

Dynamics of eicosanoids in peripheral blood cells during bronchial provocation in aspirin-intolerant asthmatics

D. Schäfer*, M. Schmid**, U.C. Göde**, H-W. Baenkler*

Dynamics of eicosanoids in peripheral blood cells during bronchial provocation in aspirin-intolerant asthmatics. D. Schäfer, M. Schmid, U.C. Göde, H-W. Baenkler. ©ERS Journals Ltd 1999.

ABSTRACT: The underlying mechanisms of bronchoconstriction in aspirin-intolerant asthmatics (AIAs) are still unknown, but the hypothesis of an altered metabolism of arachidonic acid is generally accepted. So far, no *in vitro* test for aspirin intolerance is available. The hypothesis that the profile of eicosanoid mediators is changed in AIA-even before aspirin challenge was tested.

The release of prostaglandin E₂ (PGE₂), peptidoleukotrienes and histamine was measured using competitive enzyme immunoassays in 10 asthmatics with a history of aspirin intolerance, 10 controls and eight aspirin-tolerant asthmatics (ATAs) before and after bronchial provocation with lysine-aspirin.

Comparing basal release of eicosanoids before challenge, peptidoleukotrienes were significantly elevated and PGE₂ was vastly reduced in AIAs, whereas ATAs had elevated basal peptidoleukotrienes but only slightly reduced basal PGE₂. The decrease in forced expiratory volume in one second (FEV₁) was not associated with changes in histamine release. After aspirin challenge, there was a massive increase of already elevated peptidoleukotrienes in AIAs, but not in ATAs. Arachidonic acid-induced PGE₂ release in AIAs was not significantly changed, whereas it was significantly reduced in ATAs and healthy controls. Histamine release was unaffected by aspirin challenge in all three groups.

There is a typically altered profile of eicosanoids in aspirin-intolerant asthmatics which could make *in vitro* diagnosis of aspirin intolerance possible.

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Aspirin intolerance affects ~10% of adult asthmatics. The full-blown triad of asthma, aspirin intolerance and nasal polyposis is usually associated with a severe type of asthma and frequently requires chronic treatment with glucocorticoids [1–3]. The pathogenesis of aspirin intolerance has not yet been clearly defined. On the one hand, inhibition of cyclooxygenase and lack of metabolites, *e.g.* prostaglandin E₂ (PGE₂), appears to initiate a chain of reactions [4]. On the other hand leukotrienes (metabolites of the 5-lipoxygenase pathway) are possible mediators of aspirin-induced asthma, because they have strong bronchoconstrictive effects [5]. Studies of aspirin-induced asthma focused on measuring mediators in bronchoalveolar lavage fluid [6], nasal secretion [7, 8], urinary excretion [9–11] or separated cells [12–14]. So far, no *in vitro* test for aspirin intolerance is available, and diagnosis relies on the history and different methods of provocation tests with variable sensitivity [15]. All these tests carry the risk of provoking a severe asthma attack.

Currently, many theories try to explain the symptoms of aspirin intolerance. Most theories are focused on the inhibition of cyclooxygenase [4]. Others postulate shunting of arachidonic acid from biosynthesis of prostaglandins to leukotrienes [13] or the modulation of the metabolism of

neuropeptides and other mediators by PGE₂ [16]. Furthermore, an albumin-binding property of analgesic drugs was reintroduced [17]. Thus, it is important to look not only at the lipoxygenase pathway alone, but to evaluate both interdependent pathways, *i.e.* the "profile" of eicosanoids, and also histamine, as it might play a role in the pathogenesis of asthma [18].

In the present study the dynamics of mediator release in peripheral blood cells (PBC) from aspirin-intolerant asthmatics (AIAs) during bronchial provocation with lysine-aspirin was examined. To test the hypothesis that the profile of eicosanoid mediators is changed in AIA not only after, but also before, aspirin challenge, mediator release of PBC was compared to healthy subjects and aspirin-tolerant asthmatics (ATAs). The release of PGE₂, peptidoleukotrienes (pLT) and histamine of PBC was measured to assess the role of the cyclooxygenase- and 5-lipoxygenase-pathway and the implication of histamine in a system with intact intercellular mechanisms. Thus, the long-term goal of the study was to check the possibility of a simple and cheap *in vitro* whole blood test for routine diagnosis of aspirin intolerance. For this purpose the study was not designed to identify the role of different cell types in aspirin intolerance.

Materials and methods

Subjects

Normal subjects. Ten subjects (four male, six females) were studied, aged 22–38 yrs, who had no history of asthma or sensitivity to aspirin. Allergic disease was ruled out with a prick test. Sinusitis was ruled out by endoscopy. Four subjects were smokers and none were atopic.

Aspirin-intolerant asthmatics. Ten patients (three male, seven females) aged 20–58 yrs with classic symptoms of an aspirin triad were studied. They were selected on the basis of a previous severe asthma attack after ingestion of aspirin or another nonsteroidal anti-inflammatory drug and a decrease of forced expiratory volume in one second (FEV₁) >25% after bronchial provocation. All patients had recurrent chronic polypous sinusitis on nasoendoscopy. All of the patients had undergone previous surgery for polyposis. None of the patients received topical or systemic corticosteroid therapy during the last 14 days before the study. Therapy with antihistamines or cromolyn sodium was withheld for at least 24 h prior to the study.

Aspirin-tolerant asthmatics. Eight patients (four male, four female) aged 35–58 yrs with long-term intrinsic asthma were included as an additional control group. Allergy was ruled out by careful history taking, prick-test and radioallergosorbent test (RAST). All of the ATAs used inhalative β -sympathomimetics as regular medication. Five of the patients used inhalative corticosteroids (budesonide 0.2 mg·day⁻¹ *b.i.d.*), as an additional drug, infrequently prior to the study. At the time of examination none of the patients had used inhalative corticoids for at least four days. Aspirin intolerance was ruled out by oral provocation with a single dose of 500 mg acetylsalicylic acid (ASA) under control with a pulmonary function analyser (Draeger, Lübeck, Germany). Oral provocation was only used for patients in control groups (ATAs, normal subjects) with absolutely no history of an adverse reaction to ASA.

