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Severity of exercise-induced bronchoconstriction is related to airway eosinophilic inflammation in patients with asthma

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Severity of exercise-induced bronchoconstriction is related to airway eosinophilic inflammation in patients with asthma. T. Yoshikawa, S. Shoji, T. Fujii, H. Kanazawa, S. Kudoh, K. Hirata, J. Yoshikawa. ©ERS Journals Ltd 1998.

ABSTRACT: Exercise-induced bronchoconstriction (EIB) is widely prevalent in asthmatic patients. Eosinophilic airway inflammation is considered to be a major factor in the pathogenesis of asthma. However, the effects of eosinophilic airway inflammation on EIB have been elucidated insufficiently.

To examine the relationship between the severity of EIB and eosinophilic inflammation, sputum induction and exercise challenge were performed in 21 asthmatic patients.

Significantly higher percentages of eosinophils and levels of eosinophil cationic protein (ECP) were found in induced sputum in EIB-positive asthmatics (median (range), eosinophils: 23.5 (11.0–61.0)%; ECP: 1,475 (74.8–17,701) ng·mL^{-1}) than in EIB-negative asthmatics (eosinophils: 6.0 (1.0–41.5)% (p=0.006); ECP: 270.6 (10.8–7,700) ng·mL^{-1} (p=0.049)). There was a significant correlation between the severity of EIB and the sputum eosinophil percentage (r=0.59, p=0.009) and the level of ECP (r=0.47, p=0.037). The area under the curve of the forced expiratory volume in one second for 30 min after exercise correlated with the percentage of eosinophils (r=0.60, p=0.008) and the level of ECP (r=0.45, p=0.04). There was no correlation between airway responsiveness to methacholine on the one hand and EIB, sputum eosinophils or ECP on the other.

In conclusion, these results provide evidence that the severity of bronchoconstriction evoked by exercise is more closely related to eosinophilic airway inflammation than airway hyperresponsiveness to methacholine in asthmatic patients.
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Airway inflammation is considered to be a major factor in the pathogenesis of bronchial asthma. Sputum induction has recently been widely employed in studies of airway inflammation. The percentages of eosinophils and the levels of eosinophilic cationic protein (ECP) in induced sputum of asthmatic patients are higher than in normal controls [1, 2]. It has also been reported that inflammatory profiles are significantly related to the severity of various clinical manifestations [2–4]. The level of interleukin-5 (IL-5) in induced sputum has recently been found to be a useful marker for assessing eosinophilic inflammation in atopic and nonatopic asthmatic patients [5].

Most patients with asthma develop exercise-induced bronchoconstriction (EIB) when they perform exercise of sufficient duration and intensity. It is generally accepted that the pathogenesis of EIB is closely related to changes in temperature and moisture that develop within the tracheobronchial tree during the warming and humidification of large volumes of air, although it is not entirely clear how intra-airway thermal fluxes produce bronchial narrowing. Recent observations have indicated that examination of induced sputum permits the recognition of the presence, severity and type of airway inflammation. One study has reported that EIB severity correlates with ECP levels in the serum of asthmatic patients [6]. Accordingly, the presence of sputum eosinophilia may predict airway responses evoked by exercise challenge in asthmatic patients.

The purpose of this study was to determine whether the severity of EIB in asthmatic patients is affected by eosinophilic airway inflammation evaluated by the results of sputum induction.

Materials and methods

Subjects

Twenty-one asthmatic patients were recruited from outpatients at Osaka City University Hospital (eight males, 13 females) and were enrolled after written informed consent. Nine nonsmoking normal control subjects (eight males, one female) with normal lung function were recruited from the staff of the First Department of Internal Medicine of Osaka City University. The control subjects were aged 24–35 yrs and had no history of chronic lung disease. The asthmatic subjects met the American Thoracic Society's definition of asthma [7]. Characteristics of the asthmatic subjects are shown in table 1. They were aged 16–32 yrs and all were nonsmokers. All patients had stable asthma

