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## Cigarette smoking enhances LTB<sub>4</sub> generation by leucocytes associated with bronchial hyperresponsiveness in asthma in the elderly

Fumihiro Mitsunobu, Yasuhiro Hosaki, Kozo Ashida,  
Naofumi Iwagaki, Takuya Nagata, Makoto Fujii,  
Shingo Takata, Tadashi Yokoi<sup>1)</sup>, Masanori Hamada<sup>1)</sup>, and  
Yoshiro Tanizaki

Division of Medicine, <sup>1)</sup>Division of Rehabilitation, Misasa Medical Center,  
Okayama University Medical and Dental School

**Abstract :** Influence of cigarette smoking on leukotriene B<sub>4</sub> (LTB<sub>4</sub>) generation by leucocytes associated with bronchial hyperresponsiveness was examined in 110 patients with asthma. 1. The frequency of positive RAST score for inhalant allergens and LTB<sub>4</sub> generation by leucocytes were significantly larger in previous and current smokers than in nonsmokers. 2. In nonsmokers, bronchial hyperresponsiveness was significantly higher in asthmatics under the age of 49 years compared with that in patients between 50 and 59 years, between 60 and 69 years, and those over age 70 years. In contrast, no significant differences were present between bronchial hyperresponsiveness and patient age in previous and current smokers. 3. In patients over age 60, bronchial hyperresponsiveness was significantly higher in previous and current smokers than in nonsmokers. In these patients, LTB<sub>4</sub> generation was significantly more increased in previous and current smokers than in nonsmokers. 4. A significant correlation was observed in asthmatics of nonsmokers between LTB<sub>4</sub> generation and bronchial hyperresponsiveness. 5. LTC<sub>4</sub> generation was significantly higher in previous and current smokers than in nonsmokers in patients under age 49 years. These results show that in patients over age 60 years, cigarette smoking induces an increase in the generation of LTB<sub>4</sub>, leading to an increase in bronchial hyperresponsiveness.

**Key words :** cigarette smoking, asthma, LTB<sub>4</sub> generation, LTC<sub>4</sub> generation, bronchial hyperresponsiveness

## Introduction

Bronchial asthma is a disease characteristic of airway inflammation, resulting in transient wheezing and dyspnea, which are induced by bronchial hyperresponsiveness, an increase in mucus production, edema of mucous membrane, and bronchoconstriction. It is well known that cigarette smoking influences asthma symptoms. Environmental tobacco smoke (ETS) has been found to be associated with an elevated risk of wheezing, bronchitis symptoms, symptoms of bronchitis, and physician-diagnosed asthma, and that the association of ETS with dyspnea, wheeze, and asthma is revealed as a dose-dependent increase with hours of exposure<sup>1</sup>. Recent reports have reinforced previous conclusions that exposure to ETS causes the onset of childhood asthma and exacerbation of symptoms throughout life<sup>2-4</sup>. In contrast, the effects of ETS exposure on adult asthma have not yet been investigated extensively<sup>5</sup>.

The possibility for risk of active smoking for developing adulthood asthma also remains controversial. Adult onset asthma has been reported not to be associated with ever-smoking<sup>6</sup>. However, current smoking was found to increase asthma severity<sup>7</sup>. Furthermore, several studies have supported higher incidence of asthma in current and former smokers, compared with never-smokers<sup>8-10</sup>.

Our previous studies have shown that the high resolution computed tomography (HRCT) lung densitometry, which correlated with parameters of airflow limitation and lung volume, but not with lung transfer factor, was informed by aging, disease severity, and cigarette smoking<sup>11-14</sup>.

In the present study, the influence of cigarette smoking on leukotriene B<sub>4</sub> (LTB<sub>4</sub>) generation by leukocytes, which is related to bronchial hyper-

responsiveness, was examined in patients with asthma.

## Subjects and Methods

The subjects of this study were 110 patients (43 females and 67 males) with asthma. Of all patients, 29 were previous and current smokers (mean age 65.1 years with a smoking history of 42.4 pack-year). The residual 91 patients (mean age 59.3 years) were never-smokers.

