

# Severe asthma is associated with a loss of LX4, an endogenous anti-inflammatory compound

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**Background:** Lipoxins and 15-epi-lipoxins are lipid mediators that modulate leukocyte trafficking and promote the inflammation resolution. They are produced by different enzymatic pathways. Patients with severe asthma present ongoing airway inflammation despite chronic long-term treatment including oral glucocorticoids.

**Objectives:** The aim of this study was to assess the presence of proinflammatory and anti-inflammatory mediators in the supernatants of induced sputum.

**Methods:** Induced sputum supernatants were collected from 10 normal subjects; 12 subjects with mild, 15 with moderate, and 24 with severe asthma; and 13 patients with chronic obstructive pulmonary disease. First, we validated the measurements of IL-8, leukotriene B<sub>4</sub>, lipoxin A<sub>4</sub>, and 15-epi-lipoxin A<sub>4</sub> in these samples. Then we measured these mediators by using immunoenzymatic methods.

**Results:** IL-8 levels were highly increased in patients with severe asthma ( $P < .0001$ ), and leukotriene B<sub>4</sub> levels were significantly increased in patients with severe asthma and patients with chronic obstructive pulmonary disease. Lipoxin A<sub>4</sub> was significantly increased in the supernatant obtained from patients with mild asthma ( $P < .0001$ ), whereas 15-epi-lipoxin A<sub>4</sub> levels were higher in patients with severe asthma ( $P = .05$ ). More interestingly, we found a positive correlation between the level of lipoxin A<sub>4</sub> and IL-8 in patients with mild asthma.

**Conclusion:** These results indicate that induced sputum is a suitable method to assess lipoxin and 15-epi-lipoxin measurements in bronchi. The mechanisms involved in the synthesis of these 2 eicosanoid mediators would be helpful to understand better the imbalance between proinflammatory and anti-inflammatory mediators occurring in severe asthma.

Lipoxin production involves interaction between lipoxygenases, whereas 15-epi-lipoxin production might involve CytP450 activity. (*J Allergy Clin Immunol* 2005;115:55-60.)

**Key words:** Lipoxins, asthma, induced sputum, LTB<sub>4</sub>, IL-8

Lipoxins were described for the first time by Serhan et al.<sup>1</sup> Lipoxin (LX) A<sub>4</sub> (5[S],6[R],15[S]-trihydroxy-7,9,13-trans-11-cis-eicosatetraenoic acid) is a biologically

## Abbreviations used

COPD: Chronic obstructive pulmonary disease

LO: Lipoxygenase

LT: Leukotriene

LX: Lipoxin

active eicosanoid produced by lipoxygenase (LO) interactions resulting from cellular cooperation such as epithelial cells or macrophages and eosinophils or neutrophils (15-LO and 5-LO).<sup>2-4</sup> 15-Epi-LXA<sub>4</sub> (5[S],6[R],15[R] trihydroxy-7,9,13-trans-11-cis-eicosatetraenoic acid) is also a biologically active eicosanoid, but its production results from the interaction between acetylated COX-2 in endothelial cells and 5-LO in leukocytes<sup>5</sup> and also from the cytochrome P450 pathway, which is able to produce 15(R)-hydroxyeicosatetraenoic acid.<sup>6</sup> LXA<sub>4</sub> was recovered in the bronchoalveolar and nasal lavage fluids of patients with various respiratory diseases.<sup>7,8</sup> More recently, we showed that LXA<sub>4</sub> was also recovered in supernatant of induced sputum.<sup>9</sup> There is increasing evidence suggesting that lipoxins produced endogenously might be potential mediators or modulators of inflammation within the lung.<sup>10,11</sup> LXA<sub>4</sub> has an anti-inflammatory effect on monocytes at nanomolar concentrations. It stimulates phagocytosis of apoptotic polymorphonuclear cells (PMNs) by macrophages to resolve inflammation.<sup>12,13</sup> We have shown that LXA<sub>4</sub> decreased LTB<sub>4</sub> released by human PMNs.<sup>14</sup> Moreover, LXA<sub>4</sub> and LXB<sub>4</sub> have been shown to inhibit IL-8 released by human blood mononuclear cells from patients with severe asthma.<sup>9</sup> LXA<sub>4</sub> stable analogues blocked both airway hyperresponsiveness and pulmonary inflammation in a murine model of asthma.<sup>15</sup> Glucocorticoids are potent anti-inflammatory agents and are widely used in the treatment of asthma.<sup>16</sup> Although most patients are efficiently controlled with inhaled glucocorticoid treatment, some of them are patients with severe asthma requiring a long-term systemic glucocorticoid dose in addition to their current treatment to control their disease.<sup>17</sup> These patients present an ongoing airway inflammation, usually characterized by an increased number of neutrophils,<sup>18,19</sup> activated T lymphocytes,<sup>20</sup> and eosinophils.<sup>21,22</sup> IL-8 and leukotriene (LT) B<sub>4</sub> are both potent chemoattractants of neutrophils that play an important role in mediating the inflammatory process in asthma.<sup>23,24</sup> Increased IL-8 levels

