Exhaled breath condensate cysteinyl leukotrienes are increased in children with exercise-induced bronchoconstriction

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Background: It is recognized that airway inflammation has a central role in the pathogenesis of asthma, but how it relates to exercise-induced bronchoconstriction (EIB) is not completely understood.
Objective: The aim of our study was to investigate the relationship between EIB and baseline concentrations of cysteinyl leukotrienes (Cys-LTs) and other inflammatory markers in exhaled breath condensate (EBC).
Methods: EBC was collected, and the fraction of exhaled nitric oxide (FE_{NO}) was measured in a group of 19 asthmatic children, after which they performed a treadmill exercise test. Fourteen healthy children were enrolled as control subjects.
Results: The asthmatic children were divided into the EIB group (decrease in FEV_1, ≥12%) and the non-EIB group. The EBC was analyzed for the presence of Cys-LTs, leukotriene B_4, and ammonia. Asthmatic patients with EIB (mean FEV_1 decrease, 23% ± 3%) had higher Cys-LT concentrations than either asthmatic patients without EIB or control subjects (42.2 pg/mL [median] vs 11.7 pg/mL and 5.8 pg/mL; $P < .05$ and $P < .001$, respectively). Ammonia concentrations were lower in both the EIB and non-EIB groups than in control subjects (253.2 μM and 334.6 μM vs 798.4 μM; $P < .01$ and $P < .05$, respectively). No difference in EBC leukotriene B_4 levels was found among the 3 groups. Both asthmatic groups had higher FE_{NO} levels than control subjects ($P < .001$). EBC Cys-LT ($P < .01$; $r = 0.7$) and FE_{NO} ($P < .05$; $r = 0.5$) values both correlated significantly with the postexercise FEV_1 decrease.
Conclusion: this study shows that EBC Cys-LT values are higher in asthmatic children with EIB and correlate with the decrease in FEV_1 after exercise. These findings suggest that the pathways of both Cys-LT and nitric oxide are involved in the pathogenesis of EIB. (J Allergy Clin Immunol 2005;115: 764-70.)

Key words: *Asthma, exercise-induced bronchoconstriction, exhaled breath condensate, exhaled nitric oxide, cysteinyl leukotrienes, leukotriene B4, ammonia, montelukast*

Abbreviations used
Cys-LT: Cysteinyl leukotriene
EBC: Exhaled breath condensate
EIB: Exercise-induced bronchoconstriction
$FEF_{25\%-75\%}$: Forced expiratory flow, midexpiratory phase
FE_{NO}: Fractional exhaled nitric oxide
IQR: Interquartile range
LTB_4: Leukotriene B_4
NO: Nitric oxide

Exercise-induced bronchoconstriction (EIB) is a common sign of bronchial hyperreactivity and airway inflammation, occurring in 70% to 80% of patients with current asthma.[1,2] It is more frequent in children and young adults because they are more involved in physical activities. EIB is considered a self-limiting phenomenon, but fatal asthma exacerbations precipitated by sporting activities have recently been reported.[3]

Although the mechanisms behind EIB are incompletely understood, there are 2 main hypotheses concerning its pathogenesis.[4] The thermal hypothesis is that airway cooling and rewarming cause reactive hyperemia and edema, leading to airway obstruction.[5] The osmotic hypothesis, on the other hand, is that water and heat loss from the respiratory tree causes airway surface hyperosmolarity and mast cell degranulation, with the release of rapid-acting mediators, such as histamine and leukotrienes.[4] Both these mechanisms are likely to be at work in EIB.[4]

Among the inflammatory cells, not only mast cells but also eosinophils are involved in the pathogenesis of EIB, and the latter can produce and release cysteinyl leukotrienes (Cys-LTs) as well.[6] It has been demonstrated that the maximal decrease in FEV_1 after exercise correlates with the percentage of sputum eosinophils at baseline.[7,8]

There is direct and indirect evidence that leukotrienes are involved in EIB.[1] An increase in urinary concentrations of LTE_4 after exercise has been shown in asthmatic subjects,[9,10] and the best evidence of the role of leukotrienes in EIB comes from the protection provided by antileukotriene medication.[11-13] However, although blocking the leukotriene pathway completely inhibits EIB in some patients, it has little or no effect in others,[1] suggesting that although leukotrienes play a major part, other mediators or mechanisms might contribute to EIB pathogenesis in some patients.[1] In this regard some studies

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have shown a relationship between exhaled nitric oxide (NO) levels and EIB,[14,15] suggesting a role for NO in EIB pathogenesis.

