

Original article

Bronchial hyperresponsiveness and airway inflammation in adolescents with *asymptomatic* childhood asthma

Background: About 70% of childhood asthmatics become free of asthma-related symptoms during adolescence. Little is known about bronchial hyperresponsiveness (BHR) and airway inflammation in young adults with “outgrown” childhood asthma.

Methods: We studied 61 nonsmoking medical students (18 intermittent mild asthmatics, 23 students with outgrown childhood asthma but free of asthma-related symptoms for 10 years (asymptomatic asthmatics) and 20 healthy students). BHR and lung function were measured, and induced sputum samples analyzed for eosinophil count, eosinophilic cationic protein (ECP), granulocyte-macrophage colony stimulating factor (GM-CSF), and tumor necrosis factor- α (TNF- α).

Results: BHR was still present in most asymptomatic asthmatics, but it was milder compared with healthy students. Only three subjects with previous asthma had no BHR and no signs of airway inflammation. Percentages of eosinophil, and ECP, TNF- α and GM-CSF concentrations in induced sputum of mild asthmatics and asymptomatic asthma groups were higher than in the healthy group. In asymptomatic asthmatics group, the duration of asthma, sputum eosinophil percentage, and the level of TNF- α in sputum correlated significantly with BHR.

Conclusions: Only a few subjects with *longstanding asymptomatic asthma* could be considered as cured; *most asymptomatic asthmatics* continued to exhibit BHR and signs of airway inflammation. The outcome of childhood asthma and BHR was associated with the degree of airway inflammation and the duration of childhood asthma.

Y. Obase, T. Shimoda, T. Kawano, S. Saeki, S. Tomari, K. Izaki, C. Fukushima, H. Matsuse, S. Kohnno

Second Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan

Key words: airway inflammation; bronchial hyperresponsiveness; childhood asthma; cytokine; induced sputum; outgrow.

Yasushi Obase, MD
Skin and Allergy Hospital
Helsinki University Central Hospital
PO Box 160
FIN-00029 HUS
Finland

Accepted for publication 31 October 2002

Several studies have reported that asthma-related symptoms disappear in 60–70% of childhood asthmatics after transition to adolescence, but these symptoms recur in approximately 30–40% of the children (1–4). Increased bronchial hyperresponsiveness (BHR) after disappearance of symptoms is reported in 7–63% of subjects (3–6), suggesting that BHR does not disappear readily once it is acquired. We have previously suggested that asthmatics need to be followed-up during adolescence for more than 10 years even if they seem to be free of asthma-related

symptoms, since BHR and airway inflammation often persist (7). Two important questions remain: 1) How should asthma cure or remission be defined? and 2) What factors predict outcome of childhood asthma? (1–5, 7, 8). Only a few studies have examined the relation between BHR and airway inflammation in young adolescent asthmatics with or without symptoms (9).

Previous studies have shown that eosinophil count and eosinophil cationic protein (ECP) in induced sputum or bronchoalveolar lavage from asthmatic patients increase during acute exacerbation and are increased even in patients with stable asthma compared with healthy persons (10). High levels of cytokines such as interleukin(IL)-2, IL-3, IL-4, IL-5, granulocyte-macrophage colony stimulating factor (GM-CSF), and tumor necrosis factor (TNF)- α are often detected in the airway of asthmatics (9, 11). GM-CSF is an eosinophilopoietic cytokine that activates eosinophils during an asthma attack and prolongs eosinophil survival (12, 13). Other studies have shown that eosinophil numbers and ECP

Abbreviations: BHR, bronchial hyperresponsiveness; CI, confidence interval; ECP, eosinophil cationic protein; ELISA, enzyme-linked immunosorbent assay; FEIA, fluoroenzyme immunoassay; FEV₁, forced expiratory volume in 1 s; GM-CSF, granulocyte-macrophage colony stimulating factor; IL, interleukin; PC₂₀, methacholine, provocative concentration of methacholine producing 20% fall in FEV₁ (mg/ml); RAST, radioallergosorbent assay; RIA, radioimmunoassay; RIST, radioimmunosorbent test; TNF- α , tumor necrosis factor- α .

levels in the airways correlate with BHR in asthmatics (9, 11, 14–16). BHR also correlates with atopic factors in childhood or young adolescence so, for instance, sensitivity to house dust mites (HDM) is important in this respect (17, 18).

We examined the association of BHR and airway inflammation (assessed by eosinophil count, ECP or cytokines in induced sputum) in young adolescents with normal lung function, who had outgrown childhood asthma and been free of symptoms for more than 10 years.

Material and methods

Subjects

The study was approved by the Human Ethics Committee of Nagasaki University Hospital. Two hundred and eighty-six students aged 20–29 years (mean 24.4; SD 2.2) attending the Medical School were contacted for an interview about history of childhood asthma, current asthma or allergies and smoking habits (Fig. 1). All students responded. Sixteen students (5.6%) refused to take part in further clinical studies. Three groups with predefined inclusion criteria were selected for further examinations.