Inhalative provocation

Aspirin challenges were performed as described by SCHMITZ-SCHUMANN *et al.* [19]. All subjects underwent bronchial provocation with lysine-aspirin (Aspisol; Bayer Leverkusen, Germany), which was diluted with saline to produce a range of increasing concentrations from 1.25, 2.5, 5.0, 7.5, 12.5 to 25 mg·mL⁻¹. One millilitre was placed in a Heyer nebulizer, and the subjects inhaled *via* a mouthpiece during normal breathing. Measurements of airway parameters were performed with a pulmonary function analyser (Draeger), and the subjects were excluded if baseline FEV₁ was <65% of predicted value. If the decrease of FEV₁ was <10% after inhalation of normal saline, lysine-aspirin challenge was performed. Two measurements of FEV₁ were made at 15 and 30 min after each dose of inhaled lysine-aspirin, and the maximum reading was recorded. If the decrease of FEV₁ was <15%, the next dose of lysine-aspirin was inhaled, until there was a 25%

decrease of FEV₁, or if there was a fall $\geq 15\%$ with clinical signs of bronchial obstruction. This dose was considered the threshold dose. Pulmonary function was monitored for another 24 h with a peak-flow meter and measurement of FEV₁.

Cell preparation and experimental in vitro protocol

Venous blood (10 mL) was collected before and after successful inhalative provocation in a 10 mL syringe containing 5% heparin sodium (Promonta, Hamburg, Germany). Peripheral blood cells were prepared by dextran sedimentation (5% dextran 500, GIBCO, Hamburg, Germany) for 60 min at 37°C. The top layer was recovered, washed twice with phosphate-buffered saline (PBS) by centrifugation ($900 \times g$ for 10 min at 4°C) and the pellet was resuspended in RPMI-1640 without foetal calf serum. A concentration of 10^5 PBC·mL⁻¹ (in a volume of 250 μ L·well⁻¹) was incubated as a duplicate culture in 96-well flat bottom plates which had ultra low binding interactions (Corning Costar Co., Cambridge, USA) in a humidified 5% CO₂/air mixture at 37°C for 20 min or as indicated. Adherence of cells was ruled out by light microscopy. Cells were separated using a standard Ficoll/Hypaque-gradient centrifugation ($1,000 \times g$, 40 min at 4°C). The upper layer of Percoll-gradient centrifugation ($400 \times g$, 5 min, 4°C) was removed for monocyte/lymphocyte preparation and washed twice with PBS. The lower layer was recovered for granulocyte preparation and washed three times with PBS ($400 \times g$, 5 min, 4°C) and erythrocytes were lysed by addition of distilled water (30 s on ice) and washing with PBS ($350 \times g$, 5 min, 4°C). The pellet was resuspended in RPMI-1640. The purity of the monocyte and polymorphonuclear granulocyte preparation was >97% and >95%, respectively. The viability of cells was >99% by trypan blue exclusion. Cell isolation revealed the absence of platelets, a source of PGE₂ production. For basal mediator release, cells were incubated without any stimulants; induced mediator release was achieved by addition of 10^{-5} M arachidonic acid, which proved to be an optimal concentration for this experimental procedure (fig. 1b). Additionally, calcium ionophore A23187 was tested with and without arachidonic acid. Eicosanoid release was lower using calcium ionophore alone compared to arachidonic acid. If both stimuli were used, a synergistic effect was observed resulting in a higher release of eicosanoids. However, the standard deviation of the measurements with calcium ionophore was higher, so that this stimulus was omitted.

Arachidonic acid is the main substrate for cyclooxygenase and 5-lipoxygenase and is released from its stores in tissue lipids by the action of phospholipase A₂ (PLA₂). PLA₂ are a diverse family of regulatory enzymes which play a central role in the regulation of the biosynthesis of numerous lipid pro-inflammatory mediators. Its activity is calcium dependent [21]. As this study wanted to focus on the activation of cyclooxygenase and lipoxygenase, the complex regulation and interaction of PLA₂ had to be bypassed. Therefore, arachidonic acid was chosen to be the optimal stimulus for induction of pLT and PGE₂ release in these experiments. Arachidonic acid-induced histamine release was measured, to rule out the histamine-influence on arachidonic acid-induced pLT and PGE₂ release.

For *in vitro* inhibition experiments PBC were pre-incubated for 15 min with variable concentrations of PGE₂, dibutyl-*cyclic* adenosine monophosphate (cAMP), or caffeic acid (10^{-5} M). The incubation of PBC was terminated by centrifugation ($900 \times g$, 7 min, 4°C) and supernatants were collected in 96-well flat bottom plates and frozen at -80°C for up to four weeks until mediator quantification. The chemicals were purchased from Biomol (Hamburg, Germany).

Quantification of mediators

The release of immunoreactive pLT, PGE₂ and histamine was quantified simultaneously for each sample in duplicates by highly sensitive and specific competitive enzyme immunoassays (EIA) using formerly established and validated monoclonal antibodies for pLT [22] and PGE₂ [23]. The cross-reactivities of the monoclonal pLT-antibody were 95.7%, 100%, and 88.7% for leukotriene (LT)C₄, LTD₄, and LTE₄, respectively, those for the monoclonal PGE₂-antibody were 100% and 5.54% for PGE₂ and PGE₁, respectively. For more detailed data on cross-reactivities for the other eicosanoids tested, see [22, 23]. The pLT- and PGE₂-EIA were performed according to the protocols published recently [24], diluting standard and

samples in RPMI-1640. The intra- and interassay variance for pLT-EIA were 9% and 7.5%, respectively and for PGE₂-EIA 8% and 6%, respectively. The rate of recovery for pLT and PGE₂ was $\geq 98\%$ with a sensitivity of $1 \text{ pg}\cdot\text{mL}^{-1}$ for pLT-EIA and $3 \text{ pg}\cdot\text{mL}^{-1}$ for PGE₂-EIA. Owing to the extremely low basal production of pLT these measurements were made in a range below the recommended sensitivity of the assay (values of basal pLT were in the range $>80\% B/B_0$ at $1 \text{ pg}\cdot\text{mL}^{-1}$). Histamine was measured by a commercially available histamine-EIA purchased from DIANOVA-Immunotech (Hamburg, Germany). The assay was performed according to the instructions of the company, diluting samples in RPMI-1640. The results are presented in $\text{pg}\cdot\text{mL}^{-1}$.

