

Short communication

Increased *in vitro* cysteinyl leukotriene release from blood leukocytes in patients with asthma, nasal polyps, and aspirin intolerance

Mewes T, Riechelmann H, Klimek L. Increased *in vitro* cysteinyl leukotriene release from blood leukocytes in patients with asthma, nasal polyps, and aspirin intolerance.

Allergy 1996; 51: 506–510. © Munksgaard 1996.

In vitro cysteinyl leukotriene (cLT) release from blood leukocytes was measured in eight normal individuals (NI), nine patients with nasal polyps (NP) without aspirin intolerance, and eight patients with NP, asthma, and aspirin intolerance (AI). Blood leukocytes were prestimulated with interleukin-3 (IL-3) and incubated with acetylsalicylic acid (ASA) (10 and 100 µg/ml) together with C5a (10^{-8} mol/l) for 18 h. cLT release (LTC_4 , LTD_4 and LTE_4) from blood leukocytes was measured with a competitive enzyme-linked immunoassay. Background cLT release was 259 ± 66 pg/ml (mean $\pm$ SEM) in the NI group, 185 ± 33 pg/ml in the NP group, and 578 ± 136 pg/ml in the AI group ($P=0.1$). After incubation with 10 µg/ml ASA, cLT concentration was lower in normal subjects (346 ± 72 pg/ml) and in patients with NP (209 ± 53) than in patients with AI (686 ± 75 pg/ml, $P=0.002$). After incubation with 100 µg/ml ASA, cLT concentrations were 285 ± 72 pg/ml in the NI group, 313 ± 77 pg/ml in the NP group, and 654 ± 121 pg/ml in the AI group ($P=0.04$), respectively. Simultaneous incubation with ASA 10 µg/ml and C5a (10^{-8} mol/l) resulted in a cLT concentration of 751 ± 171 pg/ml in the NI group, 343 ± 102 pg/ml in the NP group, and 2196 ± 480 pg/ml in patients with AI ($P=0.0006$), whereas simultaneous incubation with ASA 100 µg/ml and C5a (10^{-8} mol/l) resulted in 268 ± 51 pg/ml in the NI group, 412 ± 97 pg/ml in the NP group, and 1701 ± 368 pg/ml in the AI group ($P=0.005$). In patients with AI, cLT release from blood leukocytes is altered when compared with normals and patients with NP. The presented cLT-release assay could be of potential use in the *in vitro* diagnosis of AI.

**T. Mewes, H. Riechelmann,
L. Klimek**

Department of Otorhinolaryngology,
Johannes Gutenberg University, Medical
School, Mainz, Germany

Key words: aspirin intolerance syndrome;
cysteinyl leukotrienes; ELISA.

T. Mewes, MD
Johannes Gutenberg University
Medical School
Department of Otorhinolaryngology
Langenbeckstr. 1
55101 Mainz
Germany

Accepted for publication 21 March 1996

Aspirin intolerance (AI) syndrome is reported to occur in 2–20% of patients with nasal polyps (NP) (1). The pathophysiology of this condition is unknown. Diagnosis is based on history and oral (2), bronchial, or nasal (3) acetylsalicylic acid (ASA) challenge procedures. These challenge procedures entail the risk of severe asthma attacks.

In NP of patients suffering from AI, significantly increased levels of cysteinyl leukotriene (cLT) have been demonstrated (4). After ASA challenge in

AI, increased concentrations of LTC_4 and histamine have been found in nasal lavage fluid (5, 6).

In addition to altered arachidonic acid metabolism (7), facilitated releasability of histamine and cLT from basophils, mast cells, and eosinophils might be responsible for elevated cLT levels. Non-steroidal anti-inflammatory drugs (NSAIDs) have been found to enhance immunologic release of histamine and cLT from human bronchus and lung tissue (8, 9). Platelet-activating factor-induced histamine release was significantly higher in patients

with AI and atopic patients with asthma than in normal subjects (10).

Increased cLT release from human basophils after prestimulation with interleukin-3 (IL-3) has been demonstrated. After priming with IL-3, C5a can release cLT from basophils, mast cells, and eosinophils (11, 12). cLT release of IL-3-primed basophils after stimulation with C5a has recently been shown to be significantly higher in patients with AI than in normal subjects or atopic patients without AI (13).

