

A moderate and unspecific release of cysteinyl leukotrienes by aspirin from peripheral blood leucocytes precludes its value for aspirin sensitivity testing in asthma

M. PIERZCHALSKA, L. MASTALERZ, M. SANAK, M. ZAZULA and A. SZCZEKLIK

Department of Medicine, Jagiellonian University Medical School, Cracow, Poland

Summary

Background Aspirin-induced asthma (AIA) is a clinical syndrome related to cysteinyl leukotriene overproduction in airways. The confirmation of the diagnosis requires inconvenient provocation tests with acetyl salicylic acid (ASA).

Objective A study was performed to evaluate whether measurement *in vitro* of cysteinyl leukotrienes (cys-LTs) release by isolated peripheral blood leucocytes, stimulated with ASA, can be of use for diagnosis of AIA.

Methods A cellular allergen stimulation test, CAST, was adapted to measure leukotriene release from leucocytes of 32 aspirin-tolerant and 26 aspirin-intolerant asthmatics. The cells were stimulated with Lys-ASA, N-formyl-methionyl-leucyl-phenylalanine (fMLP), or both fMLP and Lys-ASA, in a buffer containing IL-3, and results compared with human leukaemia cell line (HI-60) response to Lys-ASA. Cys-LTs were measured in cell supernatant fluids by ELISA.

Results ASA had a rather weak stimulatory effect on cys-LTs release in both groups of patients. Contrary to some previous studies, no significant differences were found between cys-LTs release by leucocytes from AIA and ATA, or by differentiated HI-60 cells.

Conclusion Measurement of cysteinyl-leukotriene release by peripheral blood leucocytes pre-treated with aspirin has no value for diagnosis of AIA.

Keywords: asthma, aspirin sensitivity, cysteinyl leukotrienes, CAST, leucocytes

Clinical and Experimental Allergy, Vol. 30, pp. 1785–1791. Submitted 17 January 2000; revised 25 March 2000; accepted 18 May 2000.

Introduction

Acetyl salicylic acid (ASA) belongs to the most frequently used pharmacologically-active compounds. Adverse reactions to ASA and to other non-steroidal anti-inflammatory drugs (NSAIDs) have distinct clinical patterns and are of particular interest due to their popular use [1].

ASA intolerance affects asthmatics with unusually high frequency. About 10% of adult asthmatic patients react with acute asthma attack on administration of a single dose of ASA or other NSAIDs [2]. Perennial rhinitis and nasal polyps are main symptoms accompanying ASA

intolerance. It is now widely accepted that these symptoms constitute a clear-cut clinical syndrome, aspirin-induced asthma (AIA) [3]. Despite extensive research on the mechanism of the disease, no *in vitro* diagnostic test for AIA has been invented. The diagnosis is currently based on oral or inhalatory provocations with aspirin that are both time-consuming and potentially harmful challenge procedures [4].

It is now generally accepted that cysteinyl leukotrienes (cys-LTs) play an important role in the pathogenesis of asthma, and AIA seems to be especially dependent on leukotriene overproduction [5,6]. Patients suffering from AIA excrete excessive amounts of LTE₄ in their urine compared with aspirin-tolerant asthmatics (ATA) or healthy individuals [7]. A further increase of LTE₄

Correspondence: A. Szczeklik, Department of Medicine, Jagiellonian University Medical School, 8 Skawińska Str., 31–066 Cracow, Poland.

concentration occurs after ASA challenge in urine [8] and bronchial or nasal lavage fluids [9,10]. Expression of LTC₄ synthase—a key enzyme in cys-LTs cellular synthesis—is enhanced in the lungs of AIA patients [11]. It is possible that this distinct abnormality in cys-LTs metabolism can lead to acute asthma attack, when the protective role of PGE₂ on airways is ablated by administration of cyclooxygenase-inhibiting NSAIDs [12]. It has been recently hypothesized that it is an insufficient COX-2 expression in AIA that makes these patients more susceptible to the effect of aspirin [13,14].

