

Serum interleukin-5 in aspirin-induced asthma

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Summary

Background Eosinophilopoietic cytokine IL-5 enhances cysteinyl-leukotriene (cys-LT) synthesis in eosinophils *in vitro*. In patients with aspirin-induced asthma (AIA) bronchial biopsies revealed eosinophil infiltration and a marked increase in IL-5 positive cells.

Objective We wondered whether in AIA patients the bronchial IL-5 increase is reflected in peripheral blood, and if so, whether it is related to overproduction of cys-LT.

Methods In 11 stable patients with AIA, 32 with ATA (aspirin-tolerant asthma) and in 16 controls we measured serum IL-5 concentrations and urinary LTE₄, believed to reflect global cys-LT production.

Results Serum IL-5 was detectable in 12 of 43 asthmatics, but in none of the control subjects. It was highest in the ATA group and differed significantly from the controls. There was no significant difference in IL-5 levels between: (i) the asthmatic groups studied, and (ii) AIA patients and controls. No relationship was found between serum IL-5 and urinary cys-LT.

Conclusion Overexpression of IL-5 reported in the airways of aspirin-sensitive patients with asthma was not reflected in their blood. If IL-5 affects cys-LT production, it is rather in the bronchi of the patients than in the blood.

Keywords: aspirin-induced asthma, interleukin-5, cysteinyl-leukotrienes.

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Introduction

Interleukin (IL)-5 is the cytokine that specifically promotes terminal differentiation of eosinophil progenitors and enhances survival, migration and degranulation of matured eosinophils *in vitro* [1–4]. These properties of IL-5 have stimulated great interest, since increased eosinophil production and recruitment into airways appears to be essential for clinical manifestation of allergic inflammation [5]. IL-5 also enhances cysteinyl-leukotriene (cys-LT) synthesis *in vitro*, mimicking changes in eosinophils from asthmatic patients [6]. Enhanced capacity for cys-LT synthesis in human blood eosinophils after 6-h culture with IL-5 is associated with increased expression of 5-lipoxygenase (5-LO) activating protein and translocation of 5-lipoxygenase to the nucleus [7]. Thus, exposure to excess IL-5 *in vivo* in the bone marrow, circulation or lung may produce a

marked increase in 5-LO pathway activity leading to impaired lung function and further eosinophil migration.

Eosinophil count is elevated in blood and bronchial tissue of patients with aspirin-induced asthma (AIA), a clinical syndrome that affects about 10% of adult asthmatics [8,9]. Circulating blood eosinophils of these patients show enhanced expression of mRNA LTC₄ synthase [10]. In contrast to eosinophils from aspirin-tolerant asthmatics (ATA) and normal subjects, blood eosinophils from AIA patients are relatively insensitive *in vitro* to stimulating effects of IL-5 on mRNA LTC₄ synthase, suggesting the existence of *in vivo* priming mechanism [11]. Bronchial biopsies of AIA patients, as compared with ATA reveal a five-fold increase in LTC₄ synthase cells, which are predominantly eosinophils and a four-fold increase in IL-5⁺ cells, which are predominantly mast cells [12]. Urinary cys-leukotriene excretion in AIA patients is markedly higher than in the ATA group [9].

We hypothesized that serum IL-5 may be higher in AIA

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Table 1. Clinical characteristics of the subjects. Values presented as mean $\pm$ SD, except for IgE (median, upper and lower quartile)

Parameters studied	Healthy	AIA	ATA
<i>n</i>	16	11	32
Age (yr)	35.2 $\pm$ 10.4	47.5 $\pm$ 10.1	37.5 $\pm$ 14.3
Male/female	9/7	5/6	14/18
Duration of asthma (yr)		15.5 $\pm$ 5.9	7.30 $\pm$ 5.6
Patients on inhaled steroids		6	12
Daily inhaled steroids, dose μ g		1400 $\pm$ 456.0	1100 $\pm$ 289.0
Total IgE (IU/mL)	28.3 (28.3–40.3)†	48.5 (28.3–129)†	105.5 (50.2–254.5)†
Positive skin prick tests		5	24

* Values are mean $\pm$ SD.