Most of the studies investigating the role of leukotrienes in EIB measured these eicosanoids in plasma and urine. Some authors have reported higher leukotriene levels after exercise challenge,[9,10] but others have not.[16,17] However, biologic fluids, such as urine and blood, are collected remote from the lung; therefore, they might reflect systemic rather than lung eicosanoid production. The recent development of the exhaled breath condensate (EBC) method enables a noninvasive investigation of several mediators and metabolites directly sampled from the airways.[18,19]

A new phenomenon recently described using EBC is the potential role of airway acidification in the pathogenesis of asthma.[18] Acidopnea occurs in the airways of patients with asthma exacerbation (returning to normal after corticosteroid treatment), and it seems to correlate with airway inflammation.[20] Ammonia produced by airway epithelial cells seems to have a role in maintaining airway pH homeostasis.[21] Although a relationship between ammonia and EIB has not been evaluated as yet, a dysregulation of the acid-base equilibrium of the airways could be involved in the pathogenesis of EIB.

To date, there have been no studies evaluating the relationship between EIB and inflammatory markers measured directly in samples obtained from the airways. The main objective of the present study was to evaluate EBC Cys-LT, leukotriene B_4 (LTB_4), and ammonia concentrations and fraction of exhaled NO (FE_{NO}) levels in asthmatic children with and without EIB and to determine whether the severity of EIB is associated with these mediators. A secondary aim of the study was to assess the postexercise decrease in FEV_1, FE_{NO} level, and EBC Cys-LT concentration after a 3-day course of montelukast in children with proved EIB.

METHODS

Study subjects

Nineteen asthmatic children aged 7 to 18 years were consecutively enrolled in this study. They were recruited from patients attending the pulmonology-allergy outpatient clinic at the Pediatrics Department in Padova. The diagnosis of asthma was based on clinical history and examination, pulmonary function parameters, and response to β_2-agonists, according to international guidelines.[22] The patients had had no exacerbation or hospitalization for asthma for at least 2 months. Children were excluded from the study if they had a respiratory tract infection in the last 3 weeks. Eleven children with mild-to-moderate persistent asthma had been on maintenance therapy with low-to-medium constant doses of inhaled corticosteroids (budesonide or fluticasone)[22] for at least 2 months. Eight children with intermittent asthma were steroid free. None of the patients were being treated with leukotriene receptor antagonists. Asthmatic children used β_2-agonists on demand, but they refrained from taking this medication for at least 24 hours before the test.

All the asthmatic children were atopic and sensitized to common allergens (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, mixed grass pollen, *Artemisia vulgaris*, *Alternaria* species, dog, and cat), as evaluated by means of skin prick tests, RASTs, or both. None of the children with grass pollen allergy were studied during the grass pollen season.

The study also included 14 healthy control subjects (ie, white children with no history of asthma or atopy) who had no respiratory infections in the previous 4 weeks. None of the control subjects were taking any medication or smoked.

The ethical committee at our hospital approved the study, and all parents provided their informed consent.

Study design

The asthmatic children enrolled in the study underwent physical examination, FE_{NO} measurement, and EBC collection and then completed a standardized treadmill exercise test. EBC was collected and analyzed for the presence of Cys-LTs, LTB_4, and ammonia. Children who had EIB (decrease in FEV_1, $\geq$12% after exercise) were given montelukast treatment (5 mg at $<$13 years of age and 10 mg at $>$13 years of age).[22] After 3 days of therapy, they repeated the FE_{NO} measurement, the EBC collection for Cys-LT assay, and the treadmill exercise test.

