

Increased prostaglandin E₂ concentrations and cyclooxygenase-2 expression in asthmatic subjects with sputum eosinophilia

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Background: Prostaglandin E₂ (PGE₂) is known to be produced within human airways, but it is not clear whether in airway diseases it can play a deleterious or a beneficial role. Recently it has been reported that PGE₂ can enhance eosinophil survival in vitro.

Objective: To evaluate whether the concentrations of PGE₂ in asthmatic airways correlate with the number of eosinophils and can be responsible for eosinophil-enhanced survival and to identify the cyclooxygenase isoform contributing to the synthesis of PGE₂ by cells present in asthmatic airways.

Methods: Reversed-phase high-performance liquid chromatography and/or specific radioimmunoassay was used to measure PGE₂ concentrations in induced sputum supernatants from 14 control and 30 asthmatic subjects. Correlations between concentrations of PGE₂ and the number of eosinophils in induced sputum were evaluated. Expression of cyclooxygenase-2 (COX-2) in induced sputum cells was determined by immunocytochemistry, and the effect of COX-2 inhibition on PGE₂ production was evaluated with the use of radiolabeled arachidonic acid. The effects on eosinophil apoptosis by PGE₂ or induced sputum supernatants were studied by using peripheral blood eosinophils obtained by negative immunomagnetic selection. **Results:** PGE₂ concentrations resulted in elevated samples from asthmatic subjects and directly correlated with the percentage of eosinophils and the concentrations of eosinophilic cationic protein. Immunostaining for COX-2 showed enhanced expression in macrophages of asthmatic subjects when compared with control subjects, and the use of a specific COX-2 inhibitor provided evidence that PGE₂ synthesis was the result of COX-2 enzymatic activity in asthma-induced sputum cells. Supernatant from induced sputum of asthmatic subjects with high eosinophil counts caused a decreased apoptosis of peripheral blood eosinophils when compared with control subjects, and immunoprecipitation of PGE₂ significantly reverted this phenomenon, suggesting that PGE₂ was present in biologically relevant concentrations in induced sputum.

Conclusions: The results obtained suggest that COX-2 expression in alveolar macrophages from asthmatic subjects may contribute to enhanced eosinophil survival through an increased PGE₂ production. (*J Allergy Clin Immunol* 2003;112:709-16.)

Key words: Alveolar macrophages, apoptosis, inflammation, granulocyte-macrophage colony stimulating factor, lipopolysaccharides

Among the mediators involved in the development of airway diseases, an important role is played by arachidonic acid (AA) metabolites, such as prostaglandins, the family of metabolites resulting from enzymes possessing cyclooxygenase activity.¹ In particular, prostaglandin E₂ (PGE₂) has been shown to have potent biological activities. PGE₂ synthesis can be induced by cross-linking of IgG, IgA, and IgE receptors² and by exposure to interleukin (IL)-1, tumor necrosis factor- α , lipopolysaccharides (LPSs), components of the complement cascade, and IL-6. Synthesis of PGE₂ takes place in several different cellular types: epithelial cells, follicular dendritic cells, fibroblasts, and monocytes,³ but alveolar macrophages certainly represent a major source of PGE₂, in particular after expression of the inducible form of cyclooxygenase, namely, COX-2.⁴

The production of PGE₂ within human airways is well known and documented, but it is not clear whether PGE₂ synthesis in airway diseases can play a deleterious or a beneficial role. PGE₂ has bronchoprotective effects on allergen- and aspirin-induced asthma,^{5,6} but it also exerts potent proinflammatory activities and can cause airway smooth muscle contraction that can contribute to bronchospasm.⁷ A recent study has shown an inverse correlation between eosinophilic airway inflammation and PGE₂ concentration in induced sputum from asthmatic subjects, giving evidence of the potential for an anti-inflammatory role of PGE₂⁸; on the other hand, a recent report has suggested that PGE₂ may enhance eosinophil survival in vitro,⁹ providing a potential contribution to the proinflammatory activity of these cells in chronic asthma.