Data analysis

The eicosanoid and histamine release values are expressed as arithmetic mean \pm SEM. The two-tailed t-test was used for statistical calculation on normally distributed data. Student's paired t-test was used to compare the stimulated mediator release within the subjects. The Mann-Whitney test was used to compare AIAs, ATAs and normal subjects. A p-value of <0.05 was regarded as statistically significant.

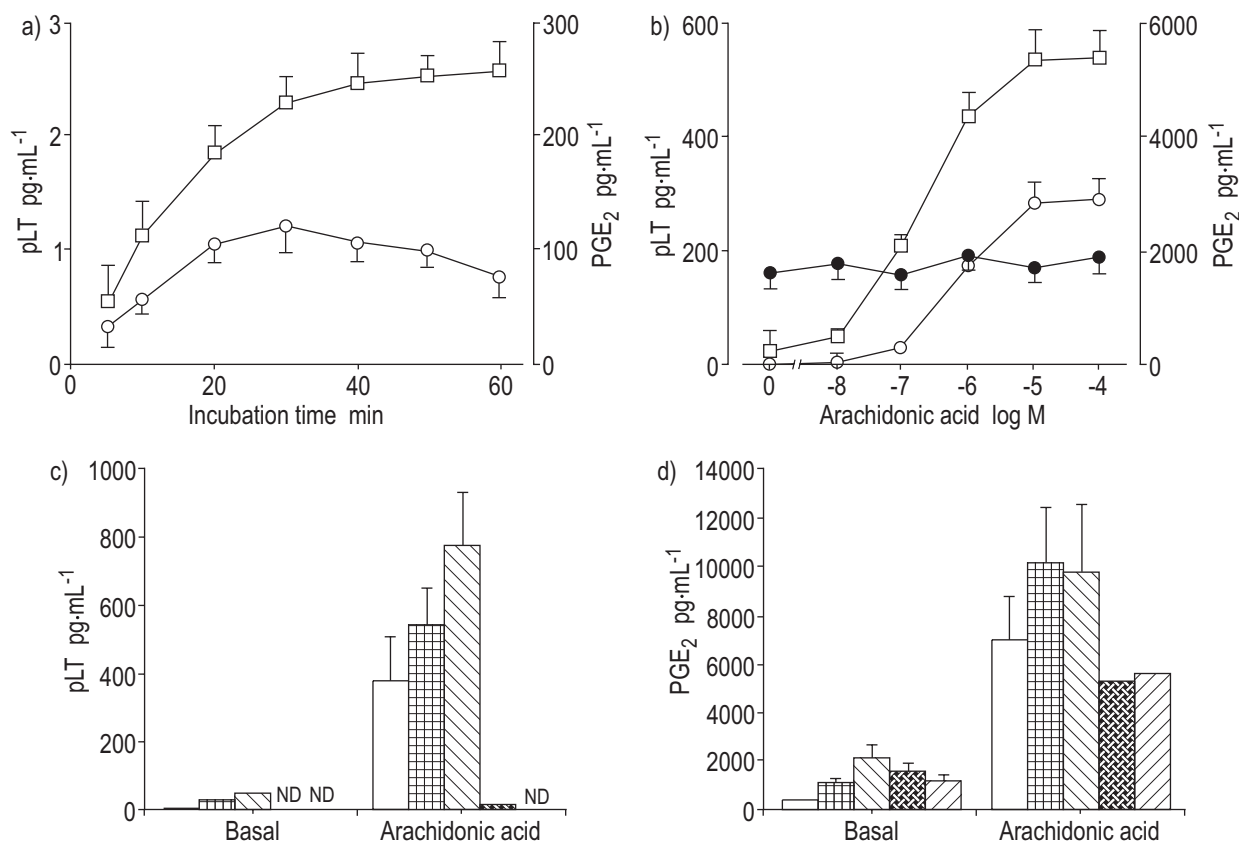


Fig. 1. – a) Kinetics of basal eicosanoid (peptidoleukotriene (pLT; ○) and prostaglandin E₂ (PGE₂; □)) release. In this experiment 10^5 peripheral blood cells (PBC)·mL⁻¹ from normal subjects (n=7) were incubated in duplicates. b) Dose response to arachidonic acid. In this experiment 10^5 PBC·mL⁻¹ from normal subjects (n=10) were incubated in duplicates. ●: histamine (His). c) pLT release from different cell types. In experiments for both (c) and (d) cells were separated from normal subjects (n=10) by Ficoll gradient centrifugation. Every cell subset (□: PBC; ▨: monocytes; ▩: polymorphonuclear granulocytes; ▤: T-lymphocytes; ▧: Jurkat human T-cell line) was standardized to 10^5 cells·mL⁻¹. Samples were incubated in duplicates. ND: No detectable levels found. Bars indicate SEM.

