**ORIGINAL ARTICLE**

# Sputum Eosinophilia, Airway Hyperresponsiveness and Airway Narrowing in Young Adults with Former Asthma

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**ABSTRACT**

**Background:** 30−80% of outgrown asthma subjects develop symptoms again later in life. We investigated inflammation and function of lower airway in adolescents with former asthma.
**Methods:** 326 never-smoking young adults (mean age 24.0 years) were interviewed with special emphasis on history of asthma. Diagnosis of asthma was based on GINA guidelines. Former asthma subjects consisted of ones with a history of physician-diagnosed childhood asthma, who had been free of asthma symptoms without the use of medication for at least 10 years prior to the study. Provocative concentration of methacholine causing a 20% fall in forced expiratory volume in 1 second ($FEV_1$) ($PC_{20}$) and eosinophil percentage in induced sputum were measured.
**Results:** 31 subjects were former asthma subjects (FBA), 11 subjects were current asthma subjects (CBA) and 284 subjects had no history of asthma (non-BA). $PC_{20}$ and $FEV_1/FVC$ ratio were significantly lower in the FBA group than in the non-BA group ($P < 0.01$). Maximal mid-expiratory flow (MMF) was significantly lower in the FBA group than in the non-BA group ($P < 0.05$). Sputum eosinophil percentage was significantly increased in the FBA group compared with the non-BA group ($P < 0.01$). $PC_{20}$ was significantly lower in the CBA group than in the FBA and non-BA groups ($P < 0.01$). $FEV_1$, $FEV_1/FVC$ ratio and MMF were significantly lower in the CBA group than in the FBA group ($P < 0.05$, $P < 0.05$ and $P < 0.05$, respectively) and the non-BA group ($P < 0.01$, $P < 0.01$ and $P < 0.05$, respectively). Sputum eosinophils were significantly higher in the CBA group than in the FBA and non-BA groups ($P < 0.01$).
**Conclusions:** This study shows that subjects with long-term outgrown asthma continue to have airway eosinophilic inflammation, airway hyperresponsiveness and airway narrowing.

**KEY WORDS**
airway hyperresponsiveness, airway inflammation, airway narrowing, former asthma, remission

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## INTRODUCTION

Asthma is a clinical entity characterized by a combination of three features: airway obstruction with spontaneous and/or pharmacological reversibility, in-

creased bronchial responsiveness, and airway inflammation. Airway inflammation has been considered the primary event leading to airway obstruction and hyperresponsiveness.[1]

Epidemiologic studies demonstrated that a consid-

erable proportion of children with asthma in remission develop symptoms again later in life.[2] It has also been shown that eosinophilic airway inflammation is present in outgrown asthma children[3] and adult atopic asthma subjects[4] in clinical remission. Furthermore, most adults with former asthma considered in remission showed a persistent increase in airway responsiveness to methacholine[5,6] and airway remodeling was present during clinical remission of atopic asthma subjects.[4] Some authors have suggested that ongoing airway inflammation is the principal cause of progressive airway abnormalities[7] and, consequently, high relapse rate.[3,4]

To our knowledge, in most previous studies except one,[8] clinical remission of asthma was defined as reported complete absence of asthma symptoms in subjects not taking any asthma medication for only a few years. We examined the inflammation and function of the lower airways in young adults who had been free of symptoms and had not taken any asthma medications for at least 10 years before the study.

## METHODS

### SUBJECTS
This study was performed between 2002 and 2005. A total of 326 never-smoking students of Kanazawa University School of Medicine, aged 21 to 34 years (mean age 24.0 years), were interviewed with special emphasis on history of asthma. Diagnosis of asthma was based on GINA guidelines.[9] Current asthma subjects consisted of students with asthma symptoms within the previous year, who had taken no asthma medications for at least 4 weeks before or during the study period. Former asthma subjects consisted of students with history of physician-diagnosed childhood asthma, who had been free of asthma symptoms and had not taken any asthma medication for at least 10 years prior to the study. No subject had a history of respiratory infections for at least 4 weeks before or during the study period. The study was approved by the Ethics Committee of Kanazawa University, and informed consent was obtained from all subjects after the purpose of the test had been explained.