Table 1. – Characteristics of asthmatic subjects

Subject No.	Sex	Age yrs	Atopy	FEV_1 % pred	PC_{20} $\mu g \cdot mL^{-1}$
1	M	25		83.3	2.94
2	F	19	+	84.6	3.33
3	F	27		84.6	3.38
4	M	25		73.7	2.80
5	F	32		120.7	3.40
6	M	28	+	78.4	3.89
7	F	27	+	83.0	3.10
8	F	29	+	105.3	3.35
9	M	19	+	81.4	2.80
10	M	31	+	99.2	3.20
11	F	24		92.7	3.99
12	F	28	+	98.9	3.16
13	F	24	+	89.5	3.31
14	F	21	+	71.4	2.80
15	F	16	+	93.7	2.88
16	F	26	+	84.5	3.40
17	M	16	+	108.7	3.45
18	M	22	+	90.3	3.91
19	F	27	+	108.7	2.98
20	M	24	+	85.3	2.80
21	F	23	+	83.8	3.20
Mean±SEM		24.4±1.0		90.6±2.7	3.2±0.1*

FEV_1: forced expiratory volume in one second; PC_{20}: provocation concentration of methacholine causing a 20% fall in FEV_1; M: male; F: female. *: Geometric mean.

with a forced expiratory volume in one second (FEV_1) >70% of the predicted normal on all study days. None of the patients had a history of asthma exacerbation or respiratory infection within the four-week period preceding entry into the study. Sixteen patients were atopic, and five were nonatopic. All atopic patients had evidence of atopy by skin-prick testing for at least one of five common aeroallergens (house dust mites, cat dander, dog dander, grass pollen and *Aspergillus fumigatus*). No patients were receiving steroids and all patients were taking oral theophylline (400 $mg \cdot day^{-1}$) and inhaled β-agonists. Medications were not changed for a one-month period preceding the study and they were withdrawn for at least 12 h before the methacholine challenge test and the exercise test.

Protocol

Sputum induction was performed following pulmonary function testing and methacholine challenge testing at approximately 13:00 h on the same visit to the hospital. Three days after the test, the exercise test was performed at approximately 13:00 h to eliminate the effects of diurnal variation. Exercise challenge testing was performed on an electrically driven treadmill (Q55xt, Series 90; Quinton Instrument Co., Seattle, WA, USA) for 6 min with a fixed workload adjusted to increase the cardiac frequency to 90% of the maximum predicted for the age of the patient [8]. All subjects breathed unconditioned room air (temperature 22–25°C) and were coached to overcome hyperventilation during testing. A single-lead electrocardiogram (ECG) and pulse oximetry (502-US; CSI, Tokyo, Japan) were monito-red continuously. The criteria for exclusion were the pre-sence of coronary artery disease or cardiac arrhythmias. A spirometer (Chestac-25F; Chest Co., Tokyo, Japan) was used to obtain spirometric measurements before and after exercise challenge. The higher of two measurements of FEV_1 obtained before exercise challenge was taken as the baseline value. Single measurements of FEV_1 were obtai-ned 1, 3, 5, 10, 15, 20, 25 and 30 min after completion of the exercise challenge. The response to exercise challenge was taken to be the maximum percentage fall in FEV_1 after exercise:

$$\% \text{ fall in } FEV_1 = ((FEV_1 \text{ (baseline)} - FEV_1 \text{ (after)}) / (FEV_1 \text{ (baseline)})) \times 100$$

Those patients whose maximum decrease in FEV_1 was >20% were considered to be EIB-positive asthmatics. The maximum fall in FEV_1 was used as a parameter of the EIB severity.

In addition to the EIB severity, the bronchoconstrictor response was assessed as the area under the curve of the percentage fall in FEV_1 plotted against time for 30 min ($AUC_{0–30}$). The $AUC_{0–30}$ was calculated using trapezoidal integration as used by MAKKER *et al.* [9].

Sputum induction and processing

Spirometry was performed with a Chestac-25F system before inhalation of 200 μg salbutamol through a metered-dose inhaler. Subjects were instructed to wash their mouths thoroughly with water. They then inhaled 3% saline solution at room temperature, nebulized by an ultrasonic nebulizer (NE-U12; Omron Co., Tokyo, Japan) at maximum output. Subjects were encouraged to cough deeply after 5 min and at 3-min intervals thereafter. Sputum was collected in a polypropylene container and one portion of the specimen was collected separately for total cell counts and slide preparation. After sputum induction, spirometry was repeated. When FEV_1 had fallen, the subject was required to wait until it had returned to baseline. The sputum samples were kept at 4°C for no more than 2 h before further processing. Sputum was processed using a modified version of the method described by HANSEL *et al.* [10]. The volume of each sample was recorded. The portion of the sample for cell counting was diluted with phosphate-buffered saline (PBS) containing 10 $mmol \cdot L^{-1}$ dithiothreitol (Sigma Chemical Co., St Louis, MO, USA) and gently vortexed at room temperature. The portions for cell counting were then centrifuged at 400×g for 10 min and the cell pellet was resuspended in PBS. Total cell counts were performed with a haemocytometer and slides were prepared with the use of a cytospin (Cytospin 3; Shandon, Tokyo, Japan) and stained with May-Grünwald-Giemsa stain for differential cell counts.