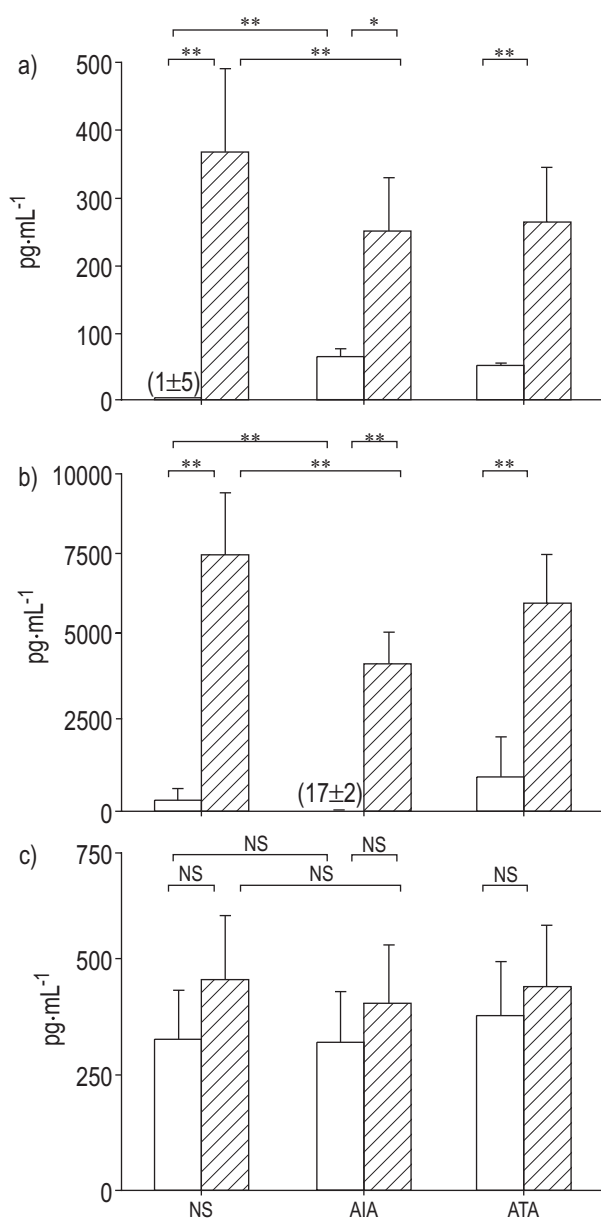


Fig. 2. – Induced mediator release of peptidoleukotriene (a), prostaglandin E₂ (b) and histamine (c). Basal (□) and arachidonic acid (▨)-induced (10^{-5} M) eicosanoid and histamine release were measured in duplicate samples of peripheral blood cells from aspirin-intolerant asthmatics (AIA; n=10), aspirin-tolerant asthmatics (ATA; n=8) and normal subjects (NS; n=10) before aspirin challenge. Numerical values in parentheses have been added where bars are not clearly visible. *: $p < 0.05$; **: $p < 0.01$.

Results

Eicosanoid release: kinetic, arachidonic acid induced release and involved cell type

The kinetic of basal pLT and PGE₂ release of PBC from normal subjects are shown in figure 1a. Basal pLT release was at a maximum after 20 min of incubation (1.2 ± 0.8 pg·mL⁻¹) and decreased after that (0.8 ± 0.3 pg·mL⁻¹ after 60 min), whereas basal PGE₂ release had a slower onset (185 ± 31.5 pg·mL⁻¹ after 20 min) and without a significant decrease during the observed incubation time (258 ± 49.1

pg·mL⁻¹ after 60 min) in this protocol. Optimal detection levels for both eicosanoids were achieved after 20 min of incubation with 10^5 PBC·mL⁻¹. Similar results were obtained by stimulation with arachidonic acid (10^{-5} M, data not shown). The production of pLT was more than ten times lower than for PGE₂ release.

For arachidonic acid a concentration dependent elevation of pLT and PGE₂ release was measured, using 20 min of incubation with 10^5 PBC·mL⁻¹ (fig. 1b). Again after stimulation with 10^{-5} M arachidonic acid, where mediator release reached stable levels, pLT release was lower (282 ± 45.7 pg·mL⁻¹) than PGE₂ release ($5,240 \pm 354.6$ pg·mL⁻¹). This concentration of arachidonic acid was selected for further study. Histamine release of PBC (181.2 ± 23.4 pg·mL⁻¹) was not influenced by stimulation with exogenous arachidonic acid.

As seen in figure 1c, basal and arachidonic acid-induced pLT and PGE₂ release of PBC (basal pLT: 1.2 ± 0.6 pg·mL⁻¹, induced pLT: 378 ± 47.2 pg·mL⁻¹; basal PGE₂: 380 ± 26.1 pg·mL⁻¹, induced PGE₂: $7,050 \pm 1,540$ pg·mL⁻¹) can be attributed mostly to monocytes (basal pLT 23 ± 9.6 pg·mL⁻¹, induced pLT 543 ± 154.3 pg·mL⁻¹; basal PGE₂: $1,080 \pm 97.5$ pg·mL⁻¹, induced PGE₂: $10,200 \pm 2,430$ pg·mL⁻¹) and polymorphonuclear granulocytes (basal pLT 45 ± 12.4 pg·mL⁻¹, induced pLT 777.6 ± 194.7 pg·mL⁻¹; basal PGE₂: $2,910 \pm 341.5$ pg·mL⁻¹, induced PGE₂: $9,780 \pm 2,610$ pg·mL⁻¹), which are part of PBC used in the other experiments. The Jurkat T-cell line did not release pLT, but PGE₂ (basal $1,560 \pm 150.7$ pg·mL⁻¹, induced $5,340 \pm 395.2$ pg·mL⁻¹) in this procedure. Also for the separated T-cells no amounts of basal pLT release were measured. But there were minute amounts after stimulation with arachidonic acid (10 ± 7.1 pg·mL⁻¹), which can be explained by the separation technique and a contamination with monocytes/granulocytes of 2%. PGE₂ release of separated T-lymphocytes was similar to that of the Jurkat T-cell line (basal $1,160 \pm 134.1$ pg·mL⁻¹, induced $5,660 \pm 428.6$ pg·mL⁻¹). The data shown supports the idea that the examination of PBC could be a valuable method to analyse basics of eicosanoid metabolism and to develop a screening test for aspirin intolerance.

Induction of eicosanoid release before aspirin challenge

Testing the synthesis capacity of 5-lipoxygenase and cyclooxygenase and getting around PLA₂-dependent mechanisms, PBC were incubated with arachidonic acid. As shown in figure 2, pLT and PGE₂ release were significantly elevated by addition of arachidonic acid with no effect on histamine release in normal subjects, AIAs or ATAs. PBC of AIAs released significantly higher levels of basal pLT (55.5 ± 8.5 pg·mL⁻¹) but significantly lower basal PGE₂ (17 ± 9.7 pg·mL⁻¹) than the normal subjects (1.5 ± 0.4 pg·mL⁻¹ for pLT and 236 ± 38.2 pg·mL⁻¹ for PGE₂). ATAs released similar amounts of basal pLT (40.2 ± 9.6 pg·mL⁻¹) as AIAs, but in contrast to AIAs a huge release of basal PGE₂ (723.8 ± 69.3 pg·mL⁻¹) was noted. Although basal PGE₂ release in AIAs was extremely low (17 ± 9.7 pg·mL⁻¹) it could be stimulated very well by arachidonic acid (by a factor of 235). Arachidonic acid-induced PGE₂ release did not differ significantly in all groups ($7,378 \pm 1,530$ pg·mL⁻¹ for normal subjects, $4,099 \pm 958$ pg·mL⁻¹

