

Release of cysteinyl leukotrienes with aspirin stimulation and the effect of prostaglandin E₂ on this release from peripheral blood leucocytes in aspirin-induced asthmatic patients

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Summary

Background The decrease in prostaglandin E₂ (PGE₂) release due to aspirin (ASA)-induced cyclooxygenase inhibition and the increment in cysteinyl leukotriene (Cys-LT) release secondary to the removal of the inhibitory effect of PGE₂ on Cys-LT release have been suggested in the pathogenesis of aspirin-induced asthma (AIA).

Objective In this study, we aimed to investigate the *in vitro* release of Cys-LT and to determine the effect of PGE₂ on Cys-LT release from peripheral blood leucocytes of patients with AIA after stimulation by ASA.

Patients and methods Patients with AIA ($n = 13$), patients with ASA-tolerant asthma (ATA) ($n = 12$) and healthy volunteers as controls ($n = 13$) were included to the study. ASA and PGE₂ at three different concentrations were applied to the peripheral blood leucocytes of the study group, and Cys-LT levels following stimulants were assessed by enzyme immunoassay method.

Results There was no difference in baseline Cys-LT levels between groups (AIA 353.4 ± 55.5 pg/mL, ATA 354.7 ± 40.3 pg/mL, and control group 368.5 ± 30.2 pg/mL; $P > 0.05$). Though not present in other groups, the Cys-LT level of 453.6 ± 70.0 pg/mL following ASA stimulation was higher than baseline in patients with AIA ($P = 0.04$). When PGE₂ was added to the ASA-stimulated samples of patients with AIA, Cys-LT levels were measured as 298.7 ± 78.6 pg/mL, 279.8 ± 79.9 pg/mL, and 243.4 ± 51.3 pg/mL at PGE₂ 10^{-7} M, 10^{-6} M and 10^{-5} M concentrations, respectively. These levels were lower than the ASA-stimulated Cys-LT values ($P = 0.03$, $P = 0.01$ and $P = 0.01$, respectively). The inhibitory effect of different PGE₂ concentrations on Cys-LT release was also present in patients with ATA and in controls.

Conclusion The increase in Cys-LT levels following ASA stimulation seems to be unique to AIA, which was not present in patients with ATA and in healthy controls. The inhibitory effect of PGE₂ on stimulated Cys-LT levels is another important finding to elucidate the role of PGE₂ in the pathogenesis of AIA.

Keywords: aspirin-induced asthma, cysteinyl leukotrienes, prostaglandin E₂

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Introduction

Aspirin (ASA), from its first introduction to date, has been

one of the most consumed over-the-counter analgesics in many countries. Shortly after it appeared in the medical markets, it was noticed that the drug could cause bronchospasm in particular asthmatic patients. In 1968, Samter & Beers described a triad associated with asthma,

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Table 1. The distribution of stimulants according to the tubes in the study protocol

Stimulants	Tube number									
	1	2	3	4	5	6	7	8	9	10
ASA		+		+	+	+	+	+	+	+
fMLP			+	+				+	+	+
Cytochalasin B			+	+				+	+	+
PGE ₂ (10 ⁻⁷ M)					+			+		
PGE ₂ (10 ⁻⁶ M)						+			+	
PGE ₂ (10 ⁻⁵ M)							+			+
Buffer solution added (µL)	50	40	30	20	30	30	30	10	10	10

aspirin intolerance (AI) and nasal polyps [1]. Aspirin intolerance is reported to occur in 5–20% of adult asthmatic patients [2].