The supernatant was stored at -70°C for subsequent assay of ECP concentration. ECP concentration was measured using a radioimmunoassay kit (Pharmacia Diagnostics, Uppsala, Sweden).

Methacholine challenge test

Methacholine inhalation challenge was performed as described by MAKINO *et al.* [11]. Methacholine chloride (Sigma) was dissolved in physiological saline and methacholine solutions at cumulative concentrations (313–10,000 $\mu g \cdot mL^{-1}$) were prepared. Patients inhaled saline or

methacholine solution from a Devilbiss 646 nebulizer (Devilbiss Co., Somerset, PA, USA) operated with compressed air at 5 L·min^{-1}. They inhaled saline or methacholine solution for 2 min by tidal breathing while wearing a noseclip. Spirometry was performed with a Chestac-25F system immediately after inhalation and FEV_1 was measured. After a fall in FEV_1 of <10% following saline inhalation, the methacholine challenge was started. Subjects successively inhaled methacholine solutions of cumulative concentrations and the test was stopped when a fall in FEV_1 of Š20% below the baseline occurred. The measured values were plotted on a semilogarithmic graph and the provocative concentration of methacholine causing a 20% fall in the FEV_1 (PC_{20}) was calculated in noncumulative units by linear interpolation between the last two points on the graph.

Statistical analysis

Data were analysed nonparametrically. Group data were expressed as median with the range given in parentheses because a normal distribution of these variables could not be demonstrated. When multiple comparisons were made between groups, significant intergroup variability was first established using the Kruskal-Wallis test. The Mann-Whitney U-test was then used for intergroup comparisons. The significance of correlations was evaluated by determining Spearman's rank correlation coefficients. A p-value <0.05 was considered significant.

Results

The 21 asthmatic subjects and nine normal control subjects produced adequate sputum specimens. All subjects tolerated the sputum induction procedure well. There was no significant decrease in FEV_1 after sputum induction in any subject and no subject experienced shortness of breath or chest tightness. There were no significant differences in the temperature and humidity during the exercise test between EIB-positive and EIB-negative asthmatics. Figure 1 shows the percentages of eosinophils and the ECP concentrations in induced sputum. The percentage of eosinophils was significantly higher in EIB-positive asthmatic subjects (median (range), 23.5 (11.0–61.0)%) than in EIB-negative asthmatic patients (6.0 (1.0–41.5)%) (p=0.006) and normal control subjects (0.0 (0.0–1.4)%) (p=0.0002). ECP concentrations were also significantly higher in EIB-positive asthmatic patients (1,475 (74.8–17,701) ng·mL^{-1}) than in EIB-negative asthmatic patients (270.6 (10.8–7,700) ng·mL^{-1}) (p=0.049) and normal control subjects (69.6 (0.0–270) ng·mL^{-1}) (p=0.001).

