels are also decreased compared with media control levels ($P < .0001$). Representative Western blots are shown above the graph.

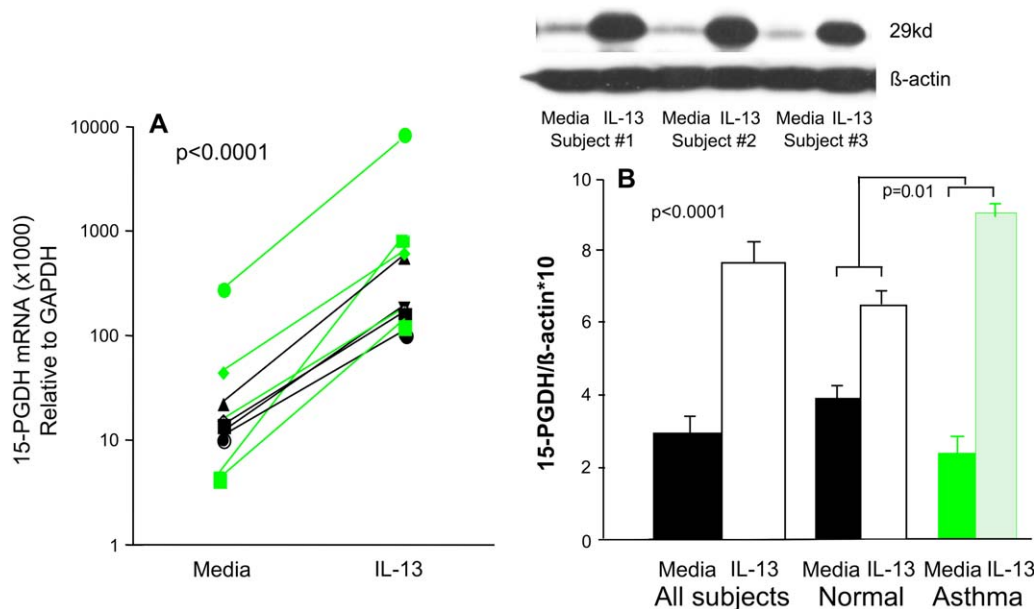


FIG 3. A, IL-13 (10 ng/mL) increases 15-PGDH mRNA levels ($P = .002$) compared with levels in cells stimulated with media alone. The mRNA responses for the asthmatic subjects are shown in green. B, 15-PGDH protein levels are increased by IL-13 ($P < .0001$). There is a greater response to IL-13 in the asthmatic subjects ($n = 5$) compared with that seen in the healthy subjects ($n = 3$, $P = .01$). Representative Western blots are shown above the graph.

8 cultures after 10 days of stimulation with IL-13 ($P < .0001$; Fig 1, A). COX-2 protein was also markedly suppressed by IL-13 in all 8 subjects, as determined by means of Western blotting ($P = .0001$; Fig 1, B). In several cases it was nearly undetectable after IL-13 exposure. There were no differences across subject groups.

The downstream synthetic enzyme PGE synthase 1 was also suppressed at the mRNA ($P = .001$) and protein levels ($P = .0001$) by IL-13 compared with media alone (Fig 2), without differences between asthmatic and healthy subjects. There was a strong correlation of changes of COX-2 protein with changes of PGE synthase