The mechanism of aspirin-induced asthma (AIA) remains unknown and has been a current topic of interest of many researchers. However, over the last years, cysteinyl leukotrienes (Cys-LTs) namely, leukotriene (LT)C₄, LTD₄ and LTE₄, have emerged as major mediators in the pathogenesis of AIA [2–5]. Cys-LTs, the most potent bronchoconstrictors yet described, induce mucus secretion, airway oedema and eosinophil influx [6–8]. Improvement in pulmonary function and inhibition of the spectrum of aspirin-associated response after administration of 5-lipoxygenase (5-LO) inhibitors or Cys-LT receptor antagonists in AIA suggested that the pathogenesis is strictly related to the elaboration of the Cys-LTs [9–11]. Cyclooxygenase (COX) enzyme inhibition seems to have a central role in this increment [2–5]. Early theories suggested that blockade of COX by ASA and/or non-steroidal anti-inflammatory drugs (NSAIDs) causes shunting of arachidonic acid into the 5-LO pathway in which Cys-LTs are synthesized and precipitate adverse respiratory reactions [3]. However, the lack of inverse relationship between fall in urinary COX products such as 11-dehydro-tromboxane B₂ and rise in urinary LTE₄ made this hypothesis unlikely [12]. On the other hand, the demonstration of bronchoprotective effect of inhaled prostaglandin E₂ (PGE₂) against aspirin-induced bronchospasm and suppression of rise in urinary LTE₄ after aspirin challenge in patients with AIA has suggested the inhibitory effect of PGE₂ on the 5-LO pathway [13]. According to this new hypothesis implied by only *in vivo* ASA provocation, it has been proposed that PGE₂ exerts direct inhibitory action on the 5-LO pathway and deficiency of PGE₂ as a consequence of COX inhibition causes the removal of suppressive effect and a rise in Cys-LTs.

In this study, we aimed to investigate the *in vitro* release of Cys-LT and to determine the effect of PGE₂ on Cys-LT release from blood leucocytes after stimulation by ASA in patients with AIA, in patients with aspirin-tolerant asthma (ATA) and in healthy controls.

Patients and methods

Patient selection

The study consisted of three groups: Group 1, 13 patients with AIA (mean age 38.8 ± 5.2 years, 11 female, two male) diagnosed by a clinically documented asthmatic response after ingestion of ASA; Group 2, 12 patients with ATA having no problem in taking ASA and/or NSAIDs (mean age 41.8 ± 9.8 years, 11 female, one male); Group 3, 13 healthy volunteers as controls (mean age 34.4 ± 8.2 years, 10 female, three male). Patients with AIA and ATA were comparable regarding the baseline pulmonary function tests (forced expiratory volume in 1 s (FEV₁), %predicted 78.9 ± 4.6 vs. 81.3 ± 3.9), duration of disease (8.6 ± 5.8 years vs. 9.7 ± 6.8 years) and presence of atopy assessed by skin prick tests (46.1% vs. 50%), respectively.

All asthmatic patients were in a stable period for at least 4 weeks and taking regular asthma treatment including inhaled anti-inflammatory drugs, and long-acting and/or short-acting β_2 agonists when necessary. None of them was taking systemic corticosteroid or antihistaminic medications. None of the asthmatic patients was on specific immunotherapy. The subjects who had taken any NSAIDs or ASA during the last 2 weeks were not included in the study. This study was approved by the local ethics committee of Ankara University School of Medicine, and each subject gave informed consent to be included to the study.

Cell preparation

A volume of 3 mL peripheral venous blood was drawn into venepuncture tubes containing EDTA and then transferred into polypropylene tubes. After addition of 1.66 mL of 3.5% dextran solution, the sample was vortexed and incubated at 37°C for 90 min. Then, the supernatant containing blood leucocytes was transferred into another tube and centrifuged at 1500 g and 4°C for 15 min. Finally, the supernatant was discarded and the cells were stimulated. The subjects having a leucocyte count $< 4000/\text{mm}^3$ were excluded from the evaluation.

