

Eotaxin in Exhaled Breath Condensate of Allergic Asthma Patients with Exercise-Induced Bronchoconstriction

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Key Words

Exercise-induced bronchoconstriction • Eotaxin • Airway inflammation • Asthma • Exhaled breath condensate • Exhaled nitric oxide

Abstract

Background: Eosinophils are the key inflammatory cells in asthma, and more and more evidence suggests their crucial role in exercise-induced bronchoconstriction (EIB). Eotaxin, as the most important chemotactic factor for eosinophils, plays an important role in the pathogenesis of asthma. **Objectives:** The aim of the study was to evaluate the changes in eotaxin levels in exhaled breath condensate (EBC) following intensive exercise in allergic asthmatics. **Methods:** The study was performed in a group of 27 asthmatics (17 with EIB, 13 without EIB) and 9 healthy volunteers. Changes induced by intensive exercise in the concentrations of eotaxin in EBC during the 24 h after an exercise test were assessed. The possible correlations of these measurements with the results of other tests commonly associated with eosinophilic airway inflammation were also determined. **Results:** In asthmatic patients with EIB, a statistically significant increase in eotaxin concentrations in EBC collected during the first 24 h after an exercise test – with maximal increase after 6 h – was revealed. A statistically significant correlation between the

maximum increase in eotaxin concentrations in EBC after exercise, and an increase in either serum eosinophil cationic protein or F_{ENO} 24 h after exercise in the group of asthmatics with EIB, was observed. **Conclusions:** Our results confirm connections between EIB and airway eosinophilic inflammation. The increase of eotaxin in asthmatic airways, by promoting the migration and activation of eosinophils, may play an important role in upregulation and sustaining of the airway inflammation observed in EIB in asthmatic patients.

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Background

There is increasing evidence that exercise-induced bronchoconstriction (EIB) in patients with asthma is associated with eosinophilic airway inflammation [1]. However, findings are still conflicting concerning the participation of different mechanisms and inflammatory mediators in either the induction of bronchoconstriction or maintenance of airway inflammation provoked by exercise [2].

The trafficking of eosinophils to the airways is a complex process that may be regulated by many mediators, such as cytokines, chemokines, adhesion molecules and lipid mediators [3, 4]. These molecules affect eosinophil

development, release from the bone marrow, accumulation, effector function and survival in tissues. Once recruited to the airways, eosinophils receive signals that promote degranulation and the secretion of cytokines [5, 6].

CC chemokine ligands, such as eotaxin and regulated upon activation normal T cell expressed and secreted (RANTES), play an important role in the allergic inflammation in asthma by promoting the migration and activation of inflammatory cells, including eosinophils [7].

In our previous studies we observed that in asthmatic patients, as a consequence of postexercise bronchoconstriction, increased expression of RANTES in the airways occurs [8]. This process, by promoting the migration and activation of inflammatory cells including eosinophils, may play an important role in upregulation of the airway inflammation observed after EIB in asthmatic patients.

In the present study (performed in a new cohort of patients and healthy volunteers) we have decided to investigate the role of eotaxin, present in asthmatic airways, in the pathogenesis of EIB. Eotaxin is one of the most important chemokines in promoting airway inflammation in asthmatic patients. Eotaxin acts together with IL-5 and increases the count of mature eosinophils in blood, producing peripheral and tissue eosinophilia [9]. In vivo, eotaxin constitutes a specific – and the strongest – chemoattractant for eosinophils. It seems to be responsible for retardation of apoptosis of eosinophil, and its interaction with a receptor promotes many intracellular alterations which lead to activation and degranulation of the cell [10]. So far, there is no evidence confirming the role of this cytokine in the pathogenesis of postexercise bronchoconstriction in asthmatic patients.

The aim of the study was to evaluate the effect of postexercise bronchoconstriction on eosinophilic inflammation in asthmatic patients. To obtain the results the changes in eotaxin in exhaled breath condensate (EBC) following intensive exercise and the possible correlation of these measurements with the parameters of airway eosinophilic inflammation and their changes after exercise were analyzed.