### STUDY DESIGN
Pulmonary function, the methacholine concentration producing a 20% fall in forced expiratory volume in 1 second ($FEV_1$) ($PC_{20}$) and eosinophil percentage in hypertonic saline-induced sputum were measured within 3 weeks in random order. The baseline of the pulmonary function test was measured after physiological saline inhalation during the methacholine provocation test. The predicted value of FVC, $FEV_1$ and MMF were calculated according to the Japanese Respiratory Society guidelines.[10]

## MEASUREMENT OF BRONCHIAL RESPONSIVENESS
The methacholine provocation test was done according to a previously published method.[11] Methacholine chloride was dissolved in physiological saline solution to make concentrations of 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40, 80 and 160 mg/ml. Saline and each methacholine solution were inhaled from a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, PA, USA) operated by compressed air at 5 L/min. The nebulizer output was 0.14 ml/min. Saline was inhaled first for 2 minutes and partial and full flow-volume curves were measured on a dry wedge spirometer (Chestac 11, Chest Co., Ltd., Tokyo, Japan). If the change in $FEV_1$ from the baseline value was 10% or less, inhalation of methacholine was started, and if the saline solution caused a change in $FEV_1$ >10%, the test was stopped or postponed. Methacholine was inhaled for 2 minutes by tidal mouth breathing with the subjects wearing a nose clip, and this was followed immediately by three measurements of partial and full flow-volume curves at 1-min intervals; the curve with the largest FVC was retained for analysis. Increasing concentrations of methacholine were inhaled until a decrease of 20% or more in $FEV_1$ occurred. $PC_{20}$ was calculated. When a 20% or greater fall in $FEV_1$ was not obtained by the final concentration of methacholine (160 mg/ml), the $PC_{20}$ value was assumed to be 320 mg/ml for statistical analysis.

## SPUTUM INDUCTION
Induction of sputum was done according to a method described previously by us.[12] Hypertonic saline (5%) was nebulized with a compressor nebulizer (Omron, Mie, Japan) for 60 minutes. When subjects felt sticking in the throat, they were asked to try to cough sputum into a Petri dish. By this procedure most subjects successfully produced sputum. Subjects who had not been able to bring up material at the end of a 60-minute period were released. The specimens were considered adequate if alveolar macrophages were detected and the percentage of squamous cells was less than 10% among nucleated cells. Sputum was transferred to slides in small amounts and finely distributed over two microscopic slides and smeared. Each smear was air-dried and stained by the May-Grunwald-Giemsa method. Differential cell counts were performed by an observer blinded to the clinical characteristics of the subjects.

## DATA ANALYSIS
Data except for $PC_{20}$ were shown as means ± standard deviation (SD). $PC_{20}$ values were expressed as geometric means with the geometric standard error of the mean (GSEM) expressed as a factor. Differences in spirometric values between groups were analyzed by parametric one-way analysis of variance

**Table 1** Characteristics of subjects

|  | Non-BA | FBA | CBA |
|---|---|---|---|
| Number of subjects | 284 | 31 | 11 |
| Age (years) | 24.2±2.2 | 23.3±1.1** | 23.9±1.5 |
| Sex (M/F) | 204/80 | 25/6 | 6/5 |
| Height (cm) | 168±8.3 | 171±7.2 | 170±9.8 |
| Body weight (kg) | 60.0±9.7 | 65.0±9.3** | 63.7±11 |
| Body mass index | 21.0±2.3 | 22.1±2.6* | 22.1±3.3 |

Non-BA: non-asthma subjects, FBA: former asthma subjects, CBA: current asthma subjects. Data are expressed as means ± SD. *$P < 0.01$ *vs.* non-BA, **$P < 0.05$ *vs.* non-BA.

