

Exhaled leukotrienes and prostaglandins in asthma

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Background: Most of the studies investigating the role of leukotrienes (LTs) and prostaglandins (PGs) in asthma have used invasive (eg, bronchoalveolar lavage fluid) or semi-invasive (eg, sputum induction) techniques. Others have measured eicosanoids in plasma or urine, probably reflecting systemic rather than lung inflammation. Collection of exhaled breath condensate (EBC) is a noninvasive method to collect airway secretions.

Objective: We sought to investigate whether eicosanoids are measurable in EBC, to show possible differences in their concentrations in asthmatic patients and healthy subjects, and to investigate whether exhaled eicosanoids correlate with exhaled nitric oxide (NO), a marker of airway inflammation.

Methods: Twelve healthy nonsmokers and 15 steroid-naïve patients with mild asthma were studied. Subjects attended on one occasion for pulmonary function tests, collection of EBC, and exhaled NO measurements. Exhaled LTB₄-like immunoreactivity, LTE₄-like immunoreactivity, PGE₂-like immunoreactivity, PGD₂-methoxime, PGF_{2α}-like immunoreactivity, and thromboxane B₂-like immunoreactivity were measured by means of enzyme immunoassays.

Results: LTE₄-like immunoreactivity and LTB₄-like immunoreactivity were detectable in EBC in healthy subjects, and their levels in asthmatic patients were increased about 3-fold ($P < .0001$) and 2-fold ($P < .0005$), respectively. Exhaled NO was increased in asthmatic patients compared with healthy subjects ($P < .0001$). There was a correlation between exhaled LTB₄ and exhaled NO ($r = 0.56$, $P < .04$) in patients with asthma. When measurable, prostanoid levels were similar in asthmatic patients and control subjects.

Conclusions: Exhaled LTE₄ and LTB₄ are increased in steroid-naïve patients with mild asthma. EBC may be proved to be a novel method to monitor airway inflammation in asthma. (J Allergy Clin Immunol 2002;109:615-20.)

Key words: Leukotrienes, prostaglandins, exhaled breath condensate, nitric oxide, asthma, airway inflammation, noninvasive markers

Abbreviations used

BAL:	Bronchoalveolar lavage
CI:	Confidence interval
cysLT:	Cysteinyl leukotriene
EBC:	Exhaled breath condensate
LT:	Leukotriene
NO:	Nitric oxide
PGD ₂ -MOX:	PGD ₂ -methoxime
TX:	Thromboxane

Eicosanoids are important inflammatory mediators in asthma.¹ These lipid mediators include leukotrienes (LTs), prostaglandins (PGs), and thromboxane A₂ (TXA₂). LTC₄, LTD₄, and LTE₄, known as cysteinyl leukotrienes (cysLTs), contract airway smooth muscle, increase vascular permeability, stimulate mucus secretion, and decrease mucociliary clearance.² These effects may be relevant to bronchial obstruction in patients with asthma. Selective cysLT₁ receptor antagonists are currently used in asthma therapy.³ LTB₄ is a potent neutrophil chemoattractant that enhances neutrophil-endothelial interactions and stimulates neutrophil activation.² LTB₄ has weak effects on smooth muscle, but it may contribute to airway narrowing by producing local edema and by increasing mucus secretion.² The importance of LTB₄ in causing asthmatic airway inflammation is not known, although this compound could play a role, particularly during some exacerbations or severe persisting asthma, which is associated with increased neutrophils in the airways.^{4,5} PGs have both deleterious and beneficial effects on the lung. PGD₂ and PGF_{2α} cause bronchoconstriction in asthmatic patients but not in healthy subjects.^{6,7} Inhaled PGE₂ attenuates allergen-induced airway responses and airway inflammation in asthmatic patients.⁸ TXA₂ is a potent bronchoconstrictor and causes airway smooth muscle hyperplasia, a characteristic feature of asthmatic airways, in vitro.⁹