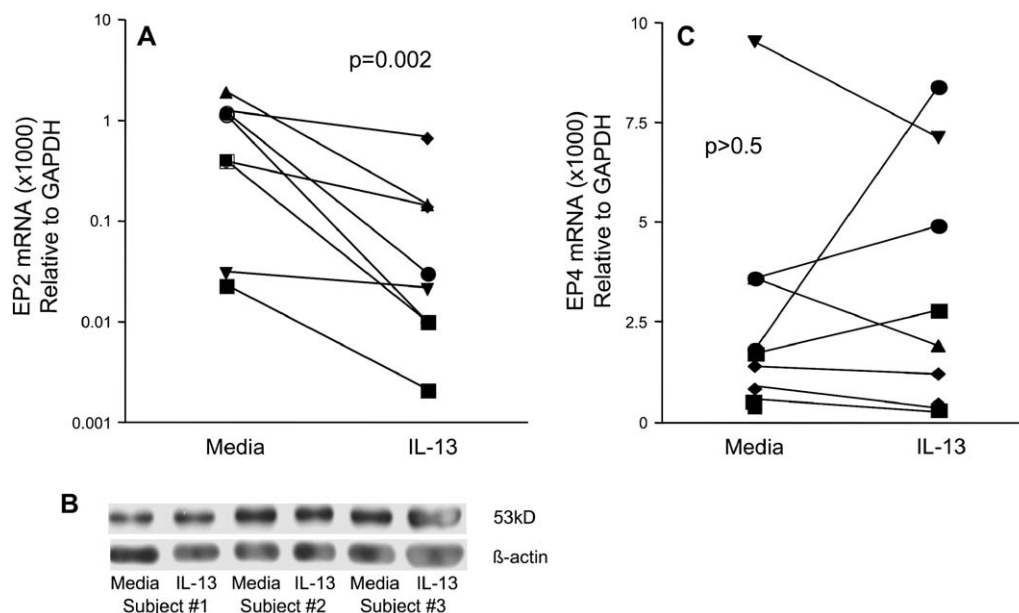


FIG 4. A, IL-13 (10 ng/mL) significantly decreases mRNA levels for the EP2 receptor ($P < .002$) compared with levels in cells stimulated with media alone. B, In contrast, EP2 protein does not differ from the media control levels ($P < .0001$). Representative Western blots are shown below the mRNA graph. C, IL-13 (10 ng/mL) has no significant effect on the EP4 receptor compared with the effect seen in cells stimulated with media alone.

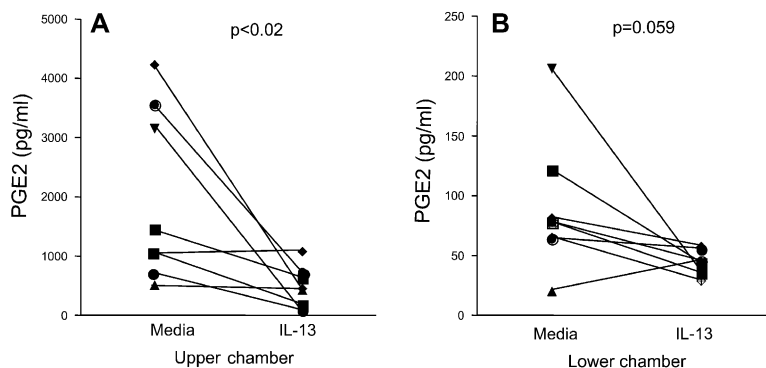


FIG 5. A, IL-13 (10 ng/mL) significantly decreases PGE₂ levels in the upper chamber supernatants compared with media control levels ($P < .02$). B, IL-13 tends to decrease PGE₂ levels in the lower chamber supernatants compared with media control levels ($P < .06$).

1 protein in response to IL-13 ($r^2 = 0.70$, $P = .02$), but no correlations were seen at the mRNA level.

The PGE₂ metabolizing enzyme 15-PGDH

In contrast to the suppression of the synthetic enzymes by IL-13, 10 days of IL-13 stimulation markedly enhanced both mRNA (nearly 30-fold, $P < .0001$) and protein ($P = .002$) levels for this enzyme compared with those seen in media alone (Fig 3). Although there were no differences in baseline 15-PGDH protein or mRNA levels ($P = .28$ and $P = .63$, respectively), the increase in response to IL-13 in the asthmatic subjects was markedly greater than that seen in the healthy control subjects ($P = .01$; Fig 3, B). There was no difference in the change in mRNA between the groups (Fig 3, A).

The changes in 15-PGDH mRNA levels were inversely correlated with the changes in COX-2 mRNA levels ($r^2 = 0.46$, $P = .0008$), suggesting overlap in the regulatory mechanisms. However, no such negative correlations were observed between COX-2 (or PGE synthase 1) and 15-PGDH protein levels.

EP2 and EP4 receptors

Similar to the effects on the PG synthetic enzymes, IL-13 decreased mRNA levels for EP2 ($P = .002$, $n = 7$; Fig 4, A). However, after 10 days of stimulation with IL-13, there were no consistent effects on EP2 at the protein level (Fig 4, B). In contrast to any other pathway elements measured, EP4 mRNA levels were not consistently changed by IL-13 in the small sample size evaluated

(Fig 4, C). The EP4 antibodies used and available (2 antibodies, both from Cayman) did not elicit specific bands at the appropriate molecular weight to provide interpretable results.