Cell priming

A volume of 3 mL buffer solution (20 mM HEPES, 0.025%

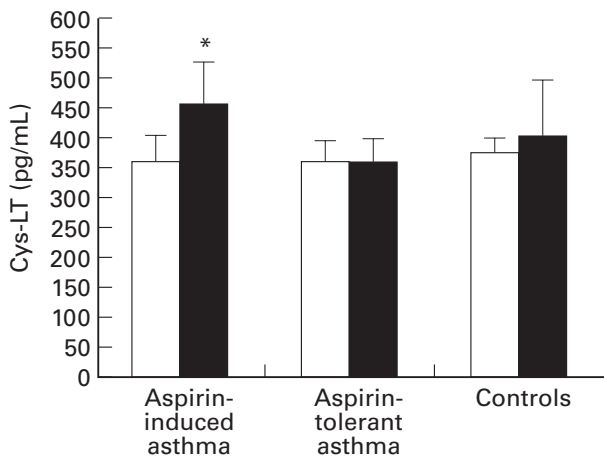


Fig. 1. Baseline (□) and aspirin (ASA)-stimulated (■) cysteinyl leukotriene (Cys-LT) levels. Values are expressed as mean $\pm$ S.E.M. *The Cys-LT level following ASA stimulation was higher than baseline only in patients with aspirin-induced asthma ($P = 0.04$).

HSA, 125 mM NaCl, 5 mM KCl, 2 mM CaCl_2 , 1 mM MgCl_2 , 0.5 mM glucose) containing 1.5 pg/mL IL-3 (human recombinant IL-3; Biosource International, Camarillo, CA, USA) was added to the tube containing the cells immediately at room temperature and vortexed. Before cell stimulation, a volume of 200 μL of this suspension was pipetted into 10 separate Eppendorf tubes and stimulated immediately.

Cell stimulation

Stimulants were applied to the tubes as shown in Table 1. No stimulant was added to the first tube and considered as baseline unstimulated level. ASA (50 $\mu\text{g/mL}$; Sigma, München, Germany) was applied to tube 2 for specific stimulation, 5×10^{-6} M of formyl-methionin-leucine-phenylalanin (fMLP; Sigma) and 5 $\mu\text{g/mL}$ of cytochalasine-B (Cyt-B; Sigma) used as non-specific leucocyte stimulants were added to tube 3. Tube 4 contained both ASA and fMLP + Cyt-B to observe the effect of ASA on fMLP + Cyt-B-stimulated leucocytes to release Cys-LT. The effect of PGE_2 on Cys-LT release was determined on tubes 5–10. Tubes 5, 6 and 7 contained both ASA and PGE_2 at concentrations of 10^{-7} M, 10^{-6} M and 10^{-5} M, respectively. Tubes 8, 9 and 10 contained both ASA, fMLP, Cyt-B and PGE_2 at concentrations of 10^{-7} M, 10^{-6} M and 10^{-5} M, respectively.

A volume of 10 μL of each stimulant was applied to the tubes. Finally, the amount of the volume was completed to the 250 μL volume by buffer solution (Table 1). After adding of stimulants, samples were incubated at 37°C for

40 min and centrifuged at 4°C, 1000 g for 5 min. The supernatant was stored at -70°C until analysis.

Enzyme immunoassay

Cys-LT release was evaluated by competitive enzyme immunoassay (EIA; SPI-BIO, Massy Cedex, France). In the measurement, Cys-LT antiserum, Cys-LT acetylcholine esterase tracer, and standard for Cys-LT were used. The minimal detectable level of Cys-LT was 30 pg/mL. Cross-reactivity for several LO and COX products was $< 0.01\%$. A volume of 50 μL each of sample, tracer and antiserum were pipetted into microplates and incubated at 4°C for 20 h. After removal of unbounded materials by washing with EIA washing solution, 200 μL of Ellmann's reagent were added to microplates. Adsorbance was measured at 414 nm after 60–90 min. A standard curve was achieved using seven concentrations between 15.6 pg/mL and 1000 pg/mL for each microplate. EIA assay was done in duplicates. Cys-LT concentration was calculated by a four-parameter logistic equation.

Statistical analysis

For comparison of baseline and post-stimulant levels in groups, paired-samples *t*-test was used. The intergroup comparisons were accomplished by one-way analysis of variance (ANOVA). In case of significant result after ANOVA, *post hoc* pairwise comparisons were performed by Tukey test. Values were expressed as mean $\pm$ S.E.M. *P*-values < 0.05 were considered statistically significant. Statistical Package for Social Sciences (SPSS) for Windows v6.0. was used for data analysis.