Subjects and Methods

Study Population

The study was performed in a group of 27 mild allergic asthma patients. Asthma was diagnosed according to the criteria recommended by GINA 2006 [11]. The diagnosis of allergic asthma was based on positive skin prick tests, history of asthma and allergic rhinitis symptom intensification after the patient's exposure to

sensitizing allergens. All patients had been in a stable condition, free from acute exacerbations and respiratory tract infections for the previous 2 months. Patients with other factors which could change exhaled nitric oxide (F_{ENO}) levels (except for asthma, features of atopy or allergic rhinitis) were excluded from the study. In all patients the tests were performed out of pollen season. Prior to the beginning of this study, patients were allowed to take short-acting β_2 -agonists, but not within 12 h before exercise test or bronchial provocation test (BPT) with histamine. Asthmatic patients who had been treated with drugs other than β_2 -agonists (inhaled steroids, antileukotrienes) in the previous 3 months were excluded from the study. F_{ENO} measurement, skin prick tests with commonly encountered aeroallergens (house dust mites, trees, weeds, grasses, cat, *Alternaria* and *Cladosporium*), total IgE, flow/volume spirometry and a BPT with histamine were performed on each asthmatic patient before qualifying for the exercise test.

Nine healthy volunteers were recruited for the study as a negative control group. All of them underwent F_{ENO} , flow/volume spirometry and skin prick tests with common aeroallergens. They had forced expiratory volume in 1 second (FEV_1) >80% predicted. They were free of respiratory tract infections for 2 months prior to the study and from other significant illnesses known to affect F_{ENO} measurements. Asthma patients and healthy volunteers were nonsmokers and during the last year had not been passive smokers.

In all asthmatic patients and healthy volunteers, an exercise test on the bicycle ergometer was performed. BPT with histamine was performed 24 h before and 24 h after the exercise test. EBC was collected before and 30 min after BPT with histamine, before, 10, 30, 60 min, 6 and 24 h after the exercise test. F_{ENO} was measured before BPT with histamine, before the exercise test and 24 h after the exercise test. Before and 24 h after the exercise test, serum eosinophil cationic protein (ECP) and peripheral blood eosinophilia were measured.

The study protocol was approved by the Ethics of Research Committee of the Medical University of Białystok, agreement No. R-I-002/265/2009. Informed consent was obtained from each patient participating in the study.

F_{ENO} Measurements

F_{ENO} was measured by the chemiluminescence technique using a Sievers 280i NO Analyzer (Boulder, Colo., USA). The measurements were performed at an expiratory flow of 50 ml/s according to ATS recommendations for on-line measurement of F_{ENO} in adults [12].

Lung Function

The baseline spirometry was performed using a MasterScreen Pneumo PC spirometer (Jaeger, Hoechberg, Germany), according to ATS standards [13]. FEV_1 was evaluated. Before the examination the patients did not take any medications that could change spirometry results.

Bronchial Hyperreactivity Assessment

Histamine challenge testing was performed using the APS Pro dosimeter technique (VIASYS Healthcare GmbH, Hoechberg, Germany), using a jet-type nebulizer (DeVilbiss Model 646) powered by compressed air [14]. The system precisely and automatically determines the administered dose of methacholine by measuring the effective nebulization time at inspiration of any breath

and referring it to drug concentration and nebulizer power. APS was calibrated to produce an output of 160 mg/min. During tidal breathing, the subject was instructed to inhale slowly and deeply from the nebulizer. Soon after the inhalation began, the dosimeter was triggered. The patient's breathing pattern is displayed on-line in a flow/time diagram to control the inhalation of the challenge substance. This was repeated for a total of 5 inhalations. 120 s after each step, FEV₁ was measured. The results were presented as PC₂₀FEV₁ – the concentration of histamine which causes a decrease in FEV₁ of exactly 20% in comparison to initial values. PC₂₀FEV₁ was calculated by logarithmic interpolation using an integrated program.

Exercise Test

An exercise test was performed on a bicycle ergometer for 9 min with a fixed workload adjusted to increase the heart rate to 85% of the maximum predicted (calculated as 220 – age in years) [15]. All exercise tests were performed in the same ambient conditions (with ambient temperature of 20–25°C and low relative humidity of 40–50%). Basic spirometric parameters were recorded before and immediately after the exercise test, and 1, 5, 10, 15, 20, 60 min and 24 h after completion of exercise. Those patients whose maximum decrease in FEV₁ was greater than 15% (in at least two consecutive measurement points) were considered to have EIB. All studied groups performed the same relative intensity of the exercise.