This study aimed to investigate the *in vitro* cLT release from blood leukocytes after stimulation by ASA and costimulation by ASA and C5a in the presence of IL-3 in normal individuals (NI), in patients with NP without AI, and in patients suffering from asthma and NP with AI.

Material and methods

Blood donors

Blood from eight healthy subjects (mean age 37 ± 13 years, two male and six female) without nasal complaints and/or bronchial asthma served as controls. They had no history of atopy, and skin prick tests for common inhalative allergens were negative. These subjects had had no medication for at least 3 weeks.

Nine patients with NP and chronic hyperplastic sinusitis (mean age 48 ± 12 years, six male and three female) without bronchial asthma were included in the NP group. All patients of the NP group had no medical history of AI. They had used aspirin for treatment of headaches several times within the last 2 years without any problems.

Eight patients with NP and AI (mean age 47 ± 16 years, three male and five female) were included in the AI group. The AI patients had suffered from severe asthma attacks after ingestion of ASA. In two patients, an oral aspirin provocation test had been performed. Six patients refused to take part in oral or bronchial aspirin challenge, because they had suffered from life-threatening bronchospasm after aspirin ingestion.

NP were confirmed by nasal endoscopy and computed tomography in NP and AI patients. Allergy to common inhalative allergens was excluded by medical history and skin prick tests in the NP and AI patients. None of the NP and AI patients had had systemic corticosteroid therapy. Three of the NP patients took a nasal topical steroid b.i.d. Five of the AI patients took a topical steroid with a bronchial inhaler once or twice daily, one patient in addition taking a nasal steroid b.i.d.

Cell preparation

A volume of 3 ml blood was drawn into EDTA venipuncture tubes and transferred into polypropylene tubes. After 0.75 ml dextran solution had been added, the sample was vortexed and incubated for 90 min at room temperature. The upper phase containing blood leukocytes was transferred into a second tube and centrifuged for 15 min at 130 g and 4°C. The supernatant was discarded, and the cells in the pellet were directly stimulated.

Cell stimulation

Cells contained in each pellet were primed by suspension in 3 ml of a buffer containing Ca^{2+} , Mg^{+} , K^{+} , Na^{+} , Cl^{-} , HSA and HEPES at pH 7.4 with 0.45 µl IL-3 (10 ng/ml, Bühlmann AG, Allschwil, Switzerland). A volume of 200 µl of the suspension was pipetted into polypropylene tubes and immediately stimulated with either 50 µl buffer (background), 50 µl monoclonal antibody to IgE (stimulation control, Bühlmann AG, Allschwil, Switzerland), 50 µl ASA (10 µg/ml, Sigma Chemicals, Deisenhofen, Germany) (ASA-1), 50 µl ASA (100 µg/ml) (ASA-2), 50 µl ASA (10 µg/ml) containing 10^{-8} mol/l C5a (C5a-ASA-1), or 50 µl ASA (100 µg/ml) containing 10^{-8} mol/l C5a (C5a-ASA-2). C5a (Sigma Chemicals, Deisenhofen, Germany) has a chemokinetic potency for human neutrophils (EC_{50}) of 1.2×10^{-10} mol/l. For stimulation, the solutions were incubated for 40 min at 37°C. They were then centrifuged for 3 min at 1000 g and room temperature. The supernatant was further used.

Enzyme-linked immunosorbent assay (ELISA)

cLT release (LTC_4 , LTD_4 , and LTE_4) was evaluated by competitive ELISA (Bühlmann Lab. AG, Allschwil, Switzerland). Microtiter plates coated with a mouse monoclonal cLT-antibody (1A-LDR1) were used. Competition of samples with alkaline phosphatase-labeled LTD_4 was evaluated (14). The minimal detectable dose of cLT was calculated to be 41 pg/ml, and the cross-reactivity at 50% binding for LTB_4 , several lipoxygenase and cyclooxygenase products, and amino acids was less than 0.25% (Bühlmann cellular antigen stimulation test, test manual, Bühlmann Lab. AG, Allschwil, Switzerland). A volume of 100 µl of the supernatant was pipetted into the microtiter plates. After 50 µl of cLT-conjugated alkaline phosphatase had been added, plates were incubated for 18 h at 4°C. Each sample was measured in duplicate. After repeated washing, substrate was added and the reaction was stopped with sodium hydroxide. Extinction was