**Table 2** Pulmonary function and methacholine bronchial responsiveness (PC$_{20}$)

|  | Non-BA | FBA | CBA |
|---|---|---|---|
| FVC (% predicted) | 112±13.4 | 117±21.0 | 114±14.1 |
| FEV$_1$ (% predicted) | 101±11.4 | 99.6±14.9 | 89.9±11.5*## |
| FEV$_1$/FVC (%) | 88.4±6.45 | 83.6±5.92* | 78.8±9.18*## |
| MMF (% predicted) | 99.7±20.8 | 90.9±24.4** | 74.4±17.9*## |
| PC$_{20}$ (mg/ml) | 41.7 (1.10) | 8.71 (1.39)* | 0.69 (1.52)*# |

Non-BA: non-asthma subjects, FBA: former asthma subjects, CBA: current asthma subjects. Abbreviations: FVC, forced vital capacity; FEV$_1$, forced expiratory volume in 1 second; MMF, maximum mid-expiratory flow; PC$_{20}$, provocative concentration of methacholine causing a 20% fall in FEV$_1$. Data are expressed as means (±SD) except for PC$_{20}$, which is expressed as the geometric mean value with geometric standard error of the mean in parentheses. *$P < 0.01$ *vs.* non-BA, #$P < 0.01$ *vs.* FBA, **$P < 0.05$ *vs.* non-BA, ##$P < 0.05$ *vs.* FBA.

(ANOVA). Differences in PC$_{20}$ between groups were analyzed by ANOVA using logarithmically transformed values. Differences in sputum eosinophils were analyzed by Mann-Whitney's *U* test. $X^2$ tests were used to make between-group comparison on the prevalence of successful sputum induction. Correlations between variables were analyzed using the Spearman's correlation coefficient. Gender difference was determined by $X^2$ tests. A *P*-value of < 0.05 was taken to indicate significant differences.

## RESULTS

### SUBJECT CHARACTERISTICS
Based on the interview, 326 non-smoking subjects were divided into three groups; current asthma subjects (CBA) (*n* = 11), former asthma subjects (FBA) (*n* = 31), non-asthma subjects (non-BA) (*n* = 284). A summary of characteristics of subjects is given in Table 1. FBA subjects were significantly lower aged than non-BA subjects (*P* < 0.05). FBA subjects had a significantly higher body weight (*P* < 0.01) and higher BMI than non-BA subjects (*P* < 0.05).

### PULMONARY FUNCTION TESTS
As shown in Table 2, no difference in FVC was detectable between the three groups. CBA subjects had a significantly lower FEV$_1$ compared with FBA (*P* < 0.05) and non-BA subjects (*P* < 0.01). FBA subjects had a significantly lower FEV$_1$/FVC ratio (*P* < 0.01) and lower maximum mid-expiratory flow (MMF) (*P* < 0.05) compared with non-BA subjects. CBA subjects had a significantly lower FEV$_1$/FVC ratio compared with FBA (*P* < 0.05) and non-BA subjects (*P* < 0.01). CBA subjects had a significantly lower MMF compared with FBA (*P* < 0.05) and non-BA subjects (*P* < 0.01).

### BRONCHIAL RESPONSIVENESS TO METHACHOLINE
FBA subjects had a significantly lower PC$_{20}$ compared with non-BA subjects (*P* < 0.01). CBA subjects had a significantly lower PC$_{20}$ compared with both FBA and non-BA subjects (*P* < 0.01) (Table 2). One subject in the CBA group had normal bronchial responsiveness (PC$_{20}$ 18.8 mg/mL). 11 subjects in the FBA group had normal bronchial responsiveness (PC$_{20}$: range 12.2−320 mg/mL). 224 subjects in the non-BA group had normal bronchial responsiveness (PC$_{20}$ >10 mg/mL).