Eicosanoids have been measured in plasma, urine, sputum, and bronchoalveolar lavage (BAL) fluid in patients with asthma. Generally, no difference has been reported between baseline urinary LTE₄ levels in healthy and atopic asthmatic subjects,¹⁰ although one study showed that the urinary LTE₄ excretion rate is increased in asthmatic patients compared with that seen in healthy subjects.¹¹ LTB₄ and LTE₄ plasma levels^{12,13} and sputum cysLT concentrations¹⁴ are higher in asthmatic patients

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TABLE I. Subject characteristics*

	Healthy subjects	Asthmatic patients
No.	12	15
Age (y)	30 ± 3	34 ± 2
Sex (F/M)	7/5	8/7
Smoking	No	No
FEV ₁ (L)	4.45 ± 0.27	3.56 ± 0.29
FVC (L)	4.62 ± 0.30	3.89 ± 0.24
FEV ₁ (% predicted)	93.7 ± 3.7	88.7 ± 3.9
FVC (% predicted)	97.4 ± 2.8	93.8 ± 3.1
Atopy	No	12
PC ₂₀ (mg/mL)	>8	1.6 ± 0.5†

FVC, Forced vital capacity.

*Data are expressed as numbers or means ± SEM.

†*P* < .01 compared with healthy subjects.

than in healthy subjects. Asthmatic patients have higher BAL fluid LTB₄ and LTC₄ concentrations than healthy subjects.¹⁵ Patients with nocturnal asthma have increased BAL cysLT and LTB₄ concentrations and urinary LTE₄ levels than control subjects.¹⁶ Recently, cysLT-like immunoreactivity and LTB₄-like immunoreactivity have been measured in exhaled breath condensate (EBC) in asthmatic patients, and the levels were higher in these individuals than in healthy subjects.¹⁷

Most of the studies investigating the role of eicosanoids in asthma have used invasive techniques, such as measurement of BAL fluid,¹⁵ or measured these compounds in plasma or urine remote from the site of production.^{11,12} Concentrations of eicosanoids in these biologic fluids probably reflect systemic rather than lung inflammation. Moreover, sputum induction itself causes inflammation because it increases neutrophil counts in sputum after repeated induction within short intervals.¹⁸ We have previously shown that 8-isoprostane, a PGF_{2α} analogue that is formed by free radical-catalyzed peroxidation of arachidonic acid, is measurable in EBC in healthy subjects and is increased in patients with asthma of different severities, reflecting the degree of airway inflammation.¹⁹ In this study we measured LTs and PGs in EBC, which is a completely noninvasive method likely to reflect inflammation in the respiratory tract. We also measured exhaled nitric oxide (NO), a well-established marker of airway inflammation in patients with asthma not treated with corticosteroids.²⁰

The aims of this study were to investigate whether LTs and PGs are measurable in EBC in healthy nonsmoking subjects, to identify possible differences in exhaled eicosanoid levels between healthy subjects and steroid-naïve patients with mild asthma, and to investigate the relationship between exhaled eicosanoids and airway inflammation in asthma, as reflected by exhaled NO levels.

METHODS

Study subjects

Two groups of subjects were studied: 12 healthy nonsmokers and 15 patients with mild asthma who were nonsmokers (Table I). Asth-

matic patients attended the outpatient clinic at the Royal Brompton Hospital in London. Informed consent was obtained from all subjects. This study was approved by the Ethics Committee of the Royal Brompton Hospital and Harefield Trust.

The diagnosis of asthma was based on the criteria of the American Thoracic Society.²¹ Severity of asthma was classified according to the National Institute of Health–World Health Organization guidelines.²² Briefly, patients with mild asthma had symptoms twice a week or less often, with an FEV₁ of 80% of predicted value or greater, reversibility of greater than 15% with salbutamol, or a PC₂₀ of less than 8 mg/mL histamine. They were receiving no regular medication but used inhaled β₂-agonists as needed for symptom relief. No asthmatic patients had received inhaled or systemic corticosteroids, theophyllines, leukotriene antagonists, nonsteroidal anti-inflammatory drugs, or other prescribed medications in the previous 4 weeks. Patients had no upper respiratory tract infections in the previous 4 weeks. Atopy was assessed by means of skin prick tests for common allergens.