Results

Baseline Cys-LT levels

Baseline Cys-LT levels were 353.4 ± 55.5 , 354.7 ± 40.3 and 368.5 ± 30.2 pg/mL in patients with AIA, in patients with ATA and in controls, respectively, with no significant difference between groups ($P > 0.05$) (Fig. 1).

Cys-LT levels after specific stimulation with ASA

Cys-LT levels were found to be 453.6 ± 70.0 , 353 ± 40.6 and 405.2 ± 86.6 pg/mL in patients with AIA, in patients with ATA and in controls, respectively. The ASA-stimulated Cys-LT level was found to be significantly higher than baseline only in patients with AIA ($P = 0.04$) (Fig. 1).

The percent changes in Cys-LT levels from baseline following ASA stimulation ((post-stimulant level – baseline

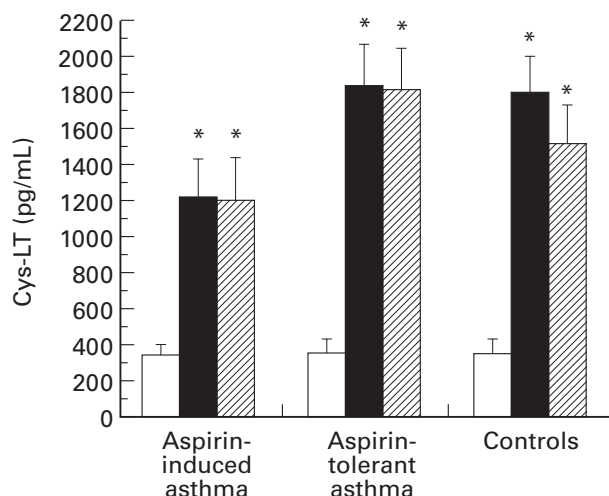


Fig. 2. Cysteinyl leukotriene (Cys-LT) levels at baseline (□) and following formyl-methionin-leucine-phenylalanine (fMLP) + cytochalasine-B (Cyt-B) (■) and aspirin (ASA) + fMLP + Cyt-B (hatched) stimulation. Values are expressed as mean $\pm$ S.E.M. *A significant increase was observed after fMLP + Cyt-B and after ASA + fMLP + Cyt-B stimulation compared with baseline levels. fMLP + Cyt-B levels were not different from ASA + fMLP + Cyt-B levels ($P > 0.05$).

level/baseline level) $\times 100$) were $22.4 \pm 6.5\%$, $-7.4 \pm 5.6\%$ and $-11.6 \pm 12.3\%$ in patients with AIA, in patients with ATA and in controls, respectively. Intergroup comparisons revealed that percent changes in Cys-LT of patients with AIA were higher than both that of patients with ATA and that of controls ($P = 0.02$). The median cut-off level for percent change in Cys-LT levels was 15.2 in patients with AIA and all samples (13/13) exhibited an increase over baseline levels following ASA stimulation where this trend was present in 50% (6/12) and 46.1% (6/13) of patients with ATA and in control subjects, respectively.

Cys-LT levels after non-specific stimulation with fMLP and Cyt-B

A significant increase over baseline levels was observed after fMLP + Cyt-B stimulation in each group (AIA 1230.1 ± 196.3 pg/mL, $P = 0.04$; ATA 1860.7 ± 600.9 pg/mL, $P = 0.004$; and controls 1832.2 ± 238.2 pg/mL, $P = 0.008$) (Fig. 2).

Cys-LT levels after stimulation with fMLP + Cyt-B and ASA

After addition of ASA to fMLP + Cyt-B, a significant increase was observed in each group when compared with baseline (AIA 1211.8 ± 130.7 pg/mL, $P = 0.001$; ATA

1843.3 ± 428.1 pg/mL, $P = 0.02$; and controls 1541.9 ± 206.1 pg/mL, $P = 0.0009$) (Fig. 2). However, these levels were not different from those of fMLP + Cyt-B-stimulated alone ($P > 0.05$) (Fig. 2).

