

Original article

Increased prostaglandin E₂ levels in the airway of patients with eosinophilic bronchitis

Background: Eosinophilic bronchitis is a common cause of chronic cough, which like asthma is characterized by sputum eosinophilia, but unlike asthma there is no variable airflow obstruction or airway hyperresponsiveness. We tested the hypothesis that the different airway function in patients with eosinophilic bronchitis and asthma could be caused by an imbalance in the production of bronchoconstrictor (LTC₄) and bronchoprotective (prostaglandin E₂; PGE₂) lipid mediators.

Methods: We measured cytokines levels, proinflammatory mediators and eicosanoids concentration in sputum from 13 subjects with nonasthmatic eosinophilic bronchitis, 13 subjects with asthma, and 11 healthy control subjects. Cytokines mRNA levels were measured by real time PCR, proinflammatory mediators, PGE₂, and LTC₄ were measured by enzyme immunoassays.

Results: The median sputum eosinophil count was not statistically different in patients with asthma (7.95%) and eosinophilic bronchitis (15.29%). The levels of mRNA specific to interleukin-5 (IL-5), IL-4, IL-10, IL-13, interferon γ (IFN- γ), IL-2, vascular endothelial growth factor and transforming growth factor β were similar in both conditions. In addition, no differences were found between asthma and eosinophilic bronchitis in proinflammatory cytokines, such as IL-8, IFN- γ and tumor necrosis factor α (TNF- α) levels. Sputum cysteinyl-leukotrienes concentration was raised both in eosinophilic bronchitis and asthma patients. We found that induced sputum PGE₂ concentrations were significantly increased in subjects with eosinophilic bronchitis (838.3 ± 612 pg/ml) when compared with asthmatic (7.54 ± 2.14 pg/ml) and healthy subjects (4 ± 1.3 pg/ml).

Conclusion: This data suggest that the difference in airway function observed in subjects with eosinophilic bronchitis and asthma could be due to differences in PGE₂ production in the airways.

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Key words: asthma; cytokines; eosinophilic bronchitis; induced sputum; prostaglandins.

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Accepted for publication 4 July 2007

The development of sputum induction has provided a safe noninvasive method for assessing airway inflammation. One of the most interesting early observations made using this method was the identification of a group of patients with sputum eosinophilia identical to that seen in asthma, but with normal airway function. The physiological features of this condition were different from those of asthma, Gibson et al. suggested that it should be known as eosinophilic bronchitis (1). The cough in patients with nonasthmatic eosinophilic bronchitis responds well to inhaled corticosteroids, in contrast with the cough in patients without sputum eosinophilia. Nonasthmatic eosinophilic bronchitis is responsible of about 10–30% of patients of isolated chronic cough referred for specialist investigations (2).

In patients with eosinophilic bronchitis, there is a clear dissociation between sputum eosinophilia and airway hyperresponsiveness. The pathophysiology of eosino-

philic bronchitis and the reason for the absence of airway hyperresponsiveness in this disease remains unclear. One possible explanation is that the eosinophilic airway inflammation is less active than in asthma, with less release of effector mediators. Several studies employing different techniques to assess airway inflammation have shown that the inflammatory component is very similar in patients with asthma and eosinophilic bronchitis (3). Indeed, asthma and eosinophilic bronchitis share many immunopathologic features including increased numbers of eosinophils and mast cells in the superficial airway. In addition to airway eosinophilia, both conditions are associated with reticular basement membrane thickening and similar numbers of subepithelial T lymphocytes, mast cells, and macrophages (3). One aspect of the inflammatory response that might be particularly important is the localization of mast cells in the airway wall, as they are present within the airway smooth muscle in asthma but

not in eosinophilic bronchitis (4). Moreover, in subjects with eosinophilic bronchitis, CXCL8 and CXCL10 concentrations were elevated in airway secretions. These chemokines may play a key role in mast cell recruitment to the superficial airway in this condition (5). Kanazawa et al. (6) found higher levels of vascular endothelial growth factor (VEGF) in asthma than in eosinophilic bronchitis. The cyclooxygenase product prostaglandin E₂ (PGE₂) is produced by several cells in human airways, including epithelium (7) and smooth muscle (8); it has inhibitory effects on inflammatory cells and bronchoprotective effects in patients with bronchial asthma. Prostaglandin E₂ has been shown to protect against exercise-induced (9), allergen-induced (10), and aspirin-induced bronchoconstriction (8), as well as against bronchoconstrictor agents, such as methacholine and histamine (11). The pharmacological actions of PGE₂ are mediated through specific E-prostanoid (EP)-1, EP-2, EP-3, and EP-4 receptors, which are encoded by different genes and expressed differentially in each tissue (12). Prostaglandin E₂ may represent an endogenous protective mechanism in the airways. In this study, we postulate that the different airway function in patients with eosinophilic bronchitis and asthma could be as a result of an imbalance in the production of bronchoconstrictor (LTC₄) and bronchoprotective (PGE₂) lipid mediators.