Collection of EBC

EBC was collected by using a commercially available condenser (EcoScreen; Jaeger) according to the current ATS/ERS guidelines [16]. All measurements were performed at the same time (between 8.00 and 10.00 AM) to avoid possible circadian rhythm of mediator concentrations in EBC. All patients were asked to refrain from eating and drinking before collection of EBC.

Exhaled air entered and left the chamber through one-way valves and the inlet and outlet, thus keeping the chamber closed. A low temperature inside the condensing chamber throughout the collection time produced a cooling down sample. The temperature of collection was around 0°C [17]. Patients were instructed to breathe tidally for 10 min with nose clip. The respiratory rate ranged from 15 to 20 breaths/min. Patients were asked to swallow their saliva periodically and to temporarily discontinue collection if they needed to cough. At the end of collection, 1.5- to 3.5-ml aliquots of condensate were transferred to Eppendorf tubes and immediately frozen. Exhaled breath collections were performed before, 10, 30, 60 min, 6 and 24 h after the exercise challenge test. Samples were stored at –80°C. The longest storage time of EBC samples did not exceed 3 months [17]. The samples were not concentrated prior to measurement. All measurements were performed in a blinded fashion. All samples were run in duplicate.

Because a marker used to correct the difference in the degree of dilution has not yet been established, in our study we made no attempt to assess the dilution of airway lining fluid (ALF) in EBC. The results (eotaxin) were well repeatable (CV = 4–7%). We did not observe results below detection limit. We performed the preliminary study, in which we measured eotaxin in EBC immediately after collection, and after 1, 2 and 3 months of storage at –80°C, and did not observe any important changes. Therefore, we suggest that eotaxin in EBC stored at –80°C remains stable for at least 3 months.

Measurements of Eotaxin, ECP and Other Laboratory Parameters

Serum total IgE and ECP concentrations were assessed using ImmunoCAPTM Technology (Pharmacia Diagnostics, Uppsala, Sweden). Blood eosinophil count was measured using a hematology analyzer (Coulter Electronics GmbH, Miami, Fla., USA). The concentrations of eotaxin (R&D Systems, Wiesbaden-Nordenstadt, Germany) in EBC were determined using ELISA. The minimum detectable level was 5.0 pg/ml.

Statistical Analysis

Because the data were not normally distributed, statistical significance was analyzed by using nonparametric tests. The Mann-Whitney U test was used for assessment of differences between groups (independent samples). The comparison between variables (dependent samples) was performed using Wilcoxon's matched pairs test. The analysis for comparison of repeated measures of eotaxin was performed using Friedman's two-way analysis of variance. All values were expressed as means ± SD; $p < 0.05$ was considered significant. PC₂₀ values were logarithmically transformed for analysis. The relationship between studied parameters was assayed by correlation. The Spearman R correlation test was used.

Results

Characteristics of studied asthmatic patients and healthy volunteers are shown in table 1. In the studied group of asthmatics, 17 patients had a positive (maximum decrease in FEV₁ after exercise: $24.76 \pm 10.70\%$; min. 15%, max. 49%) and 13 had a negative ($3.46 \pm 2.36\%$; min. 0%, max 8%) exercise test. In none of the healthy volunteers were spirometric parameters worse after exercise ($0.33 \pm 3.27\%$; min. –4%, max. 7%).

Differences between the Studied Groups of Patients and Healthy Volunteers at Rest

Blood eosinophilia, serum ECP, baseline F_{ENO} and total IgE were statistically significantly higher in both groups of asthmatics compared with healthy volunteers ($p < 0.001$). In the group of patients with positive exercise tests compared to patients without EIB, we determined a statistically significantly raised blood eosinophil count and serum ECP concentrations. We also observed higher but not statistically significant serum levels of total IgE and baseline F_{ENO}, as well as a tendency to lower values of FEV₁.

We revealed statistically significantly higher levels of eotaxin in EBC in all studied asthmatic patients compared with healthy controls (10.55 ± 1.72 vs. 6.23 ± 0.63 pg/ml, $p < 0.001$). There was no statistically significant difference between the concentrations of eotaxin in EBC