### INDUCED SPUTUM EOSINOPHILS
Sputum eosinophils were significantly greater in FBA subjects (median: 1.0%, range: 0.0−20.5%) than in non-BA subjects (median: 0.0%, range: 0.0−12.3%) (*P* < 0.01). The sputum eosinophil percentage of CBA subjects (median: 4.45%, range: 0.0−31.3%) was significantly higher than that of non-BA subjects (*P* < 0.01) but not than that of FBA subjects (Fig. 1). There was no significant difference in prevalence of successful sputum induction among groups.

8 of 31 FBA subjects (25.8%) had whole normal pulmonary function (%FVC >80%, FEV$_1$/FVC ratio >70%), normal bronchial responsiveness to methacholine (PC$_{20}$ >10 mg/mL) and normal sputum eosinophils (sputum eosinophil percentage <2.5%). 11 CBA subjects had an abnormality either in pulmonary function, bronchial responsiveness and/or sputum eosinophils.

### RELATIONSHIP BETWEEN SPUTUM EOSINOPHILS AND BRONCHIAL RESPONSIVENESS
In 326 subjects, we examined the correlation between sputum eosinophil percentage and log PC$_{20}$. The correlation between log PC$_{20}$ and percentage of sputum eosinophil was significant (*r* = −0.310, *P* < 0.01) (data
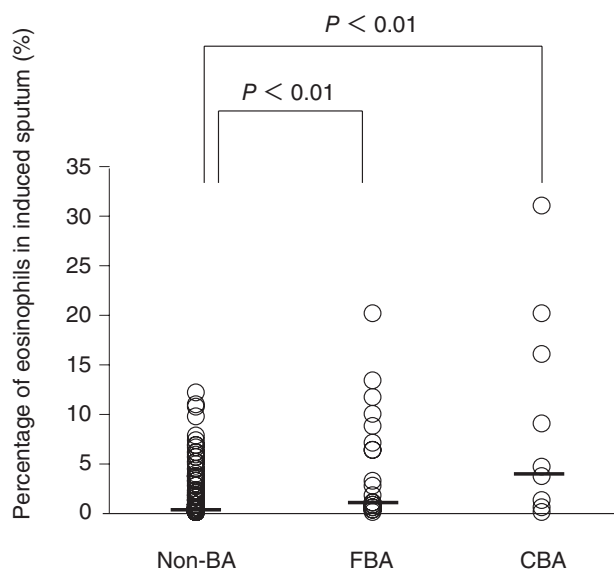
**Fig. 1** Differences in eosinophil percentage in hypertonic saline-induced sputum among former asthma subjects, current asthma subjects and non-asthma subjects (Mann-Whitney $U$ test) Horizontal bars indicate median values. The median values of percentages of eosinophils were 0.0% in non-BA, 1.0% in FBA and 4.45% in CBA subjects.
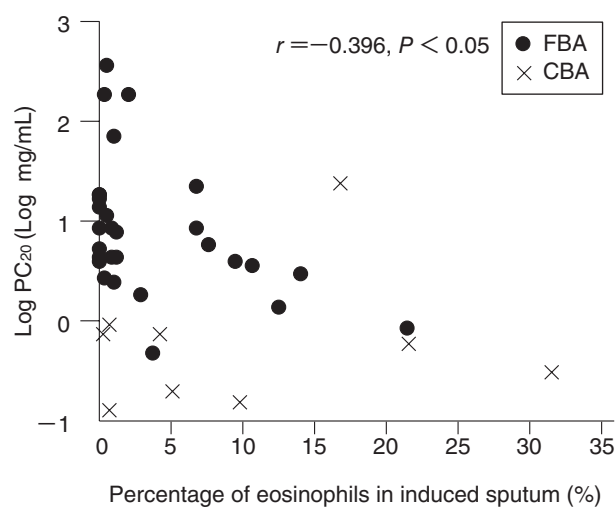