Biologically active prostaglandins can be generated by two different enzyme isoforms: COX-1 and COX-2. COX-1 can be detected in most tissue under normal conditions and is thought to be an enzyme mainly involved in homeostatic regulation, whereas COX-2 is undetectable in most mammalian tissue but its expression can

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Abbreviations used

- AA: Arachidonic acid
 COX-2: Cyclooxygenase-2
 ECP: Eosinophilic cationic protein
 LPS: Lipopolysaccharides
 PGE₂: Prostaglandin E₂

be significantly induced by proinflammatory stimuli in vitro, including cytokines, growth factors, tumor promoters, hormones, and bacterial endotoxin.¹⁰ COX-2 expression is proved to be increased at the sites of inflammation and COX-2 expression is reported in airway cells from patients with asthma and chronic obstructive pulmonary disease.³

Over the last few years, induced sputum has been widely used because it provides several advantages over different airways sampling techniques: It is reliable and safe and can be used repeatedly in patients with asthma. In this study, we have evaluated the concentration of the cyclooxygenase metabolite PGE₂, as well as the concentration of eosinophilic cationic protein (ECP) as a marker of eosinophilic inflammation, in supernatant fluids of induced sputum obtained from control and asthmatic subjects. Furthermore, we have used immunocytochemistry to determine COX-2 expression in cells obtained from induced sputum, and we have tested the effect of induced sputum supernatants on eosinophil apoptosis. The results obtained have proved that COX-2 expression is enhanced in asthmatic subjects and that PGE₂, leading to enhanced survival of eosinophils within human airways, can also contribute to the overall evolution of the inflammatory response in asthma.

METHODS**Patients**

The study included 14 control subjects and 30 patients with asthma. Control subjects (age median, 31; 25th to 75th percentiles, and 29 to 37 years) never had asthma, allergic diseases, or chronic bronchitis. All the subjects were lifelong nonsmokers, and their pulmonary functions were within the normal range.

Asthmatic subjects (median age, 38; 29 to 55 years) were diagnosed on the basis of criteria previously described in detail,¹¹ and they had objective evidence of variable airflow obstruction as indicated by one or more of the following: methacholine airway hyperresponsiveness (PC₂₀ <8 mg/mL); an improvement in FEV₁ of >15% 10 minutes after 200 g of albuterol; a maximum morning to evening variability in peak expiratory flow (PEF) measured twice daily over 14 days of >20%. None of the subjects was a current or previous smoker. Patients who had undergone a severe exacerbation of asthma requiring hospitalization during the month previous to the beginning of the study were excluded. Inhaled or oral corticosteroids were suspended at least 2 months before the beginning of the study, nedocromil sodium or cromoglycate at least 2 weeks before, and theophylline was not assumed later than 48 hours before induced sputum collection. None of the subjects had received a prior or concomitant treatment with COX-2 inhibitors.

Atopy was assessed by means of skin prick tests for common allergens.

None of the subjects had a bronchial or respiratory tract infection during the month previous to the study.

The appropriate ethics committee approved the study, and the patients gave informed consent for participation.

Induced sputum production and processing

Each subject was submitted to spirometry before the beginning of the procedure. Induced sputum production and processing were carried out according to Fahy et al,^{12,13} as previously described.^{14,15} Briefly, patients in a fasting condition were exposed for 20 minutes to an aerosol of 3% hypertonic saline solution early in the morning, and sputum was collected into previously weighted 50-mL sterile ampules. A sample of saliva was collected from all subjects before the sputum induction procedure.

The volume of the induced sputum and saliva was previously determined, and an equal volume of dithiothreitol (0.1% in saline; Sigma Chemical Co, St Louis, Mo) was added. After homogenization, sputum and saliva were centrifuged at 800g for 10 minutes to separate the supernatants from the cell pellet. The supernatants were then aspirated and frozen at -20°C in separate aliquots for the subsequent biochemical analysis.

The cell pellet was resuspended in saline solution, and cell viability was assessed by Trypan blue exclusion. The cells obtained from induced sputum were then cytocentrifuged (Cytospin 2, Shandon Instruments, Runcorn, UK) and stained with Diff-Quick (Merz-Dade, Duding, CH) for differential cell counting. The slides were read blindly by two independent investigators, who counted at least 400 cells per slide. The number of squamous cells was subtracted from the total cell count to get the corrected cell number. The differential cell counts were then expressed in percentage terms of corrected cell number.

The cytospins for immunocytochemistry were prepared on aptex-coated (3-aminopropyltriethoxysilane) slides by adding 100 µL of cell suspension (~5 × 10⁵ cells/mL) into Shandon II cytocentrifuge cups and centrifuging at 180g for 5 minutes. The air-dry slides were fixed in PLP (periodate-lysine-paraformaldehyde) for 30 minutes and in 15% sucrose in DPBS for 30 minutes. Those slides were stored at -80°C until use for immunocytochemical staining.

Analysis of prostanoids in induced sputum supernatant

The aliquots from the supernatants recovered from induced sputum of control and asthma subjects were thawed, and PGE₂ was extracted according to Powell et al.¹⁶ The recovery was evaluated by using standard PGE₂, which was added to samples prepared and extracted in parallel to the induced sputum samples. Subsequently, the extracted samples were analyzed for PGE₂ concentrations, after conversion to the corresponding methyl oximate derivative, using a commercially available radioimmunoassay (RIA) (Amersham International plc, Little Chalfont, Buckinghamshire, UK) performed according to the manufacturer's directions. The limit of detection was 50 pg/mL, and the cross-reactivity for prostaglandin D₂, prostaglandin F_{2α}, 6- keto-prostaglandin E₁, 13,14 DiHydro-15-keto prostaglandin F, 6- keto-prostaglandin F_{1α}, and arachidonic acid was <.001. Results are expressed as picograms per milliliter of induced sputum supernatant.