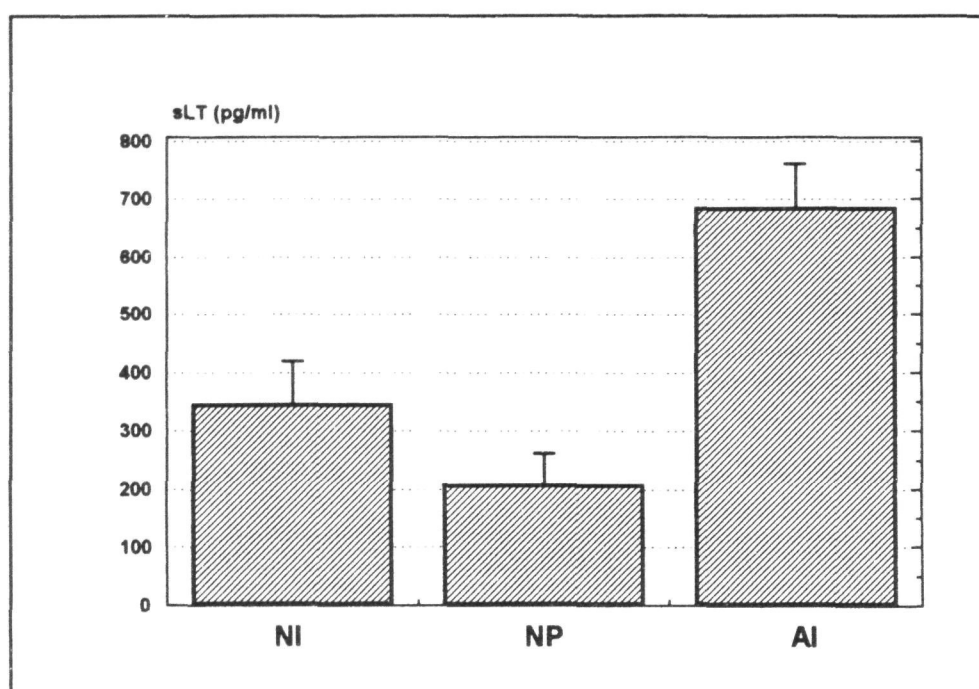


Fig. 1. Cysteinyl leukotriene release (mean ± SEM) from IL-3-primed human blood leukocytes after stimulation with ASA (10 µg/ml) in normal individuals (NI), patients with nasal polyps (NP), and patients with nasal polyps and aspirin intolerance (AI) ($P=0.002$).

recorded at 405 nm. The cLT concentration was calculated with a linear/logarithmic standard curve.

Statistics

For statistical evaluation, cLT values of each tested subject after background release, anti-IgE-stimulated release, ASA-1-stimulated release, C5a-ASA-1-stimulated release, ASA-2-stimulated release, and C5a-ASA-2-stimulated release were ranked. The ranks were grouped by the NI group, the NP group, and the AI group. For each group, the mean rank of each release method was calculated and compared by the Kruskal-Wallis test (15).

Results

Background cLT-release from blood leukocytes did not significantly differ in the three groups investigated. It was 259 ± 66 pg/ml (mean ± SEM) in the NI group, 185 ± 33 pg/ml in the NP group, and 578 ± 136 pg/ml in the AI group ($P=0.1$). cLT concentration after anti-IgE stimulation also revealed no statistically significant differences between normal subjects and patients with NP or AI, respectively. In the NI group, cLT release after stimulation with anti-IgE was 1921 ± 691 pg/ml, in the NP group 1559 ± 492 pg/ml, and in the AI group 3150 ± 643 pg/ml ($P=0.28$). Patients with AI revealed increased cLT release from blood leukocytes after stimulation with ASA. After incubation with ASA-1, cLT concentration was 346 ± 72 pg/ml in normal subjects, in patients with NP 209 ± 53 pg/ml, and in patients with AI 686 ± 75 pg/ml ($P=0.002$, Fig. 1). After incubation with ASA-2, cLT concentration was