† median, lower and upper quartile.

patients compared with control groups and that serum IL-5 may predict eosinophil count. We also tested the hypothesis that eosinophil count might influence the urinary cys-LTs excretion. Genotypes of a common LTC₄ synthase polymorphism were ascertained as a potentially confounding variable for the study. The $-444C$ allelic variant predisposes to and is more common in severe AIA patients but the molecular mechanism for overexpression of LTC₄ synthase seems not to depend on IL-5 priming [11].

Materials and methods

Study group

Studies were performed in 11 stable asthmatics with aspirin sensitivity confirmed by inhalation provocation test with aspirin and in 32 stable aspirin-tolerant asthmatics who took aspirin without any adverse effects within 6 months

preceding the study. The control group comprised 16 adult healthy volunteers. The subject characteristics are presented in Table 1. Patients were instructed to withhold inhaled short-acting β_2 agonists for 6 h, long-acting β_2 agonists for 24 h, aminophylline for 24 h, antihistamines for 48 h and inhaled steroids for 48 h prior to the study day. None of the patients was on chronic corticotherapy. Their baseline FEV₁ was $> 70\%$ of the predicted value.

Study design

Examination of each individual was performed within 1 day, between 08.00 and 10.00. First, the patients underwent basic spirometry. If their baseline FEV₁ was over 70% of predicted value, their blood and urine samples were collected. Blood was tested for eosinophil count, IL-5 concentration and genetic polymorphism of leukotriene C₄

Table 2. Serum concentration of IL-5, blood eosinophil counts, urinary baseline level of cys-LTs and the percentage of allele C⁺ and the frequency of C₋₄₄₄ transversion (q) in the study groups

Parameters studied	Healthy	AIA	ATA
<i>n</i>	16	11	32
IL-5 (pg/mL)	Non-detectable	2.25 $\pm$ 0.78	4.60 $\pm$ 2.10†
Number (<i>n</i>)		2	10
Eosinophil count /mm ³	259.4 $\pm$ 177.9	530 $\pm$ 404.9†	408 $\pm$ 314.7
Cys-LTs (pg/mg creatinine)	140.8 $\pm$ 46.6	780.8 $\pm$ 310.8†*	475.5 $\pm$ 309.1†*
Allel C ⁺ (%)	26	67	50
(q)	0.17	0.33	0.29

Values are means $\pm$ SD.*P* < 0.05: *statistically significant difference between AIA and ATA.

†Statistically significant difference between healthy and asthmatic groups.

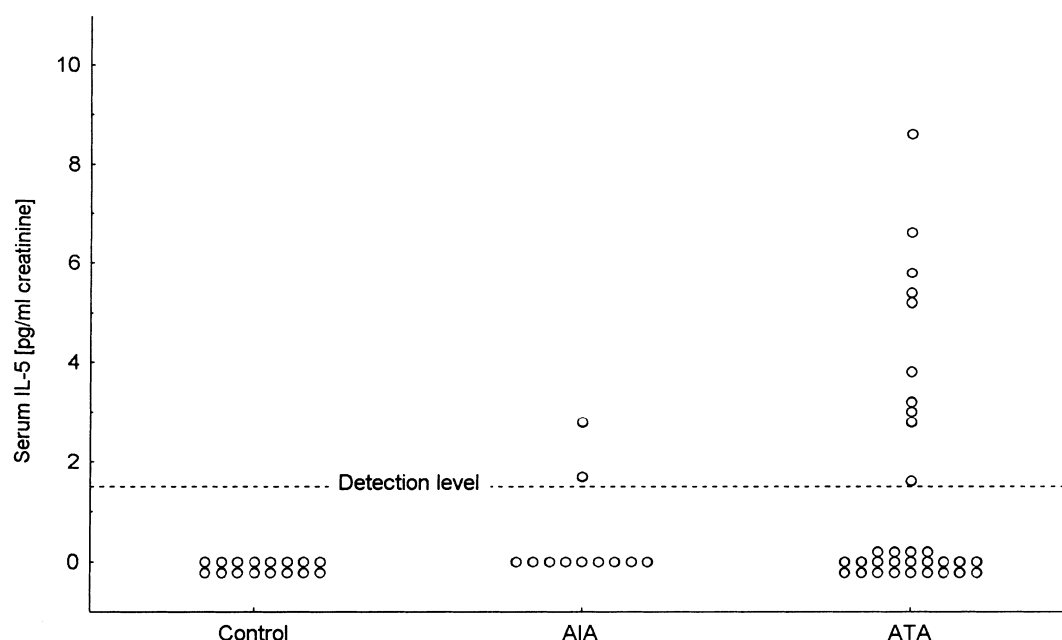


Fig. 1. Comparison of serum concentrations of interleukin-5 in control group, aspirin-induced asthma (AIA) and aspirin tolerant asthmatics (ATA).

synthase. Urine was collected for determination of cysteinyl leukotrienes at baseline (from 6.30 to 8.30).

