

# Effect of prostaglandin E<sub>2</sub> on eicosanoid release by human bronchial biopsy specimens from normal and inflamed mucosa

D Schäfer, U Lindenthal, M Wagner, P L Bölcskei, H W Baenkler

## Abstract

**Background** – Eicosanoids such as prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), thromboxane A<sub>2</sub> (TXA<sub>2</sub>), and peptidoleukotrienes (pLT) are known to be biologically highly active lipid mediators, especially in human lung epithelium. PGE<sub>2</sub> is thought to have mostly bronchoprotective effects, whereas pLT and TXA<sub>2</sub> are bronchoconstrictive. This study was undertaken to assess the release and interaction of eicosanoids in human bronchial biopsy specimens of normal and inflamed mucosa.

**Methods** – Bronchial biopsy specimens were obtained from 16 patients, seven controls without signs of inflammation and nine patients with severe inflammatory processes in the epithelium. The release of pLT, TXA<sub>2</sub> (measured as TXB<sub>2</sub>), and PGE<sub>2</sub> was investigated using a “functional in vitro test” and the addition of several stimuli.

**Results** – Specimens incubated with arachidonic acid released higher amounts of pLT, TXB<sub>2</sub>, and PGE<sub>2</sub> than unstimulated specimens. Preincubation with PGE<sub>2</sub> revealed significant inhibition of arachidonic acid-induced release of pLT and TXB<sub>2</sub> (> 50%). The inhibitory effect was higher in normal than in inflamed epithelium.

**Conclusions** – Exogenous PGE<sub>2</sub> has inhibitory effects on the release of pLT and TXB<sub>2</sub> in human bronchial biopsy specimens. This finding could explain the bronchoprotective effect of inhaled PGE<sub>2</sub> in normal subjects and asthmatic subjects as direct eicosanoid interactions. It also supports the concept of PGE<sub>2</sub> as a bronchoprotective endogenous substance. The complex effects of PGE<sub>2</sub> as a modulating mediator in inflammation may be worth investigating.

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The epithelium of the respiratory tract has a remarkably complex and diverse structure consisting of various populations of cells, although the function of many of them remains uncertain.<sup>1</sup> Bronchial inflammation is known to be associated with pathological changes in the epithelium.<sup>2,3</sup> The epithelium is also a source of several highly biologically active sub-

stances such as cytokines<sup>4</sup> and eicosanoids.<sup>5,6</sup> Eicosanoids such as peptidoleukotrienes (pLT; for example, LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub>), thromboxane A<sub>2</sub> (TXA<sub>2</sub>), and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) are highly potent lipid mediators formed from metabolites of arachidonic acid.<sup>7,8</sup>

There are numerous cellular and tissue sources of eicosanoids including inflammatory cells such as basophil, neutrophil and eosinophil granulocytes, mast cells, macrophages, and monocytes.<sup>9</sup> Several groups of investigators have provided evidence that the qualitative and quantitative nature of eicosanoid release depends on the cell type as well as on the stimulus.<sup>10-12</sup> After allergen challenge the release of peptidoleukotrienes and thromboxane B<sub>2</sub> is increased,<sup>13</sup> and both eicosanoids cause bronchoconstriction in the respiratory tract.<sup>14,15</sup> Mucus secretion<sup>16</sup> and microvascular permeability are increased by peptidoleukotrienes. In contrast to the proinflammatory properties of peptidoleukotrienes and thromboxane B<sub>2</sub>, prostaglandin E<sub>2</sub> is thought to protect against bronchoconstriction provoked by various stimuli.<sup>17</sup>

This study was undertaken to examine the role of PGE<sub>2</sub> on pLT and TXA<sub>2</sub> release using a “functional in vitro test” and native biopsy specimens of normal and inflamed human bronchial mucosa. This method was used to take into account the complex structure of the epithelium and to facilitate naturally occurring intracellular and transcellular eicosanoid metabolism. The eicosanoid release patterns of peptidoleukotrienes, thromboxane B<sub>2</sub> and prostaglandin E<sub>2</sub> were analysed in parallel using enzyme immuno assays.