Cys-LT levels after stimulation with PGE₂

Patients with AIA After addition of PGE₂ at concentrations of 10^{-7} M, 10^{-6} M and 10^{-5} M to ASA-stimulated leucocytes, Cys-LT levels were found to be 298.7 ± 78.6 , 279.8 ± 79.9 and 243.4 ± 51.3 pg/mL, respectively. These results were significantly lower than only ASA-stimulated Cys-LT levels ($P = 0.03$, $P = 0.01$ and $P = 0.01$, respectively) (Fig. 3).

Cys-LT levels, after addition of PGE₂ to the samples stimulated by both ASA and fMLP + Cyt-B, were 1104.5 ± 177.6 pg/mL for PGE₂ 10^{-7} M, 1074.1 ± 149.9 pg/mL for PGE₂ 10^{-6} M, and 882.6 ± 105.8 pg/mL for PGE₂ 10^{-5} M. The Cys-LT level after addition of PGE₂ 10^{-5} M to the samples stimulated by both ASA and fMLP + Cyt-B was significantly lower than the levels of ASA and fMLP + Cyt-B-stimulated alone samples ($P = 0.009$) (Fig. 4a).

Patients with ATA Following addition of PGE₂ at concentrations of 10^{-7} M, 10^{-6} M and 10^{-5} M to ASA-stimulated leucocytes, Cys-LT levels were 311.6 ± 82.6 , 306.6 ± 66.6 and 300.5 ± 68.0 pg/mL, respectively. Cys-LT levels obtained after addition of PGE₂ at 10^{-6} M and 10^{-5} M concentrations to the ASA-stimulated samples were significantly lower than only ASA-stimulated Cys-LT levels ($P = 0.04$ and $P = 0.03$).

Cys-LT levels, after addition of PGE₂ to the samples stimulated by both ASA and fMLP + Cyt-B, were 1375.3 ± 405.6 pg/mL for PGE₂ 10^{-7} M, 1178.2 ± 378.0 pg/mL for PGE₂ 10^{-6} M and 990.8 ± 446.1 pg/mL for PGE₂ 10^{-5} M. The Cys-LT level after adding PGE₂ at 10^{-7} M, 10^{-6} M and 10^{-5} M concentrations to the samples stimulated with both ASA and fMLP + Cyt-B was significantly lower than the level of only ASA and only fMLP + Cyt-B-stimulated samples ($P = 0.04$, $P = 0.03$ and $P = 0.02$, respectively) (Fig. 4b).

Controls After addition of PGE₂ at concentrations of 10^{-7} M, 10^{-6} M and 10^{-5} M to ASA-stimulated leucocytes, Cys-LT levels were found to be 324.8 ± 87.3 , 290.7 ± 64.1 and 258.5 ± 60.9 pg/mL, respectively. Cys-LT levels obtained after addition of PGE₂ at 10^{-7} M, 10^{-6} M and 10^{-5} M concentrations to ASA were significantly lower than only ASA-stimulated Cys-LT levels ($P = 0.03$, $P = 0.009$ and $P = 0.007$, respectively).

Cys-LT levels, after addition of PGE₂ to the samples stimulated by both ASA and fMLP + Cyt-B, were