The protocol was approved by the Jagiellonian University Ethical Committee and informed consent was obtained from each patient.

Bronchial provocation test with L-lysine aspirin

This test was performed as described previously [13].

Measurement of urinary peptido-leukotrienes

Peptido-leukotrienes were measured [14] in urine samples by direct enzyme immunoassay (Cayman Chemical, Ann Arbor, MI, USA).

Genetic polymorphism of LTC₄ synthase

Genotyping of a common single nucleotide polymorphism of the LTC₄ synthase gene was carried out on samples of genomic DNA isolated from the peripheral blood. The PCR amplified gene fragment was digested with MspI restriction endonuclease and products were resolved on acrylamide gel, as previously described [15].

Eosinophil count

Eosinophils were counted using a light microscope, after diluting the whole blood sample in Pilot's solution [13].

Serum IL-5 concentration

Serum concentration of IL-5 was measured using Enzyme Immunometric Assay (Milenia IL-5 DPC Biermann GmbH, Germany). The detection limit of the assay, defined as the concentration of two standard deviations above the response at zero dose, is approximately 1.5 pg/mL. In a reference study with 41 serum samples from healthy volunteers, using the Milenia IL-5 procedure, the absolute range of the samples was from non-detectable to 17 pg/mL.

Statistical evaluation

Summary statistics were expressed as mean (M) and standard deviation (SD) for symmetric distributed data and median, 25% and 75% percentiles for non-symmetric (skewed) distributed data, respectively.

An ANOVA was used to compare several dependent and independent groups. When the parametric ANOVA assumptions were not fulfilled (even after variance stabilizing transformation) we used Kruskal-Wallis and Friedman rank ANOVA. Similarly, for comparison of two groups we used Student's *t*-test and the Mann-Whitney U Rank Sum Test to compare two independent groups and the Wilcoxon signed rank test for matched pair studies. The relationship between variables was studied with Spearman's *r* correlation coefficient.

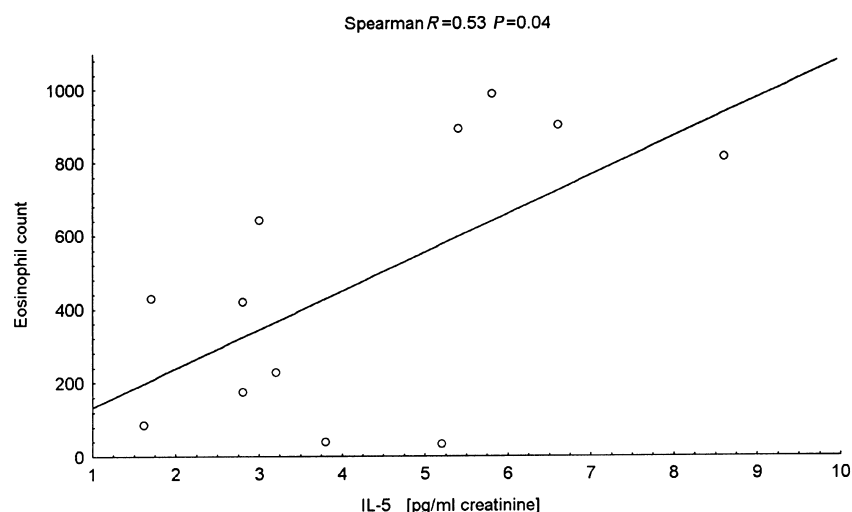


Fig. 2. The correlation between IL-5 and eosinophil counts in the 12 asthmatic subjects with measurable IL-5-values (2 AIA, 10 ATA).