## Methods

### SUBJECTS

Bronchial biopsy specimens were obtained from 16 patients aged 28-68 years during routine bronchoscopy. Indications for bronchoscopy included apparent abnormalities on the chest radiographs, inflammatory lung disease, and haemoptysis. Patients with a bleeding diathesis were excluded. None of the subjects received treatment with topical or systemic corticosteroids during the 10 days prior to the bronchoscopic examination. Bronchoscopic indications of mucosal inflammation were oedema and erythema, and the histopathological indication was granulocyte infiltration. Seven patients were examined to rule out a neoplasm. They had no inflammation, neither

Medical Clinic III,  
Department of  
Allergology,  
University of  
Erlangen-Nuremberg,  
Krankenhausstrasse  
12, D-91054 Erlangen,  
Germany  
D Schäfer  
U Lindenthal  
H W Baenkler

Medical Clinic 3,  
Department of  
Pneumology, Teaching  
Hospital Nuremberg,  
Nuremberg, Germany  
M Wagner  
P L Bölcskei

Correspondence to:  
Dr D Schäfer.

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on bronchoscopic nor on histopathological examination. This group of patients with normal epithelium served as controls. Nine patients showed signs of mucosal inflammation. Two further patients with allergic alveolitis to pigeon feathers were investigated for induced eicosanoid release using a specific allergen, immunoglobulin E, and immunoglobulin G.

Subjects gave their signed consent after receiving both written and oral information. The study design was approved by the local ethic committee.

#### FIBREOPTIC BRONCHOSCOPY

Premedication was given with atropine 0.5 mg and codeine 15 mg subcutaneously. Topical anaesthesia of the mucosa was performed with lignocaine in a concentration of 2%. Midazolam, 3–5 mg, was given intravenously as a sedative. All bronchoscopic examinations were performed using a Pentax flexible fiberoptic bronchoscope by one experienced bronchoscopist using a transnasal approach. Six biopsy specimens were taken from the mucosa of the central bronchus of each patient for the functional biopsy test and divided into pieces with a fresh weight of 0.4–0.8 mg. One additional biopsy specimen was obtained for standard histological staining to confirm the macroscopic findings.

#### FUNCTIONAL BIOPSY TEST AND SAMPLE COLLECTION

The biopsy specimens were placed immediately into 1 ml of modified Hank's balanced salt solution (HBSS; 4°C) containing 50 mM L-serine for 20 minutes, then transferred either into 500 µl fresh HBSS (37°C) containing no stimuli in order to examine the basal release of pLT, TXB<sub>2</sub> and PGE<sub>2</sub>, or into 500 µl fresh HBSS with one of the following additions: arachidonic acid (10 µM), acetylsalicylic acid (10 µM), caffeic acid (10 µM; Biomol, Hamburg, Germany), PGE<sub>2</sub> (10 µM), or rabbit anti-human immunoglobulin E (anti-IgE, dilution 1:100; DAKO Diagnostics, Hamburg, Germany). Specimens were incubated for 20 minutes at 37°C and continuously gassed with carbogen (95% O<sub>2</sub>, 5% CO<sub>2</sub>).

To test the inhibitory action of PGE<sub>2</sub>, acetylsalicylic acid, and caffeic acid on arachidonic acid-induced eicosanoid release, biopsy specimens were preincubated as indicated in table 1, arachidonic acid was added and the specimens were further incubated for 20 minutes. Three biopsy specimens were then removed and the incubation medium was stored at –80°C for up to four weeks before being analysed by enzyme immuno assays. The stimulations were performed in triplicate for each patient. Each specimen was weighed twice using an ultrasensitive weighing machine. Unless otherwise indicated, chemicals were purchased from Sigma (Daisenhofen, Germany).