Methods

Subjects

Thirteen subjects with nonasthmatic eosinophilic bronchitis, 13 subjects with asthma, and 11 healthy control subjects were recruited from Fundación Jimenez Díaz, Allergy clinic outpatients and staff. All patients with eosinophilic bronchitis attending our outpatient clinic during 2003 and 2006 were invited to enroll in the study, and all agreed to participate. The asthma group comprised consecutive adult patients with asthma (atopic or nonatopic) recruited in the outpatient allergy clinic. Control subjects were recruited from the staff at the allergy and immunology departments. Control subjects were lifelong nonsmokers, had no history of asthma, allergic diseases, or chronic bronchitis and they showed negative skin prick tests to common aeroallergens.

The subjects' clinical characteristics are shown in Table 1. The subjects with eosinophilic bronchitis had an isolated cough lasting more than 8 weeks, no symptoms suggesting variable airflow obstruction, normal spirometric values, a methacholine PC₂₀ value > 16 mg/ml, a normal chest radiograph, and sputum eosinophilia (sputum eosinophils > 3%). Subjects with asthma provided a consistent history and had objective evidence of asthma (as defined by the American Thoracic Society) (13) for at least 6 months. These patients showed > 12% improvement in forced expiratory volume in first second 10 min after administration of 500 µg of inhaled terbutaline, or had methacholine airway hyperresponsiveness (PC₂₀ methacholine < 16 mg/ml). Patients with asthma had mild persistent disease (14) and were clinically stable, nine were atopics and none had a history of respiratory infection for at least the 6-week period preceding the study. We included in the asthmatic group both atopic and nonatopic patients, as no differences had been previously observed in the parameters assessed between these patients.

Table 1. Clinical characteristics of study subjects

	Healthy controls	Eosinophilic bronchitis	Asthma
No.	11	13	13
Age (years)*	23 (21–42)	43 (31–71)	37 (16–61)
Male (%)	5 (45.5)	9 (69.2)	5 (38.5)
Atopy (%)	0	7 (53.8)	9 (69.2)
Duration of symptoms (years)*	0	2 (0.5–29)	8 (0.5–26)
Smoking history			
Current (%)	0	2 (15.4)	0 (0)
Ex-smoker (%)	0	4 (30.8)	2 (15.4)
Nonsmoker (%)	11 (100)	7 (53.8)	11 (84.6)
FEV ₁ % predicted*	106 (100–130)	105 (91–128)	109 (73–127)
FEV ₁ /FVC %*	85 (80–91)	81 (75–87)	82 (65–92)
Total serum IgE (kU/l)†	ND	48.6 (3.8–626)	135.9 (7.2–1291)
PC ₂₀ methacholine (mg/ml)†	ND	NEG	2.1 (0.9–8)

FVC, forced vital capacity; FEV₁, forced expiratory volume in the first second; ND, not done; NEG, (PC₂₀ > 16 mg/ml).

*Median (range).

†Geometric mean (range).

Seven (53.8%) of the patients with eosinophilic bronchitis and nine (69.2%) of the patients with asthma were atopics (as defined by positive skin prick test to common aeroallergens). The percentage of positive skin prick tests to common aeroallergens in patients with eosinophilic bronchitis and asthma was as follows: grass and/or tree pollen (38.5% and 61.5%, respectively), house dust mites (15.4% and 30.8%), cat and/or dog dander (7.7% and 30.8%), molds (0% and 15.4%) and cockroaches (7.7% and 15.4%). Total serum immunoglobulin E (IgE) was significantly higher ($P < 0.05$) in patients with asthma (geometric mean 135.9 kU/l, range 7.2–1291) than in patients with eosinophilic bronchitis (48.6 kU/l, 3.8–626 kU/l). For patients who were receiving inhaled corticosteroids (three subjects with eosinophilic bronchitis and 11 with asthma), these drugs were withdrawn for at least 2 weeks before sputum induction. No patient was receiving oral corticosteroids (for at least 6 months prior to the study), leukotriene receptor antagonists, aspirin, or any other cyclooxygenase inhibitor. Blood and sputum samples were obtained from adult donors after giving written informed consent. The study was blinded. Patients were classified with a number code by the clinical staff and all the analysis were developed in codified tubes without knowing the patient identity and diagnosis.

Sputum induction and processing

Sputum was induced using inhalations of increasing concentrations (3%, 4%, and 5%) of hypertonic saline (15) and processed and examined for nonsquamous cell counts, as previously described (16), and following European Respiratory Society recommendations (17). Solid sputum material was selected from saliva, divided and processed within 2 h by two parallel procedures. The protocol used was previously described (18), and slightly modified. Sputum was treated with 0.1% dithiothreitol (DTT) to improve cell dispersion. Sputum was treated with a similar volume of DTT and was incubated on a shaker at 37°C for 15 min. To prevent the DTT effect on the cell suspension, an equal volume of Dulbecco's phosphate buffer saline (PBS) was added. The fraction treated with PBS + protease inhibitors (PI) was incubated at 4°C for 30 min. Mucus was then removed by means of filtration through a 100 µm-pore nylon mesh, and the filtrate was centrifuged at 400 g for 10 min to sediment any cells present. The supernatant was

aliquoted and stored at -80°C until analysis. Pellet was resuspended in PBS/BSA/EDTA. The total cell count, viability and squamous cells (%) were determined by trypan blue exclusion staining. Cell suspension was adjusted for 1×10^6 cells/ml with PBS containing 2% fetal calf serum (FCS), to flow cytometry. The rest of cell suspension was resuspended in Trizol and store at -80°C until used.