The cellular antigen stimulation test (CAST; Bühlmann Laboratories AG, Switzerland) is a fast and simple tool, described in 1991 and subsequently applied as a diagnostic procedure in allergology and as an investigation tool in clinical studies [15]. The test is based on IL-3 priming and stimulation with the allergen of isolated human leucocytes; this procedure leads to *in vitro* generation of cys-LTs. Accumulation of cys-LTs in the supernatant fluids is measured by an enzyme immunoassay.

In view of recent findings which point to a pivotal role of cys-LTs overproduction in aspirin-induced asthma, the clinical suitability of CAST for diagnosis of AIA was evaluated. The Assay of Cys-LTs release by isolated leucocytes was also investigated to broaden our understanding of AIA pathogenesis.

Materials and methods

Cys-LT release by HI-60 cells

Promyelocytic cell line HI-60 was propagated in RPMI 1640 (Gibco) supplemented with 10% fetal calf serum (Gibco) and antibiotics (50 U penicillin and 50 µg/mL streptomycin), as a suspension culture. Cells were induced to differentiate towards granulocytes by exposure to 1.3% of DMSO (Sigma), as described previously [16,17]. After 5 days of incubation in DMSO-containing medium, cells were harvested by centrifugation (200 g, 10 min) and resuspended in stimulation buffer (Bühlmann Laboratories AG) at a density of 1×10^7 cells/mL. Arachidonic acid (Sigma) in DMSO (320 mM) was added to the stimulation buffer to a final concentration of 50 µM. Cells were incubated for 15 min in the presence of arachidonic acid at 37 °C in a gently rotating bath and harvested by centrifugation (200 g, 10 min). The cells were then stimulated for 15 min with fMLP (10 µM, Sigma) or with Lys-ASA (Bühlmann Laboratories AG) at various concentrations, 0.01 mM, 0.1 mM, 0.5 mM or 1 mM (1 mM Lys-ASA corresponds to 180 mg/mL of ASA), under the same incubation conditions. Cys-LTs release was measured in supernatant fluids, separated by centrifugation of the sample at 4 °C (10 000 g, 15 min), using the ELISA-kit

(Bühlmann Laboratories AG). The cys-LTs production by cells incubated in 10 µM fMLP was treated as a positive control.

Patients

The study included two groups with a total number of 58 individuals. The first group consisted of 26 aspirin-intolerant asthmatics (AIA), the second group of 32 aspirin-tolerant asthmatics (ATA). The groups' characteristics are given in Table 1. Patients were in a stable clinical condition. The diagnosis of aspirin intolerance was confirmed by aspirin provocation tests [9] performed during the 12 months preceding the study. All ATA patients occasionally used aspirin and other NSAIDs without any adverse reactions; 14 of 32 underwent aspirin provocation tests [9] with negative results.

Cys-LT release by isolated peripheral blood leucocytes

Blood samples were collected into the heparin-containing tubes. The measurements of cys-LTs release were performed using the CAST-ELISA kit (Bühlmann Laboratories AG), according to the manufacturer's instructions. Briefly, leucocytes were isolated by dextran sedimentation for 90 min, transferred into fresh tubes and harvested by centrifugation (130 g, 15 min). The cells were then resuspended in the stimulation buffer (Bühlmann Laboratories AG) containing 20 ng/mL IL-3. Leucocytes were stimulated with Lys-ASA (0.1 mM, Bühlmann Laboratories), 10 µM fMLP (Sigma), or both Lys-ASA (0.1 mM) and fMLP (10 µM). Cys-LTs production induced by monoclonal antibody against the high affinity IgE receptor (Bühlmann Laboratories AG) served as positive control. Cys-LT production by cells incubated in buffer alone was treated as a negative control. Cells were stimulated for 40 min at 37 °C and release of cys-LTs was measured in cell supernatant fluid using the ELISA-kit (Bühlmann Laboratories AG).

Urinary LTE₄ excretion

LTE₄ was measured in unpurified samples by direct enzyme immunoassay (Cayman Chemical, Ann Arbor, MI, USA) and standardized in ng/mg creatinine [18]. The urine samples were collected on the same day as the blood samples.