#### QUANTIFICATION OF EICOSANOID RELEASE

The release of immunoreactive pLT, TXB<sub>2</sub>, and PGE<sub>2</sub> was quantified simultaneously for

each sample in duplicate by highly sensitive and specific competitive enzyme immuno assays using monoclonal antibodies for pLT (clone 1A-LDR1),<sup>18</sup> TXB<sub>2</sub> (clone 4E-TBR1),<sup>19</sup> and PGE<sub>2</sub> (clone E2R1).<sup>20</sup> The monoclonal antibodies were a gift from Dr Reinke (Medical Clinic III, University of Erlangen-Nuremberg).

TXB<sub>2</sub> and PGE<sub>2</sub> were coupled to bovine serum albumin (BSA) and LTD<sub>4</sub> was coupled to bovine thyroglobulin using 1-ethyl-3-(3-methylaminopropyl)carbodiimide HCl.<sup>21</sup> Polystyrene microtitre plates (Nunc, Wiesbaden, Germany) were coated with pLT, TXB<sub>2</sub>, or PGE<sub>2</sub> conjugate for 18 hours (relevant conjugate in 15 mM NaCO<sub>3</sub>/35 mM NaHCO<sub>3</sub>, pH 9.6; 200 µl/well). Thereafter, remaining binding sites were blocked with 0.1% BSA in phosphate buffered saline (PBS) for 60 minutes. As standards, relevant eicosanoids were prepared in duplicate at seven different concentration levels (500 pg to 1 pg/well, in HBSS). Samples or standards (100 µl/well) were incubated for 18 hours at 4°C with monoclonal antibodies for pLT, TXB<sub>2</sub>, or PGE<sub>2</sub> (100 µl/well of 1.8 µg, 2.3 µg, or 5 µg antibody, respectively, in 1 ml PBS-BSA 1%). Subsequently, biotinylated goat-anti mouse antibody was added (1:2000 diluted stock solution in PBS-BSA 0.1%; Amersham, Braunschweig, Germany; 200 µl/well for two hours at 37°C). Enzyme immuno assays for TXB<sub>2</sub> and PGE<sub>2</sub> were then incubated for one hour at 37°C with a streptavidin-biotin complex (coupled with horseradish peroxidase; 1:1000 diluted stock solution in PBS-BSA 0.1%, DAKO Diagnostics, Hamburg; 200 µl/well). The enzyme immuno assay for pLT was incubated for 30 minutes at 37°C with streptavidin-biotin complex followed by a series of incubations with modified tyramine-biotin conjugate<sup>22</sup> (0.1% (v/v) conjugate and H<sub>2</sub>O<sub>2</sub> 0.06% in 50 mM Tris buffer, pH 8.5; 200 µl/well) for 15 minutes at 37°C, with streptavidin-biotin complex (diluted 1:750 in PBS-BSA 1%; 200 µl/well) for 30 minutes at 37°C, and finally in 2,2'-azino-di-(3-ethylbenzthiazoline-6-sulphonate) solution as chromogenic substrate (1 mg in 1 ml 70 mM Na<sub>2</sub>HPO<sub>4</sub>/6 mM sodium perborate/40 mM citric acid, pH 4.5; 200 µl/well) for 45 minutes at room temperature. Absorption was measured at 405 nm (reference filter 490 nm). Each incubation step was followed by a washing step using PBS-Tween 0.1% (v/v). The detection limit was 1 pg/well for pLT and 3 pg/well for TXB<sub>2</sub> and PGE<sub>2</sub>. The rate of recovery for pLT (> 95%), TXB<sub>2</sub> (> 98%), and PGE<sub>2</sub> (> 98%) verified the accuracy of the assays. Intra-assay and interassay coefficients of variation for pLT were 7.4% and 8.9%, for TXB<sub>2</sub> were 4.9% and 7.2%, and for PGE<sub>2</sub> were 5.6% and 8.1%.