The *mild asthmatics* group ($n = 18$; 20 eligible, 2 refused) consisted of students with current intermittent asthma that was diagnosed by a physician, who fulfilled the

following criteria: 1) daytime asthma symptoms appearing twice a week or less; 2) night-time symptoms appearing twice per month or less; 3) asymptomatic and normal peak expiratory flow rates between symptomatic periods; and 4) use of short-acting β_2 -agonist as needed (8).

The *asymptomatic asthmatics* group ($n = 23$; 23 eligible, no refusals) consisted of students with a history of physician-diagnosed childhood asthma, who had been free of symptoms for at least 10 years.

The *healthy individuals* group ($n = 20$; 34 eligible, 14 refused) consisted of students who fulfilled the following criteria: 1) no history of any allergic disease; 2) no serum specific IgE-antibodies against six common inhalant allergens tested (*Dermatophagoides farinae*, *Aspergillus fumigatus*, *Candida albicans*, dog, cat, and Japanese cedar *Cryptomeria japonica*); radioallergosorbent test (RAST) score = 0 (Pharmacia & Upjohn, Uppsala, Sweden).

For all subjects the exclusion criteria were 1) history of a respiratory infection during four weeks prior to the study; 2) being unable to take part in the methacholine challenge test; and 3) history of past or current smoking (Table 1, Fig. 1).

Tests for sensitivity to *D. farinae* indicated that all subjects that fulfilled the inclusion criteria for the groups of *mild and asymptomatic asthmatics* were positive (RAST score ≥ 2). All students were examined outside the pollen season. In the *mild asthmatics* group, inhaled or oral

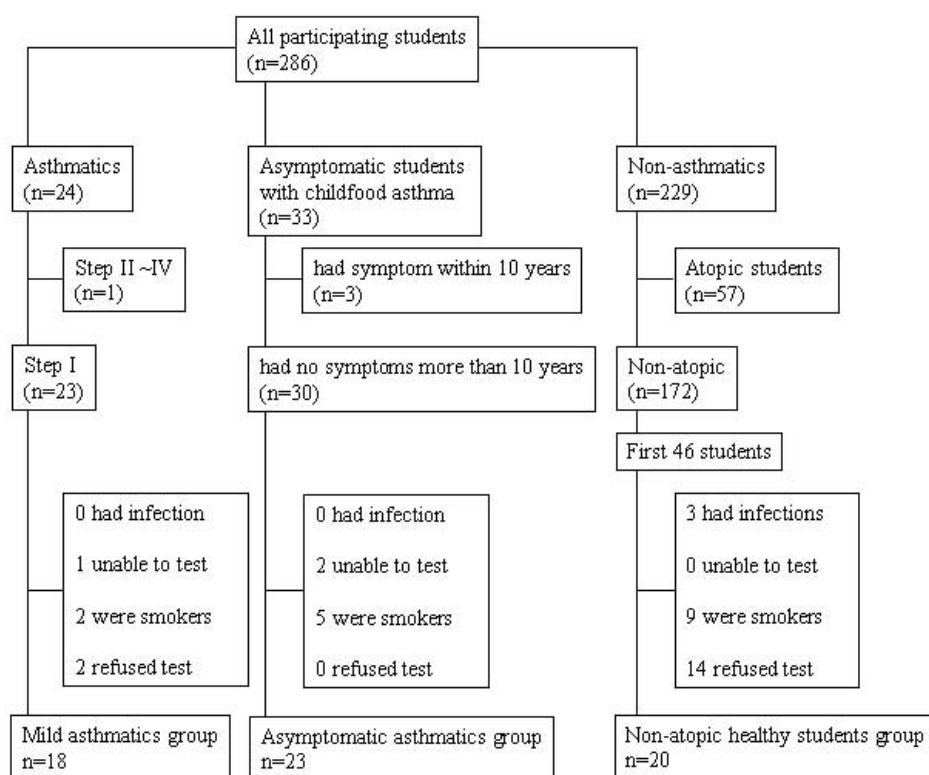


Figure 1. Subject distribution.

Table 1. Characteristics of the study subjects

	Mild asthmatics	Asymptomatic asthmatics	Healthy individuals
Number in group	18	23	20
Female/male	6/12	7/16	7/13
Mean age in years (SD)	24.1 (2.1)	24.9 (2.5)	24.2 (3.2)
Mean FVC ₁ % predicted (SD)	103.4 (9.7)	101.2 (10.3)	105.8 (11.5)
Mean FEV ₁ % predicted (SD)	97.6 (7.8)	96.3 (6.1)	90.0 (6.0)
Mean MEF ₅₀ % predicted (SD)	93.4 (10.9)	90.4 (9.6)	105.7 (9.5)
Mean MEF ₂₅ % predicted (SD)	86.3 (15.5)	85.4 (14.4)	104.7 (13.8)
Mean onset of childhood asthma in years (SD)	3.8 (1.5)	3.7 (1.9)	
Mean duration of asthma in years (SD)	20.1 (3.8)	6.1 (3.6)	

FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; MEF₅₀ and MEF₂₅, maximal expiratory flow at 50% and 25% remaining FVC.

asthma medication was withheld for 48 h prior to clinical examination, and *no students received inhaled or oral steroid therapy*.