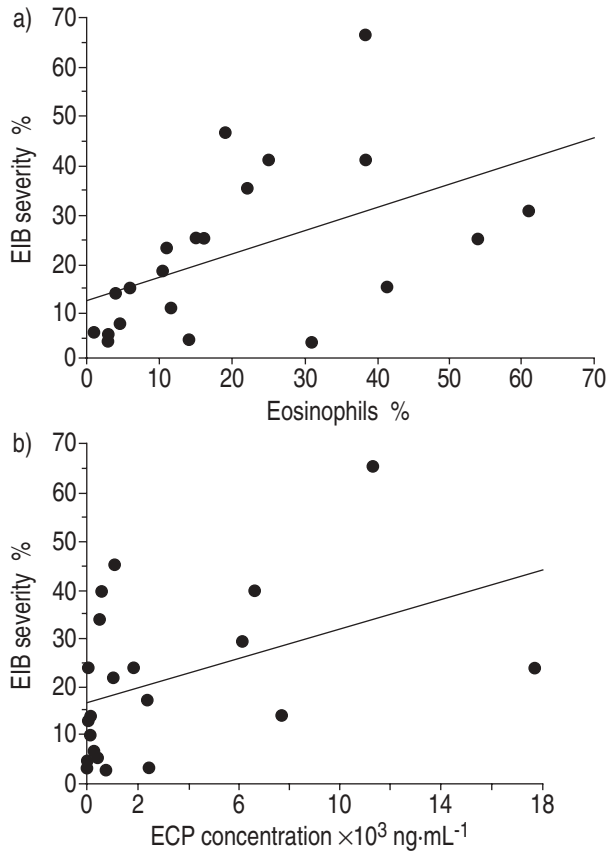

Fig. 2. – Correlation between indices of eosinophilic inflammation in induced sputum and severity of exercise-induced bronchoconstriction (EIB). a) There was a significant correlation between severity of EIB and sputum eosinophil percentage (r=0.59, p=0.009). b) EIB severity was also positively correlated with eosinophil cationic protein (ECP) concentrations (r=0.47, p=0.037).

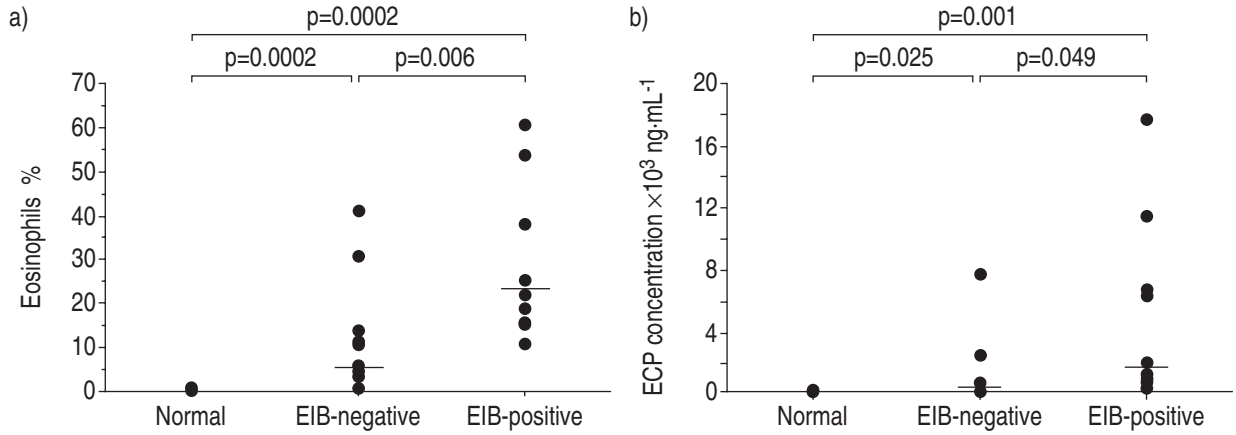

Fig. 1. – Comparison of indices of eosinophilic inflammation in induced sputum between controls and asthmatic patients with or without exercise-induced bronchoconstriction (EIB). Horizontal bars indicate the median values. a) The percentage of eosinophils was significantly higher in EIB-positive asthmatics than in EIB-negative asthmatic patients and normal control subjects. b) Eosinophil cationic protein (ECP) concentrations were also significantly higher in EIB-positive asthmatic patients than in EIB-negative asthmatic patients and normal control subjects.