Flow cytometry of sputum cells

For each test, 100 μl of sputum cell suspension was incubated with 3 μl of each monoclonal antibody (Becton Dickinson, San Jose, CA, USA) for 30 min at 4°C in the dark. Then, cells were washed with PBS/FCS 2% and resuspended in 400 μl of PBS/FCS 2%. Data were acquired with a 2-laser, 5-parameter FACSCalibur flow cytometer (Becton Dickinson) and analyzed on Cell Quest software (Becton Dickinson Bioscience). Typically, 50 000–100 000 total events were acquired to obtain adequate numbers of cellular types (10 000). All sputum cell samples were stained with Pe-Cy5 conjugated anti-human CD45 monoclonal antibody to exclude all non-leukocyte events in each test. The panel consist of: CCR3 FITC/CD16 PE, CCR3 FITC/DR PE, CD69 FITC/CD16 PE, CD14 FITC/DR PE, CD4 FITC/CD2 PE, CD4 FITC/CD25 PE, CD19 FITC/CD2 PE, CD34 FITC/CDw125 PE, CD63 FITC/IgE PE and isotype control (Becton Dickinson) that were used to establish background fluorescence for each fluorochrome.

Flow cytometry of whole blood

One hundred microliter of fresh whole blood was incubated with 3 μl of each monoclonal antibody for 30 min at room temperature in the dark. Then, erythrocytes were lysed using 1 ml of FACS-lysing solution (Becton Dickinson) for 10 min and washed with 2 ml of FACS-Flow (Becton Dickinson). Finally, cells were resuspended in 500 μl of FACS-Flow and analyzed using FACS-Calibur flow cytometer. Up to 10 000 events were analyzed per sample. The panel consist of: CCR3 FITC/CD16 PE/CD69 PeCy5, CCR3 FITC/DR PE/CD69 PeCy5, CD14 FITC/DR PE, CD4 FITC/CD2 PE/CD8 PeCy5, CD4 FITC/CD25 PE/CD8 PeCy5, CD19 FITC/CD2 PE, CD34 FITC/CDw125 PE/CD45 PeCy5, CD63 FITC/IgE PE/CD45 PeCy5 and isotype control.

Reverse transcription-PCR

Total RNA was isolated from sputum cells by Trizol[®] Reagent (Invitrogen, Carlsbad, CA, USA) and was treated with DNase I (Promega, Madison, WI, USA). RNA was measured by spectrophotometry, and 1 μg of RNA was transcribed to cDNA with first-strand cDNA synthesis with the MultiScribe transcriptase enzyme reaction (Applied Biosystems, Foster City, CA, USA).

Cytokines expression in sputum cells by real-time quantitative PCR

Quantitative real-time PCR was performed on a 7500 Real-time PCR System (Applied Biosystems). Taqman PCR was performed using a 20 μl final reaction volume containing 10 μl of TaqMan Universal PCR Master Mix (Applied Biosystems), 1 μl of 20X Assays-on-Demand Gene Expression Assay Mix and 9 μl of cDNA diluted in RNase-free water. Each assay was performed in triplicate. The PCR conditions used in all reactions were: 2 min at 50°C , 10 min at 95°C , with 40 two-step cycles (95°C for 15 s and 60°C for 60 s). Assays-on-Demand Gene Expression primers to interleukin-5 (IL-5), IL-10, IL-13, IL-2, IL-4, VEGF, transforming growth factor- β (TGF- β 1), and interferon- γ (IFN- γ) and rRNA 18s (used

as endogen) were obtained from Applied Biosystems (<http://www.appliedbiosystems.com/>; accessed 16 October 2007)

Measurement of soluble mediators, cytokines, and lipidic mediators production

We analyzed, IL-8, IFN- γ , TNF- α , and VEGF-A by ELISA Kits (Bender MedSystems GmbH, Vienna, Austria). The detection limit of these assays was between 0.99 and 13.5 pg/ml (5, 0.99, 3.83, and 13.5 pg/ml respectively). Coefficient of variation intra-assay was between 4% and 6.9%, and inter-assay between 5.7% and 10.9%. Moreover, we tested two types of lipidic mediators, PGE₂ and cysteinyl-leukotriene C4 by ELISA Kits (Cayman Chemical Company, Ann Arbor, MI, USA). Previously to test lipidic mediators, sputums were treated to remove protein and other contaminants that could interfere with the assay. In all patients, mediators are measured in neat supernatants in duplicate. Prostaglandin E₂ assay shows a detection limit of 2 pg/ml with a intra-assay coefficient of variation of 5% and a inter-assay coefficient of variation of 4% and the specificity is 100%. LTC₄ assay has a detection limit of 13 pg/ml, a specificity of 100% and a coefficient of variation intra- and inter-assay of 8% and 10%, respectively. We carried out several experiments to analyze a possible effect of DTT in these experiments and we observed that this compound had no effect in the measurement of lipidic mediators (data not shown). This conclusion has been supported by other authors (19, 20).