Repeatability for eicosanoid measurements was assessed in 15 patients with asthma in a randomized design in which a second breath condensate sample was collected while the patient was clinically stable within 7 days of obtaining the first sample.

Study design

The study was cross-sectional. Subjects attended the outpatient clinic at the Royal Brompton Hospital in London on one occasion for clinical examination, spirometry, EBC collection, and exhaled NO measurements.

Pulmonary function

Spirometry was measured with a dry spirometer (Vitalograph Ltd, Buckingham, United Kingdom), and the best value of the 3 maneuvers was expressed as an absolute value (in liters) and as a percentage of the predicted value.

Measurement of exhaled eicosanoids

EBC samples were collected with a specially designed condensing chamber (Ecoscreen; Jaeger, Hoechberg, Germany).²³ Exhaled air entered and left the chamber through 1-way valves at the inlet and outlet, thus keeping the chamber closed. Subjects breathed tidally through a mouthpiece connected to the condenser for 15 minutes while wearing nose clips. A temperature of −20°C inside the condensing chamber throughout the collection time produced immediate sample freezing. Samples were stored at −70°C before eicosanoid measurements.

LTB₄, LTE₄, PGE₂, PGF_{2α}, and TXB₂ concentrations in EBC were measured with specific enzyme immunoassay kits (Cayman Chemical, Ann Arbor, Mich). The detection limits for the individual assays were 4 pg/mL for LTB₄, 8 pg/mL for LTE₄, 30 pg/mL at room temperature for PGE₂, 8 pg/mL for PGF_{2α}, and 10 pg/mL for TXB₂. Standard concentrations that caused 50% of maximal binding values for the individual assays were 35 pg/mL for LTB₄, 55 pg/mL for LTE₄, 130 pg/mL at room temperature for PGE₂, 40 pg/mL for PGF_{2α}, and 50 pg/mL for TXB₂. Antiserum cross-reactivities greater than 1% (cross-reacting metabolite and percentage listed in parentheses) were as follows: LTB₄ (LTB₅, 100%; 6-*trans*-LTB₄, 39%); LTE₄ (LTE₅, 100%; N-acetyl-LTE₄, 20%; LTC₄, 10%; LTD₄, 9%; LTC₅, 5%; LTD₅, 4%); PGE₂ (PGE₃, 43%; PGE₁, 18.7%; 6-keto-PGF_{1α}, 15%), PGF_{2α} (PGF_{3α} and PGF_{1α}, 100%; PGD₂, 5%), and TXB₂ (2,3-dinor-TXB₂, 8.2%). Because PGD₂ is a relatively unstable eicosanoid, we measured PGD₂-methoxime (PGD₂-MOX), a stable derivative of PGD₂. The antiserum used to measure PGD₂-MOX has a 100% cross-reactivity with PGD₂-MOX and less than 1% with other eicosanoids. The sensitivity of the PGD₂-MOX assay is 3.24 pg/mL, and the concentration

that caused 50% of maximal binding values is 36.6 pg/mL. The intra-assay and interassay coefficients of variability of the eicosanoid assays were within 10% and 15%, respectively, across the range of values measured. Because of the possible cross-reactivity of the antisera against LTB₄, LTE₄, PGE₂, PGF_{2α}, and TXB₂ with structurally related eicosanoids, the values of these eicosanoids were referred to as eicosanoid-like immunoreactivity.

The possible influence of the ventilation rate on exhaled LTB₄-like immunoreactivity, LTE₄-like immunoreactivity, and PGE₂-like immunoreactivity concentrations and on breath condensate volumes collected during the 15-minute breath test was assessed. Five healthy subjects breathed at 14 and 28 breaths/min for 15 minutes, maintaining the same tidal volume. There was no difference in the concentration of LTB₄-like immunoreactivity (41.2 ± 3.7 vs 43.9 ± 4.1 pg/mL), LTE₄-like immunoreactivity (12.8 ± 1.5 vs 13.9 ± 2.3 pg/mL), and PGE₂-like immunoreactivity (38.8 ± 4.5 vs 46.1 ± 4.1 pg/mL) in the 2 samples collected from the same subject, whereas the volumes of EBC collected in the 15-minute test were higher at 28 breaths/min compared with those at 14 breaths/min (2.4 ± 0.3 vs 1.4 ± 0.2 mL, $P < .01$). Values of LTs, PGs, and TXB₂ in EBC were corrected by volume and expressed as total amount (in picograms) of eicosanoids expired in the 15-minute breath test (eicosanoid concentration $\times$ volume of EBC).