Bronchial hyperresponsiveness to methacholine was measured in all subjects by an Astograph (TCK 6100, Chest Co) when they were asymptomatic. Different concentrations of methacholine (49, 98, 195, 309, 781, 1563, 3125, 6250, 12500 and 25000  $\mu\text{g}/\text{ml}$ ) were prepared for bronchial challenge according to the method used by Chai et al<sup>15</sup>. The increase of total respiratory resistance (Rrs) after methacholine inhalation was measured by the oscillation method. A methacholine concentration causing a significant increase in Rrs was assessed as Cmin (minimum concentration). All medications were stopped 12 hours prior to examination.

The amounts of LTB<sub>4</sub> and leukotriene C<sub>4</sub> (LTC<sub>4</sub>) generated by the peripheral leukocytes were assessed as previously described<sup>16-18</sup>. Five milliliters 6% dextran (molecular weight ~200,000 kDa (Nacalai Teque, Inc.)) were added to 20 ml of heparinized peripheral blood, and the mixture was left for 1 hour at room temperature. The leukocytes-rich plasma supernatant was then removed and used. The number of cells was adjusted to  $5 \times 10^6$  cells/ml in Tris CM, and 1  $\mu\text{g}$  of calcium ionophore A23187 (Sigma, St Louis) was then added to the cell suspension. The solution was mixed and incubated for 15 minutes at 37°C. After a 4x volume of prechilled ethanol (final, 80% ethanol) was added. This was centrifuged at 3000 rpm for 30 minutes.

The filtrate through a syringe filter (Toyo Roshi Co) was decompressed and dried to solid. LTB4 and LTC4 were quantified by means of high-performance liquid chromatography as described by Lam et al<sup>19</sup>. Quantities of LTB4 and LTC4 were expressed as nanograms per  $5 \times 10^6$  cells.

Serum IgE was measured by radioimmunosorbent test (RIST), and IgE antibodies specific to aeroallergens including house dust mite, pollens, and moulds were measured using the Pharmacia CAP system (Pharmacia Diagnostics AB).

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

## Results

IgE-mediated allergy, bronchial hyperresponsiveness, and generation of LTB4 and LTC4 by leucocytes were compared in patients with asthma between previous and current smokers and never-smokers. The frequency of patients with a positive RAST score and generation of LTB4 by leucocytes were significantly larger in smokers than in nonsmokers. In contrast, serum IgE levels, bronchial hyperresponsiveness to methacholine, and LTC4 generation were not significantly different between smokers and nonsmokers (Table 1).

Table 1. IgE-mediated allergy and LTB4, LTC4 generation in patients with asthma in relation to cigarette smoking

|  | Smoker              | Nonsmoker          | Difference  |
|--|---------------------|--------------------|-------------|
| No of patients                                     | 29                  | 81                 | -           |
| Mean age (years)                                   | 65.5                | 59.3               | -           |
| Serum IgE (IU/ml)                                  | 597<br>(9-2929)     | 504<br>(2-5700)    | NS          |
| No of patients with a positive RAST score*         | 24<br>(82.8%)       | 48<br>(59.2%)      | $p < 0.001$ |
| Cmin of methacholine ( $\mu\text{g}/\text{ml}$ )   | 513                 | 471                | NS          |
| LTB4 generation ( $\text{ng}/5 \times 10^6$ cells) | 110.7<br>$\pm 26.6$ | 71.2<br>$\pm 31.0$ | $p < 0.001$ |
| LTC4 generation ( $\text{ng}/5 \times 10^6$ cells) | 54.9<br>$\pm 32.3$  | 45.1<br>$\pm 30.1$ | NS          |

\*A positive RAST for inhalant allergens. NS: not significant. Cmin: minimum concentration of methacholine inducing bronchospasm

In never-smokers of patients with asthma, bronchial hyperresponsiveness to methacholine was significantly higher in younger subjects under age 49 than in older subjects aged between 50 and 59 ( $p < 0.02$ ), between 60 and 69 ( $p < 0.05$ ), and over 70 years ( $p < 0.001$ ) (Fig. 1).