Table 1. Characteristics of study subjects and healthy volunteers

Characteristics, dimension	Patients with EIB	Patients without EIB	Differences between asthma patients with and without EIB	Healthy volunteers
Number of patients	17	13		9
Sex, F/M	10/7	8/5		6/3
Age, years	25.88 ± 6.58	27.76 ± 6.58	0.37	26.66 ± 5.04
Duration of symptoms, years	3.40 ± 4.70	3.90 ± 4.10	0.36	
Baseline FEV ₁ , % predicted	88.64 ± 11.38	92.15 ± 9.76	0.29	101.88 ± 6.79*
Maximum decrease in % FEV ₁ after exercise, min./max.	24.76 ± 10.70	3.46 ± 2.36	<0.001	0.33 ± 3.27*
Log PC ₂₀ histamine FEV ₁ , mg/ml	-0.64 ± 1.01	-0.10 ± 0.49	0.31	
Blood eosinophil count, cells/mm ³	294 ± 123	187 ± 70	0.01	46 ± 21*, +
Serum ECP, µg/l	10.97 ± 1.90	9.30 ± 1.67	0.01	3.34 ± 0.47*, +
Serum total IgE, kU/l	341 ± 223	216 ± 97	0.14	56 ± 33*, +
Baseline F _{ENO} , ppb	91.88 ± 44.42	74.00 ± 33.70	0.26	14.22 ± 5.30*, +
Baseline eotaxin (EBC), pg/ml	10.83 ± 1.67	10.18 ± 1.78	0.22	6.23 ± 0.63*, +
Positive SPT	17	13		0
Mite/cat/moulds	16/4/6	12/4/4		
Seasonal	6	5		

Data are presented as means ± SD. SPT = Skin prick tests; PC₂₀histamine FEV₁ = provocative concentration of histamine that caused a 20% fall in FEV₁. * p < 0.05, values significantly different from patients with EIB; + p < 0.05, values significantly different from patients without EIB.

before exercise in asthmatics patients with EIB in comparison to asthmatics without EIB (10.83 ± 1.67 vs. 10.18 ± 1.78 pg/ml, p = 0.22).

Changes of Studied Parameters after Exercise

A statistically significant increase in the concentrations of eotaxin in asthmatic patients with EIB was observed (p < 0.001) (10 min after exercise: 10.77 ± 1.78 pg/ml, p = 0.30; 30 min after exercise: 11.08 ± 1.68 pg/ml, p = 0.004; 60 min after exercise: 11.65 ± 1.74 pg/ml, p < 0.001; 6 h after exercise: 12.78 ± 2.30 pg/ml, p < 0.001; 24 h after exercise: 11.73 ± 2.03 pg/ml, p < 0.001). No effects of the exercise test on changes in the concentrations of eotaxin in EBC in either asthmatic patients without EIB (p = 0.21) or healthy volunteers (p = 0.29) were observed (fig. 2).

The changes in eotaxin in EBC were not observed after BPT with histamine (data not shown).

Twenty-four hours after the exercise test, in the group of asthmatics with EIB, a statistically significant increase in F_{ENO} (before exercise: 91.88 ± 44.42 ppb; 24 h after exercise: 104.23 ± 52.42 ppb; p < 0.001) and BHR to histamine (log PC₂₀FEV₁ before exercise: -0.63 ± 1.01 mg/ml; 24 h after exercise: -1.25 ± 1.42 mg/ml; p < 0.001) was revealed. Such changes were not observed in the

group of asthmatic patients without EIB (F_{ENO} before exercise: 74.00 ± 33.72 ppb; 24 h after exercise: 74.76 ± 32.58 ppb; p = 0.34; log PC₂₀FEV₁ before exercise: -0.10 ± 0.49 mg/ml; 24 h after exercise: -0.07 ± 0.50 mg/ml; p = 0.27).

In the group of asthmatic patients with EIB, we determined a statistically significant increase in serum ECP concentrations 24 h after exercise (before exercise: 10.97 ± 1.55 µg/l; 24 h after exercise: 12.77 ± 2.10 µg/l; p < 0.001). We did not observe such changes in the group of asthmatics without EIB (before exercise: 9.30 ± 1.64 µg/l; 24 h after exercise: 9.43 ± 1.69 µg/l; p = 0.43).

There were no statistically significant changes in peripheral blood eosinophilia 24 h after exercise. In neither group of asthmatics did we detect significant changes in FEV₁ 24 h after exercise.

Correlations between Studied Parameters

We found a statistically significant correlation between eotaxin levels and either F_{ENO}, serum ECP and blood eosinophil count before exercise in the group of asthmatic patients with EIB as well as without EIB. Such correlations were not observed in the group of healthy volunteers (table 2).