Study design

After confirming that FEV₁ % predicted was more than 80%, a blood sample was collected for the measurement of eosinophil count, IgE radioimmunosorbent test (RIST), IgE RAST and ECP. The provocative concentration of methacholine that produces a 20% fall in FEV₁ (mg/ml) (PC₂₀) was measured in each subject (see below). Sputum induction was performed 15 min after recovery from the methacholine challenge test. Methacholine challenge does not affect sputum results (19).

Baseline lung function

Forced vital capacity, FEV₁, maximal expiratory flow at 50% and at 25% were measured with a spirometer (Superspiro, DISCOM-21FX, Chest MI, Tokyo, Japan). Results were expressed as percentage of predicted, based on relevant reference standards (20).

Measurement of serum IgE RIST and RAST

Venous blood was obtained and serum IgE RIST was measured using fluoroenzyme immunoassay (FEIA) (CAP RIST FEIA; Pharmacia, Uppsala, Sweden). Serum IgE RAST was measured using FEIA (CAP RAST FEIA, Pharmacia). IgE RAST was scored from 0 to 6 as follows, under 0.35 IU/ml (score 0); <0.70 (score 1); <3.50 (score 2); <17.5 (score 3); <52.5 (score 4); <100 (score 5); = 100 (score 6).

Measurement of bronchial responsiveness to methacholine

The challenge test was performed using the method of Cockcroft et al. (21). All antiasthma medications were withheld for at least 48 h before measurement of bronchial responsiveness. Subjects inhaled methacholine aerosol from a hand-held nebulizer (Clinical air pump, model REB 30REPC Medicare 24; Medicare, Yokohama,

Japan) by tidal breathing for 2 min. The operating airflow rate was 5 l/min. The isotonic saline was inhaled first as a control. This was followed by doubling concentrations of methacholine from 0.049 to 25 mg/ml. The FEV₁ was measured after each inhalation with a spirometer (Superspiro, DISCOM-21FX). The percentage FEV₁ fall was calculated from the post saline-FEV₁. The test was continued until the FEV₁ had fallen > 20% or until the maximal concentration of methacholine had been administered. The bronchial sensitivity was expressed as PC₂₀. At the end of the test, any fall in FEV₁ was reversed with an inhalation of salbutamol (0.3 ml of salbutamol solution; 5 mg/mL, Venetlin® for inhalation, Sankyo Co. Ltd, Tokyo, Japan). Subjects with PC₂₀ of less than 8 mg/ml were considered to have BHR according to the criteria of the American Thoracic Society (20).

Sputum induction and processing

Sputum was induced by inhalation of hypertonic saline, as described previously (9, 10). Fifteen minutes after completion of the methacholine challenge test, the subject was instructed to gargle using tap water, then 3.6% saline was inhaled at room temperature, nebulized (output 5 l/min, particle size 1–5 µm) via an ultrasonic nebulizer (NE-U12, Omron, Tokyo, Japan). Subjects were instructed to cough deeply after 3- and 5-minute intervals. Gargling between and before each induced cough was encouraged in order to minimize salivary contamination. The initial sample from the first cough was discarded. Induced sputum was collected into a 50-ml polypropylene tube, kept at 4°C and processed within 2 h. Spirometric tests were repeated after sputum induction. If FEV₁ had dropped below 15% of the postsalbutamol value, the subject was required to stay in the laboratory until FEV₁ returned to the baseline value.

The volume of the sample was recorded, and the sample was diluted with 2 ml of Hanks' balanced salt solution containing 1% dithiothreitol (Sigma Chemicals, Poole, UK) and gently vortexed at room temperature. When uniform in consistency, samples were further diluted with Hanks' balanced salt solution and again

vortexed briefly. They were then centrifuged at 400 *g* for 15 min at 4°C, the supernatant was aspirated, and stored at -70°C for subsequent assay for ECP. An adequate specimen was defined as one in which the number of squamous epithelial cells was <30% of inflammatory cells.

Slides were then stained with May-Grünwald-Giemsa for differential cell count, which was performed by an observer blinded to the clinical characteristics of the subjects. At least two slides were used for counting and at least 300 inflammatory cells were counted in each slide. Previous studies reported no significant differences in the number of total cell counts in sputum between asthmatics and healthy individuals (21). Therefore, we did not count the total cell number in the sputum. To measure cytokine levels (TNF- α , GM-CSF and IL-5), the supernatant and five-fold concentrates were prepared (Centriprep-10, Amicon, Waltham, MA, USA). Actual values of cytokines were corrected based on albumin levels (LX-Eiken-Alb-2, Eiken, Tokyo).