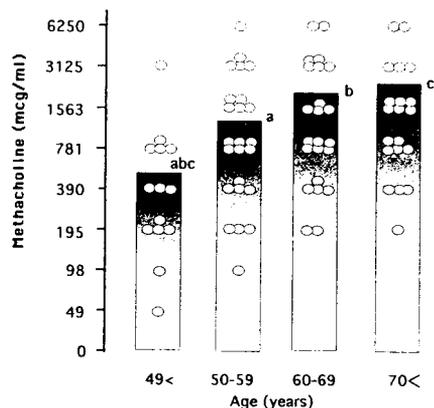


Fig. 1. Bronchial hyperresponsiveness to methacholine in nonsmokers of patients with asthma in relation to age. a:  $p < 0.02$ , b:  $p < 0.05$ , c:  $p < 0.001$ .

However, in previous and current smokers, any significant correlation was not observed between bronchial hyperresponsiveness and patient age (Fig. 2). A difference between nonsmokers and

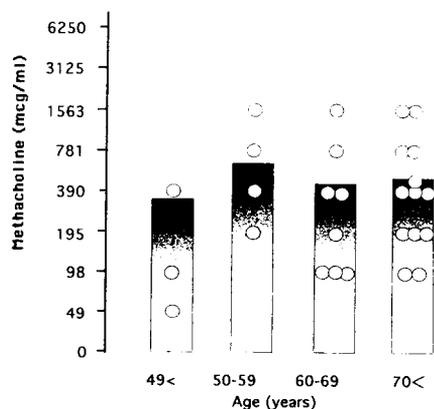


Fig. 2. Bronchial hyperresponsiveness to methacholine in smokers of patients with asthma in relation to age.

ever-smokers in bronchial hyperresponsiveness was not significant in patients under age 49, however, bronchial hyperresponsiveness in ever-smokers was significantly higher than that in

never-smokers of subjects aged between 60 and 69 ( $p < 0.05$ ) and subjects over age 70 (Fig. 3).

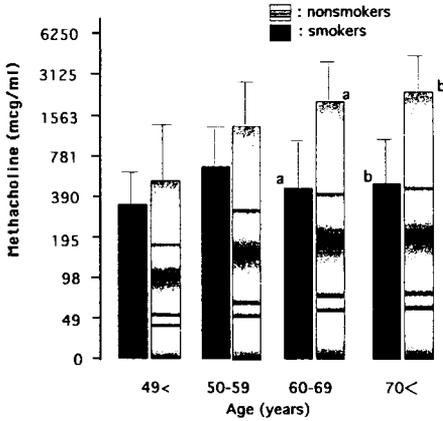


Fig.3. Comparison between nonsmokers and smokers in bronchial hyperresponsiveness to methacholine in patients with asthma in relation to age. a:  $p < 0.05$ , b:  $p < 0.001$ .

The generation of LTB<sub>4</sub> by leucocytes was not related to age in never-smokers of patients with asthma (Fig. 4). In contrast, the genera-

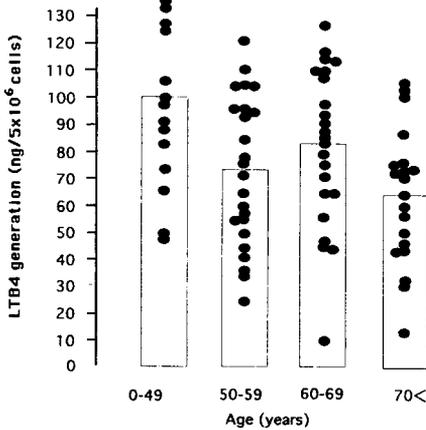


Fig.4 Leukotriene B<sub>4</sub> generation by leucocytes in nonsmokers of patients with asthma in relation to age.