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TABLE I. Demographic characteristics of patients

|                 | Number | Sex (F/M) | Age* (y)   | FEV <sub>1</sub> *<br>(% of predicted value) | Treatment        |
|-----------------|--------|-----------|------------|----------------------------------------------|------------------|
| Control         | 10     | 6 F/4 M   | 35.7 ± 3.3 | 104.7 ± 4.4                                  | —                |
| Mild            | 12     | 9 F/3 M   | 45.3 ± 4.9 | 91.1 ± 5.5                                   | —                |
| Moderate        | 15     | 6 F/9 M   | 46.2 ± 3.3 | 77.9 ± 4.7                                   | ICS‡ (800 µg/d)  |
| Severe          | 24     | 10 F/14 M | 59.2 ± 2.3 | 59.6 ± 2.5                                   | OCS‡ (15.8 mg/d) |
| COPD            | 13     | 2 F/11 M  | 62.1 ± 3.7 | 43.6 ± 4.1                                   | —                |
| <i>P</i> value† |        |           | .0002      | <.0001                                       |                  |

ICS, Inhaled corticosteroid; OCS, oral corticosteroid.

\*Mean ± SEM.

†*P* value significant difference using Kruskal-Wallis test.

‡Mean daily doses.

were found in induced sputum of patients with severe asthma<sup>9,21</sup> and of patients with chronic obstructive pulmonary disease (COPD).<sup>25</sup> Levels of LTB<sub>4</sub> are increased in induced sputum from patients with COPD,<sup>25,26</sup> but little is known about these mediators in asthma. The aim of the current study was to determine for the first time the levels of endogenous potential proinflammatory and anti-inflammatory compounds in the lungs of patients with asthma of various severities by using induced sputum. At the same time, we investigated control subjects and untreated patients with COPD, who presented another bronchial inflammatory disease with a persistent airflow impairment. For this purpose, we measured IL-8, LTB<sub>4</sub>, LXA<sub>4</sub>, and 15-epi-LXA<sub>4</sub> by using immunoassays to understand better the balance between endogenous inflammatory and potential anti-inflammatory natural mediators within the airways.

## METHODS

### Patients

Subject characteristics are listed in Table I. Fifty-one subjects with asthma were selected according to the American Thoracic Society criteria.<sup>27</sup> All had a reversible airway obstruction assessed by a 12% increase in FEV<sub>1</sub> after inhalation of 200 µg salbutamol or a positive challenge with methacholine, with at least 20% fall in FEV<sub>1</sub> and/or a 15% increase in FEV<sub>1</sub> after a short course of oral glucocorticoids. The clinical severity of asthma was assessed according to GINA guidelines.<sup>28</sup> Twelve subjects with intermittent asthma and 15 subjects with moderate asthma treated with inhaled glucocorticoids and β<sub>2</sub>-agonists as needed were recruited. Twenty-four subjects with severe asthma were selected as previously described.<sup>9,29</sup> They were described as subjects with severe asthma because we failed to wean them from oral glucocorticoids in the previous 2 years, and they presented recurrent threatening episodes of acute asthma despite optimal treatment and management. They were treated on a regular basis with prednisone, inhaled fluticasone, and long-acting β<sub>2</sub>-agonists. None of the subjects with asthma participating in this study had ever smoked.