Data analysis

Subjects characteristics were described using descriptive statistics and expressed as median and range or geometric mean and standard deviation (SD). All measurements were performed in a blinded fashion. Comparisons of mediator concentration and sputum different cell counts across the three groups and between groups were performed using Kruskal–Wallis test and the Mann–Whitney *U*-test and ANOVA and unpaired *t* test with Welch correction for nonparametric data and parametric data, respectively. Normality was analysed with the Kolmogorov–Smirnov test. A difference was considered to be significant when $P \leq 0.05$. Statistical analyses were performed using GRAPHPAD INSTAT3 (GraphPad Software Inc., San Diego, CA, USA).

Results

Differential sputum and peripheral blood cell counts

Thirty-seven sputum samples from patients with asthma, eosinophilic bronchitis and controls subjects were analysed. The sputum had $<10\%$ squamous epithelial cells and more than 85% cell viability (Table 2). No significant differences were noted between the subject groups in either total cell count or epithelial cells. Induced sputum eosinophil percentage were significantly higher in subjects with eosinophilic bronchitis and asthma than in healthy subjects (15.29%, 7.95%, and 0.95%, $P < 0.01$; median) corresponding to a total count of $1.05 \times 10^6 \pm 0.86 \times 10^6$, $0.37 \times 10^6 \pm 0.36 \times 10^6$, $0.05 \times 10^6 \pm 0.04 \times 10^6$ eosinophils/ml (geometric mean $\pm$ SD). However, no significant differences were observed in the eosinophil counts between subjects with asthma and those with eosinophilic bronchitis, as shown in Table 2.

Table 2. Total and differential cell counts in induced sputum and peripheral blood (Flow Cytometry)

	Healthy controls (<i>n</i> = 11)	Eosinophilic bronchitis (<i>n</i> = 13)	Asthma (<i>n</i> = 13)
Induced sputum			
Total cells/ml (10 ⁶)	3.9 (1.76–15.24)	3.96 (1.2–18.16)	2.69 (1.31–29.2)
Viable cells (%)	86.44 (74.03–92.4)	87.5 (74.41–95.2)	92.1 (78.6–96.3)†
Squamous cells (%)	8.57 (3.77–15.04)	3.67 (1.9–15.5)*	7.8 (0.34–16.4)
Macrophages (%)	15.21 (3.71–61.51)	10.94 (0.61–46.98)*	22.62 (0.2–62.61)†
Neutrophils (%)	10.22 (2.12–31.7)	27.77 (10.61–64.17)	24.72 (12.29–69.07)
Eosinophils (%)	0.95 (0.09–1.91)	15.29(1.98–64.03)*	7.95 (3.06–30.94)*
Lymphocytes (%)	1.49 (0.21–3.29)	0.77 (0.01–3.55)*	0.7 (0.03–5.89)*
Eosinophilic precursors	0.04 (0–0.19)	0.01 (0–7.48)	0.03 (0–0.89)
Activated basophils	0.32(0.13–2.4)	0.52 (0–26.5)	0.44 (0–5.96)
Activated eosinophils CD69 ⁺	0.39 (0.03–2.14)	1.51 (0.12–44.39)*	1.31 (0.19–8.6)*
Activated eosinophils CD25 ⁺	0.29 (0.01–2.51)	2.74 (0.19–7.1)†	1.49 (0.37–5.55)*
Peripheral blood			
Monocytes (%)	2.2 (1.27–3.38)	3.49 (1–8.2)	4.24 (2.02–6.11)
Neutrophils (%)	37.18 (13.34–50.57)	40.51 (22.46–59.65)†	49.59 (14.78–67.66)
Eosinophils (%)	0.04 (0–0.9)	4.46 (1.21–10.22)*‡	1.66 (0.57–6.48)*
Lymphocytes (%)	9.52 (0–64–20.39)	15.94 (6.31–26.4)	17.91 (10.89–30.3)
Purified eosinophils/mm ³ (10 ⁵)	2.79 (1.6–16)	22.4 (1.12–36.26)*	22.2 (3.96–52)*

Results are expressed as median (range).

**P* < 0.01 compared with healthy control subjects.

†*P* < 0.05 compared with healthy control subjects.

‡*P* < 0.01 compared with asthmatic subjects.

The percentage of activated eosinophils (CD25⁺ and CD69⁺) in sputum was significantly higher in the eosinophilic bronchitis and asthma groups than in the healthy control group, but was similar in the former two groups. No differences were seen in other differential cell counts in induced sputum between the groups, except in macrophage and lymphocytes counts, which were significantly lower in subjects with eosinophilic bronchitis and asthma than in control subjects. Median percentage of eosinophils on peripheral blood was significantly higher in the subjects with eosinophilic bronchitis and asthma than in healthy control subjects (4.46, 1.66 and 0.04 respectively; *P* < 0.0001).

Quantitative expression of cytokines genes in induced sputum cells from eosinophilic bronchitis, asthmatic patients and healthy controls.