tion of LTB<sub>4</sub> in ever-smokers was in general larger compared with that in never-smokers, however, there was no significant correlation between generation of LTB<sub>4</sub> and age (Fig. 5). A significant difference in LTB<sub>4</sub> generation was observed in patients aged between 60 and 69 ( $p < 0.01$ ), and those over age 70 ( $p < 0.001$ ):

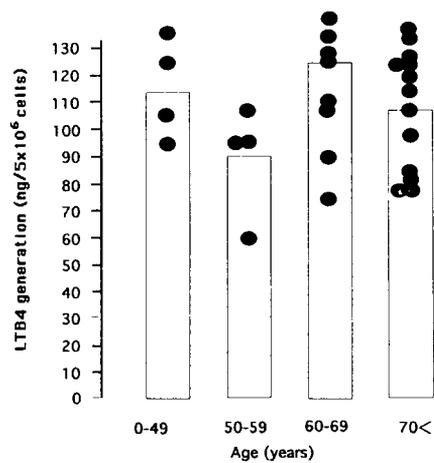


Fig.5 Leukotriene B<sub>4</sub> generation by leucocytes in smokers of patients with asthma in relation to age.

LTB<sub>4</sub> generation was significantly larger in ever-smokers than in never-smokers (Fig. 6).

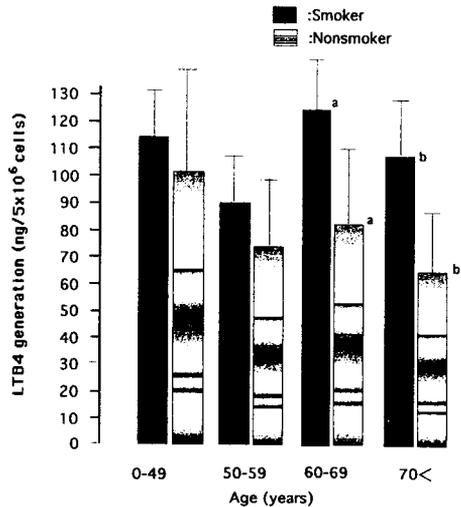


Fig.6 Leukotriene B<sub>4</sub> generation by leucocytes of patients with asthma in relation to age and cigarette smoking. a:  $p < 0.01$ , b:  $p < 0.001$ .

Bronchial hyperresponsiveness was significantly correlated with the generation of LTB<sub>4</sub> in never-smokers of asthmatics, suggesting that as the generation of LTB<sub>4</sub> is larger, bronchial hyperresponsiveness become higher, as shown in Fig. 7. The generation of LTC<sub>4</sub> by leucocytes was not related to age both in ever-smokers and never-smokers. However, LTC<sub>4</sub> generation was significantly larger in ever-smokers than in

never-smokers of younger patients under age 49 (Fig. 8).

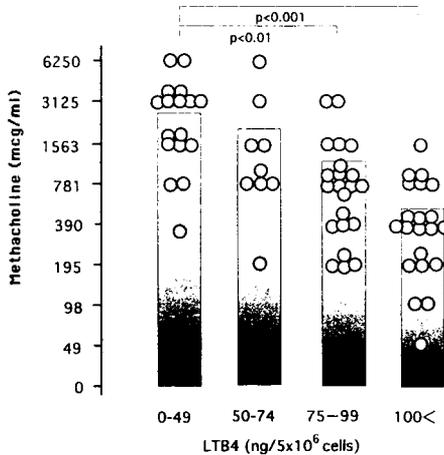


Fig. 7. Correlation between bronchial hyperresponsiveness to methacholine and LTB4 generation by leukocytes in nonsmokers of patients with asthma

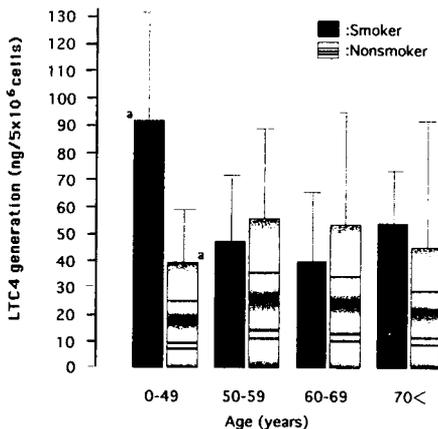


Fig. 8. Leukotriene C4 generation by leukocytes of patients with asthma in relation to age and cigarette smoking. a:  $p < 0.01$ .