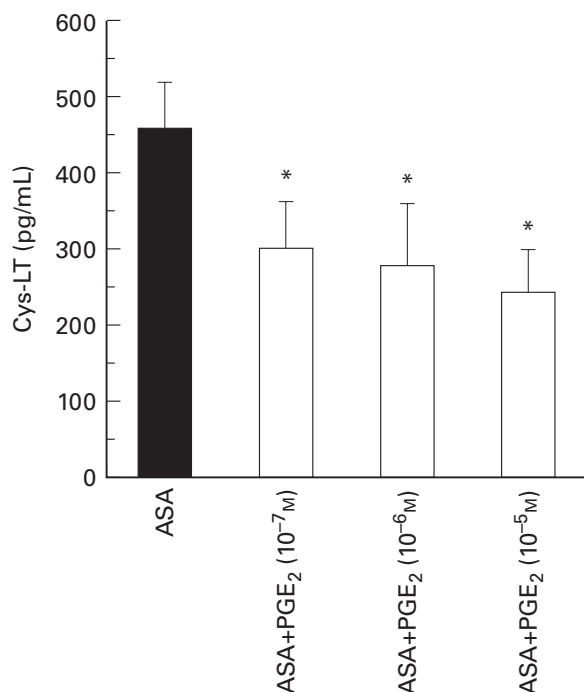


Fig. 3. Cysteinyl leukotriene (Cys-LT) levels following aspirin (ASA) and ASA + three different concentrations of prostaglandin E₂ (PGE₂) in patients with aspirin-induced asthma. Values are expressed as mean ± S.E.M. *After addition of PGE₂ at concentrations of 10⁻⁷ M, 10⁻⁶ M and 10⁻⁵ M to ASA-stimulated leucocytes, Cys-LT levels were found to be significantly lower than only ASA-stimulated Cys-LT levels ($P = 0.03$, $P = 0.01$ and $P = 0.01$, respectively).

1349.8 ± 189.3 pg/mL for PGE₂ 10⁻⁷ M, 1220.8 ± 184.6 pg/mL for PGE₂ 10⁻⁶ M and 962.3 ± 171.9 pg/mL for PGE₂ 10⁻⁵ M. The Cys-LT level, after adding PGE₂ at 10⁻⁶ M and 10⁻⁵ M concentrations to ASA and fMLP + Cyt-B-stimulated samples, was significantly lower than those of only ASA and only fMLP + Cyt-B-stimulated samples ($P = 0.04$ and $P = 0.03$) (Fig. 4c).