PGE₂ levels

PGE₂ levels were measured in both the upper and lower supernatants. PGE₂ levels were substantially higher in the upper supernatants than in the lower supernatants (10-fold higher). After IL-13 exposure, the final volume in the upper chamber was often less than that of its paired control. Despite the lower volume in the IL-13-stimulated upper chambers, PGE₂ levels were also less (mean difference, -1160 ± 483 pg/mL; $P < .02$) than those seen in the cultures stimulated with media alone (Fig 5, A). In the lower chamber fluid a similar trend with respect to IL-13 stimulation was observed ($P = .0592$; Fig 5, B). There were no differences in response by subject group. The changes in PGE₂ levels in the upper chamber were significantly correlated with the changes in COX-2 mRNA levels ($r^2 = 0.82$, $P = .0045$) and marginally with the changes in COX-2 protein levels ($r^2 = 0.45$, $P = .10$). The changes in PGE₂ levels in the upper chamber were also marginally correlated with the changes in 15-PGDH protein and mRNA levels ($r^2 = 0.47$ and 0.43 , respectively; $P = .08$ for both). There were no correlations of PGE₂ levels with mRNA or protein levels for PGE synthase 1.

Dose response and time course

Three separate dose responses and time courses were generated from 3 healthy subjects to confirm that the 10 ng/mL dose for 10 days produced the optimal degree of effects on the PG-related pathways of interest (Table II). Representative Western blots, mean mRNA, and PGE₂ levels are shown (Fig 6). PGE₂ levels in the upper chamber were progressively suppressed by increasing doses of IL-13. COX-2 protein and mRNA levels were marginally increased by low doses of IL-13 but markedly suppressed by 5 to 20 ng/mL IL-13. PGE synthase protein and mRNA levels were progressively suppressed from 0.5/1 to 20 ng/mL. 15-PGDH protein levels increased maximally at 20 ng/mL, whereas 15-PGDH mRNA levels maximally increased at 10 ng/mL IL-13. EP2 mRNA levels decreased maximally at 20 ng/mL.

The effect of IL-13 on both mRNA and protein occurred rapidly for all PG pathway elements and was marginally progressive for all elements except 15-PGDH mRNA level, which peaked at 7 days, whereas the protein level peaked at 10 days. In contrast, PGE₂ levels were suppressed progressively over the 10 days of the time course.

DISCUSSION

The data reported here are the first systematic evaluation of the effects of a T_H2 cytokine, specifically IL-13, on multiple PGE₂-related pathways and in primary human

TABLE II. Dose-response and time-course subjects

Subject	Subject group	Age (y)	Sex	FEV ₁ (%)	Corticosteroids
I	Normal	32	Female	95	None
II	Normal	38	Male	108	None
III	Normal	22	Female	111	None

airway epithelial cells. The results show a remarkably consistent, time- and dose-dependent selective inhibitory effect of IL-13 on PGE₂ pathways in 8 different primary human airway epithelial cell lines. In addition to showing that IL-13 decreases COX-2 levels (replicating results in other cell types), we show that IL-13 significantly decreases the inducible PGE synthase 1 enzyme, while markedly upregulating 15-PGDH, the enzyme preferentially metabolizing PGE₂. These changes lead to (and correlate with) significant decreases in PGE₂ levels, particularly in the upper (airway luminal) supernatants in the presence of IL-13 compared with levels seen with media alone. Interestingly, there were mixed effects on the EP receptors, with EP2 being downregulated at the mRNA (but not protein) level. There were no consistent effects on EP4 receptor mRNA expression (although detectable levels were present), whereas the protein measurements were unreliable. In toto, these findings suggest that IL-13 profoundly and selectively decreases the activity of PGE₂-related pathways in human airway epithelial cells, with potential consequences for immune regulation and host defense. Although this overview of the effects of IL-13 on PG-related pathway elements was not designed to compare responses in asthmatic versus normal epithelial cells due to the small sample sizes, the finding of a difference in the effects of IL-13 on 15-PGDH levels in asthma suggest further studies in larger subject groups should be pursued.

These consistent and correlating pathway effects implicate a similar mechanism of action behind the modulating effects. Airway epithelial cells express and respond to IL-4R α stimulation, and it is likely that the IL-4R α pathway is involved. Although IL-4R α is often thought of as acting through the signal transducer and activator of transcription (STAT) 6 pathway, several factors argue against participation of this pathway. STAT6 positively activates gene transcription, but 3 of the pathways studied here are negatively regulated by IL-13. Although involvement of STAT6 activation in the increased 15-PGDH mRNA levels cannot be ruled out, the 15-PGDH promoter does not contain a known STAT6 binding element. The transmembrane portion of IL-4R α does, however, contain an I4R (insulin growth factor response element) region with both phosphoinositol 3-kinase and extracellular signal-regulated kinase sites.¹³ Phosphoinositol 3-kinase pathway activation has also been proposed as a mechanism by which IL-13 can lower levels of COX-2 mRNA in macrophages through a destabilizing effect on mRNA.¹⁴ Studies to address the overall mechanisms of activation or suppression of these pathways (transcriptional or