#### DATA ANALYSIS

The eicosanoid release values were normalised on the mean of the corresponding fresh weight. Results are expressed as arithmetic mean, standard deviation (SD), and standard error (SE). The Student's paired *t* test was used to compare the stimulated eicosanoid release within the subjects, and the Mann-Whitney

Table 1 Mean (SE) inhibitory effect of PGE<sub>2</sub> in comparison with specific enzyme inhibitors on arachidonic acid-induced or anti-IgE-induced eicosanoid release

Source of epithelium	n	% inhibition of pLT release				% inhibition of TXB <sub>2</sub> release					
		PGE <sub>2</sub> (10 μM) + AA	PGE <sub>2</sub> (1 μM) + AA	PGE <sub>2</sub> (10 μM) + anti-IgE	CA (5 min) + AA	CA (10 min) + AA	PGE <sub>2</sub> (10 μM) + AA	PGE <sub>2</sub> (1 μM) + AA	PGE <sub>2</sub> (10 μM) + anti-IgE	ASA (5 min) + AA	ASA (10 min) + AA
Normal	7	97 (1.8)	56 (2.4)*	74 (2.8)*	36 (2.5)	48 (1.7)	55 (1.5)	23 (1.7)*	43 (2.4)*	23 (1.7)	38 (1.4)
Inflamed	9	74 (2.7)	ND**	ND**	34 (1.9)	43 (2.1)	57 (1.9)	ND**	ND**	5 (0.4)	31 (1.8)
Allergic	2	79 (3.1)	ND**	67 (2.9)	33 (2.1)	46 (1.9)	56 (3.4)	ND**	59 (3.3)	8 (0.6)	28 (2.1)

\* Only biopsy specimens of three patients were available for the functional biopsy test.

\*\* No further biopsy specimens were available for testing quantitative PGE<sub>2</sub> effects.

PGE<sub>2</sub> = prostaglandin E<sub>2</sub>; pLT = peptidoleukotriene; TXB<sub>2</sub> = thromboxane B<sub>2</sub>; AA = arachidonic acid; CA = caffeic acid; ASA = acetylsalicylic acid.

Table 2 Eicosanoid release after specific allergen challenge in two patients with allergic alveolitis

Allergen challenge	Mean (SE) eicosanoid release (pg/mg fresh weight)		
	pLT	TXB <sub>2</sub>	PGE <sub>2</sub>
Basal	0.97(0.04)	0.30(0.05)	3.60(0.6)
Arachidonic acid	1.63(0.08)	3.36(0.07)	10.69(0.3)
Pigeon feathers	1.21(0.07)	1.23(0.05)	6.18(0.3)
Anti-IgE	2.18(0.11)	1.15(0.04)	4.12(0.09)
Anti-IgG	0.42(0.02)	0.1 (0.002)	0.01(0.002)

pLT = peptidoleukotrienes; TXB<sub>2</sub> = thromboxane B<sub>2</sub>; PGE<sub>2</sub> = prostaglandin E<sub>2</sub>.

Two patients with allergic alveolitis to pigeon feathers were investigated. From each patient three biopsy specimens were taken for measurement of basal and arachidonic acid (10 μM)-induced eicosanoid release, and two biopsy specimens for allergen challenge.

test was used to determine the significance of differences in normal and inflamed tissue. A p value of < 0.05 was regarded as statistically significant.

## Results

### EICOSANOID RELEASE PATTERNS IN NORMAL AND INFLAMED EPITHELIUM

The biopsy specimens of normal epithelium released significantly larger amounts of basal and arachidonic acid-induced peptidoleukotrienes ( $p < 0.05$ ) than specimens of inflamed epithelium (fig 1A). The basal release of TXB<sub>2</sub> did not differ significantly between normal and inflamed epithelium, but arachidonic acid-induced release of TXB<sub>2</sub> was significantly higher in inflamed epithelium ( $p < 0.01$ ; fig 1B). The basal and arachidonic acid-induced release of prostaglandin E<sub>2</sub> was higher ( $p < 0.05$ ) in inflamed than in normal epithelium (fig 1C). Biopsy specimens released significantly larger amounts of eicosanoids upon stimulation with arachidonic acid ( $p < 0.01$ ) (fig 1).