285 ± 72 pg/ml in the NI group, 313 ± 77 pg/ml in the NP group, and 654 ± 121 pg/ml in the AI group ($P=0.04$), respectively. cLT release from blood leukocytes of the NI and NP groups differed most from the AI group after simultaneous stimulation with C5a and ASA. After stimulation with C5a-ASA-1, cLT concentration was 751 ± 171 pg/ml in the NI group, 334 ± 102 pg/ml in the NP group, and 2196 ± 480 pg/ml in patients with AI ($P=0.0006$, Fig. 2), whereas incubation with C5a-ASA-2 resulted in 268 ± 51 pg/ml in the NI group, 412 ± 97 pg/ml in the NP group, and 1701 ± 368 pg/ml in the AI group ($P=0.005$).

Discussion

In the NP of patients with AI, high cLT concentrations have been found in contrast to patients suffering from nasal allergy or chronic sinusitis (4). Increased concentrations of cLT were found in nasal lavages after ASA challenge in patients with AI (5, 6). Mast cells and eosinophils were abundantly present in the NP of patients suffering from AI. Increased cLT release from inflammatory cells may play an important role in the pathophysiology of AI.

Endogenous mediators such as FMLP and NAP-1 have been found to increase cLT release from various inflammatory cells and blood basophils, particularly when preincubated with IL-3, IL-5, or granulocyte/macrophage colony-stimulating factor (11, 12). C5a alone cannot induce cLT release in basophils. After preincubation with IL-3 or IL-5, leukocytes release cLT in response to C5a. Even cells from subjects who do not respond to anti-IgE

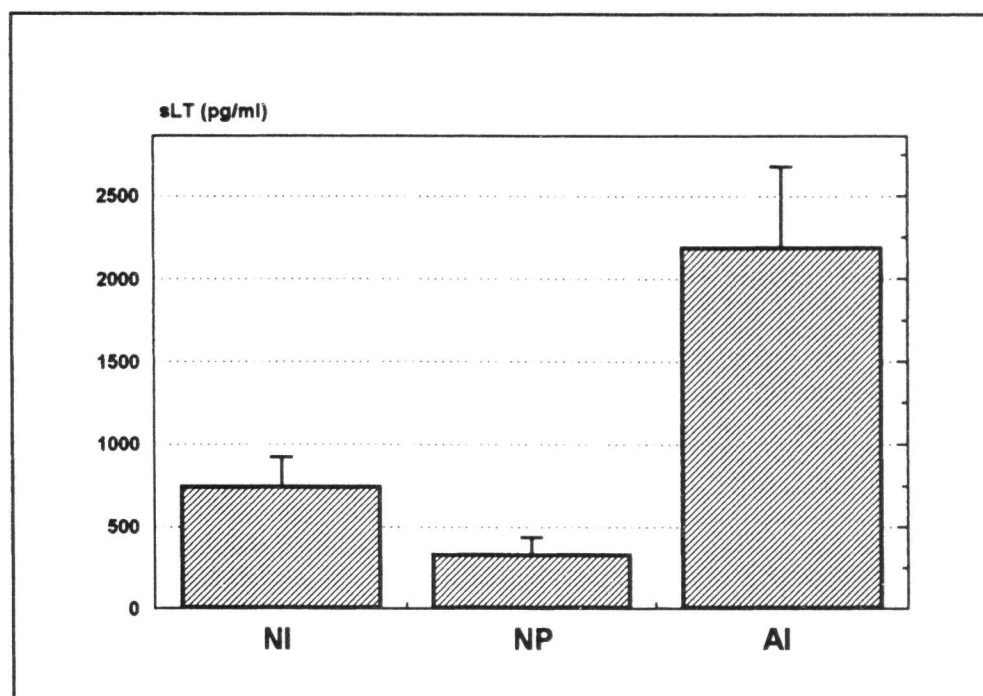


Fig. 2. Cysteinyl leukotriene release (mean $\pm$ SEM) from IL-3-primed human blood leukocytes after costimulation with ASA (10 μ g/ml) and C5a (10^{-8} mol/l) in normal individuals (NI), patients with nasal polyps (NP), and patients with nasal polyps and aspirin intolerance (AI) ($P = 0.0006$).