Healthy control subjects underwent EBC collection, FE_{NO} measurement, and spirometry.

EBC collection

EBC samples were collected in a condensing device composed of 2 glass chambers (Incofar Srl, Modena, Italy).[23] The inner glass chamber was cooled with ice and suspended in a larger glass chamber. Without using a nose clip, the children were instructed to breathe tidally through their mouths via a 2-way nonrebreathing valve for 15 minutes. The 2-way valve also served as a saliva trap. Moreover, the glass condenser was placed on a higher level than the child's mouth, with a 12-cm banded tube positioned vertically between the mouthpiece and the condensing device. This makes salivary contamination unlikely. Children were also asked to swallow their saliva periodically. The collection temperature was around 0°C. The collected EBC samples were then immediately stored in sterile tubes at −70°C.

EBC marker detection

Leukotrienes. Cys-LT and LTB_4 concentrations were measured by using specific enzyme immunoassay kits (LTC_4, LTD_4, and LTE_4: Amersham Pharmacia Biotech, Milan, Italy; LTB_4: Cayman Chemical Milan, Italy), according to the manufacturer's instructions, as described elsewhere.[23] For Cys-LT and LTB_4 EBC levels of less than the detection limit (4 pg/mL for Cys-LTs and 1 pg/mL for LTB_4), data were expressed as half of the detection limit.

Ammonia. In aqueous solution there is an equilibrium of 2 species, NH_3 and NH_4^+, which are invariably present together in a ratio determined by the pH. At the derivatization pH of 9.8, only 25% is NH_4^+, but ammonium is higher at the pH observed in EBC.[24]

NH_4^+ in EBC was measured by means of HPLC with fluorescence detection with a dansyl chloride precolumn derivatization method.[25] Briefly, 200 μL of EBC sample or standard was derivatizated in 0.7 mL of Gilson vials, adding 200 μL of borate buffer, 0.1 M at pH 9.8, followed by 200 μL of 2 mM dansyl chloride stock solution in acetone. After incubation for 90 minutes at room temperature, a 20-μL sample was injected into the HPLC. If the EBC sample was insufficient, derivatization was done with 100 μL. Chromatography of the NH_3 dansyl chloride adduct was done with an isocratic elution on a Superchrom LC18 column (250 × 4.6 mm inside diameter, 5 μm; Varian, Milan, Italy), with a mobile phase composed of acetonitrile/methanol/water (3:7:7) at a flow rate of 0.8 mL/min. Fluorometric detection was performed at 500 nm after excitation at 368 nm (Scanning Fluorescence Detector 474; Waters, Milan, Italy). The retention time was approximately 8 minutes. The dansyl

TABLE I. Subjects' characteristics, spirometric parameters, EBC biomarker values, and exhaled NO

	EIB children	Non-EIB children	Healthy children
No. (male subjects)	11 (7)	8 (7)	14 (9)
Age (y)	11.5 (8-18)	11.6 (7-14)	9.8 (6-14)
Inhaled steroids (no.)	5	6	–
FEV_1 (% predicted)*	90 ± 3	95 ± 3	96 ± 3
$FEF_{25\%-75\%}$ (% predicted)*	77 ± 5‡§	102 ± 10	101 ± 5
FEV_1 decrease (%)*	23 ± 3	5 ± 1	
Exhaled NO (ppb)†	57.5 (37.0-68.7)¶	29.5 (19.2-68.4)¶	8.8 (6.0-11.0)
EBC Cys-LTs (pg/mL)†	42.2 (19.4-97.1)‡¶	11.7 (6.1-20.2)	5.8 (2.0-15.8)
EBC LTB_4 (pg/mL)†	4.6 (3.5-23.7)	5.6 (3.3-12.9)	4.3 (2.7-24.2)
EBC ammonia (μM)†	253.2 (173.4-414.8)‖	334.6 (201.7-615.3)‡	798.4 (648.1-1263.6)

*Data are expressed as means ± SEM.
†Data are expressed as medians and IQRs.
‡$P < .05$ versus children without EIB.
§$P < .05$ versus healthy children.
‖$P < .01$ versus healthy children.
¶$P < .001$ versus healthy children.

ammonia derivative was stable in air and ambient light at room temperature for many hours.[26] An appropriate water blank was also analyzed, and a calibration curve for NH_3 was created with each batch of samples. Under the conditions described, the method's quality parameter for NH_3 was as follows: low detection limit, greater than 1 μM; recovery, greater than 98%; intraday coefficient of variation, less than 3%; interday coefficient of variation, less than 5%; operative linearity range, 20-3000 μM.