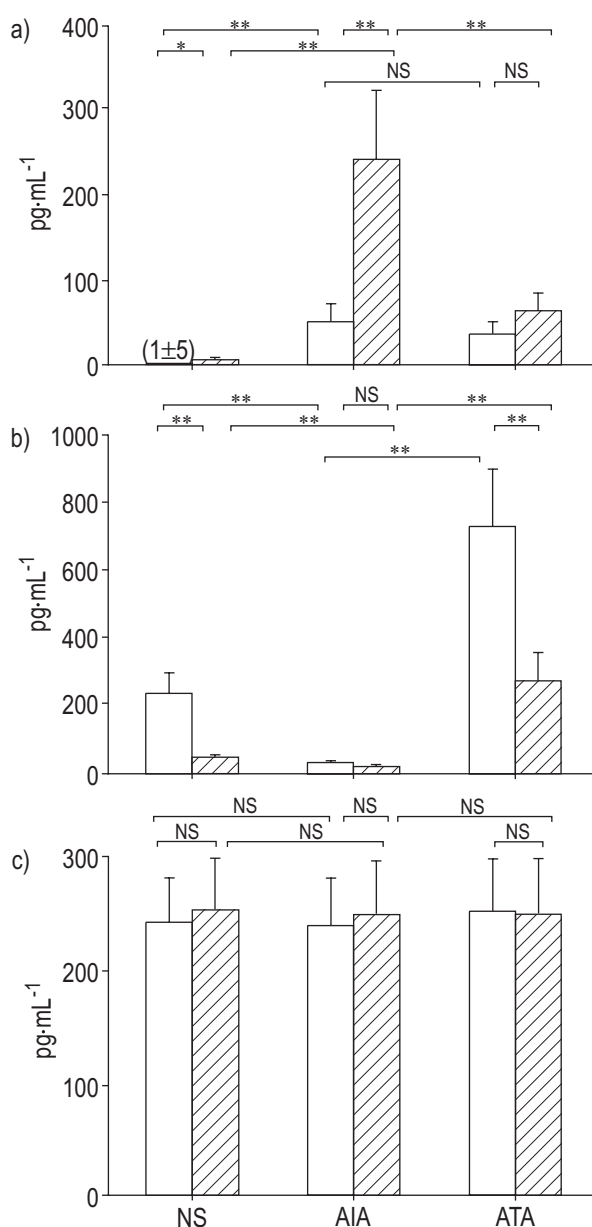


Fig. 3. – Basal mediator release influenced by aspirin challenge. Basal release of peptidoleukotriene (a), prostaglandin E₂ (b) and histamine (c) of peripheral blood cells was quantified before and after aspirin challenge in normal subjects (NS; n=10) and aspirin-intolerant asthmatics (AIA; n=10) and aspirin-tolerant asthmatics (ATA; n=8) in duplicate samples. □: before challenge; ▨: after challenge. Numerical values in parentheses have been added where bars are not clearly visible. *: p<0.05; **: p<0.01.

for AIAs, and 5,800±1,080 pg·mL⁻¹ for ATAs). Furthermore, the pLT release was stimulated by arachidonic acid to a smaller extent in AIAs (by a factor of 4.2) and ATAs (by a factor of 6.1), than in the control group (by a factor of 360). There was no significant differences in basal (241.2±31.6 pg·mL⁻¹ for AIAs, 253.4±24.6 pg·mL⁻¹ for ATAs, and 241.9±39.7 pg·mL⁻¹ for normal subjects) and arachidonic acid-induced (282±51.4 pg·mL⁻¹ for AIAs, 312±92.3 pg·mL⁻¹ for ATAs, and 241.9±19.1 pg·mL⁻¹ for normal subjects) histamine release between all groups. As expected stimulation with arachidonic acid did not influence histamine release.

Basal mediator release before and after aspirin challenge

The *in vivo* effect of lysine-aspirin on basal mediator release was investigated *in vitro*. Before aspirin provocation there was highly elevated basal pLT release in AIAs (55.5±8.5 pg·mL⁻¹) and ATAs (40.2±9.6 pg·mL⁻¹), whereas in healthy controls the initial levels were low (1.5±0.4 pg·mL⁻¹). After aspirin provocation there was a significant increase of pLT release in AIAs (237.4±34.8 pg·mL⁻¹). In contrast, in ATAs, the increase of pLT release was not statistically significant (64.7±25.3 pg·mL⁻¹) and in healthy controls the increase was minimal (7.5±1.6 pg·mL⁻¹). The basal PGE₂ release before aspirin challenge in AIAs (17±9.7 pg·mL⁻¹) was significantly lower than in normal subjects (236±38.2 pg·mL⁻¹) and ATAs (723.8±69.3 pg·mL⁻¹). After challenge, no significant change of PGE₂ release was measured in PBC from AIAs (20±8.9 pg·mL⁻¹), but a significant decrease of PGE₂ release in PBC from ATAs (268.7±57.3 pg·mL⁻¹) and normal subjects (40±11.2 pg·mL⁻¹) was found. No changes were observed on histamine release before or after lysine-aspirin challenge in AIAs (241.2±31.6 pg·mL⁻¹ before and 247.3±22.4 pg·mL⁻¹ after challenge), ATAs (253.4±24.6 pg·mL⁻¹ before and 249.5±27.2 pg·mL⁻¹ after challenge) and normal subjects (241.9±19.1 pg·mL⁻¹ before and 251.1±23.9 pg·mL⁻¹ after challenge) (fig. 3). Major eicosanoid release data including arachidonic acid-induced release and data before and after *in vivo* lysine-aspirin challenge are summarized in table 1.

The role of PGE₂ and histamine before and during the *in vivo* action of inhaled lysine-aspirin in all AIAs was investigated in more detail. Increasing doses of inhaled lysine-aspirin reduced the basal PGE₂ release and histamine release was unaffected by provocation. The relationship of basal PGE₂ release and change of FEV₁ is shown in figure 4a. The threshold aspirin dose correlated well with the biggest change in FEV₁. PGE₂ release correlated inversely with FEV₁ (r=0.87, fig. 4b and table 2).

Table 1. – Summary of major eicosanoid release data

Mediator	Challenge	NS		AIA		ATA	
		B	AA	B	AA	B	AA
PGE ₂	Before	236±38.2	7378±1530	20±9.7	4534±82.1	723±69.3	5800±361
	After	40±11.2	3793±1756	17±8.9	4089±95.8	268±57.3	3963±1132
pLT	Before	1.5±0.4	540±91.1	55.5±8.5	233±31.4	40.2±9.6	245±41.5
	After	7.5±1.6	724±174.4	237±34.8	581±149	64.7±25.3	623±153

Data presented as pg·mL⁻¹. B: basal eicosanoid release; AA: arachidonic acid-induced eicosanoid release; Before: eicosanoid release before *in vivo* challenge with lysine-aspirin; After: eicosanoid release after *in vivo* challenge with lysine-aspirin; NS: normal subjects; AIA: aspirin-intolerant asthmatics; ATA: aspirin-tolerant asthmatics; PGE₂: prostaglandin-E₂; pLT: peptide leukotriene.