Ten healthy nonsmoking subjects were recruited as a control group. None of them presented bronchial or respiratory disease and/or allergy. All had a negative methacholine challenge test and negative skin prick test results.

Thirteen subjects with COPD were selected according to the American Thoracic Society criteria and were further classified by using GOLD criteria.<sup>27,30</sup> These patients were all current smokers with a smoking history of at least 30 packs/year. There were not treated by using anti-inflammatory steroids.

The current study was approved by the ethics committee of our institution. It was carefully checked that all patients were not under current aspirin treatment, and we excluded patients who took aspirin the day before the sampling.

### Sputum induction and processing

Sputum was induced according to the method of Kips et al<sup>31</sup> and processed as described previously.<sup>9</sup> Before sputum induction, all subjects underwent spirometry with FEV<sub>1</sub> and vital capacity measurement before and 10 minutes after inhalation of 200 µg salbutamol by metered dose inhaler. Hypertonic saline was nebulized with an ultrasonic nebulizer (DP 100 Syst'am, Paris, France). Subjects inhaled hypertonic saline solution for 5-minute periods for as long as 20 minutes and were asked to rinse their mouths out with water before induction to avoid salivary contamination of induced sputum samples as much as possible. The saline concentration was 3% for subjects with severe asthma and 4.5% for all of the other groups studied according to the recently published guidelines.<sup>32</sup> Spirometry was repeated at 5-minute intervals throughout the procedure and immediately after sputum induction was completed. The nebulization was stopped after 30 minutes or earlier if a sputum sample was obtained, according to the criteria of Pin et al.<sup>33</sup> A nebulization with bronchodilators was given at the end of the test. We then analyzed selected sputum plugs from saliva.<sup>33</sup>

Sputum was processed as previously described.<sup>9</sup> The weight of the induced sputum sample was determined. A volume of 0.1% dithiothreitol (Sigma-Aldrich, Steinheim, Germany) was added equal to 4 times the sputum weight. The sample was then gently mixed with a vortex mixer and placed in a shaking water bath at 37°C for 30 minutes to ensure complete homogenization. The homogenized sample was centrifuged at 2000g for 10 minutes. The supernatant was aspirated and stocked at -80°C for later analysis. PBS was added on the cell pellets obtained after centrifugation, and cells were resuspended. Total cell counts were analyzed on May-Grunwald-Giemsa-stained cytopsins by 2 blind observers.

### LXA<sub>4</sub>, 15-epi-LXA<sub>4</sub>, IL-8, and LTB<sub>4</sub> assays

LXA<sub>4</sub>, 15-epi-LXA<sub>4</sub> (Neogen Corp, Lexington, Ky), IL-8 (Dialclone, Besançon, France) and LTB<sub>4</sub> (Cayman Chemical, Ann Arbor, Mich) were measured by ELISA kit. The detection limits were 20 pg/mL for LXA<sub>4</sub>, 15-epi-LXA<sub>4</sub>, and IL-8, and 13 pg/mL for LTB<sub>4</sub>. Spiking experiments (n = 3) were performed for each mediator. There is no cross-reactivity between LXA<sub>4</sub> or 15-epi-LXA<sub>4</sub> and LTB<sub>4</sub>. The cross reactivity for 15-epi-LXA<sub>4</sub> is 3% with LXA<sub>4</sub>.