FE_{NO} measurement

FE_{NO} was measured with the NIOX system (Aerocrine, Stockholm, Sweden) by using a single-breath online method according to the European Respiratory Society/American Thoracic Society guidelines for FE_{NO} measurement in children.[27] Children inhaled NO-free air and exhaled through a dynamic flow restrictor with a target flow of 50 mL/s for at least 6 to 7 seconds. Visual incentives provided feedback for flow-rate compliance.

Exercise challenge and spirometry

Exercise testing was done on an electrically driven treadmill (PK Morgan Ltd, Gillingham, Kent, United Kingdom) for 6 minutes under stable environmental conditions (temperature, 20°C-22°C; humidity, 50%-55%). The workload was increased until 80% of the predicted maximal heart rate was reached. All subjects were studied in the afternoon.

Spirometric measurements were performed with subjects at rest and 1, 3, 9, 15, and 20 minutes after the end of exercising by using a 10-L bell spirometer (Biomedin, Padova, Italy). The best of 3 FEV_1 values was expressed as a percentage of predicted reference values. To investigate subjects with a significant decrease in FEV_1 after exercise, a 12% postexercise decrease in FEV_1 cutoff was considered suitable because this is generally accepted to define EIB.

Heart rate was recorded continuously with a heart rate meter (Sport Tester TM PE 3000; Polar Electro, Kempelehea, Finland) during exercise and recovery.

Statistical analysis

Results are expressed as means ± SEM for normally distributed data (spirometric parameters) and as medians and interquartile ranges (IQRs) for nonnormally distributed data. For the latter, differences between groups were analyzed by using the nonparametric Kruskal-Wallis test, followed, where significant, by the Mann-Whitney *U* test for comparisons between groups. Correlations were evaluated by using the Spearman rank test. Results were considered significant at a *P* value of less than .05.

RESULTS

Nineteen consecutive asthmatic children and 14 healthy children were tested.

Eleven asthmatic children had a decrease in FEV_1 of 12% or greater after the exercise (EIB group, mean decrease in FEV_1 of 23% ± 3%), whereas 8 subjects had a less than 12% decrease (non-EIB group, mean decrease in FEV_1 of 5% ± 1%). Nine of the 11 children with EIB repeated EBC collection, FE_{NO} measurement, and exercise challenge after a 3-day course of montelukast.

Children in the EIB, non-EIB, and healthy control groups did not differ in demographic terms (Table I).

EBC leukotrienes

At baseline, the EBC Cys-LT levels were significantly higher in the EIB group than in either the non-EIB asthmatic group or the control group (42.2 pg/mL [IQR, 19.4-97.1] vs 11.7 pg/mL [IQR, 6.1-20.2] and 5.8 pg/mL [IQR, 2.0-15.8], respectively; $P < .05$ and $P < .001$, respectively; Table I and Fig 1). No significant difference in EBC Cys-LT concentrations was found between the non-EIB asthmatic group and the healthy control children ($P = .14$).

There was no significant difference in EBC values of LTB_4 among the 3 groups ($P = .67$, Table I).

After 3 days of montelukast therapy, 9 asthmatic children with EIB repeated EBC collection for Cys-LTs, which revealed a mean reduction of 32%, although this did not reach statistical significance (before therapy: 42.2 pg/mL [IQR, 17.1-136.3], after 3 days: 12.5 pg/mL [IQR, 9.5-24.5]; $P = .098$, Table II). There was no longer any difference in Cys-LT concentrations between EIB children and healthy children after 3 days of montelukast treatment ($P = .095$).