Saliva contamination of EBC was excluded by measuring amylase concentrations in 6 samples. Amylase concentrations were undetectable in all the samples tested. Eicosanoids were measured within 2 weeks after the collection of the EBC samples.

Exhaled NO measurement

Exhaled NO was measured with a chemiluminescence analyzer (model LR2000; Logan Research, Rochester, United Kingdom), which was sensitive to NO levels from 1 to 5000 ppb (by volume) and had a resolution of 0.3 ppb, which was designed for online recording of exhaled NO concentration, as previously described.²⁴ The analyzer was calibrated with certified NO mixtures (90 ppb) in nitrogen (BOC Special Gases, Guilford, United Kingdom). Measurements of exhaled NO were made by means of single-breath slow exhalations (5–6 L/min) from total lung capacity for 20 to 30 seconds against a resistance (3 ± 0.4 mm Hg) to prevent nasal contamination. Two successive recordings were made, and mean values were used in all calculations.

Statistical analysis

Data were normally distributed after logarithmic transformation. For comparing 2 groups, unpaired Student *t* tests were used. Linear regression analysis was used to assess the relationship between exhaled eicosanoids and between exhaled eicosanoids and exhaled NO or FEV₁. All data were expressed as means $\pm$ SEM, and significance was defined as a *P* value of less than .05. Repeatability of exhaled eicosanoid measurements was expressed as the intraclass correlation coefficient.²⁵

RESULTS

LTE₄-like immunoreactivity was increased in EBC in asthmatic patients (33.0 ± 3.8 pg; $P < .0001$; 95% confidence interval [CI], 24.9–41.0) compared with that in healthy subjects (13.0 ± 1.6 pg; 95% CI, 9.4–16.6; Fig 1, A). Exhaled LTB₄-like immunoreactivity was also increased in asthmatic patients (88.9 ± 10.9 pg; $P < .0005$; 95% CI, 65.5–112.3) compared with that in control subjects (38.8 ± 1.8 pg; 95% CI, 34.9–42.7; Fig 1, B). Patients with asthma (17.8 ± 2.3 ppb; $P < .0001$; 95% CI, 12.9–22.8) had higher exhaled NO levels than control