release cLT in response to IL-5- or IL-3-primed and C5a-stimulated leukocytes (11). Since cLT release in AI is assumed to be an IgE-independent event, cLT release from IL-3-primed and C5a-stimulated leukocytes might be a useful assay of increased IgE-independent cLT release in AI. Increased cLT release from basophils in patients with AI after stimulation with C5a in the presence of IL-3 has been recently found when compared with controls and patients with atopic dermatitis (13).

In this study, cLT release from blood leukocytes of patients suffering from AI was compared with cLT release from patients with NP without AI and normal subjects. The severity of paranasal sinus disease did not differ within these two groups. Differing results between these two groups could be due to the presence or absence of bronchial asthma. The asthmatic disease of the AI patients was controlled by topical steroids, and no asthma attacks had occurred within the 3 weeks before the investigation. Therefore, it is unlikely that major differences between these two groups are attributable to bronchial asthma. However, for a final answer to this question, a group of patients with bronchial asthma and NP without AI should be investigated.

No significant differences in background cLT release from human IL-3 preincubated leukocytes were found in patients with AI or NP, or in normal subjects. In addition, no significant differences in cLT-release from human leukocytes were found after stimulation with anti-IgE. This stimulation control results in a high release of cLT from blood leukocytes in most subjects (11). It confirms that

cells able to release cLT are contained in the specimens. In this study, the cells contained in the specimens were not differentiated, and it is unclear what cells were involved in the release of cLT. Because a high cLT release is obtained after stimulation with anti-IgE, it might be assumed that cLT derives mainly from basophils. The number of basophils in 3 ml whole blood has been estimated at approximately 1000 (14). Two of 25 specimens, one from the NP group and one from the AI group, revealed a weak anti-IgE-induced stimulation of cLT release (less than 100 pg/ml).

cLT release from blood leukocytes after stimulation with ASA was significantly higher in patients with AI than in patients with NP and normal subjects. The reason for this is unclear. It might be assumed that leukocytes from patients with AI have increased susceptibility to release LT also to unspecific stimuli and that the aspirin added acted as an unspecific stimulus.

The cLT assay revealed a high potency to separate patients with AI from patients with NP and normal subjects when blood leukocytes were costimulated with C5a and ASA. The best separation was obtained with ASA in a concentration of 10 μ g/ml and C5a (10^{-8} mol/l). C5a is well known to induce LT synthesis in IL-3- or IL-5-primed blood basophils (11). The cLT release after C5a incubation was significantly higher in patients with AI than in controls. Increased cLT release from human leukocytes after stimulation with C5a and ASA (10 and 100 μ g per ml) in patients with AI was recently found by Czech & Kapp (13). It can be assumed that a summation of the effects of C5a

and ASA on blood basophils contributed to the markedly increased cLT release from blood leukocytes in patients with AI.

With this assay, a large variance in values for cLT was obtained. This might be due to differing numbers of cells able to release cLT in the specimens obtained. However, the values of patients with and without AI did rarely overlap. After stimulation with ASA-C5a-2, no value of patients without AI exceeded 1100 pg cLT/ml, and only one patient with AI had a value less than 1100 pg cLT/ml. Therefore, the selectivity of this test was appropriate.

Measurement of ASA and C5a-stimulated cLT release from human blood leukocytes may improve the diagnosis of AI. Early diagnosis of AI in patients with NP allows specific therapy including a salicylic acid-free diet, aspirin desensitization, high-dose topical steroid treatment after surgery, and the use of lipoxygenase inhibitors or LT-receptor antagonists (16–20).