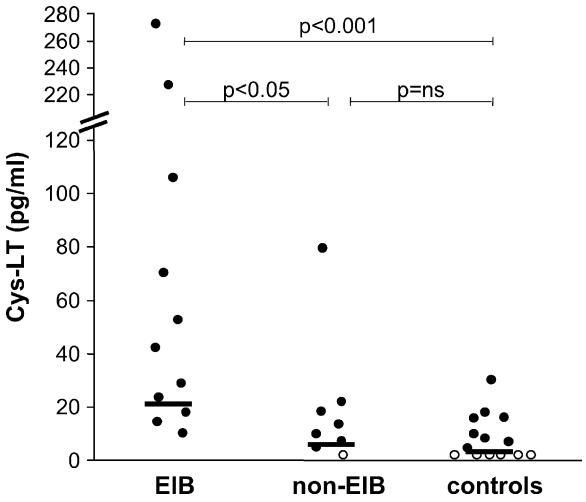

FIG 1. Cys-LT levels in EBC of asthmatic children with EIB, asthmatic children without EIB, and healthy control children. *Open circles* represent samples in which the Cys-LT concentration was less than the detection limit. *Horizontal bars* represent median values.

EBC ammonia

EBC ammonia levels before exercise were significantly greater in healthy children (798.4 μM [IQR, 648.1-1263.6 μM]) than in either the EIB (253.2 μM [IQR, 173.4-414.8 μM], $P < .01$) or non-EIB (334.6 μM [IQR, 201.7-615.3 μM], $P < .05$) asthmatic groups, whereas no significant difference in ammonia concentrations emerged between the asthmatic groups with and without EIB (Table I and Fig 2). For technical reasons, ammonia values were not measured in 8 children.

FE_{NO}

At baseline, FENO values were significantly higher in asthmatic than in healthy children (EIB group: 57.5 ppb [IQR, 37.0-68.7 ppb], non-EIB group: 29.5 ppb [IQR, 19.2-68.4 ppb], control group: 8.8 ppb [IQR, 6.0-11.0 ppb]; $P < .001$, Table I).

There was no significant change in baseline FE_{NO} values in children with EIB who repeated the exercise challenge after 3 days of montelukast treatment ($P = .99$, Table II).

Spirometric parameters

No difference in baseline FEV_1 was seen among the 3 groups ($P = .29$). At baseline, forced expiratory flow, midexpiratory phase ($FEF_{25\%-75\%}$) values were significantly lower ($P < .05$) in children with EIB (77% ± 5% of predicted value) than in either children without EIB (102% ± 10% of predicted value) or healthy children (101% ± 5% of predicted value, Table I). There was no difference between children without EIB and healthy children.

After 3 days of montelukast treatment, the children with EIB had a significant reduction in the maximal decrease in FEV_1 after exercise (21% ± 3% vs 14% ± 2%, $P < .01$), and 3 of the 9 children studied no longer had EIB (Table II and Fig 3). No significant change in FEV_1 or $FEF_{25\%-75\%}$ with respect to the baseline was found after 3 days of montelukast therapy ($P = .61$ and $P = .83$, respectively; Table II).

TABLE II. Spirometric parameters, EBC Cys-LT levels, and exhaled NO levels in children with EIB after 3 days of montelukast treatment

	Baseline	After montelukast
No. (male subjects)	9 (6)	9 (6)
FEV_1 (% predicted)*	92 ± 3	91 ± 2
$FEF_{25\%-75\%}$ (% predicted)*	77 ± 5	76 ± 4
FEV_1 decrease (%)*	21 ± 3	14 ± 2‡
Exhaled NO (ppb)†	57.5 (34.3-67.6)	53.6 (31.1-89.1)
EBC Cys-LTs (pg/mL)†	42.2 (17.1-136.3)	12.5 (9.5-24.5)

*Data are expressed as means ± SEM.
†Data are expressed as medians and IQRs.
‡$P < .01$ versus baseline.

