◎原 著

An increase in bronchial hyperresponsiveness by cigarette smoking in elderly patients with asthma

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Abstract : Influence of cigarette smoking on the pathogenesis of asthma in the elderly remains controversial. This study was undertaken to clarify the influence of cigarette smoking on IgE-mediated allergy, bronchial hyperresponsiveness, and the generation of leukotrienes B4 (LTB4) and C4 (LTC4) by leukocytes in 48 elderly patients (25 patients with a history of smoking for more than 20 years and 23 never-smokers) with asthma over the age of 70 years. 1. The incidence of positive IgE antibodies for inhalant allergens was significantly higher in asthmatics with a history of smoking than in never-smokers of asthmatics. 2. In bronchial hyperresponsiveness for methacholine, the Cmin was significantly lower in ever-smokers than in never-smokers of elderly asthmatics. 3. The generation of LB4 was significantly increased in patients with a history of smoking compared with those without a smoking history. However, the difference in LTC4 generation was not significant between ever-smokers and never-smokers of asthmatics. These results suggest that cigarette smoking increases bronchial hyperresponsiceness through IgE-mediated allergy and LTB4 generation.

Key words : cigarette smoking, IgE-mediated allergy, elderly asthmatics, bronchial hyperresponsiveness

Introduction

It has been reported that exposure of rats in utero to aged and diluted sidestream cigarette smoke (SS) induces airway hyperresponsiveness (AHR) in the offspring[1]. SS has an adjuvant effect on eosinophils, allergen-specific antibodies, and Th2 cytokines (interleukin-4 and interleukin-10) in adult mice previously sensitized by injection with ovalbumin and aluminum hydroxide[2]. These results might suggest that SS enhances IgE-mediated allergic reaction. Cigarette smoke-induced airway hyperresponsiveness is not

dependent on elevated immunoglobulin and eosinophilic inflammation in a mouse model of allergic airways disease[3].

Smoking is also known to increase the inflammatory burden of the lower respiratory tract through a number of related but separate mechanisms. These include the recruitment of increased numbers of inflammatory cells, alteration in cell subtypes, enhancement of some cellular functions, and pro-inflammatory mediator release[4]. Exposure to cigarette smoke increased sensitization to food allergens in the few years of life[5], but not associated with sensitization to inhaled allergens[6]. However, it has been shown that cigarette smoke exposure enhanced existing symptoms of allergic airway disease (i. e., airway hyperresponsiveness, AHR), as described above[3].

In this study, the influence of cigrette smoke on IgE-mediated allergy in relation to bronchial hyperresponsiveness, and the generation of leukotrienes B4 (LTB4) and C4 (LTC4) by leukocytes was examined in elderly asthmatics with a histoly of smoking for more than 20 years.

Subjects and Methods

The subjects of this study were 48 asthmatics (25 women) over the age of 70 years. Twenty five of them had a history of smoking for more than 20 years (49.1 pack-year). All of them were ex-smokers. Their mean age was 74.3 years and age at onset of the disease was 58.1 years. The remaining 23 patients were never-smokers with mean age of 74.4 years, and age at onset of 55.7 years. Asthma was diagnosed according to the definition proposed by the American Thoracic Society[7], as previously described[8]. The asthmatic subjects were stable with no changes in asthma symptoms and medication for at least 1 month, except for the use of short acting β_2 agonists.

Serum IgE was measured by radioimmunosorben test (RIST), and IgE antibodies specific to 12 common aeroallergens including house dust mite, pollens, moulds, and animal danders were measured using the Pharmacia CAP system (Pharmacia Diagnostics AB, Uppsala, Sweden).

Bronchial responsiveness to methacholine was measured by an Astograph (TCK6100, Chest Co). Different concentrations of methacholine (49, 98, 195, 390, 781, 1563, 3125, 6250, and 12500 mcg/ml) were prepared for bronchial challenge according to the method used by Chai et al.[9]. The increase of total respiratory resistance (Rrs) after methacholine inhalation was measured by the oscillation technique. A methacholine concentration causing a significant increase in Rrs was assessed as Cmin (minimum concentration). All medications was stopped 12 hours prior to examination.