We assessed different cytokine levels as IL-5, IL-10, IL-13, IL-4, and IL-2 and IFN- γ , representatives of T helper 2 (Th2) and Th1 cytokines. Moreover, we measured levels of VEGF and TGF- β 1. The results indicated that IL-5, IL-4, IL-10, IL-13, IL-2, and IFN- γ levels were similar in subjects with asthma or eosinophilic bronchitis (Fig. 1). Interleukin-5 and IL-13 expression increased in the subjects with eosinophilic bronchitis as compared with that seen in healthy controls (*P* < 0.05, Fig. 2A, *P* < 0.01 Fig. 1D). In asthmatic and eosinophilic bronchitis patients no significant differences were observed in the IL-4, IL-10, IL-2 and IFN- γ expression as compared with the healthy control group. There were not significant changes in the levels of VEGF and TGF- β between both groups with disease and the control group

(Fig. 1G,H). Thus, analysis of expression of several cytokines in the study groups showed that IL-5 and IL-13 are the predominant mediators in the local inflammatory process in the airways, with a Th2 cytokine profile in both diseases.

Cytokines levels in induced sputum supernatant

Production of several cytokines by sputum cells was evaluated. Interferon- γ and IL-8 concentrations were significantly increased in induced sputum samples from patients with respiratory disease (asthma and eosinophilic bronchitis) as compared with the control subjects (Fig. 2A,B). There was no significant difference of sputum TNF- α level between groups (Fig. 2C).

Lipidic mediators in induced sputum supernatant

An inverse relationship was observed between PGE₂ and LTC₄ concentrations in patients with asthma and eosinophilic bronchitis. Whereas LTC₄ levels were more elevated in the asthmatic group, eosinophilic bronchitis patients had increased PGE₂ levels. In eosinophilic bronchitis patients we observed significantly increased level of PGE₂ as compared with healthy and asthmatics patients; *P* < 0.005 and *P* < 0.05, respectively (Fig. 3A), whereas the latter two groups had similar values. By contrast, LTC₄ was significantly higher in the asthmatic group with respect to the others, with *P* < 0.05 between control and asthmatic subjects (Fig. 3B). Moreover, the ratio of LTC₄ to PGE₂ in induced sputum was significantly higher in asthmatics than in patients with eosinophilic bronchitis *P* < 0.005 (Fig. 4).