Analysis of ECP

The supernatants recovered from induced sputum of control and asthmatic subjects were thawed and analyzed for ECP by a commercial RIA kit (Kabi Pharmacia), with a limit of detection of 2 ng/mL. The analysis was performed according to the manufacturer's directions. Results are expressed as nanograms per milliliter of induced sputum.

Immunocytochemistry

After thawing, immunostaining of COX-2 on sputum cells was performed by using a mouse monoclonal anti-COX-2 (IgG₁) anti-

body (Cayman Chem, Ann Arbor, Mich) at a dilution of 1:10. The slides were stained by the immunoalkaline-phosphatase method (Dako LSAB, Glostrup, Denmark), with slight modification. Before incubation with the primary antibody (1:10, overnight at 4°C), the slides were incubated with blocking reagent containing a carrier protein to reduce the background. The biotin-conjugated anti-mouse IgG was incubated for 10 minutes, and the immunostaining was visualized by counterstaining with Gill II Hematoxylin (Sigma Aldrich, Milan, Italy). The endogenous alkaline phosphatase activity was inhibited by the use of levamisole (Sigma, Aldrich, Milan, Italy), and negative controls were performed with the use of an irrelevant mouse monoclonal IgG₁ antibody (Dako LSAB, Glostrup, Denmark), at a dilution of 1:10. The cell identification was based on cell morphology under light microscopy (×400 final magnification), carefully referring to the cell type distribution in corresponding Diff-Quick–stained slides; red staining identified positive cells. Two independent observers counted a minimum of 600 cells, and the mean value of the two observations was used ($\rho = .94$). The results were expressed in percentage terms of positively staining cells.

Determination of PGE₂ synthetic activity in induced sputum cells

Induced sputum cells (2×10^6 /mL) from asthmatic subjects were incubated with 0.5 μ Ci of [¹⁴C]-AA (NEN, Cologno M. Italy; 60 Ci/mmol) for 30 minutes at 37°C in RPMI-1% FCS. To determine the isoform contributing to the observed formation of PGE₂, incubations were carried out in the presence or absence of SC 58236, 10 μ mol/L at 37°C for 10 minutes, a highly specific inhibitor of COX-2 activity.^{17,18} At the end of incubation with AA, cell supernatants were harvested and stored under an argon atmosphere at –80°C until reversed-phase high-performance liquid chromatography (RP-HPLC) separation of PGE₂ as previously described.¹⁹ HPLC fractions collected at 1-minute intervals were assessed for radioactivity by scintillation counting (model LS 1801; Beckman Instruments, Fullerton, Calif) after addition of 10 mL of Ready Safe Scintillation Cocktail.

Activity of induced sputum supernatant and PGE₂ on eosinophil apoptosis

Peripheral blood polymorphonuclear leukocytes were prepared from normal subjects with the use of dextrane sedimentation and centrifugation over Ficoll cushions, as previously described.²⁰ The immunomagnetic selection of eosinophils was carried out with the use of CD16 microbeads to remove neutrophils from the polymorphonuclear cell preparations (MACS; Miltenyi Biotec, Italy); this technique consistently provided eosinophil preparations with a purity >97%, as determined by Diff-Quick differential counts on cytospin preparations. The purified eosinophils were resuspended and cultured at the concentration of 4×10^5 /mL in 24-well tissue culture plates precoated with FCS to inhibit adherence. The induced sputum supernatants (1 mL) as well as PGE₂ (1 to 25 nmol/L) were added to eosinophils for 18 hours. At the end of the incubation time, the eosinophils were harvested and apoptosis was assessed as described below.

To determine the contribution of PGE₂ present in induced sputum supernatants to the observed effect on eosinophil survival, selected samples showing the higher concentrations of PGE₂ were incubated in the presence or absence of PGE₂ affinity sorbent (mouse anti-PGE₂ IgG covalently bound to Sepharose 4B) (Cayman Chemical) and mixed gently for 2 hours; subsequently, the samples were centrifuged at 1500g and filtered on 0.2- μ m filters (Lida Manufacturing Corp, Kenosha, Wis), with the aim of separating the PGE₂ affinity sorbent and therefore eliminating PGE₂ from supernatants.