**Fig. 2A** Correlation between eosinophil percentage in hypertonic saline-induced sputum and log $PC_{20}$ in former and current asthma subjects. $r$; Spearman's rank correlation coefficient.
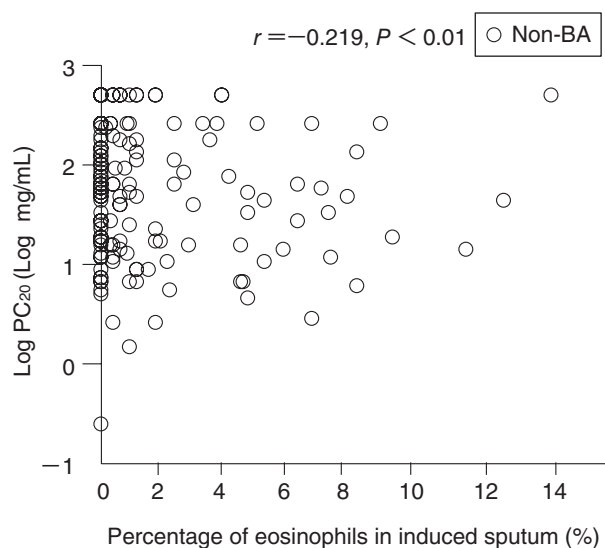


**Fig. 2B** Correlation between eosinophil percentage in hypertonic saline-induced sputum and log $PC_{20}$ in non-asthma subjects. $r$; Spearman's rank correlation coefficient.

not shown). In 42 CBA and FBA subjects, there was significant correlation between log $PC_{20}$ and percentage of sputum eosinophils ($r = -0.396$, $P < 0.05$) (Fig. 2A). In 284 non-BA subjects, there was also significant correlation between log $PC_{20}$ and percentage of sputum eosinophils ($r = -0.219$, $P < 0.01$) (Fig. 2B). There was no correlation between log $PC_{20}$ and per-

centage of sputum eosinophils in 11 CBA subjects (data not shown).

## DISCUSSION

Our study showed that young adults with long-term remission of childhood asthma continued to have airway eosinophilic inflammation and bronchial hyperresponsiveness, and the former asthma subjects had narrowing of lower airways, including small airways. It has been demonstrated that most subjects considered to be in asthma remission have a persistent increase in airway responsiveness,[5,6] airway obstruction,[5] and eosinophilia in bronchoalveolar lavage fluid,[3] bronchial biopsy specimens[4] and induced sputum.[8] To our knowledge, in most previous studies, clinical remission of asthma was defined as reported complete absence of asthma symptoms in subjects not taking any asthma medications for only 1 to 3 years. On the other hand, Obase *et al*. defined clinical remission of childhood asthma as free of asthma symptoms for at least 10 years.[8] Remission of asthma remains unsolved[13] and the duration of clinical remission of asthma is variable. In previous studies, it may be possible that because durations of childhood asthma remission were shorter, subjects with outgrown asthma had eosinophilic airway inflammation and bronchial hyperresponsiveness. Our results suggest that the longer duration of remission of childhood asthma (*i.e.* 10 years) does not result in vanishing of lower airway eosinophilic inflammation and airway hyperresponsiveness. This result may agree with a previous report showing that there was no correlation between airway responsiveness to methacholine and duration of asthma remission.[5]

The Japanese Pediatric Guidelines for The Treatment and Management of Asthma 2005 define functional remission of asthma as complete absence of symptoms and no use of medication in the previous 5 years, and pulmonary function test and bronchial responsiveness are normal. These guidelines also define clinical remission of asthma as complete absence of symptoms and no use of medication in the previous 5 years.[14] In our study, 25.8% of subjects with clinical remission of asthma, who had been free of asthma symptoms and had not taken any asthma medication, were considered to be in functional remission. We could not investigate childhood factors associated with adulthood asthma remission (*i.e.* pulmonary functions in childhood). Vonk *et al.* showed that remission was associated with a higher lung function level in childhood and a higher subsequent increase in FEV1.[15] Furthermore, we could not follow up subjects with clinical remission of asthma in this study. Ongoing airway inflammation and bronchial hyperresponsiveness may be risk factors for future recurrence in subjects with clinical remission of asthma.[3,16]