The amount of leukotrienes (LTs), LTB4 and LTC4, generated by peripheral leukocytes was assessed as described previously[11-12]. Five milliliters of 6% dextran (molecular weight ~200,000 kDa) (Nakalai Tesquc Inc., Kyoto, Japan) were added to 20 mL heparinized peripheral blood, and the resultant mixture was incubated for 1 hour at room temperature. The leukocytes-rich plasma supernatant was then removed and used. The number of cells was adjusted to $5x10^6$ cells/mL in Tris CM buffer, and the cells were then incubated with 1 μg calcium ionophore A23187 (Sigma, St Louis, Mo) for 15 minutes at 37 ℃. After a 4x volume of prechilled ethanol (final, 80% ethanol) was added, this was centrifuged at 3000 rpm for 30 minutes. A syringe filter (Toyo Roshi Co, Tokyo, Japan) was used to draw the supernatant, and the filtrate was decompressed and dried to a solid. Quantification of LTB4 and LTC4 was performed by HPLC and UV

spectroscopy, with the method of Lam et al.[13]. Quantities of LTB4 and LTC4 were expressed as nanogram per $5x10^6$ cells.

Spirometry was performed by means of a CHESTAC 33 (Chest Co, Tokyo, Japan) linked to a computer. The following measurements were peformed on all subjects : forced vital capacity (FVC), FEV_1, and FEV_1/ FVC. Informed consent was obtained from all subjects and the study protocol was approved by the ethics committee of our institution.

Statistically significant differences of the mean were estimated using the unpaired Student's t test and χ^2 test. A p value of <0.05 was regarded as significant.

Results

Serum IgE level was not significantly different between ex-smokers (451 IU/ml) and never-smokers (440 IU/ml) of elderly patients with asthma. The RAST score was positive in 21 for house dust mite, 8 for Candida albicans, and 5 for other alleregns of 48 elderly asthmatics. The positive RAST score for inhalant allergens was observed in 22 of 25 asthmatics (88.0%) with a history of smoking. In contrast, the positive RAST was found in 12 of 23 asthmatics (52.2%) without a smoking history. The frequency of patients with a positive RAST score for inhalant allergens was significantly more increased in patients with a history of smoking than those without a smoking history ($P<0.05$), as shown in Fig. 1.

Regarding bronchial hyperresponsiveness to methacholine, the mean Cmin of methacholine was significantly lower in ex-smokers (949 μg/ml) than in never-smokers (1677 μg/ml) of the elderly asthmatics. The number of asthmatics with Cmin less than 780 μg/mL was significantly larger in ex-smokers than in never-smokers ($p<0.02$) (Fig. 2).

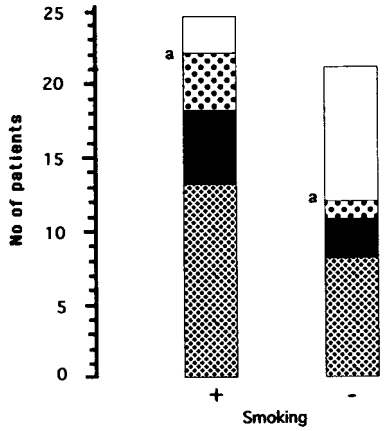

Fig. 1. Incidence of specific IgE antibodies against inhalant allergen in patients with asthma in relation to smoking. (▨): house dust mite, (■): Candida albicans, (▩): others.a:p<0.05.

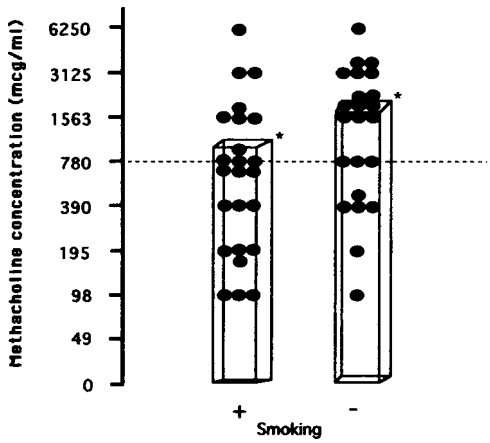

Fig. 2. Bronchial hyperresponsiveness to methacholine in elderly asthmatics in relation to cigarette smoking. *Difference in number of patients with Cmin,780mcg/ml was significant between the two groups (p<0.02).