Measurement of sputum ECP and cytokines TNF- α , GM-CSF and IL-5

The concentration of ECP in the supernatant of sputum was determined by use of radioimmunoassay (RIA) with a lower limit of detection of 2.0 μ g/l (Pharmacia ECP RIA, Pharmacia). The concentrations of TNF- α , GM-CSF and IL-5 in the supernatant of sputum were determined by use of respective enzyme-linked immunosorbent assay (ELISA), with lower limits of detection of 4, 4 and 15 pg/ml, respectively (human TNF- α , GM-CSF and IL-5 ELISA kits, Otsuka Assay, Tokyo, respectively).

Data analysis

The values of lung function are expressed as mean $\pm$ standard deviation. Data of PC₂₀ and sputum eosinophil ratio, ECP, cytokines are expressed as means and 95% confidence intervals (CI). Differences in log PC₂₀ and the concentrations of chemical mediators and cytokines between groups were analyzed using a one-way factorial ANOVA with the level of significance (*P* value) set at 0.05.

Results

Lung function and airway hyperresponsiveness

Lung volumes were normal in all subjects (Table 1). The mean values of PC₂₀ of the *mild asthmatic* group, *asymptomatic asthmatic* group and *healthy* group were 0.62 mg/ml (95% CI 0.40–1.06), 1.83 mg/ml (95% CI 0.95–3.52) and 19.63 mg/ml (95% CI 17.50–22.03), respectively (Fig. 2). The mean log PC₂₀ of *asymptomatic asthmatics* was significantly higher than of *mild asthmatic*

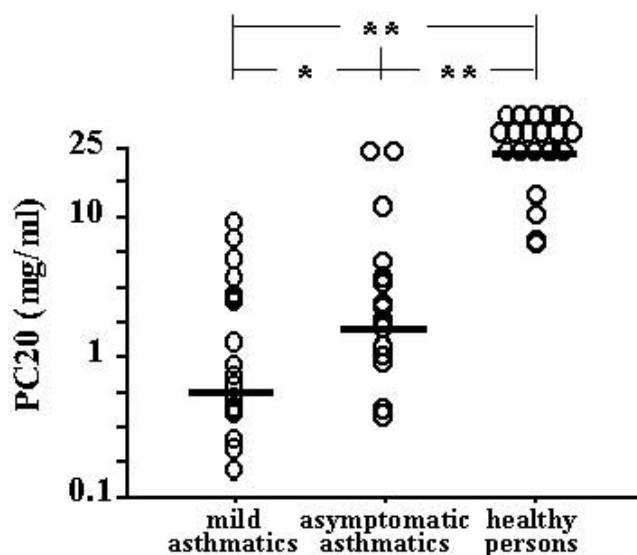


Figure 2. PC₂₀ of individual groups. Note the logarithmic scale of PC₂₀. Horizontal lines represent the mean values for each group. **P* < 0.05, ***P* < 0.01.

group (*P* < 0.05), although the two values showed that BHR existed in both groups. None of the mild asthmatics and three (13.0%) of the *asymptomatic individuals* had BHR. Three (15.0%) healthy individuals had BHR, and their mean log PC₂₀ was significantly higher compared with *mild asthmatics* and *asymptomatic individuals* (*P* < 0.01, respectively).

Eosinophils and ECP in induced sputum

Table 2 shows differences in cell counts and ECP levels in the sputum of each group. Based on the values of the *healthy group*, the upper limits of eosinophils (mean + 3 SD; 6.3%) and ECP (63.9 μ g/l) in the sputum were calculated. An increased proportion of eosinophils in the sputum was detected in all subjects of the *mild intermittent asthmatic* group and in 19 (82.6%) of the *asymptomatic asthmatic* group, but in only one (5.0%) of the *healthy* group. An increased sputum ECP level was detected in 16 (88.9%) subjects of the *mild asthmatics* group, and 16 (69.6%) of the *asymptomatic asthmatic* group, but in none of the *healthy individuals*. There were no differences between *mild asthmatics* and *asymptomatic asthmatics*, but the eosinophil numbers and ECP levels were significantly higher in these two groups compared with healthy persons (*P* < 0.01, respectively).