Correlations

In the whole group of asthmatic children, the maximal postexercise decrease in FEV_1 strongly correlated with Cys-LT levels ($r = .7$; $P < .01$; Fig 4), whereas it did not correlate with either LTB_4 ($P = .7$) or ammonia ($P = .4$) levels. There was also a significant correlation between baseline FE_{NO} values and maximal postexercise decrease in FEV_1 ($r = 0.5$; $P < .05$). No significant correlation was found between maximal decrease in FEV_1 and either baseline FEV_1 ($P = .32$) or $FEF_{25\%-75\%}$ ($P = .06$) values. There was no correlation between baseline Cys-LT levels and FE_{NO} values ($P = .50$). After 3 days of montelukast, no correlation was found between the change in Cys-LT levels and the change in baseline FEV_1 ($P = .9$), maximal decrease in FEV_1 ($P = .4$), and FE_{NO} levels ($P = .5$).

DISCUSSION

EBC collection is a noninvasive method for obtaining samples from the airways.[18,19] This is the first study showing that asthmatic children with EIB have higher levels of EBC Cys-LTs than patients without EIB and healthy control subjects. Moreover, a significant positive correlation was found between baseline Cys-LT levels and the maximal decrease in FEV_1 after exercise (Fig 4), supporting the role of these eicosanoids in the pathogenesis of EIB. Several studies have reported higher Cys-LT values in the EBC of asthmatic subjects,[23,28] and the presence of these eicosanoids in EBC has recently been confirmed by using gas chromatography–mass spectrometry, a highly specific analytic method.[29]

Although the pathogenesis of EIB is not completely understood, it has been suggested that hyperosmolarity caused by cooling and drying of the airway mucosa during exercise results in mast cell degranulation and release of mediators,[4] among which leukotrienes are considered the

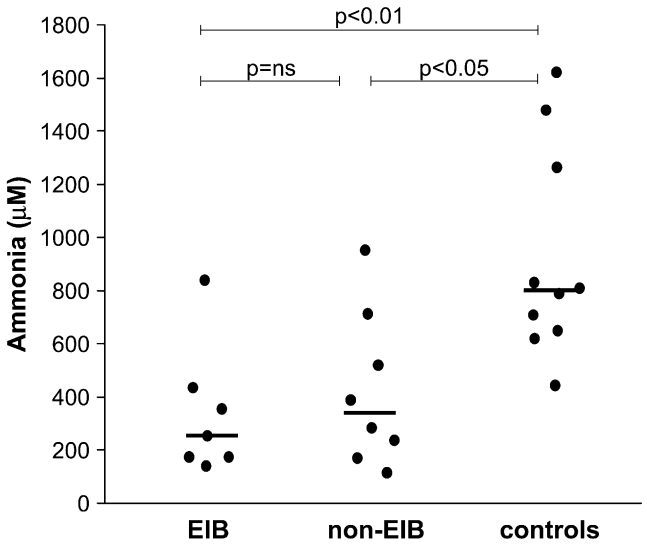

FIG 2. Ammonia levels in EBC of asthmatic children with EIB, asthmatic children without EIB, and healthy control children. *Horizontal bars* represent median values.

most important bronchoconstrictor agents, being a thousand-fold more potent than histamine.[6]

In accordance with the osmotic hypothesis, an increase in urinary LTE_4 excretion after an exercise challenge has been demonstrated in both adults[9] and children.[10] In addition, some studies have shown that antileukotriene agents have a protective effect against EIB.[9,12,13,30] It has also been reported that bronchoconstriction induced by LTD_4 inhalation is similar to exercise-stimulated bronchoconstriction.[31] Together with our finding Cys-LT levels in samples from the airways and a significant correlation between these mediators and EIB, these observations support a key role for Cys-LT levels in EIB.