### EFFECT OF PGE<sub>2</sub> ON pLT AND TXB<sub>2</sub> RELEASE COMPARED WITH CYCLO-OXYGENASE AND LIPOXYGENASE INHIBITORS

The inhibitory effect of PGE<sub>2</sub> on the release of pLT and TXB<sub>2</sub> in normal epithelium was compared with the lipoxygenase inhibitor caffeic acid<sup>23</sup> and the cyclo-oxygenase inhibitor acetylsalicylic acid.<sup>24</sup> Two different preincubation times were chosen for the enzyme inhibitors. Significant inhibition of pLT (48%;  $p < 0.01$ ) or TXB<sub>2</sub> (38%;  $p < 0.05$ ) release by the enzyme inhibitors was achieved using the longer preincubation time (10 minutes). PGE<sub>2</sub> was more potent than the enzyme inhibitors in inhibiting pLT and TXB<sub>2</sub> release using five minute preincubation. PGE<sub>2</sub> inhibited pLT and TXB<sub>2</sub> release quantitatively in normal epithelium and in mucosa from patients with allergic alveolitis (table 1). Furthermore, eicosanoid release in biopsy specimens from the two patients with allergic alveolitis was allergen specific and mediated by immunoglobulin E but not by immunoglobulin G (table 2).

### EFFECT OF PGE<sub>2</sub> ON pLT AND TXB<sub>2</sub> RELEASE IN NORMAL AND INFLAMED EPITHELIUM

Basal release of pLT and TXB<sub>2</sub> in biopsy specimens from controls was reduced by preincubation with PGE<sub>2</sub> ( $p < 0.05$ ; 23% and 29%, respectively) but no such inhibition was seen in inflamed epithelium (fig 2). Preincubation with PGE<sub>2</sub> inhibited arachidonic acid-induced pLT release in both normal (97%;  $p < 0.01$ ) and inflamed epithelium (74%;  $p < 0.01$ ) and similarly inhibited TXB<sub>2</sub> release ( $p < 0.05$ ; 55% and 57%, respectively; fig 3). Furthermore, the release of pLT and TXB<sub>2</sub> induced by anti-IgE was significantly inhibited by PGE<sub>2</sub> ( $p < 0.05$ ; 67% and 58%, respectively) (table 1).

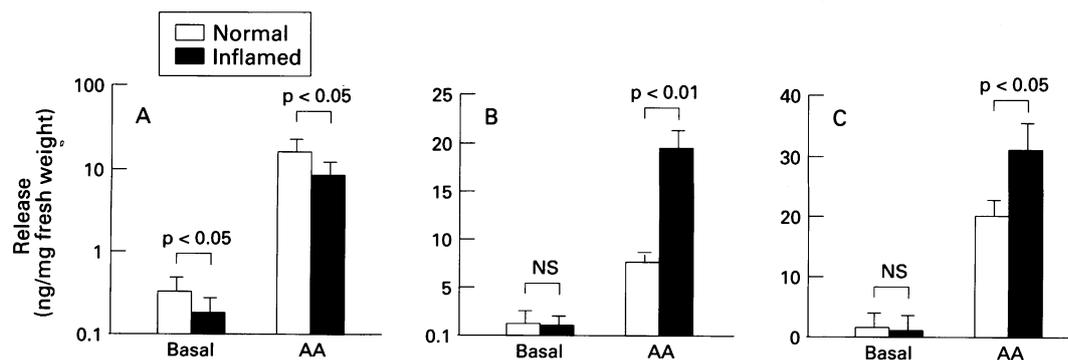
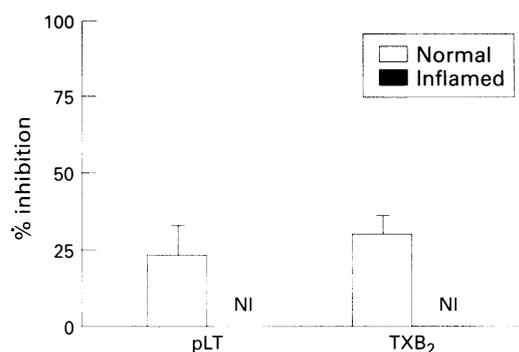
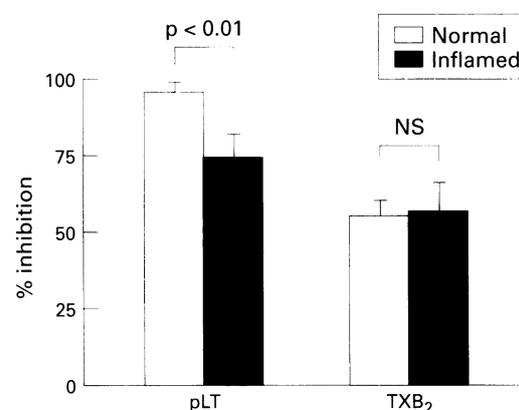