Results

Effect of ASA on Cys-LTs release by HI-60 cells differentiated into granulocytes

To test whether an increase in cys-LTs production after

Table 1. Characteristics of patients' groups

	AIA	ATA
Number	26	32
Sex (male/female)	7/19	10/22
Age, year (mean)	44.6	45.8
Duration of asthma, year	12 (1–30)	10 (1–38)
FEV1% predicted volume	71	69
PEF-base level (L/min)	348	327
Serum IgE, IU/mL (median)	107	99
Number with positive skin-prick tests	5	17
Number receiving inhaled steroids	24	29
Dosage of inhaled steroids μ g/day (mean, range)	928 (0–1800)	800 (0–2000)
Number receiving oral steroids	8	6
Dosage of oral steroids mg/day (mean, range)	2.5 (0–10)	2.0 (1–10)
Eosinophil count (mean)	416	295

ASA stimulation *in vitro* is a general or a specific phenomenon related to ASA intolerance, HI-60 cells differentiated for 5 days with 1.3% of DMSO were used. After differentiation into granulocytes, the production of cys-LTs increased about 10-fold compared with undifferentiated HI-60 cells (data not shown). All tested ASA concentrations, 0.01 mM, 0.1 mM, 0.5 mM and 1.0 mM, augmented cys-LTs production. The concentration of 0.5 mM was found to be the most effective and the increase in cys-LTs release was statistically significant (Fig. 1, Wilcoxon test for matched pairs, $P < 0.01$).

Cys-LTs release by leucocytes of aspirin-tolerant and -intolerant asthmatics

Cys-LTs release by the leucocytes of 32 patients with aspirin-tolerant asthma and by 26 patients with aspirin-intolerant asthma was measured *in vitro*. Release of cys-LTs by unstimulated cells and by cells stimulated with anti-Fc ϵ RI antibody was treated as a basic value for each individual tested. There were no differences between the AIA and ATA groups in cys-LTs release from unstimulated leucocytes, or from leucocytes stimulated by anti-Fc ϵ RI antibody (Table 2). The mean level of LTE₄ in urine was significantly higher ($P < 0.05$) in the AIA group (mean value for the AIA group was 416 ng/mg creatinine and for ATA, 200 ng/mg) (Fig. 2). Surprisingly, no correlation was found between cys-LTs released from unstimulated cells and urinary level of cys-LTs in either AIA or ATA groups

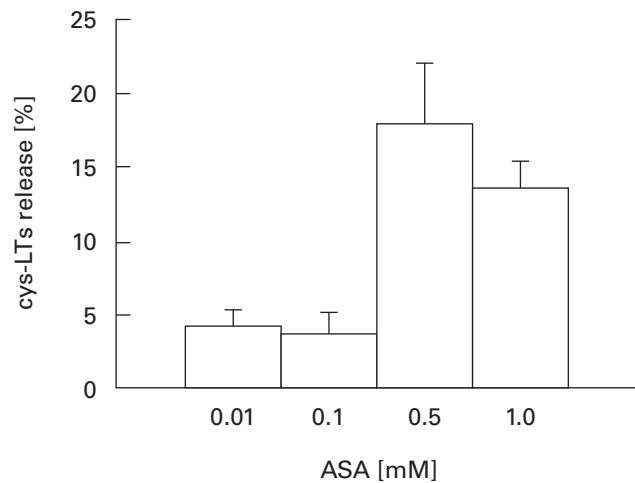


Fig. 1. The effect of various ASA concentrations on cys-LTs release from HI-60 cells. A statistically significant increase was observed for 1 mM and 0.5 mM ASA. Data are presented as a relative increase (stimulation with fMLP is treated as 100%). Statistical analysis with Wilcoxon test for matched pairs: $P < 0.01$ (0.5 mM ASA), $P < 0.05$ (1 mM ASA).

(Spearman's correlation coefficient, $R = 0.3$, ns) (data not shown). The groups did not differ in eosinophil blood counts (mean value in AIA 416/ μ L vs 295/ μ L in ATA, U -test, $P > 0.1$). No correlation was found between eosinophil blood count and cys-LTs production by isolated leucocytes in either group (data not shown).