Leukotrienes are released mainly by mast cells and macrophages in the airways. They are also produced by eosinophils recruited and delivered in large numbers to the conducting airways when asthmatic inflammation is active.[32] Primed eosinophils synthesize larger amounts of leukotrienes, which, in turn, cause further eosinophil migration into the airways. It has been demonstrated that the increased amount of leukotrienes released by the activated eosinophils can cause airway constriction, supporting a role for eosinophils as a source of Cys-LTs in asthma.[32]

Studies suggest that the degree of eosinophilic inflammation affects airway reactivity to exercise challenge, and it has been reported that sputum eosinophil counts are higher in patients with EIB and correlate with maximal percentage decrease in FEV_1.[33]

We found that Cys-LT levels are increased in the EBC of asthmatic children with EIB, and further supporting their role in EIB, a positive correlation between EBC Cys-LT concentrations and the maximal decrease in FEV_1 after exercise was demonstrated (Figs 1 and 4 and Table I). We speculate that high baseline Cys-LT EBC levels are associated with stronger bronchoconstriction in response to exercise challenge.

Some previous studies showed an increase in Cys-LT blood or urine levels after exercise, but others did not.[16,17] Likewise, we found a strong correlation between Cys-LT EBC concentration and maximal postexercise decrease in FEV_1, whereas Reiss et al[9] found no correlation between maximal decrease in FEV_1 and pre-exercise urinary LTE_4 levels in asthmatic children. A possible explanation for these discrepancies lies in that EBC is thought to reflect lung Cys-LT production better than blood or urine because these latter biologic fluids are collected far from the lung and might be influenced by systemic eicosanoid production.

When the exercise test and EBC collection were repeated in a subgroup of patients with EIB after 3 days of montelukast treatment, the maximal decrease in FEV_1 after exercise was significantly reduced (Fig 3 and Table II), which is in keeping with previous placebo-controlled studies.[11,12] This protective effect against EIB was associated with a mean 32% decrease in Cys-LT concentrations after 3 doses of montelukast, although this reduction did not reach statistical significance ($P = .098$). How can this rapid effect be explained? The anti-inflammatory effect of leukotriene modifiers is well documented because it has recently been demonstrated that they can reduce both airway eosinophilia[34] and FE_{NO} levels.[35] However, the course of anti-LT therapy was longer (3-4 weeks) in these studies[34,35] than in ours. A recent study on isolated human small bronchi shows that montelukast antagonizes the bronchial contraction induced by Cys-LTs.[36] Therefore we speculate that a few days of montelukast treatment might reduce EIB through a receptor mechanism rather than as a result of an anti-inflammatory effect. Nevertheless, our data do not allow any conclusive assertion.

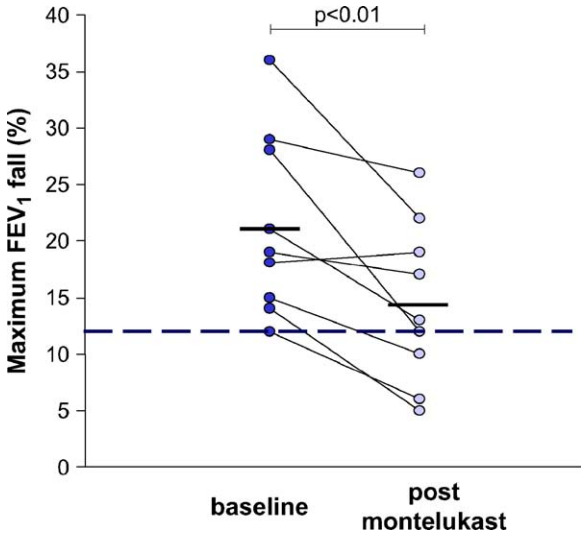

FIG 3. Maximal FEV_1 decrease after exercise in asthmatic children with EIB at baseline and after 3 days of treatment with montelukast. *Horizontal bars* represent mean values.