The efficiency of the specific extraction of PGE₂ by the PGE₂ affinity sorbent was evaluated in three separate induced sputum samples that were spiked with 1 ng of PGE₂ standard (Sigma,

TABLE I. Demographic characteristics of study subjects

	Control subjects	Asthmatic subjects	P value
No.	14	30	—
Male/female	7/7	18/12	—
Age (y)*	31 (28-37)	38 (29-55)	NS
Atopy	0	11	—
Dose inhaled steroids (mg)	0	0	—
Smoking	0	0	—

Results are expressed as median (25th to 75th percentiles). Statistical analysis was performed by Mann-Whitney *U* test. NS, Not significant.

Aldrich, Milan, Italy). Final concentrations of PGE₂ in samples incubated in the presence and absence of PGE₂ affinity sorbent were determined by specific RIA as described above.

Assessment of eosinophil apoptosis

The differential analysis of apoptotic and necrotic cells was performed by the analysis of annexin V binding, incubating the cultured eosinophils (4×10^5 cells per tube) for 15 minutes at room temperature in 100 μ L containing annexin V FITC and propidium iodide (Bender medSystem GmbH Rennweg, Vienna, Austria). Eosinophils were washed and immediately analyzed by using a FACS scan flow cytometer with CellQuest software (Beckton Dickinson Immunocytometry Systems, San Jose, Calif).

Statistical analysis

The data relative to induced sputum are expressed as medians and 25th to 75th percentiles. Statistical analysis was performed by using the nonparametric Mann-Whitney *U* test for comparison between groups. The Spearman rank test was used for correlation between data. A value of $P < .05$ was accepted as statistically significant.

The results obtained from recovery experiments are expressed as mean and SEM of *n* replication.

RESULTS

Study subjects

The median age of patients with asthma and of control subjects was not statistically different. FEV₁ values of control subjects had a median of 101% of the predicted value (25th to 75th percentiles, 100 to 105). The median of FEV₁ values for the asthmatic subjects resulted of 81% (72 to 94; $P < .001$). Fifteen untreated asthmatic subjects had a FEV₁ value of <80%. Atopy was present in 11 asthmatic subjects (Table I).

Total and differential cell counts in induced sputum samples

The median volume of recovered supernatants resulted of 4.7 mL (3 to 8.4) in control and 5.1 mL (2.6 to 8.5) in asthmatic subjects.

The differential cell counts have showed that the percentage of squamous cells was not significantly different in sputum samples obtained from control and asthmatic subjects. The percentage of eosinophils was significantly higher in asthmatic than in control subjects (5.7%, 2.1 to 16.4, and 0%, respectively; $P < .0001$) (Table II). No significant correlation between the eosinophil number and the FEV₁ was observed.

Asthma, rhinitis, other
respiratory diseases

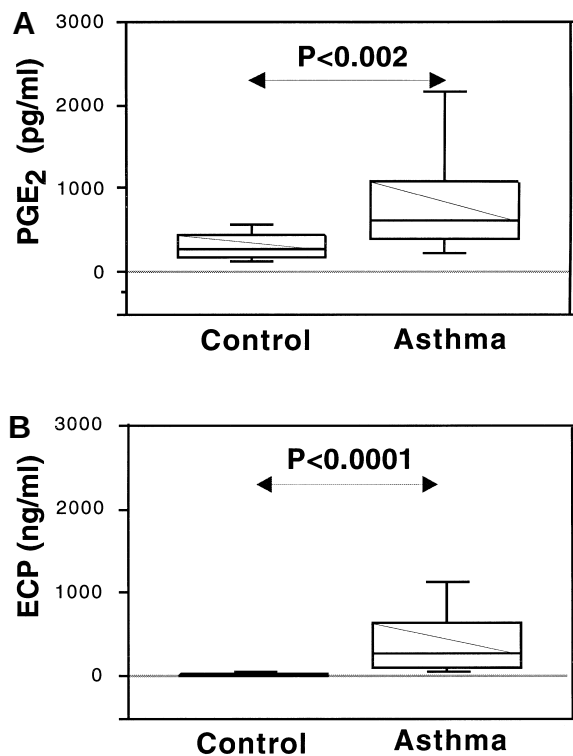


FIG 1. PGE₂ and ECP concentrations in sputum obtained from control and asthmatic subjects. PGE₂ concentrations (A) and ECP concentrations (B) in induced sputum samples were analyzed by specific RIA kits, as described in Methods section. Data are expressed as median and 25th to 75th percentiles (boxed); minimum and maximum values are represented by error bars. Statistical analysis was performed by Mann-Whitney *U* test.