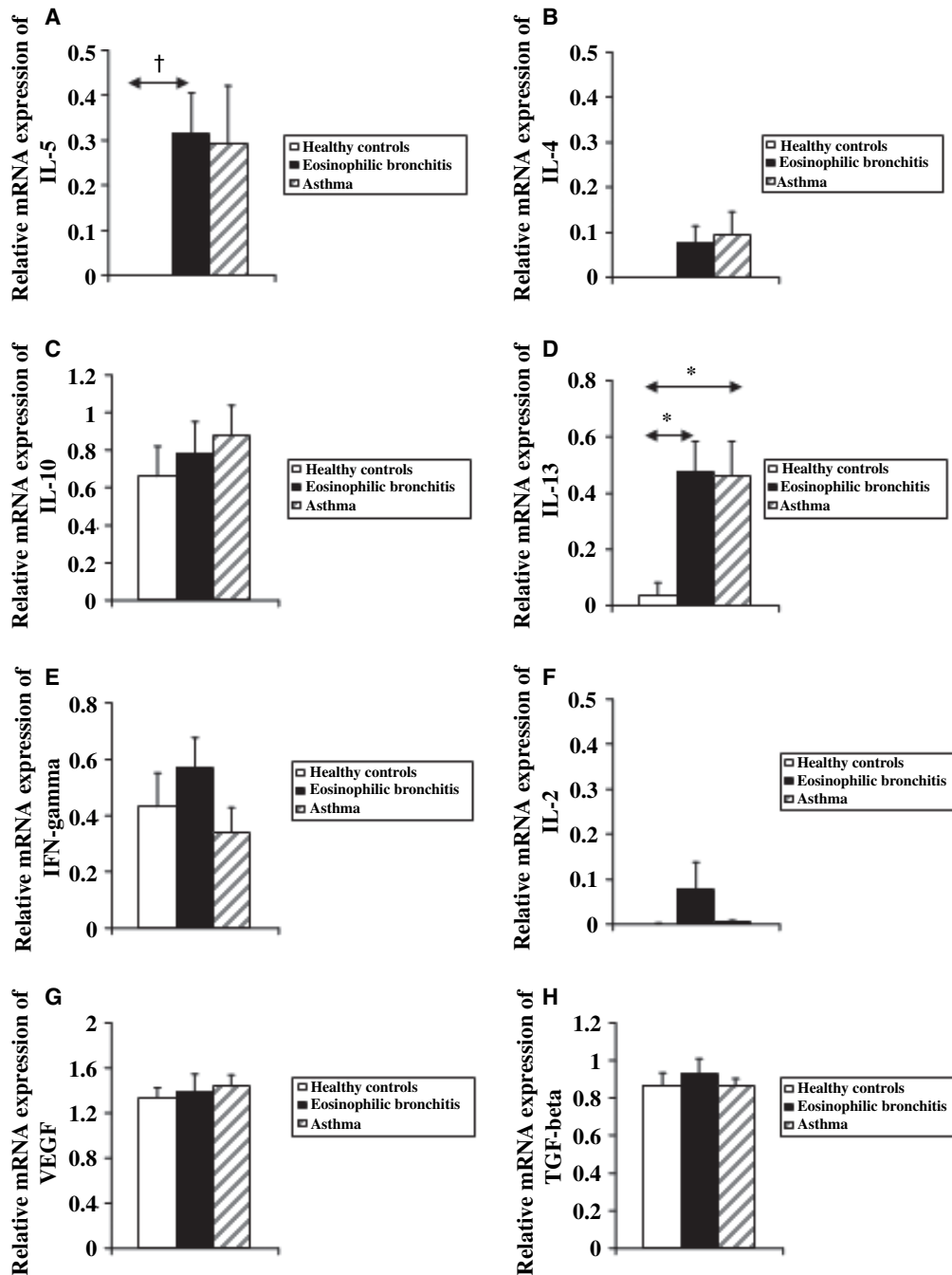


Figure 1. Cytokine mRNA expression in induced sputum from healthy subjects, asthmatic, and eosinophilic bronchitis patients. Relative mRNA levels of IL-5 (A), IL-4 (B), IL-10 (C), IL-13 (D), IFN- γ (E), IL-2 (F), VEGF (G), and TGF- β (H) gene expression in sputum cells from different groups of subjects were determined by real-time quantitative PCR. Cytokine levels were expressed as relative mRNA expression. Values were normalized with rRNA gene use as endogen. Significant differences in IL-5 ($\dagger P < 0.05$) and IL-13 ($*P < 0.01$) expression levels were obtained for eosinophilic bronchitis vs healthy controls. In the rest of cytokines studied, no significant differences were found between groups.

Discussion

Our results shows that induced sputum PGE₂ concentrations are strikingly increased (100-fold higher) in subjects with eosinophilic bronchitis as compared with asthmatic

and healthy subjects. Sputum cysteinyl-leukotrienes concentration was raised both in eosinophilic bronchitis and asthma patients. These data suggest that the differences in airway function observed in subjects with eosinophilic bronchitis and asthma might be due to differences in

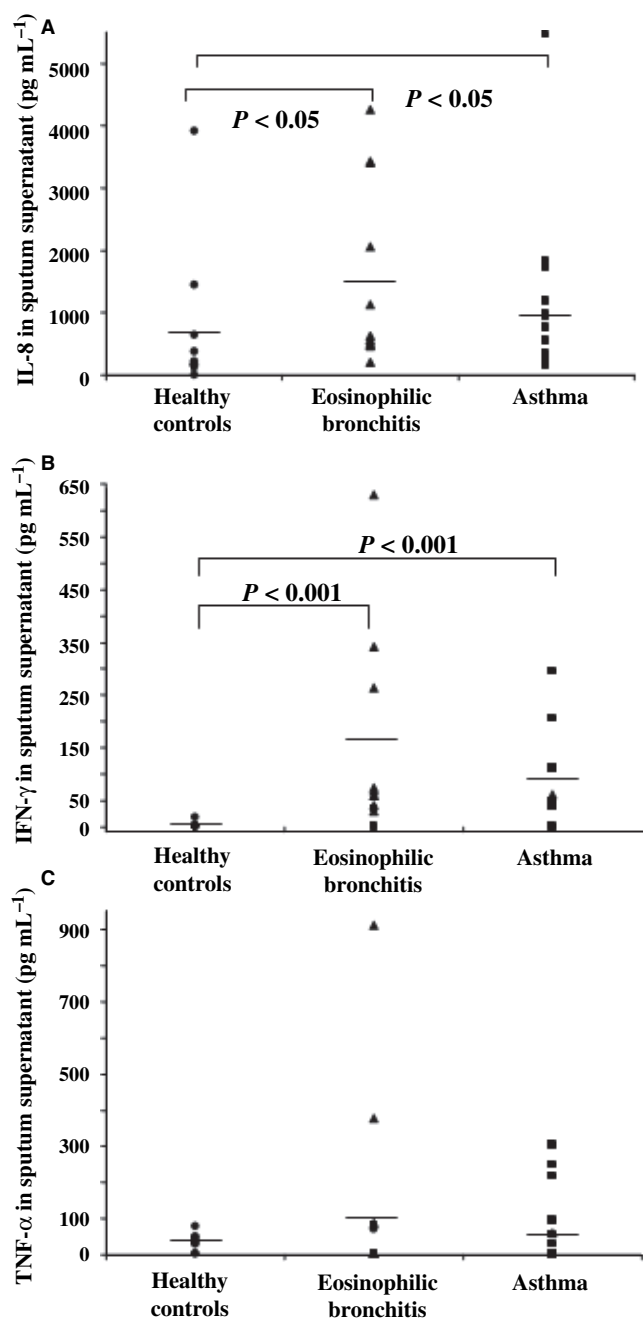


Figure 2. Cytokine levels in induced sputum supernatants. A comparison of (A) IL-8, (B) IFN- γ , and (C) TNF- α levels in induced sputum supernatants from healthy controls, eosinophilic bronchitis and subjects with asthma. Sputum from asthmatics and eosinophilic bronchitis had significant higher levels of IL-8, and IFN- γ than healthy subjects.