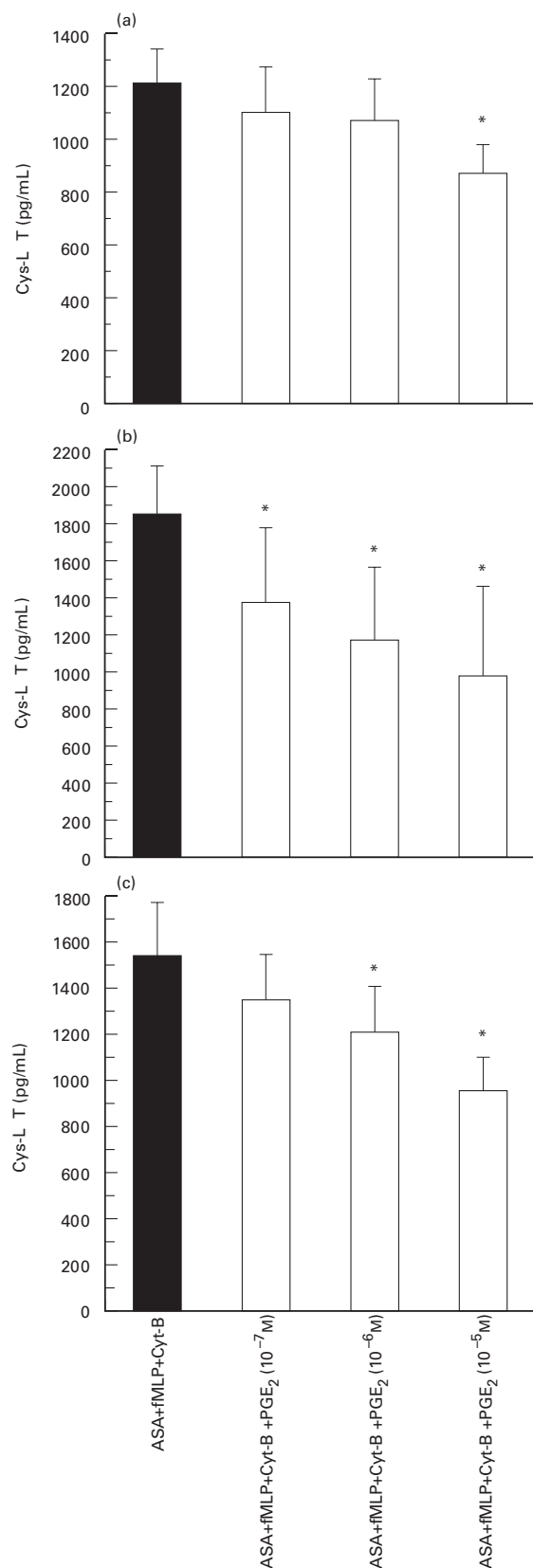
Discussion

Cys-LT over-production has been detected in bronchoalveolar lavage fluid, nasal secretions and urine of patients with AIA after challenge with ASA, but such a release of Cys-LT by ASA has not been observed in healthy subjects [14–20]. In a study conducted by Mewes and co-workers, Cys-LT release from blood leucocytes has been evaluated *in vitro* after ASA challenge in patients with asthma, nasal polyps and AI, in patients with nasal polyps without AI and in controls [21]. In this study, baseline Cys-LT levels were found to be similar and an increase in Cys-LT release after *in vitro* aspirin provocation has been observed in patients with asthma, AI, and nasal polyps. In accordance with

Mewes *et al.*, we found that baseline Cys-LT levels were similar in patients with AIA, in patients with ATA and in controls, with an increase in Cys-LT levels after *in vitro* aspirin provocation being observed only in patients with AIA. However, in the former study, as also mentioned by the authors, the study group did not consist of patients having asthma without AI, namely ATA. It has been demonstrated that Cys-LT release is increased not only in patients with AIA but also in patients with ATA, particularly after exposure to allergen and to some non-specific irritants [22,23]. As it seems necessary in such studies that the study group should also include patients with ATA, our study was designed to cover both patients with AIA and patients with ATA. We demonstrated that though baseline values were similar in patients with AIA, in patients with ATA and in healthy controls, peripheral blood leucocyte Cys-LT levels were increased only in patients with AIA after *in vitro* ASA provocation. In accordance with our data, recently May and co-workers also demonstrated the increase in Cys-LT release in patients with aspirin intolerance after *in vitro* stimulation with ASA and C5a over control groups with a similar baseline Cys-LT release in all groups [24]. Of interest, the percent increase of about 20% in Cys-LT release obtained in our study is modest compared with previous studies reporting more than two-fold increments after *in vivo* [15–19] and *in vitro* [24] aspirin challenge; this discrepancy might be due to particular methodological differences in the selection of the sample assayed (i.e. serum, urine, bronchoalveolar lavage or nasal secretions) and/or the dose of *in vivo* or *in vitro* ASA applied.

fMLP and Cyt-B are known to induce non-specific leucocyte Cys-LT release, particularly after priming by IL-3, IL-5 or granulocyte-macrophage colony-stimulating factor (GM-CSF). In our study, fMLP and Cyt-B have been shown to lead a strong Cys-LT release in all groups. However, in patients with AIA, the Cys-LT levels obtained after addition of ASA to the fMLP + Cyt-B-stimulated samples were not different from those stimulated with fMLP + Cyt-B alone. We therefore hypothesize that aspirin increases baseline Cys-LT release but has no additional effect on fMLP + Cyt-B-stimulated Cys-LT levels. Unlike the finding of aspirin-induced Cys-LT release being unique for patients with AIA in our study, fMLP + Cyt-B stimulation seemed to be a strong but non-specific inducer of Cys-LT release.