The asthmatic subjects also presented a significantly increased percentage of neutrophils and a corresponding decrease of macrophages when compared with control subjects (Table II). No significant differences between the asthmatic and the control subjects were observed with respect to the percentage of lymphocytes and epithelial cells. The median viability of the cells obtained from induced sputum samples was 79.7% (25 to 75 percentiles: 71 to 84) in control subjects and 72.9% (62 to 81.1) in asthmatic subjects.

Analysis of prostaglandin E₂ and ECP

The PGE₂ concentrations were significantly higher in supernatants of the induced sputum samples obtained from asthmatic subjects than in control subjects (619.5 pg/mL, 390 to 1089, and 282.9, 174 to 447.7, respectively) ($P < .002$) (Fig 1, A). The recovery of PGE₂ was $87\% \pm 8\%$, as assessed in induced sputum samples analyzed with and without spiking with 1 ng/mL of synthetic PGE₂.

The ECP levels in induced sputum supernatants were significantly higher in samples obtained from asthmatic patients than in control subjects (272 g/L, 118 to 652, and 12.5 g/L, 7 to 26.9, respectively) ($P < .0001$) (Fig 1, B). The ECP levels in saliva were not detectable.

ECP concentrations in induced sputum obtained from asthmatic subjects, as expected, significantly correlated

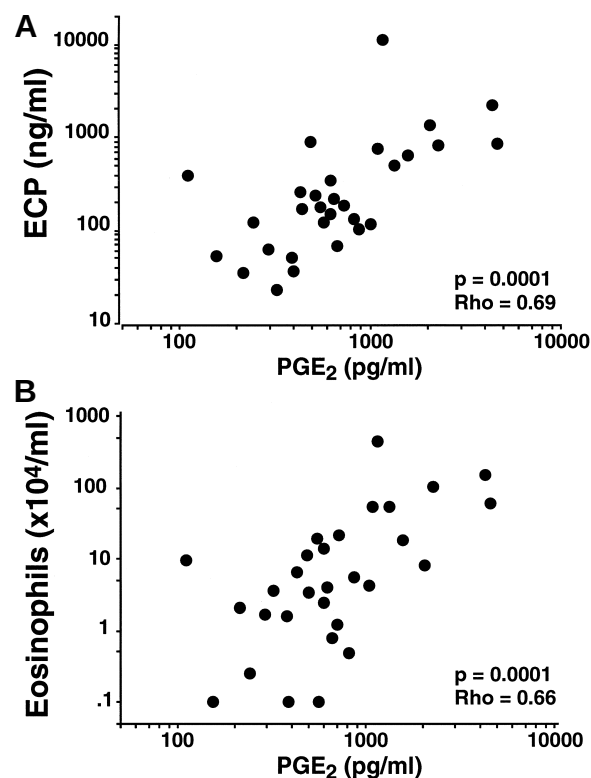


FIG 2. Correlation between PGE₂ and ECP or eosinophil concentration. Correlation between PGE₂ concentration and ECP concentration (A) or eosinophil percentage (B) in induced sputum of asthmatic subjects. Statistical analysis was performed by Spearman rank test.

with the number of eosinophils ($P < .001$) (data not shown). The PGE₂ concentrations have strikingly correlated both with the number of eosinophils ($P < .0001$) and with ECP concentrations ($P < .0001$) (Fig 2, A and B, respectively). No correlations were observed between PGE₂ concentrations and FEV₁ values.

Identification of COX-2 in cells obtained from induced sputum samples by immunocytochemistry

The COX-2 protein expression in induced sputum cells was increased in asthmatic subjects when compared with control subjects (Fig 3, B, C, and D), and the percentage of COX-2-positive cells was significantly higher in asthmatic subjects (11%, 4% to 36%) than in control subjects (4.8%, 2.5% to 5%) ($P < .01$) (Table III). Macrophages represent the main cell type expressing COX-2 in asthmatic subjects, raising from a ratio of 1 positive cell in 28 in control subjects to a ratio of 1 in 5. On the contrary, the number of neutrophils expressing COX-2 in asthmatic subjects does not increase with respect to normal subjects, despite the increased number of neutrophils, showing a ratio that declines from 1 positive cell in 15 in control subjects to 1 in 30 in asthmatic