PGE₂ production. We have also measured a wide array of cytokines and autacoids with different functions, representing the major regulator, effector and airway damaging mediators in asthma, and we did not observe significant differences in the majority of them between both disorders.

We have detected that eosinophilic bronchitis is a disease characterized by an increased expression of Th2 cytokines (IL-4, IL-5, IL-10, IL-13), as previously shown by other authors (21). These data point out a dissociation between T-cell activation and the abnormalities in airway physiology that characterize asthma (22). These results suggest that Th2-mediated cytokines are closely linked to eosinophilic inflammation, but they are not necessarily associated with the physiologic hallmarks of the asthmatic phenotype. In our study, there were no significant differences in the eosinophil counts in patients with asthma and those with eosinophilic bronchitis, and we did not find either any significant differences in activated eosinophils, eosinophil precursors and activated basophils between patients with eosinophilic bronchitis and asthma.

Recently, the involvement of VEGF in the pathogenesis of asthma has generated an active discussion. We measured this factor by two methods: quantitative real-time PCR and determination of soluble VEGF in supernatant (data not shown). We found a moderate increase of VEGF in sputum supernatant in eosinophilic bronchitis patients, whereas asthmatic patients showed similar values to healthy controls, the differences not being statistically significant. An increase in VEGF levels in the airways of asthmatic subjects as compared with control subjects or eosinophilic bronchitis patients (6) has been previously reported, but this finding was not observed in our study. Demoly et al. (23), however, reported a similar concentration of VEGF in the bronchoalveolar lavage from patients with asthma and normal controls. Thus, the role of VEGF in the pathogenesis of asthma remains unclear (24).

Cysteinyl-leukotrienes produced by eosinophils and mast cells are potent airway smooth-muscle contractile agonists, increase mucus production and vascular permeability, and may directly increase eosinophilic inflammation (25). Prostaglandin E₂ increase cough sensitivity by a direct effect on cough receptors (26). We chose induced sputum supernatant to assess airway mediator production as this method is noninvasive, simple, safe, and responsive (19). Previous studies have shown that sputum mediator concentrations are significantly higher than in bronchoalveolar lavage and can be measured repeatedly (16, 19). Cell viability was not different between groups, indicating that the observed differences in mediator levels were probably not because of cytotoxic release. Furthermore, leukotrienes and prostanoids are not stored preformed.

This study illustrates that, like asthma, there is active airway inflammation in eosinophilic bronchitis, with release of different inflammatory mediators. The main significant difference between asthma and eosinophilic bronchitis was the different ratio in the production of bronchoconstrictor (LTC₄) and bronchoprotective (PGE₂) lipid mediators. In asthma, there may be a reduction in bronchoprotective substances such as PGE₂ (27). We found much lower PGE₂ concentrations in

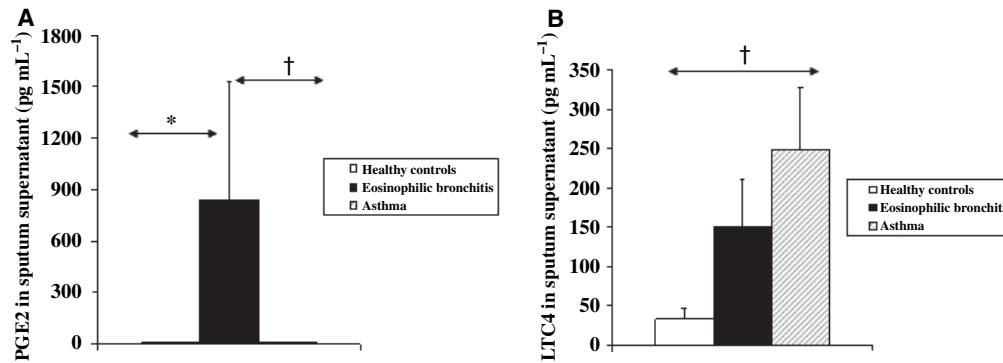


Figure 3. Sputum eicosanoids concentration in healthy controls, eosinophilic bronchitis and asthmatics. PGE₂ concentrations (A) and LTC₄ concentrations (B) in induced sputum samples were analysed by specific ELISA kits, as described in the methods section. Data are expressed as geometric mean $\pm$ SD. Subjects with eosinophilic bronchitis had significantly higher PGE₂ concentrations as compared with healthy controls and asthmatics (* P < 0.01; † P < 0.05 respectively). Statistical analysis was performed by Mann–Whitney U -test.