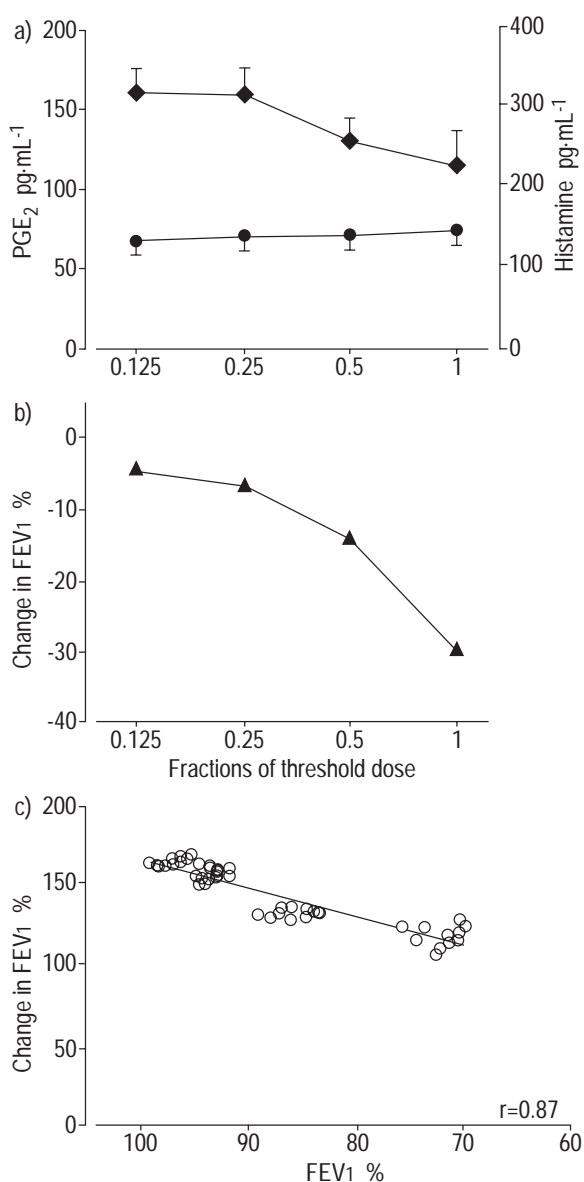


Fig. 4. – Effects of bronchial provocation and desensitization on mediator release (a and b). The relation of forced expiratory volume in one second (FEV₁; ▲) values and (b) prostaglandin E₂ (PGE₂; ◆) and histamine (●) release (a) are shown for all aspirin-intolerant asthmatics (AIA; n=10). As the threshold dose was individually different the applied dose of aspirin is shown as fractions/multiples of the threshold dose. There was no significant change in basal histamine release during provocation and desensitization. The significant fall of PGE₂ correlated with FEV₁. During inhalative provocation the time between each step of challenge was 30 min. The bars indicate SEM. The correlation of FEV₁ and PGE₂ is also shown (c), where four measurements of FEV₁ and PGE₂ release for each patient (n=10) were made.

Effect of PGE₂ on pLT release

To elucidate the interaction of PGE₂ and pLT, the effect of PGE₂ on pLT release was investigated. Therefore, PBC were preincubated with various concentrations (10⁻⁸–10⁻⁵ M) of PGE₂, dibutyl-AMP or the 5-lipoxygenase inhibitor caffeic acid [24]. In normal subjects, PGE₂ reduced pLT release in a dose-dependent fashion with 78% inhibition of arachidonic acid-induced pLT release at 10⁻⁵ M PGE₂. Similar results were obtained by preincubation of

Table 2. – Individual values of forced expiratory volume in one second (FEV₁) during bronchial provocation in aspirin intolerant asthmatics

Patient No.	Cumulative dose of aspirin mg	Decrease of FEV ₁ after provocation %
1	63.75	18
2	63.75	21
3	38.25	28
4	8.75	65
5	16.25	49
6	63.75	20
7	38.25	27
8	8.75	56
9	3.75	62
10	16.25	27

The FEV₁ was measured in triplicates. The individual decrease of FEV₁ was calculated using the individual FEV₁ value before and after inhalative bronchial provocation with lysine-aspirin. Bronchial provocation was performed as described in Materials and methods. In all the other groups (aspirin-tolerant asthmatics and normal subjects) there was no statistically decrease of FEV₁ measured after oral provocation with 500 mg of aspirin.

PBC with the membrane-penetrating analogue of cAMP, dibutyl-AMP (74% inhibition, $p < 0.01$), and caffeic acid (67% inhibition, $p < 0.01$). The inhibition was stronger on PBC from control subjects than from AIAs. The inhibitory effect on PBC from ATAs was greater compared to AIAs but slightly less than in normal subjects. These data support the hypothesis of a cAMP-dependent mechanism of action of PGE₂ (fig. 5a–c).

PGE₂/pLT relation in AIAs and control subjects

Another point of interest was the relation of PGE₂ and pLT release because of the inhibitory effect of PGE₂ as shown before. Therefore, basal release PGE₂ values were related to basal pLT values from AIAs, ATAs and normal subjects using the data described in figure 3. The results given in figure 6, clearly demonstrate differences with a very low PGE₂/pLT relation in AIAs before and even after lysine-aspirin challenge (before 0.8, after 0.3) whereas the healthy control subjects had a high PGE₂/pLT relation before (280) and a low relation after aspirin challenge (9.8). Before aspirin challenge ATAs had similar elevated pLT levels as AIAs which were compensated by excessive release of PGE₂. This resulted in a higher PGE₂/pLT relation before challenge (15.2) than in AIAs and a still positive PGE₂/pLT relation after challenge (4.8) which was similar to control subjects.