Table 2. Release of cys-LTs in ASA-tolerant (ATA, $n = 32$) and ASA-intolerant asthmatics (AIA, $n = 26$) in various stimulation conditions. The median values for each group are presented in pg/mL. The differences between groups are not statistically significant (U -test)

	Cys-LTs release (pg/mL)	
	AIA	ATA
	median (25%,75%)	median (25%,75%)
Unstimulated cells	84 (56, 146)	82 (47 100)
Stimulated control (anti-Fc ϵ RI)	930 (560, 2140)	1580 (740, 2410)
10 μ M fMLP	240 (17, 370)	290 (150, 510)
0.1 mM ASA	127 (74, 181)	122 (73, 233)
10 μ M fMLP + 0.1 mM ASA	240 (140, 460)	250 (140, 470)

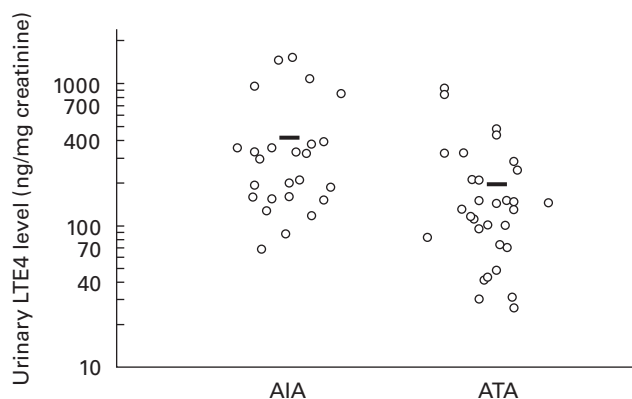


Fig. 2. The LTE_4 level secreted into urine. The points indicate values obtained for each patient calculated as ng LTE_4 /mg creatinine. Horizontal bars indicate the mean values, which were significantly higher in the AIA group (U -test, $P < 0.001$).

Effect of ASA on cys-LTs release by leucocytes of aspirin-tolerant and -intolerant asthmatics

The mean cys-LTs release from ASA-stimulated leucocytes of AIA patients did not differ from that of the ATA group (median AIA: 82 pg/mL vs ATA 84 pg/mL; $P > 0.5$, Table 2). There was a positive correlation between the levels of cys-LTs released by unstimulated leucocytes and following stimulation with ASA ($R = 0.7$, $P < 0.001$). The weak stimulatory effect of ASA at a concentration of 0.1 mM on cys-LTs release was observed in both groups (Table 2, Fig. 3, Wilcoxon test for matched pairs, $P < 0.01$). Stimulation due to ASA treatment did not differ in leucocytes isolated from the AIA compared with the ATA group (Table 2, median AIA: 127 vs ATA: 122, U -test, $P > 0.2$).