References

1. Brown BL, Harner SG, van Dellen RG. Nasal polypectomy in patients with asthma and sensitivity to aspirin. *Arch Otolaryngol* 1979;105:413-16.
2. McDonald JR, Mathison DA, Stevenson DD. Aspirin intolerance in asthma: detection by oral challenge. *J Allergy Clin Immunol* 1972;50:198.
3. Schapowall A, Hansel T, Schmitz-Schumann CM. Nasal provocation for the diagnosis of aspirin-sensitive asthma. *Am Rev Respir Dis* 1991;143: Suppl 4:434.
4. Yamashita T, Tsuyi H, Maeda N, Tomoda K, Kumazawa T. Etiology of nasal polyps associated with aspirin-sensitive asthma. *Rhinology* 1989; Suppl 8:15-24.
5. Ferreri NR, Howland WC, Stevenson DD, Siegelberg HL. Release of leukotrienes, prostaglandins, and histamine into nasal secretions of aspirin-sensitive asthmatics during reaction to aspirin. *Am Rev Respir Dis* 1988;137:847-54.
6. Kowalski ML, Sliwiska-Kowalska M, Igarashi Y, et al. Nasal secretions in response to acetylsalicylic acid. *J Allergy Clin Immunol* 1993;91:580-98.
7. Szczeklik A, Gryglewski RJ, Czerniawska-Mysik G. Relationship of inhibition of prostaglandin biosynthesis by analgesics to asthma attacks in aspirin-sensitive patients. *BMJ* 1975;1:67-8.
8. Adams GK, Lichtenstein LM. Indomethacin enhances response of human bronchus to antigen. *Am Rev Respir Dis* 1985;131:8-10.
9. Undem BJ, Pickett WC, Lichtenstein LM, Adams GK. The effect of indomethacin on immunologic release of histamine and sulfido-peptide leukotrienes from human bronchus and lung parenchyma. *Am Rev Respir Dis* 1987;136:1183-7.
10. Okuda Y, Hattori H, Takashima T, et al. Basophil histamine release by platelet-activating factor in aspirin-sensitive subjects with asthma. *J Allergy Clin Immunol* 1990;86:548-53.
11. Bischoff SC, Brunner T, de Weck AL, Dahinden CA. Interleukin 5 modifies histamine release and leukotriene generation by human basophils in response to diverse agonists. *J Exp Med* 1990;172:1577-82.
12. Kurimoto Y, de Weck AL, Dahinden CA. Interleukin 3-dependent mediator release in basophils triggered by C5a. *J Exp Med* 1989;170:467.
13. Czech W, Kapp A. Acquisition of diagnostic *in vitro* procedures in pseudoallergic reactions to food additives. 3. Statuskonferenz des PUG am 16. und 17. März 1994 im Kernforschungszentrum Karlsruhe, 1994:55-65.
14. Furukawa K, Tengler R, de Weck AL, Maly FE. Simplified sulfido-leukotriene ELISA using LTD₄-conjugated phosphatase for study of allergen induced leukotriene generation by isolated mononuclear cells and diluted whole blood. *J Invest Allergol Clin Immunol* 1994;4:110-15.
15. Siegel S, Castellan NJ. Nonparametric statistics for the behavioral sciences. Singapore: McGraw-Hill, 1988.
16. Dahlen B, Kumlin M, Margolskee DJ, et al. The leukotriene-receptor antagonist MK-0679 blocks airway obstruction induced by inhaled lysine-aspirin in aspirin-sensitive asthmatics. *Eur Respir J* 1993;6:1018-26.
17. English GM. Nasal polypectomy and sinus surgery in patients with asthma and aspirin idiosyncrasy. *Laryngoscope* 1986;96:374-80.
18. Nasser SM, Bell GS, Foster S, et al. Effect of the 5-lipoxygenase inhibitor ZD2138 on aspirin-induced asthma. *Thorax* 1994;49:749-56.
19. Settipane GA, Chafee FH. Nasal polyps in asthma and rhinitis. A review of 6037 patients. *J Allergy Clin Immunol* 1977;59:17-21.
20. Sweet JM, Stevenson DD, Simon RS, Mathison DA. Long-term effects of aspirin desensitization – treatment for aspirin-sensitive rhinosinusitis-asthma. *J Allergy Clin Immunol* 1990;85:59.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.