PGE₂, an important COX product, is produced by several cells including endothelial and epithelial cells of the respiratory tract, airway smooth muscle cells and macrophages [25]. Endogenous PGE₂ has been shown to have an important anti-inflammatory and bronchoprotective role in the airways [26]. It inhibits the release of chemoattractants such as leukotriene B₄ (LTB₄) both from alveolar



macrophages [27] and human airway epithelium [28], and has also been shown to inhibit mast cell mediator release *in vitro* [29]. Moreover, PGE₂ has variable *in vitro* smooth muscle relaxing effects in human airways. Though it causes bronchodilation in normal subjects, a more variable effect occurs in asthmatic subjects [30]. PGE₂ is likely to modulate the airway tone indirectly probably by inhibiting both acetylcholine release from cholinergic nerve endings and mast cell histamine release. In a study of Pavord *et al.* inhaled PGE₂ has been shown to inhibit the early and late asthmatic responses to inhaled allergen and to abolish the increase in bronchial reactivity [31]. In addition, inhaled PGE₂ also inhibits the response to exercise [32], ultra-sonically nebulized distilled water [33] and sodium metabisulphite in patients with asthma [34]. As far as the patients with AIA is concerned, it prevents ASA-precipitated bronchoconstriction [13,35,36] and the expected rise in urinary LTE₄ when given by inhalation of PGE₂ or as an oral PGE₁ analogue, namely misoprostol [13,36]. It seems unlikely that this protection is a result of a direct effect on airway smooth muscle, because the bronchodilation induced by inhaled PGE₂ is known to be modest [26]. Besides, the observation that PGE₂ abolishes ASA-induced urinary excretion of LTE₄, strongly suggests that the protective activity is due to the inhibition of the release of these mediators. Nevertheless, the *in vitro* demonstration of the inhibition of Cys-LT release by PGE₂ has not been achieved yet.

In the second part of this study we examined the inhibitory effect of PGE₂ on Cys-LT release. Our results demonstrate that PGE₂ inhibited Cys-LT release from blood leucocytes after both aspirin and fMLP + Cyt-B + aspirin provocations in all groups, and this inhibitory effect was more prominent at higher concentrations of PGE₂. These findings have not been previously reported, and our study is the first to demonstrate the *in vitro* inhibitory effect of PGE₂ on Cys-LT release after *in vitro* ASA provocation. Our *in vitro* findings and the recent demonstration of lower

Fig. 4. Effect of prostaglandin E₂ (PGE₂) (□) on aspirin (ASA) + formyl-methionin-leucine-phenylalanine (fMLP) + cytochalasin-B (Cyt-B) (■)-stimulated cysteinyl leukotriene (Cys-LT) release in patients with aspirin-induced asthma (a), patients with aspirin-tolerant asthma (b), and healthy controls (c). Values are expressed as mean ± S.E.M. *In patients with aspirin-induced asthma (AIA), only PGE₂ at a concentration of 10⁻⁵ M significantly inhibited Cys-LT release ($P = 0.009$) (a). In patients with ASA-tolerant asthma (ATA), all concentrations of PGE₂ at 10⁻⁷ M, 10⁻⁶ M and 10⁻⁵ M inhibited Cys-LT release ($P = 0.04$, $P = 0.03$ and $P = 0.02$, respectively) (b). In controls, Cys-LT levels after addition of PGE₂ 10⁻⁶ M and 10⁻⁵ M to ASA + FMLP + Cyt-B-stimulated samples were significantly lower than ASA + FMLP + Cyt-B-stimulated levels ($P = 0.04$ and $P = 0.03$) (c).

baseline PGE₂ levels both in peripheral blood and in nasal polyp tissues of patients with AI [37,38] are strong evidence to support the hypothesis of an altered metabolism of arachidonic acid in patients with AIA. The demonstration of an *in vitro* inhibitory effect of PGE₂ on Cys-LT release might also provide insights for subsequent trials to characterize the prostanoid receptor status of patients with asthma, and establish that a potential difference in distribution and/or function of PGE receptors on effector cells could possibly be involved in the pathogenesis of AIA [39].

In conclusion, the demonstration of increased Cys-LT release from blood leucocytes in all AIA subjects suggests that the enhanced Cys-LT production that underlies AIA may result at least in part from an abnormality in the 5-LO pathway that is expressed systemically as well as in leucocytes infiltrating the airways. The documentation of an *in vitro* inhibitory effect of PGE₂ on ASA-induced Cys-LT release is another novel finding and deserves further research directed to develop new therapeutic options for ASA-intolerant patients.

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