TNF- α , GM-CSF and IL-5 in induced sputum

The concentrations of TNF- α , GM-CSF in induced sputum are shown in Table 2. Based on the values in *healthy individuals*, the upper limits of TNF- α (mean + 3 SD; 11.5 pg/ml) and GM-CSF (11.6 pg/ml) in the sputum were calculated. High sputum TNF- α levels were

Table 2. Sputum markers in each group of subjects

Cell differences	Mild asthmatics		Asymptomatic asthmatics		Healthy individuals	
	Mean	(95% CI)	Mean	(95% CI)	Mean	(95% CI)
Eosinophils %	25.7	(11.5–39.9)*	13.6	(7.3–19.8)*	0.6	(–0.3–1.5)
Neutrophils %	3.9	(2.2–5.6)	5.3	(4.0–6.6)	2.9	(2.0–3.7)
Lymphocytes %	0.6	(0.1–1.1)	0.4	(0.1–0.7)	0.3	(0.0–0.5)
Macrophages %	73.5	(66.0–81.0)	81.6	(75.8–87.5)	96.3	(95.0–94.6)
ECP µg/l	234	(84–383)*	109	(84–255)*	16	(9–24)
TNF-α pg/ml	69.3	(38.2–100.4)*	43.8	(29.4–58.3)*	4.0	(2.8–5.2)
GM-CSF pg/ml	19.9	(7.1–32.6)*	14.5	(10.1–18.9)*	3.2	(1.9–4.5)

* $P \leq 0.01$ vs healthy individuals.

ECP, eosinophil cationic protein; GM-CSF, granulocyte-macrophage colony stimulating factor; TNF-α, tumor necrosis factor-α.

detected in all subjects of the *mild asthmatics* and 18 (78.3%) subjects of the *asymptomatic asthmatics*, but in none of the *healthy individuals*. High sputum GM-CSF concentrations were identified in 12 (66.7%) subjects of the *mild asthmatics* and 18 (78.3%) subjects of the *asymptomatic asthmatics*, but in none of the *healthy individuals*. There were no difference between *mild* and *asymptomatic asthmatics* groups, but the concentrations of both cytokines were significantly higher in the patient groups compared with the healthy persons ($P < 0.01$, respectively). The concentrations of IL-5 in the sputum were below the detection limit in all groups (data not shown).

Bronchial responsiveness, atopy and airway inflammation in students with previous asthma

Since the mean log PC₂₀ of *asymptomatic asthmatics* was lower and the number of eosinophils and concentrations of ECP and cytokines in induced sputum were higher compared with *healthy individuals*, we examined the correlations between various factors and PC₂₀ in the *asymptomatic asthmatics* group ($n = 23$) (Fig. 3). We selected gender, age of onset of asthma and duration of asthma as background factors, serum IgE and *D. farinae*-specific IgE as atopic factors, and eosinophil count and ECP in blood, as well as percentage of eosinophils and concentrations of ECP, TNF-α and GM-CSF in the sputum as markers of airway inflammation. Age and duration of the period without asthma-related symptoms were not included because we recruited only subjects aged 20–29 years and defined “outgrow” as no symptoms for more than 10 years. The correlations between log PC₂₀ and the duration of childhood asthma, percentage of eosinophils and macrophages in sputum, and ECP and TNF-α level in sputum were significant (log PC₂₀ and duration of childhood asthma, $r = -0.621$; $P < 0.02$; log PC₂₀ and percentage of eosinophils in sputum, $r = -0.569$; $P < 0.01$; log PC₂₀ and percentage of macrophages in sputum, $r = 0.540$; $P < 0.01$; log PC₂₀ and ECP in sputum, $r = -0.449$; $P < 0.05$; and log PC₂₀ and TNF-α in sputum, $r = -0.634$; $P < 0.01$). Multiple

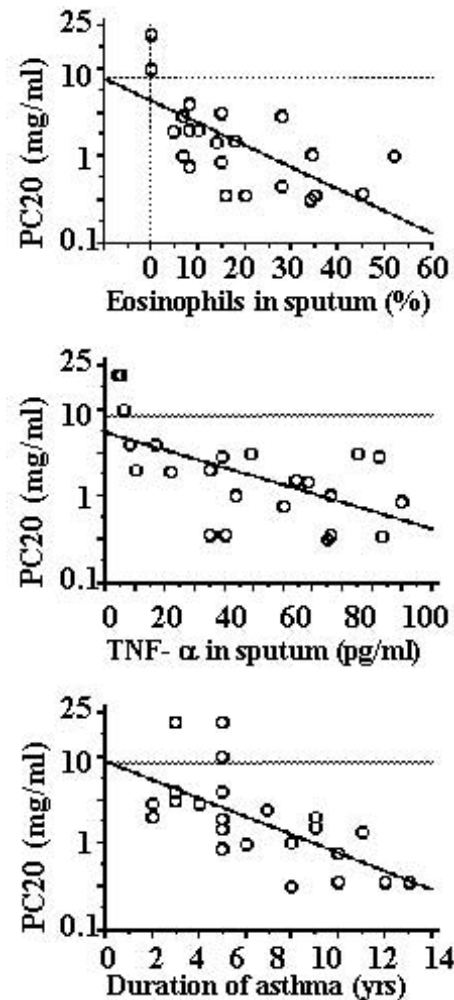


Figure 3. Relation between log PC₂₀ and percentage of eosinophil and TNF-α level in the sputum and duration of childhood asthma in asymptomatic asthmatics group. Log PC₂₀ and eosinophil percentage in sputum, $r = -0.569$; $P < 0.01$; log PC₂₀ and TNF-α in sputum, $r = -0.634$; $P < 0.01$; and log PC₂₀ and the duration of childhood asthma, $r = -0.621$; $P < 0.02$. Note the logarithmic scale of PC₂₀. The horizontal lines show the normal range (8.0 mg/ml of methacholine).