### Statistical analysis

Data were expressed as means ± SEMs. A Kruskal-Wallis test was performed for comparison among the 5 groups of subjects in addition to post hoc Bonferroni-Dunn tests. Correlations were

**TABLE II.** Differential cellular counts in induced sputum

|                                  | Controls    | Mild         | Moderate      | Severe        | COPD          | P value* |
|----------------------------------|-------------|--------------|---------------|---------------|---------------|----------|
| Total cells/mm <sup>3†</sup>     | 90.5 ± 22.6 | 300.8 ± 52.9 | 576.9 ± 134.7 | 519.2 ± 102.3 | 457.3 ± 103.8 | .0008    |
| Eosinophils, %‡                  | 0.6 ± 0.4   | 10.8 ± 6.2   | 9.7 ± 6.8     | 6.2 ± 2.1     | 2.4 ± 1.6     | NS       |
| Neutrophils, %‡                  | 40.8 ± 12.1 | 39.1 ± 7.6   | 56.7 ± 8.7    | 70.4 ± 5.0    | 85.2 ± 3.4    | .0004    |
| Lymphocytes, %‡                  | 1.0 ± 0.4   | 3.5 ± 1.3    | 3.1 ± 1.3     | 1.5 ± 0.5     | 1.7 ± 0.4     | NS       |
| Macrophages, %‡                  | 45.4 ± 10.5 | 45.4 ± 7.5   | 28.5 ± 7.6    | 17.0 ± 3.4    | 9.6 ± 2.5     | .0001    |
| Bronchial cells, %‡              | 9.0 ± 5.1   | 0.8 ± 0.5    | 1.9 ± 1.2     | 4.7 ± 2.8     | 1.0 ± 0.8     | NS       |
| IL-8 (pg/mL)‡                    | 190 ± 60    | 850 ± 150    | 2660 ± 650    | 5870 ± 1070   | 3970 ± 1010   | <.0001   |
| LTB <sub>4</sub> (pg/mL)‡        | 313 ± 76    | 458 ± 44     | 433 ± 54      | 518 ± 36      | 550 ± 46      | .03      |
| LXA <sub>4</sub> (ng/mL)‡        | 0.21 ± 0.04 | 1.01 ± 0.12  | 0.43 ± 0.07   | 0.57 ± 0.10   | 0.26 ± 0.07   | <.0001   |
| 15-Epi-LXA <sub>4</sub> (ng/mL)‡ | 0.45 ± 0.14 | 0.7 ± 0.09   | 0.81 ± 0.09   | 0.92 ± 0.09   | 0.73 ± 0.09   | .05      |

\*P value using Kruskal-Wallis test.

†Results are expressed as cell number × 10<sup>3</sup>

‡Results are expressed as percentage means ± SEMs.

determined by using a nonparametric Spearman rank test. Differences were considered significant at  $P < .05$ .

## RESULTS

### Patient characteristics

Demographic characteristics of all subjects are shown in Table I. Patients with severe asthma and COPD were older than patients with intermittent and moderate asthma and control subjects ( $P = .0002$ ). Patients with intermittent asthma and COPD were not treated with steroids. FEV<sub>1</sub> of all groups of subjects was significantly different ( $P < .001$ ), and by using Bonferroni-Dunn posttest, significant differences were observed between all of the groups except patients with mild asthma versus controls and patients with moderate asthma.

### Differential cellular contents of induced sputum

The results are presented in Table II. Among the 5 groups of subjects, the total cell counts recovered from induced sputum were significantly different ( $P = .0008$ ). Analyzing differential cell counts, we found significant differences among the 5 groups of subjects for macrophage and neutrophil percentage ( $P = .0001$  and  $P = .0004$ , respectively). Macrophage percentage was significantly lower in induced sputum of patients with severe asthma and COPD compared with patients with mild asthma ( $P = .0008$  and  $P = .0003$ , respectively) and controls ( $P = .002$  and  $P = .0005$ , respectively). In contrast, neutrophil percentage was significantly higher in induced sputum of patients with severe asthma and COPD compared with patients with mild asthma ( $P = .002$  and  $P = .0001$ , respectively) and was even higher in patients with COPD compared with control subjects ( $P = .0003$ ).