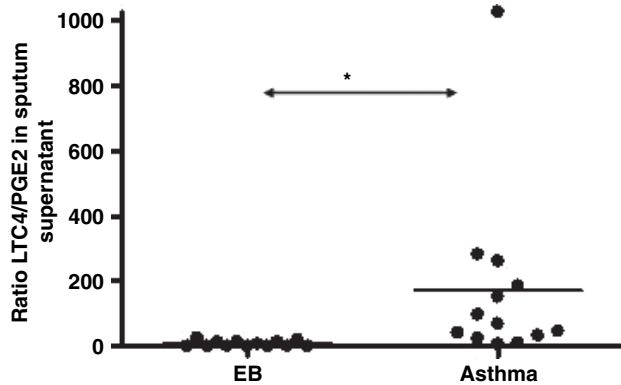


Figure 4. Ratio LTC₄/PGE₂ in induced sputum in eosinophilic bronchitis and asthmatics. Individual data and geometric mean from asthmatic and eosinophilic bronchitis subjects. Patients with asthma had a significantly higher LTC₄/PGE₂ ratio than patients with eosinophilic bronchitis (* P < 0.01).

patients with asthma than in eosinophilic bronchitis, supporting this hypothesis. We did not find any correlation between sputum eosinophil counts and PGE₂ levels in sputum supernatant. In contrast, other authors have found disparate results, reporting a negative or positive correlation between PGE₂ concentrations and sputum eosinophils (19, 28). As macrophages are a potential source of PGE₂ (29), we considered whether the low PGE₂ level in the sputum supernatants of asthmatic patients may be caused by reduced number of macrophages, but macrophage counts in eosinophilic bronchitis and asthmatic patients were similar.

Brightling et al. (30) also found numerically higher levels of PGE₂ in sputum from patients with eosinophilic bronchitis, but the differences were not statistically significant as compared with asthma patients. The discrepancy observed between this study and our results might be due to the different technique employed to

measure PGE₂, or to the different number of patients with eosinophilic bronchitis examined. Recently, elevated sputum PGE₂ concentrations have been found in all patients with chronic cough (31), although in this study cough variant asthma and eosinophilic bronchitis patients were included in the same group. One possible explanation can be that PGE₂ concentrations are elevated because of the mechanical effect of coughing *per se*, or it is possible that release of this mediator is related to the enhanced cough reflex sensitivity seen in subjects with chronic cough, because inhaled PGE₂ results in heightened cough reflex sensitivity to capsaicin (26).

The differences in sputum supernatant PGE₂ concentration between asthmatic and eosinophilic bronchitis patients may be the result of a different degradation kinetic or activity of enzymes implicated in the synthesis and/or degradation of products of arachidonic acid metabolism. It has been recently shown that Th2 cytokines have specific effects on PGE synthase 1 and 15-PGDH enzymes in airway human epithelial cells (32). Differences in these PGE₂-related pathways between patients with asthma and eosinophilic bronchitis deserve further research.

We postulate that PGE₂ elevation in patients with eosinophilic bronchitis may have protective effects against the development of bronchial hyperresponsiveness. The fact that PGE₂ and its analog have a number of bronchoprotective and anti-inflammatory effects *in vitro* (27) or after inhalation (10), as well as on allergen-induced airway responses and airway inflammation in atopic asthma (19, 33) would support such protective role. An imbalance in the ratio of bronchoconstrictor (LTC₄) and bronchoprotective (PGE₂) lipid mediators may have a role in the pathogenesis of eosinophilic bronchitis. Prostaglandin E₂ can perform contrasting activities, which can thus lead to bronchodilation and may act as an anti-inflammatory or pro-inflammatory mediator in the lung (34). Recently, several studies

showed that the PGE₂ protective actions were mediated in large part by the EP₂ receptors (35, 36). This protective action of PGE₂ might not only result in a direct effect on airway smooth-muscle relaxation, but also in the inhibition on many inflammatory processes (37).

This data can explain why patients with eosinophilic have normal tests of variable airway obstruction and airway responsiveness and experience chronic cough due to high PGE₂ levels because of its adverse effects of cough reflex and retrosternal burning (38). The pathogenesis of eosinophilic bronchitis might be the opposite to that observed in aspirin-induced asthma. There is increasing evidence that, by inhibiting cyclooxygenase-1, the protective effect exerted by endogenous PGE₂ on leukotriene generation by mast cells, eosinophils and macrophages in the airways is removed in the latter condition. In these patients with aspirin-induced bronchoconstriction, a low production of PGE₂ has been observed, seemingly because of deficient COX-2 regulation and increase expression of leukotriene-C4 synthase (39).

In conclusion, we have shown that PGE₂ is present in significantly higher concentrations in subjects with eosinophilic bronchitis than in asthmatic and control subjects. We suggest that this elevation in PGE₂ could explain the different physiological features observed in eosinophilic bronchitis and asthma, because of the protective effect of PGE₂ in airway tone and responsiveness. The findings in this study have potential implications to our understanding of the pathogenesis of eosinophilic bronchitis, although further studies are required to determine whether other mediators are involved and to determine the mechanism(s) associated with the beneficial role of PGE₂ in airway responsiveness.

Acknowledgment

This study was supported by Foundation SEAIC (Sociedad Española de Alergología e Inmunología Clínica); and fellowship grants from the Conchita Rábago Foundation (R.M) and the FIS (B.S).

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