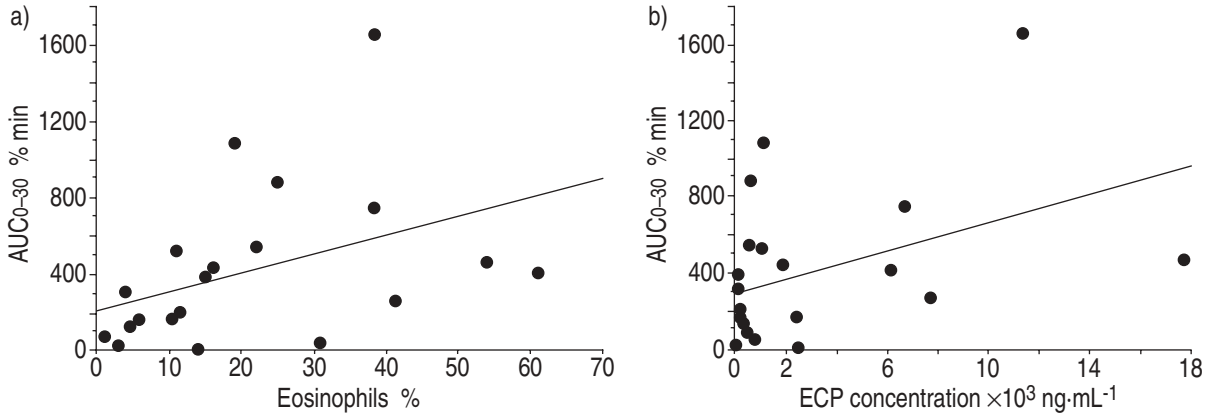

Fig. 3. – Correlation between indices of eosinophilic inflammation in induced sputum and the bronchoconstrictor response when analysed as the area under the curve (AUC) of the percentage change in the forced expiratory volume in one second from pre-exercise baseline against time over 30 min ($AUC_{0–30}$). a) There was a significant correlation between the $AUC_{0–30}$ and the sputum eosinophil percentage (r=0.60, p= 0.008). b) The $AUC_{0–30}$ also correlated positively with the eosinophil cationic protein (ECP) concentrations (r=0.45, p=0.043).

There was a significant correlation between the severity of EIB and the sputum eosinophil percentage (r=0.59, p= 0.009) (fig. 2a). EIB severity was also positively correlated with ECP concentration (r=0.47, p=0.037) (fig. 2b).

There was a significant correlation between the sputum eosinophil percentage and the bronchoconstrictor response when analysed as the $AUC_{0–30}$ (r=0.60, p=0.008) (fig. 3a). There was also a positive correlation between the levels of ECP in induced sputum and the $AUC_{0–30}$ (r=0.45, p= 0.043) (fig. 3b).

However, there was no significant correlation between EIB severity and methacholine PC_{20} (r=-0.23, p=0.314) and the $AUC_{0–30}$ did not significantly correlate with the methacholine PC_{20} (r=-0.28, p=0.203).

The methacholine PC_{20} did not correlate significantly with the sputum eosinophil percentage (r=-0.34, p=0.127) (fig. 4a) or the ECP concentration (r=-0.13, p=0.550) (fig. 4b).

Discussion

In this study, higher percentages of eosinophils and levels of ECP were found in induced sputum from EIB-positive asthmatic patients than from EIB-negative asthmatic subjects and normal controls. There was a significant correlation between the degree of eosinophilic infiltration and activation in asthmatic airways and bronchoconstrictor responses, such as the severity of EIB and the AUC of the percentage fall in FEV_1 from the pre-exercise baseline. However, there was no significant correlation between the severity of EIB and airway hyperresponsiveness to methacholine, and no correlation between airway hyperresponsiveness to methacholine and the degree of eosinophilic inflammation in asthmatics. These findings suggest that the degree of eosinophilic inflammation affects the airway obstruction evoked by exercise challenge in asthmatic patients and that the airway eosinophilic inflammation exerts an influence on airway reactivity to indirect challenge, such as exercise.

Despite the wide prevalence and clinical significance of EIB, the mechanism has yet to be described. According to one hypothesis, EIB is caused by an inflammatory mediator mechanism [12]. Hyperpnoea associated with exercise leads to increased airway water and heat loss, in addition to hyperosmolarity of the fluid interface of the mucosal surface in airways, resulting in mast cell degranulation. Since mast cell-derived mediators, such as histamine and