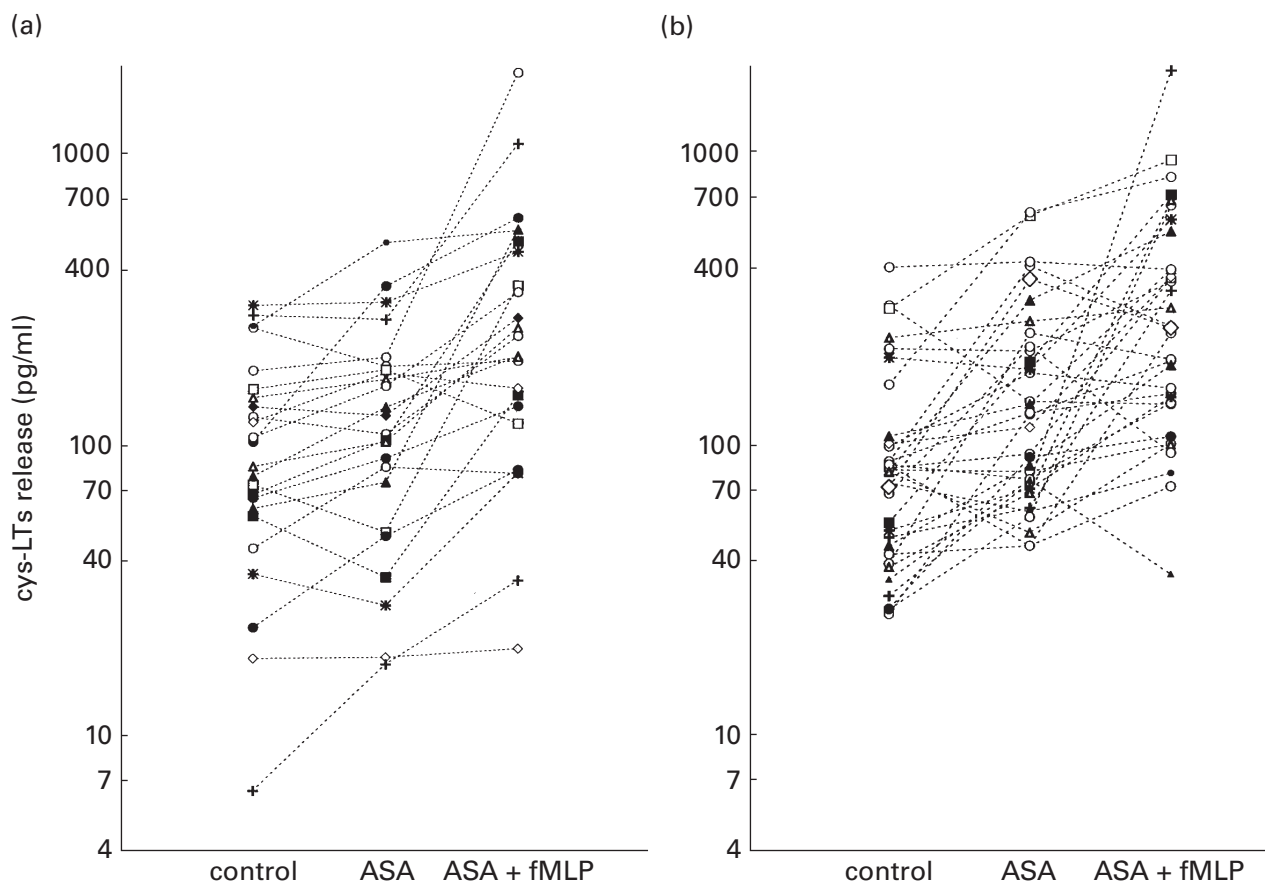


Fig. 3. The influence of 0.1 mM ASA, or 0.1 mM ASA and 5 μM fMLP, on cys-LTs release by isolated patients' leucocytes. Individual cases were marked by various symbols. ASA alone and fMLP in the presence of ASA caused a significant increase in cys-LTs in both groups (Wilcoxon test for matched pairs, $P < 0.001$). The increase observed in AIA did not differ from the increase observed for ATA (U -test, $P > 0.5$). (a) AIA; (b) ATA.

Effect of fMLP on ASA-stimulated cys-LTs release by leucocytes of aspirin-tolerant and -intolerant asthmatics

The increase in cys-LTs release both from human granulocytes and from different cell lines was observed after stimulation with various cell-surface receptor ligands such as PAF, C5a, zymosan or fMLP [19–21]. To investigate whether the differences between the AIA and ATA group would be more pronounced under proinflammatory receptor stimulation, the test was performed in the presence of 10 μ M fMLP. A significant increase in cys-LTs release after fMLP was observed in both groups (Table 2, Fig. 3, Wilcoxon test for matched pairs, $P < 0.001$), both in the presence and absence of ASA, but the magnitude of increase did not differ between the two groups (Table 2, Fig. 3, U -test, $P > 0.4$).

Discussion

No difference in production of cys-LTs by IL-3 primed leucocytes isolated from aspirin-sensitive asthmatics compared with aspirin-tolerant asthmatic patients could be demonstrated.

Differentiated HI-60 cells proved to be a good standard model for cys-LTs generation *in vitro*, in agreement with results obtained by others [20]. Within the range of ASA concentrations tested, 0.5 mM ASA was found to markedly stimulate production of cys-LTs by the cell line. As this concentration is far higher than the one achieved by administration of 300 mg ASA *per se*, which invariably precipitates asthmatic attack in susceptible patients, 0.1 mM ASA, a concentration which effectively blocks cyclooxygenases [22] and affects NF- κ B signalling [23], was tested. At this concentration, a weak stimulatory effect of aspirin on the granulocytic cell line was already evident.