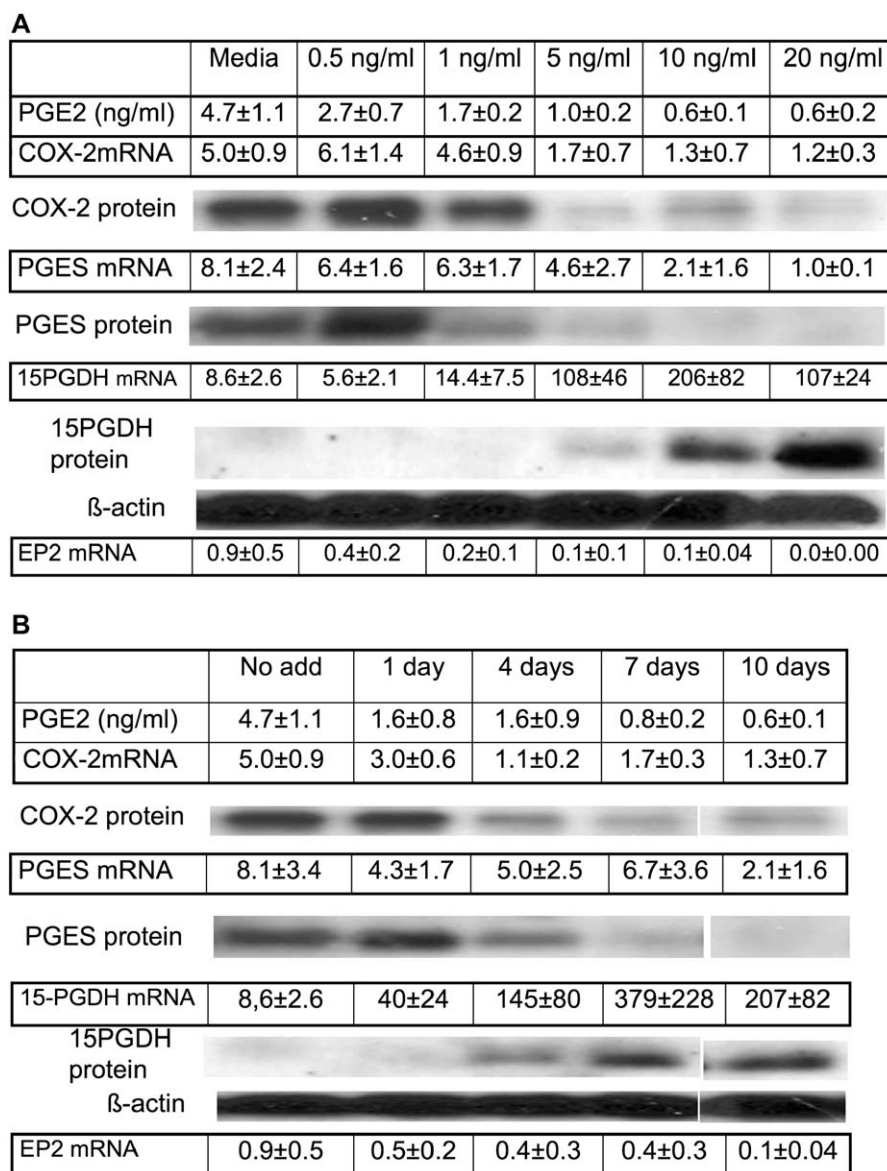


FIG 6. A, Dose response: IL-13 (0.5-20 ng/mL) was added to the cultures and PG pathway elements analyzed at day 10. **B**, Time course: IL-13 (10 ng/mL) was added to the cultures from 1 to 10 days before PG pathway analysis. Representative Western blots, mRNA $\times 1000$ (relative to GAPDH), and PGE₂ levels in the upper chambers (in nanograms per milliliter) are presented (both as means $\pm$ SEM).

posttranscriptional control and signaling elements involved) in human airway epithelial cells will require additional experiments.

The results reported here are the first to demonstrate the effect of T_H2 cytokines on expression of the PG metabolizing enzyme 15-PGDH in human airway cells. Although considerable data exist on enzymes responsible for synthesizing PGs, much less is known about their metabolism, especially in the airways. 15-PGDH levels have been shown to decrease in response to IL-1 β , but its expression has been primarily studied in relation to uterine function, with upregulation of 15-PGDH during the luteal phase of menstruation and before parturition

and subsequent downregulation believed to contribute to their onset.^{2,15} Similar to the results reported here for IL-13, progesterone has opposing effects on COX-2 and 15-PGDH, suppressing COX-2 and increasing 15-PGDH.¹⁶ Whether overlap exists in the modulatory roles of IL-13 and progesterone on PGE₂-related pathways should be studied further.¹⁷

