Original

An Analysis of Factors Associated with Airway Hyperresponsiveness in Mild to Moderate Asthma

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Abstract: The aim of this study was to examine the association between airway hyperresponsiveness (AHR) in asthmatic patients and various factors, including the duration of asthma, the thickness of the epithelial reticular basement membrane (Rbm), and eosinophilic inflammation or total IgE in blood. We measured the thickness of Rbm in bronchial biopsy specimens. Spirometric tests and histamine challenge tests were also performed to evaluate airflow obstruction and AHR. Multiple regression analysis was used to assess the correlation between PC20 histamine values with duration of asthma, thickness of Rbm, eosinophil percentage in sputum and blood, and total IgE in blood. Furthermore, we used stepwise regression analysis to determine which factors were closely correlated with AHR. This study showed that AHR was most associated with the thickness of Rbm. The results suggest that the prevention and improvement of the airway structural changes should be considered as one of the targets of asthma treatment.

Key words: asthma, airway hyperresponsiveness, airway structural change, epithelial reticular basement membrane, steroid inhalation

Introduction

One of the characteristics of bronchial asthma is airway hyperresponsiveness (AHR)¹⁻⁴⁾, therefore effective inhibition of AHR would be very useful in the treatment of asthma. Various factors have been reported to be associated with AHR: eosinophilic inflammation; structural change of airways; duration of asthma; and IgE concentration.

High levels of eosinophils in sputum and blood have been detected in asthmatic patients, even in those not having attacks⁵⁾. Furthermore, thickening of the epithelial reticular basement membrane (Rbm) of the airway wall was also found even in patients with mild asthma^{6,7)}. Some studies have suggested that the inflammation and detailed background mechanisms which induce structural changes of the airway wall might also induce AHR⁸⁻¹⁰⁾. In addition, other reports have indicated that deterioration of respiratory function is influenced by the duration of asthma and the IgE concentration¹¹⁾. It has recently been suggested that anti-inflammatory treatment is the most important treatment for asthma^{11,12)}. However, some investigators have reported that airway hyperresponsiveness could not be reduced despite intensive treatment with steroids^{7,13-15)}. Therefore, it is not clear which of these factors are strongly contributing to AHR in patients with asthma.

Clinically, we are not sure which specific therapeutic target we should aim for in the treatment of asthma: inhibition of airway inflammation; prevention or improvement of airway remodeling; reduction of IgE concentration; or other targets. We would like to clearly identify the factors which are closely associated with AHR so that therapy can be more accurately prescribed.

Materials and Methods

Subjects

We examined 32 patients (age range, 20 to 67) with mild to moderate asthma (Table 1). Ten patients had been treated with inhaled corticosteroids (200 to 800 g / day beclomethasone) and 22 patients used only bronchodilators to alleviate their asthmatic symptoms. Asthma was diagnosed and classified according to Guidelines for the Diagnosis and Management of the Second Expert Panel Report, 1997¹⁶⁾ with reversibility of forced expiratory volume in one second (FEV₁) in response to bronchodilation of > 15%. All subjects were non-smokers. No subjects were suffering from chronic obstructive pulmonary disease (COPD), bronchiectasis or inactive pulmonary tuberculosis, which was confirmed by symptom checks, physical examinations, chest roentgenographic and computed tomography (CT) examinations. All subjects were informed of the details and their consents were obtained prior to the commencement of this study.

Study design

Patients were screened by history, physical examination and respiratory function. After ascertaining that their asthma conditions were stable and their medications had remained unchanged for at least three months, a standard histamine challenge test and bronchoscopy were performed. First, we evaluated the relationship between hyperresponsiveness (PC₂₀ histamine) and other factors (duration of asthma, thickness of the epithelial reticular basement membrane (Rbm), eosinophil level in induced sputum (%spEo) and in blood (%seEo), and total IgE by multiple regression and stepwise analysis.