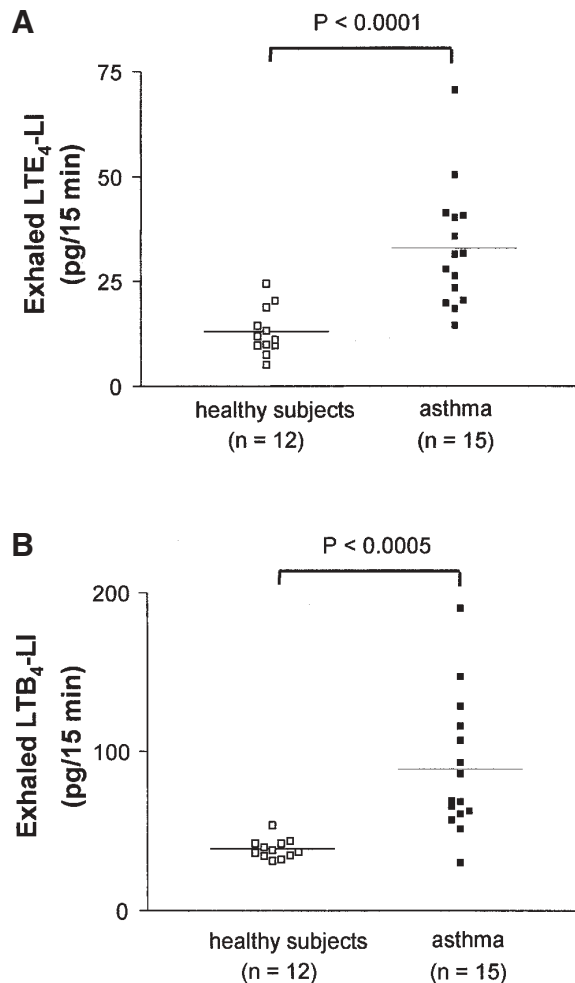


FIG 1. A, LTE₄-like immunoreactivity (LI) in EBC in healthy subjects (open squares) and asthmatic patients (filled squares). **B,** LTB₄-like immunoreactivity (LI) in EBC in healthy subjects (open squares) and asthmatic patients (filled squares). LTE₄-like immunoreactivity and LTB₄-like immunoreactivity are expressed as picograms produced during 15 minutes of breathing. Mean values are shown with horizontal bars.

subjects (5.3 ± 1.5 ppb; 95% CI, 4.3–6.2; Fig 2). There was a correlation between exhaled LTB₄ and exhaled NO ($r = 0.56$, $P < .04$) in patients with asthma (Fig 3). In these patients the correlation between exhaled LTE₄ and exhaled NO was not significant ($r = 0.48$, $P = .07$, data not shown). Exhaled PGE₂-like immunoreactivity was detectable in all subjects of each study group, and its levels were similar in asthmatic patients (47.7 ± 2.9 pg; 95% CI, 41.5–53.9) and healthy subjects (45.6 ± 3.9 pg; 95% CI, 37.0–54.1; Fig 4). Other prostanoids were detectable only in some subjects studied. PGD₂-MOX was measurable in all asthmatic patients (10.8 ± 0.7 pg; 95% CI, 9.4–12.2) and in 5 healthy subjects (12.0 ± 1.2 pg; 95% CI, 8.6–15.4; Fig 4). PGF_{2α}-like immunoreactivity was measurable in 10 patients with asthma (11.1 ± 1.5 pg; 95% CI, 7.7–14.5) and in 4 healthy subjects (8.4 ± 1.2 pg; 95% CI, 4.7–12.2; Fig 4). In those subjects in whom PGD₂-MOX and PGF_{2α}-like immunoreactivity were measurable,

Asthma, rhinitis,
other respiratory
diseases

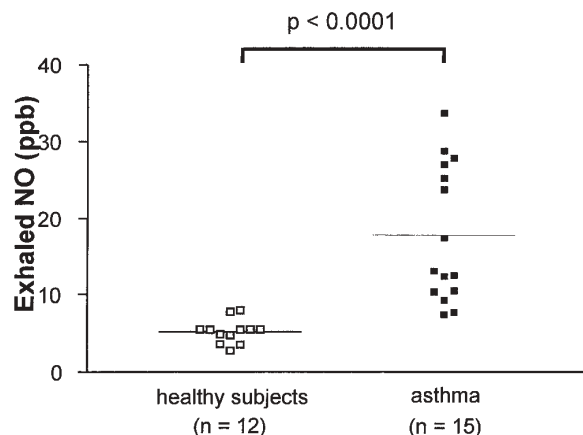


FIG 2. Exhaled NO levels in healthy subjects (open squares) and asthmatic patients (filled squares). Exhaled NO levels are expressed as parts per billion. Mean values are shown with horizontal bars.

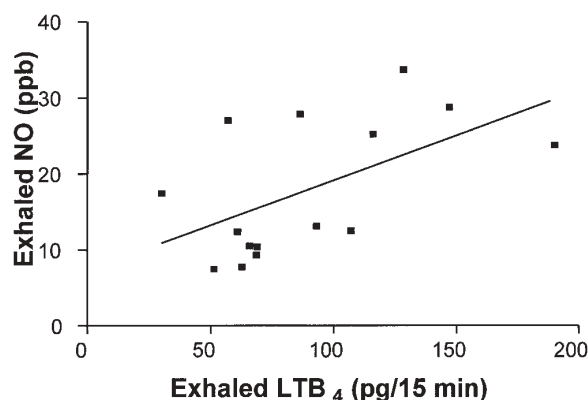


FIG 3. Correlation between exhaled LTB₄-like immunoreactivity and exhaled NO in patients with asthma ($r = 0.56$, $P < .04$, $n = 15$). LTB₄-like immunoreactivity is expressed as picograms produced during 15 minutes of breathing. Exhaled NO levels are expressed as parts per billion.