These studies are also the first to address the effect of IL-13 on membrane-associated PGE synthase 1 and EP receptors. Very little is known regarding the regulation of these factors. PGE synthase 1 has been reported to be upregulated in response to IL-1 β , but in that study of chondrocytes from patients with osteoarthritis, no effect

of the T_H2 cytokine IL-4 was observed.¹⁸ Interestingly, a study of murine macrophages found that LPS enhanced the expression of EP2 while downregulating EP4.¹⁹ The authors suggested that innate stimuli in mice were able to preferentially activate PG-related pathways in macrophages, directing the process toward EP2 activation. The studies reported here confirm that IL-13 has opposing effects on PGE₂ pathways compared with innate stimuli. In contrast to innate stimuli, IL-13 consistently suppresses PGE₂ synthetic pathways, while also strongly suppressing mRNA for EP2. One implication for these opposing effects is that T_H2 cytokines play an important counter-regulatory role in the setting of innate inflammation, perhaps suggesting that residual PGE₂ preferentially activates the EP4 receptor. Changes in the balance of expression of EP2 and EP4 (alone) in response to IL-13 could induce differing effects on epithelial cells by nature of the downstream pathways activated.²⁰

Recent studies in the lungs have suggested that PGE₂ plays an important modulatory role in inflammatory mediator production and fibrosis.²¹⁻²³ The production of PGE₂ in response to innate stimuli might be part of a positive feedback loop to "appropriately" sustain the initial inflammatory response to these factors and limit ensuing chronic inflammation and fibrosis.²⁴ Therefore background suppression of PGE₂ by IL-13 in T_H2 -associated diseases such as asthma could alter the response to inflammatory stimuli seen during a viral (or bacterial) infection in which COX-2 would normally be upregulated. This suppression of PGE₂ by IL-13 could then contribute to an inadequate or subacute innate immune response, as has been reported for asthmatic patients during viral infections.^{25,26} PGE₂ also appears to be necessary for wound healing and the prevention of fibrosis in the lung, suggesting that the negative effect of IL-13 on PGE₂-related pathways might explain some of the profibrotic effects of T_H2 cytokines.^{27,28}

Allergic diseases, such as asthma, are the diseases most commonly associated with T_H2 inflammation. In mouse models of allergic inflammation, inhibition of COX-2 markedly enhances airway inflammation and hyperresponsiveness after allergen challenge.^{5,29} However, there are also intriguing data in human asthma to implicate substantial dysregulation of COX-2 pathways, most obviously in aspirin-sensitive asthma. COX-2 and PGE₂ levels have been reported to be downregulated in nasal polyps and airway fibroblasts from these patients, but the mechanisms for this downregulation are not known.^{30,31} Ingestion of nonspecific COX inhibitors in these patients induces a rapid and often severe asthmatic reaction that can be prevented by pretreatment with PGE₂ or its analogues.⁹ Interestingly, COX-2 inhibitors do not stimulate these reactions, perhaps because of the background suppression of COX-2 by T_H2 stimuli.³² 15-PGDH levels have not been evaluated in aspirin-sensitive asthma, but if COX-2 and 15-PGDH are coordinately regulated, as suggested by these data, it is likely that 15-PGDH levels are increased. This combination of events could lead to marked suppression of PGE₂ in the airways.

Perhaps the most interesting data regarding PGE₂ in human asthma is the ability of inhaled PGE₂ to almost completely eliminate the late asthmatic reaction when it is inhaled just before allergen challenge.^{7,33} The effect on late-phase bronchoconstriction in human asthma appears to be greater than that reported for most other inhibitory agents (leukotriene receptor antagonists, theophylline, and phosphodiesterase 4 inhibitors) and on a par with that of inhaled corticosteroids.^{34,35} This effect is lost if PGE₂ is given too early before allergen.³⁶ Given the ability of an upregulated 15-PGDH to rapidly metabolize PGE₂, these issues of timing are perhaps not surprising.

In summary, the results reported here demonstrate a systematic and highly consistent inhibitory effect of IL-13 on PGE₂-related pathways in primary human airway epithelial cells. Importantly, these studies are also the first reports of the presence of the PG metabolizing enzyme 15-PGDH in primary human airway epithelial cells and its upregulation by IL-13. Although the mechanisms for these effects require further investigation, the precise targeting of these pathways by IL-13 suggests that these effects play important roles in T_H2 -mediated diseases. Further study is necessary to detail not only the mechanisms for these responses but also the clinical implications for these changes in diseases such as asthma.

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