24 h before ET – day 1	Day 2	24 h after ET – day 3
----- ---30 min-----	----ET---10 min---30 min---60 min---6 h---	----- -----
1, 2, 3 4 1	1, 2 1 1 1 1	1, 2, 3, 5, 6 4
	3, 5, 6 3 (0, 1, 5, 10, 15, 20, 60 min)	

Fig. 1. The schema of the protocol. ET = Exercise test; 1 = EBC, 2 = F_{ENO} ; 3 = FEV_{15} ; 4 = BPT with histamine; 5 = ECP; 6 = peripheral blood eosinophilia.

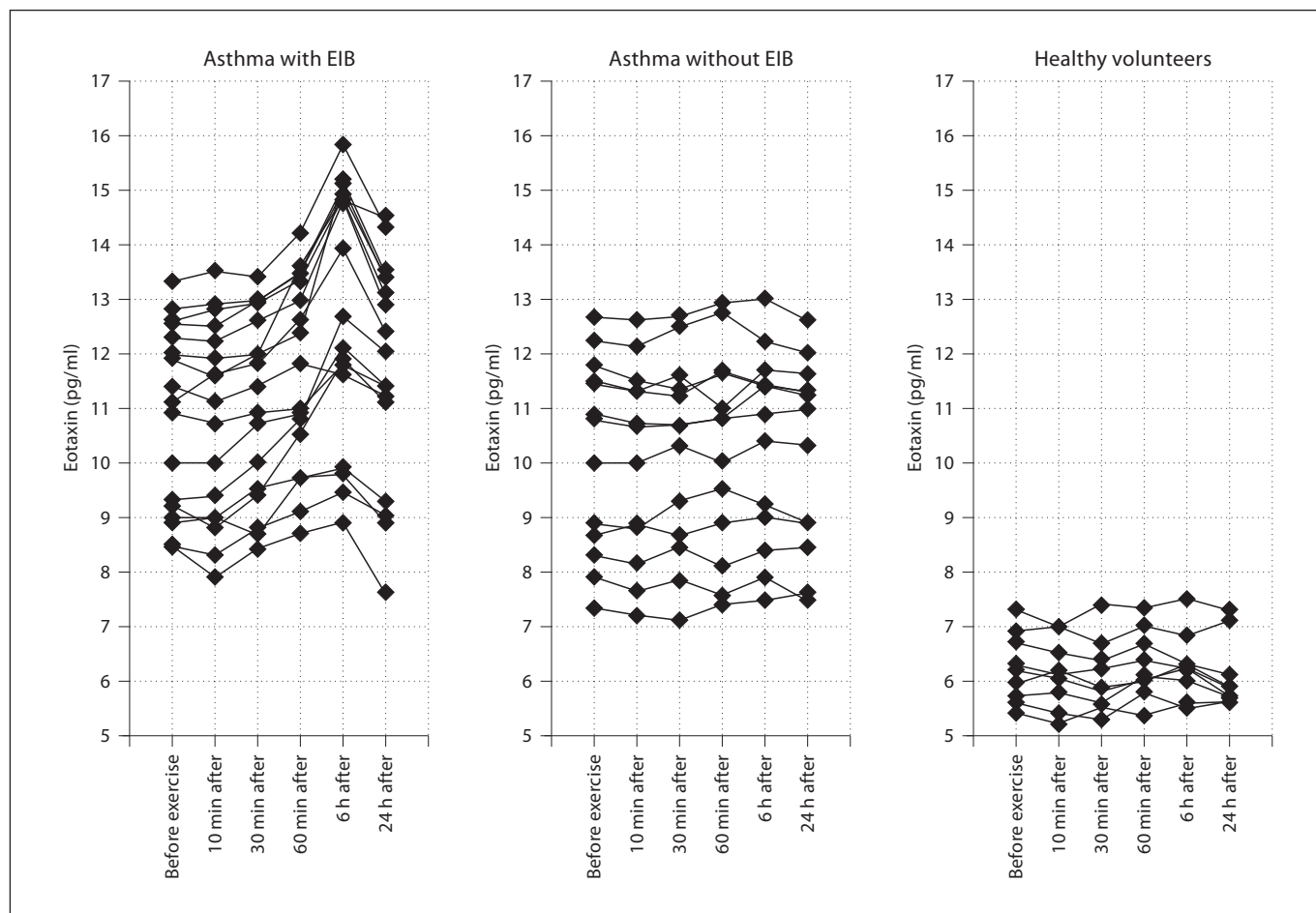


Fig. 2. Concentrations of eotaxin in EBC at rest and subsequent changes which were observed during the 24 h after exercise test in asthmatic patients and healthy volunteers.