## Discussion

It has been reported that incidence of asthma and disease severity were associated with cigarette smoke when these were compared with never-smokers<sup>9,10,20</sup>. It has been also suggested that adult onset of asthma was not associated with ever-smoking, however, current smoking

increased asthma severity<sup>7</sup>. Our previous studies have demonstrated that aging, disease severity, and cigarette smoke influence low attenuation area (LAA)  $< -950$  HU of the lungs on HRCT<sup>11-14</sup>. Regarding pulmonary function, air-flow limitation (% forced expiratory volume in one second (FEV1), FEV1/forced vital capacity), lung volume (% Residual volume (RV)), and lung transfer factor (% diffusing capacity for carbon monoxide (DLco)), of asthmatics relating to smoking, the parameters showing air-flow limitation were not found to be different between ex-smokers and never-smokers of elderly asthmatics. The %RV value was significantly larger in ex-smokers than in never-smokers of elderly asthmatics. The %DLco value was significantly lower in ex-smokers compared with that in never-smokers.

In the present study, effects of cigarette smoking on the generation of LTB4 by leukocytes was examined in patients with asthma. Our previous studies demonstrated that the leukocytes of patients with relatively more severe hyperresponsiveness to methacholine produced significantly more LTB4 than those of patients who are less hyperresponsive<sup>18</sup>. Although hyperresponsiveness to methacholine also significantly correlated with leukotriene C4 production, this correlation was weaker than that between methacholine hyperresponsiveness and LTB4 production. Another report showed that interleukin-8 induces bronchial hyper-responsiveness as well as airway neutrophil accumulation in guinea-pigs in vivo, and that this may be partly mediated by the release of LTB4<sup>21</sup>. The results obtained here suggest that long-term cigarette smoking enhanced the generation of LTB4 by leukocytes of elderly asthmatics over age 60, and that at the same time, bronchial hyperresponsiveness in these aged patients was significantly higher in ever-smokers than in never-smokers. Furthermore,

a significant correlation was observed in these patients between bronchial hyperresponsiveness and the generation of LTB<sub>4</sub>. These findings may suggest that cigarette smoking enhances LTB<sub>4</sub> generation, leading to an increase in bronchial hyperresponsiveness.

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**長期間の喫煙は高齢者気管支喘息における気道過敏性と関連した白血球LTB4産生を亢進させる**

光延文裕, 保崎泰弘, 芦田耕三, 岩垣尚史,  
永田拓也, 藤井 誠, 高田真吾, 横井 正<sup>1)</sup>,  
浜田全紀<sup>1)</sup>, 谷崎勝朗

岡山大学医学部・歯学部附属病院三朝医療センター  
内科, <sup>1)</sup>リハビリテーション科

気管支喘息110例を対象に, 長期間の喫煙の気道過敏性および白血球のロイコトリエンB4産生に及ぼす影響について検討した。1. 吸入抗原に対してRAST陽性を示す症例の頻度および白血球

のLTB4産生は, 喫煙例において非喫煙例に比べ有意に高い値を示した。2. 非喫煙例においては, その気道過敏性は49才以下の症例において50-59才, 60-69才および70才以上の症例に比べ, 有意に高い値を示した。しかし, 喫煙例においては, 年齢による気道過敏性の差は見られなかった。3. 60才以上の症例では, その気道過敏性は非喫煙例に比べ, 喫煙例で有意に高い値を示した。このような症例では, LTB4産生能も非喫煙例に比べ喫煙例で有意の亢進が見られた。4. 非喫煙例では, 気道過敏性とLTB4産生との間に有意の相関が見られた。以上の結果より, 60才以上の症例では喫煙がLTB4産生を亢進させ, その結果として気道過敏性が亢進してくることが推測された。