It has been shown that ASA causes a statistically significant but rather weak (less than twofold) increase in cys-LTs generation in leucocytes from both groups of patients. Therefore, the observation from cultured HI-60 cells was confirmed in a much more complicated *ex vivo* model. These results are in agreement with some previous studies [24,25], but contrast to some others [26,27]. Such a discrepancy can be explained by random effects observed in the relatively small numbers of subjects, non-homogeneity of the groups studied (asthma and urticaria), and by the use of a high concentration of aspirin which can unmask some intercellular mechanisms related to the higher eosinophilia in AIA. Hood and colleagues [28] recently showed that intravenous administration of corticosteroid suppresses leukotriene release from isolated leucocytes. It is possible that prolonged oral corticosteroid therapy can influence CAST results as well.

The present results indicate that isolated leucocytes of

AIA and ATA do not differ significantly in their response to 0.1 mM ASA (Table 2, Fig. 3). On the base of this fact, it is concluded that the measurement of cysteinyl-leukotrienes released by peripheral blood leucocytes following aspirin *ex vivo* stimulation is not suitable for diagnosis of aspirin-sensitive asthma.

The patients with AIA had an elevated level of LTE₄ in their urine, as previously reported [7,8]. However, the IL-3 primed leucocytes isolated from peripheral blood of AIA patients did not exhibit the enhanced capacity to produce cys-LTs (Table 2). The same result was obtained recently by May *et al.* [24]. The amount of cys-LTs released by leucocytes *ex vivo* did not correlate with patients' LTE₄ urinary levels, an observation which supports lung-specific cys-LTs biosynthesis in AIA. It indicates that total cys-LTs production cannot be judged based on measurement of cys-LTs release from isolated leucocytes.

Challenge with most NSAIDs leads to a dramatic increase in LTE₄ level in bronchoalveolar lavage, nasal secretion and urine in AIA, but not in ATA patients or healthy controls [8–10]. This rise in LTE₄ excretion reflects cys-LTs overproduction in the lungs. Eosinophils and mast cells are thought to be the main source of cys-LTs in asthmatic airways [29]. The rapid migration of eosinophils into airways tissue after cyclooxygenase inhibition appears to be the most distinctive feature of AIA [11]. In the procedure tested, the mature blood basophils, eosinophils and monocytes are the source of measured cys-LTs. No positive correlation was found between eosinophil blood count and cys-LTs production by isolated leucocytes. Therefore, it is likely that IL-3 primed eosinophils present in the sample did not contribute significantly to the observed differences. The mechanism which regulates cys-LTs synthesis by eosinophils in inflamed respiratory tract tissues is probably different from the mechanism operating in peripheral blood leucocytes [30,31]. It was recently shown that LTC₄ synthesis by eosinophils is strongly dependent on the attachment to fibronectin [32], and the expression of LTC₄ synthase is regulated by cytokines (IL-5) and growth factors (TGF β) [33,34]. Furthermore, the amount of released cys-LTs significantly depends on the interaction of different cell types [35,36]. It is possible that the differences in response to ASA between aspirin-tolerant and -intolerant patients are relevant only in the specific microenvironment of the lungs. The relationship between ASA stimulation and cys-LTs overproduction is probably complex. In the isolated mixed leucocyte preparation, some pivotal elements are missing. Although the present results are discouraging for the use of cys-LTs release by peripheral blood leucocytes in AIA diagnosis, they do not contradict the hypothesis of a predominant role of cysteinyl leukotrienes in the pathogenesis of aspirin-induced asthma.

Acknowledgements

This study was sponsored in part by the Polish State Committee for Scientific Research grant 4 P05A 057 17.