Statistically significant correlations between the maximum increase in eotaxin concentrations in EBC after exercise and the increase in F_{ENO} ($r = 0.78$, $p < 0.001$) or serum ECP ($r = 0.74$, $p < 0.001$) 24 h after exercise in the group of asthmatics with EIB were observed (table 3).

We did not find correlations between concentrations of eotaxin at rest and changes of its levels observed after exercise. Correlations between the severity of EIB and baseline levels of studied parameters and their changes after the exercise were also not demonstrated.

Table 2. Correlations between baseline levels of eotaxin in EBC and other studied parameters in the groups of asthma patients and healthy volunteers before exercise

Studied groups	F _{ENO}	Serum ECP	Blood eosinophil count	Serum total IgE	Baseline FEV ₁	BHR to histamine
Asthma with EIB	r = 0.92 p < 0.001	r = 0.64 p = 0.004	r = 0.75 p < 0.001	r = -0.13 p = 0.61	r = -0.14 p = 0.57	r = 0.47 p = 0.06
Asthma without EIB	r = 0.83 p < 0.001	r = 0.74 p = 0.003	r = 0.84 p < 0.001	r = -0.13 p = 0.65	r = -0.02 p = 0.92	r = 0.48 p = 0.08
Healthy volunteers	r = -0.30 p = 0.43	r = 0.26 p = 0.43	r = 0.10 p = 0.79	r = -0.35 p = 0.35	r = -0.25 p = 0.23	

Table 3. Correlations between changes in eotaxin levels in EBC and changes in other studied parameters after exercise

Studied groups	F _{ENO} (24 h after exercise)	Serum ECP (24 h after exercise)	BHR to histamine (24 h after exercise)	EIB severity (decrease in FEV ₁ after exercise)
Asthma with EIB	r = 0.78 p < 0.001	r = 0.74 p < 0.001	r = 0.27 p = 0.28	r = -0.18 p = 0.47
Asthma without EIB	r = -0.18 p = 0.53	r = 0.03 p = 0.91	r = -0.09 p = 0.74	r = 0.32 p = 0.28
Healthy volunteers	r = 0.43 p = 0.24	r = -0.37 p = 0.32		r = 0.17 p = 0.64

Discussion

Eosinophils are the key inflammatory cells in asthma, and there is increasing evidence to suggest their important role in postexercise bronchoconstriction [18]. Koh and Choi [19] have demonstrated that the severity of postexercise bronchoconstriction correlates with peripheral blood eosinophilia, and blood eosinophil count is a significant predictor of EIB. Venge et al. [20] have found that the serum level of ECP reflects the degree of bronchoconstriction after exercise in asthma patients. The role of eosinophils in EIB has also been described by Kivity et al. [21], who demonstrated a significant increase in sputum eosinophil counts in patients with EIB, but not in those without EIB or after BPT with metacholine.

Eotaxin is the most specific and strongest factor which could modulate the function of eosinophil. The role of eotaxin is very important at each stage of the life cycle of eosinophil, and therefore its effect can be observed during the development of allergic reaction. This chemokine affects the release of progenitors of eosinophils from the

bone marrow, and, acting together with IL-5, increases the count of mature forms in peripheral blood, leading to peripheral and tissue eosinophilia [22]. The eosinophil count in the infiltrated organ is dependent on eotaxin concentrations in this site. Eotaxin is also responsible for retardation of the apoptosis of eosinophils, and the interaction of this cytokine with a receptor elicits their activation and degranulation [7]. Eotaxin can also play a role in the migration of mast cells [23] and basophils [24].

Our results confirm the connections between postexercise bronchoconstriction and airway eosinophilic inflammation. In this study, in the group of patients in whom, as a consequence of intensive exercise, bronchoconstriction occurred, we found a statistically significant higher baseline blood eosinophil count and serum ECP levels. In this group of patients, the tendency to higher levels of F_{ENO} and eotaxin before exercise in EBC was observed; however, the differences were not statistically significant.