Table 3. Contribution of each factor to log PC₂₀ (multiple regression analysis) in asymptomatic asthmatics group

Factors	Regression coefficient	T value	P value
Gender (female 0, male 1)	0.274	1.390	0.195
Age of onset of asthma	0.132	0.737	0.478
Duration of asthma	-0.331	-2.059	0.067
Total IgE	-0.238	-1.269	0.233
<i>Dermatophagoides farinae</i> -specific IgE	0.240	1.590	0.143
Eosinophil count in blood	0.328	2.055	0.067
ECP in serum	0.216	0.909	0.385
Eosinophils % in sputum	-0.125	-0.497	0.630
ECP in sputum	-0.168	-0.847	0.417
TNF- α in sputum	-0.421	-2.171	0.055
GM-CSF in sputum	-0.083	-0.325	0.752

ECP, eosinophil cationic protein; GM-CSF, granulocyte-macrophage colony stimulating factor; TNF- α , tumor necrosis factor- α .

logistic regression analysis (log PC₂₀ vs the above factors) showed borderline significance for duration of asthma, eosinophils count in peripheral blood, and TNF- α in sputum (Table 3).

Characteristics of three students presumed "cured"

Three subjects from the *asymptomatic asthmatic* group whose BHR tests were negative could be labeled as "cured" and different from the other 20 subjects who still had a positive BHR test. Proper statistical analysis could not be performed due to the small number of subjects. However it was interesting that their durations of childhood asthma (5, 5, and 3 years) were short, and the levels of eosinophils (0, 0, and 2%), ECP (16.1, 10.7, and 120.5 μ g/l), TNF- α (6.1, 4.4, and 4.8 pg/ml), and GM-CSF (9.0, 4.7, and 6.1 pg/ml) in sputum of these three people were low compared with the other 20 who still showed BHR (duration of asthma, mean of 6.8 years, 95%CI of 3.4–10.2; eosinophils, 17.1%, 4.5–29.7; ECP, 187.4 μ g/l, 101.4–272.9; TNF- α , 52.6 pg/ml, 24.0–81.2; GM-CSF, 16.5 pg/ml, 6.5–26.5) (except for elevated ECP level in the sputum of Student 3 (> 63.9 μ g/l)).

Discussion

The main findings of the present study were that subjects who had childhood asthma, presumed to be "outgrown" without any asthma-related symptoms for more than 10 years, were significantly more responsive to methacholine than healthy ones, though less than intermittent mild asthmatic people. They had signs of airway inflammation to the same extent of those with intermittent mild asthmatics. The level of BHR in students who had not had asthma symptoms for 10 years correlated significantly not only with the present level of airway inflammation but also with the duration of childhood asthma.

Previous studies indicated that the BHR attained during childhood does not disappear easily (1, 23, 24).

We previously examined 447 young adults and demonstrated that subjects who had outgrown childhood asthma had BHR comparable to that of mild asthmatics (6). An increase in BHR to a nonspecific irritant is fundamental characteristics of bronchial asthma, together with airway inflammation (7). Only a few reports have examined the correlation between BHR and other signs of airway inflammation in individuals with outgrown asthma (1–6, 23–25). In recent years, induction of sputum by inhalation of hypertonic saline has been widely used as a noninvasive method for obtaining specimens from the airway for the evaluation of airway inflammation (9, 10). Our results showed a significant correlation between BHR and percentage of eosinophils and TNF- α level in induced sputum in the outgrown group. Percentage of eosinophils in the sputum has been recognized as an indicator for validating allergic inflammation of the airways in asthmatics (10). Ackerman et al. (26) reported that the number of TNF- α -positive cells was greater in bronchial biopsies from symptomatic than asymptomatic asthmatic subjects, suggesting that the levels of this cytokine in the airways might be related to eosinophils numbers, lung function and asthma severity. Lack of IL-5 in the sample does not always mean absence of this cytokine in the sputum, for this can be due to problems in the method used for measurement (27).

Based on the above findings, it is possible that subjects with outgrown asthma who continue to demonstrate features of airway inflammation and have BHR are potentially at risk of asthma recurrence—even if they have had no asthma-related symptoms for more than 10 years (i.e. they are in remission, but not cured) (23,24). Interestingly, our study identified only three subjects in the outgrown group who did not have BHR. In these subjects, the percentage of eosinophils and levels of ECP, TNF- α and GM-CSF in induced sputum were lower than in the other students with outgrown asthma, suggesting disappearance of airway inflammation. Panhuysen et al. (28) reported that 11% of adulthood asthmatics were no longer considered asthmatic after 25 years, suggesting

that in a substantial proportion of symptomatic asthmatics the condition can improve, and that a subset of patients may outgrow their asthma, even in adulthood. Further studies are needed to explore the differences between "cured" subjects and those considered to have achieved remission (29, 30).