References

- 1 Szczeklik A, Gryglewski RJ, Czerniawska-Mysik G. Clinical patterns of hypersensitivity to nonsteroidal antiinflammatory drugs and their pathogenesis. *J Allergy Clin Immunol* 1977; 60:276–84.
- 2 Stevenson DD, Simon RA. Sensitivity to aspirin and nonsteroidal antiinflammatory drugs. In: Middleton F, Reed CE, Ellis EF, Adkinson NF Jr, Junginger JW, Busse WW eds. *Allergy: Principles and Practice*. St Louis: Mosby-Year Book Inc., 1998: 1225–34.
- 3 Szczeklik A, Stevenson DD. Aspirin induced asthma: advances in pathogenesis and management. *J Allergy Clin Immunol* 1999; 104:5–13.
- 4 Dahlen B, Zetterstrom O. Comparison of bronchial and per oral provocation with aspirin in aspirin-sensitive asthmatics. *Eur Respir J* 1990; 3:527–34.
- 5 Drazen JM. Cysteinyl leukotrienes. In: Barnes PJ, Rodger IW, Thomson NC. *Asthma Basic Mechanisms and Clinical Management*. New York: Academic Press, 1988: 281–95.
- 6 Shuaib Nasser SM, Lee TH. Leukotrienes in aspirin-sensitive asthma. In: Szczeklik A, Gryglewski RJ, Vane JR. eds. *Eicosanoids, Aspirin and Asthma*. New York: Marcel Dekker, Inc., 1998: 317–32.
- 7 Christie PE, Tagari P, Ford-Hutchinson AW *et al.* Urinary leukotriene E₄ concentrations increase after aspirin challenge in aspirin-sensitive asthmatics subjects. *Am Rev Respir Dis* 1991; 143:1025–9.
- 8 Kumlin M, Dahlen B, Bjorck T, Zetterstrom O, Granstrom E, Dahlen SE. Urinary excretion of leukotriene E₄ and 11-dehydro-thromboxane B₂ in response to bronchial provocations with allergen, aspirin, leukotriene D₄, and histamine in asthmatics. *Am Rev Respir Dis* 1992; 146:96–103.
- 9 Szczeklik A, Sladek K, Dworski R *et al.* Bronchial aspirin challenge causes specific eicosanoid response in aspirin-sensitive asthmatics. *Am J Respir Crit Care Med* 1996; 154:1608–14.
- 10 Picado C, Remis I, Rossello J *et al.* Release of peptide leukotriene into nasal secretions after local instillation of aspirin in aspirin-sensitive asthmatic patients. *Am Rev Respir Dis* 1992; 145:65–59.
- 11 Cowburn AS, Sladek K, Soja J *et al.* Overexpression of leukotriene C₄ synthase in bronchial biopsies from patients with aspirin-intolerant asthma. *J Clin Invest* 1998; 101:834–46.
- 12 Sanak M, Simon H-U, Szczeklik A. Leukotriene C₄ synthase promoter polymorphism and risk of aspirin-induced asthma. *Lancet* 1997; 350:1599–600.
- 13 Kowalski ML, Pawliczak R, Wozniak J *et al.* Differential metabolism of arachidonic acid in nasal polyp epithelial cells cultured from aspirin-sensitive and aspirin-tolerant patients. *Am J Respir Crit Care Med* 2000; 161:391–8.
- 14 Picado C, Fernandez-Morata JC, Juan M *et al.* Cyclooxygenase-2 mRNA is downexpressed in nasal polyps from aspirin-sensitive asthmatics. *Am J Respir Crit Care Med* 1999; 160:291–6.
- 15 De Weck AL, Stadler BM, Urwyler A, Wehner HU, Bühlmann RP. Cellular antigen stimulation test (CAST): a new dimension in allergy diagnostics. *Allergy Clin Immunol News* 1993; 5:9–14.
- 16 Laouar A, Collart FR, Hubermann E. Induction of differentiation in human myeloid HL-60 leukemia cells. In: Celis JE. ed. *Cell Biology: a Laboratory Handbook*. London: Academic Press, 1998: 233–9.
- 17 Zaitzu M, Hamasaki Y, Yamamoto S. *et al.* Effect of dexamethasone on leukotriene synthesis in DMSO-stimulated HL-60 cells. *Prostaglandins Leukot Essent Fatty Acids* 1998; 59:385–93.
- 18 Kumlin M, Stensvad F, Larsson L, Dahlén B, Dahlén SE. Validation and application of a new simple strategy for measurements of urinary leukotriene E₄ in humans. *Clin Exp Allergy* 1995; 5:467–79.
- 19 Ochensberger B, Tassera L, Bifrare D, Rihs S, Dahinden CA. Regulation of cytokine expression and leukotriene formation in human basophils by growth factors, chemokines and chemotactic agonists. *Eur J Immunol* 1999; 29:11–22.
- 20 Ford-Hutchinson AW. Regulation of the biosynthesis of the sulphido-leukotrienes. *Adv Exp Med Biol* 1997; 400B: 841–71.
- 21 Tenor H, Hatzelmann A, Church MK, Schudt C, Shute JK. Effect of theophylline and rolipram on leukotriene C₄ synthesis and chemotaxis of human eosinophils from normal and atopic subjects. *Br J Pharmacol* 1996; 118:1727–35.
- 22 Gryglewski RJ. Screening and assessment of the potency of anti-inflammatory drugs in vitro. In: Vane JR, Ferreira SH eds. *Anti-Inflammatory Drugs Handbook of Experimental Pharmacology*. Berlin: Springer Verlag, 1979; 1–43.
- 23 Yin M-N, Yamamoto Y, Gaynor RB. The anti-inflammatory agents aspirin and salicylate inhibit the activity of I κ B kinase- β . *Nature* 1998; 396(5):77–80.
- 24 May A, Weber A, Gall H, Kaufmann R, Zollner TM. Means of increasing sensitivity of an in vitro diagnostic test for aspirin intolerance. *Clin Exp Allergy* 1999; 29:1402–11.
- 25 Schäfer D, Schmid M, Göde UC, Baenkler H-W. Dynamics of eicosanoids in peripheral blood during bronchial provocation in aspirin-intolerant asthmatics. *Eur Res J* 1999; 13:638–46.
- 26 Czech W, Schöpf E, Kapp A. Release of sulfidoleukotrienes *in vitro*: its relevance in the diagnosis of pseudoallergy to acetylsalicylic acid. *Inflamm Res* 1995; 44:291–5.
- 27 Wedi B, Elsner J, Kapp A. In vitro diagnosis of pseudoallergic reactions – new aspects. *ACI Int* 1996; 8:113–5.
- 28 Hood PP, Cotter TP, Costello JF, Sampson AP. Effect of intravenous corticosteroid on ex vivo leukotriene generation by blood leucocytes of normal and asthmatic patients. *Thorax* 1999; 54:1075–82.
- 29 Sampson AP, Cowburn AS, Sladek K *et al.* Profound overexpression of leukotriene C₄ synthase in bronchial biopsies from aspirin-intolerant asthmatic patients. *Int Arch Allergy Immunol* 1997; 113:355–7.