Figure 1 Eicosanoid release pattern in normal and inflamed epithelium. Basal and arachidonic acid (AA, 10 μM)-induced release of (A) peptidoleukotrienes (pLT), (B) thromboxane B<sub>2</sub> (TXB<sub>2</sub>), and (C) prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) in biopsy specimens from seven normal subjects and nine patients with inflamed mucosa. Results are given as mean values, bars indicating SE.



**Figure 2** Effect of prostaglandin  $E_2$  ( $PGE_2$ ) on basal release of peptidoleukotrienes (pLT) and thromboxane  $B_2$  ( $TXB_2$ ). Biopsy specimens from seven normal subjects and nine patients with inflamed mucosa were incubated with  $PGE_2$  ( $10 \mu M$ ). Results are presented as % inhibition of pLT and  $TXB_2$  release by  $PGE_2$  compared with the corresponding basal eicosanoid release, bars indicating SE. NI = no inhibition.



**Figure 3** Effect of prostaglandin  $E_2$  ( $PGE_2$ ) on arachidonic acid-induced release of peptidoleukotrienes (pLT) and thromboxane  $B_2$  ( $TXB_2$ ). Biopsy specimens from seven normal subjects and nine patients with inflamed mucosa were preincubated with  $PGE_2$  ( $10 \mu M$ ) for five minutes. Results are presented as % inhibition of pLT and  $TXB_2$  release by  $PGE_2$  compared with arachidonic acid ( $10 \mu M$ )-induced eicosanoid release, bars indicating SE.

## Discussion

Intensive research on eicosanoid metabolism in respiratory tract epithelium has shown that epithelial cells release high levels of eicosanoids. Several studies have attributed the release of single eicosanoids to specific cell types.<sup>25</sup> Transcellular eicosanoid metabolism has also been described.<sup>26</sup> To take into account these naturally occurring intercellular mechanisms we used intact unhomogenised tissue specimens.

In this study human bronchial specimens were found to have different basal and arachidonic acid-induced eicosanoid release patterns depending on the inflammatory status of the epithelium. Specimens of normal epithelium released higher basal levels of pLT,  $TXB_2$ , and  $PGE_2$  than inflamed mucosa, but after stimulation with arachidonic acid the inflamed tissue released more  $TXB_2$  and  $PGE_2$  than normal tissue. Incubation with  $PGE_2$  resulted in a mild degree of inhibition of basal pLT and  $TXB_2$  release in normal epithelium but not in inflamed tissue. When biopsy specimens were stimulated with arachidonic acid or anti-IgE,  $PGE_2$  inhibited pLT release most effectively and, to a smaller extent,  $TXB_2$  release in both normal and inflamed epithelium. Prostaglan-

in  $E_2$  inhibition of arachidonic acid-induced pLT and  $TXB_2$  release was more potent than specific cyclo-oxygenase and 5-lipoxygenase inhibitors. Bronchial biopsy specimens from the two patients with allergic alveolitis released higher levels of eicosanoids than those of the control group. The release was allergen specific and the mechanism was mediated by immunoglobulin E rather than by immunoglobulin G.

