

Sensitivity to the house dust mite and airway hyperresponsiveness in a young adult population

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Background: The pathogenic mechanisms of airway hyperresponsiveness (AHR) in asthma are unknown and only a few studies have examined the importance of sensitivity to antigens in AHR in young adults.

Objective: We investigated the correlation between AHR and sensitivity to specific antigens, atopy, history of childhood asthma and spirometry in a young adult population.

Methods: Based on the results of interviews with 447 students at our university, 308 non-smoker students were classified into six groups. Group 1 comprised subjects with intermittent mild bronchial asthma; group 2, subjects with history of childhood asthma; group 3, subjects with atopic disease, and a RAST score for *Dermatophagoides farinae* (*Def*) of ≥ 2 ; group 4, normal subjects with a RAST score for *Def* of ≥ 2 ; group 5, subjects with cedar pollinosis; and group 6, normal subjects. We measured AHR to methacholine (MCh), spirometry, immunoglobulin E-radioimmunosorbent test (IgE-RIST), IgE-radioallergosorbent test to six common antigens, eosinophil cationic protein (ECP), and eosinophil count in peripheral blood in each subject.

Results: Airway hyperresponsiveness to MCh did not correlate with IgE-RIST, eosinophil count, or ECP. The highest AHR to MCh was present in groups 1 and 2 and lowest in groups 5 and 6. Multiple regression analysis showed that sensitivity to *Def* was the only factor that significantly influenced AHR to MCh. Airway hyperresponsiveness to MCh of groups with a RAST score for *Def* of 0/1 was lower than groups with a RAST score of 2 to 6. Airway hyperresponsiveness to MCh did not correlate with the degree of positivity to *Def* antigen among positive sensitized groups (RAST score 2 to 6).

Conclusions: Sensitivity to mite antigen may be important in the pathogenesis of AHR and *Def* is a major contributing antigen in young adults in Japan. Once asthma occurs, AHR remains positive for a long time even after the disappearance of asthma-related symptoms.

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INTRODUCTION

Although the exact pathogenic mechanisms of airway hyperresponsiveness (AHR), the hallmark of bronchial asthma, is unknown, several hypotheses have been formulated. These include airway inflammation, disorders of respiratory epithelium, changes in neural control mechanisms of the air-

ways, functional changes in the airway smooth muscle itself, and airway occlusion.^{1–5} There are only a few studies in young adult subjects with and/or without asthma that have examined the correlation between AHR and active asthma, diagnosed asthma, history of wheezing and atopy.⁶ Although it has been reported that the presence of atopy may facilitate the development of AHR,^{7–9} the role of sensitivity to specific antigens in AHR or onset of asthma is currently a subject of vigorous investigation.^{10–13} In 1974, the incidence of pollinosis due to pollen from the Japanese cedar tree was

around 3.8%, but this value increased to 5.8% in 1977 and to 9.4% in 1981. This trend has continued and the figure is now well over 10%.¹⁴

Concerning the natural history of asthma, several studies have investigated the status of asthma in children from birth to 10 years of age and from 10 to 40 years of age at various age intervals (from 5 to 15 years).^{15–17} The definition of remission of asthma not only in children but also in adults remains controversial. Several studies have reported the resolution of asthma symptoms in 60% to 70% of children with childhood asthma, after transition to adulthood, although these symptoms recur in approximately 30% to 40% of these children.^{16,18,19} The rate of positive AHR after disappearance of symptoms is reported at 7% to 63%,^{16,17,20} suggesting that AHR does not disappear immediately once it is acquired.

In the present study, we investigated the correlation between AHR and sensitivity to specific antigens (especially house dust mite), atopy, history of childhood asthma, and spirometry in young adult subjects with and/or without asthma.

METHODS

Subjects

A total of 447 students, all members the sixth grade of our medical school aged 20 to 29 years, were contacted for an interview about histories of allergic diseases such as childhood asthma, current diseases, and smoking habits. A total of 308 subjects with or without asthma volunteered to participate in the study. All subjects were studied out of the pollination season. They fulfilled predefined inclusion/exclusion criteria and gave informed consent to participate in this study. The exclusion

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criteria included (1) history of sino-pulmonary infection during a period of 4 weeks preceding the present study; (2) presence of conditions that hindered a proper measurement of AHR, such as dental treatment that affected measurement of respiratory functions; (3) history of current or past smoking; and (4) asthmatics with mild, persistent, or severe symptoms. In all subjects, inhaled or oral antiasthmatic medications were withheld during the 48-hour period prior to the present study. The study was approved by the Human Ethics Review Committee of our University Hospital.