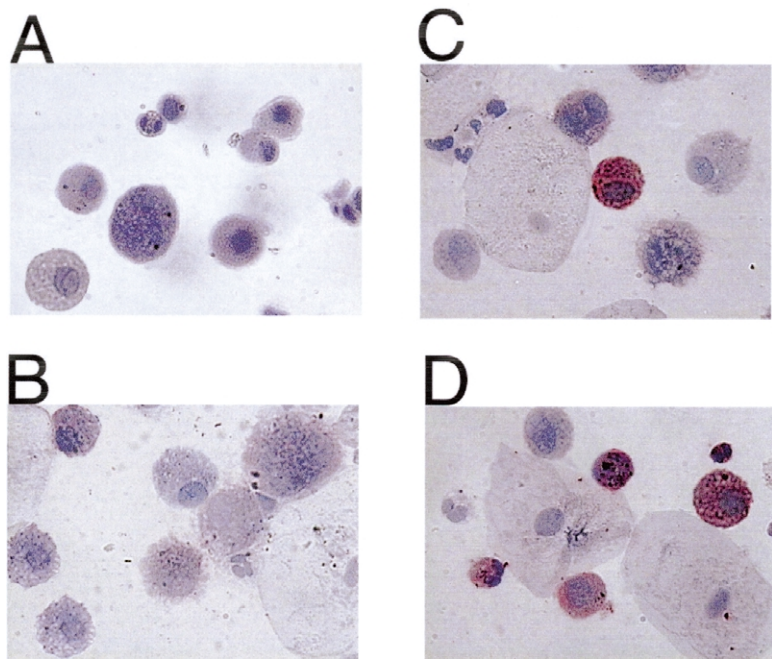


FIG 3. Expression of COX-2 in induced sputum cells. Immunocytochemistry for COX-2 protein of cells obtained from induced sputum samples was performed by the immunoalkaline-phosphatase method as described in Methods section. **A**, Negative control of induced sputum cells, performed with irrelevant mouse monoclonal IgG₁ antibody. **B**, Control subject. **C** and **D**, Asthmatic subjects.

Asthma, rhinitis, other
respiratory diseases

TABLE II. Total and differential count of cells from induced sputum samples

	Control subjects	Asthmatic subjects	P value
Squamous cells (%)	25 (14.8-32.8)	18.9 (10.1-39.1)	NS
Total cell counts (10 ⁶ cells/mL)	0.9 (0.6-1.8)	1.1 (0.7-2)	NS
Macrophages (%)	85 (81.8-92.9)	50.5 (30.9-65.3)	.0001
Neutrophils (%)	15 (6.3-15.8)	32.6 (23.2-48.8)	.0002
Lymphocytes (%)	0 (0-0.5)	0.1 (0-1.6)	NS
Eosinophils (%)	0	5.7 (2.1-16.4)	.0001
Epithelial cells (%)	0 (0-2.9)	1.2 (0-2.6)	NS

Results are expressed as median (25th to 75th percentiles).
Statistical analysis was performed by Mann-Whitney *U* test.
NS, Not significant.

subjects. A limited number of eosinophils, 1 in 10, also expressed COX-2 in asthmatic subjects.

Determination of PGE₂ synthetic activity in induced sputum cells

The determination of PGE₂ synthetic activity in induced sputum cells obtained from asthmatic subjects, performed with the use of radiolabeled AA as a substrate and RP-HPLC separation of the radioactive PGE₂, showed significant production of radioactive PGE₂ that was reduced by 70.5% ± 6.8% (n = 5) when cells were preincubated with a specific COX-2 inhibitor. This evidence has suggested that COX-2 is the isoform mainly responsible for the production of PGE₂ by the cells present within the airways of asthmatic subjects.

Effect of induced sputum supernatant and PGE₂ on eosinophils apoptosis

On the basis of the direct correlation between the PGE₂ concentrations and the percentage of eosinophils in induced sputum, and in light of the reported inhibitory effect of PGE₂ on eosinophil apoptosis,⁹ we have evaluated the effect on apoptosis of peripheral blood eosinophils of supernatant obtained from the control as well as from the asthmatic subjects showing eosinophil counts higher than 5 × 10⁴/mL (n = 17). The results obtained showed a significantly decreased number of apoptotic eosinophils upon incubation with induced sputum supernatants from the asthmatic subjects when compared with the control subjects (*P* < .005).