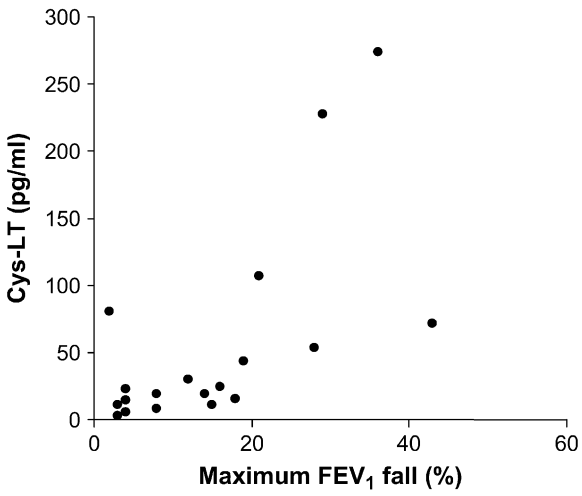

FIG 4. Correlation between EBC Cys-LT baseline concentrations and maximal postexercise FEV_1 decrease in asthmatic children ($P < .01$; $r = 0.7$).

Further placebo-controlled studies enrolling more children treated with a longer course of montelukast are needed to support our results.

Although Cys-LTs have a major role in EIB, there is evidence that other mediators or mechanisms might contribute to this phenomenon in some patients.[1] In fact, we found that some children in our study had EIB despite normal Cys-LT concentrations (Fig 1). An important challenge is to identify other inflammatory pathways that might be involved in EIB pathogenesis. We evaluated FE_{NO} levels and assessed the concentrations of LTB_4 and ammonia in the EBC, investigating the potential role of these compounds in EIB pathogenesis.

Unlike Cys-LTs, we found LTB_4 in similar concentrations in the EBC of asthmatic and healthy children. LTB_4 is released by neutrophils and is a potent neutrophil chemoattractant and activator,[32] but there is evidence that it has no effect on airway muscle tone.[37] Studies have shown higher LTB_4 concentrations in clinical conditions probably associated with neutrophilic airway inflammation (eg, acute asthma exacerbation[38] and severe persistent asthma[28]). Asthmatic children enrolled in our study, however, presented with good control of their disease, and this might explain our LTB_4 results.

In addition to Cys-LTs and LTB_4, we also measured the level of ammonia in the EBC, a compound that seems to have a role in maintaining airway pH homeostasis.[21] We found that asthmatic children had lower ammonia levels in the EBC than healthy control subjects, and this might lead to a lower pH buffering capacity (Fig 2). But ammonia levels in the EBC were no higher in asthmatic children with EIB than in asthmatic children without EIB, and therefore ammonia production seems to reflect an inflammatory pattern that is not closely related to exercise-stimulated bronchoconstriction.

Therefore among the markers we detected in the EBC, only Cys-LTs correlated with EIB, suggesting that EBC might be a valid and noninvasive tool for identifying children with inflammatory patterns more likely associated with EIB among the various asthma phenotypes.

In addition, the observation that LTB_4 levels are similar and that ammonia levels are lower than those detected in healthy control children suggests that although children with EIB had lower $FEF_{25\%-75\%}$ values, the increased Cys-LT concentration cannot be due to a more turbulent airflow, which is one of the hypothesized mechanisms for respiratory droplet production.

We also measured FE_{NO} levels and found a correlation between baseline FE_{NO} and maximal decrease in FEV_1, suggesting that EIB is greater with higher baseline FE_{NO} levels. A role for FE_{NO} in EIB has already been reported in previous studies.[14,15] It is worth noting the lack of any correlation between FE_{NO} and Cys-LT levels, suggesting that both mediators might have a role in EIB, but they reflect different aspects of its pathogenesis.

Interestingly, children in the present study with EIB had normal FEV_1 values (Table I), and no correlation was found between baseline lung function and EIB. This supports the growing conviction that more sensitive biologic markers of disease activity are needed to evaluate asthmatic children with minimal or no lung function abnormalities.[39]

In conclusion, this study shows that despite normal lung function, EBC Cys-LT and FE_{NO} levels are higher in children with EIB and correlate with the severity of EIB. Although other studies are needed to better clarify the inflammatory pattern of subjects with EIB, our data suggest that the Cys-LT and NO pathways are actively involved in the pathogenesis of EIB, stressing the need for more sensitive markers of disease activity in asthma.

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