### Mediator levels in supernatants of induced sputum

Known exogenous compounds were added in induced sputum samples from control subjects just after the collection. Spiking experiments, performed to assess the

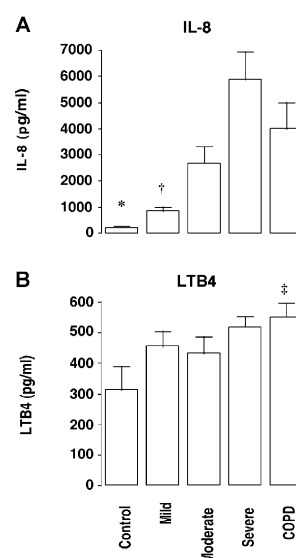


**FIG 1.** IL-8 (A) and LTB<sub>4</sub> (B) concentrations in supernatants of induced sputum from these subjects. The results were analyzed by using a Kruskal-Wallis test with a post hoc test, and values are expressed as means ± SEMs (pg/mL). A, \* $P < .0001$  for controls and † $P < .0005$  for patients with mild versus severe asthma. B, ‡ $P < .005$  for controls versus COPD.

yield recovery, were analyzed by using ELISA test for IL-8 and reverse-phase high-performance chromatography and ELISA test for eicosanoids. They showed that recovery of exogenous mediators was always more than 95%. Scheme 1 showed a characteristic HPLC profile of the LXA<sub>4</sub> spiking experiment.

The levels of IL-8 were significantly different among the 5 groups, with  $P < .0001$  (Fig 1, A). IL-8 was significantly higher in the supernatant of induced sputum from patients with severe asthma compared with controls ( $P < .001$ ) and with patients with mild asthma ( $P = .0002$ ). The levels of LTB<sub>4</sub> were significantly different among the 5 groups, with  $P = .03$  (Fig 1, B). LTB<sub>4</sub> was significantly higher in the supernatant of induced sputum from patients with severe asthma and COPD compared with controls ( $P = .005$  and  $P = .004$ , respectively).

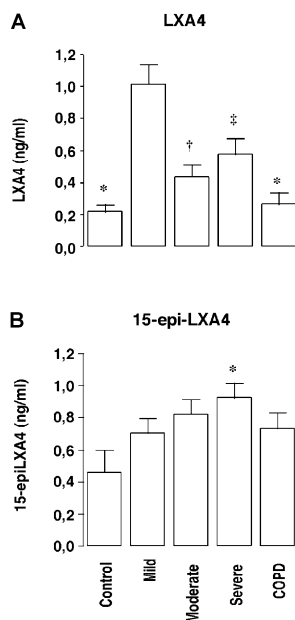


**FIG 2.** LX<sub>4</sub> (A) and 15-epi-LX<sub>4</sub> (B) concentrations in supernatants of induced sputum from these subjects. The results were analyzed by using a Kruskal-Wallis test with a post hoc test, and values are expressed as means  $\pm$  SEMs (ng/mL). A, \* $P < .0001$  for controls and COPD, † $P < .0005$  for patients with moderate asthma, and ‡ $P < .005$  for patients with severe versus mild asthma. B, ‡ $P < .005$  for controls versus patients with severe asthma.

The levels of LX<sub>4</sub> were significantly different among the 5 groups, with  $P < .0001$  (Fig 2, A). More precisely, LX<sub>4</sub> was found significantly higher in the supernatant of induced sputum in patients with mild asthma compared with patients with moderate asthma ( $P = .0002$ ), patients with severe asthma ( $P = .002$ ), controls, and COPD ( $P < .0001$ ). The levels of 15-epi-LX<sub>4</sub> were significantly different among the 5 groups, with  $P = .05$  (Fig 2, B). In contrast with LX<sub>4</sub>, the levels of 15-epi-LX<sub>4</sub> were significantly higher in supernatants of induced sputum from patients with severe asthma compared with controls ( $P = .002$ ).

Moreover, we confirmed that there was a significant correlation between IL-8 and LX<sub>4</sub> levels in supernatants of induced sputum in patients with mild asthma only ( $r = 0.699$ ;  $P = .02$  using Spearman rank test; Fig 3).

There was no relation found between the levels of either LX<sub>4</sub> or 15-epi-LX<sub>4</sub> and any of the other inflammatory mediators (IL-8, LTB<sub>4</sub>) or cellular counts and the clinical parameters (such as FEV<sub>1</sub> or steroid doses) within a patient group. Moreover, no relation was found between the levels of either IL-8 or LTB<sub>4</sub> and any of the cell counts and clinical parameters.