- 30 Bischoff SC, De Weck AL, Dahinden CA. Interleukin 3 and granulocyte macrophage-colony stimulating factor render human basophils responsive to low concentration of complement component C3a. *Proc Natl Acad Sci USA* 1990; 87:6813–7.
- 31 Rabe KF, Dent G. Pharmacological intervention in generation of leukotrienes by eosinophils. In: Szuzelelik A, Gryglewski RJ, Vane JR eds. *Eicosanoids, aspirin and asthma*. NY: Marcel Dekker, Inc., 1998: 391–419.
- 32 Brock GB, Anderson JA, Fries FP, Peters-Golden M, Sporn PHS. Decreased leukotriene C₄ synthesis accompanies adherence-dependent nuclear import of 5-lipoxygenase in human blood eosinophils. *J Immunol* 1999; 162:1669–76.
- 33 Cowburn AS, Holgate ST, Sampson AP. IL-5 Increases expression of 5-lipoxygenase-activating protein and translocates 5-lipoxygenase to the nucleus in human blood eosinophils. *J Immunol* 1999; 163:456–65.
- 34 Riddick CA, Serio KJ, Hodulik CR, Ring WL, Regan MS, Bigby TB. TGF- β increases leukotriene C₄ synthase expression in the monocyte-like cell line, THP-1. *J Immunol* 1999; 162:1101–7.
- 35 Maclouf J, Murphy RC, Henson PM. Transcellular biosynthesis of sulfidopeptide leukotrienes during receptor-mediated stimulation of human neutrophil/platelet mixtures. *Blood* 1990; 76:1838–44.
- 36 Feinmark SJ. The role of the endothelial cell in leukotriene biosynthesis. *Am Rev Respir Dis* 1992; 146:S51–5.