In a previous study, Tahan et al. [25] did not observe any significant changes in systemic chemokine levels (in-

cluding eotaxin) after exercise in asthmatic children with or without EIB. However, the authors point out some limitations of the study. Firstly, the chemokine measurements were performed in the systemic circulation and may not reflect the changes that take place in the local environment in the lungs after exercise. Secondly, taking the results of previous studies into account, in which it was shown that systemic levels of chemokines may change after marathon running [26], it is possible that a much stronger response to exercise is necessary to induce changes in eotaxin levels.

Eotaxin can be produced by many human cells, such as the airway epithelium, endothelial cells, lymphocytes, macrophages and eosinophils, as well as airway smooth muscle cells [7]. Eotaxin is recognized as the strongest chemotactic factor for eosinophils, playing a key role in the development of eosinophil infiltration and allergic inflammation [10]. Studies on animal models have revealed the local overproduction of eotaxin [27]. In the lung cells of asthmatics, an increase in mRNA for eotaxin and for the CCR-3 receptor has been shown, and a positive correlation between this increase and the degree of bronchial hyperreactivity has been observed [28]. Increased expression of mRNA for eotaxin and an increase in concentrations of this chemokine in the bronchial epithelium, as well as higher levels of eotaxin in the bronchoalveolar lavage, have been found in asthmatic patients when compared with healthy volunteers [29].

In previous studies, the possibility of measuring eotaxin levels in EBC has been confirmed both in children [30] and in adults with asthma [31]. It is worth noting that Leung et al. [32], in a study using factor analyses, show that the eotaxins measured in both peripheral blood and EBC are assigned to separate factors. The authors also suggest that inflammatory markers such as eotaxin in peripheral blood and EBC should be considered as separate dimensions. Results of our previous studies indicate that eotaxin levels in EBC were higher in asthmatic patients with different degrees of asthma severity when compared with controls. In patients with unstable asthma, these values were significantly higher compared with subjects with stable disease and correlated with other inflammatory parameters such as F_{ENO} or serum ECP [33].

This is the first study in which changes in eotaxin concentrations in EBC, which could be a consequence of the increased expression of this chemokine in asthmatic airways following intensive exercise, have been assessed, and in which possible correlations with the results of other measurements commonly associated with eosinophil-

ic inflammation were investigated. Only in the group of patients with EIB could we observe a statistically significant increase in eotaxin levels in EBC collected between 30 min and 24 h after exercise, with a maximal increase after 6 h. The maximum increase in eotaxin was significantly correlated with the increase in F_{ENO} or serum ECP 24 h after exercise.

The results of our study indicate that, in asthmatic patients, postexercise bronchoconstriction leads to increased expression of eotaxin in airways. It cannot be completely excluded that eotaxin in EBC originates from circulating blood. However, it seems to be more probable that in these patients increased eotaxin production by many inflammatory and structural cells of the airways, such as airway epithelium, endothelial cells, lymphocytes, macrophages and eosinophils, as well as airway smooth muscle cells, occurs. In the authors' opinion this is a very important observation. On the one hand, the results of this study confirm the important role of eotaxin and eosinophils in the pathogenesis of EIB. On the other hand, the obtained results could indicate the role of many inflammatory or structural cells in the pathogenesis of EIB and induce follow-up studies concerning the effect of exercise on the respiratory system in asthmatic patients.

There are some limitations of the study. An important weakness is the small group of highly selected asthmatic patients included into the study. The results should be confirmed in widespread investigations. Another weak point could concern the diagnostic methods used in the study, because the analysis of EBC is still in the experimental phase. Many questions concerning the lack of standardization for both the collection and analysis of EBC, and the effect of many factors on concentrations of EBC markers, are still unresolved. EBC volume does not depend on lung function parameters. There is no evidence to show that changes in airway caliber cause any differences in mediator's release or dilution of EBC; however, this point is still under investigations. Cytokine concentrations in EBC are usually quantified by ELISA kits. Several different cytokines have been described to be present in EBC: IL-4, IL-6, IL-10, IL-1 β , TNF- α . However, the concentrations of several cytokines are around the lower limits of detection [16, 17, 34].

Conclusion

This study was performed to establish the possible role of eotaxin, the most specific and strongest factor which could affect the function of eosinophil, in the pathogen-

esis of EIB. We found that, as a result of intensive exercise leading to bronchoconstriction, an increase in the level of eotaxin in EBC occurs. Based on our findings, it is considered that increased expression of eotaxin in the airways, through the effect on recruitment and activity of eosinophils, may play an important role in the upregulation and maintenance of the airway inflammation observed after EIB in asthmatic patients.

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