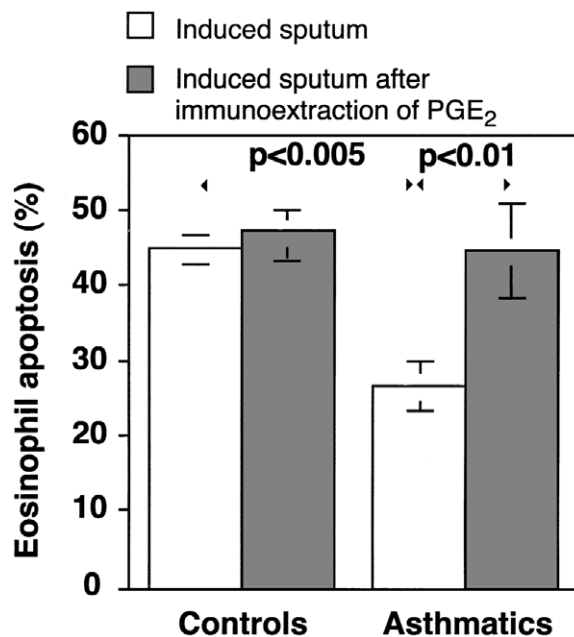


FIG 4. Apoptosis of eosinophils in the presence of supernatant from induced sputum. Eosinophil apoptosis resulting from incubation of cells with supernatant of induced sputum from asthmatic subjects with eosinophil count $> 5 \times 10^4/\text{mL}$ ($n = 17$) and control subjects ($n = 10$). Supernatant (1 mL) was used as obtained from induced sputum processing or after depletion of PGE₂ by immunoprecipitation with specific affinity sorbent (mouse monoclonal anti-PGE₂ antibody coupled to Sepharose 4B). Results are expressed as mean $\pm$ SD of percentage of annexin positive cells. Statistical analysis was performed with analysis of variance.

TABLE III. COX-2 expression by different cell types

	Control subjects	Asthmatic subjects	P value
Macrophages (%)	3.3 (1.4-5)	10.1 (3.7-28.8)	.01
Neutrophils (%)	1.1 (0-1)	1.1 (0-4)	NS
Lymphocytes (%)	0 (0-0)	0 (0-0)	NS
Eosinophils (%)	0 (0-0)	0.5 (0-1.3)	.05
Epithelial cells (%)	0 (0-0)	0 (0-0.5)	NS
Total cells	4.8 (2.5-5)	11 (4-36)	.01

Results are expressed as median (25th to 75th percentiles) of positive cells with respect to total cell counts.

Statistical analysis was performed by Mann-Whitney *U* test.

NS, Not significant.

The specific role of the PGE₂ present in induced sputum supernatants of asthmatic subjects and control subjects with respect to eosinophil survival was assessed by immunoprecipitation of PGE₂ with the use of a specific affinity sorbent. The depletion of the PGE₂ from induced sputum supernatants significantly enhanced eosinophil apoptosis in asthmatic subject samples ($P < .01$), whereas it has not caused a significant effect on control sputum samples (Fig 4).

The efficiency of the affinity sorbent to immunoprecipitate PGE₂, as assessed in samples spiked with synthetic PGE₂ and analyzed by RIA before and after immunoprecipitation, was $>80\%$ ($82\% \pm 7\%$, $n = 3$).

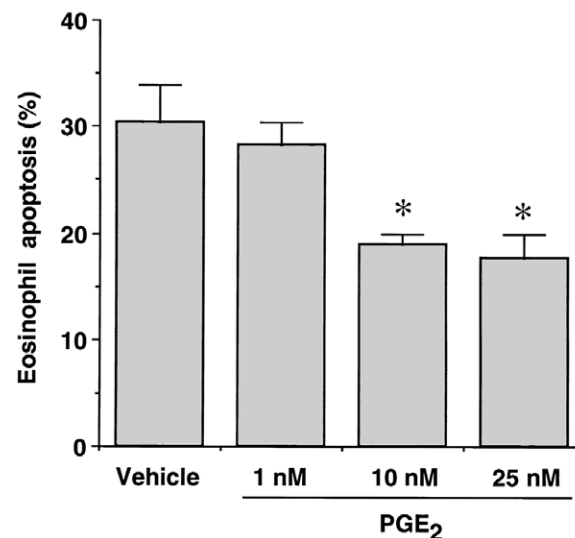


FIG 5. Effect of synthetic PGE₂ on eosinophil apoptosis. Purified eosinophils were cultured for 18 hours in the presence or absence of PGE₂ (1, 10, 25 nmol/L); apoptosis was assessed by flow cytometry as the percentage of cells binding annexin V. Results are expressed as mean $\pm$ SD of percentage of annexin-positive cells. Statistical analysis was performed by analysis of variance.

The evaluation of synthetic PGE₂ on eosinophil apoptosis provided results in agreement with published data, showing a statistically significant reduction of eosinophil apoptosis at 18 hours at concentrations as low as 10 and 25 nmol/L (Fig 5).

DISCUSSION

The results of this study showed that PGE₂ concentrations in induced sputum samples of asthmatic subjects directly correlate with eosinophilic inflammation markers such as the concentration of ECP or the number of eosinophils present, pointing out a potential link between AA cyclooxygenase metabolites and eosinophilic infiltration and inflammation.