The severity of airway inflammation in the outgrown group was similar to that observed in the still asthmatic group, but the BHR was worse in the students who still reported asthma symptoms. We might be able to explain the former by the group setting; if we had set several students in the symptomatic asthmatic group, the difference might have been larger (31), whereas if we had included atopic nonasthmatics in the healthy individual group, the difference might have been smaller (6). However, the point of the study is not only to find the difference among these three groups but also to clarify the prognosis of the "outgrown" asthmatics. We emphasize that 20 of 23 students who had childhood asthma and had been in an asymptomatic state for more than 10 years still had high BHR and airway inflammation among a group of 286 students.

The duration of childhood asthma correlated with the persistence of BHR as well as airway inflammation in the outgrown group. At present, there is no treatment to reverse structural changes already established in the

airways, but it is considered that early intervention by antiinflammatory treatment may prevent airway remodelling (32, 33). Sont et al. (33) suggested that antiinflammatory treatment by monitoring of BHR leads to a reduction in airway thickness. In our study, multiple regression analysis showed that the only factors that tended to correlate with PC₂₀ were the duration of asthma, eosinophil count in peripheral blood and TNF- α in sputum (Table 3). This suggests that we should perhaps include larger number of subjects in future studies.

Our findings indicate that only a few students with a history of childhood asthma achieve a state of "cure" as defined by lack of symptoms and BHR, whereas *most* childhood asthmatics continue to have BHR in adolescence and are rather in a remission state. The outcome of asthma symptoms and presence of BHR can be assessed by measuring inflammatory indices in induced sputum and estimation of the duration of childhood asthma. Warke et al. (24) reported that eosinophils were increased in the bronchoalveolar lavage fluid of asymptomatic asthmatics compared to controls. Hence, it must be confirmed, in the future, whether children who have had asthma for several years—but have outgrown their disease—should be regarded as potential asthmatics during adulthood.

References

- VON MUTIUS E. Progression of allergy and asthma through childhood to adolescence. *Thorax* 1996;**51** (Suppl. 1): S3–S6.
- WIESCH DG, SAMET JM. Epidemiology and natural history of asthma. In: MIDDLETON E JR, ELLIS, EF, YUNGINGER, JW, REED, CE, ADKINSON, NF Jr, BUSSE, WW, eds. *Allergy Principles and Practice*, 5th edn. St Louis, MO, USA, Mosby Year Book 1998; 799–815.
- JOHNSTONE DE. Some aspects of the natural history of asthma. *Ann Allergy* 1982;**49**:257–264.
- BALFOUR-LYNN L. Childhood asthma and puberty. *Arch Dis Child* 1985;**60**:231–235.
- GERRITSEN J, KOETER GH, POSTMA DS, SCHOUTEN JP, KNOL K. Prognosis of asthma from childhood to adulthood. *Am Rev Respir Dis* 1989;**140**:1325–1330.
- BOULET LP, TURCOTTE H, BROCHU A. Persistence of airway obstruction and hyperresponsiveness in subjects with asthma remission. *Chest* 1994;**105**: 1024–1031.
- OBASE Y, SHIMODA T, MITSUTA K, MATSUO N, MATSUSE H, KOHNO S. Sensitivity to the house dust mite and airway hyperresponsiveness in a young adult population. *Ann Allergy Asthma Immunol* 1999;**83**:305–310.
- DEPARTMENT OF HEALTH AND HUMAN SERVICES. Overview of the report. Guidelines for the Diagnosis and Management of Asthma: National Asthma Education Program Expert Panel Report 2. NIH Publication 97-4051. Bethesda, MD, USA, Department of Health and Human Services 1997;4051.
- OBASE Y, SHIMODA T, MITSUTA K, MATSUO N, MATSUSE H, KOHNO S. Correlation between airway hyperresponsiveness and airway inflammation in a young adult population: eosinophil, ECP, and cytokine levels in induced sputum. *Ann Allergy Asthma Immunol* 2001;**86**:304–310.
- PIN I, RADFORD S, KOLENDOWICZ R, et al. Airway inflammation in symptomatic and asymptomatic children with methacholine hyperresponsiveness. *Eur Respir J* 1993;**6**:1249–1256.
- ROBINSON DS, HAMID Q, YING S, et al. Predominant Th2-like bronchoalveolar T_H-lymphocyte population in atopic asthma. *N Engl J Med* 1992;**326**: 298–304.
- MOTOJIMA S, ADACHI T, MANAKA K, ARIMA M, FUKUDA T, MAKINO S. Eosinophil peroxidase stimulates the release of granulocyte-macrophage colony-stimulating factor from bronchial epithelial cells. *J Allergy Clin Immunol* 1996;**98**:S216–S223.
- LOPEZ AF, WILLIAMSON DJ, GAMBLE JR, et al. Recombinant human granulocyte macrophage colony-stimulating factor stimulates in vitro mature human neutrophil and eosinophil function, surface receptor expression, and survival. *J Clin Invest* 1986;**78**:1220–1228.
- KIRBY JG, HARGREAVE FE, GLEICH GJ, O'BYRNE PM. Bronchoalveolar cell profiles of asthmatic and non-asthmatic subjects. *Am Rev Respir Dis* 1987;**136**:379–383.
- WALKER C, KAEGI MK, BRAUN P, BLASER K. Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated disease severity. *J Allergy Clin Immunol* 1991;**88**:935–942.