## DISCUSSION

The current study demonstrates that patients with severe asthma present a persistent inflammation characterized by the proinflammatory IL-8 and LTB<sub>4</sub> over-

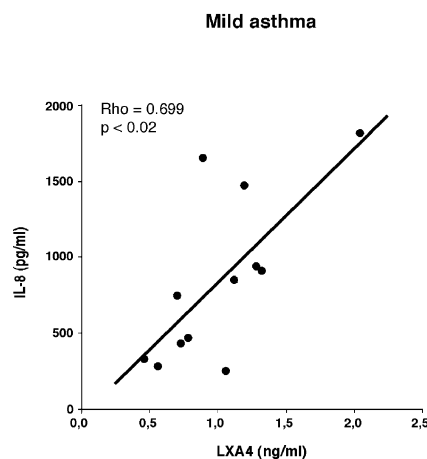


**FIG 3.** Positive correlation between IL-8 and LX<sub>4</sub> released in induced sputum from patients with mild asthma ( $n = 12$ ) was analyzed with a nonparametric Spearman test. The Spearman parameters were as indicated:  $\rho = 0.699$  with  $P = .02$ .

production detected in supernatants of induced sputum. In the same way, we also observed that LTB<sub>4</sub> was increased in supernatants of patients with COPD. Moreover, the anti-inflammatory LX<sub>4</sub> was present in supernatants of induced sputum and was increased in patients with mild asthma. Inversely, the anti-inflammatory LX<sub>4</sub> epimer, 15-epi-LX<sub>4</sub>, was increased in patients with severe asthma. We also found a positive correlation between IL-8 and LX<sub>4</sub> only in the group of patients with mild asthma, meaning that the balance between proinflammation and anti-inflammation is defective in severe asthma.

Examination of induced sputum is a minimally invasive method for studying airway inflammatory cells and mediators that has been well validated.<sup>32,34</sup> Patients with COPD and severe asthma were older than the other groups of subjects. It was very difficult to obtain age-matching groups in this study according to the etiology of these diseases that appear later in life. To our knowledge, there is no evidence that lipoxin metabolism could be different relative to age. In the current study, we showed an increase in neutrophil percentage in severe asthma and in COPD and, in contrast, a decrease in macrophage percentage. Until recently, increased neutrophil number was mainly reported in COPD.<sup>35</sup> However, an increase of neutrophil infiltration in severe asthma was reported by other studies using different tools<sup>18,21</sup> and in the ENFUMOSA (European Network for Understanding the Mechanisms of Severe Asthma) study using induced sputum.<sup>36</sup>

Numerous inflammatory mediators have been measured in the fluid phase of induced sputum, including cytokines, chemokines, granulocyte proteins, markers of vascular leakage, eicosanoids, proteases, and others.<sup>37</sup> Moreover, LX<sub>4</sub> and 15-epi-LX<sub>4</sub> are also measured in urine after extraction procedure.<sup>38</sup> In the current work, we demonstrated that the measurement of proinflammatory mediators such as IL-8 and LTB<sub>4</sub> and anti-inflammatory mediators such as LX<sub>4</sub> and 15-epi-LX<sub>4</sub> in supernatants of induced sputum are reliable in control subjects and in

patients with asthma and COPD. Indeed, the spiking experiments performed showed that the recovery of all of these mediators was at least more than 95%.