Group Classification

Subjects were classified into six different groups based on the results of a detailed interview and immunoglobulin E radioallergosorbent test (IgE-RAST) to *Dermatophagoides farinae* (Def) (Table 1). Group 1 (n = 31) subjects with mild intermittent bronchial asthma fit the criterion of $\geq 15\%$ > reversibility after bronchodilators.²¹ All subjects showed a RAST score for of Def ≥ 2 . The age of onset varied widely from 3 to 24 years, with a mean value of 9.2 ± 8.3 years. Group 2 (n = 33) comprised subjects with childhood asthma who had not developed any asthma-related symptoms for more than 10 years. All showed a RAST score for Def of ≥ 2 . Group 3 (n = 47) comprised non-asthmatic subjects who had atopic dermatitis or allergic rhinitis, with a RAST score for Def of ≥ 2 . Group 4 (n = 53) had normal subjects

who did not have either asthma or atopic disease but showed a RAST score for Def of ≥ 2 . Group 5 (n = 42) comprised non-asthmatic subjects with cedar pollinosis and a RAST score for Def of ≤ 1 , while the RAST score for Japanese cedar pollen (Jcp) was ≥ 2 . Group 6 (n = 102) was normal subjects who did not have asthma or atopic disease, and a RAST score for Def of ≤ 1 . Differences in the sensitivity to antigen were compared between subjects of groups 3 and 5 who both had atopic disease and between groups 4 and 6 consisting of normal subjects. There were no significant differences among the groups with regard to female/male ratio, age, forced vital capacity (FVC) (% predicted), forced expiratory volume in first second (FEV₁) (% predicted), forced expiratory flows at 50% (V₅₀) (% predicted), and 25% (V₂₅) (% predicted) of the flow-volume curve. Each spirometric parameter was within the normal range in all subjects.

Study Design

After confirming that FEV₁ (% pred) was >80%, a blood sample was collected for the measurement of eosinophil count, IgE-RIST, IgE-RAST and eosinophil cationic protein (ECP). PC₂₀ (an indicator of AHR) was measured in each subject (see below).

Baseline Lung Function Tests

Forced vital capacity and FEV₁ were measured with a spirometer (Superspiro, DISCOM-21FX, Chest M.I. Co., Tokyo). A maximal expiratory flow-

volume curve was recorded on a pneumotachograph spirometer (Superspiro, DISCOM-21FX, Chest M.I. Co.). V₅₀ and V₂₅ were measured from the expiratory flow-volume curve. Results were expressed as percentage of predicted normal, based on relevant reference standards.²²

Measurement of serum IgE-RIST and IgE-RAST

Serum IgE-RIST was measured using fluoroenzyme immunoassay (CAP RIST FEIA, Pharmacia, Stockholm, Sweden). Serum IgE-RAST (Def, *Aspergillus fumigatus*, *Candida albicans*, dog hair, cat hair, and Jcp) was measured using fluoroenzyme immunoassay (CAP RAST FEIA, Pharmacia).

Measurement of Serum ECP

Blood samples for measurement of serum ECP were collected in specially designed tubes (Vacutainer, Brand Blood Collection tube, Becton Dickinson), left to stand at room temperature for 1 hour, then centrifuged at 1,300 g for 10 minutes. The supernatant was cryopreserved at -40°C . The concentration of ECP in the serum was determined by use of radioimmunoassay. The lower limit of detection using this assay is 2.0 $\mu\text{g/L}$ (ECP RIA, Pharmacia).

Measurement of Bronchial Responsiveness to Methacholine (MCh)

All antiasthma medications were withheld for at least 48 hours before mea-

Table 1. Subject Characteristics

	Group*					
	1	2	3	4	5	6
n	31	33	47	53	42	102
F/M†	10/21	7/26	10/37	16/37	13/29	29/73
Age (yrs)	24 ± 3	25 ± 2	25 ± 3	23 ± 3	24 ± 3	24 ± 3
FVC (% pred)	103 ± 11	100 ± 10	102 ± 11	110 ± 12	98 ± 11	106 ± 13
FEV ₁ (% pred)	102 ± 10	94 ± 10	106 ± 10	98 ± 9	104 ± 11	102 ± 11
V ₅₀ (% pred)	93 ± 10	90 ± 10	107 ± 10	102 ± 9	109 ± 11	106 ± 10
V ₂₅ (% pred)	86 ± 14	83 ± 12	110 ± 25	95 ± 19	101 ± 14	105 ± 16

Values are expressed as mean $\pm$ SD.