Recently, a subdivision of asthma phenotype based on the presence of high or low eosinophil infiltrate has been proposed²¹ suggesting that in addition to a classic eosinophil-dependent process, a separate "pathologically unclassic" process leading to asthma may exist. Indeed, a significant variability in the number of eosinophils recovered in induced sputum has been observed among the asthmatic subjects who participated in our study. Nevertheless, a significant increase in PGE₂ concentrations has been detected in asthmatic subjects, and, whereas PGE₂ concentrations did not correlate with functional parameters such as FEV₁, a striking correlation with eosinophil or ECP concentrations in induced sputum samples was observed.

The direct correlation between PGE₂ concentrations and eosinophils prompted us to investigate a potential causal relation between cyclooxygenase metabolite synthesis and eosinophilic inflammation; recently published data reported that PGE₂ can enhance eosinophil survival

in vitro in a cAMP-dependent manner,⁹ and we have been able to confirm these data, proving a significant decrease of eosinophil apoptosis at concentrations as low as 10 to 25 nmol/L.

Incubation of peripheral blood eosinophils with asthma-induced sputum supernatant caused a significant decrease of eosinophil apoptosis at 18 hours, suggesting the presence of factor(s) capable to enhance eosinophil survival in vivo.

Interestingly, immunoprecipitation of PGE₂ with an anti-PGE₂ monoclonal antibody significantly reverted this effect, providing evidence that PGE₂ present in the airways of asthmatic subjects can contribute to increased eosinophil survival and development of eosinophilic inflammation in asthma. This effect can have important consequences in asthma pathophysiology, since we have previously shown that the number of apoptotic eosinophils inversely correlate with the clinical severity of the disease.²² It is therefore likely that an increased expression of COX-2 resulting in enhanced production of PGE₂ within the specific subset of eosinophil-dependent asthmatic subjects may contribute to the overall evolution of airway inflammatory reactions in asthma.

The direct correlation between PGE₂ concentration and eosinophilic inflammation observed in our study is not in keeping with a previous report by Pavord et al,⁸ who found an inverse correlation between PGE₂ concentration and the percentage of eosinophils in asthma-induced sputum. Nevertheless, a number of significant differences exist between the studied populations of asthmatic subjects; in particular, the subjects studied by Pavord were mostly assuming either topic or oral corticosteroids, whereas in our study, the subjects were enrolled after a period of drug treatments washout and in particular did not assume corticosteroids within 2 months before the beginning of the study. This is especially significant in light of the effect of corticosteroids both on eosinophil apoptosis²³ and on COX-2 expression²⁴ and can easily account for the differences in the obtained results.

Immunostaining of induced sputum cells for the inducible form of cyclooxygenase (COX-2) can strongly suggest that COX-2 expression in airway cells might play an important role for the synthesis of PGE₂ in asthmatic subjects. Although a potential involvement of the inducible microsomal PGE synthase cannot be ruled out,²⁵ the marked suppression of the ex vivo synthesis of PGE₂ obtained using the specific COX-2 inhibitor SC-58236 clearly supports a key role for COX-2 expression toward the PGE₂ enhanced synthesis observed in asthmatic subjects. While none of the subjects studied assumed COX-2-specific inhibitor for the duration of this study, it may be of interest to design a specific study aimed at evaluating the impact of their use on the presence of eosinophilic inflammation markers within the airways of asthmatic subjects.

Immunocytochemical analysis pointed out the macrophages as the main cellular phenotype expressing COX-2 in induced sputum samples, with minor expression observed in eosinophils and neutrophils. It is well

known that macrophages can express COX-2 in response to LPS activation,⁴ and it is therefore possible to hypothesize that bacterial infections may influence the activation state of airway macrophages, suggesting that increased COX-2 expression and PGE₂ production may contribute to the causal relation between infectious agents and eosinophilic chronic asthma.²⁶⁻²⁹

Although we have focused our attention on the potential contribution by induced sputum recovered cells, formation of PGE₂ from airway resident cells can obviously take place, as we and others have shown that cells such as bronchial smooth muscle cells can express COX-2 and synthesize PGE₂ in response to inflammatory cytokines.³⁰ Apart from its role in inflammatory responses, it is known that inhalation of PGE₂ can exert a protective role on the airway; nevertheless, no correlation whatsoever could be observed between PGE₂ concentrations in inhaled sputum and FEV₁ values within the group of studied asthmatic subjects. This evidence suggests that acute, pharmacologic administration of PGE₂ may grant effects that do not overimpose with the complex role of this lipid mediator within the inflamed airways of asthmatic subjects.

In conclusion, the results that we obtained from the study of asthma-induced sputum suggest that the COX metabolite PGE₂, enhancing eosinophil survival, may participate in the development of the "classic" eosinophilic inflammation of the airways of asthmatic subjects. Increased production of PGE₂ by alveolar macrophages expressing COX-2 in response to inflammatory cytokines or bacterial products, such as LPS, may significantly contribute to this process.

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