there was no difference in the levels of these PGs between the 2 study groups (Fig 4). TXB₂-like immunoreactivity was detectable in 7 asthmatic patients (9.1 ± 0.5 pg; 95% CI, 7.8-10.4) and was below the sensitivity of the assay in the other patients with asthma and in the healthy subjects (Fig 4).

There was no correlation between exhaled eicosanoids and age, sex, and lung function in any study group. There was no correlation between the different exhaled eicosanoids in any study groups. Apart from LTB₄, there was no correlation between exhaled eicosanoids and exhaled NO. The intraclass correlation coefficient was 0.69 for LTB₄-like immunoreactivity, 0.79 for LTE₄-like immunoreactivity, 0.75 for PGE₂-like immunoreactivity, 0.81 for PGD₂-MOX, and 0.73 for PGF_{2 α} -like immunoreactivity. The intraclass correlation coefficient for TXB₂-like immunoreactivity was not calculated because this eicosanoid was undetectable in most (20/27) of the subjects studied.

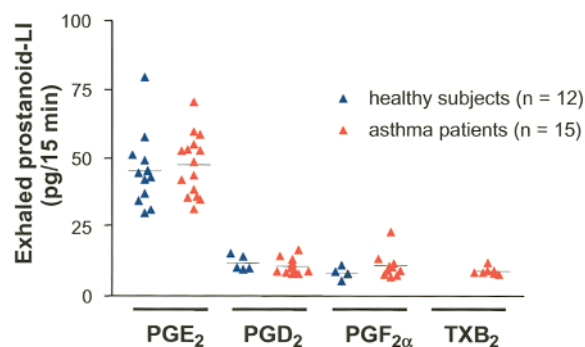


FIG 4. PGE₂-like immunoreactivity (LI), MOX-PGD₂, PGF_{2 α} -like immunoreactivity, and TXB₂-like immunoreactivity in EBC in healthy subjects (blue triangles) and asthmatic patients (red triangles). Immunoreactivities of prostanoids are expressed as picograms produced in 15 minutes of breathing. PGD₂ (as the stable derivative PCG₂-methoxine) was undetectable in 7 healthy subjects, PGF_{2 α} -like immunoreactivity was undetectable in 8 healthy subjects and in 5 asthmatic patients, and TXB₂-like immunoreactivity was undetectable in 8 asthmatic patients and in all healthy subjects. Mean values are shown with horizontal bars.

DISCUSSION

In this study we have shown that LTE₄-like immunoreactivity is increased in EBC in steroid-naïve patients with mild asthma, who have about 3-fold higher levels than healthy subjects. Increased LTE₄-like immunoreactivity levels in patients with asthma are consistent with a role for cysLTs in the pathophysiology of this disease. Most of the previous studies failed to show any increase in LTE₄ levels in patients with mild asthma.¹⁰ However, LTE₄ concentrations were generally measured in plasma or urine, biologic fluids that are likely to reflect systemic, rather than local, production of this eicosanoid. Indeed, a recent study measured increased LTE₄ concentrations in sputum of patients with asthma, indicating increased local production of cysLTs.¹⁴ In the present study, we have also shown that exhaled LTB₄-like immunoreactivity is increased in patients with mild asthma. A recent study reported increased exhaled cysLTs and LTB₄ levels in patients with moderate and severe asthma but not in patients with mild asthma.¹⁷ These discrepancies may be due to a higher degree of airway hyperresponsiveness in our group of patients with mild asthma, as reflected by lower PC₂₀ values (1.6 ± 0.5 vs 4.5 ± 0.6 mg/mL).