Table 1. Subject characteristics

	32 asthmatics
Age (yrs)	37.1 (12.5)
Sex M : F	18 : 14
Atopy : Non-atopy	14 : 18
BDP (+) : (-)	10 : 22
%FEV ₁ (%)	77.4 (14.1)
PC ₂₀ histamine (μg/ml)	1881 (2833)

Definition of abbreviations :

BDP=beclometasone dipropionate

PC₂₀ histamine=concentration of histamine causing 20% decrease in FEV₁

FEV₁ forced expiratory volume in one second

%FEV₁=FEV₁/predicted×100

Values are expressed as mean (SD).

Respiratory function tests

Spirometric tests were performed with a spirometer (Autospiro AS-300, Minato Co., Osaka, Japan) to measure forced vital capacity (FVC) and forced expiratory volume in *one* second (FEV1). The tests were repeated at least three times and the highest acceptable measurement was used for analysis.

Histamine challenges

Bronchial responsiveness to histamine was measured by a method described by guidelines produced by the Japanese Society of Allergology¹⁷⁾. Subjects inhaled doubling concentrations of histamine by De Villbis nebulizer for two minutes by tidal breathing. Increasing concentrations of histamine were administered until FEV1 decreased by >20% of the baseline value. Results were expressed as the provocative concentration that caused a 20% decrease in FEV1 from the post-saline baseline (PC20 histamine). PC20 histamine values were log-transformed before analysis.

Bronchial biopsy

Bronchial biopsy was performed in all subjects using a bronchofiberscope examination (BF type240, Olympus Co., Tokyo, Japan). Before bronchofiberscopic examination, we ensured an absence of asthma attacks in the subjects. We used lidocaine (20 ml of 2 % solution for local anesthesia), atropine (0.5 mg intramuscularly) and midazolam (0.1 mg/kg by slow intravascular infusion) as premedications. We started bronchofiberscopy by monitoring the percutaneous oxygen saturation and pulse by pulse oximeter (Pulsox, Teijin Co., Tokyo, Japan), as well as respiratory rate and blood pressure. Four to five biopsy specimens were taken from the subsegmental bronchial bifurcation in the right lower lobe in each subject. The outer sites of biopsy specimens were stained by eosin to ensure proper orientation for cross-sectioning. After fixation in 10% buffered formalin solution at room temperature, the specimens were embedded in paraffin, cut into five micrometer sections with a microtome, and stained with hematoxylin-eosin. Three measurable stained sections were obtained per subject. Rbm thickness was quantified by measuring the area of the whole Rbm (from the base of the bronchial epithelium to the outer limit of the reticular lamia) and the length of the true basement membrane of the Rbm area using a computerized image (Apple Computer, Cupertino, CA, NIH image). Rbm thickness was calculated by the Rbm area/the length of the basement membrane. We used the average value from three to five measurements in three to five stained sections per subject for analysis. A single pathologist examined all sections blindly.

Sputum induction and processing

Sputum induction was performed as described previously¹⁸⁾. Briefly, sputum was induced by inhalation of increasing concentrations of hypertonic saline (0.9, 1.8, 3.0, 4.0, and 5.0%) until an adequate sample was collected. Subjects were instructed to cough deeply in five-minute intervals. Gargling before and between each induced cough was encouraged to minimize salivary contamination. In sputum, cell plugs were separated from saliva and treated with Wright-Giemsa stain so that inflammatory cells could be counted. The percentage of inflammatory cells was determined by counting 900 cells under a light microscope.

Table 2. Factors for airway hyperresponsiveness —multiple regression analysis—

	mean (SD)	regression coefficient	95%low	95%high	p value	F value
duration (years)	8.5(12.5)	−0.008	−0.028	0.011	0.39	0.78
Rbm thickness (μm)	7.1(2.3)	−0.149	−0.281	−0.016	0.03	5.33
%spEo (%)	23.9(22.6)	−0.008	−0.021	0.004	0.19	1.85
%seEo (%)	6.8(7.7)	0.017	−0.018	0.052	0.33	0.97
IgE in serum (IU)	558.6(790.8)	0.00017	−0.00016	0.0005	0.31	1.10

Definition of abbreviations:

Rbm=epithelial reticular basement membrane

%spEo=percentage of eosinophils in induced sputum

%seEo=percentage of eosinophils in blood

Values are expressed as mean (SD)

Fig. 1. Relationships between bronchial Rbm thickness and logPC20 histamine in patients with mild to moderate asthma.