The generation of leukotriene B4 (LTB4) by peripheral leukocytes was significantly larger in ex-smokers (107.7 ng/$5x10^6$ cells) than in never-smokers (77.3 ng/$5x10^6$ cells) of elderly asthmatics. However, any significant difference was not found in the generation of leukotriene C4 (LTC4) between ex-smokers and never-smokers (Fig. 3).

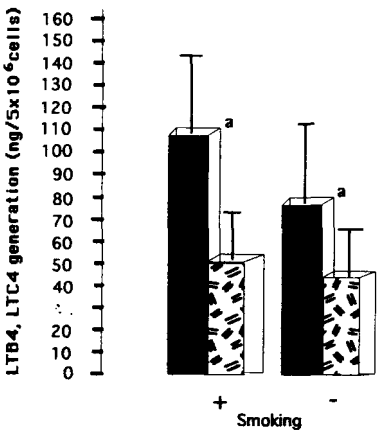

Fig. 3. The generation of LTB4 (■) and LTC4 (▨) by leukocytes in elderly patients with asthma in relation to smoking. a; p<0.001

Discussion

It has been reported that cigarette smoke exposure is associated with the development of asthma in children. Environmental tobacco smoke (ETS) exposure is found to be associated with respiratory symptoms in children[6,14]. The prevalence of physician-diagnosed asthma was significantly larger in never-smokers with childhood ETS exposure compared with that in nonexposed subjects[15].

It has been found that higher incidences of asthma were observed in current and former smokers, compared with never-smokers. In these cases, what role does cigarette smoke play?

Exposure of rats in utero to aged and diluted sidestream cigarette smoke (SS) induces airway hyperresponsiveness (AHR) in the offspring[1]. Cigarette smoke-induced airway hyperresponsiveness was not associated with elevated immunoglobulin and eosinophilic inflammation[3].

In this study, significantly higher incidence of patients having IgE antibodies specific for inhalant allergens was found in patients with a history of smoking compared with those without a smoking history. Bronchial hyperresponsiveness to methacholine was also significantly more increased in asthmatics with a history of smoking compared with those without a smoking history. Furthermore, the generation of LTB4 by leukocytes was significantly more increased in ex-smokers than in never-smokers of elderly asthmatics. Our previous studies have shown that the presence of specific IgE antibodies for inhalant allergens enhances LTC4 generation, bronchial hyperresponsiveness and the relationship between LTC4 and airway obstruction[12], and further that increased generation of LTB4 is closely related to bronchial hyperresponsiveness in patients with asthma[11]. These results may suggest that cigarette smoke influences IgE-related allergy including production of IgE antibodies, bronchial hyperesponsiveness, and generation of LTs in elderly patients with asthma.

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高齢者喘息における喫煙による気道過敏性の亢進について

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高齢者喘息の病態におよぼす喫煙の影響についてはなお不明な点が多い。本論文では，IgEにmediateされるアレルギー反応，気道過敏性，ロイコトリエンB4（LTB4），C4（LTC4）産生に及ぼす喫煙の影響について，48例（20年以上の喫煙歴のある症例25例，非喫煙例23例）の70歳以上の高齢者喘息症例を対象に検討した。1．吸入抗原に対する特異的IgE抗体の陽性頻度は，喫煙歴のある症例で，非喫煙例と比べ有意に高い値を示した。2．メサコリンに対する気道過敏性の検討では，平均Cminは，喫煙例（949 μg／ml）において非喫煙例（1677 μg／ml）に比べ有意に低い値を示し，喫煙例でより気道の過敏性が高いことが示された。3．LTB4の産生は喫煙例で非喫煙例に比べ有意に高い傾向を示したが，LTC4に関しては両群間に有意の差は見られなかった。以上の結果より，長期間の喫煙は，IgEにmediateされるアレルギーやLTB4産生の亢進などを通じて気道過敏性を亢進させる可能性が示唆された。