* see text ("Group Classification").

† F = female and M = male. FVC = forced vital capacity, FEV₁ = forced expiratory volume in 1 sec, V₅₀ = maximal expiratory flow at 50% remaining FVC, and V₂₅ = maximal expiratory flow at 25% remaining FVC.

surement of bronchial responsiveness. The methacholine challenge test was performed according to the standardized procedure recommended by the Japanese Society of Allergology.²³ The challenge test was performed if the subject's FEV₁/FVC value was more than 70% in the initial pulmonary function test. Methacholine aerosol was administered from a hand-held DeVilbiss No. 5650N nebulizer (DeVilbiss Co., Somerset, PA) during tidal breathing for 2 minutes. The operating airflow rate was 5 L/min. Isotonic saline was inhaled first as a control. This was followed by doubling concentrations of MCh from 0.049 to 25 mg/mL. FEV₁ was measured after each inhalation with a spirometer (Superspiro, DISCOM-21FX, Chest M.I. Co.). The percentage FEV₁ fall was calculated from the post-saline FEV₁. The test was continued until FEV₁ had fallen >20% or until the maximal concentration of MCh had been administered. Bronchial sensitivity was expressed as the concentration of MCh that would provoke a fall in FEV₁ of 20% (PC₂₀). At the end of the test, any fall in FEV₁ was reversed with inhalation of salbutamol.

Statistical Analysis

PC₂₀ is expressed as the mean value with 95% confidence interval (CI). All other data are expressed as mean $\pm$ SD. Comparisons between groups were assessed by one-way factorial ANOVA and multiple comparison tests. Correlations between parameters (PC₂₀, total-IgE, ECP, and eosinophil number) were analyzed with Pearson's correlation coefficient. The influence of sensitivity to a specific antigen on PC₂₀ was determined by multiple logistic regression analysis. The contribution ratio was calculated using Stat-View software. A *P* value <.05 denoted the presence of significant difference.

RESULTS

Analysis of Peripheral Blood

There was a significant correlation between log IgE-RIST and eosinophil count ($r = 0.40$, $P < .01$), between log

IgE-RIST and ECP ($r = 0.21$, $P < .05$) and between eosinophil count and ECP ($r = 0.66$, $P < .01$) (data not shown).

PC₂₀ in Different Groups

The mean values of PC₂₀ of groups 1 and 2 were 0.91 (95% CI: 0.64 to 1.29, $n = 31$) and 2.14 mg/mL (95% CI: 1.80 to 2.53, $n = 33$), respectively, and both groups were AHR-positive. The mean values of PC₂₀ of groups 5 and 6 were 15.52 (95% CI 13.24 to 18.20, $n = 42$) and 13.56 mg/mL (95% CI: 12.50 to 14.83, $n = 102$), respectively, and both groups were AHR-negative. The mean values of PC₂₀ of groups 3 and 4 were 5.41 (95% CI 4.23 to 6.92, $n = 47$) and 6.12 mg/mL (95% CI 5.21 to 7.19, $n = 53$), respectively, which were not significantly different (Fig 1). Both values were midway between those of groups 1 and 2 and groups 5 and 6 (all groups, $P < .05$; group 3

versus group 1, 2, 5, and 6, group 4 versus groups 1, 2, 5 and 6). In addition, examination of the correlation between PC₂₀ and each of IgE-RIST, eosinophil count and ECP showed insignificant correlations (data not shown).

Influence of Sensitivity to Antigen Sensitization on AHR

We also examined the effect of sensitivity to one or more antigens (six antigens: *Def*, *A. fumigatus*, *C. albicans*, dog hair, cat hair, Jcp) on PC₂₀ (log PC₂₀) by using multiple regression analysis (Table 2). Multiple logistic regression analysis (log PC₂₀ versus sensitivity to these six specific antigens) showed a correlation coefficient of 0.34 ($P < .01$), and contribution ratio of 15.9%. Only the contribution of *Def* to PC₂₀ was significant.