Results

The two asthma groups studied did not differ with respect to main clinical characteristics (Table 1). Mean serum IL-5 concentration was the highest in ATA group and differed significantly from the controls, who had no detectable IL-5 (Fig. 1, Table 2.). There was no significant difference in IL-5 levels between: (i) the asthmatic groups studied, or (ii) AIA patients and controls. In a population of asthmatic patients with measurable serum IL-5 there was a positive correlation between IL-5 and eosinophil count ($n = 12$, $R = 0.53$, $P = 0.04$), but not between IL-5 and urinary cys-LT or IL-5 and LTC₄S polymorphism. Urinary cys-LT levels were the highest in AIA patients, differing significantly from the other two groups studied. There was no relationship between eosinophil count and cys-LT values or between LTC₄S genetic polymorphism and urinary excretion of cys-LTs. Frequency of the C₋₄₄₄ variant of LTC₄ synthase was 0.17 in controls, 0.29 in ATA and 0.33 in AIA patients; differences between the groups were insignificant (Table 2). For asthmatics stratified according to LTC₄ genotypes, IL-5 levels, eosinophilia and urinary cys-LTs excretion were not different.

Discussion

Our results confirm previous findings that IL-5 is detectable in serum of a proportion of both atopic and non-atopic patients with asthma. In a study by Alexander and his colleagues [16], IL-5 was found in the serum of 15 of 29 patients, but in none of the seven control subjects. Motojima *et al.* [17] reported measurable concentrations of IL-5 in 27 of 39 asthmatics, but only in seven of 49 controls. No relationship was observed between atopic status and IL-5 levels, while correlation

with clinical status was found by the latter [17], though not the former [16], authors. The origin of serum IL-5 is uncertain. The most likely source appears to be peripheral blood T lymphocytes and mast cells [18–22]. The possibility of spill-over from the airways has also been discussed [17].

In AIA, as compared with ATA, IL-5 expression is high in bronchial tissues and BAL, and there is a four-fold increase in the number of eosinophils and mast cells expressing IL-5 [12,23]. Blood eosinophilia is also a prominent feature, as was the case in the population here reported. However, the results of serum estimations were contrary to our expectations. It was the ATA and not the AIA group that had increased serum IL-5 concentrations as compared with controls. Thus, changes reported in the airways were not reflected in the blood. The presence of IL-5 within the bronchial mucosa rather than in blood appears to be more relevant to pathogenesis of AIA.

There was a trend for higher prevalence of C₋₄₄₄ allele in AIA as compared to ATA and healthy controls, as observed by us earlier [15]. It did not reach statistical significance, probably because of the limited number of subjects within the groups studied. In the study of patients in the United States of America [24], no difference in prevalence of the allelic variant was observed between AIA and ATA patients. However, homozygotes C₋₄₄₄ were more frequent in the AIA group, which also showed deviation from the Hardy–Weinberg equilibrium [24]. While in AIA patients LTC₄S genetic polymorphism affects the response of urinary LTE₄ to inhaled lysine-aspirin [11], it clearly does not influence the LTE₄ excretion at base.

In vitro, IL-5 enhances the capacity for cys-LT synthesis in human blood eosinophils [6]. This effect is exerted via

gene transcription and protein synthesis [7]. We measured urinary LTE₄ excretion, believed to reflect total cys-LT production [25], and looked for its possible relationship to serum IL-5. As expected, cys-LT excretion was highest in the AIA group. However, no correlation between this variable and serum IL-5 could be found, suggesting again that if IL-5 affects cys-LT production it is in the bronchi rather than in the blood.