16. BENTLEY AM, MENZ G, STORZ CHR et al. Identification of T lymphocytes, macrophages, and activated eosinophils in the bronchial mucosa in intrinsic asthma. *Am Rev Respir Dis* 1992;**146**:500–506.
17. PEAT JK, WOOLCOCK AJ. Sensitivity to common allergens. relation to respiratory symptoms and bronchial hyper-responsiveness in children from three different climatic areas of Australia. *Clin Exp Allergy* 1991;**21**:573–581.
18. SEARS MR, HERBISON GP, HOLDAWAY MD, HEWITT CJ, FLANNERY EM, SILVA PA. The relative risks of sensitivity to grass pollen, house dust mite and cat dander in the development of childhood asthma. *Clin Exp Allergy* 1989;**19**: 419–424.
19. GERSHMAN NH, FAHY JV. The effect of methacholine challenge on the cellular composition of induced sputum. *J Allergy Clin Immunol* 1999;**103**: 957–959.
20. AMERICAN THORACIC SOCIETY. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;**136**: 225–244.
21. COCKCROFT DW, KILLIAN DN, MELLON JJ, HARGREAVE FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;**7**: 235–243.
22. VAN DEN TOORN LM, OVERBEEK SE, DE JONGSTE JC, LEMAN K, HOOGSTEDEN HC, PRINS JB. Airway inflammation is present during clinical remission of atopic asthma. *Am J Respir Crit Care Med* 2001;**164**:2107–2113.
23. WARKE TJ, FITCH PS, BROWN V, et al. Outgrown asthma does not mean no airways inflammation. *Eur Respir J* 2002;**19**:284–287.
24. GIBSON PG, WLODARCZYK JW, HENSLEY MJ, et al. Epidemiological association of airway inflammation with asthma symptoms and airway hyperresponsiveness in childhood. *Am J Respir Crit Care Med* 1998;**158**:36–41.
25. METSO T, RYTILÄ P, PETERSON C, HAAHTELA T. Granulocyte markers in induced sputum in patients with respiratory disorders and healthy persons obtained by two sputum-processing methods. *Resp Med* 2001;**95**:48–55.
26. ACKERMAN V, MARINI M, VITTORI E, BELLINI A, VASSALI G, MATTOLI M. Detection of cytokines and their cell sources in bronchial biopsy specimens from asthmatics patients. Relationship to atopic status, symptoms, and level of airway hyperresponsiveness. *Chest* 1994;**105**:687–696.
27. KELLY MM, LEIGH R, HORSEWOOD P, GLEICH GJ, COX G, HARGREAVE FE. Induced sputum: validity of fluid-phase IL-5 measurement. *J Allergy Clin Immunol* 2000;**105**:1162–1168.
28. PANHUYSEN CI, VONK JM, KOETER GH, et al. Adult patients may outgrow their asthma: a 25-year follow-up study. *Am J Respir Crit Care Med* 1997;**155**: 1267–1272.
29. VAN ESSEN-ZANDVLIET EE, HUGHES MD, WAALKENS HJ, DUIVERMAN EJ, KERREBIJN KF. Remission of childhood asthma after long-term treatment with an inhaled corticosteroid (budesonide). Can it be reached? *Eur Respir J* 1994;**7**:63–68.
30. HAAHTELA T, JÄRVINEN M, KAVA T, et al. Effects of reducing or discontinuing inhaled budesonide in patients with mild asthma. *N Engl J Med* 1994;**331**: 700–705.
31. TEN BRINKE A, ZWINDERMAN AH, STERK PJ, RABE KF, BEL EH. Factors associated with persistent airflow limitation in severe asthma. *Am J Respir Crit Care Med* 2001;**164**:744–748.
32. BOUSQUET J, LACOSTE JY, CHANEZ P, VIC P, GODARD P, MICHEL FB. Bronchial elastic fibers in normal subjects and asthmatic patients. *Am J Respir Crit Care Med* 1996;**153**:1648–1654.
33. SONT JK, WILLEMS LN, BEL EH, VAN KRIEKEN JH, VANDENBROUCKE JP, STERK PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. *Am J Respir Crit Care Med* 1999; **159**:1043–1051.