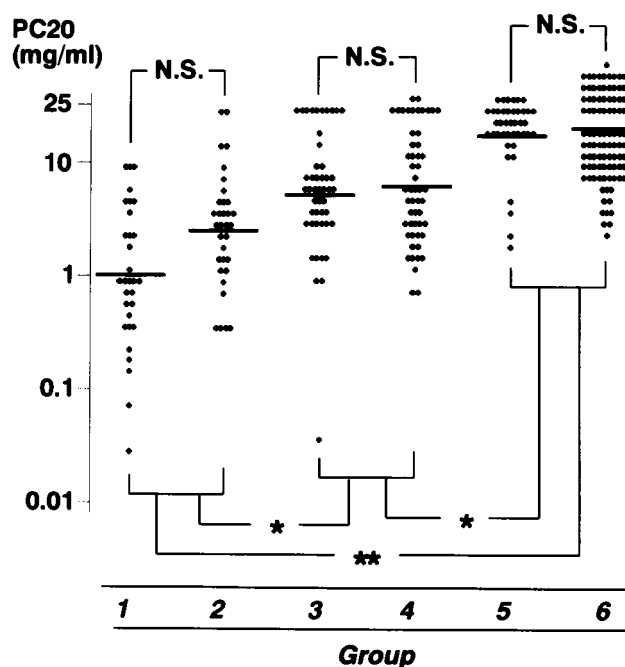


Figure 1. PC₂₀ of individual groups. Group 1 ($n = 31$): subjects with intermittent or mild asthma. Group 2 ($n = 33$): subjects with asymptomatic childhood asthma who had not demonstrated any asthma-related symptoms for more than 10 years. Group 3 ($n = 47$): non-asthmatic subjects who had atopic dermatitis or allergic rhinitis, with a RAST score for *Dermatophagoides farina* (*Def*) of ≥ 2 . Group 4 ($n = 53$): normal subjects who did not have either asthma or atopic disease but a *Def* RAST score ≥ 2 . Group 5 ($n = 42$): non-asthmatic patients with cedar pollinosis a RAST score for *Def* of ≤ 1 and for Japanese cedar pollen of ≥ 2 . Group 6 ($n = 102$): normal subjects without asthma or atopic disease, with a RAST score for *Def* of ≤ 1 . Note the logarithmic scale of PC₂₀. Each data point represents one subject, while horizontal lines represent the mean values of each group. * $P < .05$, ** $P < .01$.

PC20 and RAST Score for Def

We also examined differences in PC₂₀s by RAST score for *Def* in groups 1 to 4 sensitized by *Def* (Fig 2a) (RAST score 2 to 6, n = 164). The mean values of PC₂₀ of each group of RAST score for *Def* 2 to 6 were 8.49, 5.88, 5.12, 4.22 and 6.40 mg/mL, respectively, which were not significantly different. We also examined serum-ECP classified by RAST score for *Def* (2 to 6). The mean values were not significant (Fig 2b).

DISCUSSION

The main findings of the present study were (1) sensitization to mite antigen may be important for the development of AHR in young adults, and (2) following the development of asthma, AHR does not disappear after a long period even if asthma-related symptoms disappear.

Differences in sensitivity to house dust mite antigen were compared between subjects of groups 3 and 5 who both had atopic disease and between groups 4 and 6 consisting of normal subjects. In groups 3 and 5, we investigated whether IgE-RAST for *Def* was positive or negative in subjects with atopy. During the pollen season, Jcp is an antigen known to cause pollinosis, clinically manifested by allergic con-

Table 2. Contribution of Each Antigen to PC₂₀ (multiple regression analysis)

Antigen	Regression Coefficient	t-Value	P Value
<i>Dermatophagoides farinae</i> *	-0.468	-5.221	<0.0001
<i>Aspergillus fumigatus</i>	-0.090	-0.213	0.8314
<i>Candida albicans</i>	0.277	1.198	0.2326
Dog hair	-0.229	-1.062	0.2896
Cat hair	0.034	0.245	0.8064
Japanese cedar pollen	-0.004	-0.037	0.9701

* Only *Dermatophagoides farinae* was a significant antigen contributing to AHR.