In our study, several non-BA subjects had bronchial hyperresponsiveness and/or sputum eosinophilia. 60 (18.4% of 326 subjects) non-BA subjects had bronchial hyperresponsiveness in this study. It was shown that 34% of Japanese university students with atopy, without asthma, had bronchial hyperresponsiveness to methacholine (PC20 <8 mg/mL).[17] The prevalence of bronchial hyperresponsiveness in the general population varies from 4 to 35%.[18] Asymptomatic bronchial hyperresponsiveness in non-asthma subjects is considered to be a risk factor for developing asthma in future.[19-25] Though we did not investigate neither personal nor familial allergic disease, these two factors may contribute to the relationship between bronchial hyperresponsiveness and development of asthma.[21-25] Several studies have analyzed induced sputum[17,26-28] and bronchial biopsy[23] to investigate airway inflammation in non-asthma subjects with bronchial hyperresponsiveness. Airway eosinophils in these subjects were higher than in non-asthma subjects without bronchial hyperresponsiveness[23,27] and were not different from those in asthma subjects.[26] The mechanism by which the asymptomatic phase of bronchial hyperresponsiveness becomes overt symptomatic asthma remains unclear, but the associated increased inflammatory cell infiltrate may play an important role in this phenomenon.

In our study, former asthma subjects had significantly higher body weight and higher BMI than those of non-asthma subjects. Cross-sectional epidemiologic studies have indicated a modest association between obesity and prevalence of asthma.[29-32] A meta-analysis of prospective epidemiologic studies showed that asthma incidence increases by 50% in overweight and obese individuals.[33] This study also demonstrated a dose-response relationship between BMI and asthma, suggesting that asthma risk increases further as body weight increases.[33] The present study may support the association between asthma and obesity.

MMF was used to assess small airway function.[34] Several studies showed that subjects with former asthma had abnormal small airway functions.[5,35-38] But, to the best of our knowledge, in those reports, the periods of asthma remission were defined only from 1 month to 2 years. Furthermore, in some reports,[5,35,38] subjects with a smoking history were not excluded. In our study, subjects with asthma remission having no smoking history showed decreased MMF compared with control subjects. Hamid *et al.* demonstrated that similar but more severe inflammatory and structural changes occurred in the small airways of asthma subjects.[39,40] Wagner *et al.* showed that, in asthma subjects, the peripheral airway resistance was increased up to sevenfold compared with that of control subjects, and these measurements correlated with responsiveness to methacholine.[41] Small airways are considered to be predominant sites of airflow obstruction in asthma subjects.[42] There is no evidence suggesting that small airway remodeling is present in subjects with asthma remission. Furthermore, it is unclear whether abnormality of small airways in subjects with asthma remission could be a target of medication as in asthma subjects. Koh *et al.* demonstrated that 9 months of inhaled budesonide could not cause a significant improvement in bronchial hyperresponsiveness to methacholine and FEV1 in adolescents with long-term asthma remission.[43] Van den Toorn *et al.* found that 3 months of treatment with a salmeterol/fluticasone propionate combination could reduce airway hyperresponsiveness, nitric oxide concentration in exhaled air and tryptase density in the airway mucosa, but not major basic protein, FEV1, FEV1 reversibility or quality of life of subjects with remission of asthma.[44] De Kluijver *et al.* reported that repeated low-dose allergen exposure in asymptomatic asthma subjects could lead to airway inflammation without worsening of symptoms, which could be prevented by inhaled budesonide treatment.[45] Whether prolonged anti-inflammatory treatment alters the long-term prognosis in subjects with remission of asthma remains to be determined by future studies.

In conclusion, we found that former asthma subjects, who had been free of symptoms and medications of childhood asthma for at least 10 years, had eosinophilic airway inflammation, bronchial hyperresponsiveness and airway narrowing. Long-term studies are needed to elucidate the necessity of intervention and its efficacy and safety on airway inflammation, airway hyperresponsiveness and airway narrowing in subjects with remission of asthma.

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