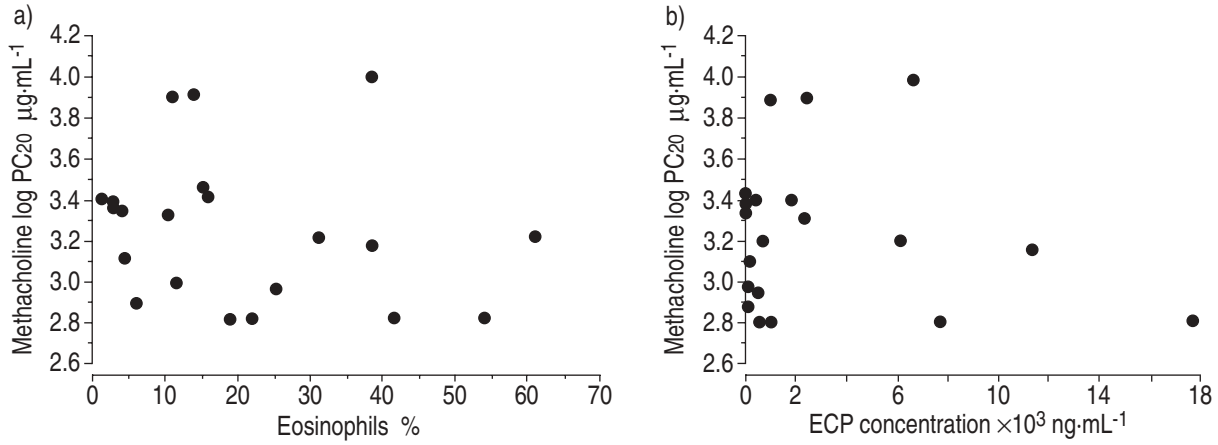

Fig. 4. – a) Correlation between indices of eosinophilic inflammation in induced sputum and the provocative concentration of methacholine causing a 20% fall in the forced expiratory volume in one second (PC_{20}). Methacholine PC_{20} did not correlate significantly with the sputum eosinophil percentage (r=-0.34, p=0.127). b) Methacholine PC_{20} did not correlate significantly with the eosinophil cationic protein (ECP) concentration (r=-0.13, p= 0.550).

leukotrienes, may cause airway smooth muscle contraction and microvascular leakage, it is possible that the airway narrowing following exercise in asthmatics is due to these mediators. The finding that agents which inhibit the release of histamine [13] and leukotrienes [9, 14–16] from mast cells attenuate EIB supports this hypothesis. However, the effects of eosinophilic airway inflammation on EIB have not been elucidated. It has been reported that serum levels of ECP before exercise correlate significantly with the maximal fall in peak expiratory flow (PEF) after exercise [6] and speculated that EIB may reflect a state of eosinophilic inflammation of the lung. This study found for the first time that the degree of eosinophilic inflammation was related to the severity of EIB directly in asthmatic airways. Eosinophils in asthmatic airways also release leukotrienes, which cause contraction of airway smooth muscle and microvascular leakage. It has previously been shown that the degree of eosinophilic inflammation reflects the amount of toxic radicals produced in the airway which damage airway epithelium [17]. Accordingly, it is possible that bronchoconstriction, stimulated by various types of mediators from mast cells, is markedly potentiated in the presence of eosinophilic inflammation.

No correlation was demonstrated between airway hyperreactivity to methacholine and EIB severity and there was no significant correlation between airway hyperreactivity to methacholine and airway eosinophilic inflammation in asthmatics. In some studies, it has been reported that there are weakly significant correlations between airway responsiveness and eosinophilic inflammation in asthmatic airways [18, 19]. In contrast, many studies have demonstrated no correlation between eosinophilic airway inflammation and airway hyperresponsiveness to direct [20, 21] or indirect [22] challenge in patients with asthma. Direct challenges, such as methacholine and histamine, are more likely to reflect smooth muscle responsiveness and/or airway remodelling than acute inflammation. A more recent cross-sectional study demonstrated, in a large sample of asthmatic patients, that chronic airway hyperresponsiveness to methacholine is not associated with the number of eosinophils in the airway lumen and mucosa, as quantified by induced sputum or bronchoalveolar lavage and bronchial biopsy [23]. The authors speculated that factors other than acute airway inflammation (*e.g.* airway wall remodelling or autonomic dysfunction) may be responsible for most of the individual variability in airway responsiveness to methacholine in asthma [24]. Indirect challenges, such as exercise, may also reflect aspects other than smooth muscle responsiveness and airway geometry. The present study demonstrates that one of these aspects is eosinophilic airway inflammation.

It is reasonable to assume that there is a difference in the mechanism of airway reactivity induced by various stimuli. The heterogeneity of airway hyperresponsiveness has been clearly defined [25] and airway hyperresponsiveness is more complex than was once thought. Even methacholine and histamine, which are both directly reactive to smooth muscle, show differences in airway responsiveness. The present study demonstrates that there is no significant correlation between airway hyperreactivity to exercise and to methacholine, and that the hyperreactivity to exercise is more closely related to the magnitude of eosinophilic inflammation than to airway hyperresponsiveness to methacholine. These findings indicate different manners of contribution of airway eosinophilic inflammation to bronchial response to each stimulus. Further studies will be required to determine the reason for the absence of a relationship between eosinophilic airway inflammation and airway hyperresponsiveness to methacholine.

In conclusion, the results of this study provide evidence that the severity of bronchoconstriction evoked by exercise is more closely related to eosinophilic airway inflammation than to airway hyperresponsiveness to methacholine in asthmatic patients. The use of induced sputum to assess eosinophilic airway inflammation will lead to a better understanding of the relationship between airway inflammation and disorders of airway function evoked by exercise.

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