junctivitis and allergic rhinitis. All subjects were studied out of the pollination season. It is known that Jcp antigen causes pollinosis but does not asthma.²⁴ We suggest that Jcp antigen does not increase AHR. The reason could be the size of the pollen. The cedar pollen is about 20 to 40 μm in diameter and particles larger than 10 μm in diameter do not usually deposit into the lower respiratory tract as they are usually filtered in the nasal or oral cavity.²⁴ Furthermore, Jcp might not cause allergic inflammation in the respiratory tract through an antigen-antibody reaction. This may explain the negative AHR in patients with cedar pollinosis in subjects of Group 5 who were only Jcp RAST-positive. Since we did not show in this study any data that support a correlation between airway inflammation and a specific anti-

gen, such organ-specific sensitization of antigens was not very clear. In groups 4 and 6, we examined differences in sensitivity to *Def* in normal subjects who did not suffer from asthma or atopic disease. In group 4, consisting of normal subjects sensitized by *Def*, although some subjects were AHR negative, many were AHR positive. This tendency was similar to that of group 3; however, the distribution of PC₂₀ of these subjects was not bi-modal. It is possible that in AHR-positive subjects the inflammatory process in the airways and lung induced by the mite antigenic material may have by itself triggered AHR. It would have been perhaps better to study a control group of asthmatics who had mild intermittent asthma, but were dust-mite negative, and also asthmatics who were asymptomatic who were dust-mite negative. All subjects who had asthma in this study were dust-mite positive.

In some countries, such as Australia and New Zealand, immediate allergy manifested by skin reaction is positive in no less than 45% of children.¹⁰ Furthermore, the percentage of subjects positive for antibodies against indoor allergens (eg, *Def* and cat hair) correlates positively with both the frequency and severity of asthma,^{11,12} suggesting that antigen sensitization is an important factor in the onset of asthma. Although subjects of groups 1–4 were all *Def* RAST positive, AHR in groups 1 and 2 with current and past histories of asthma was higher than in subjects of groups 3 and 4 who did not have a history of asthma; therefore, developing asthma requires AHR

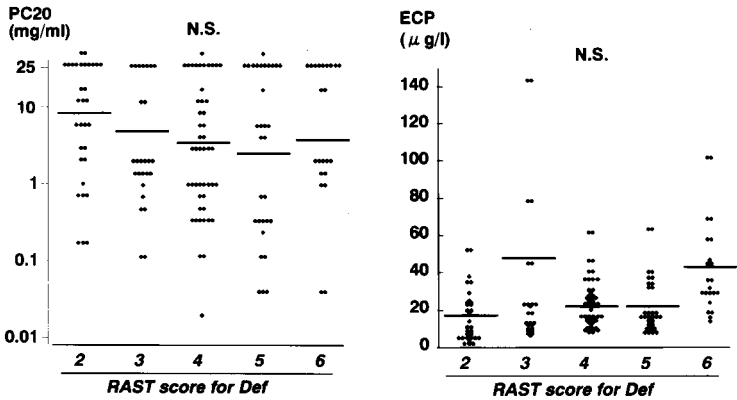


Figure 2. Left panel: Relationship between the degree of RAST positivity and PC₂₀ in *Def* positive subjects. Note the logarithmic scale of PC₂₀. Each data point represents one subject, while horizontal lines represent the mean values of each group. There was no significant correlation between positive sensitized groups (scores 2 to 6). Right panel: Relationship between the degree of RAST positivity and serum ECP in *Def* positive subjects. There was no significant correlation between positive sensitized groups (scores 2 to 6). Each data point represents one subject, while horizontal lines represent the mean values of each group.

greater than a set threshold. Konno et al²⁵ indicated that airway eosinophilic inflammation of asthmatics is greater than that in non-asthmatics.²⁵ These results suggest that the greater AHR of non-asthmatics sensitized *Def*, like groups 3 and 4 is established by increasing airway inflammation, asthmatic symptoms then appear, leading to a diagnosis of "bronchial asthma." It is predictable that subjects with mild active asthma should have more AHR than those who are asymptomatic. Our results showed that PC₂₀s were not different between young adults with intermittent mild asthma (group 1) and those with a history of childhood asthma (group 2). The reason for this finding is that the severity of asthma of group 1 was mild intermittent, and the subjects were examined at the time when asthma-related symptom were absent. If we set up more severe asthmatics as group 1, significant differences might have been obtained. However, the mean values of PC₂₀ in these two groups were significantly lower than those of groups 3–6, ie., subjects of groups 1 and 2 had a higher AHR. These results indicate that even in the absence of asthma-related symptoms at the age of 20 to 29 years, subjects with histories of childhood asthma seem to have persistently high AHR for several years (more than 10 years), which was equivalent to that of subjects with intermittent mild asthma. The definition of remission of asthma is controversial. Many studies have investigated the natural history of asthma including those who "outgrow" of asthma by comparing the state of asthma symptoms during two periods (0 to 5 years of age and 5 to 40 years of age with duration of 5 to 15 years).^{15–17} No study, however, has provided a proper definition of "outgrow" of asthma.