Exhaled NO reflects airway inflammation in patients with asthma not treated with corticosteroids.²⁰ In agreement with previous studies, exhaled NO levels in the present study were increased in steroid-naïve patients with mild asthma. In these patients exhaled LTB₄ correlated with exhaled NO, suggesting that NO released from inflammatory cells is increased by some conditions that lead to the production of this eicosanoid. The relationship between exhaled LTE₄ and exhaled NO was weak and did not reach statistical significance. These findings may be partly explained by the limited number of asthmatic patients studied. Alternatively, exhaled LTE₄ and exhaled NO may reflect different aspects of airway

inflammation, as suggested by unchanged levels of exhaled NO after LTE₄ inhalation in patients with asthma.²⁶ There was a wide interindividual variability in exhaled LTE₄ and LTB₄ levels in patients with mild asthma. These findings could be explained by a different degree of airway inflammation, as indicated by a similar high variability of exhaled NO levels in these patients. We demonstrated for the first time that PGE₂-like immunoreactivity is measurable in EBC in healthy subjects and patients with asthma. Considering that PGE₂ has bronchoprotective and inhibitory effects on inflammatory cells at concentrations known to occur in the airways,²⁷ impaired production of PGE₂ has been proposed to contribute to the pathogenesis of asthma. Our data do not support this hypothesis and are in agreement with a previous study that reported similar PGE₂ levels in sputum in asthmatic patients and healthy subjects.¹⁴

The cellular source of the eicosanoids measured in EBC is not yet known. EBC analysis cannot provide any information on the cellular origin of mediators for which invasive techniques, such as bronchial biopsies, are required. Among cells constitutively present in the airways, cysLTs are mainly produced by mast cells and macrophages.²⁸ Eosinophils, which are not present in the airways of healthy subjects, migrate into the airways of asthmatic patients in large numbers and may synthesize cysLTs, as well as LTB₄.²⁸ Neutrophils may produce LTB₄,¹ but not cysLTs, because they lack the synthase to convert LTA₄ to LTC₄.²⁸ PGE₂ is largely produced by epithelial and airway smooth muscle cells, but all cells in the airways have the capacity to release prostanoids.¹ Assessment of inflammatory cell types in the lung can be done with sputum induction. However, this technique itself causes airway inflammation, as reflected by increased neutrophil cell counts after repeated sputum induction¹⁸ and increased exhaled LTB₄ levels after a single induction in healthy subjects.²⁹ These findings indicate that sputum induction may affect the concentrations of inflammatory markers in sputum supernatants. An integrated approach consisting of measurements of eicosanoids in EBC collected before sputum induction is likely to achieve a better noninvasive quantification of inflammation in asthma. Studies aiming at measuring eicosanoid levels in bronchial biopsy specimens from asthmatic patients may provide information on the cellular source or sources of these mediators.

We were unable to assess the effects of corticosteroids on EBC eicosanoids because asthmatic patients were steroid naive. Controversial results have been reported on the effects of corticosteroids on eicosanoids in asthma, and controlled studies with these drugs are required. Measurement of eicosanoids in EBC is a noninvasive method that could clarify the actions of corticosteroids and other anti-inflammatory drugs on these important inflammatory mediators in asthma.

Measurement of eicosanoids in EBC may have important implications in asthma. First, characterization of selective patterns of exhaled eicosanoids may help differentiate between inflammatory airway diseases. Sec-

ond, evidence for increased airway inflammation, as reflected by exhaled LTE₄ and LTB₄ levels, in asthmatic patients with mild symptoms and good lung function test results may be an indication to start anti-inflammatory treatment before lung function deterioration. Third, considering that EBC analysis of eicosanoids is completely noninvasive, this method can be particularly useful for longitudinal studies, in children with asthma, and in patients with severe asthma.

In conclusion, we have shown that exhaled LTE₄ and LTB₄ levels are increased in patients with asthma not treated with corticosteroids. Identification of selective profiles of eicosanoids in EBC, a novel noninvasive approach to monitor lung inflammation, may prove to be relevant for the diagnosis and management of asthma.

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