We showed in a previous study that higher levels of IL-8 were detected in supernatants of induced sputum from patients with severe asthma compared with all of the other groups studied.<sup>9</sup> Both chemoattractants, IL-8 and LTB<sub>4</sub>, are present in higher concentrations in supernatants of induced sputum of patients with severe asthma and would be expected to provide the major drive of neutrophil influx, as was found in our previous study. Moreover, we have shown that LXA<sub>4</sub> was able to decrease the production of LTB<sub>4</sub> and IL-8 in PMN and human peripheral blood monocytes, respectively, *in vitro*.<sup>9,14</sup> IL-8 was shown to contribute to chemotactic activity in several chronic conditions affecting airways.<sup>39</sup> In contrast, the role of LTB<sub>4</sub> has received little attention in patients with asthma, in contrast with patients with COPD.<sup>25</sup> In previous reports, LXA<sub>4</sub> was detected *in vivo* in bronchoalveolar lavage fluid from patients with respiratory diseases, including patients with asthma,<sup>7</sup> and in a more recent study in induced sputum from patients with asthma.<sup>9</sup> In our current study, we were able to detect LXA<sub>4</sub> and 15-epi-LXA<sub>4</sub> not only in the supernatants of induced sputum of controls and patients with asthma of various severities but also in patients with COPD. We previously showed that human alveolar macrophages and blood PMNs from subjects with mild asthma synthesized higher levels of lipoxins than those from normal subjects.<sup>3,4</sup> In a recent study, we demonstrated that blood PMNs from patients with severe asthma produced less lipoxin than untreated patients with asthma and control subjects showing decreased 5-LO activity.<sup>14</sup> These findings are related to the hypothesis of an imbalance regulation between proinflammatory and anti-inflammatory compounds.

Lipoxins are recognized as endogenous anti-inflammatory lipid mediators of resolution. These compounds are a stop signal for PMNs and eosinophils during bronchial inflammation.<sup>11</sup> In an animal model of allergic asthma, intravenous administration of a LXA<sub>4</sub> analogue before airway antigen challenge decreased pulmonary eosinophil accumulation as well as T<sub>H</sub>2 cytokines involved in their recruitment in bronchi.<sup>15</sup> In our study, there is no correlation between PMN or eosinophil number and the lipoxin levels in bronchi. However, it seems that in severe asthma, the deficit in lipoxin is related to an increase in the PMN number and IL-8 levels and is not related to an eosinophilic inflammation.

Interestingly, patients with severe and moderate asthma presented lower LXA<sub>4</sub> levels in supernatants of induced sputum than patients with mild asthma. Moreover, we highlighted a positive correlation between IL-8 and LXA<sub>4</sub> concentrations in the supernatants of induced sputum from patients with mild asthma and not from any other group studied, thus demonstrating that the increased levels of inflammation might be counterbalanced by an increase in anti-inflammatory lipoxin synthesis. Interestingly, we observed that the LXA<sub>4</sub> and 15-epi-LXA<sub>4</sub> should have distinguished synthesis pathways within the lung because

no relation between these 2 mediators was found. 15-epi-LX synthesis needs an interaction between the COX-2 enzyme, which is acetylated in presence of aspirin, and the 5-LO present in leukocytes. It was shown that aspirin treatment of various cell types *in vitro* enhances the production of 15-epi-LXA<sub>4</sub> during cell-cell interaction.<sup>40</sup> It was reported that COX-2 expression is increased in sputum cells as well as in the bronchial epithelium in asthma.<sup>41</sup> These results could explain the higher levels of 15-epi-LXA<sub>4</sub> in severe asthma that we observed in the current study. However, no patients with asthma and COPD included in our study were taking aspirin on a regular basis, and we did not include patients who recently absorbed aspirin. On the other hand, it seems that, without aspirin, another pathway is involved in 15-epi-LX synthesis. The cytochrome P450 is highly expressed in human lung<sup>42</sup> and is able to produce 15(R)-hydroxyeicosatetraenoic acid from arachidonic acid.<sup>43</sup> In our study, 15-epi-LXA<sub>4</sub>, which could derive from Cyp450 activity, was detected in asthma and COPD. The Cyp450 activity could be increased in severe asthma; however, the 15-epi-LX synthesized was not sufficient to resolve inflammation in these patients.

In summary, we provide evidence of increased formation of IL-8 and LTB<sub>4</sub>, proinflammatory mediators, with a defect in the synthesis of LXA<sub>4</sub> but not of 15-epi-LXA<sub>4</sub>, which are both anti-inflammatory compounds with distinct biosynthesis pathways. Our results pointed out that persistent inflammation in severe asthma is also associated with a possible deficiency in anti-inflammatory mediators such as LXA<sub>4</sub>.

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