Subjects with allergic rhinitis have been shown to have a greater AHR than those without histories of atopic symptoms, but have specific IgE antibodies. Our results, however, showed that PC₂₀s were not different between group 3 and group 4. This might be due to a similar degree of antigen-

antibody reaction in the airway of individuals of these two groups.

There is convincing evidence for a significant correlation between atopy and incidence of asthma.²⁶ Sears and coworkers⁷ reported that AHR to MCh correlated with IgE-RIST in 562 children aged 11 years in New Zealand. Grainger et al,²⁷ however, indicated that IgE-RIST could explain only 10% of AHR. Our results showed a good correlation between IgE-RIST in peripheral blood and eosinophil count as well as serum ECP. There was, however, no correlation between AHR and any of these factors. IgE-RIST, eosinophil count, and ECP in peripheral blood do not reflect the state of asthma or AHR as also indicated by Marks et al²⁸; however, ECP in airways may correlate to AHR.²⁹ This topic is currently being investigated in our laboratory using induced sputum samples.

With regard to the relationship between antigen specificity and AHR, Soriano et al³⁰ recently measured AHR by methacholine challenge test in normal European subjects aged 22 to 44 years, and determined the presence of sensitivity to antigens of cat hair, *Dermatophagoides pteronyssinus*, Cladosporium, Alternaria, timothy grass, olive, birch and Parietaria or ragweed by IgE-RAST or skin test. Their results showed a strong correlation between AHR and three specific antigens: timothy grass, *D. pteronyssinus*, and olive. In most cases, asthma in children or young adults is of the atopic type,³¹ and the major allergen that causes asthma all over the world is considered to be house dust mite.^{10,31} Our present study examining the involvement of six specific antigens showed that only *Def* was a significant antigen contributing to AHR. While cat hair is one of the important specific antigens in AHR, the contribution of this antigenic material to PC₂₀ was insignificant in this study. This finding may probably be due to the low prevalence of cat ownership in Japan and the positive rate of sensitization of subjects. The other antigens (eg, cockroach antigen) have also been shown to be contributory, but they may not explain all of

AHR even if it is assumed that we examined more antigens.³⁰ Only 15.9% of AHR could be explained even if the contribution of all six tested antigens was combined together, as shown by multiple regression analysis. Although sensitization with *Def* is important for AHR, it can not explain all of AHR. Thus, after sensitization with *Def*, airway inflammation must occur to develop a positive AHR.²⁹

In this regard, Takeda et al³² examined the correlation between AHR and IgE-RAST score to mite in children, but the results were negative. Similarly, in this study, the correlation between the degree of positivity to *Def* antigen and AHR was insignificant. The reason for the lack of correlation is not clear, but it may be that IgE-RAST shows the allergic state of the system as well as other peripheral indices (eosinophil counts, ECP and IgE-RAST).

Finally, it should be cautioned that development of AHR is not synonymous with the onset of asthma,³³ and this study was an approach to examine the mechanisms of AHR only. Furthermore, a positive IgE-RAST does not always indicate that the antigen also causes asthma.³⁴ The present work showed that most of mite-sensitized subjects even those who had no asthmatic symptom were AHR-positive. Asthmatics had greater AHR than subjects without asthmatic symptoms. We suggest that those subjects with greater AHR who develop airway inflammation would show asthma-related symptoms and will be diagnosed as "bronchial asthma." Sensitization to *Def* antigen may therefore be one of the risk factors for the development of airway hyperresponsiveness and asthma. Because asthma is a chronic inflammatory disorder of the airways, airway inflammation is also a key role in developing AHR and asthma.²¹ While no data were presented in this study on airway inflammation, our next step in this direction is to investigate the interaction between airway inflammation, AHR and asthmatic attacks in young adult subjects.

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