The logPC20 histamine was negatively correlated with Rbm thickness
($p=0.0009$ $R=-0.56$)

Statistical analysis

All statistical analyses were done using Stat View software version 4.5 (Abacus Concepts, Berkeley, CA) on a Macintosh computer. Values were expressed as the mean $\pm$ standard deviation (SD). Multiple regression analysis was performed to assess the contributions of duration of asthma, thickness of Rbm, percentage of eosinophils in sputum or blood, and total IgE to AHR (logPC20 histamine). Differences with p values less than 0.05 were considered significant.

Results

Subject characteristics

The characteristics of the subjects are presented in Table 1 including: age; sex; presence of atopy; use of beclomethasone dipropionate (BDP); %FEV₁; and PC20 histamine value in 32 patients with asthma. Bronchial biopsy was performed in all subjects. PC20 histamine values ranged from 12 to 10,000 $\mu\text{g/ml}$ (mean $\pm$ SD: $1881 \pm 2833 \mu\text{g/ml}$).

Correlation between PC20 histamine and duration of asthma, thickness of Rbm, %spEo, %seEo, and IgE

We examined the correlations between PC20 histamine and five factors (duration of asthma, thickness of Rbm, %spEo, %seEo, and IgE) using multiple regression analysis. The value of PC20 histamine was significantly correlated with the thickness of Rbm ($F=5.33$, $p=0.03$). However, the other factors showed no significant correlation (Table 2). We adopted only the thickness of Rbm for the linear formula by stepwise regression analysis ($\log\text{PC20 histamine} = 4.11 - 0.19 \times \text{thickness of Rbm}$; $p=0.0009$ $R = -0.56$) (Fig. 1).

Discussion

In this study, we analyzed 32 mild or moderate asthmatic patients by a stepwise regression method to clarify which factors are correlated most closely with AHR. We examined the duration of asthma, the thickness of Rbm, eosinophil percentage in sputum, eosinophil percentage in peripheral blood and IgE in serum. These results showed that the thickness of Rbm most affected PC20 histamine, which was the index for AHR, considering the regression coefficient, p value and F value of the multiple regression analysis. These results also suggested that the thickening of Rbm might be strongly associated with AHR. Previous studies also indicated that the structural changes of the bronchial wall might induce airflow limitation and AHR in asthma^{9, 10}. Furthermore, thickening of the Rbm was reported to parallel the thickness of the whole airway wall¹⁹. The findings of the present and previous studies suggest that the airway structural changes could play a very important role in the induction of AHR and airflow limitation.

Several previous reports have suggested that airway eosinophilic inflammation is an important factor in deterioration of respiratory functions and development of persistent airway narrowing in patients with asthma^{20, 21}. We also speculate that eosinophil inflammation in airways may cause hyperresponsiveness by inducing structural changes. In this study, eosinophil percentage in sputum had the second largest correlation with PC20 histamine after the thickness of bronchial Rbm, however the correlation was not statistically significant. We evaluated the eosinophil percentage in sputum at only one time point. While eosinophilic inflammation induces airway structural changes, these changes may depend on both the intensity and duration of inflammation. This study did not show a statistically significant association between eosinophil percentage in sputum and AHR. We are also aware that some examinations have suggested that AHR did not improve with steroid therapy while eosinophilic inflammation of airway was decreasing^{11, 22-24}. To obtain conclusive evidence, we would need new strictly designed studies to examine whether eosinophilic inflammation can be associated with AHR.

In this study we suggested that the thickening of bronchial Rbm might be the factor

most closely associated with AHR. The structural change of airway walls could be associated with the deterioration of respiratory function of asthma through AHR. The results suggest that the prevention and improvement of the airway structural changes should be considered as an important target for asthma treatment.

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[Received January 18, 2002 : Accepted February 20, 2002]