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References

- Sanderson CJ, Warren DJ, Strath M. Identification of a lymphokine that stimulates eosinophil differentiation *in vitro*: its relationship to interleukin-3 and functional properties of eosinophils produced in cultures. *J Exp Med* 1985; 162:60–74.
- Lopez AF, Sanderson CJ, Gamble JR, Campbell HD, Young IG, Vadas MA. Recombinant human interleukin-5 is a selective activator of human eosinophil function. *J Exp Med* 1988; 167:219–24.
- Kay AB. Asthma and inflammation. *J Allergy Clin Immunol* 1991; 87:893–909.
- O'Byrne PM, Wood L. Interleukin-5 and allergic inflammation. *Clin Exper Allergy* 1999; 29:573–5.
- Holgate ST. The inflammation-repair cycle in asthma: the pivotal role of the airway epithelium. In: Holgate ST, Boushey HA, Fabbri L eds. *Difficult Asthma*. London: Martin Dunitz, 1999:13–22.
- Takafuji S, Bischoff SC, De Weck AL, Dahinden CA. IL-3 and IL-5 prime normal human eosinophils to produce leukotriene C₄ in response to soluble agonists. *J Immunol* 1999; 147:3855.
- 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.
- Kowalski ML, Sliwiska-Kowalska M, Igarashi Y et al. Nasal secretions in response to acetylsalicylic acid. *J Allergy Clin Immunol* 1993; 91:580–98.
- Szczeklik A, Stevenson DD. Aspirin-induced asthma: advances in pathogenesis and management. *J Allergy Clin Immunol* 1999; 104:5–13.
- Sanak M, Bazan-Socha S, Szczeklik A. Leukotriene C₄ synthase (LTC₄S) expression in blood eosinophils of patients with asthma. *Eur Respir J* 1998; 12 (Suppl. 28):373s.
- Sanak M, Pierzchalska M, Bazan-Socha S, Szczeklik A. Enhanced expression of the leukotriene C₄ synthase due to overactive transcription of an allelic variant associated with aspirin-induced asthma. *Am J Respir Cell Molec Biol* 2000; 23:290–6.
- 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.
- Szczeklik A, Sladek K, Dworski R et al. Bronchial aspirin challenge causes specific eicosanoids response in aspirin sensitive asthmatics. *Am J Respir Crit Care Med* 1996; 154:1608–14.
- Kumlin M, Stensvad F, Larsson L, Dahlen B, Dahlen S-E. Validation and application of a new simple strategy for measurements of urinary leukotriene E₄ in humans. *Clin Exp Allergy* 1995; 25:467–79.
- Sanak M, Simon HU, Szczeklik A. Leukotriene C₄ synthase promoter polymorphism and risk of aspirin-induced asthma. *Lancet* 1997; 350:1599–600.
- Alexander AG, Barkans J, Moqbel R, Barnes NC, Kay AB, Corrigan CJ. Serum interleukin-5 concentrations in atopic and non-atopic patients with glucocorticoid-dependent chronic severe asthma. *Thorax* 1994; 49:1231–3.
- Motojima S, Tateishi K, Koseki T, Makino S, Fukuda T. Serum levels of eosinophil cationic protein and IL-5 in patients with asthma without systemic corticosteroids. *Int Arch Allergy Immunol* 1997; 114:55–9.
- Walker C, Virchow J-C, Bruijnzeel PLB, Blaser K. T cell subsets and their soluble products regulate eosinophilia in allergic and non-allergic asthma. *J Immunol* 1991; 146:1829–35.
- Corrigan CJ, Haczku A, Gemou-Engesaeth V et al. CD₄ T-lymphocyte activation in asthma is accompanied by increased serum concentrations of interleukin-5. *Am Rev Respir Dis* 1993; 147:540–7.
- Bradding PJ, Roberts A, Britten KM et al. Interleukin-4,-5,-6 and tumor necrosis factor- α in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. *Am J Respir Cell Mol Biol* 1994; 10:471.
- Till SJ, Durham SR, Rajakulasingam K et al. Allergen-induced proliferation and interleukin-5 production by bronchoalveolar lavage and blood T cells after segmental allergen challenge. *Am J Respir Crit Care Med* 1998; 158:404–11.
- Borger P, Ten Hacken NHT, Vellenga E, Kaufman HF, Postma DS. Peripheral blood T lymphocytes from asthmatic patients are primed for enhanced expression of interleukin (IL-4) and IL-5 mRNA: associations with lung function and serum IgE. *Clin Exper Allergy* 1999; 29:772–9.
- Sousa AR, Lams BE, Pfister R, Christie PE, Schmitz M, Lee TH. Expression of interleukin-5 and granulocyte-macrophage colony-stimulating factor in aspirin-sensitive and non-aspirin-sensitive asthmatic airways. *Am J Respir Crit Care Med* 1997; 156:1384–9.
- Van Sambeek R, Stevenson DD, Baldasaro M et al. 5'Flanking region polymorphism of the gene encoding leukotriene C₄ synthase dose not correlated with the aspirin-intolerant asthma phenotype in the United States. *J Allergy Clin Immunol* 2000; 106:72–6.
- Kumlin M. Measurements of leukotrienes in urine: strategies and applications. *Allergy* 1997; 52:124–35.