Discussion

In this study, the arachidonic acid metabolism and histamine release of PBC from AIAs, ATAs and healthy controls were investigated with respect to bronchoconstrictive pLT [5] and bronchoprotective PGE₂ [4, 16] before and after aspirin challenge. The underlying mechanisms of bronchoconstriction in AIAs are not known, but

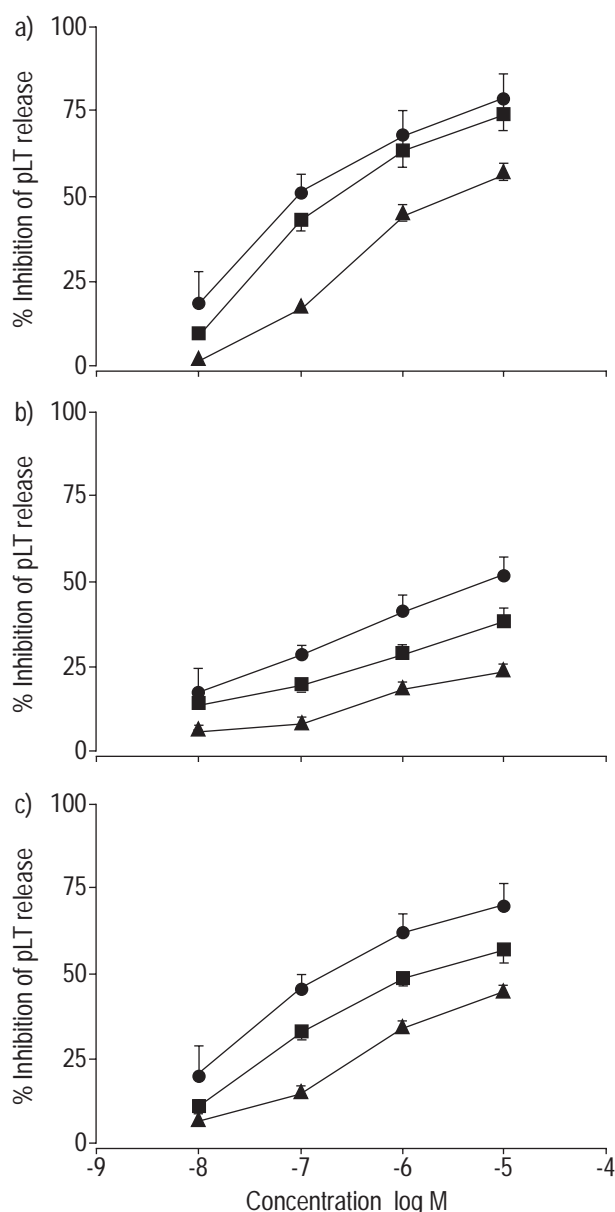


Fig. 5. – Effect of prostaglandin E_2 (PGE_2) on peptidoleukotriene (pLT) release. a) Inhibition of pLT release in peripheral blood cells (PBC) from normal subjects; b) inhibition of pLT release in PBC from aspirin-intolerant asthmatics (AIAs); c) inhibition of pLT release in PBC from aspirin-tolerant asthmatics (ATAs). PBC from normal subjects ($n=10$), AIAs ($n=10$) and ATAs ($n=8$) were preincubated for 15 min with variable concentrations of PGE_2 (●), dibuteryl-cyclic adenosine monophosphate (■) or the 5-lipoxygenase inhibitor caffeic acid (▲). Thereafter, PBC were incubated with arachidonic acid (10^{-5} M) for another 20 min. Samples were performed in duplicates and the bars indicate SEM.

the hypothesis of an altered metabolism of arachidonic acid metabolism is generally accepted [4, 6–18].

For the first time comparative measurements of pLT, PGE_2 and histamine in PBC of AIAs were performed, using a "functional cell test" which allowed transcellular metabolism as well as complex interactions of mediators [25]. An optimum of eicosanoid release could be found after 20 min, which corresponds well to previous studies [13, 25]. In a former study with monocytes, which focused on thromboxane- B_2 and LTB_4 release [13], the blood was

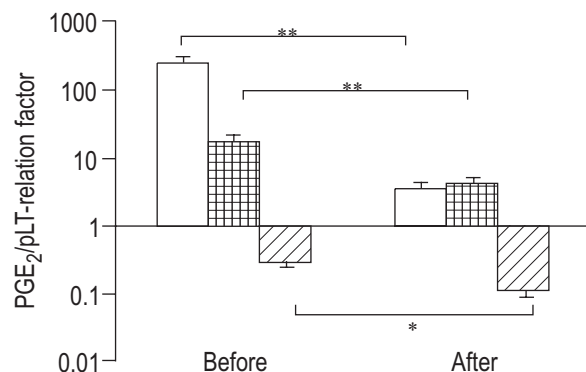


Fig. 6. – Relation of prostaglandin E_2 (PGE_2) and peptidoleukotriene (pLT) release. The quotient for basal release of PGE_2 and pLT was calculated in normal subjects (□; $n=10$), in aspirin-intolerant asthmatics (▨; $n=10$) and aspirin-tolerant asthmatics (▩; $n=8$). This was performed for values before and after aspirin challenge. *: $p<0.05$; **: $p<0.01$. Bars indicate SEM.

collected in syringes containing the calcium chelator ethylenediamine tetraacetic acid (EDTA) and the cells were stimulated with the calcium ionophore A23187 in a calcium reconstituted medium; furthermore these patients received topical and/or systemic corticosteroids. In contrast to that study, the "functional cell test" could demonstrate significant differences in basal pLT release and a significant increase of all eicosanoids after stimulation with the arachidonic acid. This can be explained by the design of the "functional cell test" using PBC from patients without relevant corticosteroid therapy and a preparation technique not affecting the calcium and corticosteroid sensitivity [21, 26] of enzymes. In addition the "functional cell test" allows naturally occurring multicellular interactions [25]. The concentration-dependent inhibition of pLT release by PGE_2 was most likely caused by an increase of intracellular cAMP (fig. 5), which was shown before in neutrophil leukocytes for chemotactically active LTB_4 [27]. The inhibition of arachidonic acid-induced pLT release by PGE_2 was more potent on a molar basis than the specific 5-lipoxygenase inhibitor caffeic acid. Lower sensitivity of PBC from AIAs to PGE_2 (fig. 5) might be caused by downregulation or diminished binding to PGE receptor subtypes (EP_x), reduced availability of endogenous PGE_2 or by elevated cAMP degradation.