Peptidoleukotrienes and thromboxane  $A_2$  are known to be potent bronchoconstrictors.<sup>14, 15</sup> The bronchoprotective effects of  $PGE_2$  inhaled by normal and asthmatic subjects using variable antigen challenge have been published. Only slight side effects such as initial cough and retrosternal soreness were observed, which rapidly subsided as inhalation of  $PGE_2$  continued. The inhibitory effect of  $PGE_2$  was assumed to occur on the neuronal pathway by inhibition of afferent and efferent mediator release.<sup>17, 27</sup> Inhibition of leukotriene  $B_4$  release by  $PGE_2$  has also been reported for activated human neutrophils. The action of  $PGE_2$  was shown to be associated with increased levels of cyclic AMP, implying that prostaglandin E was acting at the level of the prostaglandin receptor.<sup>28</sup> Furthermore, leukotriene  $B_4$  is known to activate 5-lipoxygenase.<sup>29</sup> Using arachidonic acid in the functional biopsy test,  $PGE_2$  inhibited pLT release to a greater extent in normal (97%) than in inflamed epithelium (74%), whereas  $TXB_2$  was inhibited similarly in both. There are some parallels between this functional biopsy test and clinical studies in which inhaled  $PGE_2$  provoked bronchodilatation in normal subjects. However, no changes could be shown in patients with asthma,<sup>30</sup> which is associated with inflamed bronchial epithelium.<sup>2, 3</sup> Thus, the reduced effectiveness of  $PGE_2$  on inflamed epithelium could be due to altered prostaglandin receptor expression and/or activity or  $PGE_2$  uptake. Further studies are to be done in this area.

As seen in our study, eicosanoids were released in an allergen specific manner in patients with allergic alveolitis to pigeon feathers. Specific inhibition of cyclo-oxygenase by aspirin was shown to have a bronchoprotective effect in asthmatic subjects which was explained by inhibition of bronchoconstrictive  $PGD_2$  and  $TXA_2$  synthesis.<sup>31, 32</sup> As shown in our study, arachidonic acid-induced  $TXB_2$  release was significantly higher in inflamed epithelium and could be inhibited by  $PGE_2$ , but we have found no publication describing the inhibition of  $TXB_2$  release by  $PGE_2$ . This effect remains to be clarified. The inhibition of this potent and quantitative predominant bronchoconstrictor,  $TXA_2$ , is most likely to cause beneficial effects to patients as bronchoprotective effects were shown using thromboxane receptor antagonists.<sup>33</sup> Furthermore, the inhibition of leukotriene synthesis by specific 5-lipoxygenase inhibitors and the use of a specific  $LTD_4$  receptor antagonist caused bronchoprotection.<sup>34</sup> The probability of slowing down leukotriene biosynthesis by  $PGE_2$  has been also proposed<sup>35</sup> and modulation of cytokine production by  $PGE_2$  is known.<sup>36</sup>

Our data, which provide additional aspects on the action of PGE<sub>2</sub> as a bronchoprotective mediator, in combination with the results of other studies on the effects of inhaled PGE<sub>2</sub>, can be explained in several ways: (1) modulation of cytokine and neuronal mediator release, (2) inhibition of chemotactic leukotriene B<sub>4</sub> synthesis and therefore no activation of 5-lipoxygenase by leukotriene B<sub>4</sub>, and/or (3) inhibition of synthesis of bronchoconstrictive peptidoleukotrienes and TXA<sub>2</sub> (measured as TXB<sub>2</sub>).

In conclusion, this study has shown that PGE<sub>2</sub> has inhibitory effects on the release of bronchoconstrictive pLT and TXA<sub>2</sub>, measured as TXB<sub>2</sub>. This supports the transcellular action of eicosanoids and the hypothesis of bronchoprotective effects of PGE<sub>2</sub>. The efficiency of PGE<sub>2</sub> is modulated by the inflammatory status of the tissue. The use of human biopsy specimens in a functional test is a good method for investigating these effects.

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