Before lysine-aspirin challenge, basal release of pLT was significantly higher in AIAs and ATAs than in healthy controls. This confirms previous measurements of increased urinary pLT, which reflect the whole leukotriene-metabolism of the body [9–11] and studies of bronchial [6] or nasal [7, 8] lavage fluids. The "functional cell test" presented here, demonstrated a vastly reduced basal release of PGE_2 in PBC of AIAs. These results underline the importance of measuring eicosanoid release in intact cells, as previous studies could not find any difference of PGE_2 levels in blood serum [17]. PGE_2 release in PBC from AIAs could be stimulated very well, but could not reach the level of stimulated release in controls (fig. 2). These results are in contrast to former reports focusing on platelet function [12].

The most striking difference occurred in basal PGE_2 release comparing AIAs with ATAs and normal subjects before aspirin challenge. On the one hand there was an unexpected reduced basal PGE_2 release from AIAs in

contrast to the higher levels of PGE₂ in ATAs and normal subjects. On the other hand PGE₂ release in AIAs could be stimulated very well with exogenous arachidonic acid. There was no significant difference between all three groups tested. There are three possible interpretations for this phenomenon. Firstly, in AIAs there might be reduced availability of arachidonic acid presumably *via* diminished release of arachidonic acid from phospholipid membranes. Secondly, a lowered affinity of cyclooxygenases to arachidonic acid in AIAs, and finally decreased basal metabolic activity of cyclooxygenases. Regarding the other possible pathway of arachidonic acid metabolism, both, AIAs and ATAs showed similar highly elevated basal pLT release.

After bronchial challenge, there was a massive increase of already elevated basal pLT in AIAs but not in ATAs and normal subjects. The latter showed only a slight increase of pLT release. These findings support the "shunting theory" of arachidonic acid metabolism [13]. "Shunting" seems to be more typical for AIAs than for ATAs investigated in this study. The prior decrease of PGE₂ release during lysine-aspirin challenge, which was pharmacologically expected, correlated with a time shifted decrease in FEV₁ ($r=0.87$, fig. 4b) but was not associated with changes in histamine release (figs. 2 and 3). This leads to the assumption that histamine is not involved in an asthma attack of AIAs and again pointed to the role of PGE₂. After *in vivo* lysine-aspirin challenge there was a different response of basal PGE₂ and pLT release in each group. After stimulation with exogenous arachidonic acid it was surprising to find no significant difference of eicosanoid release between AIAs, ATAs and normal subjects (fig. 3 and table 1).

Combining the role of reduced basal PGE₂ release in AIAs as discussed above and similar arachidonic acid-induced eicosanoid release after aspirin challenge in all three groups (AIAs, ATAs, normal subjects) might lead to the following explanation. It is known that exogenous arachidonic acid in human cells is incorporated into cytoplasmic "lipid bodies" [28]. These lipid bodies are surrounded by cyclooxygenase, which is not membrane-bound and could not yet be differentiated into the two isoenzymes of cyclooxygenase [29]. The lack of response of the AIAs to exogenous arachidonic acid on PGE₂ production might be due to the following. 1) Lack of endogenous substrate (arachidonic acid) in AIAs by insufficient formation of lipid bodies; only if there is a surplus of exogenous arachidonic acid, PGE₂ will be synthesized in sufficient quantities, as it was demonstrated here for ATAs and normal subjects. 2) Defect of the cyclooxygenase with higher metabolic activity in AIAs; as two isoenzymes are known [4, 15, 25, 29]; also a different sensitivity of cyclooxygenases towards aspirin is assumed [30]. This would explain the finding that there was no significant change of arachidonic acid-induced PGE₂ release after *in vivo* aspirin exposure in AIAs but a significant reduction in ATAs and healthy controls.

Summarizing the results, there is a typical "profile" of eicosanoids in the AIAs, ATAs and normal subjects, which might be brought into line with current theories of pathogenesis [4, 13, 14, 16]. It was concluded that the reduced basal PGE₂ release in AIAs plays a key role, as the lack of PGE₂ and consequent loss of endogenous inhibition of 5-lipoxygenase leads to an increase of basal pLT. This might be of interest in developing new, more

effective strategies in the drug therapy of aspirin-induced asthma, *i.e.* combined drugs of PGE₂ and 5-lipoxygenase inhibitors or leukotriene-receptor antagonists. The broncho-protective effect of inhaled PGE₂ [4, 16] is most likely mediated by direct inhibition of pLT release as it has been recently reported for human bronchial biopsy specimens [24]. When AIAs are challenged with aspirin, pLT release increases most likely because of further reduction of inhibitory PGE₂ and/or partially by "shunting" of arachidonic acid metabolism and/or release of other broncho-constrictive mediators. This becomes clinically evident as an asthma attack in AIAs, whereas in ATAs a surplus of PGE₂ before challenge can possibly prevent a reduction of PGE₂ under a critical threshold after aspirin ingestion. This also might provide some evidence for the protective properties of PGE₂ discussed above [4, 16]. Looking at the pathogenesis of this syndrome, the relation of pLT and PGE₂ seems to be more important than protein-binding properties of analgesic drugs in AIAs. Previous theories focusing on high pLT levels of bronchoalveolar [6], nasal [7, 8] or urinary leukotrienes [9–11], or on the lack ("depletion") of PGE₂ [4] alone cannot explain all phenomena. Other mediators, *e.g.* neuropeptides, are suggested to be modulated by PGE₂ [16]. In addition reduced PGE₂ synthesis found in AIAs might explain complex changes in the immune system of aspirin-sensitive patients, such as reduced immunoglobulin-G₁ (IgG₁)-synthesis [31], as PGE₂ is known to modulate immunoglobulin synthesis [32].

Considering the biochemical interdependence of prostaglandin-E₂ and peptidoleukotrienes and the typical "profile" of both mediators in aspirin-intolerant asthmatics and controls, the mathematical relation of both eicosanoids was analysed (fig. 6). In all aspirin-intolerant subjects this relation was below 1.0, before and after challenge. Before challenge, the relation was over 200 in normal subjects and in aspirin-tolerant asthmatics over 15. After challenge the relation was over 5.0 in both groups. A prostaglandin-E₂/peptidoleukotriene relation below 1.0 was typical for aspirin-intolerant asthmatics and distinguished them from all other groups, *e.g.* aspirin-tolerant asthmatics.

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